Factors Affecting Biodegradation Of 2-Chlorophenol Under Various Redox Conditions

BY

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FACTORS AFFECTING BIODEGRADATION OF 2-CHLOROPHENOL UNDER VARIOUS REDOX CONDITIONS

BY

SAIBAL KUMAR BASU

A Practicum submitted to the Faculty of Graduate Studies of the University of Manitoba in partial fulfillment of the requirements of the degree of

DOCTOR OF PHILOSOPHY

ABSTRACT

The aim of this thesis was to study factors potentially affecting the biodegradation of the toxic compound 2-Chlorophenol (2-CP) under anaerobic, aerobic and anoxic conditions. The work covered anaerobic batch and semi-continuous studies and continuous aerobic and anoxic experiments in sequencing batch reactors (SBR's). The 2-CP concentration ranged from 30 to 50 mg/L.

Under **anaerobic conditions**, digester sewage sludge exhibited a better dehalogenation potential than either the bleached kraft mill (BKM) effluent lagoon sediment or biomass from an anaerobic bioreactor treating BKM wastewater. Phenol was detected as the principal intermediate product which degraded via the benzoate pathway. Adapted sludge transformed 2-CP without a lag although dehalogenation rates did not increase linearly with the increase in biomass concentration. Sulphate reduction did not inhibit 2-CP dehalogenation and methanogenesis in batch cultures amended with 0.1% yeast extract. 10 mM bromoethanesulphonic acid (BESA) slowed the rates of 2-CP dehalogenation and partly inhibited methanogenesis while acetate and phenol accumulated in the cultures. Molybdate at concentrations above 10 mM inhibited both sulphate reduction and 2-CP dehalogenation with accumulation of propionate. Yeast extract at 0.1% was the preferred electron donor compared to 10 mM each of acetate,propionate, ethanol while hydrogen slowed 2-CP removal. 2-CP acclimated cultures dehalogenated 30 mg/L of 2,4,6-Trichlorophenol and 2,4-Dichlorophenol to 4-Chlorophenol (4-CP) which persisted. 4-CP removal was found feasible only with post aerobic treatment.

Under **aerobic conditions**, the presence, nature and concentration of supplemental substrates influenced the specific 2-CP removal in sequencing batch reactors (SBR's) by mixed microbial cultures. An optimal dextrose and phenol concentration of 40 and 60 mg soluble organic carbon (SOC)/L effected maximum 2-CP removal. An un-aerated but mixed period in the react phase of the SBR cycle enhanced the subsequent aerobic removal of 2-CP removal over continuous aerobic conditions. 2-CP removal rates decreased with the decrease in temperature and complete removal at 5 °C was feasible only in the presence of phenol. Specific biodegradation rates of 2-CP were linear according to Arrhenius equation over the 10 °C to 24 °C and 5 °C to 10 °C range. Lower sludge retention time (SRT) enriched the 2-CP degrading population and increased specific 2-CP removal rates. Biomass yield (Y) value was calculated to be 1.27 and 0.76 mg VSS/mg SOC removed/day for the dextrose and phenol systems respectively.

Biodegradation of 2-CP under **anoxic** NO_3^- reducing conditions was demonstrated. At an hydraulic retention time (HRT) of 4 hours, maximum anoxic removals of 54-58% and 66-68% for 2-CP occurred in presence of dextrose and phenol. The residual 2-CP was rapidly removed in the subsequent aerobic period and at rates which were higher than 2-CP removal rates in fully aerobic reactors.

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NOMENCLATURE AND ABBREVIATIONS

$[A^-]$	Ionic form of chlorophenols
α_o	Fraction of total chlorophenol present in molecular form
AOX	Adsorbable organic halide
ASBR	Anoxic sequencing batch reactor
BESA	Bromoethanesulphonic acid
BKM	Bleached kraft mill
BMP	Biochemical methane potential
BOD	Biochemical oxygen demand (mg/L)
COD	Chemical oxygen demand
CSTR	Continuous stirred tank reactor
C_t	Total amount of phenol in solution
DCP	Dichlorophenol
E_A	Arrhenius energy
4-CP	4-Chlorophenol (or *para*-chlorophenol)
g/L	grams/L

GAC	Granular activated carbon
GC	Gas chromatography
[HA]	Molecular form of chlorophenols
HPLC	High pressure liquid chromatography
HRT	Hydraulic retention time
K_a	Dissociation constant
K_{ow}	Octanol-water partition coefficient
mM	milli molar
NEWPCC	North End Water Pollution Control Centre, Winnipeg
NO_3-N	Nitrate nitrogen
ORP	Oxidation reduction potential
PCP	Pentachlorophenol
PHB	Poly-β-hydroxy-butyrate
pK_a	- Log of dissociation constant (K_a)
ppb	parts per billion
ppm	parts per million
r^2	Linear regression co-relation factor
SBR	Sequencing batch reactor
SOC	Soluble organic carbon
SRT	Sludge retention time
SVI	Sludge volume index
T_4CP	Tetrachlorophenol

TCP	Trichlorophenol
Θ_{min}	Minimum sludge retention time (SRT)
3-CP	3-Chlorophenol (or *meta*-chlorophenol)
TOC	Total organic carbon
TVFA	Total volatile fatty acids
2-CP	2-Chlorophenol (or *ortho*-chlorophenol)
UASB	Up-flow anaerobic sludge blanket
US. EPA	United States Environmental Protection Agency
UVR	Ultra violet radiation
VFA	Volatile fatty acids
VSS	Volatile suspended solids
w/v	weight to volume
WHO	World Health Organization

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Chapter 1.

INTRODUCTION AND SCOPE

2-Chlorophenol (2-CP) belongs to a special group of halogenated compounds (chlorophenols) that have been identified as chemicals of concern due to their inherent toxicity to a broad spectrum of organisms (Ruckdeschel and Schwarz, 1987; WHO, 1993), widespread use and resulting presence in the environmental media and biota (Health and Welfare Canada, 1989; US EPA, 1993). Active research in recent years have shown that a variety of bacteria or bacterial consortia are able to mineralize and transform chlorophenols under different conditions although degradation of these compounds in nature is often slow (Allard et al., 1987).

Under anaerobic conditions, poly-chlorophenols are degraded by reductive dehalogenation to monochlorophenols such as 2-CP, 3-CP or 4-CP or completely dehalogenated to phenol (Woods et al., 1989; Hale et al., 1990; Armenante, 1992b; Madsen and Aamand, 1992; Häggblom et al., 1993a). There are mixed reports in literature as to the relative persistence of 2-CP, 3-CP or 4-CP under anaerobic conditions. While studies conducted by Boyd et al. (1983), Boyd and Shelton (1984), Hrudey et al. (1987a) and Häggblom et al. (1993b) indicated that 2-CP was relatively easier to remove compared to either 3-CP or 4-CP, several others have concluded that 2-CP was the most recalcitrant monochlorophenol tested under anaerobic conditions (Lu and Tsai, 1993; Lu and Tsai, 1992; Liu and

Pacepavicius, 1990; Häggblom et al., 1990). Potential alternative electron acceptor such as sulphate was found to have a mixed and site specific effect on the anaerobic dehalogenation of these compounds (Colberg, 1990). Much remains to be seen however as to how such dehalogenation activity can be sustained on a long term basis, what effect does acclimation have on the overall performance of the treatment system and how such an activity is influenced by stimulating or inhibiting various metabolic groups within the bacterial consortium. This study focusses on answering some of these questions.

Aerobic degradation of 2-CP and other chlorophenols is better understood as several bacterial species responsible for degradation have been isolated and the pathways of degradation and enzyme systems have been studied in detail (Häggblom, 1992). There is however a gap in the literature as to the degradation of multiple substrates and the influence of supplementary substrates since in actual practice, a multicomponent mixture such as wastewater undergoes simultaneous biodegradation. Of the three redox environments, knowledge concerning the fate of toxic compounds such as 2-CP under anoxic conditions is the least well developed although such environments have become an important component of many treatment systems in recent years. This study aimed to gain insight to the potential factors that affect the biodegradation of 2-CP as a model compound under anaerobic, aerobic and anoxic conditions. The differences in biodegradability under various environments can be very important for ultimate process selection since the use of multiple redox environments may be the key to successful degradation of this class of compounds.

Chapter 2.

LITERATURE REVIEW

2.1 Synthesis and Properties of Chlorophenols

Chlorophenols (CP's) are organic chemicals derived from phenol (1-hydroxybenzene) by substitution in the phenol ring with one or more atoms of chlorine. Nineteen congeners are possible, ranging from monochlorophenols (2-CP, 3-CP and 4-CP) to the fully substituted pentachlorophenol (PCP). There are several methods by which chlorophenols can be synthesized, including: direct chlorination, hydrolysis of the chlorinated benzenes, conversion of diazonium salts of various chlorinated anilines, and chlorination of phenosulphonic acids and benzenesulphonic acids followed by the removal of the sulphonic acid group (Doedens, 1963). Although manufacture of chlorophenols in Canada ceased as of July 1983, large quantities of imported chlorophenols continue to be used (Health and Welfare Canada, 1989).

Some of the important properties of four different chlorophenols that were tested in this study are summarized in Table 2-1. All of the CP's are solids at room temperature, except for 2-CP which is a liquid. They have strong odours that have been described as pungent or medicinal, particularly those of 2-CP and 2,4-DCP (WHO, 1993). Taste and odour thresholds are so low that Maximum

Acceptable Concentrations (MAC) of chlorophenols in drinking water are based on organoleptic rather than toxicological criteria (WHO, 1984). The solubility of all chlorophenols in water is generally poor, varying from 2.1 X 10^{-1} mol/L for 2-CP to 7.9 X 10^{-1} for 2,3,4,6-T_4CP (WHO, 1993). In contrast, the sodium or potassium salts of chlorophenols are up to four orders of magnitude more soluble in water than the parent compounds.

Table 2 -1. Physical and chemical properties of some chlorophenols

Property	2-CP	4-CP	2,4-DCP	2,4,6-TCP
Melting Point (°C @ 760 mm)	9.0	43.2-43.7	45	69.5
Dissoc. Cont. (K_a)	3.2 x 10^{-9}	6.6 x10^{-10}	2.1 x 10^{-8}	3.8 x 10^{-8}
pK_a	8.48 - 8.65	9.42 - 9.37	7.85	6.1 - 6.62
Odour threshold in Air (ppb @ 25°C)	2	250	2	1000
Taste threshold H_20 (ppb @ 25 °C)	0.1 - 4	0.1	0.3	Not reported
Solubility (g/L @ 20 °C)	28.5	27.1	4.6	0.8
Appearance	Light amber liquid	Needle-like white to straw crystals	Colourless needles or grey flakes	Colourless crystals

Source: From Health and Welfare Canada, 1989

CP's are weak organic acids with pK_a (i.e. log dissociation constant) ranging from 4.81 for PCP to 8.49 for 2-CP. The acidity of chlorophenols increases as the

number of chlorine substitution increases. The polarity of a chemical is strongly correlated with K_{ow}, the *octanol-water partition coefficient.* K_{ow} is the ratio of a chemical's concentration in octanol to its concentration in water at equilibrium. In general, smaller molecules and more polar molecules (such as phenol) dissolve more readily in water and thus have low K_{ow} values and tendency to be adsorbed compared to larger and less polar molecules with higher K_{ow} values. K_{ow} values for chlorophenols increase with chlorination (log K_{ow} ranges from 2.15 to 5.01), also indicating a propensity of the higher chlorophenols to bioaccumulate in tissue, represented by octanol in the K_{ow} determination (WHO, 1993).

2.2 TOXICITY OF CHLOROPHENOLS

The available information on the effects of chlorophenols relates primarily to the aquatic habitats (WHO, 1993). Chlorophenols are toxic to all prokaryotic and eukaryotic organisms (Kozak et al., 1979) although a considerable overlap exists in the concentrations that are toxic to bacteria, phytoplankton, plants, invertebrates, and fish (several studies but summarized by WHO, 1993). Moreover, the toxicology of chlorinated phenols is complicated by the presence of several toxic impurities like dibenzo-p-dioxins and dibenzofurans in technical grade formulations (Ahlborg and Thunberg, 1980). Assessment of toxicity studies with chlorophenols therefore requires a knowledge of types, levels, and effects of micro-contaminants present in the formulation studied, because some of these

compounds are extremely toxic themselves.

Toxicity generally increases with the degree of chlorination of the phenol ring, presumably as a result of increasing lipophilicity but chlorophenols with chlorine in the *meta-* positions (i.e. 3 and 5 position) are often more toxic than can be expected solely on the basis of their chlorine numbers (Kobayashi et al., 1979; WHO, 1993). In addition, pH can significantly affect the toxicity of chlorophenols. At low pH, a given chlorophenol is relatively toxic, because the compounds are present mainly in the molecular form that can readily cross biological membranes. As pH increases, chlorophenol toxicity is reduced because the ionic form is more prevalent.

Exposure to chlorophenols affected algal biomass, composition, and activity (summarized by WHO, 1993). When 5 mg/L of 2,4,6-TCP was added to enclosures in a pond, oxygen level declined from initial levels of 3 - 4 ppm to less than 1 ppm within 6 days of treatment indicating a shift in the balance between heterotrophic and autotrophic metabolism (Schauerte et al., 1982). Significant amounts of both tetra- and pentachlorophenol residues were detected in several resident varieties of fishes in the Fraser river estuary of British Columbia known for its lumber and kraft pulp discharges containing organo-chlorines (Rogers et al., 1992). Studies of the effects of chlorophenols on fish have indicated fish kills and tainted flesh as a result of spills from lumber treatment and kraft pulp mill

discharges (WHO, 1993). The potential of chlorophenol bioaccumulation in a species of chinook salmon exposed to bleached kraft effluents is also known (Servizi et al., 1988). The investigators (Servizi et al., 1988) also observed enlarged nuclei in these species under study and concluded that it could be due to chronic stress.

Chlorophenol toxicity may impact on secondary biological wastewater treatment since many industrial wastes contain a variety of such compounds in large quantities. El-Gohary and Nasr (1984) have indicated that the exposure of microorganisms to 50 mg/L 2,4-DCP in bench-scale trickling filter units treating wastewater had reduced the BOD and COD removal efficiency. While studying the biodegradation of chlorinated aromatics by un-acclimated activated sludge, unexpected and anomalous behaviour was noted with chlorinated phenols. These compounds were not degraded, but instead they showed substantial use of oxygen in Warburg studies without substrate assimilation (Okey and Stensel, 1993). The anomalous behaviour was identified as uncoupling of oxidative phosphorylation.

In studies of chlorophenol toxicity on specific organisms, Keweloh et al. (1991) showed that growth of *E. coli* was inhibited in presence of 1 mM 4-CP, presumably due to the disturbances caused in the arrangement of the fatty acids residues by a lipophilic compound like 4-CP. However, addition of palmitic acid to the culture medium alleviated the toxicity effect by imparting rigidity of *E. coli*

membrane. Bryant and Schulz (1994) evaluated the effect of PCP and lower chlorophenols on the ciliate *Tetrahymena pyriformis*. They determined that decreasing chlorine substitution led to lower toxicity due to decreasing hydrophobicity and reactivity.

Under anaerobic conditions, the effects of toxicity of chlorophenols has been studied to address the treatability of various chemical wastes containing such compounds. Hrudey et al. (1987) reported that 2-CP at concentrations up to 100 mg/L (0.78 mM) did not inhibit methanogenesis. They also found that phenol degradation was inhibited by 3-CP and 4-CP at concentrations greater than 30 mg/L, while concentrations up to 97 mg/L of 2-CP were not inhibitory for ultimate phenol degradation. Extensive work on anaerobic toxicity of chlorophenols was later carried out by O'Connor and Young (1989) by applying the biochemical methane potential (BMP) and anaerobic toxicity assay techniques in 10% (vol/vol) fresh municipal digester sludge. 2-CP was found to be recalcitrant above 20 mg/L and also inhibited methanogenesis which contradicts the findings of Hrudey et al. (1987). Presence of methyl substituents in the ring appeared to reduce the toxicity effects on methanogenesis.

Further studies were conducted by Wang et al. (1991) on the effects of concentrations and type of nine monosubstituted phenols on methanogenic fermentation of acetate in batch anaerobic toxicity assays. The rate of methane

production decreased progressively with increasing concentration of phenolic compounds and the inhibitory effect toward acetate utilizing methanogens was more correlated to substituent type rather than substituent position. The authors concluded that the nitrophenols were more toxic than chlorophenols while the latter exhibited a greater toxicity compared to either hydroxyphenols or phenol. Recent observations by Kim et al. (1994) also supports previous findings and indicated that chlorophenols are most toxic toward acetoclastic compared to hydrogenotrophic methanogens. Based on anaerobic toxicity assays and upflow column studies, Jain and Bhattacharya (1994) however showed that up to 85 ppm of 2,4-DCP did not significantly inhibit acetate enriched methanogenic culture.

Due to the toxicity of chlorophenols and its widespread use, there is a concern for human health and exposure to these compounds and their associated contaminants. Figure 2-1 outlines the major distribution pathways in the environment. On the basis of preliminary estimates from literature of total chlorinated phenol residues in food, water, air and miscellaneous sources, the Canadian Department of National Health and Welfare estimated typical non - occupational exposure to all chlorophenols to be 0.18 µg/kg body weight/day for a 70 kg adult (NHW, 1988). The major identifiable sources of these chlorophenols were food and drinking water. Worker exposure is a major concern in industries in which chlorophenols are used extensively as respiratory and dermal absorption of these compounds resulted in measurable levels in the blood and urine of

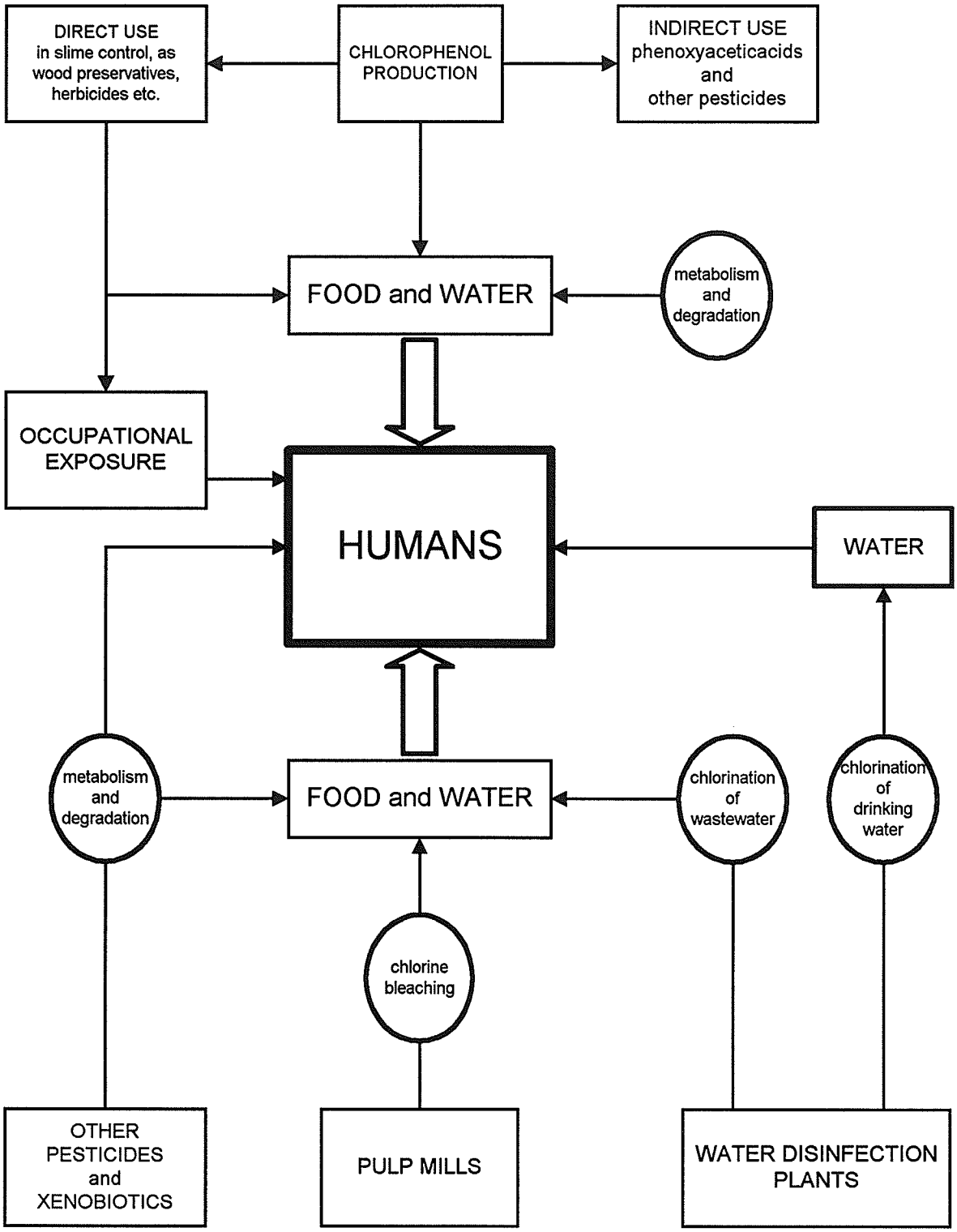


Figure 2-1 Potential Pathways of Human Exposure to Chlorophenols
(Ahlborg and Thunberg, 1980)

exposed workers (WHO, 1993).

Studies on woodworker exposure to chlorophenols are of particular relevance to Canada due to widespread use of T_4CP and PCP in the lumber industry. Saw mill workers and those associated with manufacture of chlorophenols experienced abnormal liver function, respiratory irritation and general effect on nervous system (summarized by : NRCC, 1982 and WHO, 1993). Lampi et al. (1991) have also indicated that long term exposure to chlorophenols increase the cancer risk in humans. Almost all studies on lethal doses of chlorophenols have been performed on animal models to gauge its likely impacts on humans under acute exposure.

Long term studies implicated liver and kidney as organs that accumulated high levels of chlorophenols and were often adversely affected, perhaps reflecting the role of such organs in the detoxification and elimination of xenobiotics (several studies summarized by WHO, 1993). In general, the major mode of action in the acute toxicity of chlorophenols involves the uncoupling of oxidative phosphorylation and the inhibition of electron transport system which prevents the formation of ATP (WHO, 1993). Due to this uncoupling, the increased catabolism yield higher rates of oxygen consumption, heat release and leads to depletion of metabolic reserves (Health and Welfare Canada, 1988).

2.3 TRANSPORT AND DISTRIBUTION

2.3.1 Atmospheric movement

While chlorophenols are considered to be primarily water and soil contaminants, atmospheric movements of chlorophenols also occurs. Very little is known on the ambient levels of lower chlorophenols such as 2-CP in the atmosphere but the few studies conducted on PCP provided a useful indication of the potential for atmospheric distribution of other chlorophenols (WHO, 1993). It was not known whether such compounds were present as a vapour or adsorbed on airborne particulates. Measurable quantities of chlorophenols have never the less been detected in air, rainfall, and snow, sometimes far from obvious point sources (Paasivirta et al., 1985). Furthermore, considerable quantities of chlorophenols are released with incinerator emissions. The relative contributions of volatilization and adsorption on particulate to these atmospheric levels are not known.

2.3.2 Volatilization

The degree to which any compound volatilizes depends upon several factors that include pH, temperature, mixing, wind velocity (Krijgsheld and van der Gen, 1986). Not much is known about the rates of volatilization of chlorophenols especially the lower chlorophenols in the environment. It appears that losses through this process are minimal in natural waters. The half-times for volatilization of 2-CP and 4-CP at 1 m depth were estimated to be 395 h and 3421 h

respectively by extrapolating data from 0.38 cm of still water (Krijgsheld and van der Gen, 1986). On the contrary, Saez and Rittmann (1991) observed significant abiotic loss of 2-CP via stripping compared to either phenol or 4-CP. In their studies on PCP volatilization, Kloppfer et al. (1982) determined that the half-life for PCP disappearance, through volatilization from their apparatus, was 167 h at pH 3.3 and 3120 h at pH 6. No evaporation was detected at pH 8. Ettala et al. (1992) have also shown that out of 4-120 µg/L of chlorophenols entering a full-scale activated sludge plant treating municipal wastewater in Finland, volatilization accounted for only 1% removal of these compounds.

2.3.3 Adsorption

Adsorption to sediments and soils is probably the most prevalent mechanism through which chlorophenols persist in the environment. Such deposition is quite variable in nature. In acidic soils, chlorophenols are bound strongly, while adsorption is minimal under alkaline conditions. While studying the adsorption characteristics of chlorophenols to anaerobic reactor solids, Woods (1984) concluded that the pH of the solvent was an important parameter to consider in adsorption of such compounds. Presence of organic matter also increased the wide availability of adsorption sites and is probably significant in sludge and organic soils compared to mineral soils (Isaacson and Frink, 1984).

Boyd (1982) highlighted the effect of functional groups on the adsorption of chlorophenols in an anaerobic system. The author postulated that a solute's

affinity for soil could likely affect its bioactivity, persistence, mobility and volatility. Presence of a chlorine atom in *ortho*-, *meta*- or *para*- position increased adsorption compared to phenol (Boyd, 1982) and this phenomenon was also noticed when activated carbon was present as the sorbent (Knettig et al., 1986). In their studies on biosorption of chlorophenols to anaerobic granular sludge, Kennedy et al. (1992) observed neither a good empirical correlation between increased biosorption with increasing octanol-water partition coefficient (K_{ow}) nor a clear relationship between sorption and the number or positions of chlorine substituents although the affinity of PCP to more strongly adsorb than the lesser chlorinated phenols were seen. Tsezos and Bell (1989) however showed excellent co-relation of uptake by living and dead biomass to octanol-water partition of the organic pollutants tested (lindane, diazinon, PCP and 2-chlorobiphenyl).

A major factor influencing adsorption of chlorophenols onto biomass is the distribution of ionic and molecular forms which is subsequently a function of the suspension pH (Davis and Vohra, 1994). This is due to the fact that chlorophenols are weak acids with pKa ranging from 4.81 for PCP to 9.9 for unsubstituted phenol, the distribution of the molecular form (HA) and the ionic conjugate base (A^-) varies as the solution pH is altered. The concentration of the molecular species is given by:

$$[HA] = \alpha_o C_t \qquad [2\text{-}1]$$

where C_t is the total amount of phenol in solution (i. e $[HA] + [A^-]$) and α_o is the

fraction of the total chlorophenol present in the molecular form given by :

$$\alpha_0 = \frac{1}{1 + (K_a / [H^+])} \qquad [2\text{-}2]$$

Lee et al. (1990) examined the adsorption of PCP on clays and soils at different pH values and ionic strengths. A significant difference was noted between the adsorption of PCP below pH 4 and above pH 7 on these natural materials. Above pH 7 most of the PCP was present in the ionic form which was less adsorbable (about 2 orders of magnitude) than the molecular form. These findings are supported by an earlier work by Davis and Huang (1990a) who investigated the adsorption of substituted phenols onto CdS(s) as a function of pH. Their experimental results indicated that only the molecular species were adsorbed. At pH values greater than pK_a, the adsorption decreased essentially to zero. Also, highly substituted chlorophenols adsorbed more strongly onto CdS(s), following a trend of increasing K_{ow} .

Although these results have succeeded in predicting overall removal of these compounds in wastewater treatment systems maintained under controlled conditions (Jacobsen et al., 1993), it was often difficult to assess the impact of adsorption on chlorophenol transport in the environment. Seip et al. (1986) suggested that chlorophenols bound to soils are continually turned over, and that binding sites may be saturated under the appropriate conditions leading to increased mobility and a decreased residence time of chlorophenols in the soil body.

2.3.4 Tansport in aquatic environment

While a strong potential exists for a large portion of chlorophenols entering the waters to be degraded *in situ*, they are nonetheless moderately soluble and fairly persistent and may be transported for considerable distance. Fox and Joshi (1984) detected elevated levels of trichlorophenols (TCP) and PCP as far as 82 km from the wood preserving plant where they originated. Chlorophenols that are not degraded are concentrated in the sediments, most likely through adsorption on sediment particulate. Since the majority of such sediments are either anoxic or anaerobic, there is a strong possibility of the chlorophenols undergoing transformation by microorganisms under such environments. Biological degradation potential of chlorophenols is discussed in later sections.

2.4 ABIOTIC DEGRADATION OF CHLOROPHENOLS

Two of the major processes responsible for abiotic degradation of chlorophenols are photodecomposition and chemical degradation. Although the emphasis of this thesis is on biological degradation of chlorophenols, a brief overview of two such non-biological approaches to the destruction of these compounds is presented in the next two sections.

2.4.1 Photodegradation

Many of the chlorophenol isomers are degradable abiotically to some extent by exposure to ultraviolet radiation (UVR). The breakdown often involved an oxidation reaction that dechlorinated the molecule, though a variety of reactions have been proposed (Boule et al., 1982). 2,4-DCP in aqueous solution was decomposed in a matter of minutes by irradiation from a UV lamp. The breakdown of chlorophenols and pathways of degradation were affected markedly by the position of chlorine substituents on the molecule. Among dichlorophenol isomers, substitutions at the *ortho* and *meta* positions made the chemical more reactive than *para* position (Boule et. al., 1982).

Davis and Huang (1989) studied the photocatalytic oxidation of several chlorophenols by CdS. Their results indicated that an increase in degree of chlorination rendered the phenols more oxidizable and increased in the order : 2,4,6-TCP greater than 2,4-DCP greater than 2-CP greater than phenol. In a later study, the authors (Davis and Huang, 1990) further concluded that the oxidation rates of chlorophenols by photo-degradation using aqueous CdS not only depended upon the degree of chlorination but also on the position of chlorine in the ring as previously reported by Boule et al. (1982). Miller et. al. (1988) had earlier demonstrated that a brief UV (300 nM) photolysis greatly facilitated the removal of 2,4,5-TCP and 2,4-DCP from sewage through accelerated mineralization of its products by subsequent biological treatment indicating that such sequential treatment may be more effective for destructions of such compounds.

2.4.2 Chemical degradation

There are only few reports that suggest the possibility of chlorophenols to be degraded in the environment by chemical processes. Baker and Mayfield (1980) had observed losses of 2-CP, 4-CP, and 2,4-DCP from sterile silica sand and soils. Since microbial contamination, photolysis, and volatilization were eliminated as the potential cause for such losses, the authors suggested that the chlorophenols were auto-oxidized, or broken down at catalytic sites, but did not eliminate the possibility of polymerization.

The feasibility of pre-ozonation with subsequent biodegradation as an alternate treatment to 2-CP was investigated by Stowell et al. (1992). The major ozonation by product of 2-CP was chlorosuccinic acid which was oxidized to oxalic acid upon further ozonation. However, it was chlorosuccinic acid that supported the growth of *Pseudomonas putida* during batch biodegradation studies. Although the system showed potential, the authors emphasized the importance of control of such a chemical oxidation process to prevent formation of secondary refractory intermediates. On the other hand, the beneficial effect of ozone/UV application for treatment of water containing 2-CP was shown to be inconclusive by Skorska (1993).

2.5 MICROBIAL DEGRADATION OF CHLOROPHENOLS

Although chlorophenols are quite toxic to microorganisms in general, they were nonetheless metabolized by a large number of microbes residing in soils, natural waters, sediments, and sewage sludge (WHO, 1993). Biodegradation of chlorophenols was shown to be difficult because of the presence of chlorine atoms in the phenolic ring (Reineke, 1984). Research on the potential of biodegradation of chlorophenols was started primarily under aerobic conditions in the early 60's and work on several chlorophenols was done by Alexander and Aleem (1961) and Tabak et al. (1964).

In the years that followed, aerobic degradation of chlorinated phenols has been extensively studied. Several bacterial cultures have been isolated and examined in detail, metabolic pathways and key enzymes have also been identified as reviewed and summarized by Häggblom (1990, 1992); Reineke and Knackmuss (1988). Earlier efforts to the study the anaerobic degradation of chlorophenols turned up little or no information on the behaviour of these compounds at low redox conditions (Baker and Mayfield, 1980; Horowitz et al., 1982). The persistence of PCP and T_4CP in anaerobic sediment cores were also recorded over decades by Fox and Joshi (1984). It was Boyd and his fellow researchers who first published their successful results of anaerobic metabolism of chlorophenols in fresh and acclimated sewage sludge (Boyd et al., 1983).

Before presenting an overview of the research conducted on the biodegradation of chlorophenols under aerobic, anaerobic and anoxic conditions, the key factors that may affect the biodegradability as well as biodegradation rates of these compounds are summarized below based on several references summarized by Ali (1991) and WHO (1993).

(1) Chemical factors such as solubility, size of molecule, the type, number, and position of substituents, and stereochemistry which were largely determined by the substituence.

(2) Biological factors such as age, effect of adaptations of the microbial culture, degree of toxicity of the compound to the microbial culture, and the kind and numbers of microbes present.

(3) Environmental factors such as pH, temperature, dissolved oxygen level, availability of inorganic nutrients, presence of organic matter, and degree of dispersion of the compound in the system.

2.5.1 Aerobic Biodegradation

Microbial degradation of chlorinated phenols under aerobic conditions has been studied more extensively than anaerobic or anoxic metabolism. The relative rate of aerobic biodegradation of chlorophenols generally decreased as the number of chlorine atoms on the phenolic ring increased (Tabak et al., 1964; Pitter, 1976; Baker and Mayfield, 1980). Chu and Kirsch (1973) however showed that their culture was able to utilize PCP as the sole carbon source but poorly metabolized the dichlorophenols and did not metabolize the monochlorophenols at all. Rates of biodegradation were further shown to be affected by the relative position of the chlorine atoms on the phenolic ring. Compounds with a chlorine in the meta position were generally more stable than those without (Baker and Mayfield, 1980, Chu and Kirsch , 1973). These early observations are backed by current findings of Liu and Pacepavicius (1990) who conducted a systemic study of 18 chlorophenols. The authors concluded that nonspecific generalizations such as the degree of chlorination could not be reliably correlated to the persistence of chlorophenols in their system. A key finding of their research was that position (or positions) of the chlorine substitution in the ring was the major factor affecting biodegradation of chlorophenols and followed the descending order of 2,4 > 4 > 3,5 > 2,6 > 3, or 5, or 2.

Alexander and Lustigman (1966) had previously studied the effect of chlorine substitution on degradation potential in soil. They found that both 3-CP and 2-CP

persisted for greater than 64 days while 4-CP was degraded in only 16 days. Phenol was degraded even faster compared to any of the chlorophenols demonstrating that Cl substitution significantly affected biodegradability. Haller (1978) also used soil as the inoculum to study the biodegradation of 2-, 3-, and 4-CP. While 4-CP was completely degraded in 14 to 25 days, neither 2- nor 3-CP could be degraded in that time. The potential of higher degradability at *para* position (i.e. 4-) compared to either *ortho-* or *meta-* was contradicted in the study by Baker and Mayfield (1980). They found that 2-CP and 4-CP were degraded at the same rate in soil with lag times of only 1-2 days compared to 3-CP which exhibited a lag time of 80 to 160 days to degrade > 70% which agrees with the observations made by other researchers (Alexander and Lustigman, 1966; Haller, 1978). Pitter (1976) had also shown that 2-CP degraded at a faster rate than 4-CP in an activated sludge system although different organisms could have been involved in the process.

Microorganisms that have been previously exposed to a compound are able to metabolize it immediately when re-exposed, and at a faster rate than unexposed organisms (Alexander and Aleem, 1961; Tyler and Finn, 1974), presumably because exposure induces the enzymes necessary to metabolize the chlorophenol. Microorganisms not previously acclimated often exhibited lag time of as much as several days before they begin to be degraded (Spokes and Walker, 1974; Lee and Ryan, 1979). A wide scale difference in lag times and responses was seen

by Liu and Pacepavicius (1990) in their study on biodegradation of eighteen different chlorophenols.

Similarly, prior exposure to structurally related compounds facilitated the metabolism of chlorophenols, indicating that the enzymes induced by the original compound are somewhat nonspecific. A PCP-adapted microorganism utilised T_3CPs and T_4CPs readily (Chu & Kirch, 1973), while bacteria grown on phenol, chlorophenols, or phenoxyacetic acids were able to metabolize various other lower chlorophenols (Tabak et al., 1964; Shimp and Pfaender, 1987; Saez and Rittmann, 1991). However, this was not always true since cultures enriched on toluene were able to degrade PCP, 2,4,6-TCP and 2,4-DCP compared to a phenol enrichment cultures which degraded only 2,4-DCP (Ryding et al., 1994).

Impact of supplementary substrates on aerobic biodegradation. A major concern in the engineering and design of a biological facility for the destruction of toxic compounds such as chlorophenols is the impact of secondary substrates on the overall performance of the process. Contaminated soils and groundwater subjected to either in-situ or pump and treat methods of treatment contain various sources of carbon compounds in addition to target organics. The presence of such easily degradable carbon sources may either enhance, decrease or have no effect on the biodegradation of target contaminants like chlorophenols.

Addition of lysine to a continuous stirred tank reactor (CSTR) containing a 2-chlorophenol degrading pure culture, reduced the effluent 2-CP concentration (Loven, 1985) while the mineralization of dinitrophenol by a defined bacterial consortium was enhanced by the presence of glucose in a SBR (Hess et al., 1990, 1993). In a batch study, adaptation of a microbial community obtained from a lake, to increasing concentrations of amino acids, carbohydrates or fatty acids enhanced the metabolism of monosubstituted phenols (Shimp and Pfaender, 1985).

On the other hand, the presence of alternative substrates either inhibited or repressed the biodegradative enzymes or led to the selection of strains that lacked the necessary pathways for degradation (Frick et al., 1987; Topp et al., 1988). Liu et al. (1981) studied PCP degradation in an aquatic environment. They found that the concentration of PCP decreased to a negligible amount in three days under aerobic conditions. They also noted that the addition of glucose or sodium 4-chlorophenate inhibited degradation rather than acting as inducers as was originally believed. It was also shown that addition of co-substrates had no effect on the removal rate of high concentrations (100 -150 mg/L) of 2-CP in aerobic SBR's (Oleszkiewicz et al., 1991). A majority of the work done with chlorophenols so far has been with either batch or continuous systems with pure cultures. Information on the use of a mixed microbial culture like activated sludge to degrade chlorinated phenols with different types and concentrations of supplementary substrates in a continuous system is limited.

Aerobic biodegradation pathway. Chlorophenols are aerobically biodegraded by one of two mechanisms. Either the halogen substituent is replaced with a hydroxyl group after ring cleavage, or the ring is first cleaved, then dehalogenated (Berry et al., 1987). The cleavage is accomplished through enzymes known as mono- and dioxygenases, and oxygen is assimilated into the resultant products (Evans and Fuchs, 1988). For fission of the very stable benzene nucleus to occur, the ring is first dihydroxylated by an oxygenase (or oxygenases) such that two hydroxyl groups are situated either ortho- or para- to one another on the ring producing the corresponding catechol (Steiert and Crawford, 1985; Paris et al., 1982). A pathway for aerobic biodegradation of 2-CP as proposed by Steiert and Crawford (1985) is shown in Figure 2-2.

Ring cleavage proceeds through another oxygenase-catalyzed reaction when the dioxygenase inserts molecular oxygen into the ring. A lactonizing enzyme leads to an unstable intermediate. At this point, the chlorine is released. The lactone is cleaved by a hydrolase resulting in maleylacetate which can enter the tricarboxylic acid (TCA) cycle (Steiert and Crawford, 1985). During the catabolism of lesser-chlorinated phenols such as monochlorophenols and dichlorophenols, bacteria responsible for such degradation usually open the dihydroxylated aromatic ring (using a dioxygenase) before they remove the chlorines. With more highly chlorinated phenols like PCP, at least some of the halogen atoms were removed prior to ring cleavage since chlorine-mediated ring deactivation would be sufficient to prevent the ring-opening reactions (Steiert and Crawford, 1985).

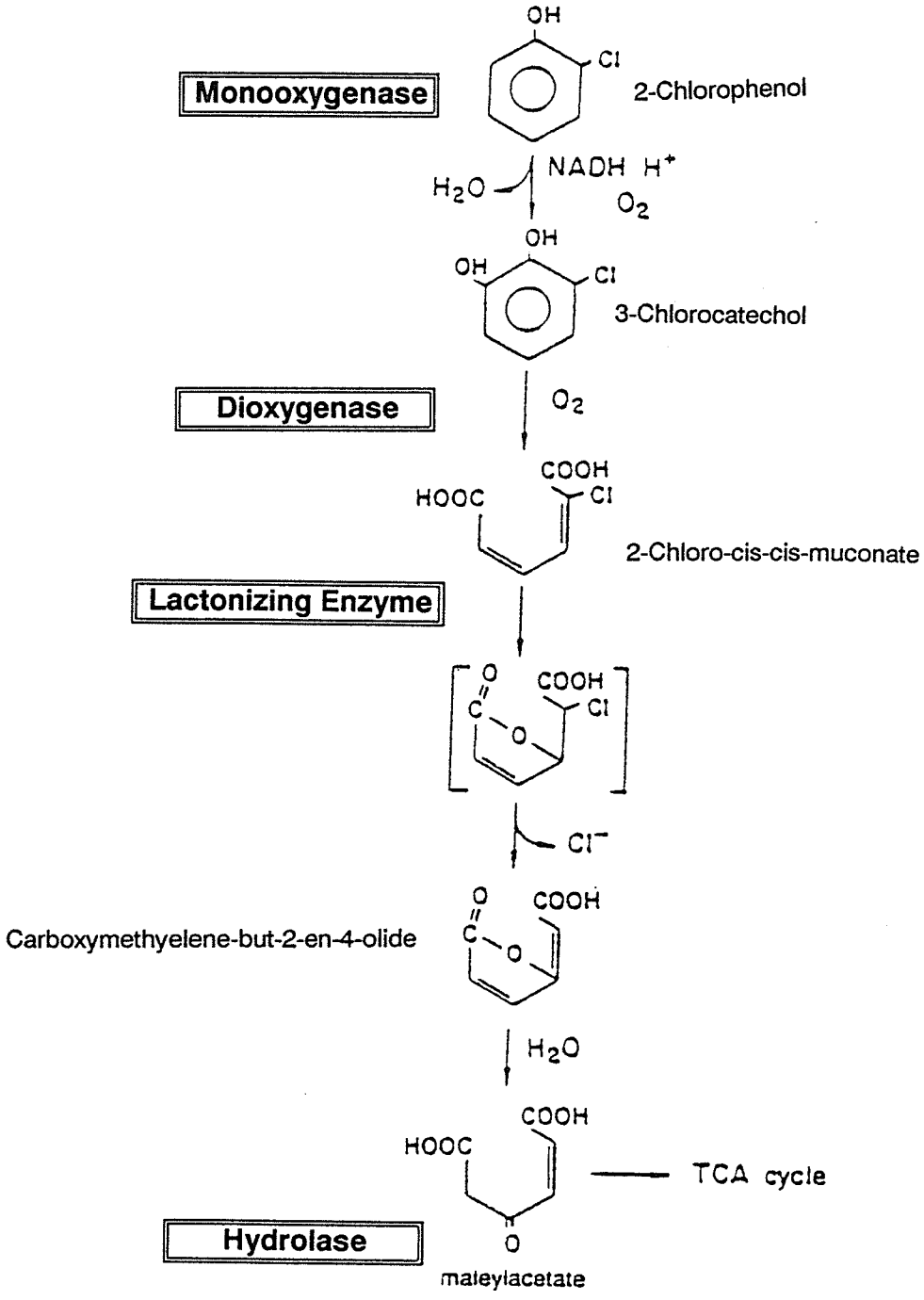


Figure 2-2 Pathway for the degradation of 2-CP using an *ortho*- ring cleavage dioxygenase (Adapted after Steiert and Crawford, 1985)

Aerobic degradation in biological reactors. While most studies on chlorophenol were conducted in batch modes there is a need to study application of these results in continuous biological reactors. Due to the generation of wastes containing such compounds, there is also a requirement by the industries as well as municipal treatment plants to be retro-fitted with the technology which can handle such compounds. The following section discusses some of the treatment processes that have proved successful or have shown promise for degradation of chlorophenolic compounds.

Biodegradation of seven chlorophenolic compounds were studied in a fixed film reactor (Salkinoja-Salonen et. al, 1983). PCP was found to be the most recalcitrant compound. The ability of immobilized cells grown under aerobic and fluidized conditions to degrade 4-CP and 2,4-DCP under the conditions of different dilution rate were investigated by Shieh et al. (1990). Micro-carriers employed for cell immobilization and retention were shown to efficiently remove up to 36 mg/L of 4-CP and 45 mg/L 2,4-DCP even at an HRT of 1 hour. In another study, Puhakka and Järvinen (1992) used a continuous-flow fluidized bed immobilized acclimated activated sludge reactor and pure oxygen to degrade polychlorinated phenols like 2,3,4,6-T_4CP and 2,4,6-TCP. Over 99% removal efficiency at HRT of 3 to 5 h was established. In a recent study the same authors presented two aerobic laboratory scale continuous flow fluidized bed reactors for bioremediation of contaminated ground water (Järvinen and Puhakka, 1994). 99% removal of

chlorophenol was observed at a HRT of just 45 minutes indicating very high process efficiency. Sofer et al. (1990) also demonstrated complete mineralization of 2-CP in an air-sparged reactor under different temperature conditions. The value for temperature activity coefficient (calculated to be 1.16) indicated higher temperature dependence of the process compared to conventional biological treatment systems.

The feasibility of RBC (Rotating Biological Contactor) technology to treat a synthetic wastewater containing several toxic compounds including 2-CP; 2,4-DCP; 2,4,6-TCP and PCP was examined by Tokuz (1991). Average hydraulic loading varied from 0.04 to 0.08 m^3/m^2. d and a constant organic loading of 0.1 Kg/m^3 of disc surface per day was maintained throughout the study period. Removal efficiencies of the compounds tested was found to be a function of hydraulic loading rate. Sequencing batch reactors (SBR's) were operated to see the effect of 2-CP removal in presence of various combination of co-substrates including phenol under different SRT's using mixed microbial culture (Oleszkiewicz et al., 1991). Addition of co-substrate was seen to have no effect on aerobic degradation rate and the increase in volumetric rates was proportional to increase in SRT.

Removal up to 71 % of the influent chlorophenol (4 - 120 µg/L) was observed in municipal sewage treatment plants in Finland indicating the potential of indigenous activated sludge micro-organisms to degrade halogenated aromatic

molecules (Ettala et al., 1992). Chudoba et al. (1989) had also used a conventional activated sludge system with constant volumetric loading to study the kinetics of 2,4-DCP degradation by adapted mixed microbial population. The maximum specific rate of 2,4-DCP removal increased with decreasing SRT which resulted from the enrichment of mixed culture with responsible microorganisms and washed out the predatory protozoan population. Armenante et al. (1992a) tested several reactors for optimizing 2-CP degradation using the fungus *Phanerochaete chrysosporium*. Of the three different configurations tested, the packed bed reactor with the fungus immobilized on balsa wood particles proved to be the most effective configuration due to low shear and presence of solid support compared to either a batch fermenter or a chemostatic reactor with the fungus immobilized on a silica-based porous biocatalyst support.

Aerobic degradation by pure cultures. The ability of microorganisms to degrade naturally occurring aromatic compounds is an ancient metabolic trait (Steiert and Crawford, 1985). One of the earliest work in isolating bacterium capable of degrading chlorophenols was demonstrated by Chu and Kirsh (1973). The bacterium designated KC3 utilized PCP as the sole carbon source but was also able to grow on 2,3,4,6-T_4CP. However the bacterium poorly metabolized 2,4-DCP and monochlorophenols were not metabolized at all. Saber and Crawford (1985) isolated PCP-mineralizing strains of *Flavobacterium* from different PCP-contaminated dump sites. All strains used PCP as the sole source of carbon and

energy, tolerating at least 20 ppm of PCP in their growth media. Steiert and Crawford (1985) also reported the ability of resting cells of one PCP-mineralizing *Flavobacterium* strain to fully or partially dechlorinate a variety of chlorinated phenols. The authors concluded that the enzyme activities for catabolism of PCP and other chlorinated phenols by *Flavobacterium* appear to be under the control of an inducible enzyme system.

Pseudomonas sp. B13 was isolated from sewage by Knackmuss and Hellwig (1978). The organism which grew in continuous culture on 4-CP as the sole carbon and energy source readily transformed 2- and 3- CP and several dichlorophenols except 2,6-DCP. Knackmuss and Hellwig (1978) also reported the ability of *Alcaligenes eutrophus* grown on phenol to co-oxidize both mono- and dichloro- phenols. The potential of using a strain *Pseudomonas putida* PpG4 to degrade 4-CP after pre-growing on phenol was presented by Saez and Rittmann (1991).

Häggblom et al. (1988) were instrumental in isolating the bacterium, *Rhodococcus chlorophenolicus* from pulp bleaching effluents which degraded 18 different polychlorinated phenolic compounds that were present in spent bleaching liquor of kraft pulp. Subsequently Valo et al. (1990) demonstrated the feasibility of two *Rhodococcus* strains for bioremediation of a simulated groundwater containing chlorophenols. Briglia et al. (1994) later studied *Rhodococcus*

chlorophenolicus PCP-1, which had the potential to be used for bioremediation of PCP contaminated soils. Apajalahti (1987) had earlier shown *Rhodococcus chlorophenolicus* degraded PCP to non-chlorinated 1,2,4-trihydroxybenzene that subsequently served as a carbon and energy source, however no evidence is presented as to the ability of the bacterium to degrade either of the monochlorophenols. Singh et al. (1993) had also recently isolated *Bacillus* sp. , *Pseudomonas* sp., and *Klebsiella* sp. from 2-CP degrading activated sludge. *Azotobacter* sp. strain GP1 was isolated from soil which utilized 2,4,6-TCP as the sole carbon and energy source (Li et al.,1991). Resting cells of *Azotobacter* sp. strain GP1 also transformed monochlorophenols; 2,6-DCP and 2,3,6-TCP.

While bacteria are most frequently studied as the agents responsible for chlorophenol biodegradation, they are not alone in this capability. The basidiomycete *Phanerochaete chrysosporium* which is a naturally occurring fungus was shown to degrade very high concentrations of 2-CP in biological reactors (Armenante et al., 1992a). The fungus was capable of producing a ligininolytic enzyme which was primarily responsible for imparting the degradation capacity to the microorganism.

The above results suggests the applicability of such a broad spectrum of microroganisms in treatment processes for destruction of chlorophenols or as innoculum for bioremediation. Although, laboratory experiments under controlled

conditions showed great potential in microbial decontamination of polluted soils and waters, much remains to be seen and learned concerning scale-up of such processes to the environmental level. With rapid advances been made in area of genetic engineering a potential exists for the use of novel microbial strains for decontaminating a wide-variety of chlorophenols. However, environmental release of such organimsms is still very much controversial and will be subjected to extreme scrutiny for its benefits and thus requires a close collaboration of microbiologists and environmental engineers.

2.5.2 Anaerobic Degradation

The rates of biodegradation of chlorophenols are in general dependent on the oxidation reduction potential of the environment (ORP). Studies have shown that multi-halogenated aromatic compounds that are recalcitrant to biodegradation and mineralization by aerobic microorganisms may be often degraded by anaerobic organisms by means of reductive dehalogenation (Goulding et al., 1986; Quensen et al., 1988).

Reductive dehalogenation as a novel pathway for anaerobic biodegradation of haloaromatic compounds was initially proposed by Suflita et al. (1982). Their conclusions were based on the work on anaerobic metabolism of halobenzenes by sediment and sewage sludge organisms. Parker et al. (1993) indicated that the electronegative nature of chlorine substituents of chlorophenols acts to increase

the oxidation level of the organic compounds, making them more susceptible to reduction reactions. For a given aromatic ring, a greater number of substitutions results in higher oxidation level and consequently a greater tendency to be reduced. Indeed as a result of reductive dehalogenation, individual chlorines of chlorophenols with multiple substitutions were well removed, while monochlorophenols such as 2-CP, 3-CP or 4-CP accumulated (Woods et al., 1989; Hale et al., 1990; Armenante et al., 1992; Madsen and Aamand, 1992; Nicholson et al., 1992). The results from several laboratory studies suggested that biodegradation of non-halogenated or lightly halogenated compounds like the monochlorophenols was most rapid and complete under aerobic environments compared to either anoxic or anaerobic habitats (Baker et al., 1980; Baker and Mayfield, 1980; Knackmuss, 1982; Chaudhry and Chapalamadagu, 1991).

The question now arises as to whether the anaerobes obtain energy from reductive dehalogenation or whether such process is co-metabolic or whether the reaction while specific is not coupled to energy conservation reactions. Zitomer and Speece (1993) presented an extensive review of this topic which showed that the biologically mediated dehalogenation reaction are thermodynamically favourable to bacteria and is a more complex process than abiotic mechanisms of dehalogenatio such as electrophilic and nucleophilic substitutions. Figure 2-3 shows schematically two possible routes for reductive dechlorination.

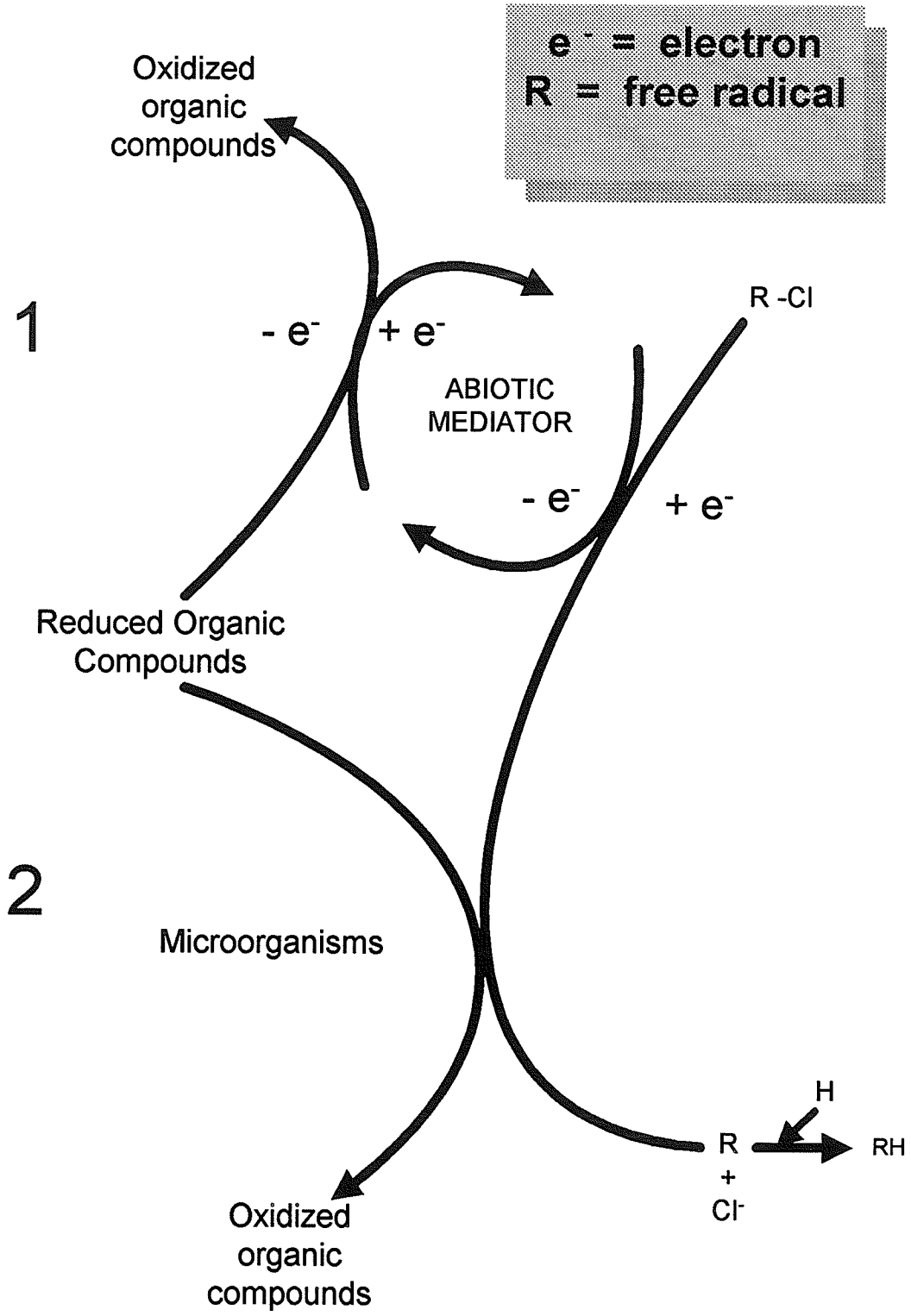


Figure 2-3 Two Possible Routes for Reductive Dechlorination
(Kobayashi and Rittmann, 1982)

Sequential anaerobic-aerobic biodegradation. The difference in biodegradability of chlorophenols as discussed in the previous section presents a feasible approach to devise a sequential anaerobic - aerobic treatment systems for complete mineralization of multi-halogenated chlorophenols. The pulp and paper industry has shown considerable interest in the removal of chlorinated phenolic compounds formed in bleaching chemical pulps by means of sequential anaerobic and aerobic biological treatment and excellent reviews on this topic are presented by Ferguson et al. (1990); Zitomer and Speece (1993).

Armenante et al. (1992b) had demonstrated an integrated anaerobic-aerobic process comprised of an anaerobic down-flow packed bed (silica beads) reactor followed by aerobic suspended system for complete removal of 2,4,6-TCP. 4-CP and phenol which were the final persistent products of first-stage anaerobic treatment could be successfully mineralized under aerobic conditions. On the contrary, Madsen and Aamand (1992) reported accumulation of 4-CP in their single stage anaerobic batch systems dehalogenating 2,4,6-TCP indicating the requirement for a post aerobic treatment.

The need for engineers to devise strategies employing such sequential environments for optimizing enhanced biodegradation of chlorinated compounds has stimulated research using two stage systems employing anaerobic and aerobic bacteria contained in separate unit operations in series (Armenante et. al., 1992b).

This is of vital importance to the wastewater industry because of the stringent effluent toxicity standards require removal of specific organic compounds like chlorophenols at the treatment plant. One such economical approach would be to implement anaerobic-aerobic single-sludge activated sludge process. The knowledge of single bacterial cultures exposed to oscillating oxidation reduction potential (ORP) sequence is presently lacking. The viability of anaerobic bacteria during aerobic period and vice-versa needs to be determined.

Anaerobic dehalogenation in methanogenic batch systems. The potential of anaerobic metabolism of chlorophenols was initially demonstrated by Boyd et al. (1983) using 10% (vol/vol) un-acclimated digested sewage sludge as the inoculum source. 2-CP was removed at a faster rate compared to 3-CP while 4-CP amended cultures showed lag periods of 8 weeks before being removed completely in an additional 8 weeks time. While 2-CP was mineralized >90% to methane in 3 to 4 weeks time followed by 3-CP in 8 weeks, 4-CP fed cultures showed no methane formation indicating inhibitory effect on the methanogenic community. Phenol was identified as an intermediate indicating dechlorination as the initial step in the reductive metabolism of chlorophenols.

In the following year, Boyd and Shelton (1984) further extended the understanding of anaerobic dehalogenation of chlorophenols by comparing the

degradation potential of these compounds (by monitoring substrate disappearance) by acclimated and un-acclimated systems. 100% (vol/vol) un-acclimated sewage sludge degraded about 50 ppm of monochlorophenols in the order of 2-CP > 3-CP > 4-CP while, of the dichlorophenols tested, dechlorination of the Cl group *ortho* to phenolic OH was observed first. Degradation results for sludge acclimated to 2-, 3-, or 4-CP however gave patterns of degradation completely different from un-acclimated sludge. Sludge acclimated to 2-CP degraded 4-CP at the same rate but failed to degrade 3-CP. 3-CP acclimated sludge degraded 4-CP but at a slower rate but not 2-CP, while sludge acclimated to 4-CP degraded 2- and 3-CP. 2-CP removal took place at a slower rate than 3-CP. Based on this response of un-acclimated and acclimated sludge to the three monochlorophenols, the authors hypothesized the involvement of two different microbial species involved in dechlorination reactions. Mineralization of 2-CP and 2,4-DCP to methane was also shown.

Complete reductive dechlorination and mineralization of PCP was demonstrated by Mikesell and Boyd (1986) by using anaerobically digested municipal sewage sludge acclimated to three monochlorophenols isomers simultaneously or added in equal volumes. On the contrary, anaerobic mixed cultures also derived from sewage sludge could only dechlorinate PCP to 3-CP under methanogenic conditions (Madsen and Aamand, 1991). The potential of anaerobic sewage sludge organisms to dechlorinate very high concentrations of 2-, 3-, and 4-CP was also seen with or without phenol addition in 100% (vol/vol)

batch and semi-continuous cultures by Hrudey et al. (1987a). They observed that up to 285 mg/L of 2-CP could be readily degraded and mineralized while 3- and 4-CP required extended lag times which agrees with the earlier observations by Boyd et al. (1983).

Hrudey et al. (1987a) also indicated that presence of > 30 mg/L 3- or 4-CP inhibited phenol degradation while up to 97 mg/L of 2-CP was required to produce the same effect. Methanogenesis was found to be un-inhibited by up to 100 mg/L of 2-CP. The preference for removal of ortho-chlorine from 2,4-; 2,3- and 2,6-DCP by 50% un-adapted anaerobic sludge was demonstrated by the same group (Hrudey et al., 1987b). A study of 2-CP degradation by anaerobic mixed cultures from sewage sludge was enriched in a yeast extract and peptone medium capable of complete mineralization of 2-CP to methane and carbon dioxide (Dietrich and Winter, 1990). Presence of yeast extract and peptone in the culture medium was found to be mandatory for 2-CP dehalogenation to occur. The enrichment also exhibited the capability to remove Cl only at the *ortho*- position relative to the OH group in the ring from the di-chlorophenols tested which agrees with the observations made previously by Hrudey et al. (1987b). While 2,6-DCP was completely dechlorinated to phenol via 2-CP, the cultures receiving 2,4-DCP accumulated 4-CP. Incidently, the potential of complete anaerobic dechlorination of 2,4-DCP to phenol was also shown by Boyd and Shelton, (1984); Wiegel et al. (1990) which indicated that the specificity of dechlorination of chlorophenols was site-specific.

Besides digested sludge, anaerobic dehalogenation of chlorophenols by freshwater and marine sediments has been also evaluated by several researchers. Freshwater sediments obtained from three sites were evaluated for degradation of monochlorophenols under variety enrichment conditions (Genthner et al., 1989a, 1989b). 2-CP degradation was most easily maintained in laboratory transfers compared to 3-CP while 4-CP was the most difficult compound to sustain biodegradation. Hale et al. (1990) reported that fresh and DCP-adapted sediments from two ponds exhibited different dechlorinating activities with respect to relative rates of dechlorination and substrate specificity (relative affinity towards 2,3-; 2,4- or 2,6-DCP). The difference in response to dechlorination observed in this study (Hale et al., 1990) further strengthen the argument on site-specificity mentioned in the previous paragraph.

All the DCP's tested by Hale et al. (1990) accumulated monochlorophenols. Sequential degradation of 2,4-DCP to 4-CP and phenol and finally to methane and carbon dioxide in freshwater pond sediments was shown to be feasible by Zhang and Wiegel (1990) at 31 °C and at different temperatures by Kohring et al. (1989a). The potential of chlorophenol degradation was recently shown in both freshwater and estuarine sediments under a variety of enrichment conditions (Häggblom et al., 1993a, 1993b) including methanogenic. Time required for initial utilization of monochlorophenols was faster in freshwater compared to estuarine sediments. Based on this result, the authors speculated that the biodegradability

of such compounds (chlorophenols) to be more a function of the presence of competent microbial populations than the molecular structure.

It is clear from the above review that indigenous bacteria from a variety of anaerobic environments show a variable response to dehalogenation of chlorophenols under adapted and un-adapted conditions. Therefore there is a definite need to study further such responses and the environmental factors that may affect the fate of chlorophenols on a long-term basis. Information obtained from such studies will be helpful to environmental engineers to device strategies for degradation these compounds in anaerobic ecosystems.

Effect of Sulphate on anaerobic dehalogenation. Although microbially mediated sulphate reduction is responsible for the mineralization of at least half of the organic matter in aquatic habitats, the knowledge of chlorophenols under-going transformation under sulphate-reducing conditions is extremely limited (Colberg, 1990). Since sulphate is commonly present in anaerobic habitats such as aquatic sediments, soil, and wastewater sludge, there is a need to study its influence on the fate of toxic compounds like chlorophenols. Although the number of studies that have explored these potential reactions is not very high, a number of reasons have been given to account for what some investigators speculate may be a metabolic limitation in sulfidogenic communities.

Results from several laboratory studies suggested that sulphate may inhibit the anaerobic degradation of chloroaromatic compounds by preventing dehalogenation (Genthner et al., 1989a; Gibson and Suflita, 1986, 1990; Kohring et al., 1989b; Kuhn et al., 1990, Madsen and Aamand 1991). Gibson and Suflita (1986) in their work with 2,4,5-trichlorophenoxyacetic acid (2,4,,5-T) had observed that addition of inorganic sulphate to active 2,4,5-T dehalogenating methanogenic enrichments resulted in significant inhibition of degradative activity. They also noted a significant increase of acclimation time and reduction in the amount of 2,4,5-T degraded compared to control cultures without any added sulphate. Similar observations were made by Khun et al. (1990) who reported significant reduction in conversion rate of 2,3,4,5-tetrachloroaniline to 2,3,5-trichloroaniline when sulphate was amended to acclimated methanogenic aquifer slurries. This inhibitory effect was also seen to be more pronounced with less chlorinated anilines. In a later study on the degradation of PCP under sulphate-reducing conditions by anaerobic mixed culture derived sewage sludge, Madsen and Aamand (1991) reported that dechlorination of PCP and its metabolites were inhibited in presence of sulphate. The inhibitory effect was relieved by either adding molybdate - a competitive inhibitor of sulphate reduction or maintaining hydrogen in the head-space.

The mechanism of this suspected sulphate inhibition of dehalogenation remains unclear. Some researchers have suggested that such an effect is

possibly due to a competition for electron donors between sulphate-reducing bacteria and the dehalogenating population such that sulfidogens were able to out compete the dehalogenators for available hydrogen or a competition exists for electron donors by various enzyme systems within the bacterial cells (Gibson and Suflita, 1986; Kuhn et al., 1990; Madsen and Aamand, 1991) . Based on rather consistently observed disparity in transformation potential of chlorophenols between sulphate reducing and methanogenic regions within a shallow aquifer study site, Khun et al. (1990) had postulated that the inhibitory effect of sulphate on anaerobic dehalogenation potential might be lower in the environments that maintain a higher redox potential (i.e., denitrifying and sulfidogenesis) and this was particulary applicable for lower chlorinated compounds. It is however worth noting that *a* pure culture of sulphate reducing bacteria - *Desulfomonile tiedjei* is capable of aryl-dehalogenation of chlorophenols in presence of sulphate (DeWeerd et al., 1986, 1990; Mohn and Kennedy, 1992a).

With sulphate as the terminal acceptor there are no thermodynamic barriers to the oxidation of chlorophenols or other chlorinated compounds (Colberg, 1990). Biodegradation of these compounds is indeed very much favourable under sulphate-reducing conditions. Energy obtained from oxidation of mono-chlorophenols and chlorobenzoates is illustrated in Table 2-2.

Table 2-2. Thermodynamic calculations for complete oxidation of mono-chlorophenols and chlorobenzoates with SO_4^{2-} as the terminal electron acceptor .

Compound	Reaction	ΔG° (kJ/mol carbon)
CHLOROBENZOATES :		
	$2C_7H_5O_2Cl + 7SO_4^{2-} + 5H^+ \Rightarrow 14CO_2 + 7HS^- + 2Cl^- + 4H_20$	
2-Chlorobenzoate		- 65.7
3-Chlorobenzoate		- 62.2
2-Chlorobenzoate		- 61.6
CHLOROPHENOLS :		
	$4C_6H_5OCl + 13SO_4^{2-} + 9H^+ \Rightarrow 24CO_2 + 13HS^- + 4Cl^- + 8H_20$	
2-Chlorophenol		- 67.0
3-Chlorophenol		- 70.4
4-Chlorophenol		- 71.8

Source: From Colberg, 1990.

Several studies as mentioned before have seen transformations to be faster and un-inhibited in methanogenic conditions with bicarbonate (HCO_3^-) as the terminal electron acceptor. However as seen from Table 2-3, conversion of 4-CP as a model compound coupled to methane formation is significantly less favoured thermodynamically compared to any of the three sulfidogenic conditions shown. This probably suggests that there are non-thermodynamic reasons which perhaps affects the transformation of chlorophenols under sulphate-reducing conditions.

Table 2-3. Comparison of terminal electron acceptors for complete oxidation of 4-CP using Sulphate(SO_4^{2-}), Sulphite(SO_3^{2-}), Thiosulphate($S_2O_3^{2-}$), or Bicarbonate(HCO_3^-).

Reaction	ΔG^o (kJ/mol carbon)
$4C_6H_5OCl + 13SO_4^{2-} + 9H^+ \Rightarrow 24CO_2 + 13HS^- + 4Cl^- + 8H_2O$	- 71.8
$3C_6H_5OCl + 13SO_3^{2-} + 10H^+ \Rightarrow 18CO_2 + 13HS^- + 3Cl^- + 6H_2O$	- 121.2
$4C_6H_5OCl + 13S_2O_3^{2-} + 5H_2O \Rightarrow 24CO_2 + 26HS^- + 4Cl^- + 4H^+$	- 60.2
$4C_6H_5OCl + 18H_2O \Rightarrow 11CO_2 + 13CH_4 + 4Cl^- + 4H^+$	- 39.4

Source: From Colberg, 1990.

Despite the fact linking sulphate with reductive dehalogenation of chlorinated compounds, there are now a growing number of reports that show chlorophenol degradation under sulphate reducing conditions. Genthner et al. (1989b) was able to show a general stimulation of the degradation of 4-CP by adding inorganic sulphate to their estuarine enrichments which indicated that sulphate reduction probably served as the terminal electron process in this case although they failed to co-relate the 4-CP removal to this reduction.

Kohring et al. (1989b) also observed the reductive dehalogenation of 2,4-DCP to 4-CP in freshwater sediments during sulphate-reduction although a longer acclimation time was needed in cultures amended with sulphate. It was also found that transformation of 4-CP to phenol under sulfidogenic conditions increased after adaptation under methanogenic conditions suggesting that the same organism may have catalyzed the dechlorination reaction under both conditions. Although

sulphate concentrations were monitored, results did not conclusively link sulphate reduction to the observed chlorophenol degradation activity.

The first report of chlorophenol degradation coupled to sulphate reduction was presented by Häggblom and Young (1990). The authors showed complete mineralization of 2-CP, 3-CP and 4-CP in freshwater and saline sediments concomitant to sulphate reduction. Inhibition of sulfidogenesis by molybdate stopped dehalogenation of these compounds totally and since methane was never detected in their enrichments, it was concluded that sulphate reduction was the main electron sink. The authors also reported dechlorination of 2,4-DCP to 4-CP and complete removal of 2,6-DCP via 2-CP and phenol during sulfidogenesis. Häggblom et al. (1993a) compared dehalogenation of monochlorophenols using three different electron acceptors in both estuarine and freshwater sediments. Dehalogenation of these compounds was dependent on the electron acceptors used and the relative position of the chlorine substituent.

In a later study, Häggblom et al. (1993a); Häggblom and Young (1995) further investigated three different forms of inorganic sulphates as potential electron acceptors for dechlorination for 4-CP and 2,6-DCP in aquatic sediments. 4-CP was degraded in presence of sulphate, sulphite and thiosulphite in the descending order. However sulphate slowed down 2,6-DCP degradation, while sulphite and thiosulphite completely inhibited reductive dehalogenation under these

conditions. Further studies are required before the process of sulphate mediated dehalogenation of chlorinated compounds like chlorophenols could be used as a viable alternative in the bioremediation of contaminated water, soils and sludges. These should include the control and factors affecting the interactions of various metabolic groups present in the system before attempting to extrapolate some of the sucessfull results achieved under laboratory conditions for large scale clean-up operations. As we have seen from this review, there is a possibility that such transformation processes are likely to be site-specific.

Anaerobic Dehalogenation by Pure Cultures. Unlike several pure cultures of aerobic microorganisms isolated so far (refer section 2.5.1: Aerobic degradation by pure cultures), reductive dehalogenation reactions mediated by a single microbial species is limited. *Desulfomonile tiedjei* formerly strain DCB-1 is the best described dechlorinating anaerobic bacterium to date (Shelton and Tiedje, 1984). *D. tiedjei* is a gram-negative sulphate reducing bacterium that obtains energy for growth from reductive dehalogenation of 3-Chlorobenzoate (DeWeerd et al., 1990).

The capability of *D. tiedjei* to dechlorinate a range of chlorophenols was investigated by Mohn and Kennedy (1992a). PCP, tetra- and tri- chlorophenols were dechlorinated only at the *meta*- position and chlorines at either *ortho*- or *para*- position were never removed. None of the monochlorophenols including 3-CP was

dechlorinated which is in contrast to the ability of this organism to dehalogenate 3-CB. In all cases, the organism had to be induced by 3-CB for dehalogenation of chlorophenols. Additionally, *D. tiedjei* has not been shown yet to obtain energy from chlorophenol dechlorination.

Dietrich and Winter (1990) suggested that a spirochaete-like organism was perhaps responsible for the dechlorination of 2-CP. This suggestion was based on the observation that their mixed culture (which comprised of three different morphologically distinctive micro-organisms) failed to dechlorinate 2-CP when the spirochaete-like organism was lost. However, any attempts to isolate this organism failed. Zhang and Wiegel (1990) proposed that a spore-forming bacterium was possibly responsible for transforming 2,4-DCP to 4-CP in a sediment free consortium. However, a sediment-free enrichment culture dechlorinating 4-CP was not obtained. Madsen and Aamand (1992) also speculated that the organisms responsible for dechlorination of 2,4,6-TCP to 4-CP via 2,4-DCP was a spore former since the methanogenic enrichment culture was still able to sustain dechlorination after heat treatment for 1 h at 80 °C.

Madsen subsequently isolated a gram positive endospore forming bacterium related to genus *Clostridium* that transformed several di- and tri-chlorophenols but not monochlorophenols (Madsen and Licht, 1992). Dechlorination occurred at primarily ortho- position although removal of meta- substituted chlorine was also

observed for 3,5-DCP. The bacterium designated strain DCB-2 unlike *D. tiedjei* did not reduce sulphate. However DCB-2 has not been shown to benefit from the dechlorination process. Another gram positive isolate, *Desulfitobacterium dehalogenans*, *ortho*- dechlorinates a wide range of chlorophenols and related compounds and appears to benefit from the dechlorination reaction when grown on pyruvate and yeast extract (Utkin et al., 1994).

It was only recently that Cole et al. (1994) finally isolated a bacterium capable of anaerobic dehalogenation of a monochlorophenol. The organism, designated strain 2CP-1 is a gram-negative facultative anaerobe that dehalogenates 2-CP to phenol. The organism also grew aerobically indicating that its use could be extended to treatment processes oscillating between two extreme ORP. Only the *ortho*- position was dechlorinated and additional chlorines at other positions either decreased or blocked *ortho*- dechlorination. The organism 2CP-1 could also grow with fumarate as an electron acceptor. Analysis of the organism's 16S rRNA sequence revealed that it was a member of the delta proteobacteria, more closely related to the myxobacteria than the sulfidogenic bacteria. The isolation of such pure cultures of bacteria has provided a better opportunity to study closely and strengthen our present knowledge on the mechanisms associated with the removal of these class of compounds under anaerobic conditions.

Anaerobic dehalogenation in bioreactors. Almost all the studies on anaerobic dehalogenation of chlorophenols and associated compounds discussed so far in this review have been carried out in batch or semi-continuous cultures. Due to the presence of these compounds in industrial wastes and a rapid development of anaerobic bioreactor technology, the feasibility of using a continuous system has been investigated by a hand-full of research groups. From some of the earlier work done in this area, Hakaulinen and Salkinoja-Salonen (1982) had indicated the potential use of anaerobic bioreactors to treat chlorophenolic wastes. An anaerobic fluidized bed system coupled to an aerobic trickling filter was shown to mineralize PCP although the pathways and extent of chlorophenol of degradation in each system was not determined.

Woods (1985) and Woods et al. (1989) used an up-flow anaerobic sludge blanket reactor to degrade a wide range of chlorophenols including PCP in presence of high concentrations of readily biodegradable substrates. Although *ortho-* and *meta-* chlorines were removed, compounds with chlorines at *para-* position persisted and ring cleavage was not observed. Krumme and Boyd (1988) on the other hand were able to demonstrate complete mineralization of all the three monochlorophenols in an upflow bioreactor system. However, only about 40% of the added monochlorophenols were removed and the system failed to dehalogenate either 2,4,6-TCP or PCP.

Use of up-flow anaerobic sludge bed reactor was further carried out by Hendriksen et al. (1992) to dehalogenate PCP in presence or absence of glucose. Although PCP dehalogenated to dichlorophenols, the removal of PCP was found to be much higher in presence of glucose compared to the un-amended reactor. In a later study, the authors (Hendriksen and Ahring, 1993) also compared UASB systems inoculated with granular sludge with fixed film reactors started with anaerobic digested sewage sludge for degradation of PCP. The UASB reactors showed a greater potential in dehalogenating PCP compared to the fixed film reactors. PCP dehalogenated primarily to dichlorophenols in the UASB system while an accumulation of 3,4,5-TCP was observed in the fixed-film reactors. Mineralization of PCP in anaerobic granular sludge using an UASB reactor was also shown by Wu et al. (1993). The authors indicated that removal of PCP was via biodegradation rather than adsorption. Radiotracer assay was used to demonstrate mineralization of [^{14}C]PCP to $^{14}CH_4$ and $^{14}CO_2$.

Lu (1992) studied the fate, toxicity and degradative pathway of five trichlorophenols and 3,5-DCP in anaerobic sludge blanket reactors. Although acclimation and alternate carbon sources reduced the inhibitory effect, monochlorophenols such as 3-CP and 4-CP accumulated as the major products. Flora et al. (1994) recently demonstrated the potential of an anaerobic fluidized-bed granular activated carbon (GAC) to treat a simulated high-strength industrial wastewater containing chlorophenols. A COD elimination of over 98% was achieved for the waste comprised of 2-CP; 2,4-DCP and 2,4,6-TCP at a GAC solid mean retention time of 100 days with an accumulation of 4-CP .

The above studies indicate that biological reactors could be successfully applied for the treatment and bioremediation of a variety of chlorophenolic wastes. Most of the studies report the versatility of the UASB system especially operating with a granular sludge although alternative bioreactors were not evaluated. Such continuous experiments should be complemented with batch studies to determine the fate and extent of chlorophenol degradation. Due to the dynamic nature of all reactor systems and the complex behaviour of chlorophenols, accurate mass balance determinations can be very difficult to make (Mohn and Kennedy, 1992b).

2. 5.3 Anoxic degradation

Compared to the studies on biodegradation of chlorophenols under aerobic conditions (Refer section 2.5.1) or anaerobic conditions (Refer section 2.5.2), very little is known about the fate and biodegradability of chlorophenols under anoxic conditions. The term anoxic conditions is frequently used in environmental engineering literature especially in the area of biological nutrient removal and selector technology to differentiate from anaerobic conditions. Most microbiologists will probably disagree with this terminology, but it is used ubiquitously in engineering. Randall et al. (1992) defines anoxic as a condition with no added oxygen or air but with total NO_x-N $\geq$ 0.5 mg/L.

Hu and Shieh (1987) tested the dehalogenation potential of 2,4-DCP in an anoxic up-flow biofilter. At maximum efficiency, the system was able to maintain a 80% removal efficiency of 2,4-DCP (as TOC) and influent nitrate respectively at

an empty bed HRT of 145 minutes. However the study failed to indicate the intermediates of chlorophenol degradation. Oleszkiewicz et al. (1991) investigated the feasibility of 2-CP degradation in presence co-substrates in an anoxic-aerobic sequencing batch reactors (SBR's). Although nitrate reduction took place, 2-CP was not removed during the anoxic part of the SBR cycle. All of the influent 2-CP was removed during the aerobic phase which followed the anoxic period. The aerobic rate for 2-CP removal following the anoxic phase was significantly greater than the rates observed for 2-CP in fully aerobic reactors. The reason for such enhancement of biodegradation rate was not investigated.

Puhakka et al. (1992) developed an anoxic biofilm which was shown to use 4-CP as the sole electron donor in denitrification reactions. Mineralization was confirmed through chloride release which agreed well with the theoretical value. The authors however failed to demonstrate dechlorination of 2,4-DCP or PCP under these conditions. A comparison of biodegradation potential in batch cultures for 2-CP, 3-CP and 4-CP was recently reported by Häggblom et al. (1993) under denitrifying conditions. Significant removal of 2-CP was observed compared to negligible amounts observed for either 3- or 4-CP. 2-CP dehalogenation however could not be sustained with refeeding. No intermediates were detected for either of these monochlorophenols nor any data given on nitrate reduction. The authors suggested the following equation for removal of monochlorophenol under anoxic conditions:

$$C_6H_5OCl + 5.2\ NO_3^- + 4.2\ H^+ \text{ =====> } 6CO_2 + 2.6\ N_2 + 4.6\ H_20 + Cl^- \qquad [2\text{-}3]$$

2.6 CONCLUSIONS

Knowledge of the mechanisms involved in the degradation of halogenated aromatic compounds such as chlorophenols is essential for the design of effective treatment processes. Biodegradation was the major route for detoxification of these compounds and has been extensively researched although several abiotic processes such as photodegradation and adsorption were also found responsible for its removal.

The pathways of biodegradation of chlorophenols varied considerably with the redox potential of the environment. Under aerobic conditions these compounds are metabolized by enzymes which incorporated oxygen atoms in the aromatic ring, followed by halogen loss from a non-aromatic intermediate. Most studies on aerobic degradation have been restricted to batch cultures employing pure or mixed microbial populations under controlled conditions. A gap exists in the literature as to the ultimate fate of chlorophenols in multi-substrate biological treatment systems.

Our understanding of anaerobic metabolism of chlorophenols has increased in the last decade. Although these compounds were shown to undergo reductive dehalogenation in primarily methanogenic systems, alternative electron acceptors such as sulphate were often reported to be inhibitory. The reason for such sulphate mediated inhibition is presently not known. In other instances,

transformation of chlorophenols were observed in presence of and coupled to sulphate reduction indicating that such a mechanism in reductive dehalogenation of chlorophenols is a site-specific phenomenon. The present understanding of the interactions taking place between metabolic groups present in a mixed culture dehalogenating chlorophenols is also limited.

Several studies had indicated that under anaerobic conditions the polychlorophenols undergo reductive dehalogenation to less substituted chlorophenols such as the monochlorophenols which either accumulated or were removed slowly. The monochlorophenols however were readily removed under aerobic conditions which led to the development of sequential anaerobic-aerobic processes for complete mineralization of chlorophenols. A single-biomass system degrading chlorophenol under oscillating ORP is however yet to be developed.

As seen from this review, degradation of chlorophenols under anoxic conditions is extremely limited. The need to further our understanding of the fate of chlorophenols under nitrate reducing conditions is important for remediating polluted soil, sediments and aquifers. An in-depth examination of the factors influencing biodegradation potential under the three redox potentials discussed in this review is essential to further our understanding of the fate of such toxic compounds under these environments so that suitable treatment strategies could be devised for abating the problem of chlorophenol contamination in our ecosystems.

Chapter 3.

RESEARCH OBJECTIVES

Degradation of 2-CP has been studied mainly under aerobic conditions with recent emphasis on degradation in anaerobic conditions. Very little is known about its fate in anoxic environments. The effects of acclimation, presence of electron donors and acceptors and the interactions of various metabolic groups present in the degradation system are not well understood. This study was initiated to address the problem of 2-CP degradation under three different redox potentials.

I. The anaerobic experiments were conducted to examine the role of following in influencing the degradation of 2-Chlorophenol (2-CP) by a mixed microbial consortium :

- the effects of the various sources of inocula (unacclimated) on acclimation and long term performance in batch systems.

- presence of alternative electron acceptors such as SO_4^{-2} and the role of various inhibitors such as BESA and molybdate on 2-CP dehalogenation.

- response of cultures to 2-CP after prior acclimation.

- effects of various electron donors such as yeast extract, acetate, propionate, ethanol and hydrogen on 2-CP dehalogenation.

- the potential degradation of other chlorophenols such as 2,4,6-TCP; 2,4-DCP and 4-CP by cultures that were adapted to 2-CP.

II. Under aerobic conditions, the experiments on degradation of 2-CP were carried out in sequencing batch reactors (SBR's) employing a suspended growth mixed microbial cultures to study the following factors:

- effect of the presence, nature and concentrations of supplemental substrates such as dextrose and phenol on specific 2-CP removal.

- effect of random batch un-aerated periods during the react phase of the SBR cycle on the subsequent aerobic degradation rates of 2-CP.

- effect of different temperatures such as 24, 15, 10 and 5 °C on specific removal rates of 2-CP in presence of either dextrose or phenol.

- effect of different SRT (20, 16, 12, 8 and 4 days) on volumetric and specific 2-CP rates in presence of either dextrose or phenol.

III. The feasibility of anoxic 2-CP removal in presence of supplementary substrates such as dextrose or phenol was investigated in SBR's programmed to operate on a sequential anoxic - aerobic mode.

- effect of anoxic HRT of 2, 3 and 4 hours on specific 2-CP removal.

- effect of anoxic HRT on the aerobic volumetric removal rates of 2-CP by mixed microbial cultures adapted to sequential anoxic-aerobic operation.

Chapter 4.

MATERIALS AND METHODS

4.1 ANAEROBIC EXPERIMENTS

The following sections discuss the experiments that were designed to evaluate the effects of different inocula sources, electron acceptors, electron donors and adaptation on anaerobic dehalogenation of 2-CP, and other chlorophenols.

4.1.1 Source of inoculum

To test the feasibility of 2-CP dechlorination in un-adapted cultures, the following samples for inoculum were obtained fresh : (i) anaerobic sludge from primary mesophillic digesters treating combined primary and activated sludge at the North End Water Pollution Control Centre (NEWPCC), Winnipeg (ii) anaerobic sediment from an aerated lagoon treating bleached kraft mill (BKM) wastewater, Fort Francis (Ontario) and (iii) biomass from a laboratory scale anaerobic bioreactor treating the wastewater from the same mill. The Winnipeg NEWPCC receives most of its wastewater from residential sources and operates the digesters at a retention time of 12 to 14 days. The inoculum typically contained 8 to 10 g/L of total volatile solids. The total chlorinated compounds as AOX (adsorbable organic halide) present in the influent kraft mill wastewater to either the lagoon or anaerobic bioreactor was typically 30 mg/L. Although chlorophenols are normally present in BKM wastes, no background 2-CP was detected in the

inoculum from either the lagoon sediment or biomass from the anaerobic bioreactor. The total volatile solids content of lagoon sediment and bioreactor biomass were 30 g/L and 14 g/L respectively. Samples from the digesters and the lagoon were collected in plastic jars filled to capacity and transported to the laboratory where they were used immediately. Samples from the laboratory anaerobic reactor were collected just before use to small glass vials provided with air tight caps.

4.1.2 Establishment of cultures

Strict anaerobic techniques based on the methods described in Daniels *et al.*(1986) were followed throughout the entire study for medium preparation, culture handling, and sampling. Experiments were conducted generally with sludge samples that were added either as 10% (vol/vol) inoculum to aluminium seal type anaerobic culture tubes (no. 2048-00150; Bellco Glass, Inc., New Jersey) or 50% (vol/vol) inoculum in 160 mL Wheaton serum bottles.

For the culture tubes, the following procedures were followed at the onset of setting up cultures. Prior to inoculation, mineral medium (prepared aerobically, see next section for details) was dispensed into the tubes which were subsequently sealed with lipped butyl rubber stoppers (aluminium seal tube stoppers, no. 2048-11800; Bellco) and crimped with aluminium seals (no. 2048-11020, Bellco). After sealing, the head-spaces were repeatedly (three times) evacuated for 3 mins. and refilled with a gas mixture of either 80% nitrogen and

20% carbon dioxide or 80% hydrogen and 20% carbon dioxide. This process of evacuating and refilling with respective gas mixtures were done using a syringe connected to a gas manifold (design modified but based on Balch and Wolfe, 1976) as shown in Fig. 4-1. The tubes were kept under positive pressure (approx 20 psi) of the appropriate gas mixture. The medium in culture tubes was subsequently reduced by the addition of 0.35 mM titanium (III) citrate (Zehnder and Wuhrmann, 1976) and the tubes were finally sterilized by autoclaving for 15 minutes at 121 °C.

The culture tubes now containing the anaerobic mineral medium were then inoculated with respective sludges by direct injection using a syringe that had been previously flushed with sterile anaerobic deionized water to a final volume of 10 mL. 2-CP (Sigma Chemicals, St. Louis, MO) was injected from a stock solution of 20 g/L (about 156 mM) to give a target initial concentration of about 50 mg/L (0.385 mM) in the tubes. Tubes were established either in duplicates or triplicates along with sterile controls. For the sterile controls, the tubes containing medium were first inoculated with respective sludges following which they were autoclaved at 121 °C for 1 h, prior to injecting 2-CP.

A slightly different procedure was adopted for experiments with 50% (vol/vol) cultures in 160 mL serum bottles. The mineral medium was concentrated two-folds and dispensed at 40 mL per bottle. The bottles were subsequently sealed, pressurised with gas mixture, reduced and sterilized as explained above. The bottles were then inoculated with 40 mL of sludge to final volume of 80 mL

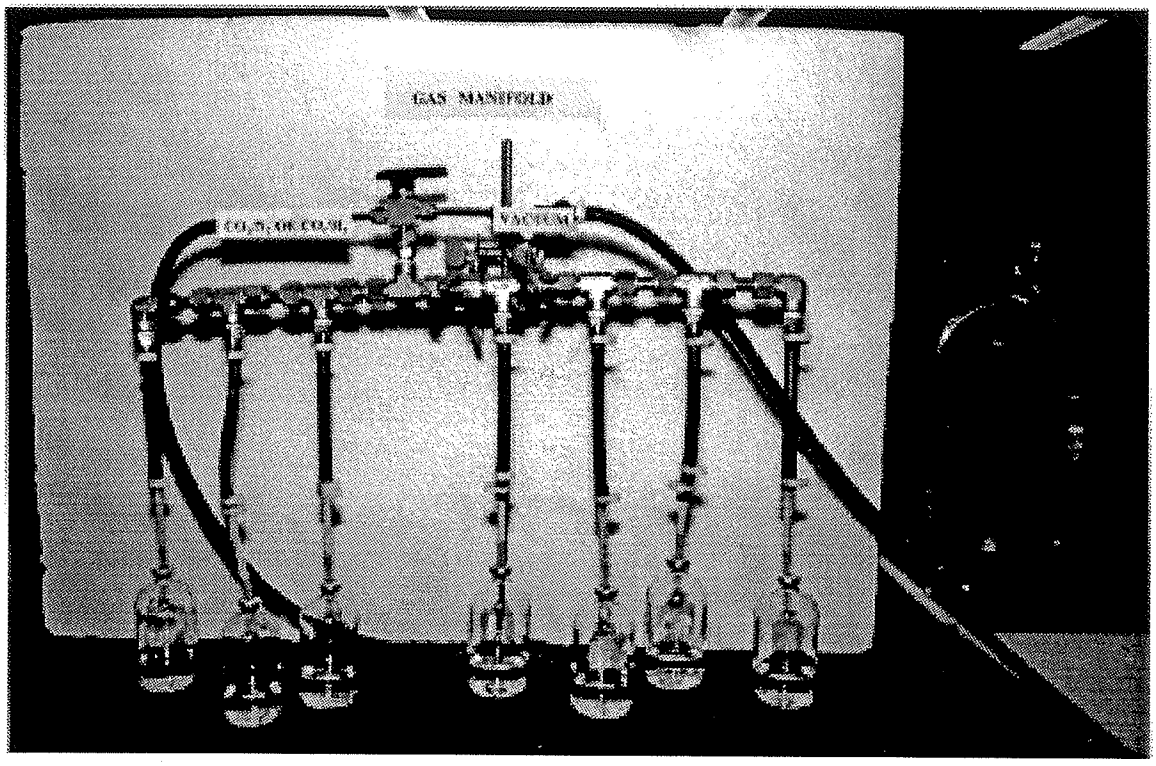


Fig. 4-1 A view of the Gas-Manifold used in this study

using a 60 mL sterile syringe (Becton Dickinson & Co., Rutherford, New Jersey) previously flushed with sterile anaerobic water and fitted with 20 gauge 1½ inch size needle. A similar procedure was also followed for the only experiment done with 25 % (vol/vol) culture in serum bottles. After establishing the cultures, all tubes or bottles were incubated statically in the dark in a walk-in chamber maintained at 35°C. Fig. 4-2 gives a view of incubating serum bottles and culture tubes.

4.1.3 Medium composition and preparation

The basic medium was prepared using the following constituents per litre of deionized water : 0.27 g KH_2PO_4, 0.35 g K_2HPO_4, 0.53 g NH_4Cl, 0.05 mg $NaSeO_3$, 0.1% yeast extract, 10 mL each of mineral elixir (see Table 4-1) and vitamin supplement (see Table 4-2). For experiments under sulfidogenic conditions, the above medium was supplemented with 5 mM SO_4^{-2} using Na_2SO_4. When either methanogenic or sulfidogenic inhibitors were used the medium was supplemented with either 1 - 10 mM of BESA (bromoethanesulphonic acid, Sigma Chemical Corp.) or 1 - 20 mM molybdate respectively. Several drops of resazurin (from a stock solution of 50 mg/L) was also added as a redox indicator. All the components were mixed by a bar magnet in a beaker mounted on a magnetic mixer while the medium was saturated with either 80% N_2 and 20% CO_2 or 80% H_2 and 20% CO_2. The pH which usually dropped to around 6.1 during this operation was finally adjusted between 7.0 to 7.1 with solid sodium bicarbonate before dispensing in culture tubes or serum bottles.

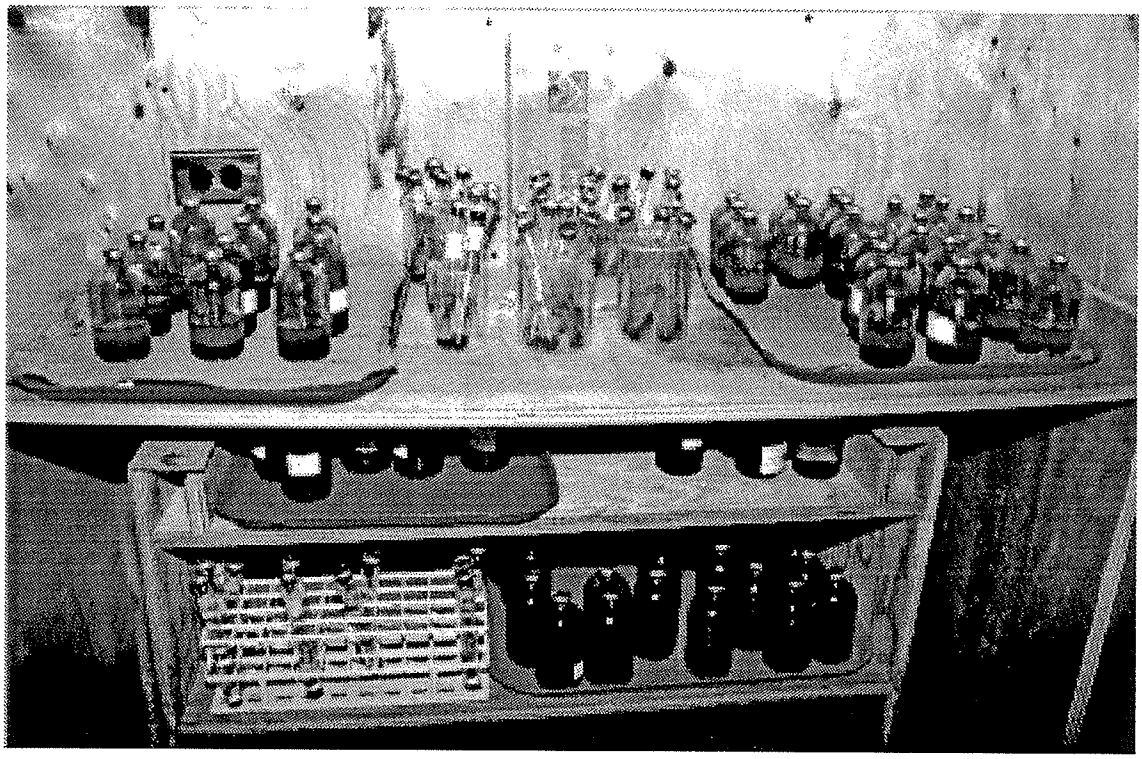


Fig. 4-2 Incubating serum bottles and culture tubes at 35 °C

Table 4-1 Mineral elixir used for the anaerobic medium

Compound	Amount (g/L)
Nitrilotriacetic acid	1.5
$MgSO_4$	3.0
$MnSO_4$	0.5
NaCl	1.0
$FeSO_4$	0.1
$CaCl_2.2H_2O$	0.1
$CoCl_2$	0.1
$ZnSO_4$	0.1
$CuSO_4.2H_2O$	0.01
Na_2MoO_4	0.01
$NiCl_2.6H_2O$	0.10

All quantities were dissolved in 1 L deionized water.

Table 4-2 Vitamin supplement adopted from Wolin *et al.*, 1963

Compound	Amount (mg/L)
Biotin	2.0
Folic Acid	2.0
Pyridoxine hydrochloride	10.0
Riboflavin	5.0
Thiamine	5.0
Nicotinic Acid	5.0
Pantothenic Acid	5.0
Vitamin B_{12}	0.1
p - aminobenzoic acid	5.0
Thioctic acid	5.0

All quantities were dissolved in 1 L deionized water.

4.1.4 Maintenance of batch and semi-continous anaerobic cultures

After complete disappearance of 2-CP (although experiments were also done to test dehalogenation of 4-CP; 2,4-DCP; and 2,4,6-TCP), the culture tubes were centrifuged at 4000 rpm for 45 minutes using a Damon/IEC Division Centrifuge, Model HN - S (Needham Hts., Massachusetts). The tubes were gently inverted to avoid displacing the bacterial pellet and the clear medium was withdrawn using a sterile syringe. The culture was subsequently washed by re-suspending the bacterial pellet in pre-reduced 0.1 M sodium phosphate buffer (pH 7), vigorously shaken and centrifuged again as above. Following removal of the buffer, the cultures were refed with a fresh dose of sterile anaerobic medium (10 mL) containing 0.1% yeast extract (as described before). The cultures were then subsequently spiked with respective chlorophenols to their initial concentrations and the experiment was repeated. Most of the serum bottles that were used in this study were operated as single-run batch reactors. However in some experiments the serum bottles were also maintained on a semi-continuous mode by refeeding yeast extract medium (from a concentrated stock) to a final volume of 80 mL before respiking with 2-CP. Anaerobic experiments conducted in this study under a variety of conditions are summarized in Table 4-3.

Table 4-3 Summary of Anaerobic Experiments

Inoculum Source	Inoculum Size	Type of Enrichment	Head-space Composition	Enrichment Condition	Media Composition
DS	10 %	un-adapted	CO_2/N_2	M	Y.E
	10 %	un-adapted	CO_2/H_2	M	Y.E
	50 %	un-adapted	CO_2/N_2	M and S	Y.E
	50 %	un-adapted	CO_2/N_2	M	BESA
	50 %	un-adapted	CO_2/N_2	M and S	Molybdate
LS	10 %	un-adapted	CO_2/N_2	M	Y.E
BB	10 %	un-adapted	CO_2/N_2	M	Y.E
DS	10 %	adapted	CO_2/N_2	M	Y.E No Y.E HAC HPr Ethanol
	10 %	adapted	CO_2/H_2	M	Y.E
	25 %	adapted	CO_2/N_2	M	Y.E
	50 %	adapted	CO_2/N_2	M	Y.E
DS	10 %	adapted	CO_2/N_2	M	Y.E
	50 %	un-adapted	CO_2/N_2	M	Y.E
(for 4-CP; 2,4-DCP & 2,4,6-TCP)					

DS : Digested Sewage Sludge; LS : Lagoon Sediment; BB : Biomass from bioreactor.

Inoculum size shown are vol/vol. 10% cultures were set-up in balch tubes while remaining in 80 mL serum bottles. Y.E means 0.1% yeast extract medium. HAC and HPr is acetic and propionic acid.

Adapted implies pre-exposure to 2-CP.

Head-space composition were 20% CO_2 + 80% of either N_2 or H_2.

Cultures were established in duplicates or triplicates and incubated statcailly in the dark at 35°C.

S and **M** indicates cultures amended with or without 5 mM $SO_4^=$ respectively.

4.1.5 Operation of Acclimation Reactor

Acclimation of anaerobic digester sewage sludge to 2-CP was carried out in two cylindrical plexiglass batch reactors of volume 2 litres. Fig. 4-3 shows a typical view of one such reactor. The reactors were provided with a sampling port which had a special rubber septum for the ease of sample withdrawal, feeding 2-CP or medium using a syringe. The two systems were initially started by transferring 1.5 litres of fresh sludge (12.3 g/L as total volatile solids) from primary anaerobic digesters of the North End Pollution Control Centre, Winnipeg following strict anaerobic techniques. The head-spaces were flushed with nitrogen before closing the reactors. Each reactor was provided with a gas outlet system connected to water lock jars. A sampling tap was provided in the gas outlet line for determining gas composition. Complete mixing was ensured by mounting each reactor on magnetic mixers and the two systems were set-up at 35°C in a walk-in environmentally controlled chamber.

One reactor received about 0.39 mM (approx 50 ppm) 2-CP as the sole carbon source along with necessary minerals and vitamins (refer Tables 4-1 and 4-2) but without any yeast extract. The second reactor on the other hand received in addition to 2-CP, mineral salts, vitamins and 0.1% of yeast extract. 2-CP and its metabolites were monitored using HPLC by regularly withdrawing samples from each reactor. When the concentration of 2-CP was below our detection limit (< 0.5 ppm), mixing was stopped and the reactors contents allowed to settle. A portion

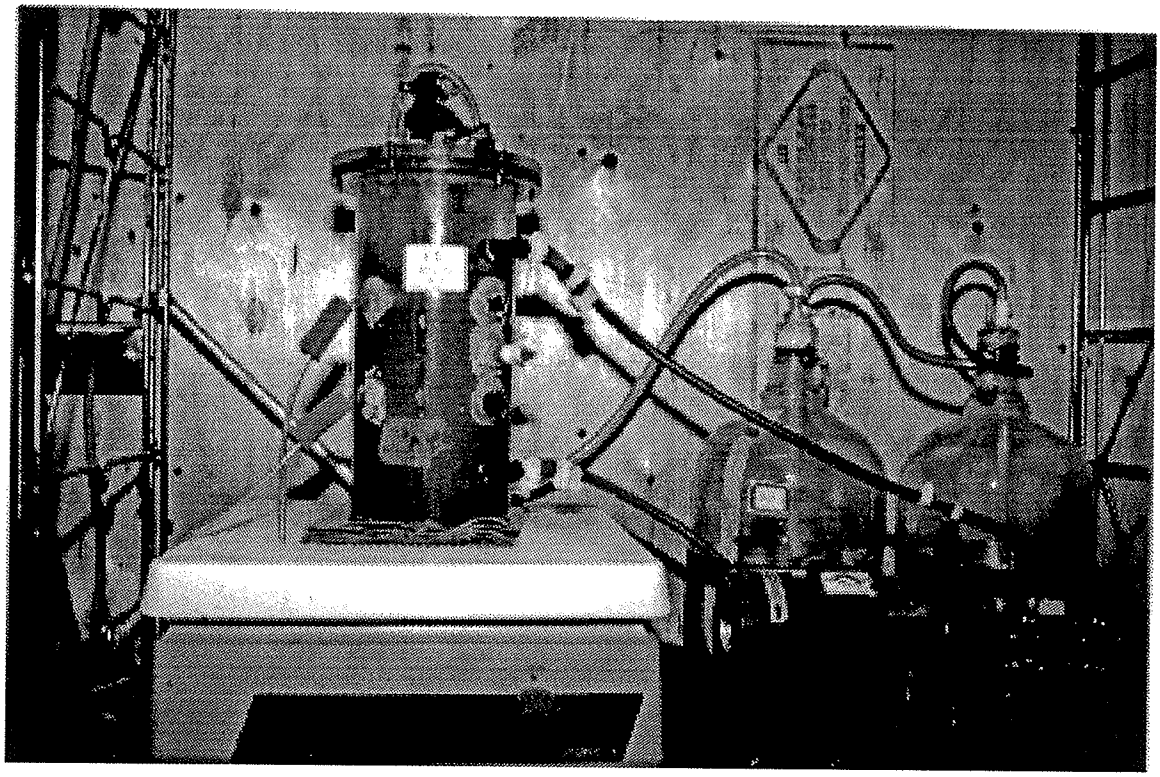


Fig. 4-3 Typical view of the acclimation reactor

of the supernatant was subsequently withdrawn before feeding 2-CP and fresh mineral medium. The amount of reactor liquid withdrawn during regular sampling was also considered at this time thereby maintaining the volume of the reactors at 1.5 litres and the initial 2-CP concentration at 0.39 mM.

Although gas production was not monitored regularly, methane was detected in the head-space of both the systems. After four months of operation, the reactor which received 2-CP as the sole carbon source accumulated phenol and the 2-CP dehalogenation rate decreased. The situation improved by supplementing the feed with yeast extract. This process was continued for a year at which time both reactors, operating in the presence of 0.1% yeast extract were capable of complete dehalogenation of 0.39 mM (50 mg/L) 2-CP in seven days.

4.1.6 Analysis of Volatile Fatty Acids (VFA)

Samples for VFA were analysed using a Hewlet Packard HP 5890A Gas Chromatograph (GC). Culture liquid was first withdrawn from tubes or bottles into 1.5 mL eppendorf polypropylene micro test tubes. The tubes were centrifuged for at least 15 minutes in Micro Centrifuge Model 235C (Fisher Scientific Co.) before transfering the clear supernatant for further analysis. Using a pippette 1 mL of the clear supernatant was transferred to another glass vial where it was diluted with deionized water whenever the expected Total-VFA exceeded the calibration range of 0 - 100 mg/L to which the GC was calibrated prior to injecting any samples.

All such samples were finally acidified with concentrated phosphoric acid and 1 µL was injected into the GC. The system was equipped with a flame ionization detector (FID) and HP - FFAP (cross linked) caplillary column of size 10 m x 0.53 mm x 1.0 µm. The injection, oven and detector temperatures was set at 150 °C, 85 °C and170 °C respectively with hydrogen as a carrier gas at a flow rate of 13 - 14 mL/min.

4.1.7 Analysis of head-space methane gas

The head-space of culture tubes and serum bottles were monitored for methane. 1 mL of the head-space gas was injected into a Gow-Mac gas chromatograph with series 550 thermal conductivity detector. The GC was equipped with Porapak Q Column (80/100 mesh) using helium as a carrier gas at a flow rate of 60 mL/min. The instrument was calibrated using standard methane gas at different (known) percentage composition.

4.1.8 HPLC analysis

Under anaerobic conditions, chlorophenols (2-CP; 4-CP; 2,4-DCP and 2, 4, 6-TCP), its metabolites phenol and benzoate were analyzed by reversed phase high pressure liquid chromatography (HPLC). Samples withdrawn aseptically from culture tubes or serum bottles were centrifuged as in VFA analysis using a microcentrifuge to remove solids. The supernatant was injected into a Millipore-Waters HPLC system. The HPLC used a Millipore-Waters Lambda Max 481 LC Spectrophotometer measuring at a wavelength of 254 nm and PRP-1

column (Hamilton Co.. Reno, Nevada). The system was operated in an isocratic mode for 2-CP while the remaining chlorophenols were analyzed in a program gradient mode. The optimal conditions for this study were developed in the lab.

In the isocratic mode, the mobile phase consisted of 2% acetonitrile (1:1) and 98% sodium triphosphate buffer (pH 12) at a flow rate of 2 ml/min. The program gradient conditions used in this study for optimal separation of the remaining chlorophenols and its metabolites are summarized in Table 4-4. The system was calibrated by using solutions of known concentrations of 2-CP (or 4-CP; 2,4-DCP and 2,4,6-TCP), phenol and benzoate. The metabolites were identified by comparison with the retention times of the known standards in the HPLC. No individual peaks corresponding to the retention times of known standards were observed for background samples (amended with 0.1% yeast extract but no 2-CP).

Table 4-4. Program gradient mode in HPLC for 4-CP; 2,4-DCP; 2,4,6-TCP

Run Time (minutes)	**Flow (mL/min)**	**Acetonitrile %**	**Buffer %**	**Curve**
Initial	2.0	5	95	---
9.0	3.0	50	50	5
10.0	5.0	5	95	5
12.0	2.0	5	95	5

Curve indicates the change of profile for both flow-rate and mobile phase composition with time as per the manufacturers specifications. Refer to APPENDIX A (pages A14 and A15) for details.

4.4.9 Analysis of Sulphate (SO_4^{2-})

Sulphate concentration as SO_4^{2-} was monitored using a automated methylthymol blue, according to standard method (APHA, AWWA and WEF, 1992) no. 4500-SO_4^{2-} F. Samples withdrawn from the serum bottles were handled the same way as described before for VFA analysis except that no acid was added. All samples were generally diluted four times with deionized water before analysis.

4.2 AEROBIC EXPERIMENTS

The study of factors affecting aerobic degradation of 2-CP were done in sequencing batch reactors (SBR's). The following sections describes the conditions under which SBR's were operated along with instrumental and chemical analysis.

4.2.1 Sequencing batch reactors (SBR's)

The aerobic biodegradability studies of 2-CP were conducted in identical laboratory scale sequencing batch reactors (SBR's). Each reactor had an operating volume of 2 L and was constructed of 6 mm thick, 100 mm internal diameter plexiglass tube. The reactors were mounted on magnetic stirrers to maintain complete mixing in the system and were operated in parallel on a 8 hour cycle consisting of 0.25 h fill, 6 h react, 1.5 h settle and 0.25 h decant. Air was supplied during the react phase through submerged diffusers to maintain a

dissolved oxygen level greater than 3 ppm in the reactor. At the end of the settling period, the reactor contents were decanted to a volume of 1 L which diluted the incoming feed by half. All operations were controlled through a GE Series One Programable Controller. The temperature varied from 23 ± 1 °C during the study period. A typical schematic of the set-up is shown in Fig. 4-4.

4.2.2 Effect of supplementary substrates and feed composition

The initial part of this study was to investigate whether the potential use of supplemental substrates like dextrose and phenol enhances the rate of 2-CP removal and to find an optimal substrate concentration for further experiments. Three parallel reactors were operated of which one received no supplemental substrates (i.e. 2-CP served as the only carbon source), the second and third reactors received varying concentrations of dextrose and phenol respectively as either 20, 40, 60, 80, or 200 mg SOC/L during separate experiments. A view of the actual set-up is shown in Fig. 4-5. In between changes of dextrose or phenol feed concentration, both the SBR's which received such supplements were allowed to operate for at least three weeks to ensure stable reactor performance prior to any experimentation and data collection. The 2-CP removal profiles were monitored over the next few weeks until consistent patterns were observed.

Besides 30 mg/ L 2-CP (Sigma Chemical Company, St. Louis, MO) and the respective supplements like dextrose or phenol the feed to the reactors contained

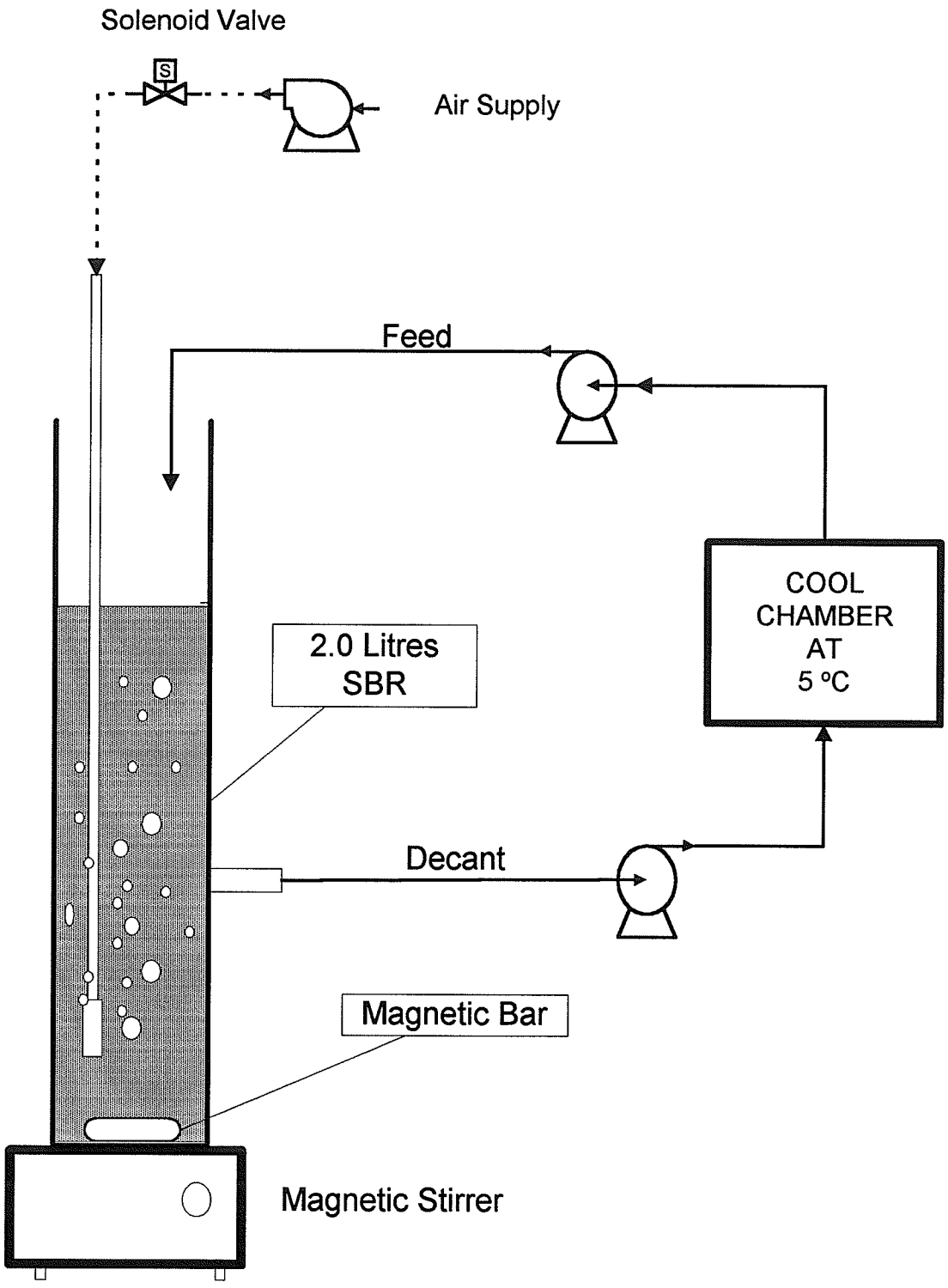


Fig. 4-4 Typical schematic of the SBR system used in this study

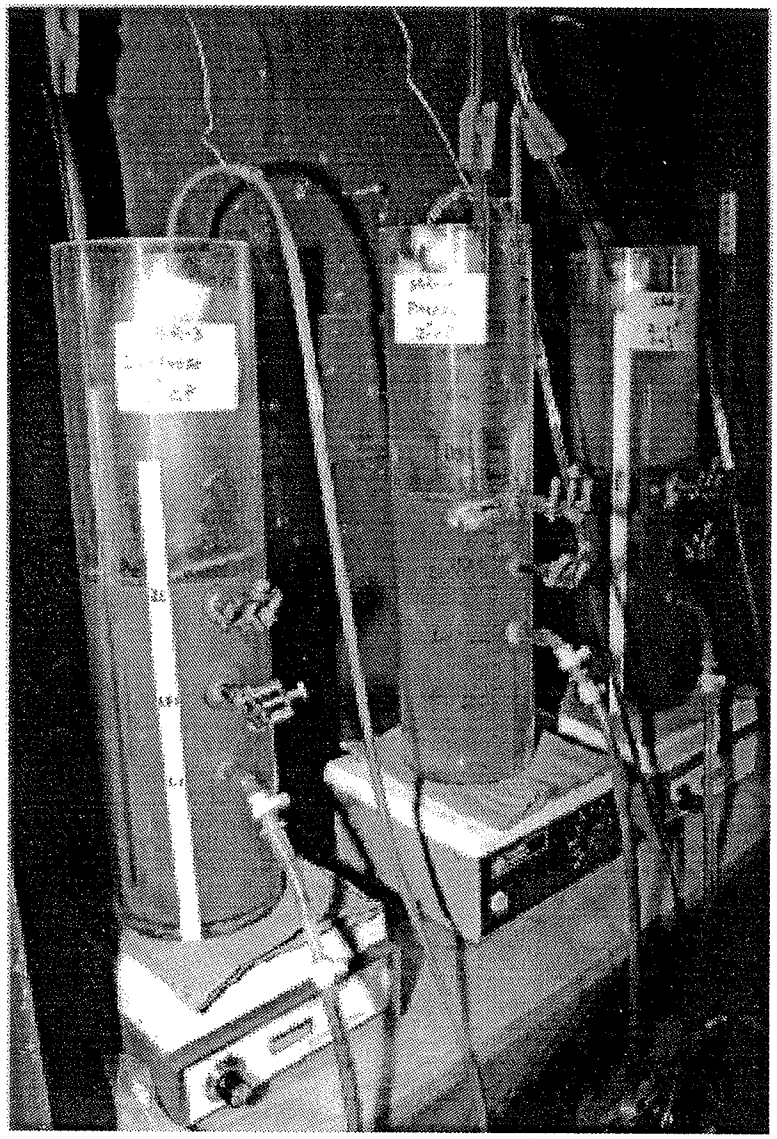


Fig. 4-5 View of aerobic SBR's degrading 2-CP with various supplemental substrates

sufficient amounts of NH_4Cl (nitrogen source) and phosphorous (in the form of $NaHPO_4$ and NaH_2PO_4) to maintain a C:N:P ratio of 100:10:3. The feed was prepared double the required strength to acount for the dilution in system. All components were dissolved in tap water and the pH was adjusted to a 6.8 - 7.0 range with solid $NaHCO_3$.

4.2.3 Start-up of sequencing batch reactors

The reactors were inoculated initially with activated sludge from the North End Water Pollution Control Centre (NEWPCC), Winnipeg. The NEWPCC operates a pure oxygen activated sludge plant with a 2.5 h HRT and a 1.5-2.0 d SRT. The target 2-CP concentration of 30 mg/L was reached by gradually increasing the influent concentration of 2-CP over a 3 week period. Periodic checks were made to avoid any unnecessary 2-CP build-up. Biomass concentration in each reactor was maintained around 2.0 g VSS/L throughout the study period by periodically wasting sludge. For the reactors receving the supplements, this maintained the same influent SOC to biomass ratio at each concentration of supplements (dextrose or phenol) tested.

4.2.4 Batch Aerobic Experiments

Several batch experiments were conducted throughout the study period using sludge from the SBR's. A system using 500 mL glass cylinders as batch reactors were generally employed. Sludge from respective units to be tested were

collected after the settling and decant period of the SBR cycle. By manually operating the magnetic mixers to ensure complete mixing, 250 mL of sludge was transferred from the SBR to 500 mL cylinders which were also mounted on magnetic mixers. The final volume of 500 mL was made up by using the respective feed. The batch reactors were positioned under diffusers for air supply.

4.2.5 Chemical analysis

2-CP and phenol concentrations were analyzed by reversed phase high pressure liquid chromatography (HPLC) as explained before. Before analysis the samples were centrifuged for at least 5 minutes before injecting the clear supernatant into the HPLC. The system was calibrated by using solutions of known concentrations of 2-CP and phenol. The performance of the reactors were also monitored by measuring the soluble organic carbon (SOC). The SOC measurements indicated the degree of mineralization of 2-CP, phenol and dextrose.

For the SOC determination, samples of mixed liquor from the reactors were initially centrifuged as mentioned above. The supernatant was passed through a cellulose acetate membrane filter cartridge and first few mL's were rejected. The filtrate was finally acidified with phosphoric acid, purged with pure oxygen for 2 minutes and finally injected in a TOC analyzer (Dohrmann DC 80, Dohrmann Div., Santa Clara, California). pH was measured directly in each reactor using a Fisher Scientific, Acumet 50 pH meter. Temperature measurements were done using a

standard thermometer with a minimum reading of 1 °C. Mixed liquor solids and sludge volume index were monitored according to the standard methods (APHA et al., 1992) following sections 2540 D, 2540 E and 2540 F respectively.

4.3 ANOXIC SEQUENTIAL BATCH REACTOR (ASBR)

The experimental set-up consisted of two identical SBR's as described before. SBR 1 and SBR-2 received 40 mg SOC/L of either dextrose or phenol respectively as supplemental carbon source besides 2-CP, nitrogen and phosphorous sources like the aerobic SBR's mentioned before. The feed was also supplemented with 30 - 40 mg NO_3-N/L. The nitrate concentration was adjusted regularly (due to incomplete denitrification and consequently nitrate buildup) to the above range. NO_3- N was analyzed according to the standard methods (APHA et al., 1992) follwing section 4500-NO_3- F : automated cadmium reduction method. Samples were filtered as per SOC procedure discussed in section 4.2.5.

Both reactors were operated in parallel on a 8 hour cycle consisting of 0.25 h Fill, 6 h React, 1.5 h Settle and 0.25 h Decant. However unlike the complete aerobic system, the reactors were equipped with a built-in, non-aerated (stirred) denitrifying period immediately following the Fill period. The anoxic period varied from 2 h in Phase I, 3 h in Phase II and 4 h in Phase III. During the aerated mixed conditions, conditions similar to fully aerobic SBR's as described before were maintained. All these operations were again controlled through a GE Series

One Programable Controller. The sequence of operation of the ASBR is shown in Fig. 4-6. The temperature varied from 22 ± 1 ^{o}C during the study period. The start-up protocol was similar to the aerobic reactors and fresh activated sludge from North End Pollution Control Centre, Winnipeg was used. The target 2-CP concentration was reached by gradually increasing the influent concentration of 2-CP up to 15 mg/L over a 2 week period. Biomass in both the reactors was maintained around 2000 mg VSS/L throughout the study period by wasting sludge two to three times a week.

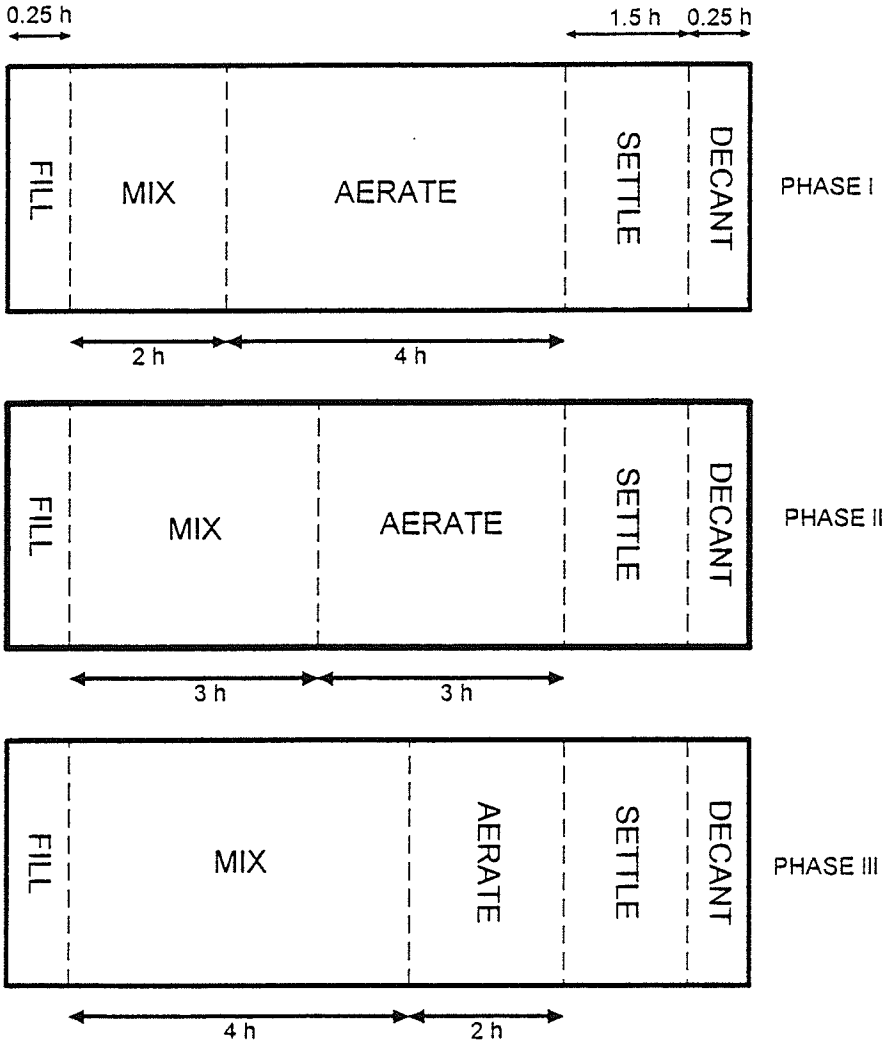


Fig. 4-6 Operational Sequences of ASBR

Chapter 5.

RESULTS AND DISCUSSION

5.1 ANAEROBIC DEGRADATION OF 2-CP

The difficulty of anaerobic degradation of monochlorophenols (2-CP, 3-CP and 4-CP) has opened an active area of research in the recent years and has produced a large amount of somewhat conflicting information about the degradability of these compounds. While researchers observed the persistence and accumulation of 2-CP as a dehalogenation product of higher chlorophenols (Wood et al., 1989; Hale et al., 1990), others have reported its relative recalcitrance over either 3-CP or 4-CP (Liu and Pacepavicius, 1990; Häggblom et al., 1990; Lu and Tsai, 1992; Lu and Tsai, 1993). 2-CP was therefore selected as a model compound for this study with an aim to address several factors which possibly affect its removal under anaerobic conditions. Factors like different sources of inocula, electron donors including hydrogen, electron acceptors like sulphate and the effect of adaptation are thoroughly examined and presented in the following sections in an effort to understand how the environmental and cultural conditions affect dehalogenation of 2-CP by mixed anaerobic cultures.

5.1.1 Effect of various inocula (unacclimated sludge)

There is presently a lack of understanding of the significance of microbial acclimation to the persistence of a chemical in the environment (Liu and

Pacepavicius, 1990) and generalizations are often made in predicting its overall removal from the environment due to limited data, (Pfaender and Shimp, 1985). Wiggins et al. (1987) have shown that extended lag times can cause the chemicals to be widely disseminated causing significant effects on susceptible species before it is destroyed.

The ability of inocula to degrade 2-CP varied considerably with the source and size of the inocula with lag periods varying from 97 days in case of digested sludge to about 250 days for either sediment or biomass from a lagoon and anaerobic bioreactor respectively treating bleached kraft mill (BKM) waste. The cultures had an inoculum size of 10 % (vol/vol) and were maintained in 0.1% yeast extract under a 20% CO_2 + 80% N_2 atmosphere. Digester sludge was chosen since chlorophenolic wastes from many industrial discharges to publicly owned treatment plants (POTW) are not removed in the conventional processes and finally end up in the sewage sludge digesters (Wild et al., 1993). There is therefore a definite need to study the fate of compounds like 2-CP in digester sludge. Sediment from a lagoon and bioreactor biomass treating BKM wastewater were also chosen for comparison purposes. BKM wastes are known to contain a multitude of chlorinated compounds and therefore such system present a feasible repository for organisms that are likely to have been exposed to compounds similar to 2-CP.

Following the extended lag period of 97 days the cultures with digested

sludge inoculum showed signs of dehalogenation of 2-CP. The appearance of two distinct metabolite peaks occurring only in cultures receiving 2-CP were identified as phenol and benzoate respectively by comparison with the retention times of these compounds in the known standards in the HPLC. No similar peaks were however identified in either background samples (amended with yeast extract but no 2-CP) or the sterile controls. Methane was detected in the head-space of all cultures degrading 2-CP.

Dehalogenation of 2-CP recurred in all cultures subjected to repeated spiking with 2-CP and steady removal was observed after five feeding operations. The response of unadapted digested sludge to such repeated process of washing and re-feeding as described before with fresh medium containing 0.1% yeast extract and 2-CP is shown in Figure 5-1. Data points are the mean of two replicate cultures. After seven feedings of medium and 2-CP and an incubation period of 436 days, the apparent rates of 2-CP dehalogenation (based on amount of 2-CP removed in a given time) was enhanced from 9 μM/d (± 1 μM/d) to 24 μM/d (± 2 μM/d), suggesting acclimation and growth of the chlorophenol-utilizing microbial population. Figure 5-2 shows a typical profile of 2-CP removal when consistent dehalogenation rates were observed. The subsequent production of phenol is also shown.

Similarly, for inocula taken from either lagoon sediments or the laboratory bioreactor treating BKM waste, positive signs of dehalogenation were seen only

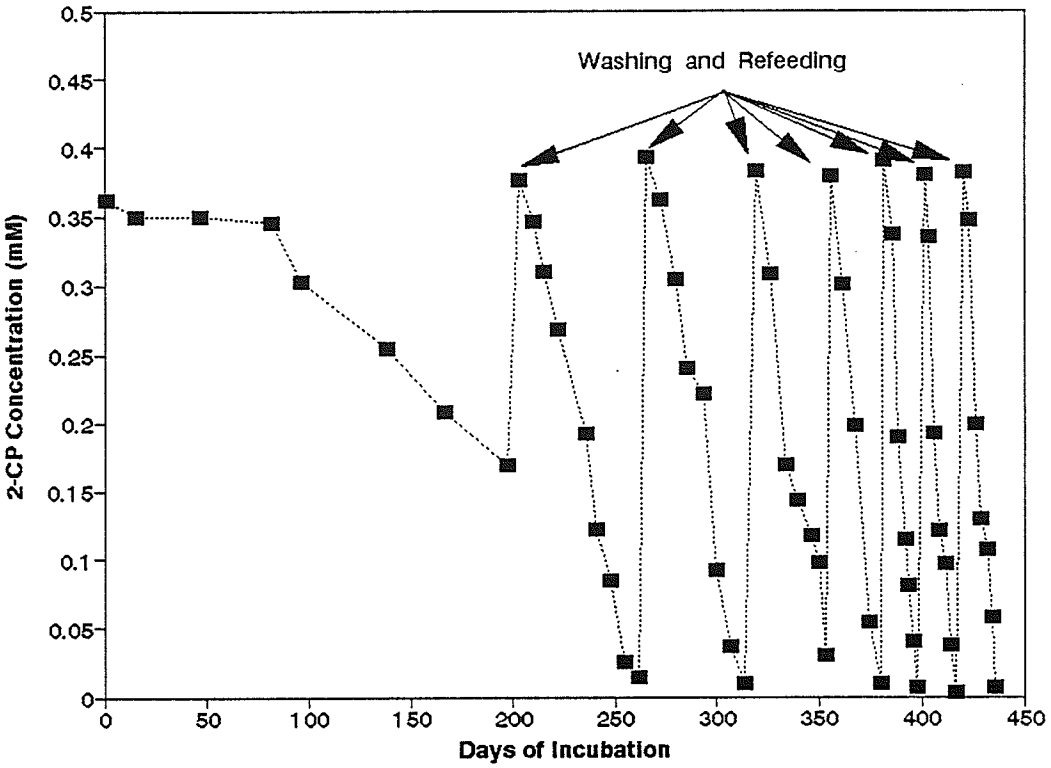


Fig. 5-1. Removal of 2-CP in batch cultures of unacclimated digested sewage sludge 10% (vol/vol) in mineral medium containing 0.1% yeast extract under 20% CO_2 + 80% N_2 atmosphere. Data points are the mean of two replicate cultures.

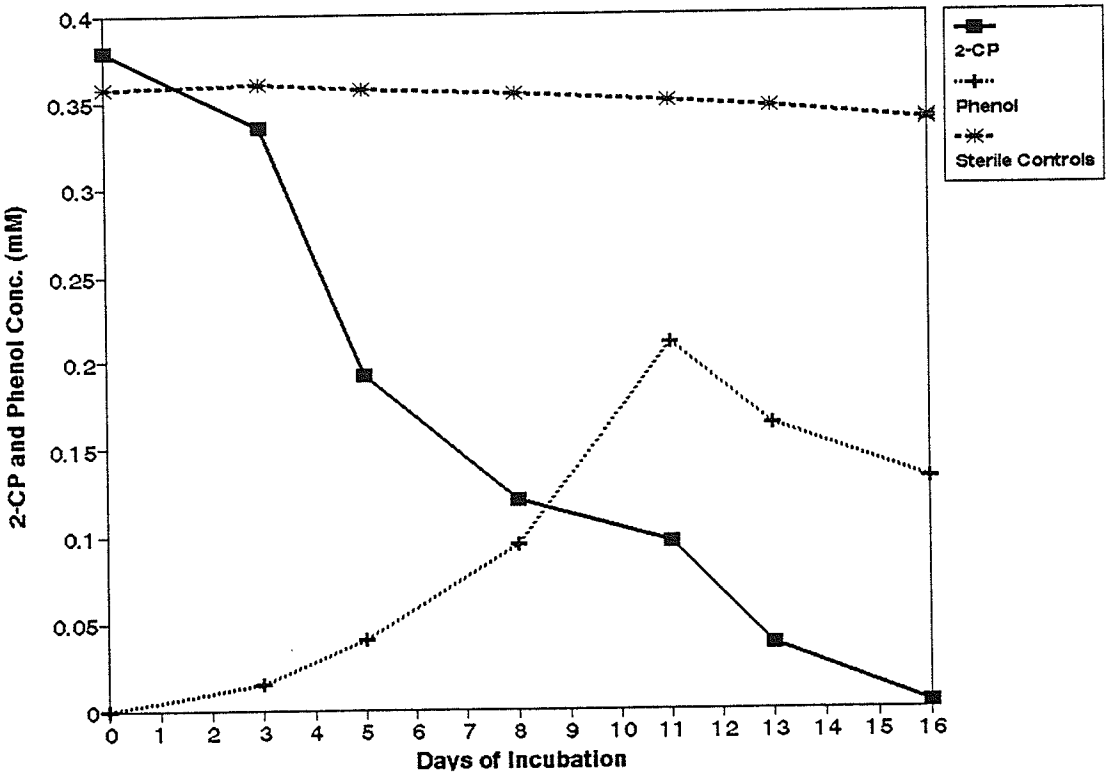


Fig. 5-2. Typical dechlorination profile of 2-CP to phenol in batch cultures of unacclimated digested sewage sludge 10% (vol/vol) in mineral medium containing 0.1% yeast extract under 20% CO_2 + 80% N_2 atmosphere. The profile is based on at least five washing (with anaerobic phosphate buffer of pH 7) and refeeding operations with yeast extract medium and 2-CP.

after 250 days of incubation. Phenol was identified as an intermediate product. As with the digested sewage sludge inoculum, the transformation rates of 2-CP in both these cultures were greatly enhanced with successive washing and re-feeding (Figures 5-3 and 5-4). Methane was identified in the head-space of all cultures degrading 2-CP. Initially the transformation of 0.38 mM 2-CP took over 70 days in both cultures (lagoon sediment and biomass from bioreactor). However after repeated re-feeding, 0.38 mM 2-CP was removed in 33 days by the lagoon cultures and 65 days by the bioreactor culture. This corresponded to an increased transformation rate apparently from 6 μM/d (± 0.5 μM/d) to 12 μM/d (± 1 μM/d) for lagoon sediment and 5.4 μM/d (± 0.7 μM/d) to 7 μM/d (± 0.6 μM/d) for bioreactor sludge. A summary of the above results using three different inocula sources are shown in Table 5-1.

Table 5-1 Comparison of acclimation time and degradation potential of 0.39 mM 2-CP by 3 different sources of inoculum.

Inoculum Source	Time reqd. for initial degradation (days)	2-CP removal rate after : 200 days (μM/d)	365 days (μM/d)	Intermediates detected
DS	97	6	24	Phenol and benzoate
LS	257	5	12	Phenol
BB	249	4	7	Phenol

DS: Anaerobic digester sludge, LS: Lagoon sediment, BB: Anaerobic bioreactor biomass

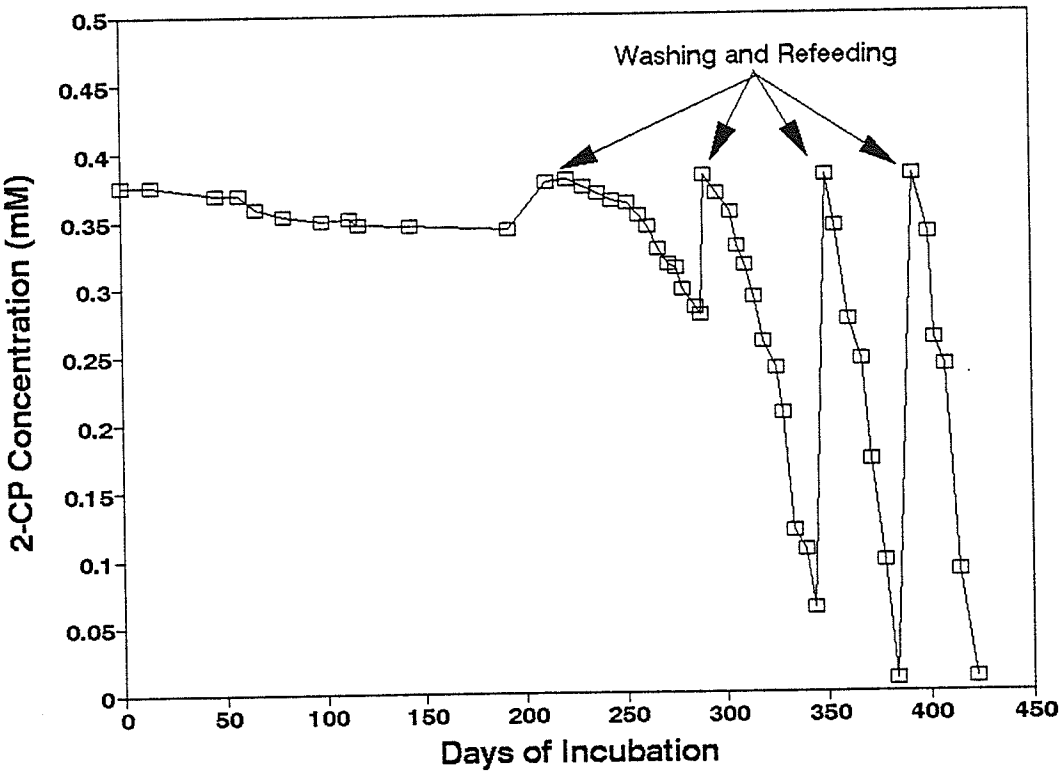


Fig. 5-3. Removal of 2-CP in batch cultures of unacclimated sediment 10% (vol/vol) originating from a lagoon in mineral medium containing 0.1% yeast extract under 20% CO_2 + 80% N_2 atmosphere. Data points are the mean of two replicate cultures.

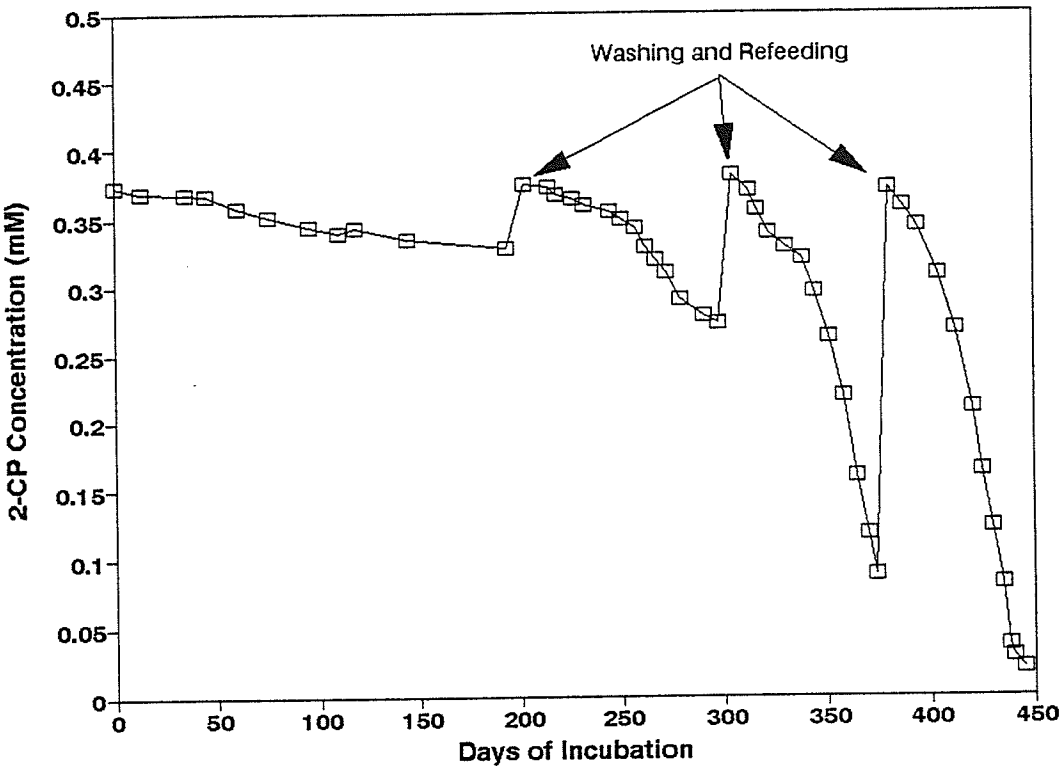


Fig. 5-4. Removal of 2-CP in unacclimated biomass 10% (vol/vol) from an anaerobic bioreactor treating bleached kraft paper waste. The mineral medium contained 0.1% yeast extract under 20% CO_2 + 80% N_2 atmosphere. Data points are the mean of two replicate cultures.

The above results indicate that anaerobic digester sludge provided the best inoculum source for 2-CP dehalogenation compared to either the sediment from a lagoon or bioreactor biomass. The response of the system containing lagoon sediment is unexpected because the microorganisms in that environment were pre-exposed to BKM waste which is known to contain several chlorinated compounds. The biomass from the lab scale anaerobic bioreactor which provided the third source of inoculum for our batch cultures had the slowest response. This may be due to poor operating conditions and improper mixing in the bioreactor (indicated by low gas production and AOX removals). Figure 5-5 shows the best 2-CP removal profile achieved by the three sources of inocula after more than one year of incubation. Methane in the head-spaces of cultures dehalogenating 2-CP indicated that methanogens were not inhibited at 0.39 mM 2-CP which is supported by the work by Hrudey *et. al.* (1987a) observed methane production in semi-continuous cultures degrading up-to 0.78 mM 2-CP.

Throughout the experimental period, the sterile controls showed negligible loss of 2-CP abiotically. Although no separate experiments were conducted to address the problem of biosorption of 2-CP on to biomass, losses due to adsorption cannot be ruled out. Chlorophenols like many hazardous organic pollutants are prone to biosorption (Kennedy *et al.*, 1992). However, errors in the analysis due to adsorption in our cultures were minimized by the use of low biomass and a lower chlorophenol like 2-CP. The tendency of lower chlorophenols (CP's) to adsorb less than the higher substituted CP's has been previously

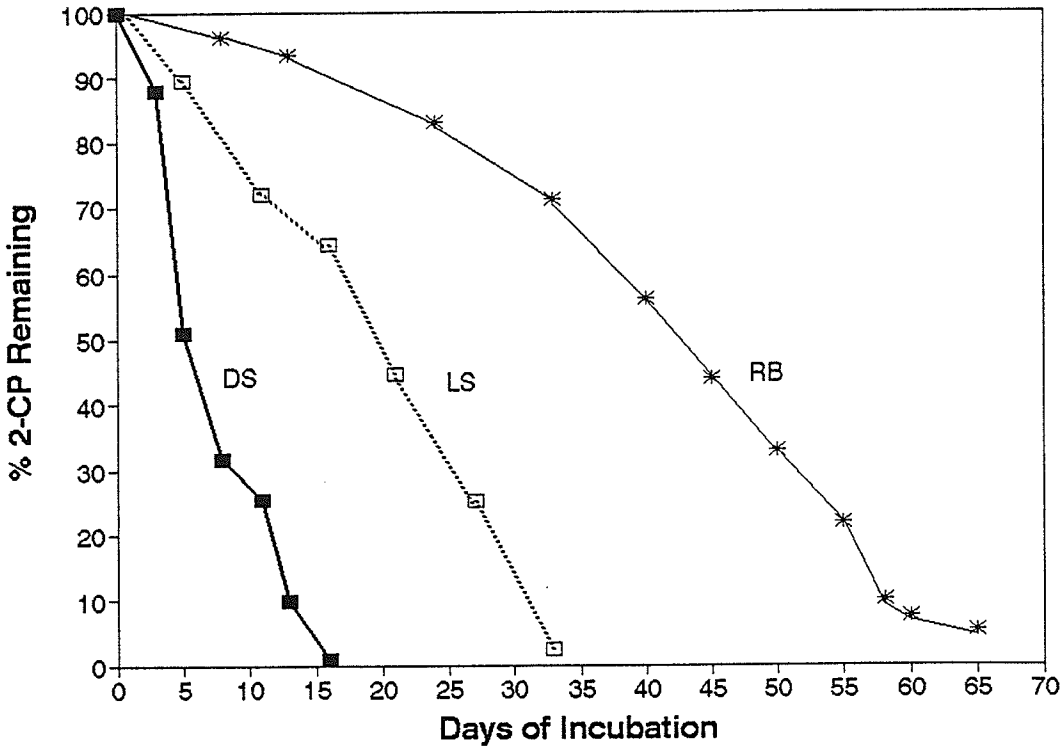


Fig. 5-5. Comparison of biodegradation potential of 0.385 mM 2-CP in anaerobic digested sewage sludge (DS), sediment from a lagoon (LS) receiving bleached kraft mill wastewater and biomass from an anaerobic bioreactor (BB) treating the same wastewater. Cultures were set-up as 10% vol/vol in mineral medium containing 0.1% yeast extract under 20% CO_2 + 80% N_2 atmosphere. Each data point is a mean of two replicate cultures.

reported (Bell and Tsezos, 1987; Davis and Huang, 1990a). In our experiments, a comparison of the amounts of 2-CP actually recovered to the target initial concentrations indicate a loss up to a maximum of 2.0 % . A summary of the major findings is given at the end of this sub-section.

2-CP Dehalogenation in 50% (vol/vol) un-adapted sludge. Since experiments with digester sludge indicated the best viable source of 2-CP dehalogenators, cultures were simultaneously set up in triplicate with 50% inoculum (vol/vol) in serum bottles under CO_2/N_2 atmosphere. Significant decrease in lag times were noticed compared to 10% inocula (vol/vol) and the first sign of 2-CP dehalogenation activity (based on detection of phenol) was observed after only 8 days of incubation. The cultures were maintained semi-continuously and after complete disappearance of initial 0.39 mM 2-CP (average of three cultures) in 21 days, the bottles were re-fed with 0.1 % yeast extract medium to maintain the final volume to 80 mL before spiking with 0.38 mM 2-CP. It took 14 days for complete removal of 0.38 mM 2-CP indicating increased dehalogenation activity in the cultures. The volume of medium added was based on the amounts withdrawn from these bottles for analysis.

This process was continued for another month during which time the cultures were able to remove about 0.39 mM 2-CP in 12 days accounting for an apparent removal rate of 32 µM 2-CP/d. These rates are higher than that

observed previously by Boyd and Shelton (1984). Lu and Tsai (1993) had previously indicated the difficulty of 2-CP dehalogenation by unacclimated microorganisms even after an incubation period lasting 12 days in a batch reactor. The results of 2-CP dehalogenation by un-adapted digested sewage sludge as seen from this study is illustrated in Figure 5-6. Phenol along with trace amounts of benzoate (refer APPENDIX A, Table A-4 for benzoate data) were identified as the intermediates of 2-CP dehalogenation. Methane was also detected in these cultures. A summary of the findings is noted below.

Observation Summary. Dehalogenation of 2-CP to phenol was observed for all cultures. Detection of benzoate in some cultures indicated the potential pathway of phenol degradation. Un-adapted digested sludge (10% vol/vol) showed better potential to dehalogenate 0.39 mM 2-CP compared to the other inocula tested: lagoon sediment or laboratory scale anaerobic bioreactor biomass receiving BKM waste. Dehalogenation efficiency improved considerably with successive refeeding of yeast extract medium and 2-CP. Significant reduction in lag times were noticed with 50% (vol/vol) cultures of un-adapted anaerobic digester sludge operated semi-continuously compared to 10% cultures. Methanogenesis was found to occur in all cultures in presence of 0.39 mM (50 mg/L) of 2-CP. Abiotic losses of 2-CP were negligible.

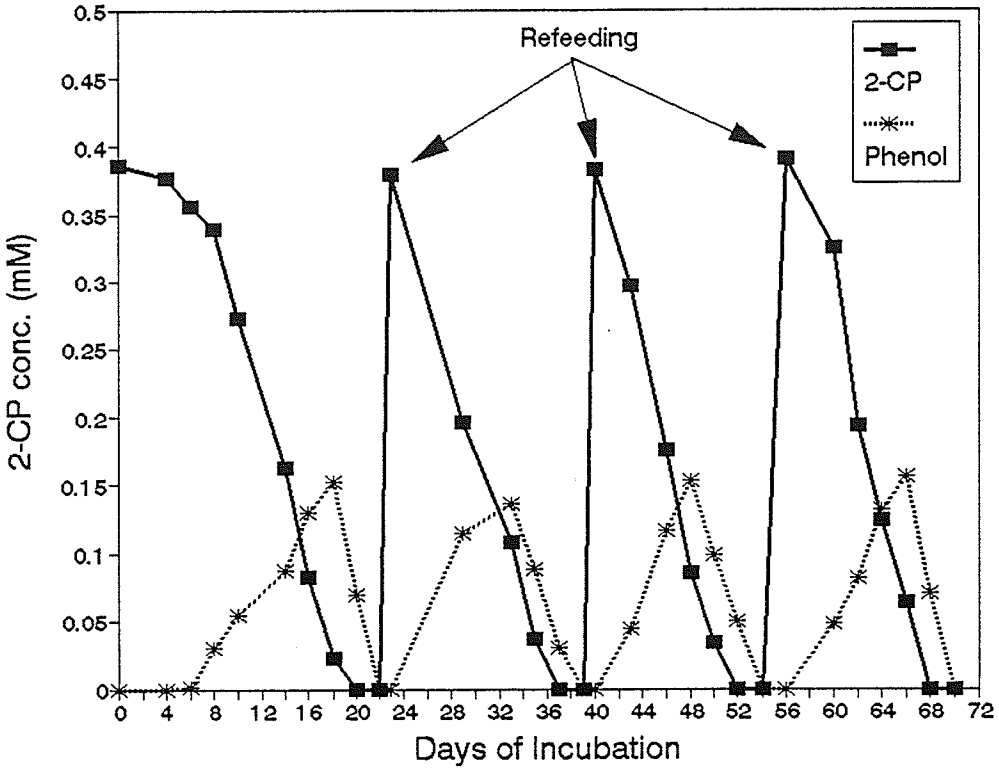


Fig. 5-6. Dehalogenation of 0.385 mM 2-CP in batch cultures (50% vol/vol) of un-adapted anaerobic digested sewage sludge and 0.1% yeast extract medium. Besides phenol, trace amounts of benzoate was also detected in these cultures. Each data point is a mean of three replicate cultures incubated in serum bottles.

5.1.2 Influence of alternative electron acceptors

Experiments with 50% un-adapted digested sewage sludge indicated that lag times associated with dehalogenation of 2-CP could be significantly shortened. This set-up was chosen to compare dehalogenation potential of 2-CP in un-adapted digested sewage sludge (50% vol/vol) using sulphate and carbonate as alternative electron acceptors.

The biodegradation of a number of chlorophenols has been studied successfully under methanogenic conditions with carbonate serving as the electron acceptor (Boyd and Shelton, 1984; Dietrich and Winter, 1990; Häggblom *et al.*, 1993a and 1993b, Hale *et al.*, 1990). However, In presence of sulphate there are conflicting reports in literature as to its impact on chlorophenol degradation. Häggblom *et al.* (1993a); Kohring et al. (1989a) and Madsen and Aamand (1992) have all observed inhibition of chlorophenol degradation in presence of sulphate while other studies although few demonstrated the transformation of chlorophenols in the presence of sulphate (Genthner et al., 1989a; Häggblom and Young, 1990; Kohring et al., 1989b).

This investigation was therefore initiated to address the potential influence of sulphate as an electron acceptor in dehalogenation reactions since it appears that degradation of compound such as 2-CP in sulphate amended systems is poorly understood. Moreover for the studies cited above on chlorophenol

degradation in the presence of sulphate, the experiments were conducted using sediments obtained from either freshwater or estuarine sites and no information is available literature as to the effect of sulphate on 2-CP dehalogenation in sewage sludge from anaerobic digesters. The effects of either bromoethanesulphonic acid (BESA) or molybdate as inhibitors of methanogenic and sulfidogenic microbial populations respectively at different concentrations in the cultures are also poorly addressed. Results of batch experiments conducted to address these issues using anaerobic digester sewage sludge (50% vol/vol) are described in the following sections.

Dehalogenation of 2-CP in cultures amended with or without sulphate. The first batch experiment consisted of four sets of serum bottles (each set in triplicates) using 50% (vol/vol) of un-adapted anaerobic digester sewage sludge and 0.1% (wt/vol) yeast extract medium under 20% CO_2 + 80% N_2 atmosphere. No sulphate was added to set number 1 and the total background sulphate (as SO_4^{2-}) from inoculum and mineral medium was found to be around 0.6 mM. Set number 2 was amended to a total initial sulphate concentration of about 5 mM. Medium in set 3 was the same sulfidogenic medium as in Set 2 but was amended with 6 mM $Fe(II)Cl_2$. Fe(II) was used to reduce possible inhibition of sulphide formed due to sulphate reduction. Set 4 served as sterile controls (autoclaved for 1 hour before addition of 2-CP) and were comprised of a combination of sulphate amended and un-amended cultures.

The results of batch experiment # 1 are shown in Figure 5-7. 2-CP removal in cultures un-amended with sulphate followed the trend as observed before and it took 19 days for complete removal of 0.38 mM (49.3 mg/L) 2-CP. No difference was noted in 2-CP removal in presence of sulphate and about 48.4 mg/L (0.38 mM) 2-CP was removed in the same time along with sulphate reduction. Sulphate was reduced from an initial concentration of 5.1 mM (491 mg/L) SO_4^{2-} to 2.7 mM (256 mg/L) indicating that sulphate reduction was not inhibitory to 2-CP removal.

Sulphate concentrations monitored even after complete removal of 2-CP (in 19 days) indicated that the process of sulphate reduction continued although at a very slower rate. Surprisingly, the presence of Fe(II) slowed the removal of 2-CP and it took 25 days for complete removal of 50.3 mg/L (0.39 mM) 2-CP suggesting that the dehalogenators were perhaps sensitive to Fe(II) Cl_2. The amount of sulphate removed in presence of Fe(II) was marginally greater (about 4%) than the sulfidogenic cultures without Fe(II). 2-CP degradation or sulphate reduction were not observed for the sterile controls indicating that metabolism in the active cultures (Sets 1, 2 and 3) were indeed biologically mediated.

Phenol was detected as a product of reductive dehalogenation of 2-CP in all the cultures except sterile controls along with trace amounts of benzoate indicated that phenol degraded via the benzoate pathway. Percentage of methane in the head-space of all cultures were monitored as a control measurement. As

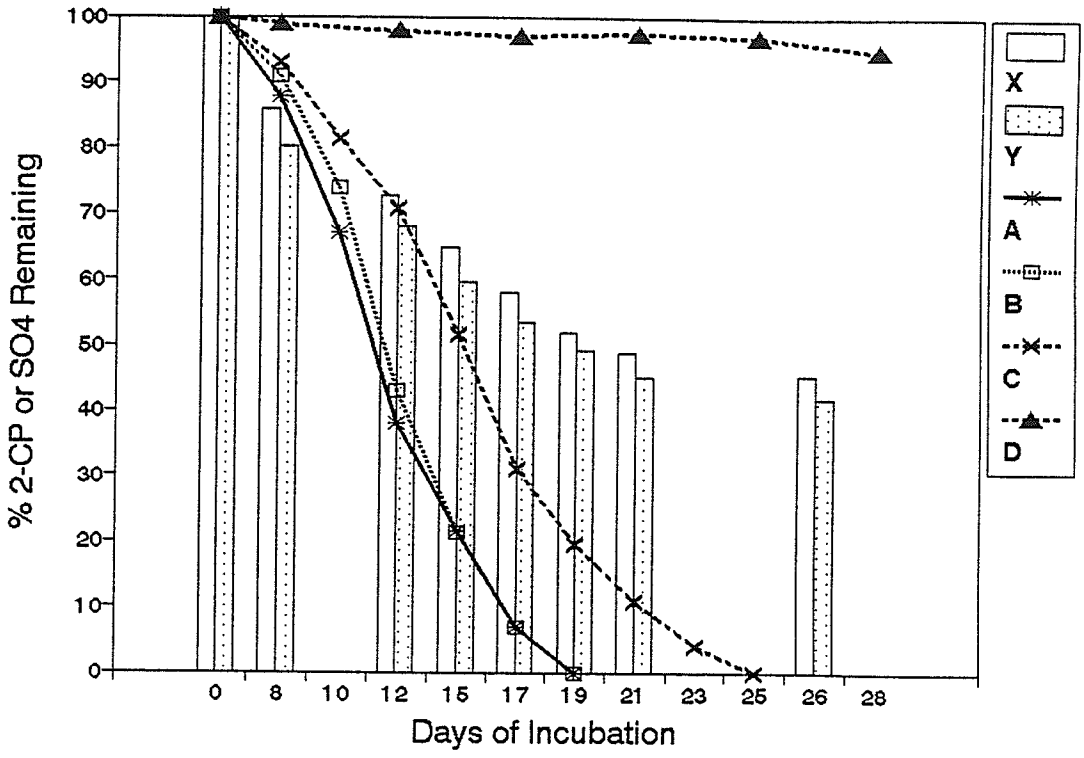


Fig. 5-7. Degradation of 0.39 mM 2-CP in batch cultures containing fresh anaerobic digester sludge 50% (vol/vol) in (**A**) absence of added sulphate, (**B**) amended with 5 mM SO_4^{2-}, and (**C**) amended with 5 mM SO_4^{2-} and 6 mM Fe(II). Sterile controls (**D**) are the means of sulphate amended and un-amended cultures. Background SO_4^{2-} from inocula and mineral medium measured about 0.6 mM. Histograms (**X**) and (**Y**) shows SO_4^{2-} removal in presence or absence of $Fe(II)Cl_2$. No SO_4^{2-} reduction was observed in sterile controls. A head-space of 20% CO_2 + 80% N_2 was maintained. Data points are means of three replicate cultures.

shown in Figure 5-8, methanogenesis was not greatly affected by sulphate reduction. Kohring et al. (1989a) had previously reported that sulphate did not inhibit methanogenesis in cultures degrading 2,4-DCP at 44 °C. The presence of Fe(II) also appeared to inhibit methanogenesis slightly but this was probably due to a greater competition for substrates by methanogenic and sulfidogenic communities in the system as more sulphate reduction was observed in the presence of Fe(II). No methane was detected in the sterile controls.

The batch experiment # 2 was conducted to re-examine batch # 1 results. To further investigate the inhibitory effect of $Fe(II)Cl_2$ on 2-CP dehalogenation, cultures were set up using 6 mM $Fe(II)Cl_2$ in presence or absence of added sulphate. Moreover, background cultures were also setup in duplicates without the addition of 2-CP and in presence or absence of added sulphate using the same 0.1% yeast extract medium. Figure 5-9 summarizes the batch # 2 results.

As observed before, Fe(II) in presence or absence of added sulphate slowed 2-CP degradation compared to the cultures maintained under the same conditions without the Fe(II) addition. Similar to our previous observation, 2-CP was removed at the same rate in presence or absence of sulphate. No significant difference in sulphate reducing activity in the background samples (amended with 0.1% yeast extract but not 2-CP) was noticed.

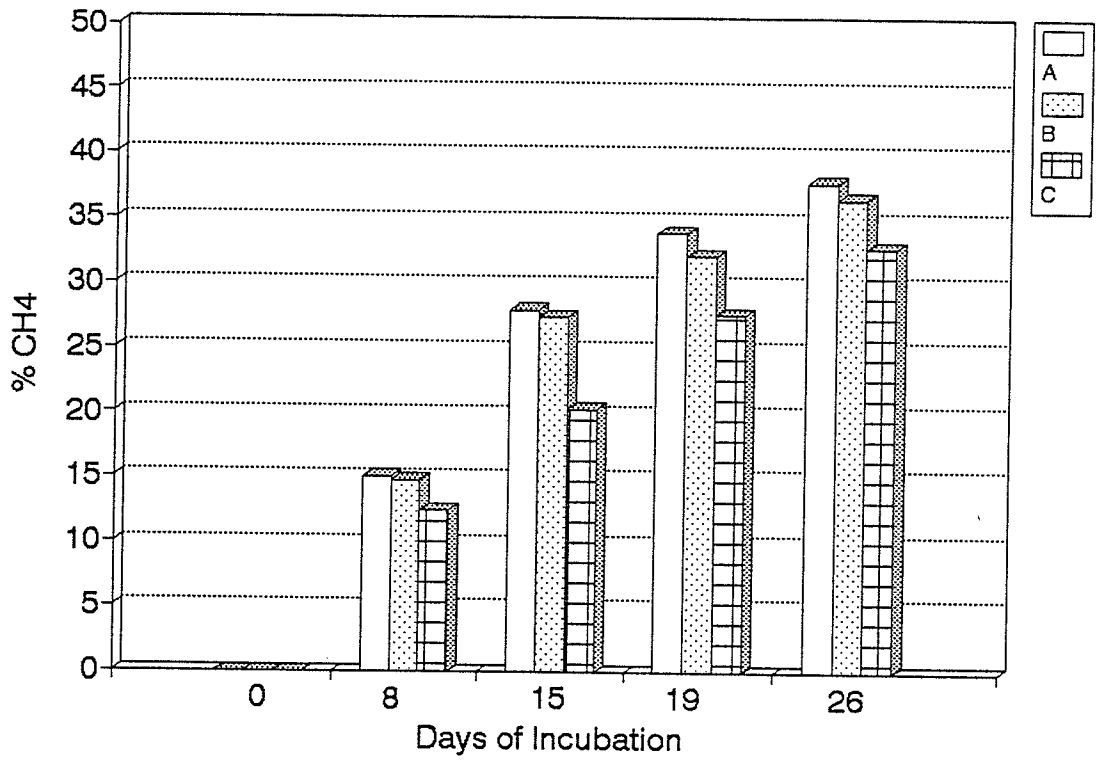


Fig. 5-8. Head-space methane in cultures (**A**) without added sulphate, (**B**) amended with 5 mM SO_4^{2-},and (**C**) amended with 5 mM SO_4^{2-} and 6 mM Fe(II) during Batch 1 experiments. Methane was absent in the sterile controls. Data points are means of three replicate cultures.

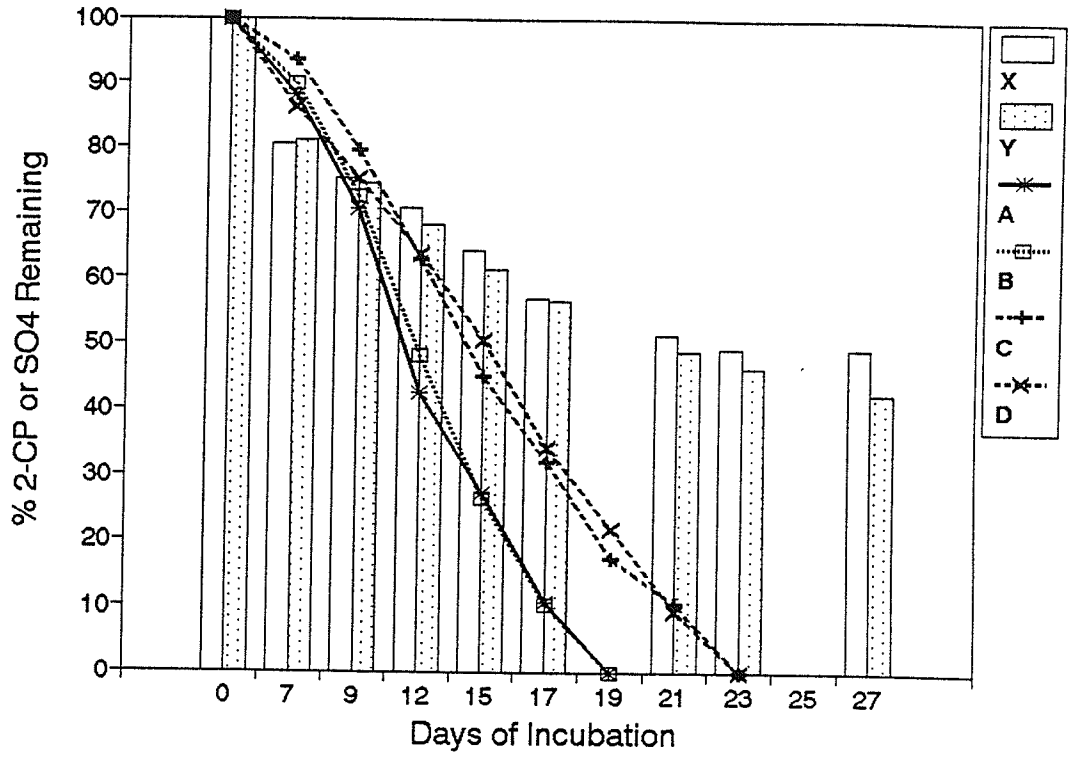


Fig. 5-9. Batch 2 experiments showing removal of 0.39 mM 2-CP in fresh anaerobic digester sludge 50% (vol/vol) (**A**) without added sulphate, (**B**) amended with 5 mM SO_4^{2-} (**C**) no added sulphate + 6 mM Fe(II), and (**D**) amended with 5 mM SO_4^{2-} + 6 mM Fe(II) under 20% CO_2 + 80% N_2 atmosphere. Histograms (**X**) and (**Y**) shows SO_4^{2-} removal in presence or absence of $Fe(II)Cl_2$. Background SO_4^{2-} from inocula and mineral medium was about 0.6 mM. No loss of 2-CP or SO_4^{2-} occurred in the sterile controls (data not shown). Data points are means of three replicate cultures.

The percentage change of methane in the head-space is shown in Figure 5-10. Formation of methane from 2-CP was difficult to quantify due to relatively higher concentrations of background substrates compared to 0.39 mM 2-CP. As seen before methane composition was lower in the head-space of cultures with Fe(II) compared to methanogenic and sulfidogenic cultures without Fe(II). Phenol was produced in all cultures which was subsequently degraded.

In summary, this experiments demonstrates that 2-CP dehalogenation is feasible under sulphate-reducing conditions with methanogenesis in un-adapted batch cultures derived from digested sewage sludge. Addition of Fe(II) was seen to slow the rate of 2-CP dehalogenation and marginally increase the total sulphate reduction. 2-CP occurred at the same rate in cultures with or without sulphate. Methane was detected in the head-space of all 2-CP dehalogenating cultures.

Effect of BESA and Molybdate. Dehalogenation of 2-CP has been primarily observed in mixed anaerobic cultures although it is only recently that a pure culture has been isolated which dehalogenates 2-CP to phenol (Cole et al., 1994). Since 2-CP dehalogenation was observed under sulphate amended and un-amended conditions, it was important to see the effect of BESA and molybdate on 2-CP dehalogenation and activities associated with it by inhibiting various metabolic groups present in the mixed culture derived from anaerobic digester sludge. In addition, knowing which groups of organisms are responsible for

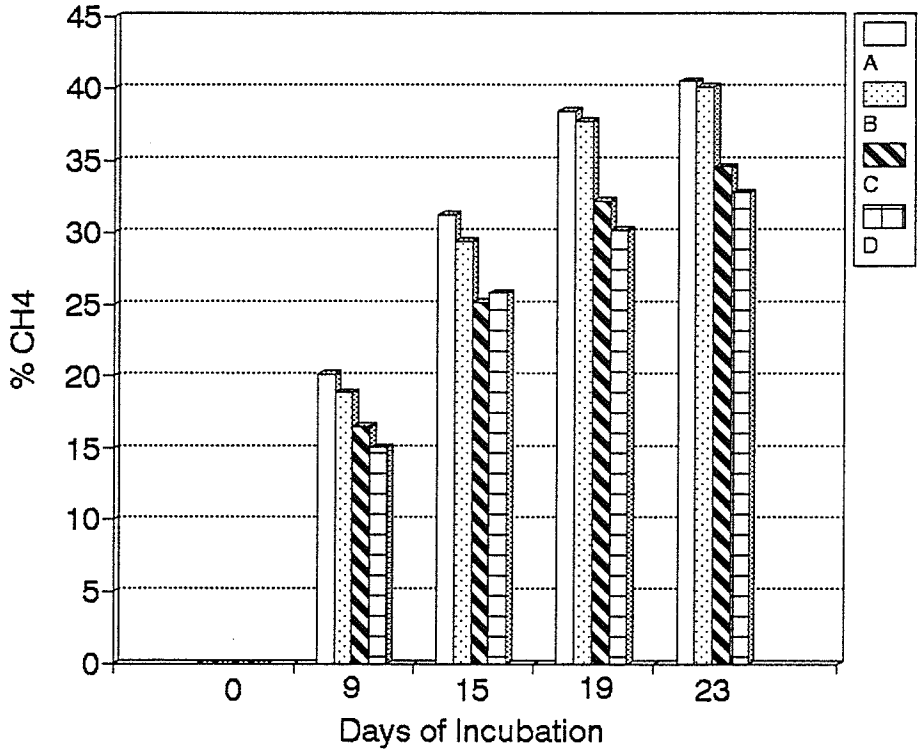


Fig. 5-10. Head-space methane in cultures (**A**) without added sulphate, (**B**) amended with 5 mM SO_4^{2-} (**C**) no added sulphate + 6 mM Fe(II), and (**D**) amended with 5 mM SO_4^{2-} + 6 mM Fe(II) during Batch 2 experiments. Methane was not detected in the sterile controls. Data points are means of three replicate cultures.

mediating the transformations may be useful for devising efficient strategies for biodegradation of compounds like 2-CP.

Cultures in serum bottles (50 % vol/vol) were set up as before with and without 10 mM bromoethanesulphonic acid (BESA) under methanogenic conditions. BESA is an analog to co-enzyme M (2-mercaptoethanesulphonic acid) which mediates the last step in methane formation (Santoro and Konisky, 1987; Ellerman et al., 1987). BESA inhibits the reduction of methyl-coenzyme M to methane. Similarly under sulfidogenic conditions triplicate cultures with an initial sulphate concentration of 5 mM received either 0 mM or 20 mM molybdate (using Na_2MoO_4). Molybdate is known to inhibit the microorganisms associated with sulfidogenesis by cessation of sulphate transport (Newport and Nedwell, 1988) and is also reported to be responsible for destruction of cellular ATP balance of *Desulfovibrio* sp. (Taylor and Oremland, 1979). A fifth set of cultures were also set-up to see the effect of molybdate (20 mM) on dechlorination of 2-CP in absence of added sulphate.

10 mM BESA slowed down 2-CP removal considerably compared to methanogenic cultures without BESA and the results are shown in Figure 5-11. In presence of BESA phenol produced as a product of 2-CP dehalogenation accumulated in the cultures. There are mixed reports in literature as to the effect of BESA on reductive dehalogenation of chlorinated compounds such as

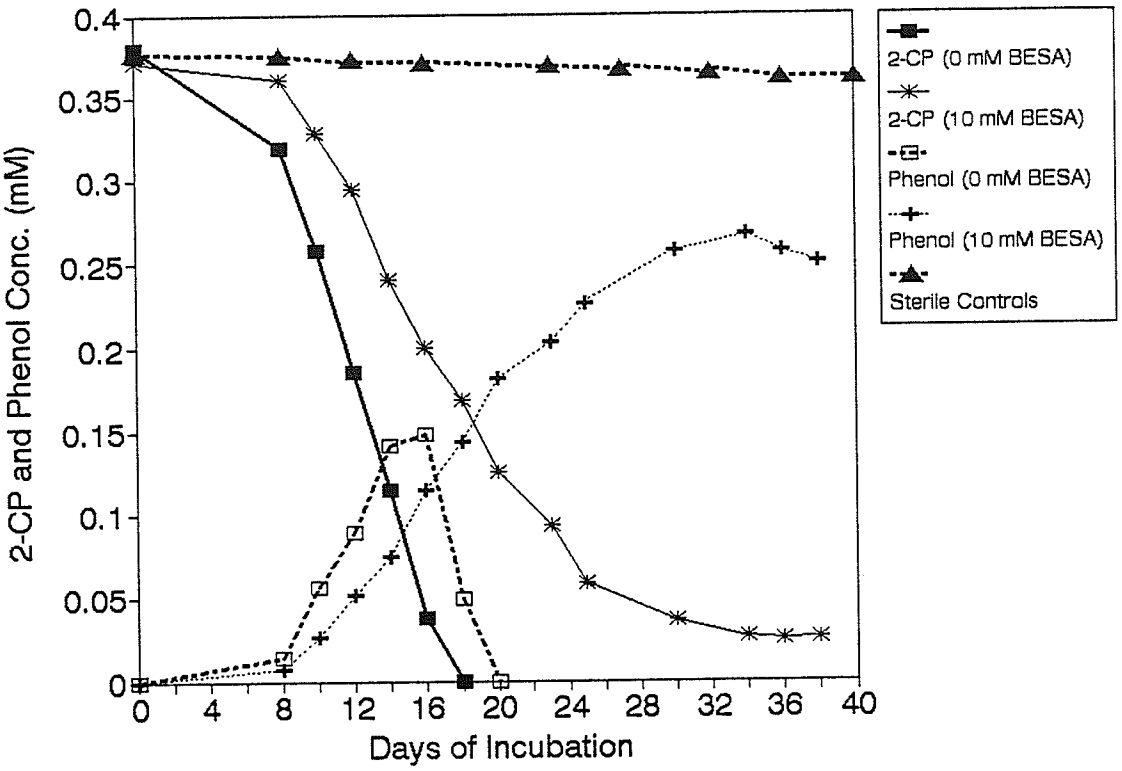


Fig. 5-11. Dehalogenation of 0.385 mM 2-CP in anaerobic digester sludge 50% (vol/vol) in presence or absence of 10 mM bromoethanesulphonic acid (BESA) under 20% CO_2 + 80% N_2 atmosphere. Data points are means of three replicate cultures.

chlorophenols. Genthner et al., (1989a) observed that while 10mM BESA prevented degradation of 3-CP and 4-CP, it delayed the degradation of 2- and 3-chlorobenzoate respectively. They explained that the effect was likely due to inhibition of methanogenesis (no data was presented) or the inhibition of reductive dechlorination by BESA. In their studies with chlorinated aliphatics, Freedman and Gosset (1989) had observed that 0.5 mM BESA immediately stopped TCE (trichloroethylene) degradation and methane production while repeated BESA addition was required before PCE (tetrachloroethylene) degradation ceased. BESA strongly affected methanogenesis but not dechlorination of 2,4,6-TCP in anaerobically digested sewage sludge with hydrogen and fructose as electron donors (Perkins et al., 1994). In the presence of acetate however, BESA completely inhibited methanogenesis and substantially slowed down dehalogenation although the results were not compared with cultures where BESA was absent. Zhang and Wiegel (1990) had also demonstrated the dechlorination of 2,4-DCP in presence of 1 mM BESA. Cole et al. (1994) recently showed that a pure culture dehalogenated 2-CP to phenol irrespective of the presence or absence of 1 mM BESA. Phenol as a product accumulated in all cultures and no data on methane formation was reported.

In summary, results from several previous studies with BESA indirectly indicated that methanogens perhaps play a key role in the reductive dehalogenation reactions involving mixed bacterial cultures. On the other hand

other studies including this one have shown reductive dehalogenation to occur even though methanogenesis was either strongly affected or completely stopped. The results of Hrudey et al. (1986a), Themel et al. (1993), Themel (1994) and Cole et al. (1994) strongly support the argument that methanogens are not involved in the dehalogenation of toxic organic chemicals like 2-CP. These investigators have been able to demonstrate the dehalogenation of 2-CP to phenol in non-methanogenic systems by pure and mixed cultures respectively.

Figure 5-11 indicates that BESA inhibited phenol degradation. The tailing of the 2-CP removal curve especially beyond day 24 and the gradual accumulation of phenol can be clearly seen. It is therefore possible that the accumulation of phenol as a dehalogenation product slowed the removal of 2-CP considerably. This is supported by results of Hrudey et al. (1986a) who had indicated that adequate phenol conversion was necessary to maintain a maximum rate for the dechlorination of 2-CP. Themel (1994) had also observed significant reduction in 2-CP dechlorination due to phenol accumulation while the addition of phenol slowed the rate of 4-CP dechlorination in active cultures (Wiegel et al., 1990).

20 mM molybdate on the other hand completely inhibited sulphate reduction and 2-CP degradation. This inhibitory effect on 2-CP removal was also observed for the set of cultures which received no additional sulphate and the results are summarized in Figure 5-12. The simultaneous inhibition of sulphate reduction and

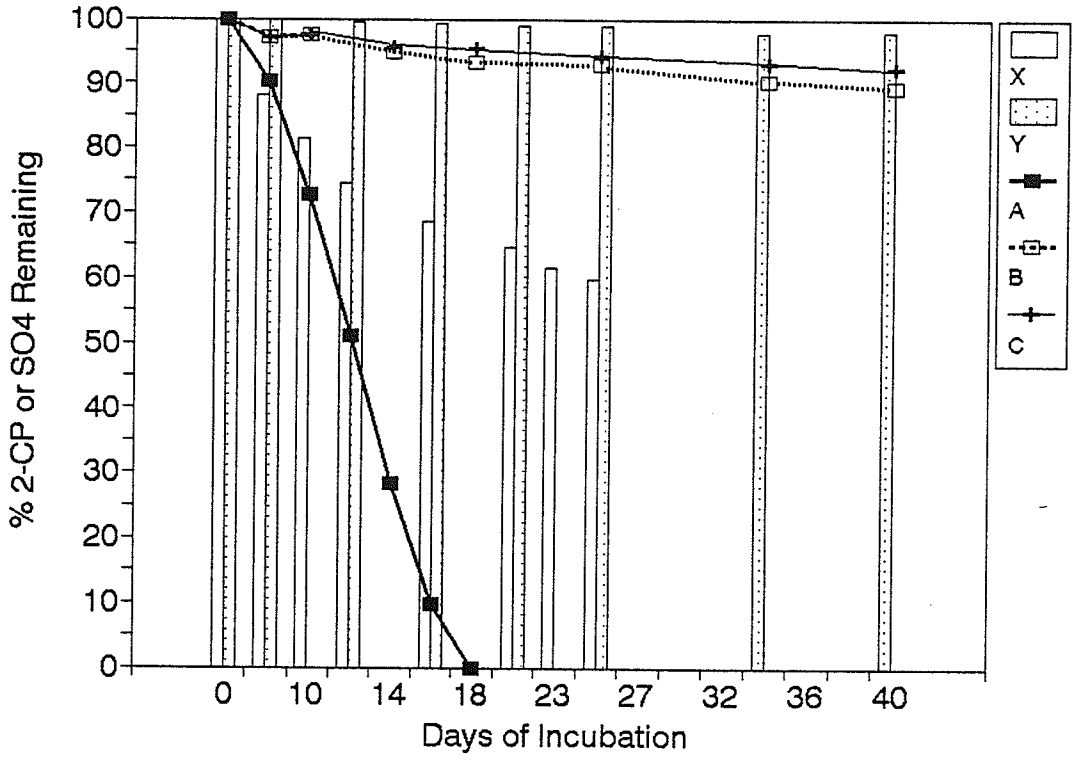


Fig. 5-12. Effect of 20 mM molybdate on degradation of 0.385 mM 2-CP and sulphate reduction in batch cultures containing anaerobic digester sludge 50% (vol/vol). SO_4^{2-} reduction in (**X**) absence of molybdate (**Y**) presence of molybdate. 2-CP removal in cultures ammended with 5 mM SO_4^{2-} in (**A**) absence of molybdate (**B**) presence of molybdate. (**C**) 2-CP removal in cultures without added sulphate but in presence of molybdate. Cultures unamended with sulphate contained typically a background sulphate concentration of about 0.6 mM. Data points are means of three replicate cultures.

2-CP removal by molybdate tends to indicate that the organism/organisms responsible for 2-CP degradation may be closely related to sulphate reducing bacteria. However such a conclusion would then contradict previous observations where it was shown that irrespective of sulphate reduction and presence of sulphate, 2-CP dehalogenation proceeded at the same rate (refer Figs. 5-7 and 5-9) with very negligible difference in methanogenesis (refer Figs. 5-8 and 5-10). Sulfidogenic cultures enriched from both saline and freshwater sediments and capable of 2-CP dehalogenation were inhibited by 20 mM molybdate (Häggblom and Young, 1990). Those authors were able to show that dechlorination reactions were indeed mediated by sulphate reducers since stoichiometric loss of sulphate with respect to 2-CP occurred.

Most of the information on the inhibitory action of molybdate has been linked to sulfidogenesis and the knowledge on the specificity of such compounds (molybdate) for other organisms is limited. Molybdate has been however shown to be bacteriostatic to methane - producing bacteria (Puhakka et al., 1990). The researchers reported that molybdate (20 mM) inhibited methanogenesis of both synthetic wastewaters and neutral spent sulphite liquor. Acetate accumulated in glucose plus molybdate media and molybdate was found to have a direct inhibitory effect on enriched acetoclastic methane-producing bacteria.

Although the exact reason for molybdate inhibition of 2-CP degradation could not be determined so far in this study, there is a strong indication that

molybdate is also capable of inhibiting organisms such as those responsible for 2-CP dehalogenation. These conclusions are based on our observed results and previous experiments in batch studies on chlorophenol dehalogenation by mixed microbial cultures since it is not possible to specify accurately the class of organisms (methanogens, sulfidogens, other obligate or facultative anaerobes etc.) responsible for 2-CP degradation.

Figure 5-13 and Figure 5-14 illustrates the effects of BESA and molybdate on 2-CP removal and methanogenesis respectively in cultures amended with or without 5 mM sulphate. The summary of the data on 2-CP removal is also shown below in Table 5-2.

Table 5-2 Comparison of 2-CP dehalogenation in unadapted anaerobic digester sludge 50% (vol/vol) under various enrichment conditions.

Enrichment type	Time for total removal of 0.39 mM 2-CP.	% 2-CP left after 14 days	Intermediates detected
Un-amended with SO_4	18 days	29.9	phenol and benzoate
Amended with SO_4	18 days	28.2	phenol and benzoate
BESA	38 days	64.8	phenol and benzoate
Molybdate (+ SO_4)	NR	94.9	ND
Molybdate (- SO_4)	NR	95.5	ND

NR : 2-CP removal was negligible; ND : No intermediates were detected

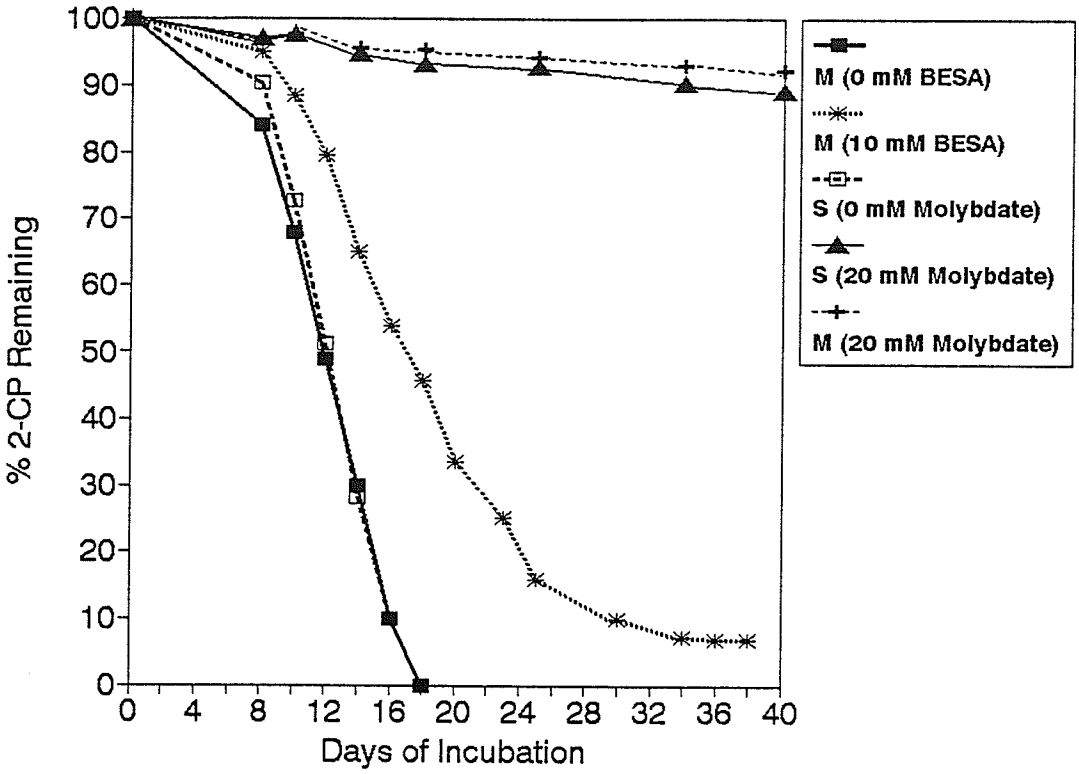


Fig. 5-13. Comparison of 2-CP removal in anaerobic digester sludge in presence or absence of either 10 mM BESA or 20 mM molybdate cultures un-amended with sulphate (**M**), amended with 5 mM sulphate (**S**). Background SO_4^{2-} was measured around 0.6 mM. All cultures were established in triplicates under 20% CO_2 + 80% N_2 atmosphere.

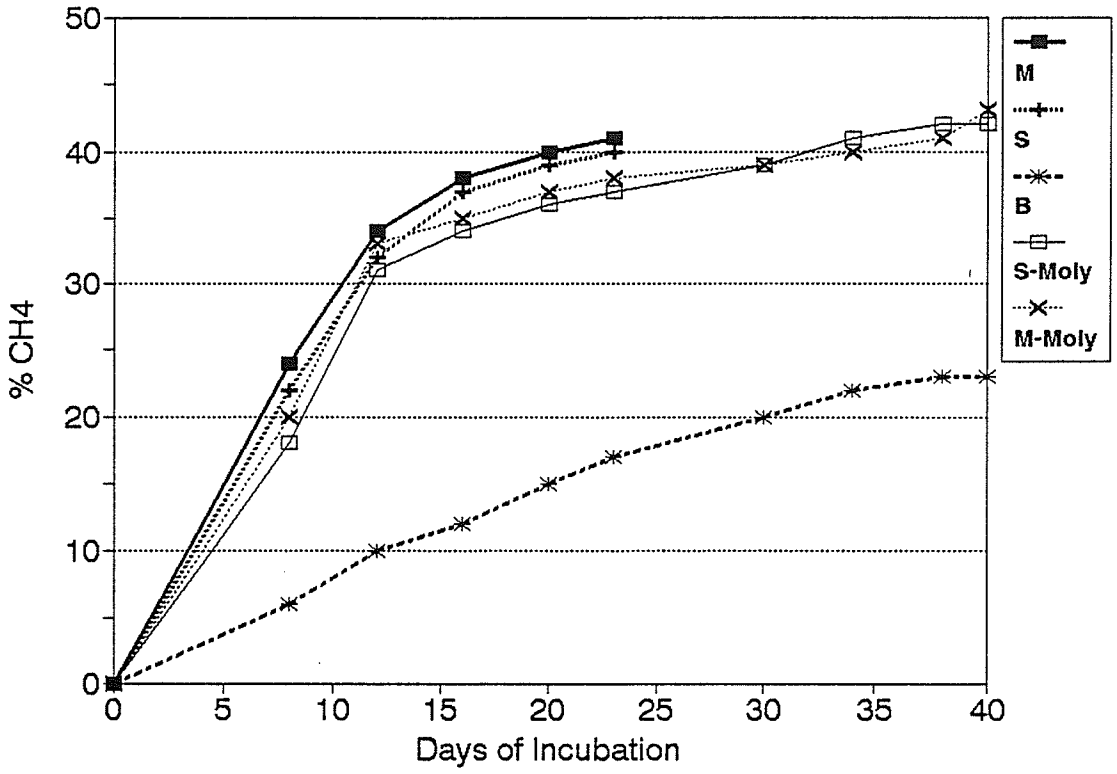


Fig. 5-14. Effect 10 mM BESA or 20 mM Molybdate on methanogenesis in batch cultures (50% vol/vol) in 0.1% yeast extract medium containing 0.385 mM 2-CP. (**M**) 0 mM BESA and unammended with sulphate (**S**) 0 mM Molybdate and ammended with 5 mM sulphate (**B**) 10 mM BESA and unammended with sulphate (**S-Moly**) 20 mM Molybdate and 5 mM sulphate (**M-Moly**) 20 mM Molybdate but un-ammended with sulphate. Background sulphate in unammended cultures was around 0.6 mM.

As evident from Fig. 5-14, methane production was identical with or without 5 mM sulphate and was not affected much by sulphate reduction. No methanogenesis was observed in the study on chlorophenol degradation under sulphate -reducing condition (Häggblom and Young, 1990). Addition of 10 mM of BESA affected methanogenesis considerably in this study but did not inhibit methane production totally. Presence of molybdate also had a very slight effect on methanogenesis in this study which therefore contradicts the only reported case of complete inhibition of methanogenesis by molybdate (Puhakka et al., 1990).

The effect of BESA and molybdate on 2-CP degradation were further examined with second-generation enrichments maintained on 0.1% yeast extract medium without addition of sulphate and 0.39 mM 2-CP. Six such cultures were operated semi-continuously in the usual manner. After two such feedings of yeast extract medium and 2-CP, duplicate bottles were injected with (i) standard 0.1% yeast extract medium, either (ii) yeast extract medium containing 10 mM BESA, or (iii) yeast extract medium containing 20 mM molybdate. All bottles were subsequently spiked with 2-CP to an initial target concentration of 0.39 mM.

The response of the three sets of culture are shown in Figure 5-15. As evident from the figure, the culture set receiving no inhibitors (i.e either BESA or molybdate) removed the initial 0.39 mM (49.7 mg/L) 2-CP in 11 days. 10 mM BESA slowed the removal rate of 2-CP and an average 2-CP concentration of 0.37 mM (47.8 mg/L) was reduced to 0.03 mM (3.7 mg/L) in about 26 days time. 2-CP

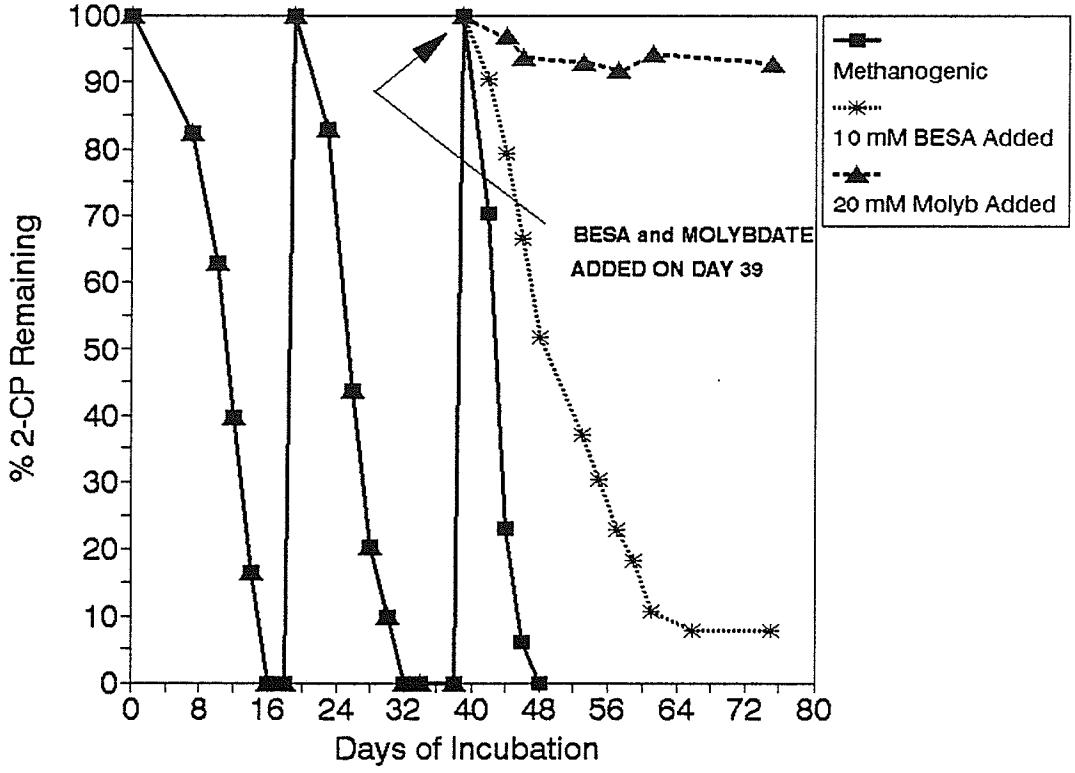


Fig. 5-15. Effect of BESA or Molybdate on degradation of 2-CP in cultures that were pre-exposed to 0.39 mM 2-CP. Before addition of respective inhibitors (BESA or Molybdate), six sets of cultures were maintained on 0.39 mM 2-CP and 0.1% yeast extract medium under 20% CO_2 + 80% N_2 atmosphere. On day 39, duplicate bottles were refed with medium containing 10 mM BESA or 20 mM Molybdate respectively followed by 0.39 mM 2-CP. The remaining bottles received medium without any inhibitors. The data points after 35 days represents average of two replicate cultures. No additional sulphate was added to the cultures and background sulphate measured on day 0 averaged around 0.65 mM.

removal in cultures which received 20 mM molybdate on the other hand was completely inhibited and a total 2-CP loss of approximate 6% were observed over a 40 day incubation period.

Phenol was identified as a dehalogenation product in all cultures except those containing molybdate. All these cultures before they were exposed to the respective inhibitors had removed about 49.5 mg/L (0.39 mM) 2-CP in 13 days. At the start of the experiment (while the cultures were set-up without the addition of sulphate and maintained on 2-CP and 0.1% yeast extract medium), the background sulphate concentration from inoculum and mineral medium was found to be around 0.6 mM (60 mg/L) SO_4^{2-}. Based on our previous observation of sulphate removal in background samples (no sulphate added), this concentration was usually reduced to about 0.3 to 0.4 mM in around 20 days. It may be possible based on this observation that the dehalogenating organism/organisms was/were sulphate reducers which utilized 2-CP instead of sulphate as the terminal electron acceptor and used sulphate in the absence of 2-CP.

The results of this also indicated that the 2-CP degradation was affected by both the inhibitors (BESA and molybdate). However molybdate at 20 mM significantly inhibited 2-CP degradation by a mixed anaerobic culture in this study compared to BESA amended cultures which had a strong impact on phenol degradation and to some extent on methanogenesis. To what extent molybdate

affected the overall activities of various metabolic groups present in the system (besides methanogens and sulfidogens) consequently affecting 2-CP degradation could not be determined nor was the reason for phenol accumulation.

In conclusion, the results of this experiment indicate that molybdate had a much stronger inhibitory effect on 2-CP dehalogenation compared to BESA. Molybdate at 20 mM completely inhibited dechlorination of 2-CP in the presence or absence of sulphate, as well as sulphate reduction. BESA partially inhibited methanogenesis and slowed 2-CP dechlorination with accumulation of phenol. A similar response of molybdate and BESA was observed for cultures previously exposed to 2-CP in absence of sulphate.

Effect of various concentrations of BESA on 2-CP dehalogenation. There is limited information in the literature on the effect of inhibitors like BESA and molybdate at different concentrations. Only Genthner et al. (1989a) had previously shown the effect of two different BESA concentration on degradation of chlorophenols and chlorobenzoates in aquatic sediments. The authors (Genthner et al., 1989a) observed that in contrast to 1 mM BESA, the presence of 10 mM BESA prevented the degradation of 3-chlorophenol and 4-chlorophenol and delayed the degradation of 2-chlorobenzoate and 3-chlorobenzoate. The inhibitory effect was believed to be due to inhibition of methanogenesis or reductive dechlorination. To investigate the effect of different concentrations of BESA on 2-CP dehalogenation, cultures were set up in triplicates with either 0 mM, 1 mM, 5

mM or 10 mM of BESA. The background sulphate from inoculum and 0.1% yeast extract medium was around 0.6 mM. The cultures were analyzed for 2-CP, Phenol, VFA (mainly acetate and propionate) and methane in the head-space.

The removal of 2-CP in presence of 0 mM, 1 mM, 5 mM and 10 mM BESA is shown in Figure 5-16. Presence of even 1 mM BESA slowed the removal of 0.39 mM 2-CP from 17 days to 23 days which is in contrast to what was reported earlier for aquatic sediments enrichments (Genthner et al., 1989a). 5 mM BESA further delayed 2-CP removal and 0.39 mM 2-CP was removed in 32 days. 2-CP removal in presence of 10 mM BESA followed the trend observed in previous experiments of this study with a distinct tailing pattern indicating inhibition towards the end and 0.38 mM 2-CP was only reduced to 0.03 mM in about 39 days.

Phenol produced from 2-CP dehalogenation was subsequently removed in all cultures except those with 5 mM and 10 mM BESA and is shown in Figure 5-17. This accumulation of phenol in BESA amended cultures were also seen in previous batch experiments of this study (refer section 5.1.3). As seen from the figure, the tendency of phenol accumulation increased with the increase in BESA concentration in the cultures and the corresponding decrease in 2-CP removal as also observed before in this study. In summary, presence of even 1 mM BESA slowed the dechlorination of 2-CP suggesting some inhibition of the culture dehalogenating 2-CP.

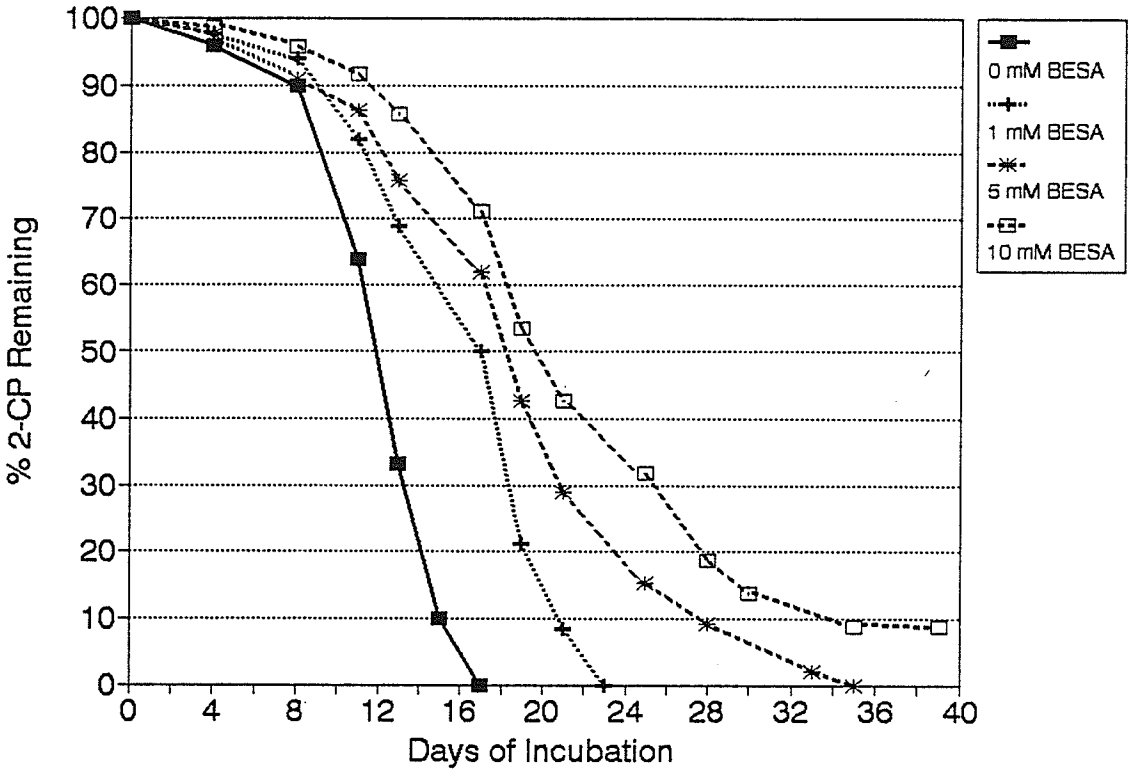


Fig. 5-16. Removal of 0.385 mM 2-CP in presence of 0 mM, 1 mM, 5 mM and 10 mM bromoethanesulphonic acid (BESA) under 20% CO_2 + 80% N_2 atmosphere in batch cultures containing 50% (vol/vol) anaerobic digester sludge and 0.1% yeast extract medium.

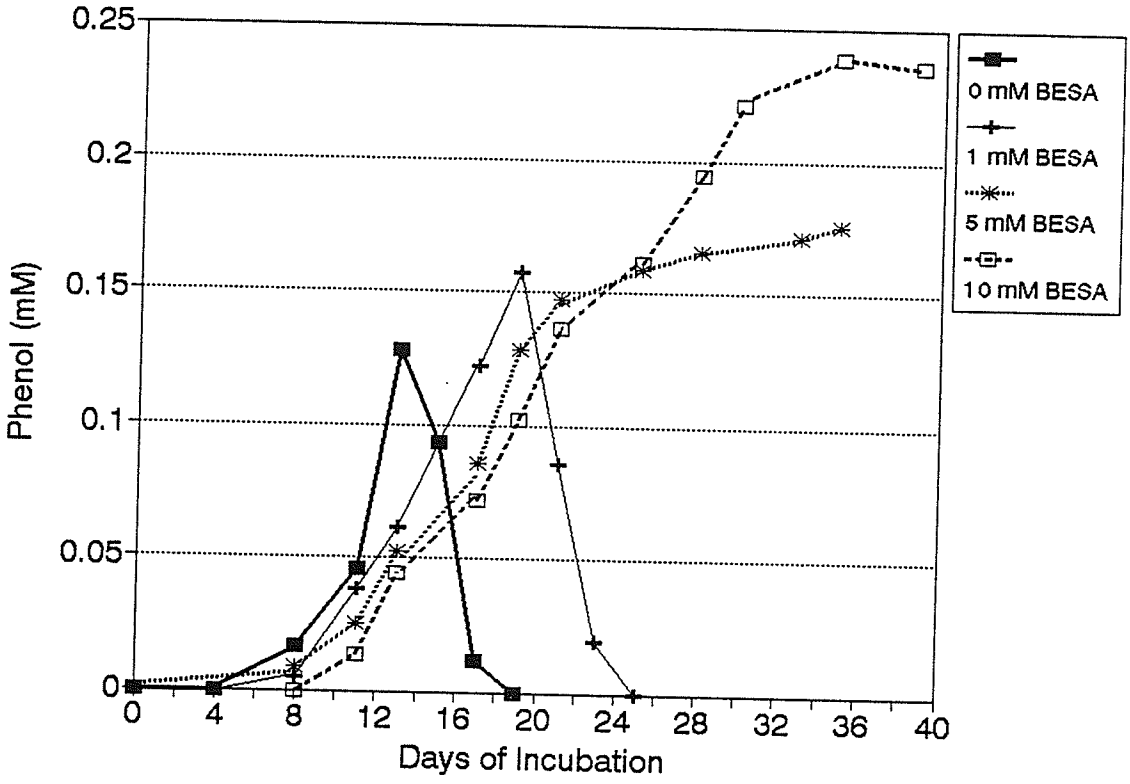


Fig. 5-17. Effect of 0 mM, 1 mM, 5 mM and 10 mM bromoethanesulphonic acid (BESA) on generation of phenol from 0.385 mM 2-CP in batch cultures containing 50% (vol/vol) anaerobic digester sludge and 0.1% yeast extract medium under 20% CO_2 + 80% N_2 atmosphere.

Effect of BESA on methanogenesis and VFA. The effect of BESA on methanogenesis is shown in Figure 5-18. Methane in the head-space of all cultures were monitored over the entire incubation period. As expected, the percentage of methane in the head-space decreased with the increase in concentrations of BESA in the cultures although complete inhibition of methanogenesis did not occur. Some authors had observed however complete inhibition of methanogenesis in presence of BESA at concentrations of even 0.5 mM (Freedman and Gosset, 1989) and 5 mM (Perkins et al., 1994) respectively. On the other hand, Zinder et al. (1984) had demonstrated that the complete inhibition of methanogenesis by BESA occurred at 50 mM. The same authors had also observed partial inhibition by even 1 mM BESA..

Volatile fatty acids were monitored and the total volatile fatty acids (TVFA) production with time in these cultures are shown in Figure 5-19. Acetate as the major fermentative product accumulated primarily in the cultures with BESA although a maximum propionate concentration was observed around day 11. The results are shown in Figure 5-20 and 5-21 respectively. The accumulation of volatile fatty acids especially acetate is expected since BESA inhibits the reduction of methyl-coenzyme M (which mediates the last step in methane formation) to methane (Perkins et al., 1994). Acetate accumulation in cultures without BESA was negligible. Studies by Sparling (1995) on the specificity of BESA on the growth of several organisms indicated that except for the methanogens other species of bacteria were not affected by the presence of up to 20 mM BESA.

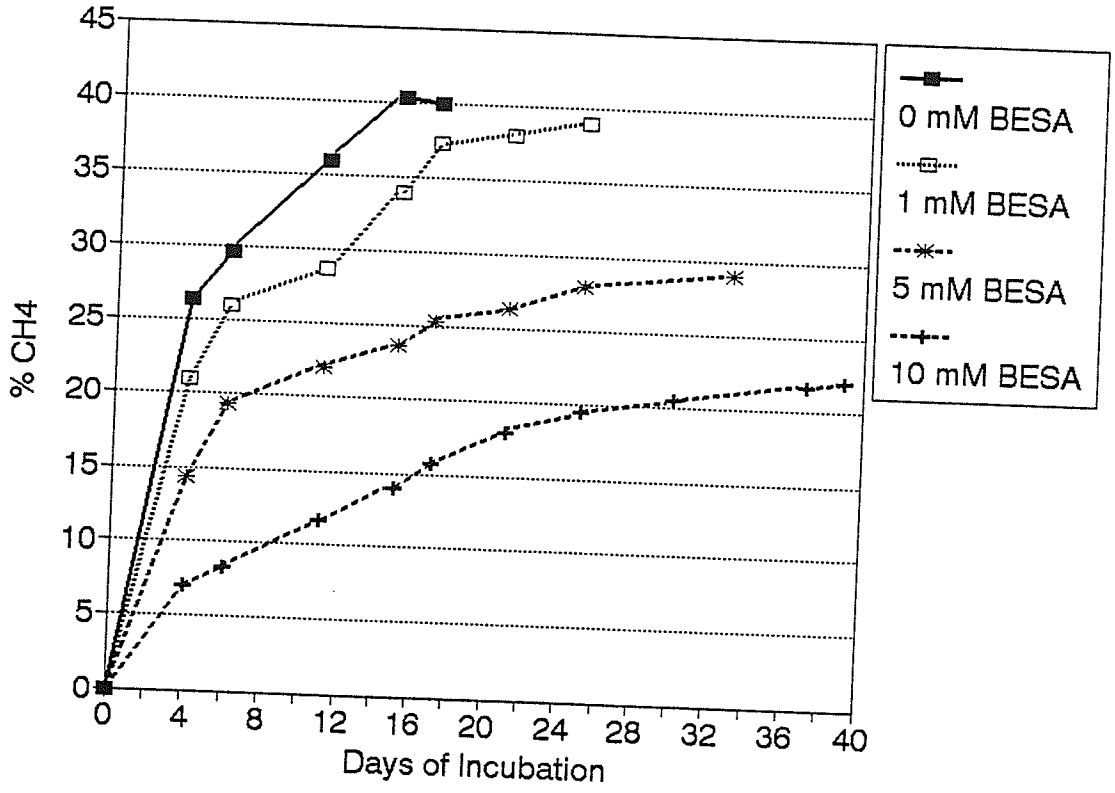


Fig. 5-18. Percentage of methane in the head-space of cultures containing 0, 1, 5, 10 mM BESA. Result are based on triplicate cultures.

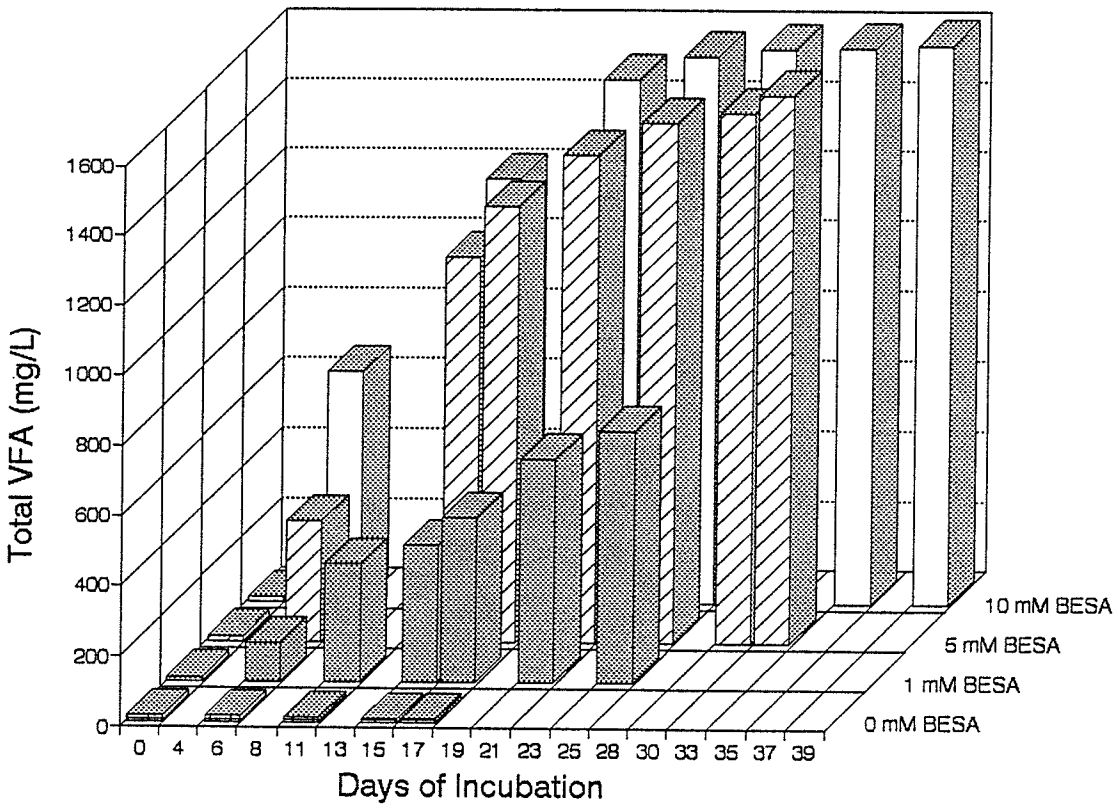


Fig. 5-19. Effect of 0, 1, 5, 10 mM BESA on total volatile fatty acids (TVFA) generation in batch cultures of (50% vol/vol) anaerobic digester sludge in presence of 0.385 mM 2-CP and 0.1% yeast extract medium under 20% CO_2 + 80% N_2 atmosphere.

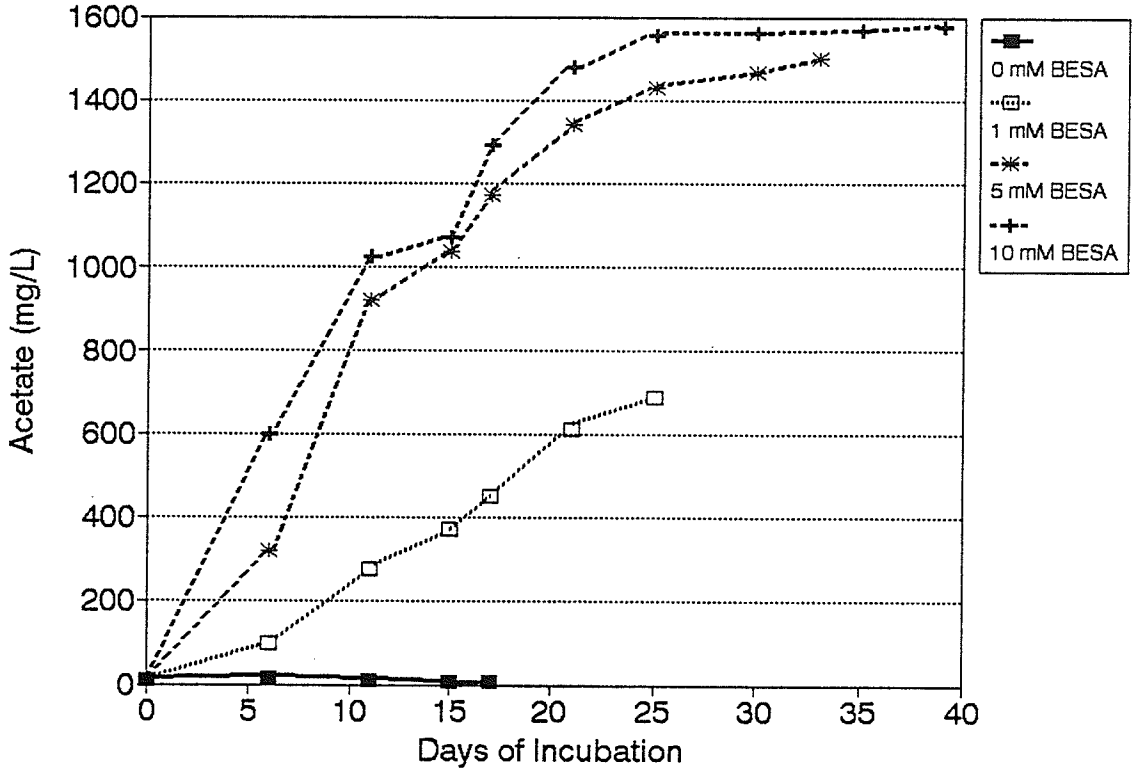


Fig. 5-20. Acetate formation in batch cultures (50% vol/vol of anaerobic digester sludge in 0.1% yeast extract medium) degrading 0.385 mM 2-CP in presence of 0mM, 1 mM, 5 mM and 10 mM BESA.

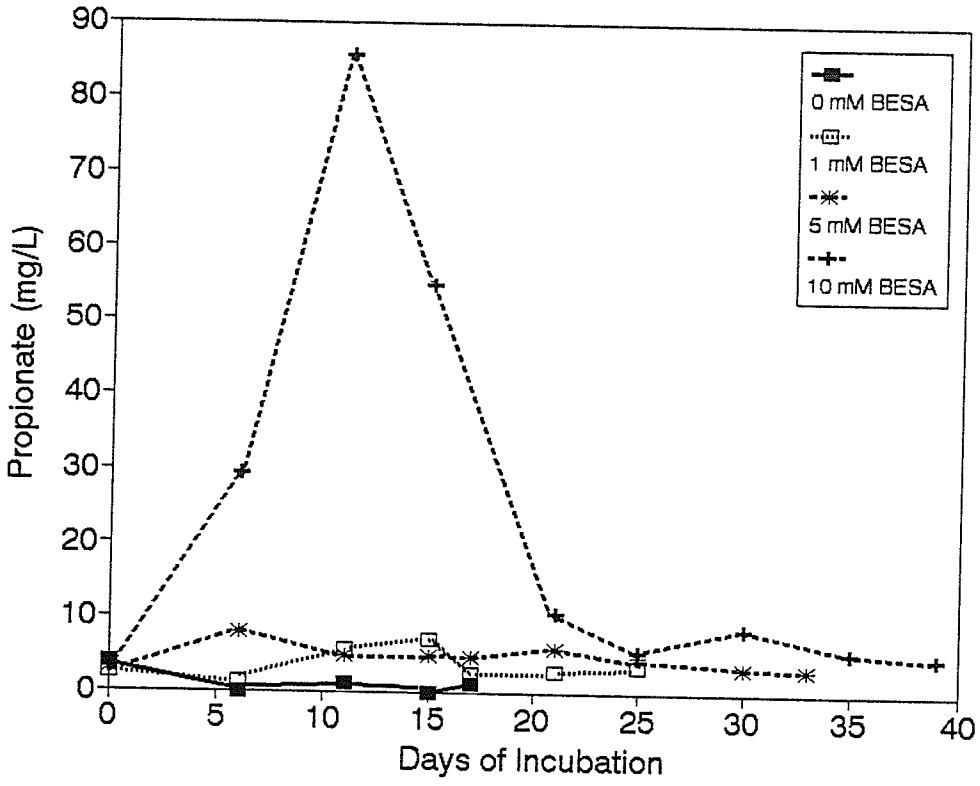


Fig. 5-21. Propionate formation in batch cultures (50% vol/vol of anaerobic digester sludge in 0.1% yeast extract medium) degrading 0.385 mM 2-CP in presence of 0mM, 1 mM, 5 mM and 10 mM BESA.

The accumulation of acetate with increasing BESA concentration also explains the tendency of these cultures to accumulate phenol. The fermentation of phenol to acetate becomes thermodynamically unfavourable (ΔG° = +5.6 kJ/reaction) in acetate accumulated cultures (Knoll and Winter, 1987) which consequently affected 2-CP removal. There was very little indication of propionate accumulation although a peak concentration was noticed around day 11 (for cultures with 10 mM BESA) but was subsequently transformed to acetate since a sharp rise in acetate concentration was observed after day 11. In summary, the accumulation of acetate, TVFA and decrease in the percentage of methane in the head-space was observed with the increase in concentration of BESA .

Effect of various concentrations of molybdate on 2-CP dehalogenation. Cultures amended with initial sulphate concentrations around 5.2 mM (500 mg/L) SO_4^{-2} were also set-up in triplicates with either 0 mM, 1 mM and 10 mM molybdate. The removal of 2-CP in these cultures in presence of 0 mM, 1 mM and 10 mM molybdate is shown in Figure 5-22. In presence of 1 mM molybdate, 2-CP degradation in the cultures slowed down considerably and 0.39 mM (50.2 mg/L) 2-CP was removed in 27 days compared to 17 days when no molybdate was present. Presence of 10 mM molybdate on the other hand completely inhibited 2-CP removal. A comparison of sulphate reduction in these cultures (Figure 5-23) indicated that very little sulphate removal took place in 1 mM molybdate cultures beyond 15 days, after some 28% removal has been

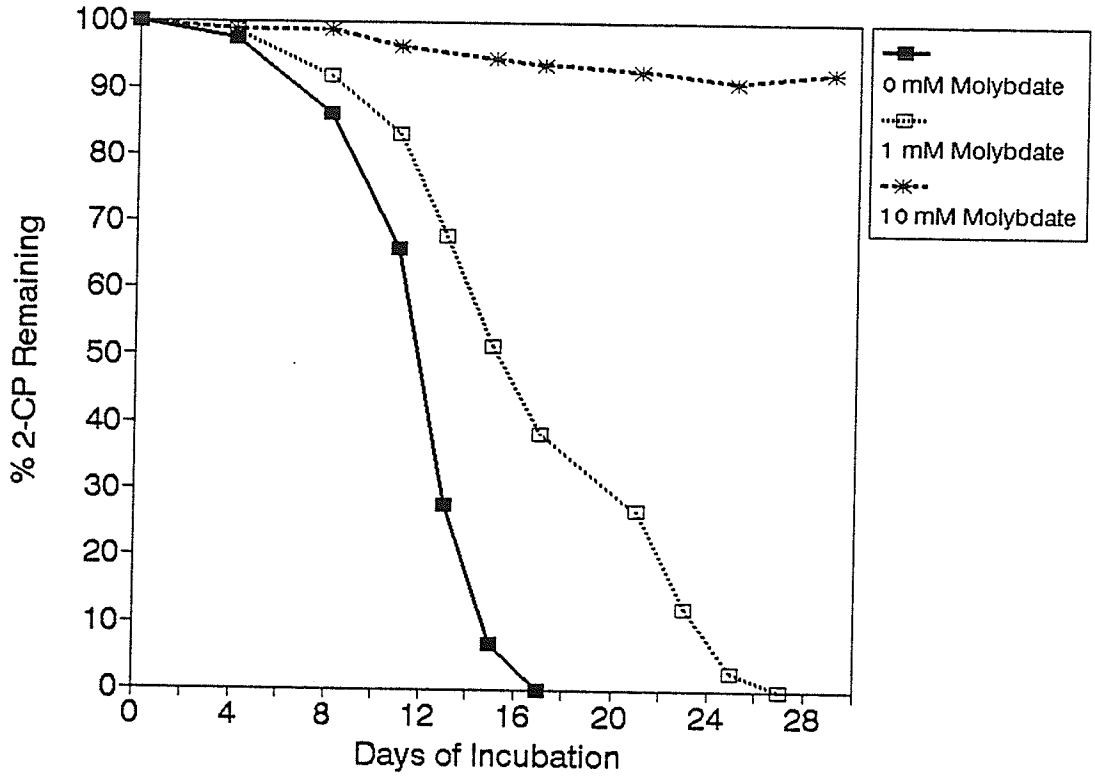


Fig. 5-22. Removal of 0.385 mM 2-CP in batch culture of anaerobic digester sludge (50% vol/vol) in presence of 0 mM, 1 mM and 10 mM Molybdate. The medium contained 0.1% yeast extract and an initial SO_4^{2-} concentration of about 5 mM. All cultures were set-up in triplicates.

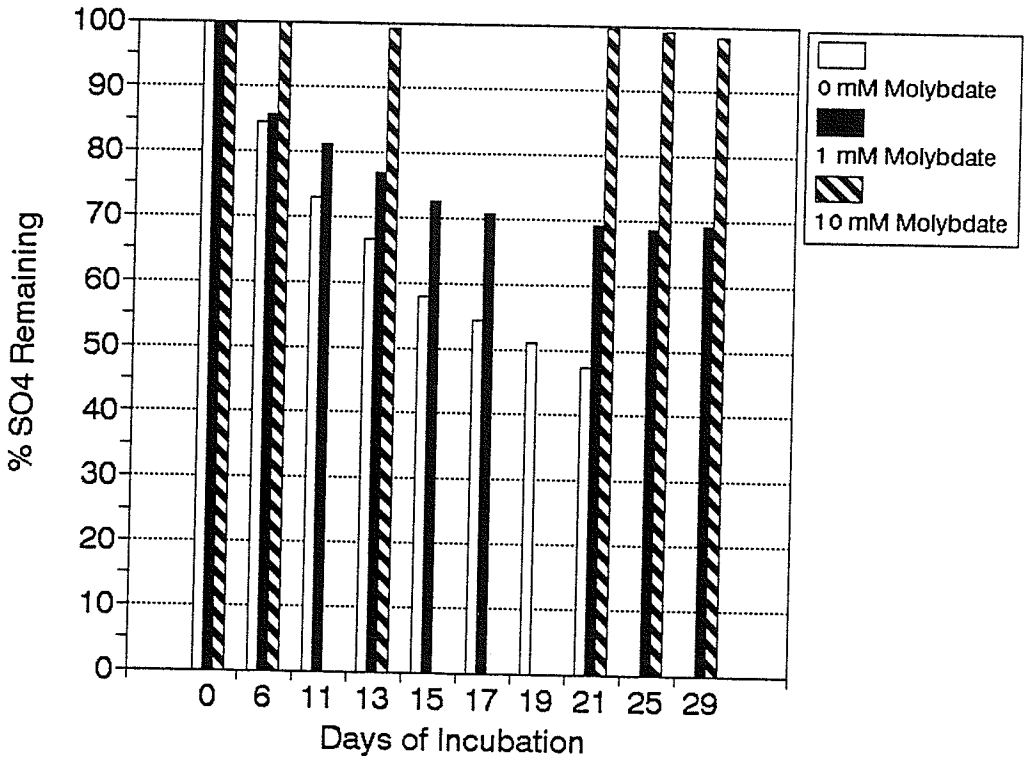


Fig. 5-23. Effect of 0 mM, 1 mM and 10 mM Molybdate on SO_4^{2-} reduction in batch cultures of anaerobic digester sludge (50% vol/vol) in presence of 0.39 mM 2-CP. Sulphate concentrations on day 15,17 and 19 were not monitored for cultures amended with 10 mM molybdate.

accomplished. The removal of 2-CP during the period when practically no sulphate reduction occurred (beyond day 15, cultures amended with 1 mM molybdate showed only 3% reduction in sulphate) indicated that 2-CP degradation in this system was not coupled to sulphate reduction. Unlike 1 mM molybdate, the cultures amended with 10 mM molybdate completely inhibited sulphate reduction.

Effect of molybdate on VFA and methanogenesis. The effect of different molybdate concentration on TVFA is shown in Figure 5-24. The graph also shows the corresponding percentage of TVFA as propionate. It is interesting to note that in presence of 10 mM molybdate, most of the VFA's were in the form of propionate indicating that molybdate at 10 mM concentration perhaps inhibits syntrophic bacteria responsible for propionate metabolism. Hilton and Archer (1988) had also reported a rapid increase in propionate concentration when molybdate was added to an anaerobic bioreactor treating molasses wastewater. A comparison of methane formation in presence of different molybdate concentration is outlined in Figure 5-25. In presence of 10 mM molybdate the concentration of methane in the head-space is slightly lower than 0 mM molybdate cultures and is like due to incomplete conversion of propionate to acetate which is finally converted to methane.

The results of this experiment with different concentrations of molybdate indicated that although sulfidogens may not be involved directly in the dehalogenation of 2-CP, electron sink through processes such as sulfidogenesis

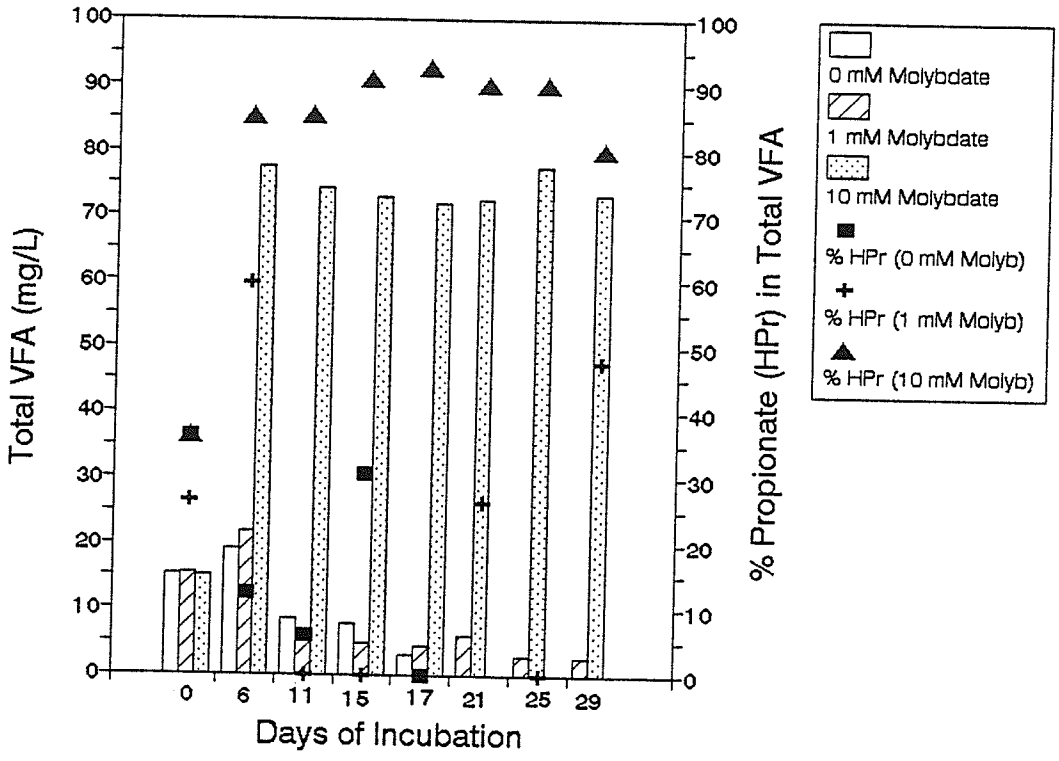


Fig. 5.24. Effect of molybdate (0 mM, 1 mM and 10 mM) on total volatile fatty acids (TVFA) production in batch cultures of anaerobic digester sludge. The graph also shows the percentage of TVFA as propionate which accumulated in the bottles. Results are based on triplicate cultures.

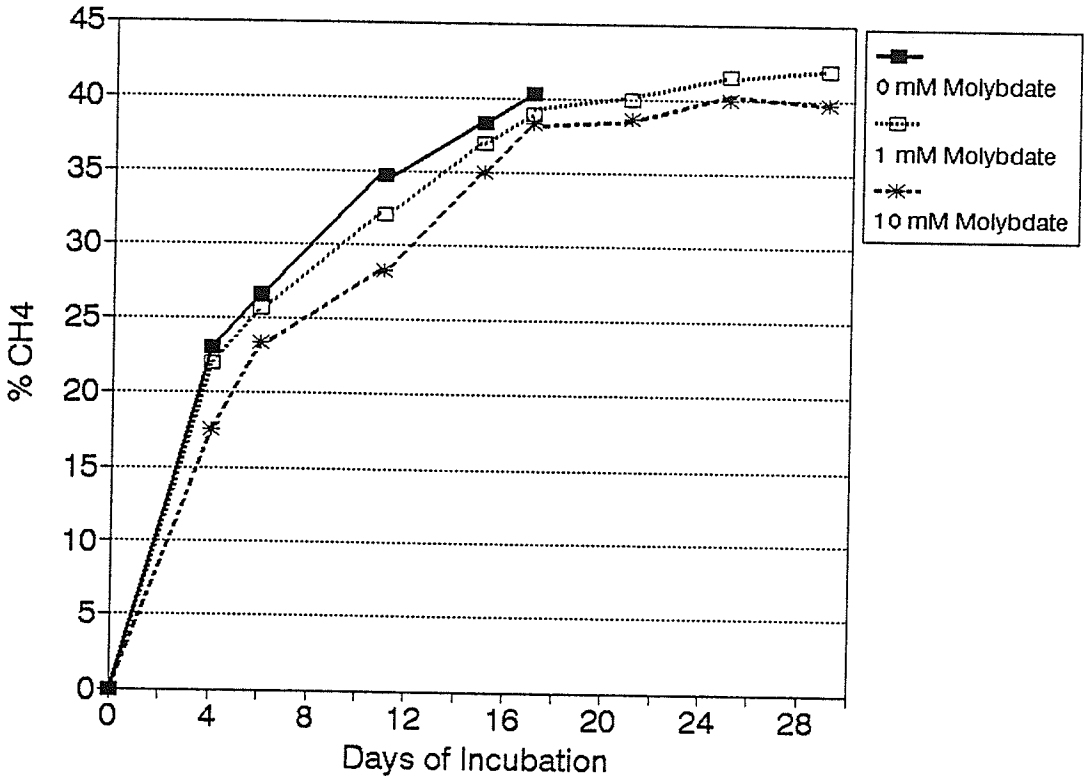


Fig. 5-25. Percentage of methane in the head-space of cultures containing 0 mM, 1 mM and 10 mM Molybdate. Result are based on triplicate cultures.

is never-the-less important in effecting 2-CP removal in anaerobic mixed bacterial cultures. The accumulation of propionate in cultures amended with 10 mM molybdate with simultaneous inhibition of 2-CP removal suggests the role of syntrophic bacteria in influencing dehalogenation of 2-CP. The classic example of degradation of xenobiotic compounds by syntrophic association is the dehalogenation of 3-CB (DeWeerd et al., 1990; Mohn and Kennedy, 1992a). An overall summary of this subsection dealing with the dechlorination of 2-CP in presence or absence of sulphate along with the effect on dechlorination, methanogenesis and VFA production by the respective inhibitors (BESA and molybdate) at various concentration is given below.

Observation summary. 2-CP dehalogenation was observed in cultures simultaneously with sulphate reduction and methanogenesis. Presence of BESA a known inhibitor of methane producers partially inhibited methanogenesis and slowed 2-CP dehalogenation rate with phenol and acetate accumulation. This accumulation increased with the increase in concentration of BESA in the cultures. Molybdate on the other hand completely inhibited both sulphate reduction and 2-CP dehalogenation at concentrations $\geq$ 10 mM. Complete dehalogenation of 2-CP continued even when sulphate reduction was inhibited after sometime by 1 mM molybdate indicating that sulphate reducing bacteria were not directly involved in the dehalogenation of 2-CP in this study. Inhibition of 2-CP dehalogenation along with accumulation of propionate at high levels of molybdate in the cultures strongly suggests the role of syntrophic association in the dehalogenation reaction.

5.1.3 Effect of prior acclimation to 2-CP

This study with un-acclimated inocula from three different sources clearly showed that the anaerobic digester sludge tested exhibited a better potential than either the lagoon sludge or the biomass from the anaerobic bioreactor treating BKM waste (refer section 5.1.1). Experiments with digester sludge adapted to 2-CP over a one year period resulted in immediate and more rapid biotransformation rates of 2-CP. The long adaptation times as observed for unadapted samples (10% vol/vol) are assumed to result from the time required for the low number of dehalogenating organisms to increase in population before they can exhibit a detectable rate of dehalogenation. Literature suggests the wide variability in dechlorinating performance for different inocula.

Experiments were conducted subsequently to investigate degradation of 2-CP with various dilutions of adapted sludge in the mineral medium containing 0.1% yeast extract to correlate biomass concentration with dehalogenation rates. Figure 5-26 shows 2-CP removal profiles in 10%, 25% and 50% (vol/vol) cultures respectively which correspond to an approximate 2-CP removal rates of 29 µM/d, 35 µM/d and 55 µM/d respectively. These rates were calculated based on the time required for complete removal of 0.38 mM 2-CP and were consistent over subsequent feeding of yeast extract medium and 2-CP. Increasing biomass concentration by 2.5 (i.e 10% to 25% vol/vol) and 5 folds (10% to 50% vol/vol), increased dehalogenation rate of 2-CP only 1.2 and 2 folds respectively indicating a non linear relationship between biomass concentration and dehalogenation rates.

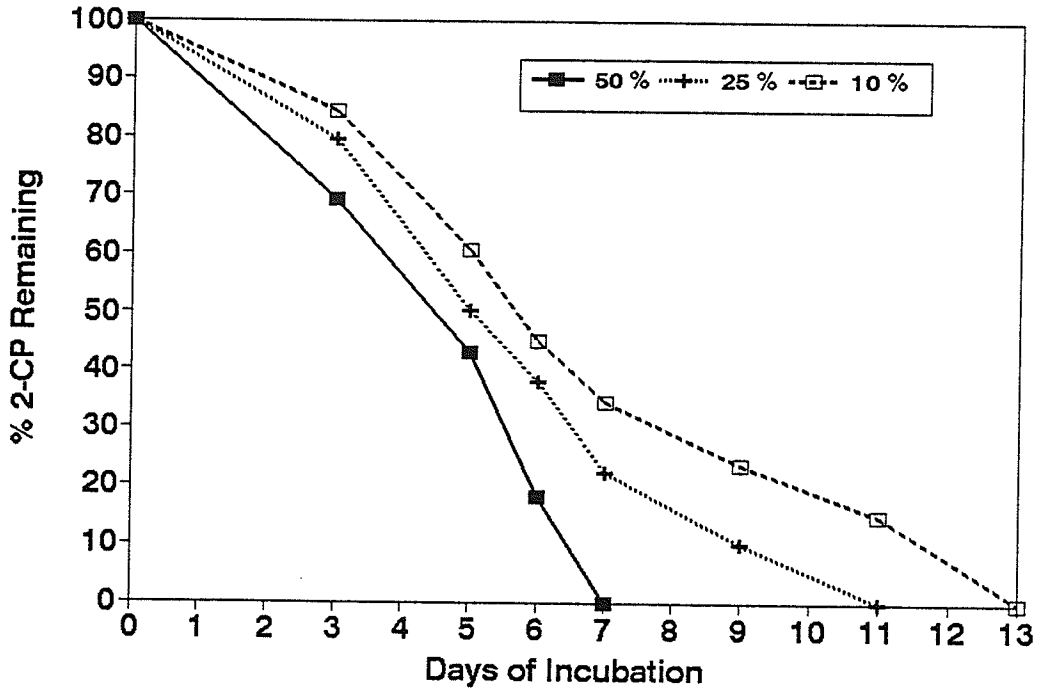


Fig. 5-26. Degradation profile of 0.385 mM 2-CP in mineral medium containing 0.1% yeast extract inoculated with various concentrations of adapted digested sewage sludge under 20% CO_2 + 80% N_2 atmosphere. Data points are the mean of two replicate cultures.

Phenol initially accumulated and degraded at a slower rate. Traces of benzoate were also observed sporadically. Detection of benzoate in cultures as an intermediate of phenol degradation indicated that the phenol degraded through the benzoate pathway. The difference in degradation rates for 2-CP and phenol in our cultures has two implications. It is possible that two very different organisms in the cultures are responsible for the removal of 2-CP and phenol respectively or that both the reactions (removal of 2-CP and phenol) were catalyzed by one organism. Experiments with anaerobic cultures (derived from digested sewage sludge) grown on phenol demonstrated that they could not dehalogenate 0.38 mM 2-CP (Fig. 5-27) and support the conclusion that organisms responsible for phenol degradation are different from 2-CP dehalogenators. This hypothesis is supported by the results of Wiegel et al. (1990) who found that phenol enrichments did not dehalogenate 4-CP. Pure cultures of bacteria dehalogenating 2-CP have been shown to accumulate phenol without further degradation (Cole *et al.*, 1994). Based on this experiment and literature it appears that two different organisms were responsible for 2-CP and phenol degradation.

Observation summary. Anaerobic digested sewage sludge adapted to 2-CP converted it to phenol without any visible lag period which was observed for un-adapted sludge. Degradation of 2-CP with various dilutions of adapted sludge indicated that dehalogenation activity did not increase linearly with biomass concentrations. Digested sewage sludge adapted to phenol under various conditions did not dehalogenate 2-CP indicating that organisms responsible for 2-CP and phenol degradation in this mixed culture were different.

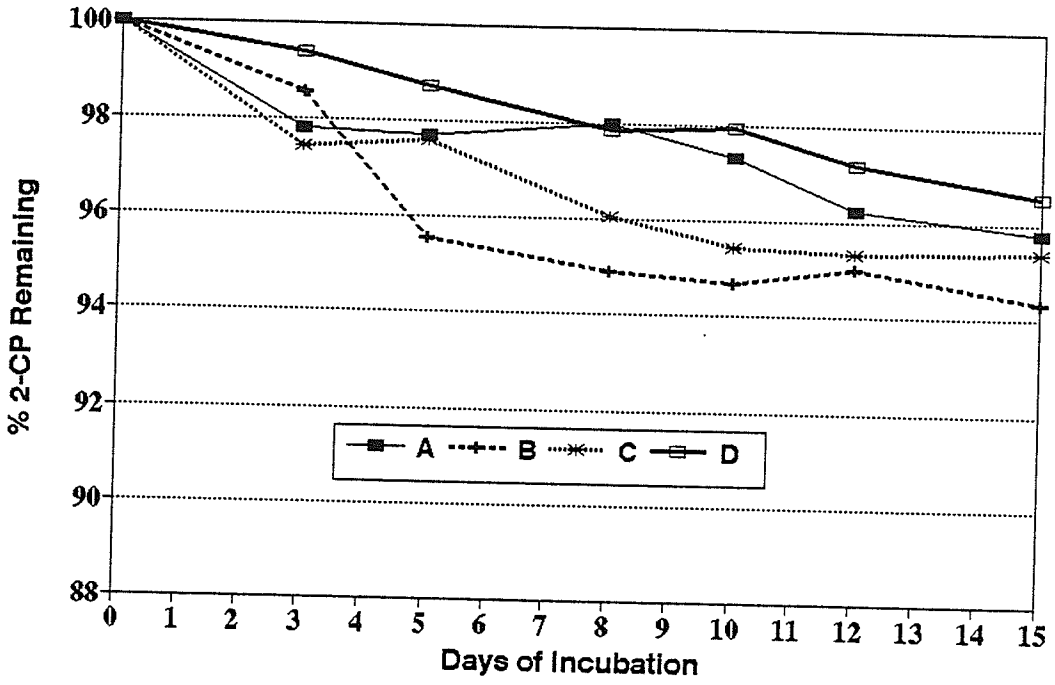


Fig. 5-27. Response of cultures exposed to 0.385 mM 2-CP. The cultures were derived from anaerobic digester sludge and adapted to (A) 1 mM phenol only (B) 1 mM phenol in mineral medium containing 0.1% yeast extract under 20% CO_2 + 80% N_2 atmosphere (C) 1 mM phenol in 0.1% yeast extract medium under 20% CO_2 + 80% H_2 atmosphere (D) Sterile controls. No phenol as a dehalogenation product was detected in any of these cultures. Data points are the mean of two replicate cultures.

5.1.4 Effect of various electron donors on anaerobic dehalogenation of 2-CP in acclimated digested sludge

Hydrogen. Very few studies have examined the use of hydrogen as an electron donor in dehalogenation of chlorinated phenols. The ability of both unadapted and adapted sewage sludge to degrade about 0.39 mM 2-CP was investigated in a mineral medium containing 0.1% yeast extract under 80% H_2 + 20% CO_2 atmosphere. The lag period for unadapted sludge before any visible dehalogenation activity (detection of phenol) was noticed lasted 97 days. After several re-feeding of yeast extract medium and 2-CP, a significant increase in dehalogenation activity was noticed (Figure 5-28) and the cultures after 400 days of incubation could maintain a degradation capacity of 16 µM/d which was still less than 24 µM/d observed under CO_2/N_2 atmosphere.

Using sludge adapted to 2-CP under CO_2/N_2 atmosphere on the other hand, dehalogenation of 2-CP started without a lag in presence of hydrogen. Similar to what was observed for cultures with adapted sludge under CO_2/N_2, some phenol accumulation was noticed which was degraded subsequently. Figure 5-29 shows the response of this adapted sludge to 2-CP in presence of 80% H_2. Figure 5-30 compares the typical profiles of 2-CP removals in adapted sludge under CO_2/N_2 and CO_2/H_2 atmosphere. These profiles are based on consistent 2-CP removal after successive feeding of yeast extract medium and 2-CP. The results indicate that cultures maintained under similar sources of carbon and energy show different

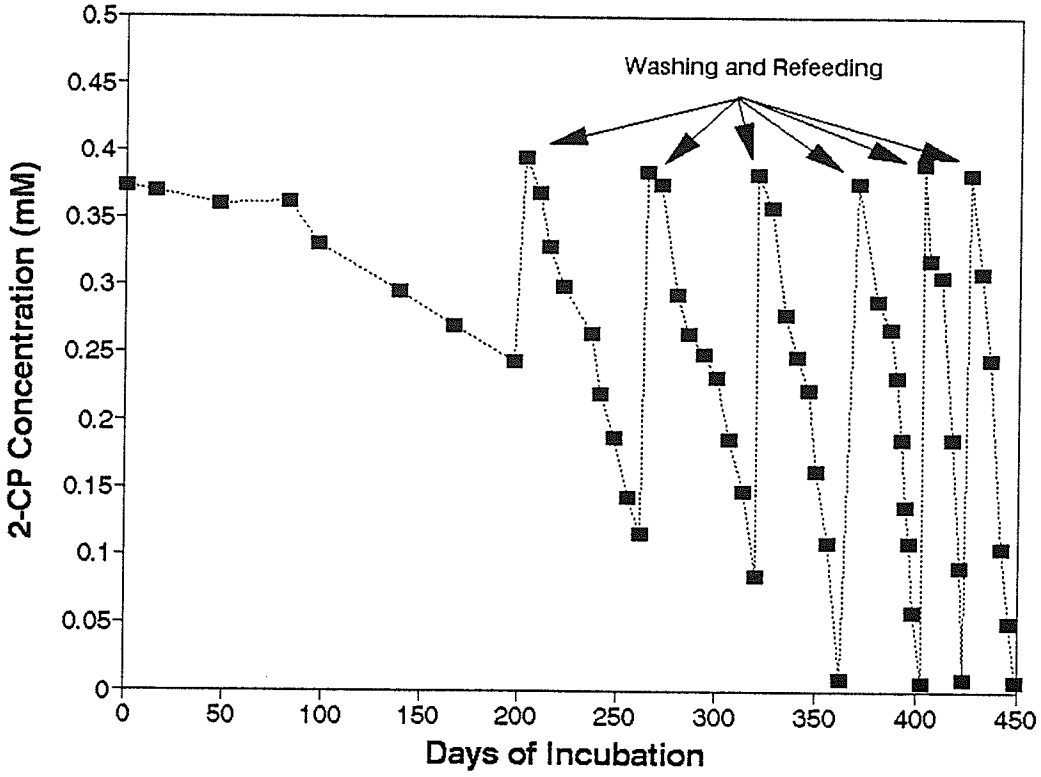


Fig. 5-28. Removal of 2-CP in batch cultures of fresh anaerobic digester sludge 10% (vol/vol) in mineral medium containing 0.1% yeast extract under 20% CO_2 + 80% H_2 atmosphere. Data points are the mean of two replicate cultures.

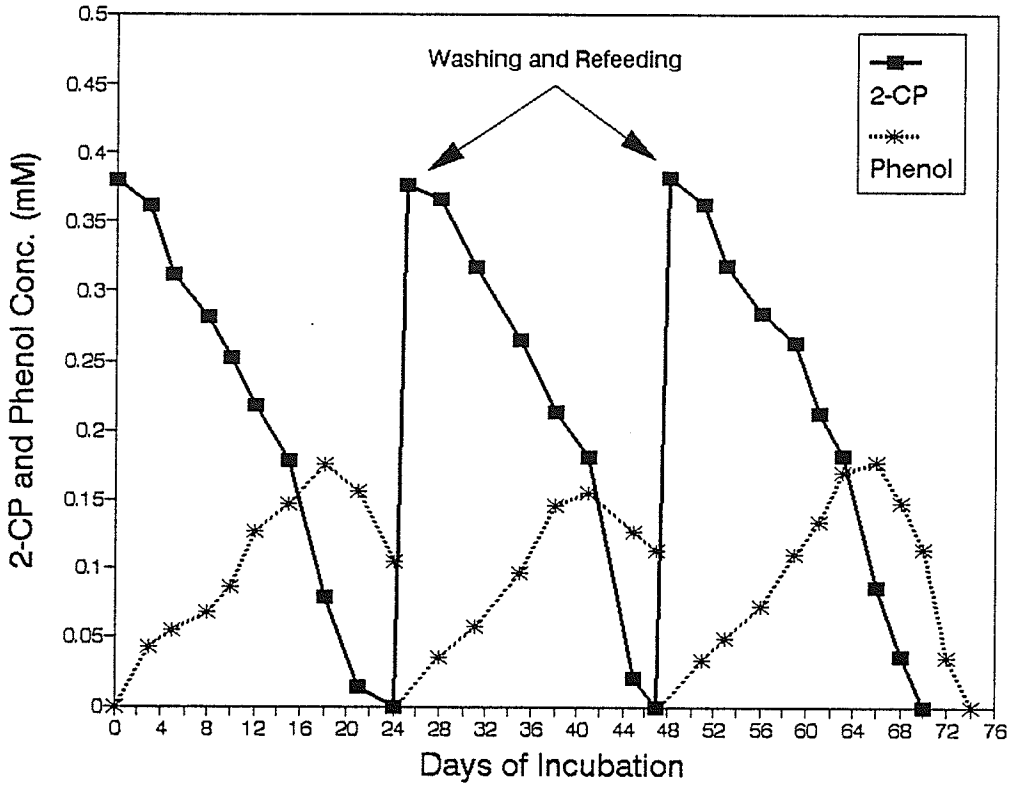


Fig. 5-29. Dehalogenation of 0.39 mM 2-CP by the adapted anaerobic digester sludge 10% (vol/vol) in mineral medium containing 0.1% yeast extract under 20% CO_2 + 80% H_2 atmosphere. The cultures were centrifuged to remove phenol and other metabolic products before re-feeding with fresh medium and 2-CP. Data points are the mean of two replicate cultures.

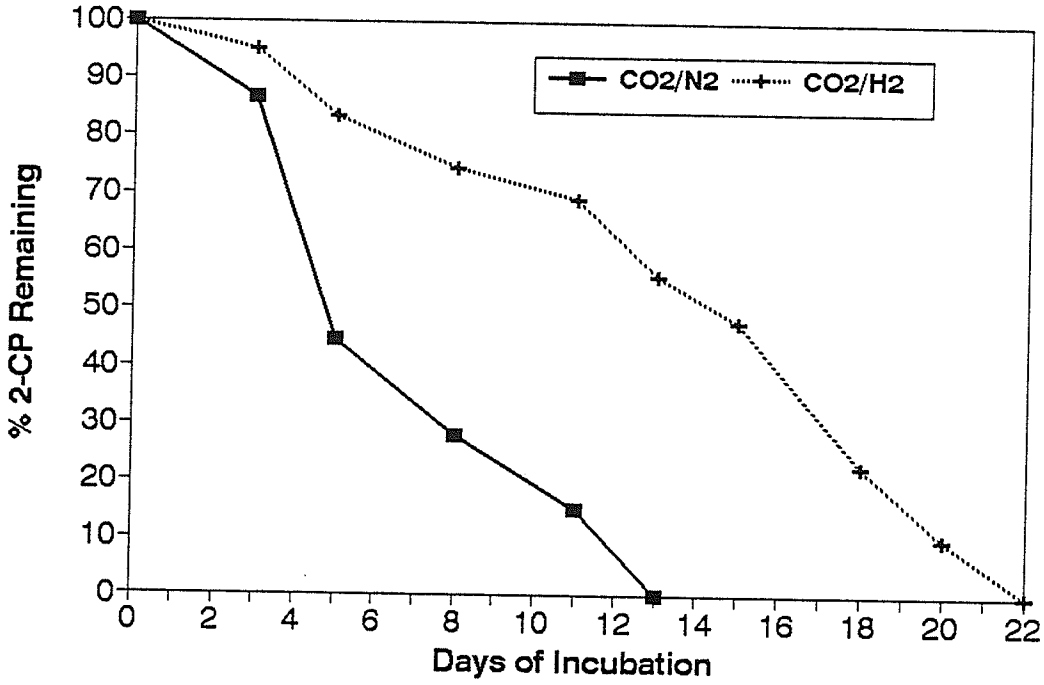


Fig. 5-30. Comparison of 2-CP removal (0.385 mM) by adapted digested sewage sludge in mineral medium containing 0.1% yeast extract under two different headspace conditions (20% CO_2 + 80% N_2 or 20% CO_2 + 80% H_2). Data points are the mean of two replicate cultures.

capacity of dehalogenation under two different head-space conditions. Cultures incubated under 20% CO_2 + 80% N_2 appeared to exhibit a higher dehalogenation capacity compared to 20% CO_2 + 80% H_2.

Zhang and Wiegel (1990) and Wiegel et al. (1990) have shown that dechlorination of 4-CP, the transformation of phenol to benzoate, and the degradation of benzoate were totally inhibited by the presence of even 10% hydrogen in the head-space. Complete mineralization of 4-CP on the other hand was observed under CO_2/N_2 atmosphere. Hydrogen at elevated concentrations is also reported to be inhibitory for the dechlorination of 3-chlorobenzoate by strain DCB-1. Accumulation of benzoate occurred in cultures degrading phenol under 80% H_2 + 20% CO_2 whereas benzoate was a transient metabolite of phenol degradation under 80% N_2 + 20% CO_2 (Kobayashi et al., 1989; Knoll and Winter, 1987). In contrast to the above results, hydrogen neither inhibited nor stimulated dehalogenation of 2,4,6-TCP (Perkins et al., 1994). Madsen and Aamand (1991) were able to show a general stimulation of pentachlorophenol (PCP) dehalogenation activity in presence of hydrogen. PCP dehalogenated directly to 3,5-DCP under 30% CO_2 + 70% H_2 while under 30% CO_2 + 70% N_2, build-up of intermediate 3,4,5-TCP was noticed.

Hydrogen can affect the syntrophic consortia and hydrogen-producing fermentative bacteria. In this experiment, with mixed cultures dehalogenating 2-CP, there is a strong possibility that the presence of 80 % hydrogen in the head-

space influenced the overall metabolic balance in our cultures thereby affecting the organisms present in the consortium. It was already presented in this study the potential involvement of syntrophic population in influencing 2-CP dehalogenation. As seen before, propionate accumulated in presence of molybdate with simultaneous inhibition of 2-CP. Although VFA was not monitored when this experiment was conducted, there seems to be a common link between these two results directly implicating the role of syntrophic population in the mixed culture.

Organic electron donors. Since reductive dehalogenation in the system was not stimulated by hydrogen as an electron donor, various organic electron sources were tested. Duplicate cultures (10% vol/vol inocula) were set up separately in basic mineral medium with 10 mM acetate, propionate, ethanol or 0.1% yeast extract using adapted sewage sludge under 20% CO_2 + 80% N_2. These organic electron donors were chosen because to study the effect on dehalogenation of 2-CP by stimulating the various metabolic groups present in the consortia. Acetate, propionate and ethanol were selected since they were non-fermentative substrates which often acts as electron donors for various forms of anaerobic respiration including sulphate reduction, and therefore pose as potential electron donors for organisms which use 2-CP as the terminal electron acceptor. Yeast extract was chosen since previous studies have shown its effectiveness in enhancing dehalogenation of other chlorophenols (Hendriksen and Ahring, 1993; Madsen and Aamand, 1992).

Another set of duplicate cultures was set up which received only the basic mineral medium (devoid of any of the organic supplements mentioned above) and 0.38 mM 2-CP. After complete removal of 2-CP, all the culture tubes were centrifuged, the old medium was withdrawn and the bacterial pellet washed with phosphate buffer (pH 7) and re-suspended again in mineral medium containing the respective electron source and a fresh spike of 2-CP. After three such successive re-amendments, the overall dehalogenation rates stabilized for all cultures receiving carbon supplements. A typical profile for cultures amended with and without organic electron sources is shown in Figure 5-31.

As seen from Figure 5-31, yeast extract or its fermentative products apparently provided the electron source for dehalogenation as well as other growth factors and cultures supplemented with 0.1% yeast extract usually took 12 to 13 days for complete removal of 0.38 mM 2-CP. No significant difference in degradation of 2-CP were noticed among cultures amended with acetate, propionate or ethanol. In all cases 0.38 mM 2-CP could be degraded in about 15 days. Along with yeast extract, these organic supplements were also effective as electron donors in the dehalogenation of 2-CP. Previous researchers have noticed a similar trend in behaviour with other chlorophenols. Presence of either 0.9 g/L of glucose or 0.1 g/L of yeast extract improved the performance of an UASB reactor treating pentachlorophenol (Hendriksen et al., 1992; Hendriksen and Ahring, 1993). The addition of p-cresol or propionate enhanced the rate of 4-CP degradation in methanogenic batch cultures (Häggblom *et al.*, 1993a). The highest

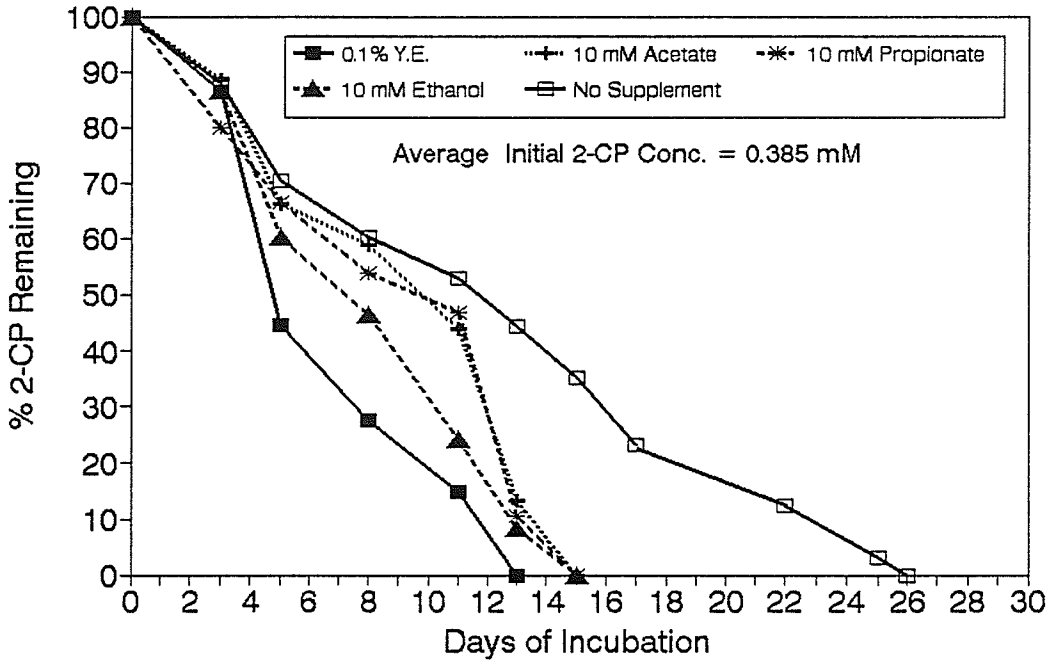


Fig. 5-31. Effect of various organic electron donors on the degradation of 0.385 mM 2-CP by adapted sewage sludge (10% vol/vol) in basic mineral medium containing 10 mM of acetate, propionate, ethanol or 0.1% yeast extract under 20% CO_2 + 80% N_2 atmosphere. The graph also shows 2-CP removal in cultures containing no such supplement. The profiles shown are based on steady state removal data. Each data points are the mean of two replicate cultures.

dechlorination rates were observed for TCP's in cultures that received complex carbon sources such as yeast extract, peptone or cas-amino acids (Madsen and Aamand, 1992).

In this study, the slower rate of 2-CP removal in the cultures which received 2-CP only, suggest that there is a requirement for additional compounds by the consortium. Also, 10 mM electron donor is better than the traces of electron donor produced from 2-CP dehalogenation to act as further source of electron donor for continued 2-CP degradation. Similar observations have been made in other studies with either original or primary enrichments (Genthner *et al.*, 1989a; Bryant *et al.*, 1991). This indicates that the presence of organic electron donors stimulated dehalogenation of 2-CP. In this study yeast extract 0.1% (w/v) appeared to be marginally superior compared to 10 mM of acetate, propionate or ethanol in sustaining an active 2-CP degrading population. Yeast extract being a complex organic substrate also provides essential growth factors besides serving as an electron donor. Alternatively other microorganisms in the consortium could have produced necessary co-factors or vitamins which are of vital importance to the microorganisms responsible for 2-CP dehalogenation.

Studies have shown that many highly enriched cultures using defined electron sources can only be grown in the presence of highly complex carbon source (Dietrich and Winter, 1990; Madsen and Aamand, 1991). Results from this study reveal that there is a general stimulation of a microbial consortium capable

of degrading the aromatic structure in presence of these organic compounds although the reasons for this stimulation are unclear. An important significance of this work is in the development of appropriate bioremediation technologies for enhanced removal of toxic compounds like chlorophenols in either polluted waters and soils by addition of such readily metabolized carbon sources. The methodology adopted in this study allowed for direct measurement of biodegradation potential of the acclimated system.

Observation summary. The nature of head-space composition in batch cultures showed that dechlorination activity was higher under CO_2/N_2 atmosphere compared to CO_2/H_2. Presence of hydrogen slowed 2-CP removal in both adapted and un-adapted cultures. The presence of 0.1% yeast extract (w/v) was more effective as an electron source compared to other organic electron donors such as acetate, propionate and ethanol. 2-CP dehalogenation rate decreased considerably in the absence of any organic electron donors.

5.1.5 Degradation of 2,4,6-TCP; 2,4-DCP and 4-CP in cultures adapted to 2-CP.

To examine the potential of cultures adapted to 2-CP to degrade other chlorophenols, active 2-CP dehalogenating cultures were exposed to about 30 mg/L of 2,4,6-TCP, 2,4-DCP and 4-CP respectively. The cultures were set-up in duplicates (10% vol/vol in 0.1% yeast extract medium) under CO_2/N_2 head-space. The second objective was to see whether the susceptibility to attack was a function of the *Cl* position on the phenolic ring. Figure 5-32 and Figure 5-33

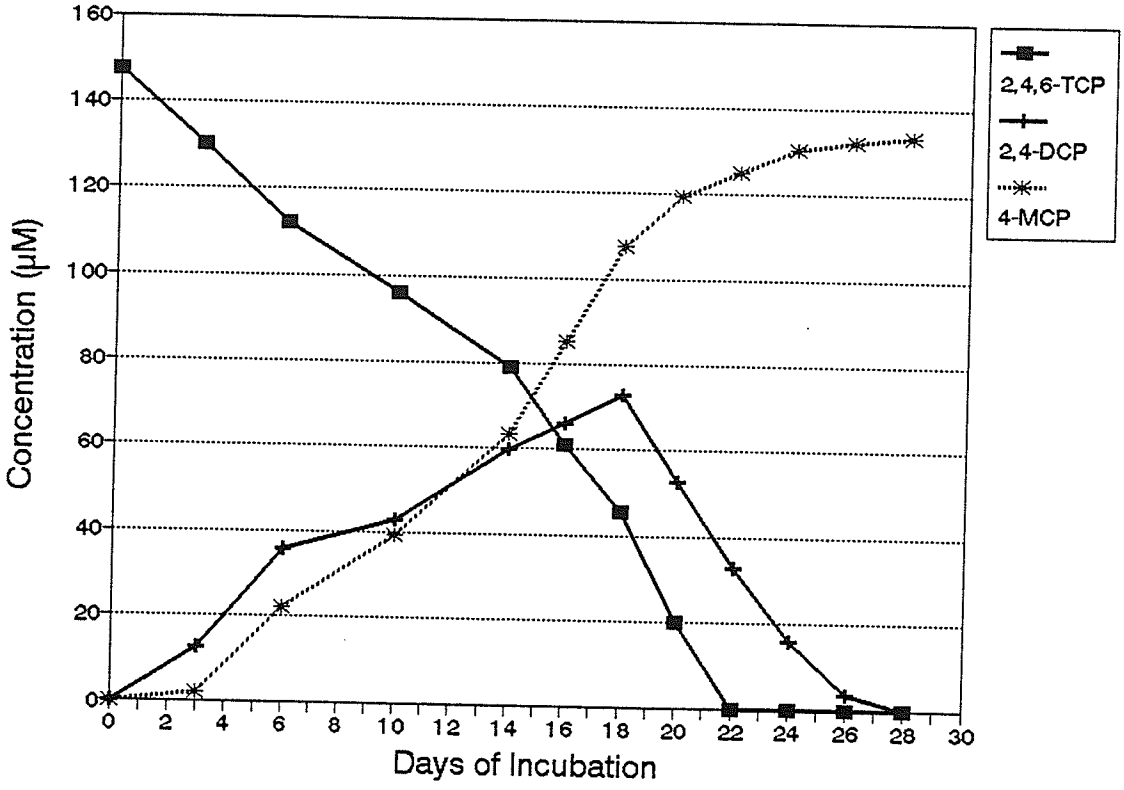


Fig. 5-32. Dehalogenation of 150 µM (30 mg/L) 2,4,6-TCP by batch cultures of anaerobic digester sludge pre-exposed to 2-CP in 0.1% yeast extract medium under 20% CO_2 + 80% N_2 atmosphere. Data points are the mean of two replicate cultures.

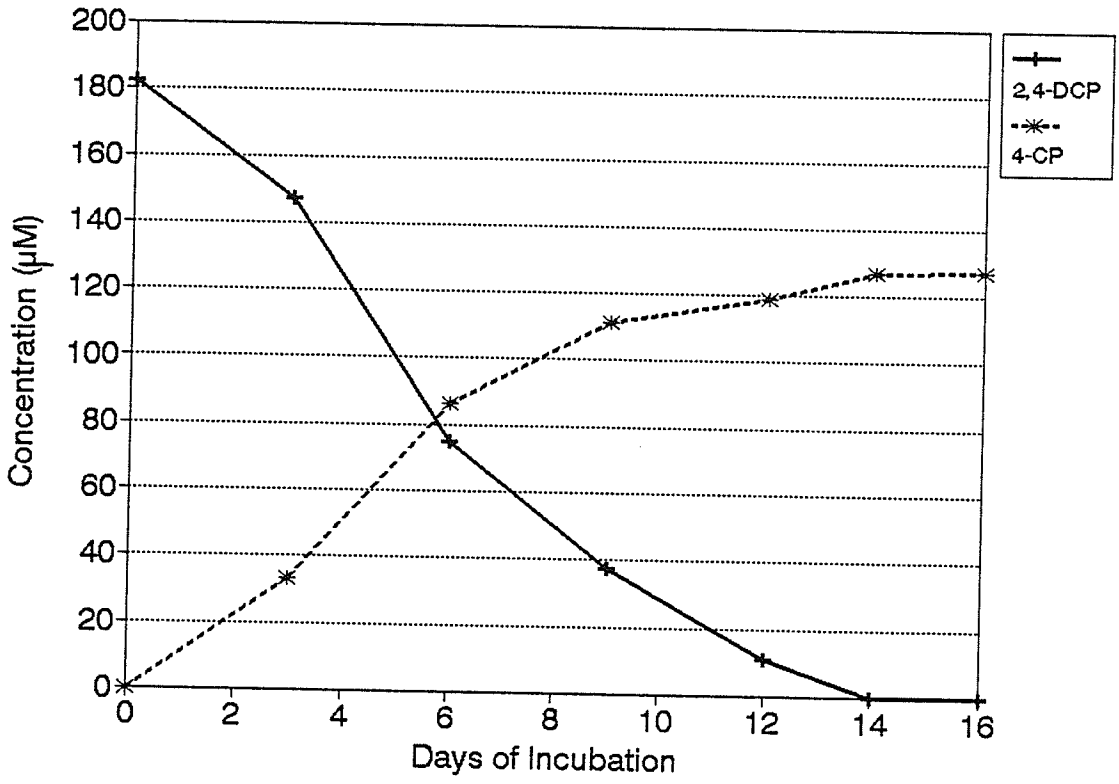


Fig. 5-33. Dehalogenation of 180 μM (30 mg/L) 2,4-DCP by batch cultures of anaerobic digester sludge pre-exposed to 2-CP in 0.1% yeast extract medium under 20% CO_2 + 80% N_2 atmosphere. Data points are the mean of two replicate cultures.

shows a typical dehalogenation profile of about 150 µM (30 mg/L) of 2,4,6-TCP and 180 µM (30 mg/L) of 2,4-DCP respectively.

About 133 µM of 4-CP persisted in the cultures dosed with 2,4,6-TCP while 134 µM of 4-CP accumulated in the 2,4-DCP dosed system indicating some of the 4-CP was further degraded although phenol as its dehalogenation product was never detected. Cultures exposed to about 230 µM (30 mg/L) of 4-CP did not dehalogenate as phenol was never detected and there was a little change in initial 4-CP concentration over an incubation period lasting for about a month. Some of the preliminary work done with these compounds in un-adapted digested sewage sludge (50% vol/vol) had also indicated that 4-CP was significantly recalcitrant to biodegradation by anaerobic organisms present in sewage sludge from NEWPCC, Winnipeg (data is shown in Table A-17 of APPENDIX A for reference purpose). Dietrich and Winter (1990) had earlier reported that their enrichment culture (exposed to 2-CP) could only dechlorinate at *ortho*- position as a result 2,4-DCP feed cultures accumulated 4-CP while 2,6-DCP was completely removed via 2-CP. Accumulation of 4-CP as a dehalogenation product of TCP's and DCP's has been also indicated by Boyd and Shelton (1984), Hrudey et al. (1987b) Armenante et al. (1992), Madsen and Aamand (1992) and Perkins et al. (1994).

Results from this study and previous reports indicated above strongly suggest the capability of both adapted and un-adapted organisms to degrade higher chlorophenols like 2,4-DCP or 2,4,6-TCP although the dechlorination

process appear to be limited to only *ortho* chlorine position. Boyd and Shelton (1984) on the contrary showed that biomass acclimated to 2-CP could transform 4-CP at the same rate although it failed to degrade 3-CP suggesting such a generalization of ortho- dechlorination pattern is difficult to establish. The authors noted that the sludge acclimated to 3-CP degraded 4-CP but not 2-CP while 4-CP adapted sludge degraded both 2 and 3-CP indicating an existence of an non-specific enzyme system. The authors concluded that the specific acclimation pattern perhaps selected two unique microbial population. However, the enrichment of a different class of dehalogenating organisms in their culture when compared to this work is strongly possible which might explain the variability of the results.

The variability in response to different chlorophenols as a function of size and source of inocula is also suggested. Hrudey et al. (1987a) had summarized reports by other researchers which indicated that monochlorophenols like 3- and 4-CP were only mineralized by undiluted sewage sludge and not by diluted sludge (10% vol/vol) compared to 2-CP. However such findings may not be generalized as seen from this study since 4-CP could not be dehalogenated in either un-adapted (50% vol/vol) or 2-CP adapted (10% vol/vol) sewage sludge from NEWPCC within the observed period of 30 days.

Mikesell and Boyd (1986) investigated the feasibility of pentachlorophenol degradation with 2-CP, 3-CP and 4-CP acclimated sludges individually or together.

Results indicate that the 2-CP system removed chlorine primarily at the *ortho* position and at a faster rate than the remaining two systems and eventually accumulated 3,4,5-TCP and 3,5-DCP in the ratio of 2:1. Cole et al., (1994) recently isolated an organism (2CP-1) closely related to the myxobacteria that dehalogenates 2-CP to phenol. Parallel cultures were exposed to 100 µM of 2-CP, 3-CP and 4-CP, 2,3,4-monobromo and monoflurophenols; 2,5-DCP, 2-chlorophenoxyacetic acid and 2,4-dichlorophenoxyacetic acid. Except 2-CP which was removed completely in 5 days, none of the other compounds were degraded in 34 days.

Dehalogenation of several other chlorophenols (3-CP; 4-CP; 2,3-DCP; 2-4 DCP; 2,6-DCP and PCP) by 2CP-1 was also tested by Cole et al. (1994). Only 2,6-DCP was dechlorinated to 2,CP and phenol while 2,5-DCP was dechlorinated by resting cells but did not support growth. Cole et al. (1994) thereby concluded that chlorophenol dechlorination was not a fortuitous or cometabolic reaction but instead is a reaction that has evolved for the use of *ortho*- chlorophenols or close natural analogs. Results from this study indicated a much higher substrate specificity which may be due to the use of mixed cultures rather than pure cultures although dechlorination only occurred at the *ortho*- position. Figure 5-34 compares the removal profile of 2,4,6-TCP and 2,4-DCP. 4-CP was not degraded. In actual practice, two-stage continuous flow bioreactors could be designed to operate on a sequential anaerobic - aerobic mode such that anaerobic effluents containing 4-CP could be further subjected to aerobic degradation. Subsequently, batch

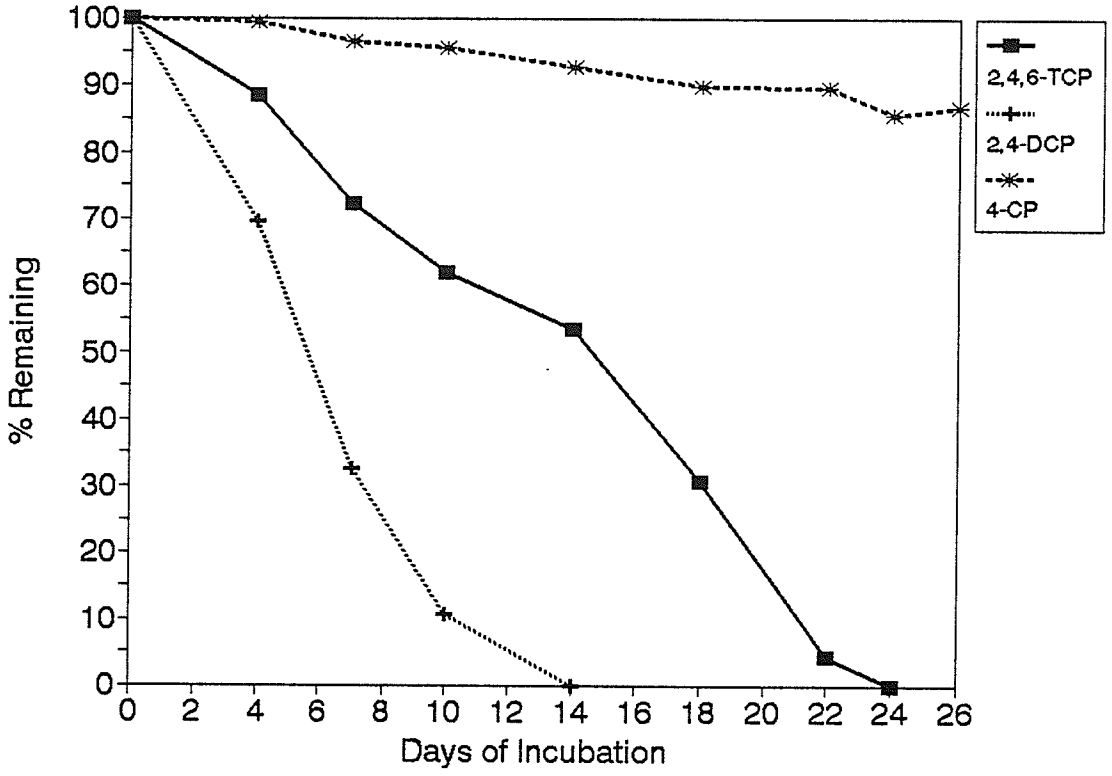


Fig. 5-34. Comparison of 2,4,6-TCP; 2,4-DCP and 4-CP removal by anaerobic digester sludge pre-exposed to 2-CP in 0.1% yeast extract medium under 20% CO_2 + 80% N_2 atmosphere. Data points are the mean of two replicate cultures.

experiments were conducted to demonstrate the feasibility of post aerobic degradation of 4-CP by mixed aerobic cultures adapted to 2-CP. The data and graphs are shown as Fig. A-1, Fig. A-2 and Table A-16 in Appendix A.

Observation Summary. Biomass adapted to 2-CP showed dechlorination of higher chlorophenols at *ortho*- position only. 2,4,6-TCP was sequentially dehalogenated via 2,4-DCP to 4-CP which persisted in the culture. 2,4-DCP fed cultures also accumulated 4-CP whereas 4-CP fed cultures did not under go any further dehalogenation either in 2-CP adapted or fresh anaerobic digested sludge. 4-CP as an anaerobic by-product could be removed by post-aerobic treatment suggesting sequential anaerobic-aerobic phases for complete removals of 2,4,6-TCP and 2,4-DCP.

5.2 AEROBIC DEGRADATION OF 2-CP

A major concern in the engineering and design of a biological facility for the destruction of toxic compounds such as chlorophenols is the impact of secondary substrates on the overall performance of the process. Contaminated soils and groundwater subjected to either in-situ or pump-and-treat methods of treatment contain various sources of carbon compounds in addition to target organics. The presence of such easily degradable carbon sources may either enhance, decrease or have no effect on biodegradation of target contaminants like chlorophenols.

Although microbial degradation of chlorophenols under aerobic conditions is well documented, information on the use of a mixed microbial culture like activated sludge degrading chlorinated phenols with different types and concentrations of supplementary substrates in a continuous system is lacking. A majority of the work done so far especially with chlorophenols have either used a batch system or pure cultures in their experiments and these have been studied in detail along with the identification of key enzymes and metabolic pathways (Steiert and Crawford, 1985; Reineke and Knackmuss, 1988; Häggblom, 1990).

In this section, activated sludge SBR system is presented as a model system for the degradation of 2-CP in presence or absence of either dextrose or phenol as two very different supplemental substrates. An important aspect of this study was to investigate how the nature of such supplemental substrates may affect the specific biodegradation rates of 2-CP and to what extent does dextrose and phenol enhance or suppress 2-CP biodegradation. Dextrose was chosen because it has been successfully used in the past for enhancing the removal of other toxic compounds (Schmidt et al., 1987; Hess et al., 1990; Hess et al., 1993). Phenol was selected based on its similarity to 2-CP carbon skeleton. Sequencing batch reactors (SBR's) were selected due to their ease of operation. Although their non-steady state regime leads to initially high concentration of the target pollutant (the nature of batch process), they have been demonstrated as applicable to degradation of toxic wastes (Hezbrun, 1985; Weber, 1986; Schmidt et al., 1987; Hess et al., 1990; Hess et al., 1993).

5.2.1 Effect of presence, nature and concentration of supplementary substrates on aerobic degradation of 2-CP in SBR's.

The experiments were conducted using variable concentration of either dextrose or phenol as supplemental substrates in order to maximize 2-CP degradation rates in a sequencing batch reactor (SBR). While operation of conventional activated sludge processes by control of SRT is an accepted practice, the use of MLVSS as a control parameter (MLVSS was kept at the same concentration) in this study experiments maintained the SBR's receiving supplements under a similar condition. Use of mixed liquor solids as a control parameter in the treatment of low concentration of toxic compounds like 2-CP has been suggested in the past (Irvine, 1977).

The degradation profile of 2-CP in SBR-1 when 2-CP served as the only carbon source is shown in Figure 5-35. The profile is based on six track studies (2-CP monitored with time) over the acclimated steady state period when consistent 2-CP removal was noticed. In comparison the 2-CP removal in SBR-2 and SBR-3 receiving dextrose and phenol respectively at concentrations of 20 to 200 mg as soluble organic carbon (SOC) per litre are shown in Figures 5-36 (A-E). The results shown are based on at least four sets of experiments conducted during steady-state operation in each reactor for individual concentrations of supplemental substrates. A typical single test run of SOC profile at dextrose concentration of 40 mg SOC/L is shown in Figure 5-37 while Figure 5-38 shows corresponding removal of phenol in SBR-3.

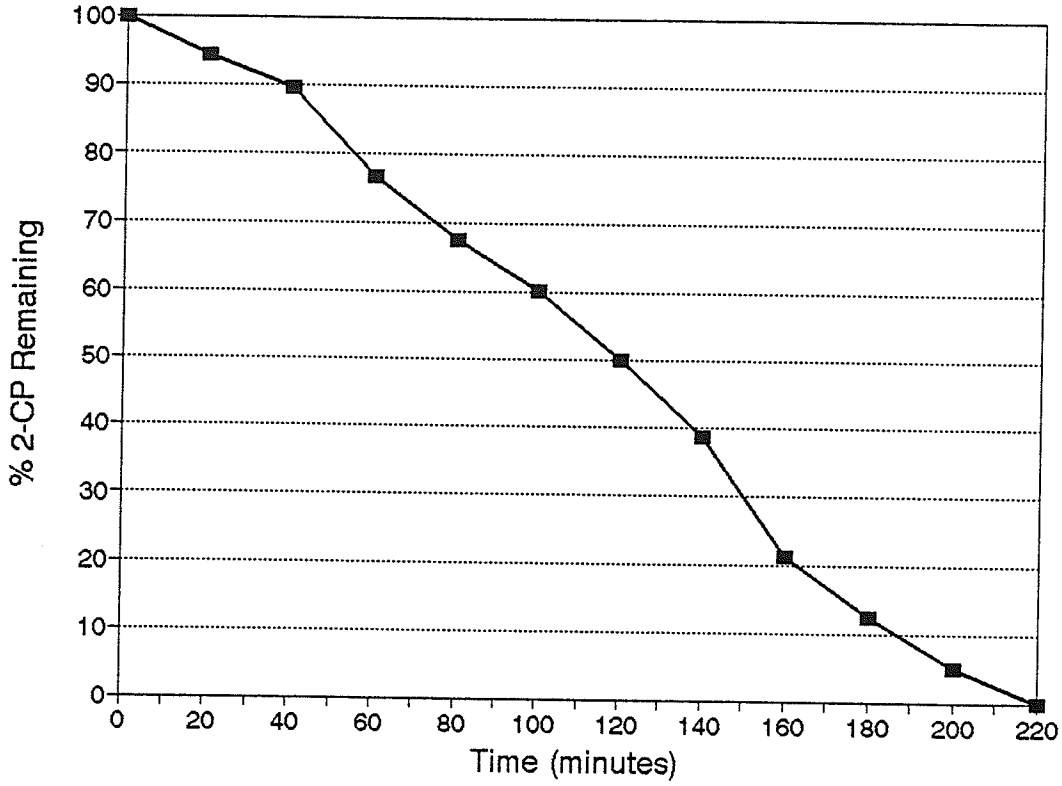


Fig. 5-35. Degradation profile of 30 mg/L of 2-CP in reactor SBR-1 which received no supplemental substrates (dextrose or phenol). Average of six acclimated steady-state runs are shown.

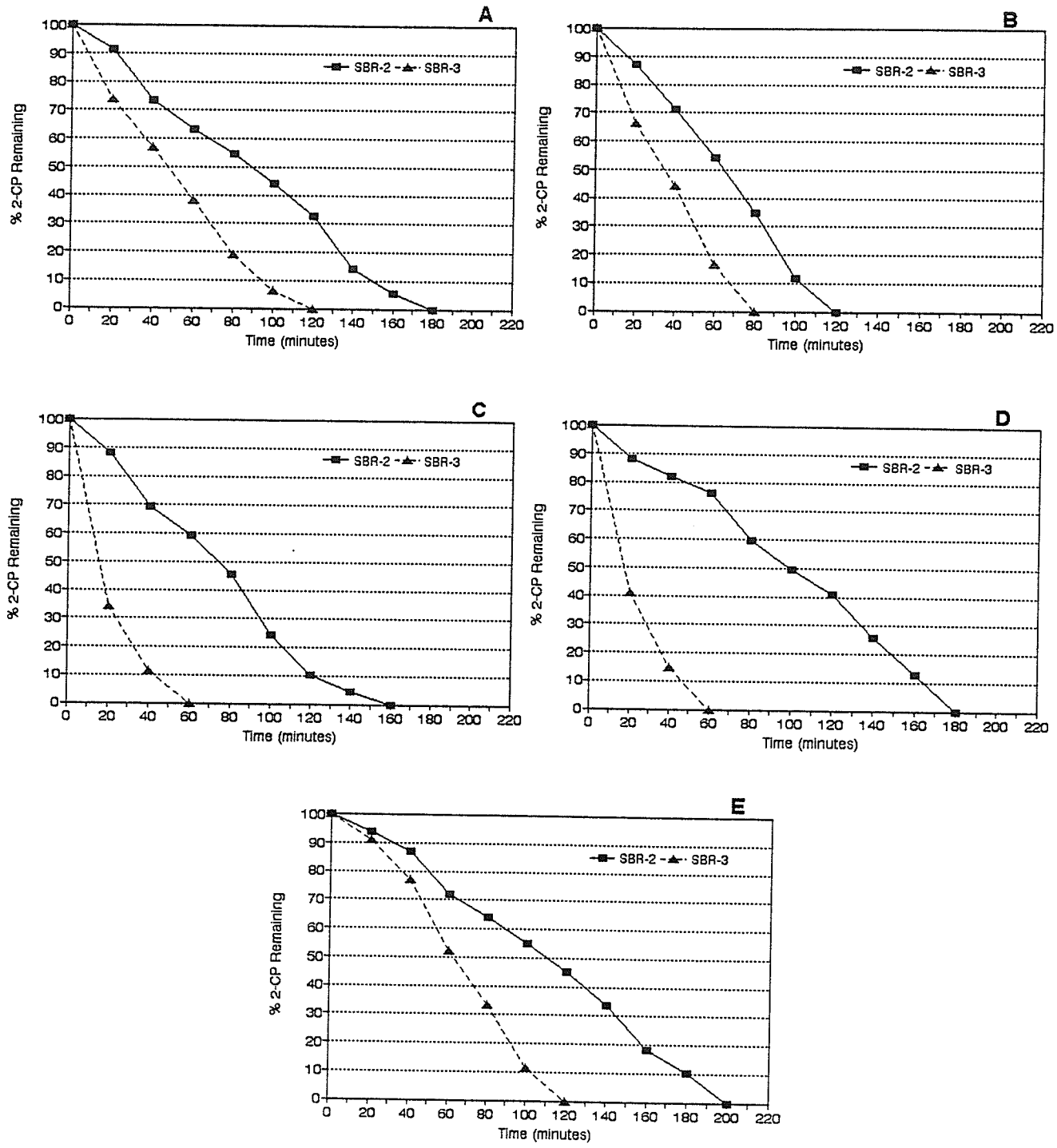


Fig. 5-36. Degradation of 30 mg/L of 2-CP in presence of **(A)** 20, (**B**) 40, (**C**) 60, (**D**) 80 or (**E**) 200 mgSOC/L of either dextrose (in SBR-2) or phenol (in SBR-3). Average of at least four acclimated steady-state runs are shown.

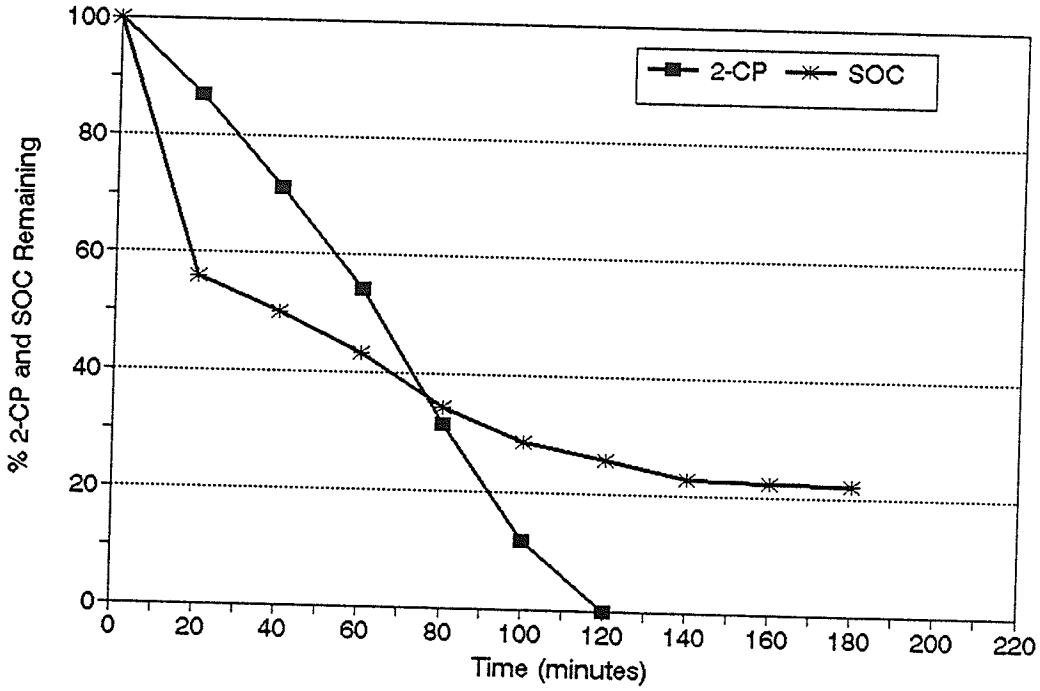


Fig. 5-37. A typical degradation profile of 2-CP and dextrose during acclimated steady-state operation of SBR-2. Concentrations of 2-CP and dextrose at the start of the cycle were around 30 mg/L and 40 mgSOC/L respectively. SOC shown in this graph includes both 2-CP and dextrose. The residual SOC represents soluble microbial products and the background SOC from tap water.

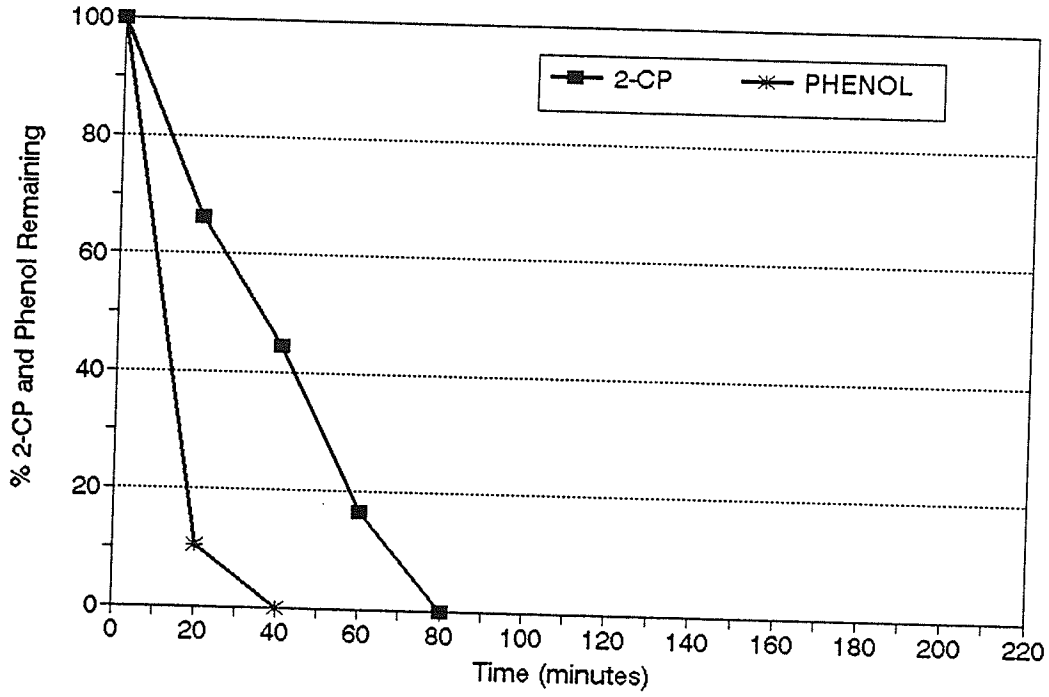


Fig. 5-38. A typical degradation profile of 2-CP and phenol during acclimated steady-state operation of SBR-3. Concentrations of 2-CP and phenol at the start of the cycle were around 30 mg/L and 52 mg/L respectively.

Throughout the experimental period, simultaneous utilization of supplemental substrates (dextrose in SBR-2 and phenol in SBR-3) and 2-CP occurred. After complete removal of 2-CP, samples collected from all reactors were analyzed for SOC to see whether any by-products of 2-CP degradation accumulated in the system (data not shown). Effluent SOC values at this time were recorded to be in the range of 10 - 13 mg SOC/L which corresponded to the background concentrations for tap water. SOC levels were however measured at regular time intervals during the 2-CP degradation studies in SBR-2 to monitor the dextrose removal in the system. To investigate the abiotic loss of 2-CP through stripping or other means, batch tests were conducted with biomass taken from all the three reactors (SBR-1 to 3). The respective biomass were autoclaved separately and aerated for six hours. The total loss was less than 5% of the initial 2-CP concentration.

Comparing Figure 5-35 with Figure 5-36, it is evident that the stimulation of 2-CP removal occurred in presence of supplemental substrates like dextrose (in SBR-2) or phenol (in SBR-3) were present at a concentration of 20 mg SOC/L or greater in all experiments. The specific 2-CP biodegradation rates (based on time required for complete removal of 30 mg/L of 2-CP) increased slightly from 4.0 mg 2-CP/gVSS.h in absence of any supplement (SBR-1) to 5.2 mg 2-CP/gVSS.h in presence of 20 mgSOC/L of dextrose.

The presence of the same concentration of supplement (i.e. 20 mgSOC/L) as phenol doubled the removal rate to 8.2 mg 2-CP/gVSS.h indicating that phenol was more effective as a supplement to the mixed microbial population compared to dextrose. The specific rates further increased to 7.9 and 11.5 mg 2-CP/gVSS.h in presence of 40 mg SOC/L of dextrose (in SBR-2) and phenol (in SBR-3) respectively.

When the supplement concentration was increased to 60 mg SOC/L, it decreased the specific removal rate to 5.9 mg 2-CP/gVSS.h in SBR-2 but further increased the biodegradation rate in SBR-3 to 14.9 mg 2-CP/gVSS.h for phenol supplement. When concentration of dextrose or phenol was raised to 80 mg SOC/L the rate of 2-CP removal in SBR-2 decreased slightly to 5.3 mg 2-CP/gVSS.h while the rate in SBR-3 remained the same at 14.8 mg 2-CP/gVSS.h.

At a supplement concentration of 200 mg SOC/L the 2-CP specific removal rate in SBR-2 reduced further to 4.5 mg 2-CP/gVSS.h while there was a sharp fall in rate in SBR-3 to 7.6 mg 2-CP/gVSS.h. The effect of various concentrations of supplemental substrates on 2-CP specific biodegradation rates are shown in Figure 5-39. Table 5-3 summarizes the values of specific removal rates along with steady-state VSS levels for various concentrations of supplemental substrates tested in SBR-1, 2 and 3.

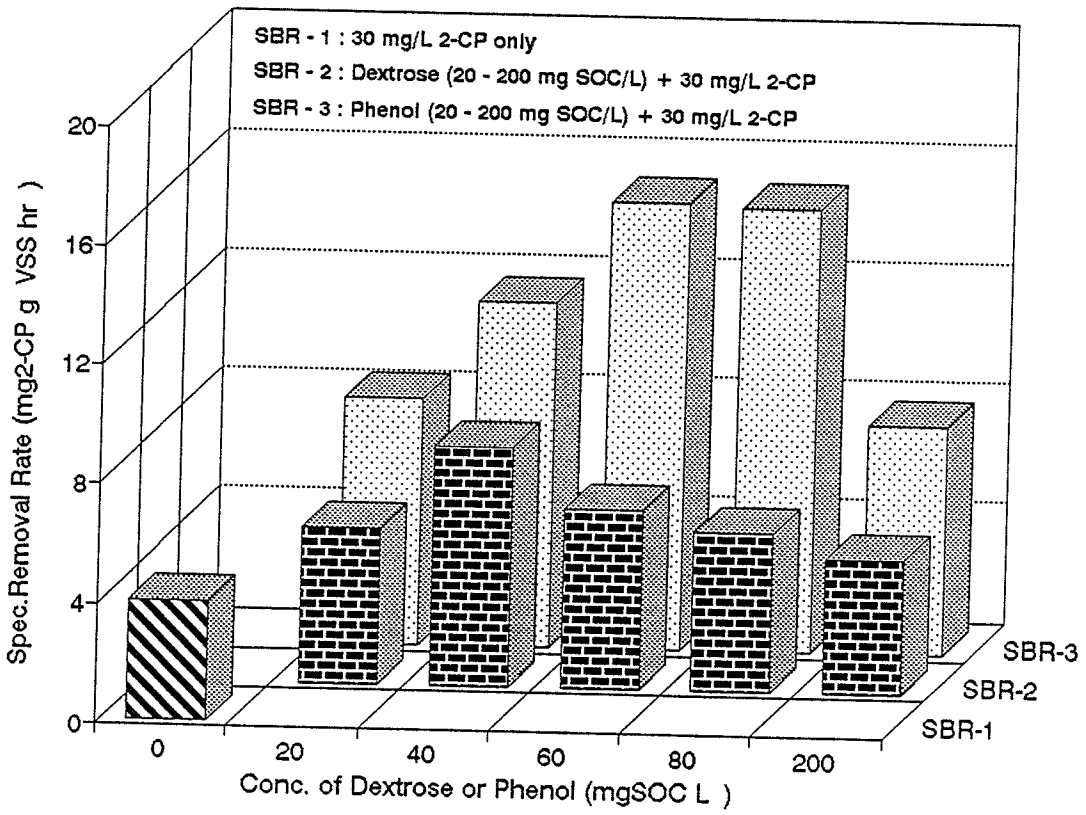


Fig. 5-39. Effect of different dextrose and phenol concentrations on specific 2-CP removal in sequencing batch reactors.

TABLE 5-3 Summary of specific 2-CP removal rates and VSS levels for various concentrations of dextrose or phenol in SBR-1, 2 and 3

Dextrose or Phenol Conc. (mg SOC/L)	Average Specific 2-CP Removal (mg 2-CP/gVSS.h)		Average VSS (mg/L)	
	With Dextrose	With Phenol	Dextrose	Phenol
0	4.0	4.0	2030	2030
20	5.2	8.2	1995	1955
40	7.9	11.5	1970	1990
60	5.9	14.9	1995	2010
80	5.3	14.8	1980	2015
200	4.5	7.6	2015	1990

SBR-1 : 30 mg/L 2-CP only (i.e dextrose or phenol absent)
SBR-2 : 20 - 200 mgSOC/L of dextrose + 30 mg/L 2-CP
SBR-3 : 20 -200 mgSOC/L of phenol + 30 mg/L 2-CP

The results indicated that the enhancement of 2-CP removal by a mixed microbial population was dependent on the presence, nature and concentration of supplemental substrates. Enhancement was noticed for dextrose concentration up to 40 mg SOC/L and phenol up to 60 mg SOC/L. Further increase of supplements especially beyond 80 mg SOC/L was found to reduce specific 2-CP removals. Although the specific rates for 2-CP varied with the concentrations of supplements (dextrose or phenol) tested, the specific rates at any given concentration of a supplement were always higher than when no supplements were present. Phenol as a supplemental substrate was more effective in

enhancing the degradation rate of 2-CP compared to the same concentration of dextrose (as soluble carbon).

To further investigate the mechanism of such enhancement, fresh activated sludge was adapted in separate systems to 40 mgSOC/L of dextrose and phenol as the sole susbstrate. Batch experiments were conducted during which these sludges were exposed to 30 mg/L of 2-CP only to see whether microorganisms grown on dextrose or phenol could degrade 2-CP. The results are shown in Figure 5-40. It was seen that organisms pre-grown on phenol have the capability of 2-CP degradation compared to dextrose grown sludge where 2-CP was not degraded after four hours of aeration.

These results are consistent with the reports in the literature on potential metabolism of chlorophenols by biomass adapted to phenol (Tabak et al., 1964; Shimp and Pfaender, 1987; Saez and Rittmann, 1991). While studying the cometabolic transformation of 4-CP with a culture grown on phenol, Saez and Rittmann (1991) indicated that the enzyme responsible for converting phenol to catechol (phenol hydroxylase - a monooxygenase enzyme) was non-specific and was therefore effective in 4-CP removal. This hypothesis is supported by previous work by Spain and Gibson, 1988 who explained that the low substrate specificity of oxygenases makes it possible to attack substrates with similar chemical structures.

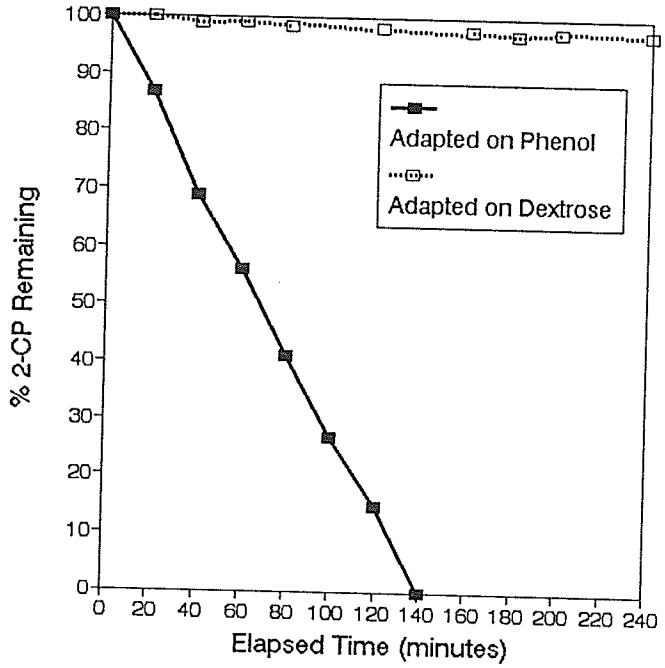


Fig. 5-40. Response of activated sludge grown on 40 mgSOC/L of either dextrose or phenol and subsequently exposed to 30 mg/L of 2-CP. The experiment was conducted on a batch mode and average results of at least four such studies are shown.

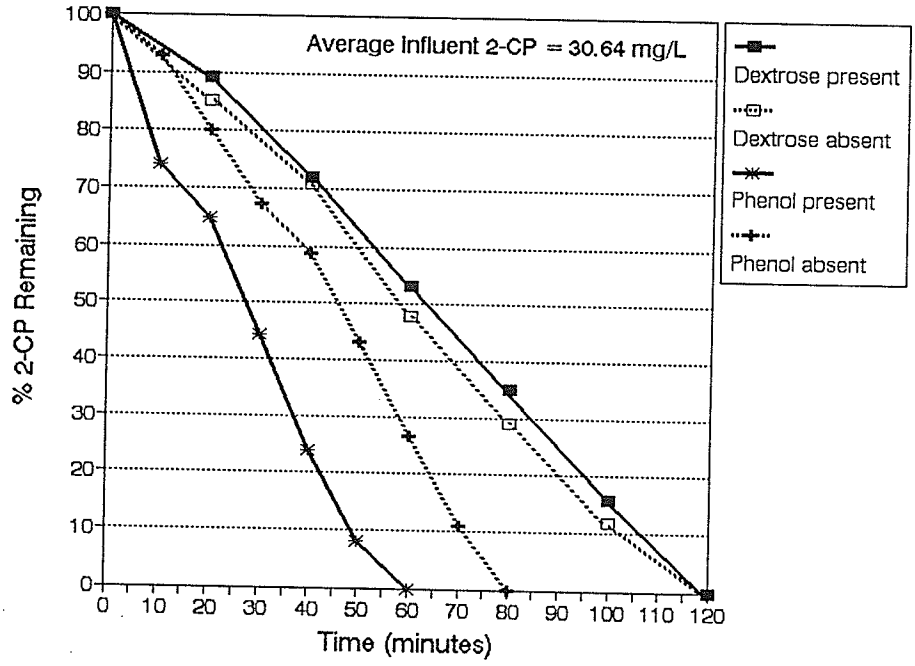


Fig. 5-41. The effect of deleting supplemental substrates: dextrose or phenol in the feed at random cycles on 2-CP degradation. The reactors were normally operated to degrade 30 mg/L 2-CP in presence of either 40 mgSOC/L of dextrose or phenol respectively.

Based on the results of this study and previous reports cited before, it is clear that the mechanisms responsible for 2-CP removal in the present system are quite different when phenol was present as a supplemental substrate compared to dextrose. An experiment was conducted to see if the deletion of dextrose or phenol from one cycle of operation of the SBR's receiving the respective supplemental substrates would cause a reduction in 2-CP removal. Settled sludge from each reactor was transferred to two sets of parallel batch reactors. For the dextrose sludge, one received the usual feed containing 2-CP and dextrose while the other received 2-CP only. A similar arrangement was used for the phenol system which also received a similar feed combination (i.e. 2-CP and phenol in one while 2-CP only in the other). The feed for both systems contained the necessary nitrogen and phosphorous elements as described before.

Figure 5-41 shows the average results of three such experiments. In the presence of or absence of dextrose, the 2-CP removal profiles are almost identical which indicates that the role of dextrose was not directly related to the growth-rate of 2-CP mineralizing organisms. In contrast to the dextrose system, absence of phenol slowed down 2-CP degradation rate. As seen from our previous studies, organisms responsible for phenol degradation participate in 2-CP degradation due to non-specific enzyme system. Due to a rapid transformation of phenol these systems become more active to influence 2-CP removal also. The dextrose amended reactor exhibited larger floc sizes visually compared to the phenol

system or the reactor which received no supplement although no direct measurement of floc sizes were done.

These observations strengthen the hypothesis put forward by Silverstein (1990) and Schmidt et al. (1992) on 2,4-dinitrophenol (DNP) degradation in presence of dextrose. The authors suggested that the role of dextrose was related to its effect on increased flocculation rather than increased growth rate of 2,4-DNP mineralizing bacteria during any one cycle of the SBR operation and that the resulting complex biomass was able to harbour an increased number of active 2-DNP degrading population. Schmidt et al. (1992) also compared the population dynamics on their system amended with or without dextrose and concluded that dextrose stimulated a higher diversified microbial community resulting in a greater microbial interactions which were absent in their un-amended reactor.

The increase in removal rate of 2-CP in this study with the increase in concentration of respective supplemental substrates (dextrose or phenol) may also be due to greater specific growth rate of the biomass at the higher feed strengths. Studies have shown that biomass grown at higher growth rates exhibits higher specific removal rates than the biomass grown at lower growth rates (Powell, 1967; Sokol, 1987). The decrease in specific 2-CP removal observed when dextrose concentration was increased from 40 to 80 mg SOC/L and beyond may be likely due to the decreasing community of 2-CP degraders since each time the

concentration of the supplemental substrate was increased, the proportion of carbon and energy derived from 2-CP was decreased. However detailed study of population dynamics of the system was not in the scope of this study. The fact that equivalent increase in phenol actually increased the 2-CP removal rates to a greater extent suggests overlap in the communities degrading these two compounds due to their structural similarity and non-specific enzymes effecting conversion to respective catechols. This is supported by the results of our batch studies where activated sludge pre-grown on phenol actively degraded 2-CP.

The findings of this study have implications in the design of bioreactors for industrial wastes or on-site bioremediation of contaminated groundwater containing 2-CP. Use of a simple treatment technology like SBR and addition of readily metabolized supplements like dextrose or phenol can be applied for enhancing the removal of target toxic compounds such as 2-CP.

Observation summary. Specific aerobic biodegradation rates for 2-CP in SBR's were influenced by the presence and nature of supplemental substrates like dextrose or phenol. Increasing the supplement concentration increased the 2-CP removal rates only to a certain limit beyond which the rates declined. Phenol was found to be more effective than dextrose at all concentrations tested and its presence was found necessary in acclimated steady-state operation for enhancement. When dextrose was randomly omitted from the feed it did not alter

the 2-CP removal pattern. Biomass grown only on dextrose did not biodegrade 2-CP while biomass adapted to phenol only readily removed 2-CP indicating different mechanisms involved in 2-CP removal when these compounds are present as supplemental substrates. Abiotic removal of 2-CP was negligible.

5.2.2 Effect of batch un-aerated periods on the subsequent aerobic degradation of 2-CP by biomass originally adapted under complete aerobic conditions.

It was seen from the studies of 2-CP degradation in the presence of dextrose and phenol (discussed in the previous section) that complete removal of the compound was possible within the 6 h aeration period with biomass concentration around 2 gVSS/L.

The existing aerobic SBR's were operated in batch cycles with various periods of un-aerated but mixed conditions to see its effect on subsequent aerobic degradation of 2-CP. Because a maximum specific removal for 2-CP was observed under aerobic conditions at 40 mgSOC/L of dextrose as a supplemental substrate, an equivalent concentration (as SOC) of phenol was used in this study. Previous studies in this lab have indicated that aerobic volumetric removal rates for 2-CP increased following an un-aerated period (Oleszkiewicz et al., 1991).

The first objective of this study was to see whether a co-relation between the duration of un-aerated periods and the subsequent aerobic removal rates could

be established and secondly if there was a possible explanation for this phenomenon. The study had to be done primarily with the dextrose system since the phenol sludge was accidently washed out by a timer failure during the time these experiments were conducted. Appendix B, Table B-26 summarizes some of the results obtained with phenol.

Aerobic volumetric removal rates of 2-CP increased with the increase in un-aerated periods from 0 h, 1 h, 2 h and 3 h respectively. 30 mg/L of 2-CP was completely removed in 120 minutes under fully aerobic conditions (i.e. 0 h un-aerated period) thus accounting for a removal rate of 15 mg/L. h. With 1 h un-aerated period 29.9 mg/L 2-CP (average based on four experiments) could be removed in 100 minutes i.e. at a removal rate of 17.9 mg/L.h. With 2 hour un-aerated period 29.4 mg/L 2-CP was removed in the same time although 2-CP removal appeared to be accelerated. Increasing the un-aerated period to 3 hour resulted in a removal of 29.0 mg/L of 2-CP in 80 minutes increasing the volumetric rates to 21.8 mg/L.h. An increase in un-aerated period to 4 hour did not increase the volumetric 2-CP removal rate any further. During all un-aerated periods tested, negligible removal of 2-CP was observed. For the phenol system (studies with 1 h un-aerated period only) neither 2-CP nor phenol was removed, while in presence of dextrose, very little SOC was removed (maximum 4 mg/L) during the un-aerated periods.

In order to compare the removal of 2-CP with the four un-aerated periods tested (1,2 3, and 4 hour) and compare it with the completely aerobic conditions (i.e. 6 hour aerobic with no un-aerated period), linear regression analyses were done to correlate 2-CP removal (%) with time (minutes). The correlation factor r^2 varied from 0.96 to 0.99 indicating a fairly good relationship between % removal and time. The results of the plot are summarized in Figure 5-42. The predicted versus actual removal data for different conditions are also shown in Figure 5-43. The results clearly indicate that 3 hour un-aerated period was optimal for subsequent aerobic degradation of 2-CP.

It is well known that when bacteria which are normally present in activated sludge plants are temporarily deprived of one or more key elements such as nitrogen, sulphur, phosphorus, or oxygen they acquire a unique survival mechanism of storage of carbon in the form of poly-β-hydroxy-butyrate (PHB) and/or glycogen. Many chlorophenol degrading populations like the *Pseudomonas* and *Alcaligenes* species are capable of PHB accumulation under similar stress conditions (summarized by Anderson and Dawes, 1990). In this case, oxygen is important for 2-CP removal since metabolism of such compounds requires the participation of oxygenases, enzymes that incorporates oxygen in the ring (Steirtert and Crawford, 1985). To investigate whether the phenomenon of enhanced 2-CP removal as observed in this system is linked to PHB further experiments were done.

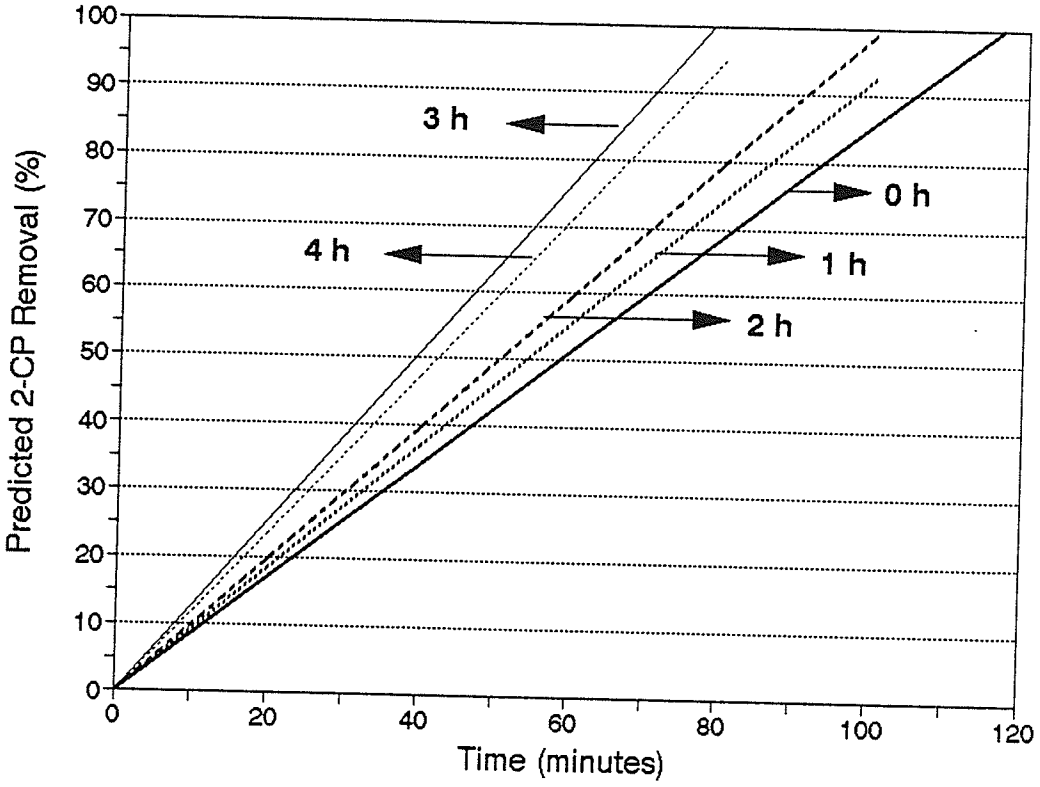


Fig. 5-42. Predicted removal of 2-CP in presence of 40 mgSOC/L of dextrose under aerobic conditions following various un-aerated periods as shown by arrows in batch operations. The results are based upon four studies done at each condition. 2-CP removal during the respective un-aerated periods were negligible.

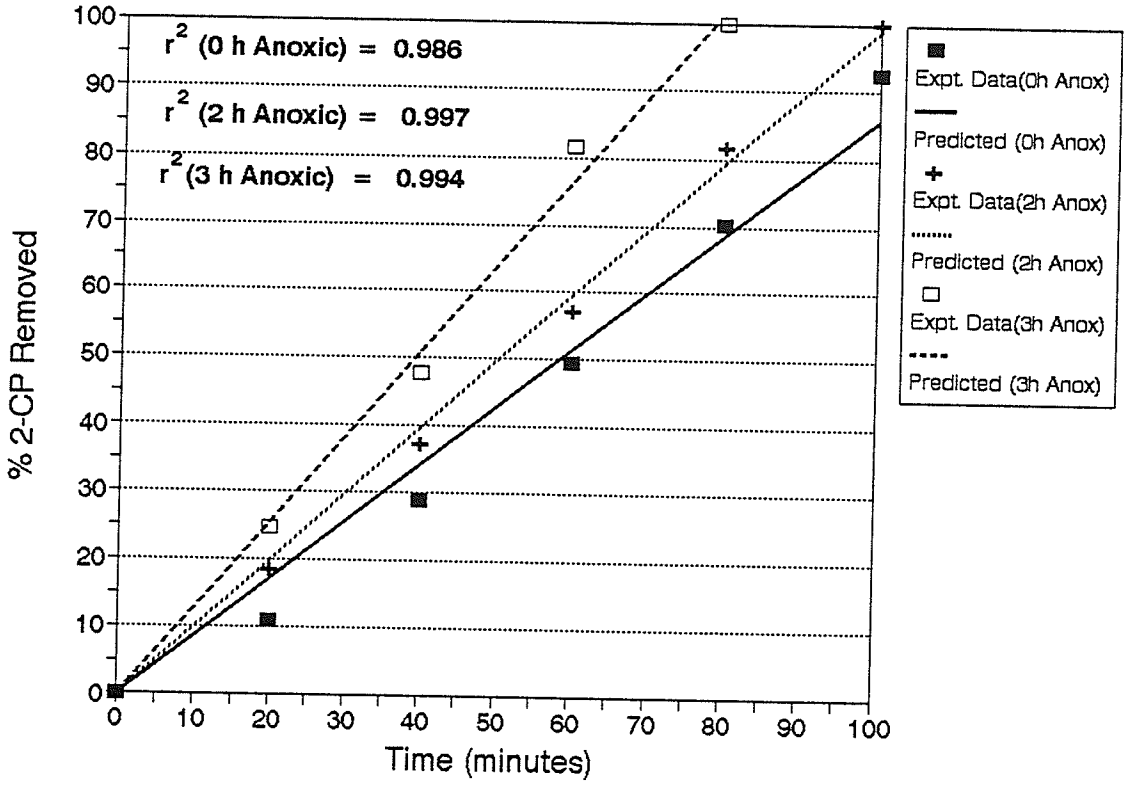


Fig. 5-43. Predicted versus actual removal profile of 2-CP under aerobic conditions in batch operation of SBR following various anoxic periods.

Using the biomass from the SBR, direct batch experiments were conducted under 3 h un-aerated period using the following feed composition in presence of 2-CP: (1) standard feed with dextrose, nitrogen and phosphorous (2) nitrogen source in the feed was replaced with 25 mg/L of NO_3-N but still contained dextrose and phosphorous and finally (3) a nitrogen free feed but with dextrose and phosphorous. The removal profiles were analyzed by regression analysis and the predicted data is shown in Figure 5-44 (based on two sets of experiments). As a comparison, the data showing 2-CP removal under fully aerobic conditions is also shown.

As evident from the Figure 5-44, no difference in 2-CP removal pattern in the three systems described above was observed. In presence of NO_3-N one would have expected not to see an enhancement if such a phenomenon is attributable to PHB accumulation. As observed before, no major change in SOC was also observed during the 3 h un-aerated period that would otherwise indicate an uptake of carbon. Negligible SOC and NO_3-N were removed during this period. Although the exact mechanism influencing 2-CP removal after an un-aerated stress period to the mixed culture is not clear, It is nevertheless of interest to note that such a phenomenon can be applied to improve the efficiency of a treatment system besides significant energy savings by switching of the air supply.

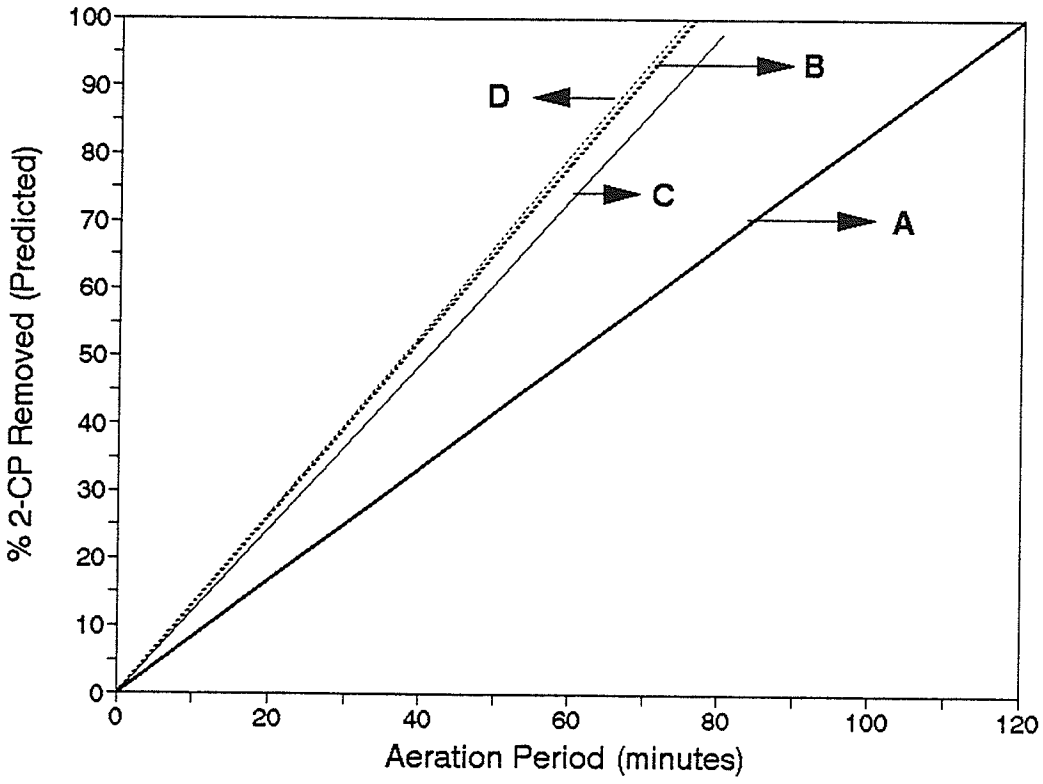


Fig. 5-44. Effect of various feed composition and operating conditions on predicted aerobic removal of 2-CP in batch operations. (**A**) normal feed with no un-aerated period (**B**) normal feed with 3 h un-aerated period (**C**) normal feed supplemented with NO_3^- and operated with 3 h un-aerated period (**D**) feed with no nitrogen source and operated with 3 h unaerated period. The results are average of two sets of experiments.

Observation summary. Biomass adapted in aerobic SBR's to 2-CP in presence of dextrose as supplemental substrate exhibited a higher aerobic volumetric removal rates when preceded by an un-aerated period. The aerobic rates increased with increase of un-aerated period although un-aerated removals of both target compound 2-CP and supplement (dextrose) was negligible. An optimal un-aerated period of 3 h was found to effect maximum volumetric 2-CP removal rate. Batch experiments conducted with various feed composition did not support a role for PHB in influencing the enhancement effect observed in this study.

5.2.3 Effect of different temperatures on aerobic degradation of 2-CP in SBR's.

Temperature in wastewater can vary widely over seasons. In Canada for example, spring thaw wastewater temperatures can fall below 10 °C while temperatures of 15 - 20 °C are usually encountered during summer time. These fluctuations influence the performance of aerobic processes that are vital for degradation of many hazardous organic chemicals like chlorophenols released to the water environment. Therefore, to predict the fate of compound like 2-CP, it is necessary to include the influence of temperature on aerobic biodegradation.

There is a paucity of information on the effect of low temperature on aerobic degradation of chlorophenols in bioreactors. The effects of temperature on biological processes in general were reviewed by Viraraghavan et al. (1993). The

microbial breakdown of lower chlorophenols as a function of temperature was investigated in water, soil, and sediment by Baker et al. (1980) using batch cultures. As expected, biodegradation was higher at 20°C or 4 °C than at 0 °C. On the contrary, Tyler and Finn (1974) found that the growth of *Pseudomonads* on 2,4-dichlorophenol fell sharply above 25 °C, indicating that higher temperatures may also limit microbial decomposition of chlorophenols. The primary objective of this study was to investigate the feasibility of the SBR process to treat a toxic compound like 2-CP at different temperatures (24, 15, 10, 5 °C) in the presence of two different supplemental substrates (dextrose and phenol).

The two systems had been previously operating at 24 °C for over a year. Figure 5-45 and Figure 5-46 shows the removal profile of 2-CP at different temperatures in the presence of 40 mgSOC/L of dextrose and phenol respectively. 2-CP biodegradation rates decreased with decrease in temperature although complete removal of 30 mg/L 2-CP was observed in both systems within the 6 h HRT period up to 10 °C. A further decrease in temperature to 5 °C severely affected the system receiving 2-CP in the presence of dextrose, and biomass washout was observed. 2-CP accumulated over cycles due to incomplete degradation. Any attempt to wash the biomass of residual 2-CP subsequently failed with periodic wash-out of the viable 2-CP degrading population. However, 2-CP could still be removed in the phenol system even at 5 °C as shown in Figure 5-46.

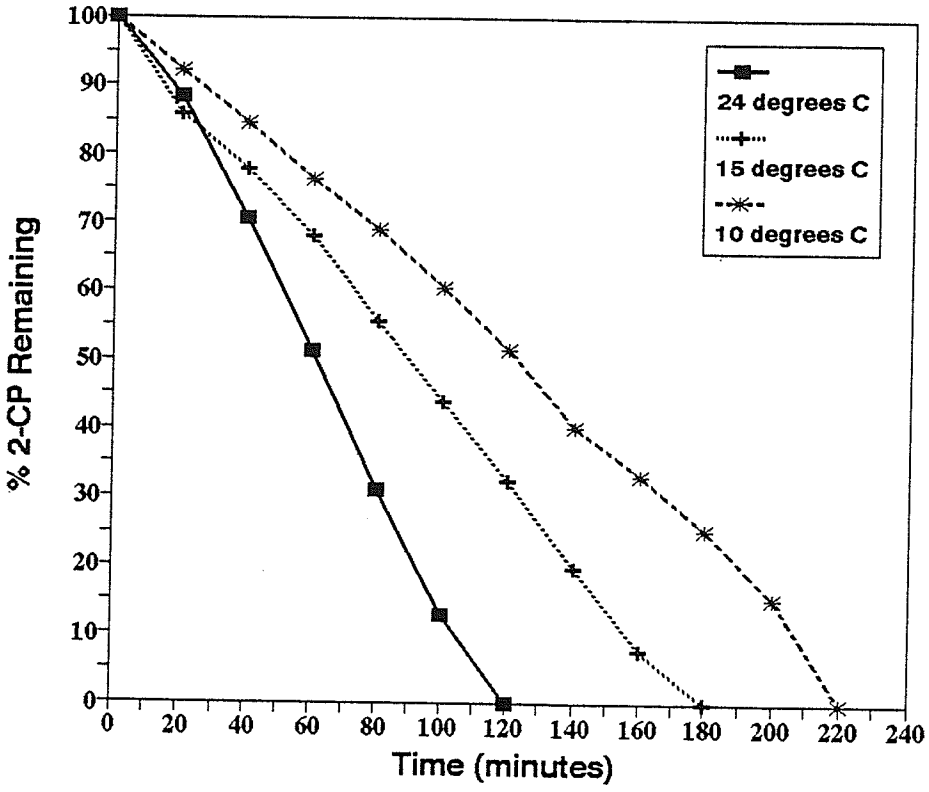


Fig. 5-45. Removal of 30 mg/L 2-CP in presence of 40 mgSOC/L of dextrose in aerobic SBR's operated at different temperatures. The results shown are based on average of four profiles during steady-state operation at each temperature.

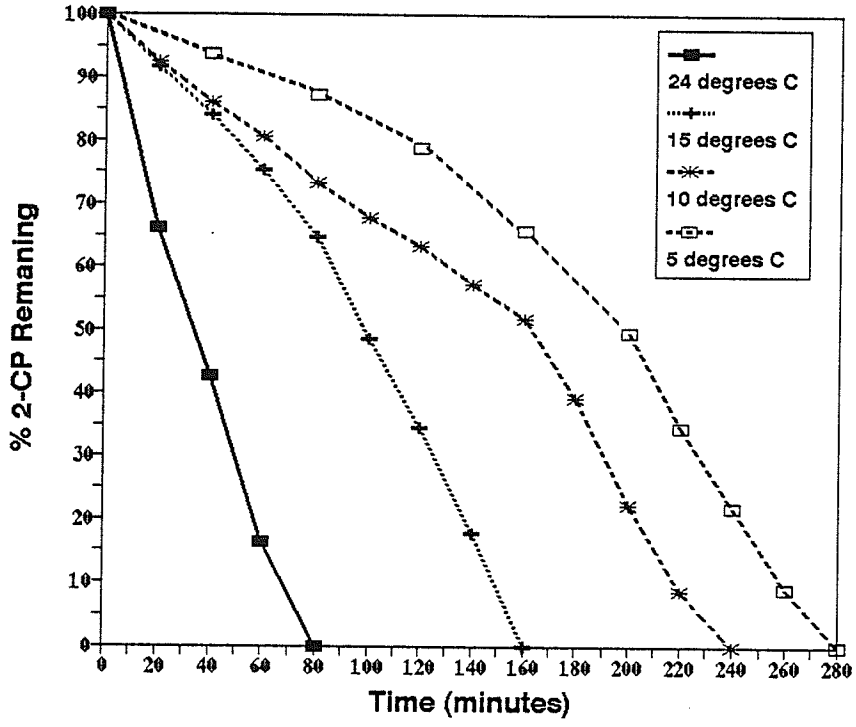


Fig. 5-46. Removal of 30 mg/L 2-CP in presence of 40 mgSOC/L of phenol in aerobic SBR's operated at different temperatures. The results shown are based on average of four profiles during steady-state operation at each temperature.

Figure 5-47 summarizes the specific 2-CP biodegradation rates in presence of dextrose or phenol respectively at different temperatures. Since a profile for 2-CP removal in presence of dextrose at 5 °C could not be obtained, the specific removal rate shown in the figure is an approximate value based on total 2-CP removed in the dextrose system in a day (i.e. 3 cycles of operation). The amount of 2-CP removed was calculated from the total 2-CP fed to the reactor to the 2-CP amount recovered in the effluent at the end of the day. Although some inconsistency was observed in the removal (possibly due to sporadic washout of the viable biomass) and a short period for data collection this result is never the less shown in Figure 5-47 for comparison purposes.

The removal rates of 2-CP decreased with the decrease in temperature in presence of both the supplements. At 24 °C, the specific removal rate of 2-CP in presence of phenol was significantly higher at 11.5 mg 2-CP/gVSS.h compared to 7.8 mg 2-CP/gVSS.h which was also seen before in this study under aerobic conditions. When the temperature was reduced to 15 °C, the specific removal rate for 2-CP decreased to 5.2 mg 2-CP/gVSS.h in presence of dextrose. However, in the presence of phenol the specific 2-CP removal rate decreased significantly to 5.8 mg 2-CP/gVSS.h. Judging by the phenol removal data (refer Table B-31 and Fig. B-5 in Appendix B), this decrease in 2-CP removal rate is probably due to the reduction in rate of phenol removal from the system at 15 °C.

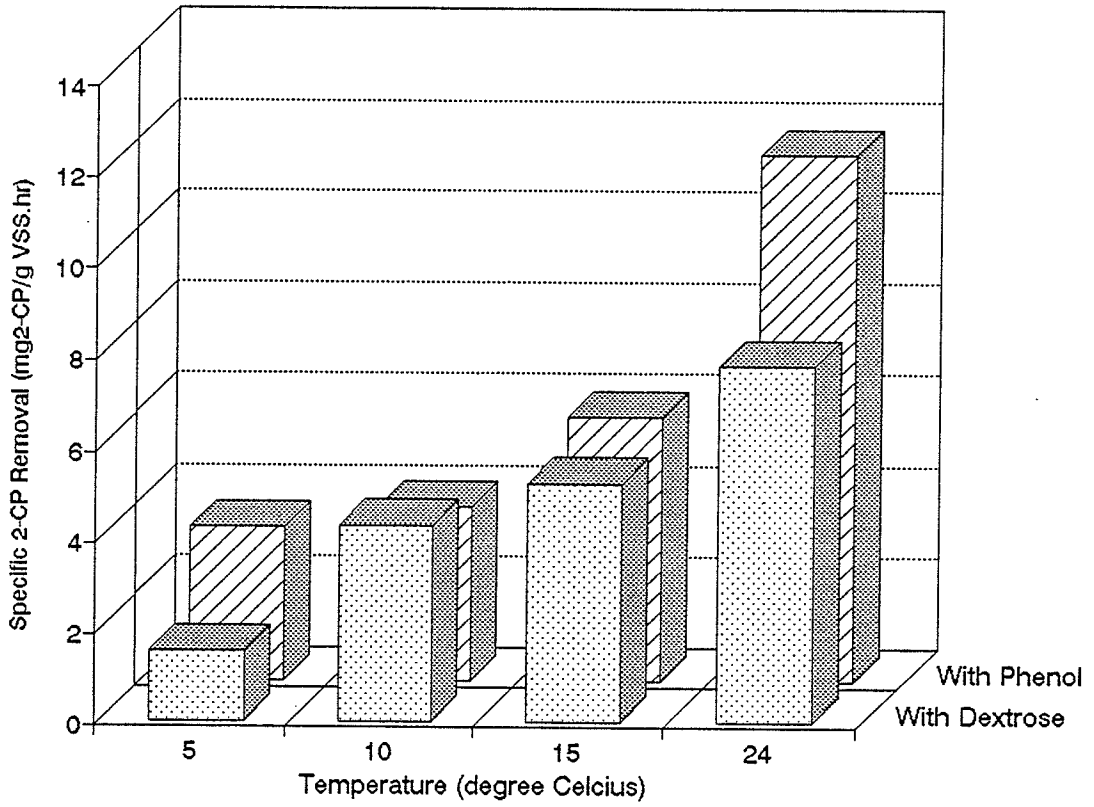


Fig. 5-47. Effect of different temperature on specific 2-CP degradation rates in SBR's operated in presence of 40 mg SOC/L dextrose or phenol respectively. Average results from four experiments conducted over three weeks during steady-state operation of the SBR's are shown. The results from dextrose reactor at 5 °C is an approximate rate based on 2-CP removal over several cycles.

When temperature was further reduced to 10 °C during phase-III, a further decrease in 2-CP removal rates were noticed in both the reactors. The specific removal rate observed in presence of dextrose was 4.3 mg 2-CP/gVSS.h compared to 3.8 mg 2-CP/gVSS.h in presence of phenol indicating that the removal of 2-CP in presence of dextrose at this temperature was marginally superior. At 5 °C 2-CP accumulated in the dextrose system at concentrations as high as 52.4 mg/L due to incomplete 2-CP within the 6 h aeration period. Based on 2-CP fed to the dextrose system and 2-CP recovered from effluent an approximate specific removal of 1.5 mg2-CP/gVSS.h was determined.

2-CP was however completely removed even 5 °C in presence of phenol and the specific rate was found to be 3.4 mg 2-CP/gVSS.h. It is interesting to note from Figure 5-46 that the 2-CP removal profile in presence of phenol at temperatures $\leq$ 15 °C shows similar rates of removal once the 2-CP concentrations had reached around 16 mg/L. The rates were actually higher beyond the points when the supplement phenol was completely removed suggesting a greater participation of the microbial population for 2-CP removal. This is based on our previous experiments where the capability of 2-CP was shown to be also mediated by phenol utilizing organisms. The specific rates of 2-CP in presence of phenol shown in Figure 5-47 are therefore an apparent rate calculated based on time required for total removal of target 2-CP concentrations.

The quality of effluent and biomass characteristics also affected by the temperature in this study. At 24 °C effluent suspended solids in both the system were less than 8 mg/L with the sludge volume index (SVI) in the range of 45 - 55 mL/g and 50 - 65 mL/g in the dextrose and phenol amended reactors respectively indicating very good settleability. At 15 °C, effluent SS due to biomass washout increased to 25 - 30 mg/L in the dextrose reactor and 10 - 15 mg/L in the presence of phenol. During this phase, the SVI values (45 - 60 mL/g) showed little change for the dextrose system while it increased to 80 -100 mL/g in presence of phenol. The effluent quality deteriorated further especially for the dextrose system at 10 °C with SS increasing to 40 - 65 mg/L compared to 10 -24 mg/L in phenol system. The sludge settleability was further affected with SVI increasing to 60 - 80 mL/g (dextrose reactor) and 125 - 140 mL/g (phenol reactor). As discussed before, reducing the temperature further to 5 °C severely affected the dextrose reactor. A severe biomass washout occurred and 2-CP accumulated and the system had to be shut down. However, a very minor changes in effluent quality and SVI were observed for the phenol system.

The trend of increasing SVI with decrease in operating temperature observed in this study contradicts the findings of Benefield and Randall (1980). Based on the SVI data of conventional activated sludge systems operated within the range of 4 - 32 °C, the authors concluded that the settleability of mixed liquor

was not adversely affected by lowering the temperature. Since the work reported by Benefield and Randall (1980) was restricted to conventional activated sludge systems, it is difficult therefore to extrapolate their results to other systems especially those treating hazardous wastes such as this. A greater effect of temperature on sludge settleability was observed in the presence of dextrose as compared to phenol throughout this study.

After complete disappearance of 2-CP, the contents of each reactor was analyzed for SOC to see whether any metabolic bi-products accumulated in the system. The SOC values in both reactors at all temperatures were found in the range of 11.2 to 13.1 mg/L (except for dextrose system at 5 °C which varied from 28.7 to 35.3 mg/L and reflected the 2-CP residue). The background SOC from tap water which was used for preparing the synthetic feed varies from 10 to 12 mg/L indicating complete degradation of 2-CP in this system. The effects of temperature on specific 2-CP removal rate, effluent suspended solids and sludge volume index (SVI) in both systems are summarized in Table 5-4.

Temperature effects upon biological reactions are generally adequately described by the Arrhenius equation. The effects of temperature can be extrapolated from one environment to another through the Arrhenius energy (E_A) of the reaction. E_A can be determined by plotting the logarithm of the

transformation rate (specific removal in this case) against the reciprocal temperature, measured in Kelvin, and then determining the slope of the linear portion of the curve. Results can then be extrapolated over the temperature range defined by the linearity of the Arrhenius plot.

TABLE 5-4 Summary of experimental results at different temperatures

Parameters	Reactor	Temperatures			
		24 °C	15 °C	10 °C	5 °C
Specific Removal Rate (mg 2-CP/gVSS.h)	SBR-1	7.8	5.2	4.3	1.5
	SBR-2	11.5	5.8	3.8	3.4
Effluent SS (mg/L)	SBR-1	< 8	25-30	40-65	70 - 100
	SBR-2	< 8	10-15	10-24	15-25
SVI (mL/g)	SBR-1	45-55	45-60	60-80	70-85
	SBR-2	50-65	80-100	125-140	120-150

SBR-1 : 2-CP degradation in presence of dextrose
SBR-2 : 2-CP degradation in presence of phenol

Figure 5-48 and 5-49 shows the Arrhenius plots for specific 2-CP removals in presence of dextrose and phenol respectively between 5 °C and 24 °C. The figures represents best fit lines based on points that were averaged from steady-state runs at different temperatures. The data was found to be linear only over the 10 °C - 24 °C range for both systems. It has been also reported that many

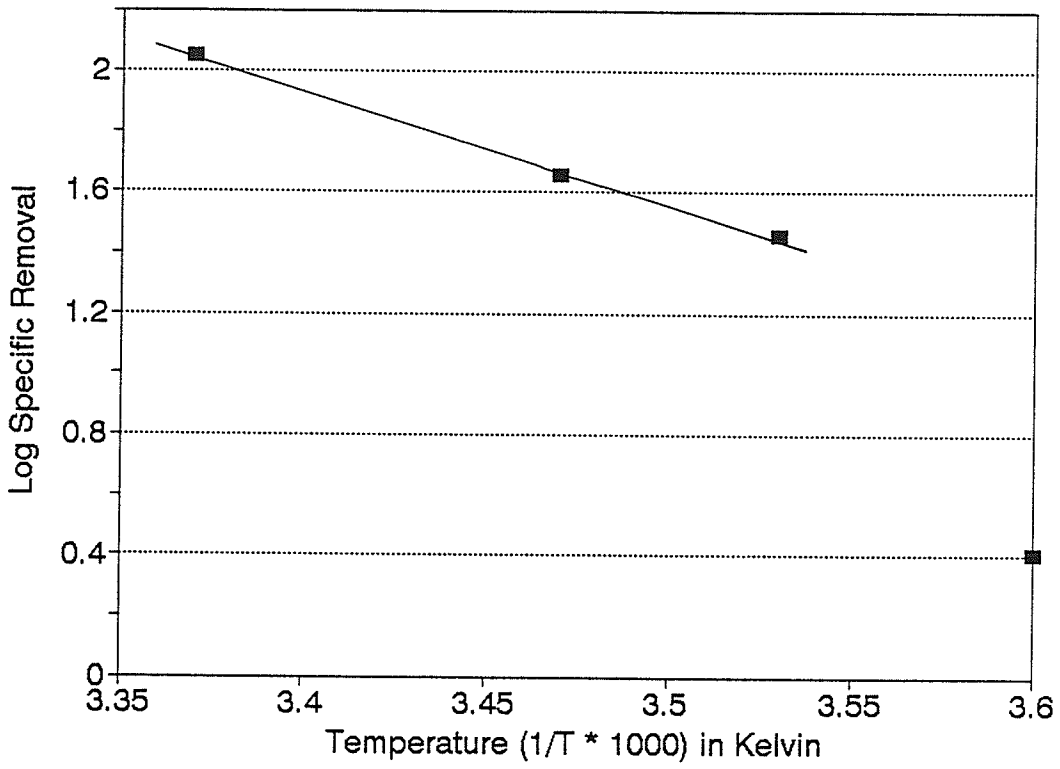


Fig. 5-48. Arrhenius plot of specific 2-CP degradation rate in SBR receiving 40mgSOC/L dextrose as a supplemental substrate at different temperatures.

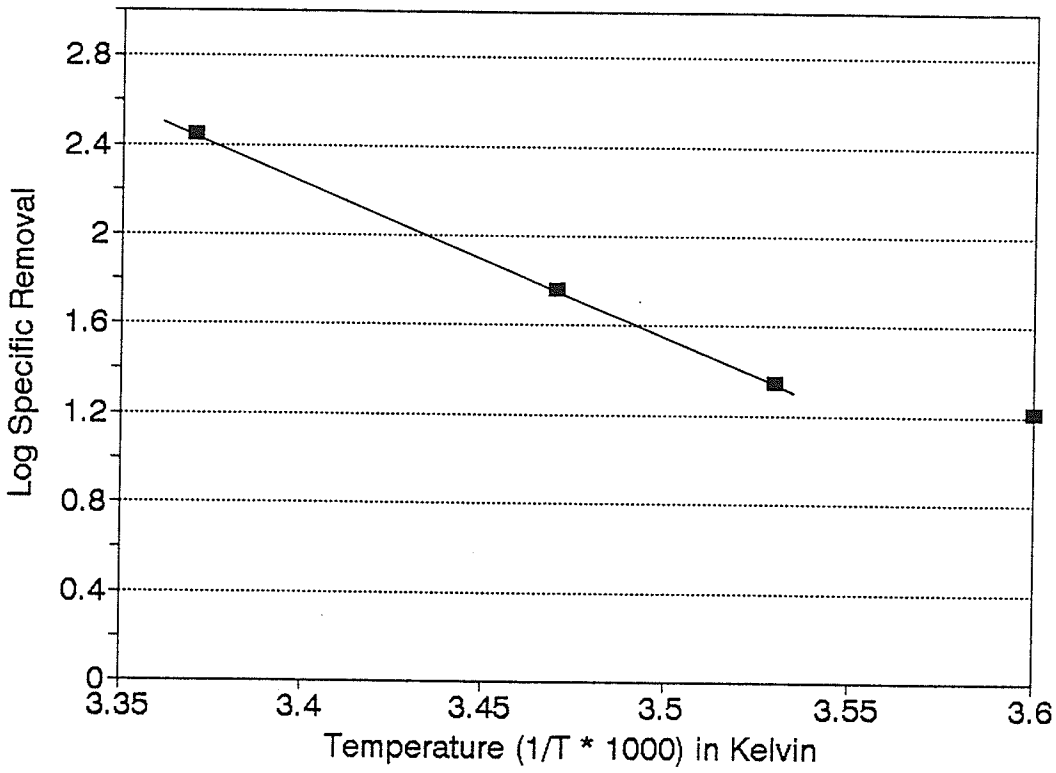


Fig. 5-49. Arrhenius plot of specific 2-CP degradation rate in SBR receiving 40mgSOC/L phenol as a supplemental substrate at different temperatures.

biological reactions have been known to fit the above plot over a small temperature range only (Grady and Lim, 1980; Oleszkiewicz and Berquist, 1988). Temperature can exert an effect upon biological systems in two ways : by influencing the rate of enzymatically catalyzed reactions and by affecting the rate of diffusion of the substrate to the cell (Grady and Lim, 1980).

Temperature can also affect composition and interactions of microbial community in microbiological processes (Kohring et al., 1989a). The effects of population become even more pronounced when more than one organism is involved in the activity (Kohring et al., 1989a). It was noticed in this study that the removal of phenol (which also stimulates 2-CP removal) is severely impaired at lower temperature such as 5 °C. Microbial interactions and enzymatic activities of different responsible organisms vary at different rates with the lowering of temperature which probably explains the variability of correlation between the effect of temperature and specific 2-CP removal rates.

Observation Summary. Low temperature was found to reduce the rates of 2-CP removal in SBR's in presence of both dextrose or phenol. Below 10 °C however, significant washout of biomass occurred in the dextrose system which resulted in incomplete 2-CP removal in the system within the design aeration period although complete 2-CP removal was observed in presence of phenol.

Temperature co-relation factors based on Arrhenius plots indicates non-linearity over the entire 24 °C - 5 °C range tested in this study.

5.2.4 Effect of SRT on aerobic degradation of 2-CP in SBR's.

Studies with different concentrations of supplemental substrates revealed that optimal removals of 2-CP took place at a dextrose concentration of 40 mg SOC/L and a phenol concentration of 60 mg SOC/L. To study the effect of SRT on 2-CP removal in presence of dextrose and phenol, the concentration of the supplemental substrates (dextrose or phenol) were fixed at 40 mg SOC/L. The role of SRT in operation of biological wastewater facility has been summarized by Lawrence and McCarty (1970). A minimum SRT (Θ_{min}), was defined as that below which microorganisms responsible for degradation of given organic compound are totally washed from the cultivation system (Lawrence and McCarty, 1970).

The reactors were started using the biomass acclimated to 2-CP in presence of either dextrose or phenol (at 40 mgSOC/L) which resulted in rapid and stable performance of the systems. Four reactors (two for 2-CP in presence of dextrose and two for 2-CP with phenol) were used simultaneously to operate at SRT of 4, 8, 12, 16 and 20 days respectively for each supplemental substrate (i.e. dextrose or phenol).

The desired SRT was maintained by wasting a specified portion of the SBR contents every day at the end of react cycle. The wasting procedure suggested by Marais and Ekama (1976) was followed throughout the experiment. Biomass was wasted once a day for SRT's $\geq$ 16 d, twice daily for SRT of 8 and 12 d and four times daily for SRT of 4 d. The reactors were allowed up to 3 SRT's before any steady state data were collected.

MLVSS increased with increase in SRT and the effect of SRT on volumetric and specific 2-CP removal in presence of dextrose and phenol are summarized in Figures 5-50 and 5-51 respectively. The value of specific 2-CP removal increased with decreasing SRT which may indicate enrichment of the mixed microbial culture with 2-CP degraders and gradual washout of other slow growers from the system. On the other hand volumetric removal efficiency increased with the increase of SRT - concomitant with the increase in steady state biomass concentration in the SBR's.

Chudoba et al. (1989) had also observed an increase in maximum specific 2,4-DCP removal rates with the decreasing SRT in a conventional activated sludge system. The authors explained that this was due to the gradual washout of predators which resulted in the proportional increase of chlorophenol degrading organisms. A compare of the values of specific 2-CP removal rates achieved in

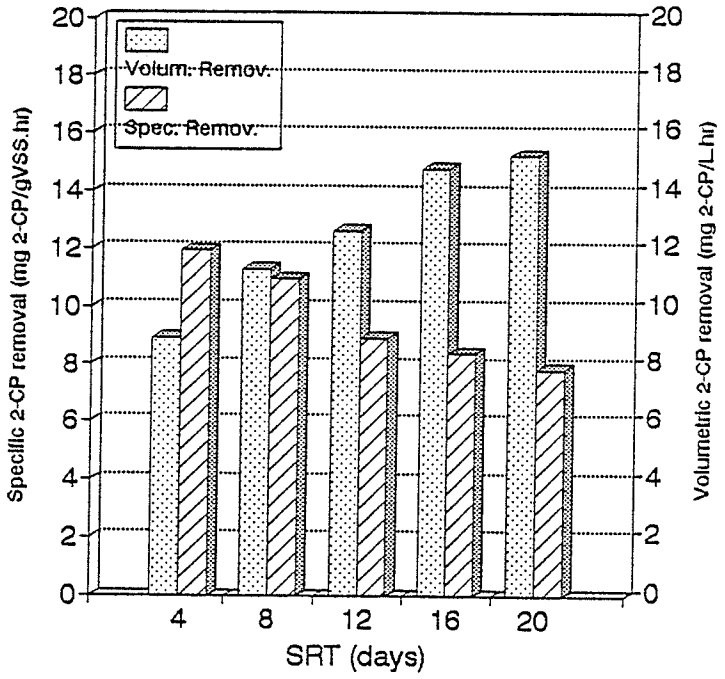


Fig. 5-50. Volumetric and specific removal rates of 2-CP in aerobic SBR's receiving 40mgSOC/L dextrose as supplemental substrate at various SRT's.

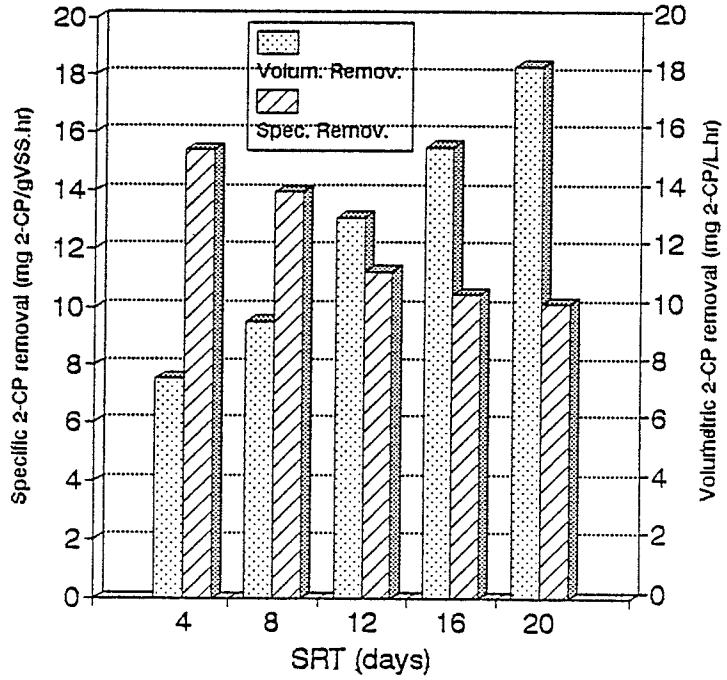


Fig. 5-51. Volumetric and specific removal rates of 2-CP in aerobic SBR's receiving 40mgSOC/L phenol as supplemental substrate at various SRT's.

presence of either dextrose or phenol at five different SRT's is summarized in Figure 5-52. As observed before, 2-CP removals at each SRT were higher in the presence of phenol compared to those in the presence of dextrose. A summary of the removal rates and steady-state biomass concentration at each SRT's are shown in Tables 5-5 and 5-6 respectively.

In order to estimate the value of yield coefficient Y for the systems degrading 2-CP in presence of dextrose or phenol, linear regression analysis were done from the plots of the inverse SRT and specific substrate utilization rate (based on SOC) and are shown in Figure 5-53 respectively. The values of Y for the systems degrading 30 mg/L 2-CP in presence of dextrose or phenol as the supplemental substrates were calculated to be 1.27 and 0.76 mgVSS/mgSOC removed. d respectively.

Observation Summary. Specific removal rates of 2-CP increased with decrease in SRT in presence of both dextrose and phenol due to enrichment of responsible organisms in SBR's. However, these rates were higher in presence of phenol compared to dextrose at each SRT. Volumetric removal rates on the other hand increased with increase in SRT and concomitant increase in steady state biomass concentration. Y values for dextrose and phenol system were determined.

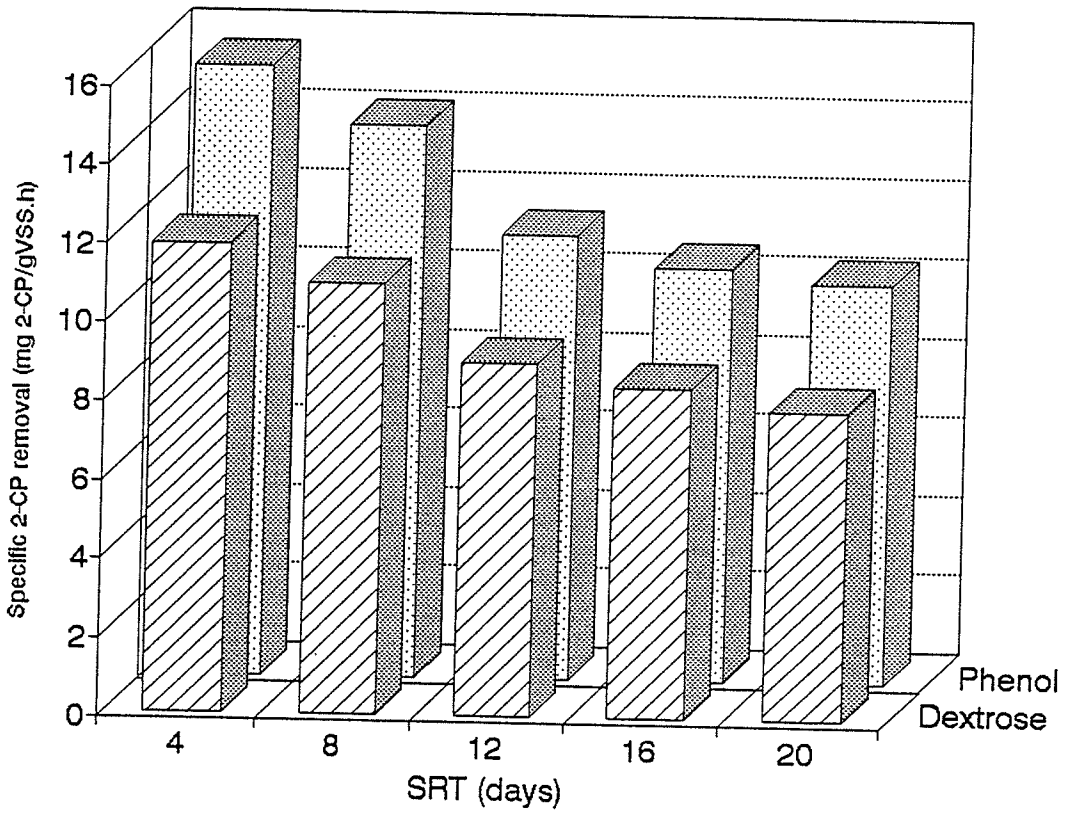
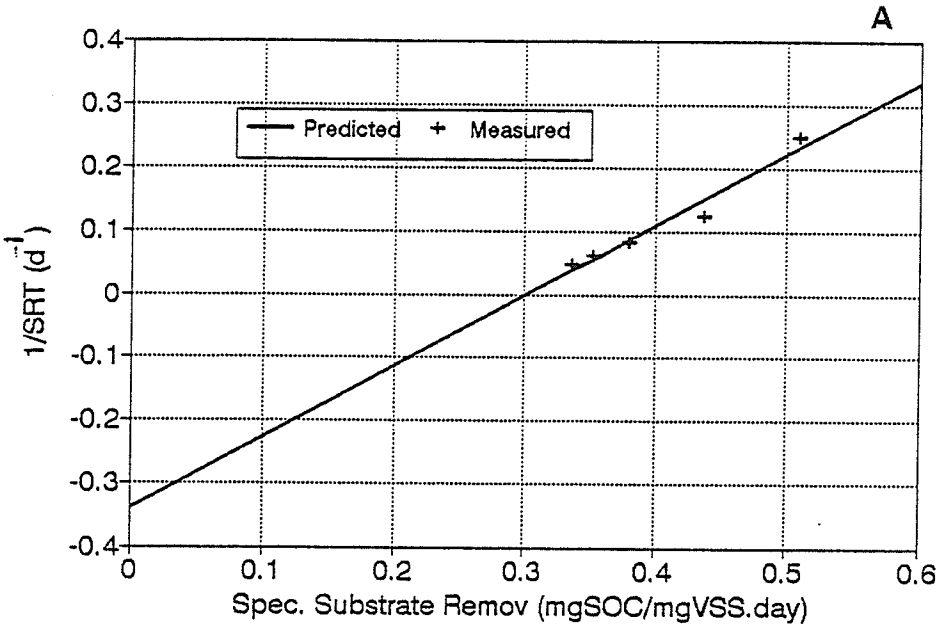


Fig. 5-52. Comparison of average specific 2-CP removal rates at various SRT's in SBR's receving either dextrose or phenol as supplemental substrates at 40 mg SOC/L.



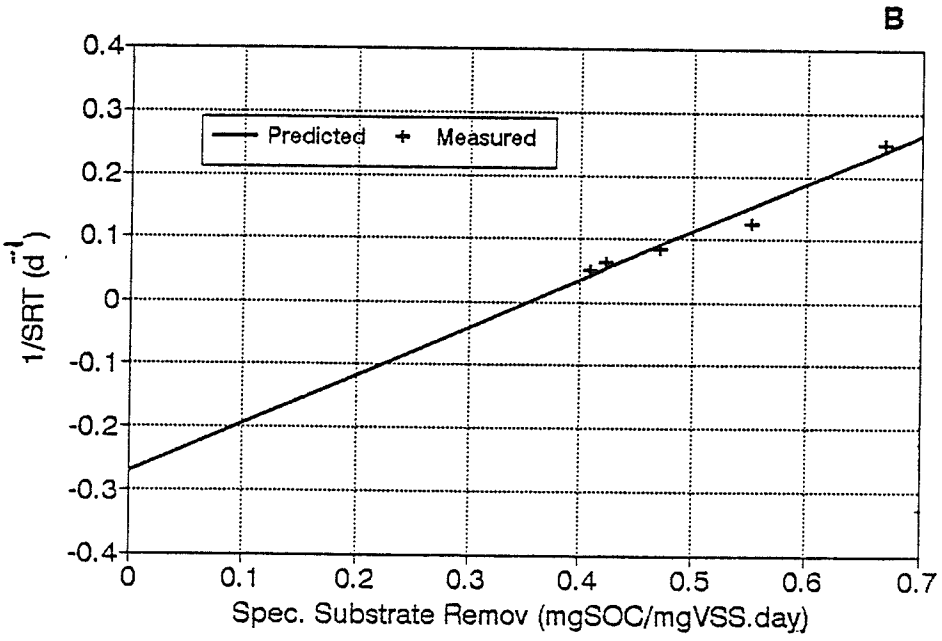


Fig. 5-53. Relationship between bacterial growth and specific substrate utilization rates in SBR's operated at different SRT's in presence of 40 mgSOC/L of (**A**) dextrose and (**B**) phenol.

Table 5-5 Operational data for the dextrose reactor

Parameters	Sludge Retention Time (SRT)				
	4 d	8 d	12 d	16 d	20 d
HRT (hours)	6	6	6	6	6
Avg. Influent 2-CP (mg/L)	29.8	30.1	29.5	29.6	30.5
Avg. Effluent 2-CP (mg/L)	0.0	0.0	0.0	0.0	0.0
Avg. MLVSS (mg/L)	750	1030	1410	1765	1955
Avg. Vol. Removal (mg/L.h)	8.9	11.3	12.6	14.8	15.3
Avg. Spec. Removal (mg/gVSS.h)	11.9	10.9	9.0	8.4	7 . 8

Table 5-6 Operational data for the phenol reactor

Parameters	Sludge Retention Time (SRT)				
	4 d	8 d	12 d	16 d	20 d
HRT (hours)	6	6	6	6	6
Avg. Influent 2-CP (mg/L)	30.2	31.6	30.5	31.0	30.5
Avg. Effluent 2-CP (mg/L)	0.0	0.0	0.0	0.0	0.0
Avg. MLVSS (mg/L)	490	680	1165	1485	1810
Avg. Vol. Removal (mg/L.h)	7.5	9.5	13.1	15.5	5.3
Avg. Spec. Removal (mg/gVSS.h)	15.4	14.0	11.2	10.4	10.1

5.3 FEASIBILITY OF ANOXIC 2-CP REMOVAL IN ANOXIC - AEROBIC SBR's

There is limited information in the literature on anoxic removal of chlorophenols. This section discusses the study undertaken to investigate the degradation potential of 15 mg/L of 2-CP by a mixed bacterial culture adapted in anoxic-aerobic sequential batch reactors. Besides 2-CP, the reactors received either dextrose or phenol at a concentration of 40 mg SOC/L throughout the entire study period. The anoxic periods varied from 2 h in Phase I, 3 h in Phase II and 4 h in Phase III. Both reactors were inoculated with fresh activated sludge from the North End Pollution Control Centre, Winnipeg and the target 2-CP concentration of 15 mg/L was reached by gradually increasing the influent concentration of 2-CP over a 3 week period. Similar to the operation of the fully aerobic SBR's discussed in section 5.2.1, the biomass concentration in both reactors was maintained around 2 gVSS/L by periodically wasting sludge from the bioreactors. This way the MLVSS was maintained fairly constant during steady state operations.

The performance of SBR's to remove 2-CP, SOC and NO_3-N under anoxic conditions throughout the study period is shown in Figures 5-54 (in the presence of dextrose and phenol respectively). During Phase-I which lasted for 65 days, both reactors were operated under 2 hours anoxic + 4 hours aerobic period. After

53 days of operation, anoxic 2-CP removal in SBR-1 (dextrose system) varied from 19 - 21%, NO_3-N removal 16 - 19% and overall SOC from 60 - 63%. For the system with phenol, the corresponding values of anoxic 2-CP, NO_3-N and phenol removals were: 45 - 48%, 26 - 32%, and 41 - 47% respectively. The residual 2-CP concentrations at the end of each anoxic period were completely removed upon transition to aerobic phase in both the systems and the removal occurred at a higher rate. Track studies were conducted at the end of each phase during which 2-CP concentrations were monitored with time during the anoxic and aerobic period for both the systems. A typical profile for the two systems during Phase I is shown in Figure 5-55(A). 2-CP removal during the anoxic period was greater when phenol was present as the supplemental substrate as compared to dextrose.

To investigate the effect of anoxic HRT on 2-CP degradation, the anoxic period was increased on day 66 from 2 hours to 3 hours during Phase II thereby reducing the aerobic portion of the reaction cycle (6 hours in total) to 4 hours. A significant increase in 2-CP degradation was noticed and after 20 days of operation, it generally ranged from 45% - 50% in presence of dextrose and 57 - 60% in presence of phenol. More NO_3-N was also reduced which ranged from 26 - 31% in presence of dextrose while 32 - 42 % in presence of phenol. SOC removals in the dextrose system increased to 67 - 70% while phenol removal increased to 55% -60%.

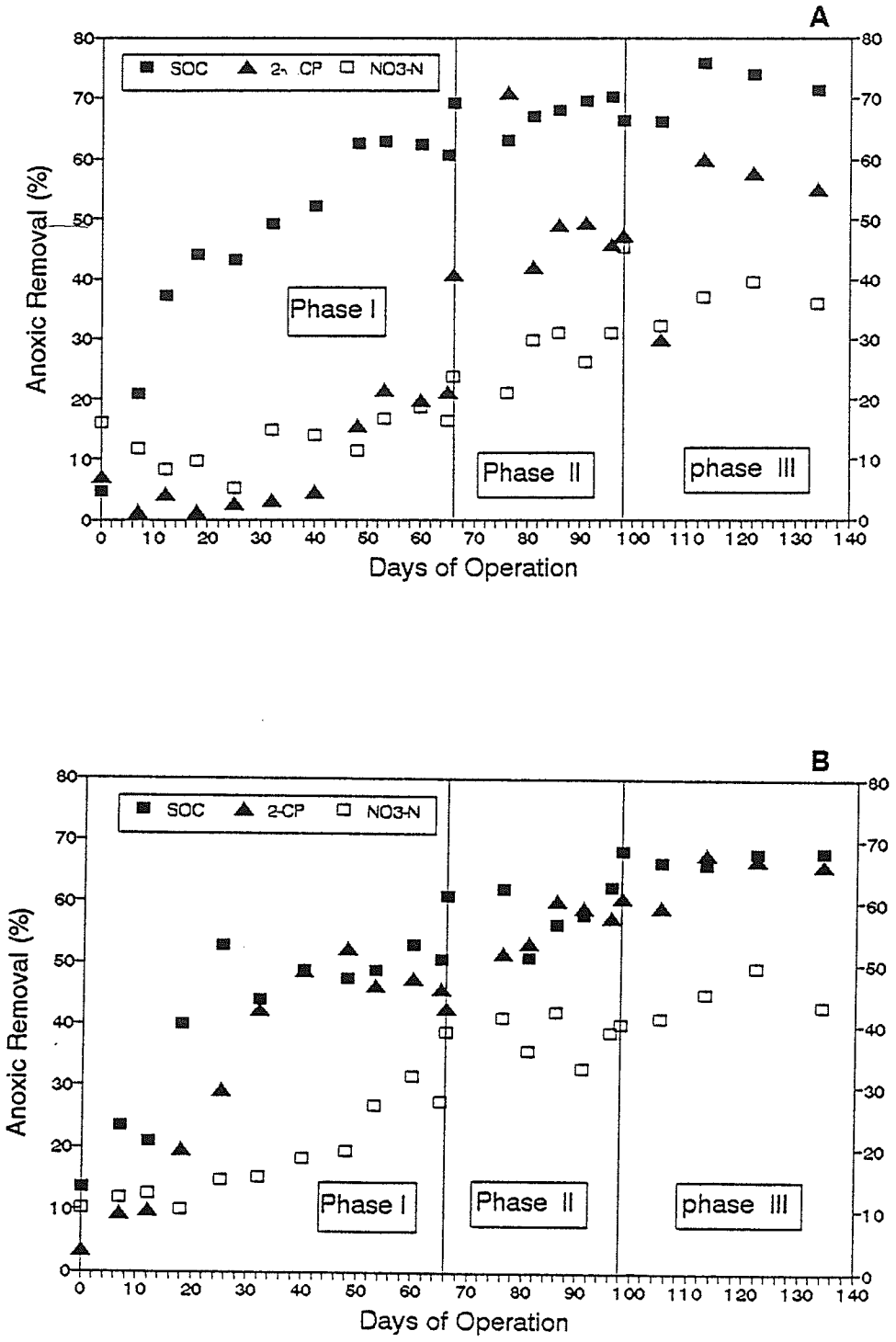


Fig. 5-54. Histograph of anoxic removals of SOC, 2-CP and NO_3-N in sequential Anoxic - Aerobic SBR's receiving 40 mg SOC/L of (**A**) dextrose and (**B**) phenol during the three phases of operation. Phase I, II and III were operated with 2 h, 3 h and 4 h anoxic periods. The remaining time was maintained under aerobic conditions during the 6 h react period in the whole cycle (i.e. it was equal to 4, 3 and 2 h aerobic respectively).

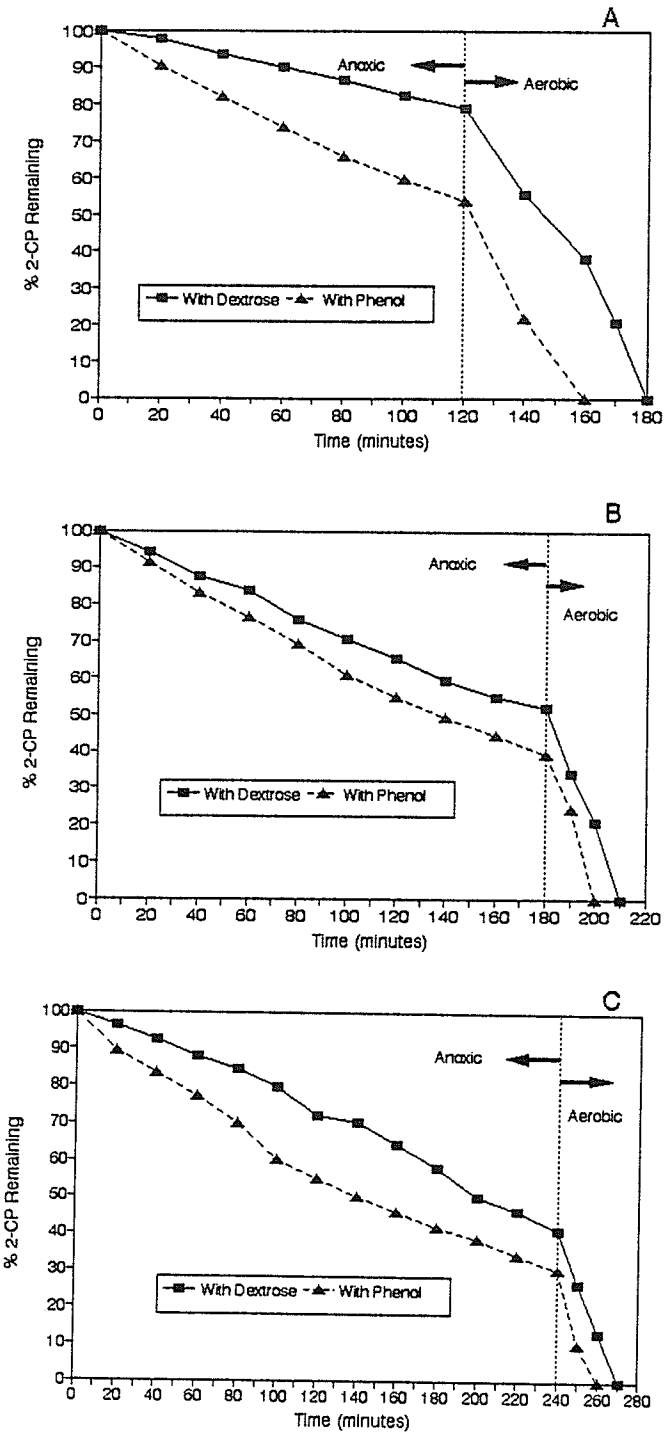


Fig. 5-55. Removal of 2-CP in anoxic-aerobic reactors in presence of 40mgSOC/L of either dextrose or phenol during (**A**) phase I, (**B**) phase II and (**C**) phase III operations. Profiles shown for both the systems are based on average results of three track studies conducted during the end of each phase.

A typical 2-CP removal profile during this phase is shown in Fig. 5-55(B). During Phase-III, the anoxic period was further increased to 4 hours reducing the corresponding aerobic period to 2 h. Anoxic 2-CP removals increased to 54 - 58% and 66 - 68% in presence of dextrose and phenol respectively. This overall increase was however not as high as observed when conditions were changed for the phase-II operations. 2-CP removals in both the systems during phase-III is illustrated in Fig. 5-55(C). The NO_3-N reduction were 36 - 40% and 43 - 50% in presence of dextrose and phenol respectively. A summary of anoxic removals during the three phases are summarized in Table 5-7.

The results of this study indicate that 2-CP removal can be mediated through denitrifying mechanisms in the SBR. Higher rates observed in the presence of phenol suggest that enzymes required for anoxic phenol removal are non-specific due a structural similarity with 2-CP. The effect of an anoxic phase prior to the aerobic period seems to enhance the 2-CP removal rates (during aerobic phase) compared to fully aerobic SBR's. Previous studies Oleszkiewicz et al. (1991) have reported a similar phenomenon where denitrifying SBR's operated sequentially on a anoxic -aerobic mode exhibited higher aerobic volumetric rates for chlorophenol removal compared to completely aerobic reactors.

Table 5-7 Summary of anoxic removals of 2-CP, SOC, Phenol and NO_3- N

Parameters	Reactor	Phase I	Phase II	Phase III
2-CP	SBR-1	19 - 21%	45 - 50%	54 - 58%
	SBR-2	45 - 48%	57 - 60%	66 - 68%
SOC	SBR-1	60 - 63%	67 - 70%	71 - 76%
Phenol	SBR-2	41 - 47%	55 - 60%	63 - 68%
NO_3-N	SBR-1	16 - 19%	26 - 31%	36 - 40%
	SBR-2	26 - 32%	32 - 42%	43 - 50%

SBR-1 : Reactor received 15 mg/L 2-CP, 40 mg SOC/L dextrose and about 40 mg NO_3-N/L

SBR-2 : Reactor received 15 mg/L 2-CP, 40 mg SOC/L phenol and about 40 mg NO_3-N/L

Based on the track studies (2-CP concentration monitored with time during a cycle) done at the end of each phase, the specific removal rate for 2-CP during the anoxic period was shown in Fig. 5-56. The values of average (based on three runs) specific removal rates and biomass concentrations are also summarized in Table 5-8. The results indicate that increasing the anoxic period of the react phase of the cycle did not necessarily increase the specific or volumetric rates of 2-CP removals. For the dextrose system, the rates actually increased during phase II (3 h anoxic + 3 h aerobic) and then decreased again once the anoxic period during the start of phase II was increased to 4 hours.

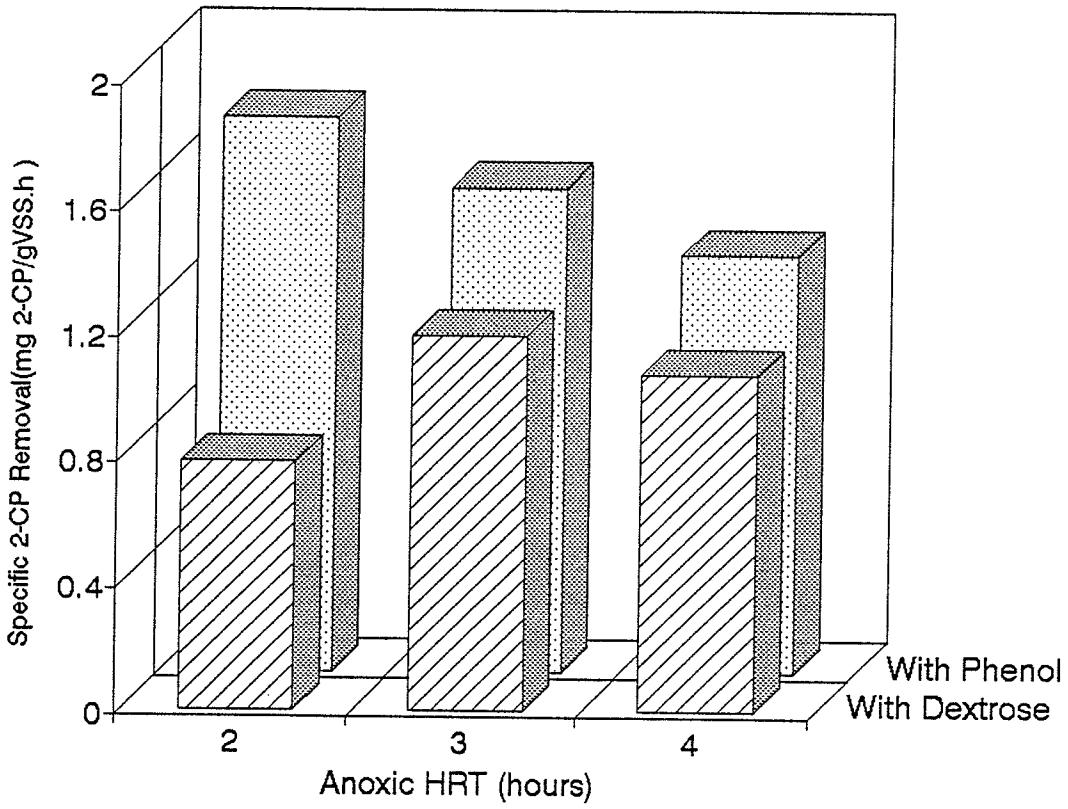


Fig. 5-56. Specific 2-CP removal rates of SBR's operated in presence of 40 mgSOC/L of either dextrose or phenol under different anoxic retention times during the three phases.

For the phenol system on the other hand, the rates declined as the anoxic period HRT was increased from 2 h to 3 h and finally to 4 h. The results also indicate that phenol could once again serve as a superior supplement compared to dextrose possibly due to structural similarity and a non-specific enzyme system that participates in 2-CP removal during both anoxic and aerobic phases of a cycle.

Table 5-8 Summary of anoxic removal rates and biomass concentrations in reactors receiving either dextrose or phenol as supplements.

Parameters	Reactor	Phase I	Phase II	Phase III
Anoxic HRT (hours)	SBR-1 and SBR-2	2	3	4
Volume Removal Rate (mg 2-CP/L.h)	SBR-1	1.6	2.4	2.1
	SBR-2	3.4	3.0	2.6
Specific Removal Rate (mg 2-CP/gVSS.h)	SBR-1	0.8	1.2	1.1
	SBR-2	1.8	1.5	1.3
Avg. Biomass Conc. (mg VSS/L)	SBR-1	1980	1990	1980
	SBR-2	1950	1960	1970

SBR-1 : Reactor receiving 2-CP and 40 mg SOC/L of dextrose
SBR-2 : Reactor receiving 2-CP and 40 mg SOC/L of phenol

Observation Summary. Biodegradation of 2-CP under denitrifying conditions was demonstrated in sequencing batch reactors. Anoxic removal of 2-CP was higher in the presence of phenol as compared to dextrose which agrees with the results obtained with fully aerobic SBR's in this study. 2-CP residuals from anoxic period was quickly removed during the aerobic period that followed. The increase of anoxic HRT increased the amounts of 2-CP removed in presence of both dextrose and phenol but did necessarily increase the specific 2-CP biodegradation rates.

Chapter 6.

RESEARCH OVERVIEW

6.1 Conclusions

The following conclusions are based on the results obtained from this study on 2-CP biodegradation under different redox conditions.

1. Sewage sludge from the anaerobic digester tested exhibited a better potential for reductive dehalogenation of 0.39 mM 2-CP along with shorter lag times compared to either biomass from a laboratory anaerobic bioreactor treating bleached kraft mill (BKM) waste or BKM lagoon sediment. Phenol was identified as the principal product which degraded via the benzoate pathway. Methane was identified in the head-space of all cultures.

2. Reductive dehalogenation of 2-CP occurred at the same rate in the presence or absence of 5 mM sulphate (SO_4^{2-}) along with sulphate reduction and methanogenesis. Presence of $Fe(II)Cl_2$ slowed the dehalogenation but a greater amount of sulphate was reduced in cultures amended with Fe(II).

3. The presence of BESA slowed 2-CP dehalogenation and partly inhibited methanogenesis even at 10 mM concentration. Phenol as a product of dehalogenation accumulated along with acetate as the major VFA.

4. Molybdate inhibited 2-CP dehalogenation and sulphate reduction at concentrations $\geq$ 10 mM while propionate accumulated in the cultures. With 1 mM Molybdate, sulphate reduction practically stopped after 15 days although 2-CP removal continued indicating that sulphate reducing bacteria were not involved in dehalogenation of 2-CP. Results also indicate a major involvement of syntrophic bacteria in influencing 2-CP dehalogenation by mixed bacterial cultures in this study.

5. Anaerobic digested sewage sludge adapted to 2-CP could dehalogenate 0.39 mM 2-CP without any visible lag period. Degradation of 2-CP with various dilutions of adapted sludge indicated that the dehalogenation rate (μM/d) did not increase linearly with biomass concentration.

6. Hydrogen as a potential electron donor slowed 2-CP dehalogenation rate by adapted batch cultures. A study of the effects of several organic electron donors on dechlorination activity showed that 0.1% yeast extract was more effective in providing the electron source for dehalogenation of 2-CP by the methanogenic consortium. Presence of other electron donors like acetate, propionate and ethanol at 10 mM also stimulated 2-CP dehalogenation. The dehalogenation rate was the slowest when neither of such organic electron donors were present in the medium.

7. Sludge adapted to 2-CP dechlorinated 2,4,6-TCP and 2,4-DCP to 4-CP as end product. 4-CP was not removed indicating that organism/organisms were capable of dechlorinating compounds at the *ortho-* position. 4-CP was however removed readily by aerobic metabolism indicating the requirement for sequential anaerobic - aerobic environments for complete mineralization of such compounds.

8. Aerobic degradation of 2-CP by adapted mixed microbial culture derived from activated sludge grown in SBR's was influenced by the presence, nature and concentration of supplemental substrates such as dextrose or phenol. Phenol was found to be more effective in the enhancement of specific 2-CP degradation rate when compared to the same concentration of dextrose. An optimal substrate concentration of 40 mgSOC/L for dextrose and 60 mgSOC/L for phenol resulted in maximum specific rate of 2-CP removal of 7.9 and 14.9 mg 2-CP/gVSS.h respectively. Un-adapted activated sludge degraded 2-CP when grown on phenol but not on dextrose.

9. Biomass adapted aerobically to 2-CP in presence of dextrose exhibited a higher aerobic volumetric rates when preceded by batch anoxic periods. Maximum aerobic rates for 2-CP removal occurred under 3 h anoxic conditions. Negligible SOC or 2-CP was removed during the anoxic phase of the cycle. Addition of nitrate in the feed did not induce any denitrification. PHB was not found to be the factor influencing such bacterial enhancement of 2-CP degradation.

10. Feasibility of complete degradation of 30 mg/L 2-CP was seen at temperatures as low as 5 °C particularly when phenol was present as a supplemental substrate. Rates of specific 2-CP removal decreased as the temperature was decreased successively from 24 °C to 15 °C, 10 °C and finally to 5 °C. Specific 2-CP removals were not linear according to the Arrhenius equation over the entire 5 °C and 24 °C range.

11. Specific rates of 2-CP removal increased in SBR's operating in presence of either dextrose or phenol when SRT was decreased from 20 d, 16 d, 12 d, 8 d and finally to 4 d indicating enrichment of mixed microbial culture with 2-CP degraders. Biomass yield (Y) was calculated to be 1.27 and 0.76 mg VSS/mg SOC removed per day for the system with dextrose and phenol respectively.

12. The feasibility of 2-CP removal under denitrifying conditions was demonstrated in SBR's operated on continuous anoxic-aerobic mode. A maximum 2-CP removal under anoxic conditions of 54-58% and 66-68% was observed in presence of dextrose and phenol respectively at an anoxic HRT of 4 h. Denitrification was confirmed through NO_3^- reduction in both the systems. Phenol was more effective than dextrose in enhancing the removal of 2-CP. The residual 2-CP after the anoxic period was rapidly removed during the subsequent aerobic period. The volumetric rates for 2-CP removal under aerobic conditions following the anoxic period were higher than the 2-CP removal rate in fully aerobic SBR's.

6.2 Engineering significance and recommendations for future work

In this study, most of the work with anaerobic dehalogenation of chlorophenols was conducted in batch serum bottles or aluminium seal type culture tubes. The culture tubes resembled an anaerobic sequencing batch reactor since the spent medium was withdrawn at regular intervals without disturbing the biomass pellet - a technique that was used successfully for repeatability of the experiments conducted. The results from several batch studies provided information for conceptual basic design of continuous-flow anaerobic reactors for treatment of chlorophenolic wastes. A major finding of this study was the capability of a mixed culture to dehalogenate under sulphate reducing conditions. Since these studies were conducted in single run batch serum bottle reactors, the response of the system to acclimation and its subsequent impact on 2-CP dehalogenation remains to be seen. The study on dehalogenation of polychlorinated phenols like 2,4,6-TCP and 2,4-DCP indicated a potential for sequential anaerobic-aerobic treatment systems for complete removal of these toxic compounds. Pollution control engineers and scientists currently employ sequential environments primarily to facilitate biological nutrient removal.

The anaerobic experiments in this study were conducted with mixed cultures. In order to have a better understanding of the organism/organisms responsible for 2-CP dehalogenation it is recommended that an isolation procedure

be carried out. Other investigators have attempted to categorize bacteria that accomplish reductive dechlorination. It is most important to distinguish whether they are strict anaerobes, or whether they are facultative anaerobes. Cole et al. (1994) recently isolated a pure culture capable of 2-CP dehalogenation. Interestingly, the organism named 2CP-1 turned out to be a facultative anaerobe that readily grew under aerobic conditions. If that is the case a single sludge system could be developed for treatment of chlorophenolic wastes unlike the two-stage system suggested in this study. Naturally the question arises: can this microbe survive in mixed culture, mixed feed conditions? Much work remains to be done before sequential environments are employed to their fullest potential.

Aerobc experiments were conducted entirely in a continuous mode in SBR's with some batch experiments in presence or absence of dextrose or phenol. Although phenol was highly effective as a supplemental substrate for aerobic removal of 2-CP, results from this study and previous studies show that its persistence or addition under anaerobic conditions can hinder the removal of removal efficiency of target organic compound such as 2-CP. The SBR system adopted in this study can serve as potential above - ground treatment systems for bioremediation of contaminated water or as selectors in POTW receiving a industrial wastes containing chlorinated compounds.

Chlorophenol degradation under anoxic conditions has not been as extensively investigated as its removal in aerobic processes. The importance of anoxic conditions becomes an issue while dealing with environmental problems related to groundwater pollution because soil-water systems are often anoxic. This study looked into the effect of anoxic HRT and nature of supplements (dextrose or phenol) on 2-CP degradation in anoxic-aerobic SBR's. The sequential anoxic-aerobic mode indicated a greater efficiency of 2-CP removal. Although the exact reason to cause such an enhancement needs to be investigated further and the results verified for with other biological treatment systems, the suggested mode of operation can help to economize the overall energy usages and sizes of biological reactors in the treatment plant.

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APPENDIX A

Data showing 2-CP removal in unadapted digested sewage sludge
(10 % vol/vol cultures under 20 % CO2 + 80 % N2 atmosphere)

TABLE A-1

		DS 1			DS 2			AVERAGE		
Total Day	Day	2-CP	Phenol	Benzoate	2-CP	Phenol	Benzoate	2-CP	Phenol	
0	0	0.369	0.000		0.355	0.000		0.36	0.00	
15	15	0.360	0.000		0.340	0.000		0.35	0.00	
47	47	0.360	0.000		0.340	0.000		0.35	0.00	
82	82	0.353	0.000		0.338	0.000		0.35	0.00	
97	97	0.305	0.046		0.300	0.033		0.30	0.04	
139	139	0.252	0.095		0.256	0.078		0.25	0.09	
167	167	0.206	0.111	0.050	0.211	0.113	0.065	0.21	0.11	
198	198	0.162	0.135		0.177	0.152		0.17	0.14	WASH &
203	0	0.379	0.000		0.372	0.000		0.38	0.00	REFEED
210	7	0.347	0.012		0.345	0.010		0.35	0.01	
215	12	0.312	0.018		0.309	0.016		0.31	0.02	
222	19	0.271	0.046		0.265	0.047		0.27	0.05	
236	33	0.195	0.118		0.189	0.201		0.19	0.16	
241	38	0.125	0.205		0.119	0.211		0.12	0.21	
248	45	0.090	0.172	0.110	0.080	0.190	0.080	0.09	0.18	
255	52	0.040	0.140	0.060	0.010	0.160	0.032	0.03	0.15	
262	59	0.020	0.130		0.008	0.151		0.01	0.14	WASH &
265	0	0.398	0.000		0.388	0.000		0.39	0.00	REFEED
272	7	0.364	0.014		0.361	0.013		0.36	0.01	
280	15	0.311	0.019		0.298	0.024		0.30	0.02	
286	21	0.241	0.051		0.239	0.038		0.24	0.04	
294	29	0.226	0.201		0.216	0.198		0.22	0.20	
300	35	0.084	0.216		0.100	0.204		0.09	0.21	
307	42	0.031	0.148	0.038	0.042	0.132	0.041	0.04	0.14	
314	49	0.007	0.130		0.012	0.121		0.01	0.13	WASH &
320	0	0.389	0.000		0.376	0.000		0.38	0.00	REFEED
327	7	0.311	0.019		0.305	0.009		0.31	0.01	
334	14	0.175	0.048		0.164	0.055		0.17	0.05	
340	20	0.142	0.062		0.145	0.078		0.14	0.07	
346	26	0.114	0.119		0.120	0.129		0.12	0.12	
350	30	0.090	0.108		0.105	0.119		0.10	0.11	
353	33	0.009	0.087		0.050	0.101		0.03	0.09	WASH &
356	0	0.376	0.000		0.381	0.000		0.38	0.00	REFEED
362	6	0.296	0.019		0.305	0.017		0.30	0.02	
368	12	0.195	0.132		0.201	0.129		0.20	0.13	
375	15	0.047	0.112	0.021	0.062	0.116	0.026	0.05	0.11	
380	20	0.008	0.097		0.010	0.106		0.01	0.10	WASH &
382	0	0.388	0.000		0.391	0.000		0.39	0.00	REFEED
386	4	0.336	0.029		0.337	0.022		0.34	0.03	
389	7	0.189	0.075		0.191	0.088		0.19	0.08	
392	10	0.112	0.149		0.118	0.155		0.12	0.15	
394	12	0.078	0.215		0.084	0.219		0.08	0.22	
396	14	0.034	0.112	0.034	0.045	0.121	0.022	0.04	0.12	
398	16	0.004	0.085		0.009	0.103		0.01	0.09	WASH &
402	0	0.383	0.000		0.376	0.000		0.38	0.00	REFEED
404	3	0.339	0.018		0.331	0.012		0.34	0.02	
406	5	0.197	0.034		0.188	0.048		0.19	0.04	
409	8	0.128	0.088		0.114	0.102		0.12	0.10	
412	11	0.101	0.207	0.027	0.092	0.215	0.018	0.10	0.21	
414	13	0.041	0.126		0.033	0.201		0.04	0.16	
417	16	0.006	0.108		0.000	0.156		0.00	0.13	WASH &
421	0	0.379	0.000		0.384	0.000		0.38	0.00	REFEED
423	3	0.341	0.017		0.352	0.012		0.35	0.01	
426	6	0.198	0.039		0.201	0.045		0.20	0.04	
429	9	0.126	0.097		0.132	0.103		0.13	0.10	
432	12	0.102	0.218	0.004	0.113	0.204	0.008	0.11	0.21	
434	14	0.051	0.119		0.065	0.131		0.06	0.13	
436	16	0.004	0.098		0.008	0.112		0.01	0.11	

All concentrations of 2-CP, Phenol and Benzoate are in mM

TABLE A-2

Data showing 2-CP removal in unadapted sediment of an aerated lagoon treating bleached kraft waste.
(10 % vol/vol cultures under 20 % CO2 + 80 % N2 atmosphere)

		LS 1			LS 2			AVERAGE		
Total Day	Day	2-CP	Phenol	Benzoate	2-CP	Phenol	Benzoate	2-CP	Phenol	
0	0	0.37	0.00		0.38	0.00		0.38	0.00	
15	15	0.37	0.00		0.38	0.00		0.38	0.00	
46	46	0.37	0.00		0.37	0.00		0.37	0.00	
58	58	0.36	0.00		0.38	0.00		0.37	0.00	
66	66	0.35	0.00		0.37	0.00		0.36	0.00	
80	80	0.35	0.00		0.36	0.00		0.35	0.00	
99	99	0.35	0.00		0.35	0.00		0.35	0.00	
113	113	0.35	0.00		0.36	0.00		0.35	0.00	
118	118	0.34	0.00		0.35	0.00		0.35	0.00	
144	144	0.34	0.00		0.35	0.00		0.35	0.00	
193	193	0.34	0.00		0.35	0.00		0.34	0.00	WASH &
212	0	0.38	0.00		0.38	0.00		0.38	0.00	REFEED
222	10	0.38	0.00		0.38	0.00		0.38	0.00	
230	18	0.37	0.00		0.38	0.00		0.37	0.00	
237	25	0.37	0.00		0.37	0.00		0.37	0.00	
244	32	0.37	0.00		0.36	0.00		0.36	0.00	
252	40	0.37	0.00		0.36	0.00		0.36	0.00	
257	45	0.35	0.01		0.35	0.01		0.35	0.01	
262	50	0.35	0.03		0.34	0.02		0.34	0.02	
267	55	0.32	0.03		0.33	0.02		0.33	0.02	
272	60	0.31	0.03		0.32	0.03		0.32	0.03	
276	64	0.31	0.03		0.32	0.03		0.31	0.03	
279	67	0.30	0.03		0.29	0.03		0.30	0.03	
285	72	0.29	0.03		0.28	0.03		0.28	0.03	
288	75	0.28	0.04		0.28	0.03		0.28	0.04	WASH &
290	0	0.39	0.00		0.38	0.00		0.38	0.00	REFEED
296	7	0.37	0.01		0.37	0.02		0.37	0.02	
303	14	0.35	0.03		0.36	0.02		0.35	0.02	
306	17	0.33	0.03		0.33	0.03		0.33	0.03	
310	21	0.31	0.03		0.32	0.03		0.31	0.03	
315	26	0.29	0.03		0.30	0.04		0.29	0.03	
319	30	0.27	0.04		0.25	0.04		0.26	0.04	
325	36	0.24	0.05		0.24	0.04		0.24	0.04	
329	40	0.20	0.04		0.21	0.05		0.21	0.05	
334	45	0.12	0.05		0.12	0.06		0.12	0.05	
339	50	0.10	0.05		0.11	0.06		0.11	0.05	
344	55	0.06	0.07		0.07	0.06		0.06	0.06	WASH &
350	0	0.39	0.00		0.38	0.00		0.38	0.00	REFEED
355	6	0.34	0.03		0.35	0.03		0.34	0.03	
361	12	0.27	0.04		0.28	0.04		0.27	0.04	
367	16	0.24	0.05		0.25	0.05		0.25	0.05	
372	21	0.18	0.06		0.17	0.06		0.17	0.06	
378	27	0.10	0.06		0.09	0.07		0.10	0.06	
384	33	0.01	0.07		0.01	0.07		0.01	0.07	WASH &
393	0	0.38	0.00		0.38	0.00		0.38	0.00	REFEED
400	7	0.33	0.02		0.34	0.04		0.34	0.03	
403	13	0.26	0.03		0.26	0.04		0.26	0.03	
408	20	0.24	0.05		0.24	0.06		0.24	0.05	
414	28	0.09	0.06		0.10	0.07		0.09	0.06	
423	33	0.01	0.07		0.01	0.08		0.01	0.08	

All concentrations of 2-CP and Phenol are in mM

TABLE A-3

Data showing 2-CP removal in unadapted biomass from an anaerobic bioreactor treating bleached kraft waste. (10 % vol/vol cultures under 20 % CO2 + 80 % N2 atmosphere)

		RB 1			RB 2			AVERAGE		
Total Days	Day	2-CP	Phenol	Benzoate	2-CP	Phenol	Benzoate	2-CP	Phenol	
0	0	0.360	0.000		0.388	0.000		0.37	0.00	
13	13	0.354	0.000		0.384	0.000		0.37	0.00	
35	35	0.358	0.000		0.378	0.000		0.37	0.00	
45	45	0.351	0.000		0.382	0.000		0.37	0.00	
60	60	0.343	0.000		0.372	0.000		0.36	0.00	
75	75	0.338	0.000		0.365	0.000		0.35	0.00	
95	95	0.334	0.000		0.354	0.000		0.34	0.00	
110	110	0.336	0.000		0.342	0.000		0.34	0.00	
118	118	0.337	0.000		0.348	0.000		0.34	0.00	
144	144	0.330	0.000		0.338	0.000		0.33	0.00	
193	193	0.325	0.000		0.330	0.000		0.33	0.00	WASH &
202	0	0.380	0.000		0.369	0.000		0.37	0.00	REFEED
214	12	0.378	0.000		0.367	0.000		0.37	0.00	
218	16	0.372	0.000		0.361	0.000		0.37	0.00	
226	24	0.370	0.000		0.359	0.000		0.36	0.00	
232	30	0.365	0.000		0.354	0.000		0.36	0.00	
244	42	0.360	0.000		0.349	0.000		0.35	0.00	
250	48	0.354	0.005		0.343	0.006		0.35	0.01	
257	55	0.348	0.005		0.337	0.007		0.34	0.01	
262	60	0.334	0.008		0.323	0.010		0.33	0.01	
267	65	0.321	0.010		0.319	0.012		0.32	0.01	
272	70	0.312	0.015		0.308	0.008		0.31	0.01	
279	77	0.298	0.019		0.284	0.015		0.29	0.02	
290	88	0.286	0.025		0.271	0.019		0.28	0.02	
297	95	0.281	0.029		0.265	0.022		0.27	0.03	WASH &
304	0	0.377	0.000		0.386	0.000		0.38	0.00	REFEED
312	8	0.368	0.000		0.373	0.000		0.37	0.00	
316	12	0.355	0.009		0.356	0.012		0.36	0.01	
322	18	0.341	0.015		0.338	0.015		0.34	0.02	
330	26	0.334	0.018		0.325	0.022		0.33	0.02	
338	34	0.331	0.024		0.311	0.029		0.32	0.03	
344	40	0.305	0.028		0.286	0.033		0.30	0.03	
351	47	0.254	0.031		0.270	0.028		0.26	0.03	
358	54	0.216	0.037		0.221	0.035		0.22	0.04	
364	60	0.154	0.035		0.165	0.030		0.16	0.03	
370	66	0.112	0.038		0.123	0.032		0.12	0.04	
374	70	0.081	0.024		0.094	0.027		0.09	0.03	WASH &
380	0	0.376			0.368			0.37	0.00	REFEED
387	7	0.365	0.004		0.352	0.006		0.36	0.01	
394	14	0.348	0.008		0.340	0.012		0.34	0.01	
404	24	0.302	0.012		0.315	0.016		0.31	0.01	
412	32	0.261	0.016		0.275	0.026		0.27	0.02	
420	40	0.202	0.028		0.216	0.030		0.21	0.03	
425	45	0.158	0.025		0.169	0.034		0.16	0.03	
430	50	0.115	0.031		0.131	0.028		0.12	0.03	
435	55	0.068	0.032		0.095	0.031		0.08	0.03	
438	58	0.021	0.030		0.052	0.028		0.04	0.03	
440	60	0.024	0.032		0.031	0.026		0.03	0.03	
445	65	0.018	0.025		0.022	0.025		0.02	0.03	

All concentrations of 2-CP and Phenol are in mM

TABLE A-4

Dehalogenation of 2-CP by unadapted anaerobic digester sludge (50 % vol/vol) under CO2/N2 head-space

Total Day	Day	Sample # 1			Sample # 2			Sample # 3			Average			
		2-CP mg/L	Phenol mg/L	Benz mg/L	2-CP mg/L	Phenol mg/L	Benz mg/L	2-CP mg/L	Phenol mg/L	Benz mg/L	2-CP mM	Phenol mM	Benz mM	
0	0	50.21	0.00		48.88	0.00		49.80	0.00		0.386	0.000	0.000	
4	4	48.87	0.00		48.02	0.00		48.10	0.00		0.376	0.000	0.000	
6	6	45.64	0.42		45.22	0.00		46.28	0.00		0.356	0.001	0.000	
8	8	43.19	2.65		43.31	2.91		44.32	3.12		0.339	0.031	0.000	
10	10	36.73	5.37		32.86	5.18		35.48	4.95		0.273	0.055	0.000	
14	14	22.14	7.79		20.11	7.88		20.60	8.87		0.163	0.087	0.000	
16	16	12.52	11.58		9.28	12.36		10.24	12.94		0.083	0.131	0.000	
18	18	4.28	13.94	3.85	2.47	14.15	2.62	2.19	14.87	2.17	0.023	0.152	0.024	
20	20	0.00	5.57	1.22	0.00	6.62		0.00	7.65	1.47	0.000	0.070	0.011	
22	22	0.00	0.00		0.00	0.00		0.00	0.00		0.000	0.000	0.000	Refeed
23	0	49.53	0.00		48.63	0.00		48.37	0.00		0.380	0.000	0.000	
29	6	23.55	10.12		26.81	12.47		25.42	9.88		0.197	0.115	0.000	
33	10	13.49	13.11		12.87	13.52		15.41	11.74		0.108	0.136	0.000	
35	12	5.21	8.16	4.56	4.03	9.31	3.15	4.76	7.49	2.89	0.036	0.089	0.029	
37	14	0.00	2.24		0.00	3.76		0.00	2.58		0.000	0.030	0.000	
39	16	0.00	0.00		0.00	0.00		0.00	0.00		0.000	0.000	0.000	Refeed
40	0	48.65	0.00		49.38	0.00		49.80	0.00		0.383	0.000	0.000	
43	3	36.62	3.67		39.17	4.18		38.75	4.68		0.297	0.044	0.000	
46	6	20.44	9.49		24.85	10.73		22.62	12.60		0.176	0.116	0.000	
48	8	10.21	13.10		11.97	14.75		10.86	15.43		0.086	0.153	0.000	
50	10	4.12	7.83	6.61	5.33	9.42	4.37	3.57	10.50	5.26	0.034	0.098	0.045	
52	12	0.00	3.05		0.00	4.29		0.00	6.88		0.000	0.050	0.000	
54	14	0.00	0.00		0.00	0.00		0.00	0.00		0.000	0.000	0.000	Refeed
56	0	49.10	0.00		50.37	0.00		51.19	0.00		0.391	0.000	0.000	
60	4	40.20	4.61		42.14	4.23		42.85	4.97		0.325	0.049	0.000	
62	6	24.60	8.80		23.88	6.93		26.19	7.42		0.194	0.082	0.000	
64	8	15.20	12.61	4.95	16.04	11.20	6.6	16.86	13.37	8.15	0.125	0.132	0.054	
66	10	9.10	14.42	2.66	8.41	14.08	2.3	7.28	15.65	3.49	0.064	0.157	0.023	
68	12	0.00	5.42		0.00	6.71		0.00	7.81		0.000	0.071	0.000	
70	14	0.00	0.00		0.00	0.00		0.00	0.00		0.000	0.000	0.000	

Cultures were fed with 0.1% y.Extract medium + 2-CP

TABLE A-5

Data showing 2-CP removal in unadapted digested sewage sludge
(10 % vol/vol cultures under 20 % CO2 + 80 % H2 atmosphere)

		DSH 1			DSH 2			AVERAGE		
TOT-Day	Day	2-CP	PHENOL	Benz	2-CP	PHENOL	Benz	2-CP	PHENOL	
0	0	0.360	0.000		0.386	0.000		0.373	0.000	
15	15	0.359	0.000		0.380	0.000		0.370	0.000	
47	47	0.347	0.000		0.373	0.000		0.360	0.000	
82	82	0.349	0.000		0.374	0.000		0.362	0.000	
97	97	0.317	0.015		0.345	0.013		0.331	0.014	
139	139	0.279	0.044	0.081	0.313	0.040	0.065	0.296	0.042	
167	167	0.262	0.060		0.278	0.051		0.270	0.056	
198	198	0.252	0.067		0.236	0.058		0.244	0.063	WASH &
203	0	0.393	0.000		0.397	0.000		0.395	0.000	REFEED
210	7	0.375	0.012		0.361	0.018		0.368	0.015	
215	12	0.324	0.018		0.334	0.022		0.329	0.020	
222	19	0.304	0.047		0.294	0.061		0.299	0.054	
236	33	0.272	0.089		0.258	0.096		0.265	0.093	
241	38	0.226	0.123		0.214	0.129		0.220	0.126	
248	45	0.194	0.157		0.182	0.165		0.188	0.161	
255	52	0.147	0.148	0.035	0.139	0.154	0.044	0.143	0.151	
262	59	0.114	0.138		0.118	0.147		0.116	0.143	WASH &
265	0	0.380	0.000		0.389	0.000		0.385	0.000	REFEED
272	7	0.371	0.008		0.379	0.010		0.375	0.009	
280	15	0.297	0.019		0.290	0.024		0.294	0.022	
286	21	0.268	0.081		0.261	0.089		0.265	0.085	
294	29	0.254	0.098		0.244	0.106		0.249	0.102	
300	35	0.236	0.112		0.229	0.119		0.233	0.116	
307	42	0.190	0.149		0.183	0.152		0.187	0.151	
314	49	0.151	0.153	0.021	0.146	0.160	0.038	0.149	0.157	
320	55	0.090	0.134	0.005	0.079	0.142		0.085	0.138	WASH &
320	0	0.388	0.000		0.377	0.000		0.383	0.000	REFEED
327	7	0.362	0.018		0.354	0.016		0.358	0.017	
334	14	0.284	0.047		0.273	0.051		0.279	0.049	
340	20	0.253	0.109		0.241	0.114		0.247	0.112	
346	26	0.227	0.128		0.219	0.120		0.223	0.124	
350	30	0.168	0.097	0.068	0.158	0.089	0.057	0.163	0.093	
356	36	0.112	0.070		0.108	0.065		0.110	0.068	
362	42	0.010	0.054		0.009	0.060		0.010	0.057	WASH &
370	0	0.372	0.000		0.380	0.000		0.376	0.000	REFEED
380	10	0.287	0.042		0.291	0.035		0.289	0.039	
386	16	0.266	0.064		0.270	0.065		0.268	0.065	
389	19	0.229	0.119		0.235	0.122		0.232	0.121	
392	22	0.184	0.147		0.190	0.150		0.187	0.149	
394	24	0.133	0.172		0.140	0.178		0.137	0.175	
396	26	0.107	0.154		0.112	0.160		0.110	0.157	
398	28	0.056	0.138	0.033	0.061	0.143	0.021	0.059	0.141	
402	32	0.005	0.126		0.008	0.134		0.007	0.130	WASH &
402	0	0.388	0.000		0.392	0.000		0.390	0.000	REFEED
406	5	0.316	0.037		0.322	0.041		0.319	0.039	
412	11	0.254	0.078		0.361	0.085		0.308	0.082	
417	16	0.183	0.125		0.190	0.130		0.187	0.128	
421	20	0.087	0.149		0.095	0.154		0.091	0.152	
423	24	0.008	0.130	0.016	0.010	0.141	0.010	0.009	0.136	WASH &
426	0	0.379	0.000		0.386	0.000		0.383	0.000	REFEED
432	7	0.307	0.026		0.312	0.031		0.310	0.029	
436	13	0.242	0.119		0.250	0.123		0.246	0.121	
442	19	0.101	0.133		0.109	0.123		0.105	0.128	
446	21	0.048	0.122	0.102	0.054	0.128	0.087	0.051	0.125	
449	24	0.009	0.117	0.011	0.006	0.123	0.027	0.008	0.120	

All concentrations of 2-CP, Phenol and Benzoate are in mM

TABLE A-6

2-CP dehalogenation in 0.1 % yeast extract medium
using adapted sludge under 20% CO2 + 80% H2

Total-Days	Day	Sample#1 2-CP	Sample#1 Phenol	Sample#2 2-CP	Sample#2 Phenol	AVERAGE 2-CP	AVERAGE Phenol	
0	0	0.388	0.000	0.372	0.000	0.380	0.000	
3	3	0.365	0.045	0.359	0.041	0.362	0.043	
5	5	0.316	0.057	0.308	0.052	0.312	0.055	
8	8	0.289	0.065	0.274	0.071	0.282	0.068	
10	11	0.258	0.084	0.247	0.089	0.253	0.087	
12	12	0.223	0.130	0.212	0.123	0.218	0.127	
15	15	0.183	0.145	0.175	0.150	0.179	0.148	
18	18	0.087	0.179	0.071	0.172	0.079	0.176	
21	21	0.018	0.152	0.011	0.160	0.015	0.156	WASHING
24	24	0.000	0.093	0.000	0.115	0.000	0.104	AND
25	0	0.380	0.000	0.373	0.000	0.377	NA	REFEEDING
28	3	0.370	0.032	0.362	0.040	0.366	0.036	
31	6	0.320	0.060	0.314	0.055	0.317	0.058	
35	10	0.272	0.091	0.258	0.103	0.265	0.097	
38	13	0.226	0.140	0.200	0.151	0.213	0.146	
41	16	0.188	0.150	0.174	0.160	0.181	0.155	
45	20	0.025	0.133	0.016	0.120	0.021	0.127	
47	22	0.000	0.118	0.000	0.108	0.000	0.113	WASHING
48	0	0.380	0.000	0.384	0.000	0.382	NA	AND
51	3	0.358	0.037	0.368	0.030	0.363	0.034	REFEEDING
53	5	0.321	0.048	0.315	0.051	0.318	0.050	
56	8	0.281	0.077	0.288	0.068	0.285	0.073	
59	11	0.261	0.106	0.265	0.115	0.263	0.111	
61	13	0.208	0.138	0.217	0.129	0.213	0.134	
63	15	0.185	0.174	0.179	0.166	0.182	0.170	
66	18	0.091	0.181	0.082	0.173	0.087	0.177	
68	20	0.042	0.152	0.032	0.144	0.037	0.148	
70	22	0.000	0.118	0.000	0.109	0.000	0.114	
72	24	NA	0.042	NA	0.029	NA	0.036	
74	26	NA	0.000	NA	0.000	NA	0.000	

TABLE A-7

2-CP dehalogenation with various concentrations of adapted digested sewage sludge in 0.1 % yeast extract medium

50 %							25 %					
		Sample#1			Sample#2		Sample#1			Sample#2		
Total-Days	Day	2-CP	Phenol	Benz	2-CP	Phenol	Benz	2-CP	Phenol	Benz	2-CP	Phenol	Benz
0	0	0.380	0.000		0.387	0.000		0.383	0.000		0.379	0.000	
3	3	0.268	0.050		0.262	0.043		0.306	0.107		0.300	0.111	
5	5	0.167	0.087		0.163	0.079		0.196	0.165		0.188	0.170	
6	6	0.072	0.125	0.025	0.068	0.118	0.017	0.150	0.176		0.140	0.180	
7	7	0.000	0.108		0.000	0.112		0.088	0.211		0.081	0.216	
9	9	0.000	0.000		0.000	0.000		0.041	0.238	0.072	0.035	0.243	0.048
11	11							0.000	0.218		0.000	0.207	
13	13								0.156			0.140	
15	15								0.118	0.015		0.102	0.024
17	17								0.000			0.000	
19	19												
20	0	0.388	0.000		0.382	0.000		0.381	0.000		0.400	0.000	
24	4	0.188	0.063		0.179	0.070		0.233	0.129		0.221	0.133	
26	6	0.081	0.114	0.037	0.071	0.116		0.171	0.147		0.165	0.153	
27	7	0.000	0.096	0.034	0.000	0.090	0.038	0.096	0.214		0.086	0.204	
29	9	0.000	0.000		0.000	0.000		0.052	0.224		0.041	0.216	
31	11							0.000	0.200	0.048	0.000	0.190	0.063
33	13								0.142			0.130	
35	15								0.049			0.032	
37	17								0.000			0.000	
39	19												

All concentrations of 2-CP, Phenol and Benzoate shown are in mM.

TABLE A-7 (... contd)

2-CP dehalogenation with various concentrations of adapted digested sewage sludge in 0.1 % yeast extract medium .....contd

		10 %						AVERAGE		
		Sample#1			Sample#2			50 %	25 %	10 %
Total-Days	Day	2-CP	Phenol	Benz	2-CP	Phenol	Benz	2-CP	2-CP	2-CP
0	0	0.385	0.000		0.380	0.000		0.384	0.381	0.383
3	3	0.326	0.032		0.320	0.028		0.265	0.303	0.323
5	5	0.230	0.091		0.234	0.096		0.165	0.192	0.232
6	6	0.169	0.108		0.175	0.114		0.070	0.145	0.172
7	7	0.130	0.112		0.134	0.119		0.000	0.085	0.132
9	9	0.095	0.132		0.087	0.134		0.000	0.038	0.091
11	11	0.055	0.159	0.046	0.059	0.162	0.037	0.000	0.000	0.057
13	13	0.000	0.132	0.022	0.000	0.140	0.021	0.000	0.000	0.000
15	15		0.118			0.120				
17	17		0.065			0.070		REFEED Y.E. MEDIUM AND 2-CP		
19	19		0.000			0.000				
20	0	0.382	0.000		0.380	0.000		0.385	0.391	0.381
24	4	0.290	0.042		0.295	0.037		0.184	0.227	0.293
26	6	0.172	0.123		0.170	0.127		0.076	0.168	0.171
27	7	0.109	0.142		0.111	0.140		0.000	0.091	0.110
29	9	0.090	0.150		0.081	0.147		0.000	0.047	0.086
31	11	0.041	0.128	0.053	0.032	0.133	0.044	0.000	0.000	0.037
33	13	0.000	0.098		0.000	0.086		0.000	0.000	0.000
35	15		0.045			0.030				
37	17		0.000			0.000				
39	19									

All concentrations of 2-CP, Phenol and Benzoate shown are in mM.

TABLE A-8

2-CP degradation in mineral medium containing 0.1% Yeast Extract

Total-Days	Day	Sample#1		Sample#2		AVERAGE (mM)		
		2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	
		(mg/L)		(mg/L)				
0	0	45.12	0.00	45.34	0.00	0.352	0.000	
3	3	38.15	5.06	40.22	6.10	0.305	0.059	
5	5	18.92	14.80	21.39	15.80	0.157	0.163	
8	8	11.02	12.80	14.18	13.70	0.098	0.141	
11	11	6.85	10.30	6.65	11.60	0.053	0.116	
13	13	0.00	8.50	0.00	7.20	0.000	0.084	WASH
15	15	NA	NA	NA	NA	NA	NA	AND
16	0	48.86	0.00	47.72	0.00	0.376	0.000	REFEED
20	4	41.22	6.30	38.68	5.77	0.311	0.064	
22	6	20.15	13.32	17.51	14.90	0.147	0.150	
24	8	13.41	15.47	11.64	16.42	0.097	0.170	
27	11	5.79	16.88	6.26	17.91	0.047	0.185	
29	13	0.00	10.20	0.00	8.85	0.000	0.101	
31	15	NA	4.32	NA	2.80	NA	0.038	
33	17	NA	0.00	NA	0.00	NA	0.000	WASH
35	19	NA	NA	NA	NA	NA	NA	AND
36	0	47.39	0.00	48.82	0.00	0.374	0.000	REFEED
39	3	40.55	2.32	42.89	3.37	0.325	0.030	
41	5	20.02	10.91	22.82	12.36	0.167	0.124	
43	7	15.12	13.49	16.80	14.58	0.124	0.149	
45	9	11.56	14.39	10.94	16.02	0.088	0.162	
47	11	5.35	18.28	6.10	19.71	0.045	0.202	
49	13	0.00	12.10	0.00	12.65	0.000	0.132	
51	15	NA	5.22	NA	6.18	NA	0.061	
53	17	NA	0.00	NA	0.00	NA	0.000	
55	19	NA	NA	NA	NA	NA	NA	

TABLE A-9

2-CP degradation in mineral medium containing 10 mM Acetate

Total-Days	Day	Sample#1		Sample#2		AVERAGE (mM)		
		2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	
		(mg/L)		(mg/L)				
0	0	44.56	0.00	45.34	0.00	0.350	0.000	
3	3	38.79	2.88	41.12	3.59	0.311	0.034	
5	5	30.84	6.41	28.79	8.57	0.232	0.080	
8	8	27.02	12.32	26.12	14.58	0.207	0.143	
11	11	20.62	16.16	18.83	16.94	0.154	0.176	
13	13	6.58	16.65	5.46	16.35	0.047	0.176	WASH
15	15	0.00	12.75	0.00	11.80	0.000	0.131	AND
16	0	49.25	0.00	48.14	0.00	0.379	0.000	REFEED
20	4	42.85	3.66	43.17	2.89	0.335	0.035	
22	6	33.79	6.95	30.64	7.54	0.251	0.077	
24	8	30.17	13.65	28.68	14.38	0.229	0.149	
27	11	21.95	16.71	19.24	17.42	0.160	0.182	
29	13	7.13	18.55	7.87	19.39	0.058	0.202	
31	15	0.00	10.16	0.00	12.44	0.000	0.120	
33	17	NA	5.12	NA	4.89	NA	0.053	WASH
35	19	NA	0.00	NA	0.00	NA	0.000	AND
36	0	49.27	0.00	48.14	0.00	0.379	0.000	REFEED
39	3	44.15	2.12	42.37	1.86	0.337	0.021	
41	5	35.53	5.33	33.28	4.85	0.268	0.054	
43	7	30.44	8.74	28.92	7.37	0.231	0.086	
45	9	25.42	14.19	23.19	14.50	0.189	0.153	
47	11	18.18	16.71	17.09	17.05	0.137	0.180	
49	13	8.24	17.87	7.11	18.38	0.060	0.193	
51	15	0.00	11.49	0.00	12.62	0.000	0.128	
53	17	NA	5.60	NA	6.00	NA	0.062	
55	19	NA	0.00	NA	0.00	NA	0.000	

TABLE A-10

2-CP degradation in mineral medium containing 10 mM Propionate

Total-Days	Day	Sample#1		Sample#2		AVERAGE (mM)		
		2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	
		(mg/L)		(mg/L)				
0	0	45.25	0.00	44.12	0.00	0.348	0.000	
3	3	34.81	1.15	36.77	1.42	0.279	0.014	
5	5	28.35	7.18	31.22	6.32	0.232	0.072	
8	8	25.18	14.09	23.08	15.19	0.188	0.156	
11	11	20.69	18.32	21.18	18.08	0.163	0.194	
13	13	4.35	9.32	5.12	11.24	0.037	0.109	WASH
15	15	0.00	7.65	0.00	6.10	0.000	0.073	AND
16	0	50.04	0.00	47.31	0.00	0.379	0.000	REFEED
20	4	40.88	2.64	37.82	2.80	0.306	0.029	
22	6	32.19	6.88	29.55	8.10	0.240	0.080	
24	8	24.35	13.73	21.47	15.52	0.178	0.156	
27	11	18.64	16.19	16.69	18.47	0.137	0.184	
29	13	7.11	18.94	6.54	20.27	0.053	0.209	
31	15	0.00	12.68	0.00	13.34	0.000	0.138	
33	17	NA	6.15	NA	6.68	NA	0.068	WASH
35	19	NA	0.00	NA	0.00	NA	0.000	AND
36	0	46.88	0.00	47.59	0.00	0.368	0.000	REFEED
39	3	38.29	1.88	36.44	2.24	0.291	0.022	
41	5	33.38	4.68	30.87	5.17	0.250	0.052	
43	7	28.77	8.62	26.50	10.80	0.215	0.103	
45	9	23.14	11.34	21.75	12.55	0.175	0.127	
47	11	16.64	14.20	15.02	15.16	0.123	0.156	
49	13	7.19	16.13	7.40	17.80	0.057	0.180	
51	15	0.00	10.60	0.00	8.95	0.000	0.104	
53	17	NA	4.24	NA	1.97	NA	0.033	
55	19	NA	0.00	NA	0.00	NA	0.000	

TABLE A-11

Table 11: **2-CP degradation in mineral medium containing 10 mM Ethanol**

Total-Days	Day	Sample#1		Sample#2		AVERAGE (mM)		
		2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	
		(mg/L)		(mg/L)				
0	0	44.87	0.00	45.73	0.00	0.353	0.000	
3	3	37.88	3.28	40.79	2.91	0.306	0.033	
5	5	26.17	5.93	28.46	6.37	0.213	0.065	
8	8	20.04	10.96	22.14	9.28	0.164	0.108	
11	11	11.75	15.16	10.14	12.32	0.085	0.146	
13	13	4.06	12.91	3.48	10.49	0.029	0.124	WASH
15	15	0.00	12.12	0.00	7.86	0.000	0.106	AND
16	0	46.85	0.00	48.92	0.00	0.373	0.000	REFEED
20	4	36.22	4.02	41.13	3.54	0.301	0.040	
22	6	23.41	6.16	25.67	6.82	0.191	0.069	
24	8	18.05	13.85	20.24	12.64	0.149	0.141	
27	11	10.18	16.52	8.73	16.17	0.074	0.174	
29	13	4.26	20.55	3.89	19.37	0.032	0.212	
31	15	0.00	12.12	0.00	13.86	0.000	0.138	
33	17	NA	6.58	NA	7.11	NA	0.073	WASH
35	19	NA	0.00	NA	0.00	NA	0.000	AND
36	0	47.76	0.00	48.95	0.00	0.376	0.000	REFEED
39	3	38.14	3.85	40.50	4.57	0.306	0.033	
41	5	24.52	6.20	26.19	7.14	0.197	0.052	
43	7	19.68	10.91	20.43	11.72	0.156	0.088	
45	9	14.87	13.32	15.71	14.58	0.119	0.109	
47	11	10.20	16.06	10.85	17.25	0.082	0.130	
49	13	3.82	18.20	4.79	18.87	0.034	0.144	
51	15	0.00	11.37	0.00	10.50	0.000	0.085	
53	17	NA	4.90	NA	3.47	NA	0.033	
55	19	NA	0.00	NA	0.00	NA	0.000	

TABLE A-12

Table EU : **2-CP degradation in basic mineral medium (without supplements)**

Total-Days	Day	Sample#1 2-CP (mg/L)	Sample#1 Phenol (mg/L)	Sample#2 2-CP (mg/L)	Sample#2 Phenol (mg/L)	AVERAGE (mM) 2-CP	AVERAGE (mM) Phenol	
0	0	46.90	0.00	48.70	0.00	0.372	0.000	
6	6	45.23	1.13	43.43	1.22	0.345	0.013	
8	8	36.88	3.95	34.82	3.20	0.279	0.038	
12	12	23.39	6.39	25.70	7.43	0.191	0.074	
16	16	18.63	10.81	15.55	10.25	0.133	0.112	
20	20	9.51	11.28	16.71	13.44	0.102	0.132	
22	22	4.11	12.69	5.14	14.38	0.036	0.144	
24	24	1.80	11.66	2.31	12.41	0.016	0.128	WASHING
26	26	0.00	10.90	0.00	9.87	0.000	0.111	AND
28	0	48.44	0.00	48.96	0.00	0.379	0.000	REFEEDING
33	5	45.36	0.75	46.26	1.22	0.357	0.011	
36	8	37.78	3.38	38.68	3.57	0.298	0.037	
40	12	25.57	5.73	26.47	5.45	0.203	0.060	
43	15	20.17	9.21	21.20	8.37	0.161	0.094	
46	18	14.65	10.53	15.42	9.96	0.117	0.109	
48	20	10.28	11.28	11.82	10.81	0.086	0.118	
51	23	6.43	9.40	8.10	7.61	0.057	0.091	
53	25	1.16	7.99	1.80	6.96	0.012	0.080	WASHING
54	26	0.00	6.58	0.00	6.11	0.000	0.068	AND
55	0	47.29	0.00	46.52	0.00	0.365	0.000	REFEEDING
58	3	40.86	1.32	41.63	0.94	0.321	0.012	
60	5	33.41	2.26	32.64	2.63	0.257	0.026	
63	8	28.78	5.92	27.76	5.17	0.220	0.059	
64	9	NA	NA	NA	NA	NA	NA	
66	11	25.44	6.96	24.42	6.39	0.194	0.071	
68	13	21.07	8.55	20.43	7.90	0.162	0.088	
70	15	16.96	10.53	16.19	11.09	0.129	0.115	
72	17	11.44	11.09	10.54	11.37	0.086	0.119	
74	19	NA	NA	NA	NA	NA	NA	
77	22	6.17	9.59	5.40	8.46	0.045	0.096	
80	25	1.16	8.27	1.67	6.96	0.011	0.081	
81	26	0.00	5.73	0.00	4.89	0.000	0.057	

Gradient elution is dependent on time controlled changes of two or more solvents. Each solvent contributes varying percentages of the total mobile phase throughout the separation. The Gradient Table constructed by the operator determines these percentages. Flow rate and the time each set of conditions is in effect are used to generate a chromatogram of well-defined peaks in a minimum amount of time.

The gradient program enables the operator to control the rate of change of the individual solvents in each segment. Each segment is described through the use of concave, convex, and linear composition profiles.

Beyond gradient separations, programs can be devised to prepare a system (equilibration, solvent changeovers, cleanup following buffer use, regulated flow rate increase for column warmup, etc.) using the four reservoirs in the Waters 600E. Operating as a system controller, the Waters 600E can control entire methods including detector and auto-injector sampling parameters.

To prepare a Gradient Table:

Press **PROGRAM TABLES.** The Waters 600E can permanently store up to 15 tables (numbered 1-15). A Gradient Table can contain up to 15 lines automatically ordered by time. Each time entered in the table must be unique.

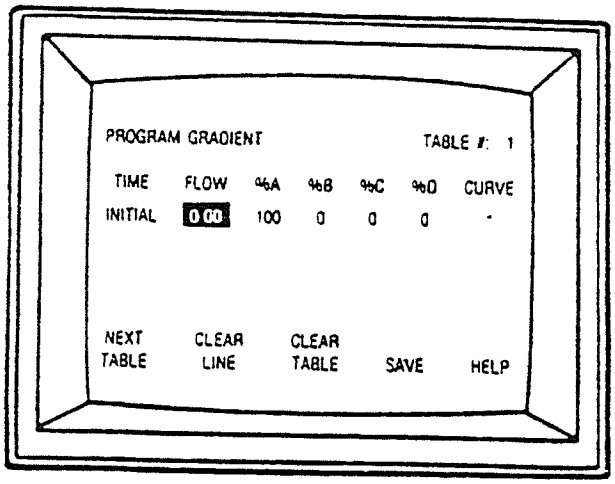

FIGURE 3-30 PROGRAM GRADIENT TABLE

TABLE 3-16
GRADIENT PROGRAM SCREEN PARAMETERS

REQUIRED ENTRIES	RANGE	COMMENTS
TIME FLOW	0.00-655.34	Time
	0-20 ml/min	Flow (100 ul heads)
	0-45 ml/min	Flow (225 ul heads)
	0-100	%A, %B, %C, %D; must total 100
Curve	1	Go to end condition immediately
	2-5	Convex
	6	Linear
	7-10	Concave
	11	Maintain start conditions until end of segment (used to create step gradients)
Table Number	Programs 1-15	Enter number of the program you wish to edit or create

The function keys displayed while in this mode of operation are:

CLEAR LINE	Clears the line on which the cursor rests. Does not delete the line permanent memory.
CLEAR TABLE	Clears the entire table currently displayed on the screen. Initial conditions line returns to default status. Does not delete the table from permanent memory.
SAVE	Saves the table currently displayed on the screen. To store a modified table for use or to delete a table (press the appropriate CLEAR key and then SAVE.)
HELP	Provides a Help message.

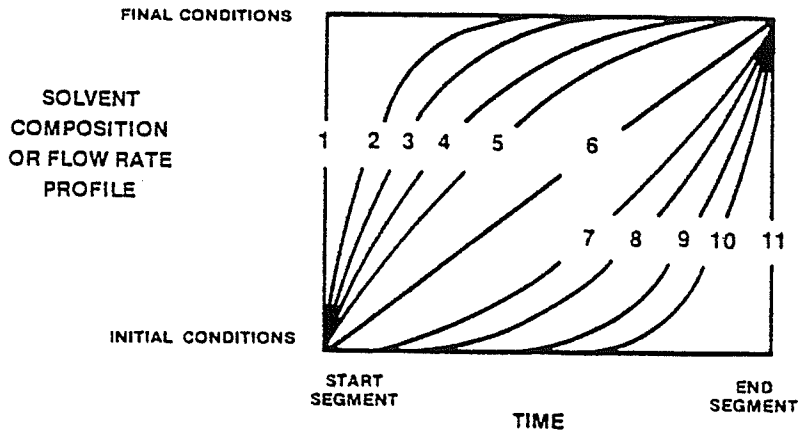

FIGURE 3-31 WATERS 600E GRADIENT CURVE SHAPES

In the example below the curve number refers to the shapes shown in Figure 3-31. Each curve illustrates a different proportional rate of change over the time specified on each line of the Gradient Table for the solvent composition or the flow rate. In the example, mobile phase flows as soon as the pump is started with 100% Solvent A at 2 ml/min. The solvent composition changes to 80% Solvent A and 20% Solvent B in a steady, gradual fashion over the next three minutes using Curve 6. The flow rate remains unchanged. The next segment could use the same curve or choose one that changes composition more radically (1-5) or less radically (7-11) at the outset before establishing an isocratic plateau.

Generally whenever you want resolution at specific conditions within an analysis, couple the last curve with an opposite shape in the next timed segment. Whenever you wish to expedite the solvent changeover (no resolution is taking place), use a steep curve.

1. Develop the Gradient Table:
   - o Make a photocopy of the Analysis Plan and File Sheet found in Appendix D to develop and permanently preserve your methods.

GRADIENT TABLE

TIME *	FLOW *	% A	% B	% C	% D	CURVE
INITIAL	2.0	100	0	0	0	*
3.00	2.00	80	20	0	0	6

FIGURE 3-32 GRADIENT TABLE

TABLE A-13

Dehalogenation of 2,4,6-TCP with cultures adapted to 2-CP
under 20% CO2 + 80% N2 atmosphere in 0.1% Y.Extract medium

Total-Days	Day	SAMPLE # 1				SAMPLE # 2				AVERAGE				
		2,4,6-TCP	2,4-DCP	4-CP	Phenol	2,4,6-TCP	2,4-DCP	4-CP	Phenol	2,4,6-TCP	2,4-DCP	4-CP	Phenol	
			(mg/L)				(mg/L)				(mg/L)			
0	0	30.12	0.00	0.00	0.00	28.78	0.00	0.00	0.00	29.45	0.00	0.00	0.00	
4	4	27.38	1.51	0.00	0.00	26.32	0.75	0.00	0.00	26.85	1.13	0.00	0.00	
6	6	23.54	3.88	0.50	0.00	21.08	3.69	0.96	0.00	22.31	3.79	0.73	0.00	
10	10	20.25	5.49	1.01	0.00	19.11	6.28	1.33	0.00	19.68	5.89	1.17	0.00	
14	14	17.16	7.12	4.47	0.00	16.03	7.85	4.89	0.00	16.60	7.49	4.68	0.00	
18	18	13.55	8.86	5.56	0.00	14.25	9.04	6.01	0.00	13.90	8.95	5.79	0.00	
20	20	10.92	11.72	8.21	0.00	11.76	12.26	8.75	0.00	11.34	11.99	8.48	0.00	
22	22	5.41	8.11	11.94	0.00	6.35	9.34	11.64	0.00	5.88	8.73	11.79	0.00	
24	24	1.02	5.82	13.75	0.00	2.81	5.47	13.83	0.00	1.92	5.65	13.79	0.00	
26	26	0.00	1.97	15.85	0.00	0.00	2.35	15.46	0.00	0.00	2.16	15.66	0.00	WASHING
30	30	0.00	0.00	16.79	0.00	0.00	0.00	16.34	0.00	0.00	0.00	16.57	0.00	AND
31	0	29.03	0.00	0.00	0.00	30.14	0.00	0.00	0.00	29.59	0.00	0.00	0.00	REFEEDING
35	4	25.30	2.50	0.61	0.00	27.12	1.74	0.70	0.00	26.21	2.12	0.66	0.00	
38	7	20.20	6.80	1.22	0.00	22.49	6.33	1.45	0.00	21.35	6.57	1.34	0.00	
41	10	17.60	8.40	4.28	0.00	19.05	7.85	4.83	0.00	18.33	8.13	4.56	0.00	
45	14	15.03	10.30	6.13	0.00	16.55	10.97	6.67	0.00	15.79	10.64	6.40	0.00	
49	18	7.84	12.48	10.62	0.00	10.28	13.11	11.19	0.00	9.06	12.80	10.91	0.00	
53	22	0.00	6.71	14.19	0.00	2.50	7.49	15.17	0.00	1.25	7.10	14.68	0.00	
55	24	0.00	2.29	15.40	0.00	0.00	3.34	15.87	0.00	0.00	2.82	15.64	0.00	WASHING
57	26	0.00	0.00	15.95	0.00	0.00	0.00	16.26	0.00	0.00	0.00	16.11	0.00	AND
59	0	28.65	0.00	0.00	0.00	29.54	0.00	0.00	0.00	29.10	0.00	0.00	0.00	REFEEDING
62	3	25.21	1.88	0.00	0.00	26.16	2.18	0.55	0.00	25.69	2.03	0.28	0.00	
65	6	21.37	5.65	2.35	0.00	22.88	5.97	3.31	0.00	22.13	5.81	2.83	0.00	
69	10	18.80	6.87	4.87	0.00	19.13	7.14	5.18	0.00	18.97	7.01	5.03	0.00	
73	14	15.22	8.92	8.38	0.00	16.02	10.61	7.93	0.00	15.62	9.77	8.16	0.00	
75	16	11.76	10.21	10.61	0.00	12.31	11.28	11.28	0.00	12.04	10.75	10.95	0.00	
77	18	7.16	11.37	13.85	0.00	10.74	12.36	13.79	0.00	8.95	11.87	13.82	0.00	
79	20	3.75	9.36	15.85	0.00	4.10	7.76	14.84	0.00	3.93	8.56	15.35	0.00	
81	22	0.00	5.63	14.83	0.00	0.00	4.97	15.28	0.00	0.00	5.30	16.06	0.00	
83	24	0.00	2.86	14.80	0.00	0.00	2.28	15.76	0.00	0.00	2.57	16.75	0.00	
85	26	0.00	0.71	14.62	0.00	0.00	0.50	16.14	0.00	0.00	0.61	17.02	0.00	
87	28	0.00	0.00	14.73	0.00	0.00	0.00	16.20	0.00	0.00	0.00	17.14	0.00	

TABLE A-14

Dehalogenation of 2,4-DCP with cultures adapted to 2-CP under 20% CO2 + 80% N2 atmosphere in 0.1% Y.Extract medium

Total-Days	Day	SAMPLE # 1 2,4-DCP (mg/L)	SAMPLE # 1 4-CP (mg/L)	SAMPLE # 1 Phenol	SAMPLE # 2 2,4-DCP (mg/L)	SAMPLE # 2 4-CP (mg/L)	SAMPLE # 2 Phenol	AVERAGE 2,4-DCP (mg/L)	AVERAGE 4-CP (mg/L)	AVERAGE Phenol	
0	0	29.75	0.00	0.00	28.69	0.00	0.00	29.22	0.00	0.00	
4	6	21.11	6.88	0.00	22.41	4.69	0.00	21.76	5.79	0.00	
8	8	15.46	11.62	0.00	16.78	10.06	0.00	16.12	10.84	0.00	
12	12	8.63	14.89	0.00	10.06	14.46	0.00	9.35	14.68	0.00	
14	14	4.75	16.68	0.00	6.64	16.17	0.00	5.70	16.43	0.00	
16	16	1.02	17.14	0.00	2.35	16.89	0.00	1.69	17.02	0.00	WASHING
18	18	0.00	17.20	0.00	0.00	17.24	0.00	0.00	17.22	0.00	AND
20	0	30.45	0.00	0.00	29.86	0.00	0.00	30.16	0.00	0.00	REFEEDING
24	4	20.56	6.07	0.00	21.42	5.78	0.00	20.99	5.93	0.00	
27	7	9.48	12.35	0.00	10.18	12.78	0.00	9.83	12.57	0.00	
30	10	3.50	15.65	0.00	2.87	16.16	0.00	3.19	15.91	0.00	
34	14	0.00	16.29	0.00	0.00	16.75	0.00	0.00	16.52	0.00	WASHING
38	18	0.00	16.35	0.00	0.00	16.60	0.00	0.00	16.48	0.00	AND
39	0	29.27	0.00	0.00	30.15	0.00	0.00	29.71	0.00	0.00	REFEEDING
42	3	23.24	4.17	0.00	24.76	4.37	0.00	24.00	4.27	0.00	
45	6	11.77	11.35	0.00	12.64	10.81	0.00	12.21	11.08	0.00	
48	9	5.42	14.68	0.00	6.76	13.85	0.00	6.09	14.27	0.00	
51	12	1.28	15.37	0.00	2.18	15.19	0.00	1.73	15.28	0.00	
53	14	0.00	16.14	0.00	0.00	16.50	0.00	0.00	16.32	0.00	
55	16	0.00	16.25	0.00	0.00	16.42	0.00	0.00	16.34	0.00	

TABLE A-15

2-CP adapted cultures exposed to 4-CP
under 20% CO2 + 80% N2 atmosphere in 0.1% Y.Extract medium

Total-Days	Day	SAMPLE # 1 4-CP (mg/L)	SAMPLE # 1 Phenol (mg/L)	SAMPLE # 2 4-CP (mg/L)	SAMPLE # 2 Phenol (mg/L)	AVERAGE 4-CP (mg/L)	AVERAGE Phenol (mg/L)	
0	0	30.51	0.00	29.67	0.00	30.09	0.00	
4	4	30.20	0.00	29.60	0.00	29.90	0.00	
7	7	28.89	0.00	29.16	0.00	29.03	0.00	
10	10	28.72	0.00	28.82	0.00	28.77	0.00	
14	14	27.60	0.00	28.15	0.00	27.88	0.00	
18	18	26.65	0.00	27.35	0.00	27.00	0.00	
22	22	26.32	0.00	27.55	0.00	26.94	0.00	
24	24	26.01	0.00	25.49	0.00	25.75	0.00	WASHING
26	26	26.24	0.00	26.02	0.00	26.13	0.00	AND
28	0	29.72	0.00	28.65	0.00	29.19	0.00	REFEEDING
31	3	NA	0.00	NA	0.00	NA	0.00	
34	6	27.76	0.00	28.82	0.00	28.29	0.00	
38	10	27.25	0.00	28.41	0.00	27.83	0.00	
42	14	27.33	0.00	28.06	0.00	27.70	0.00	
44	16	NA	0.00	NA	0.00	NA	0.00	
46	18	27.15	0.00	27.66	0.00	27.41	0.00	
48	20	NA	0.00	NA	0.00	NA	0.00	
50	22	25.42	0.00	26.86	0.00	26.14	0.00	
52	24	NA	0.00	NA	0.00	NA	0.00	
54	26	25.92	0.00	26.14	0.00	26.03	0.00	
56	28	25.29	0.00	26.39	0.00	25.84	0.00	

- Post- Aerobic Treatment -
of 4-CP

Fig. A-1 4-CP removal profile in cultures adapted to 2-CP with dext or phenol

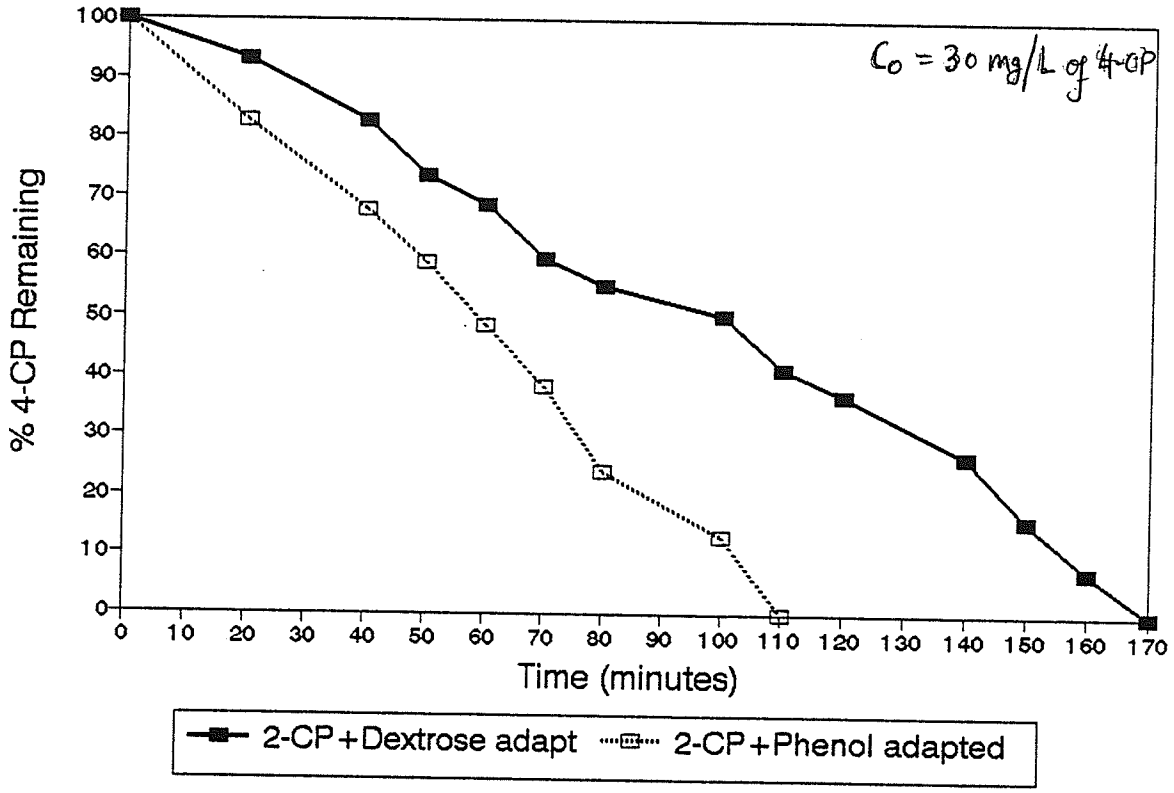

Fig. A-2 Specific 4-CP removal in cultures adapted to 2-CP with Dext or Phenol

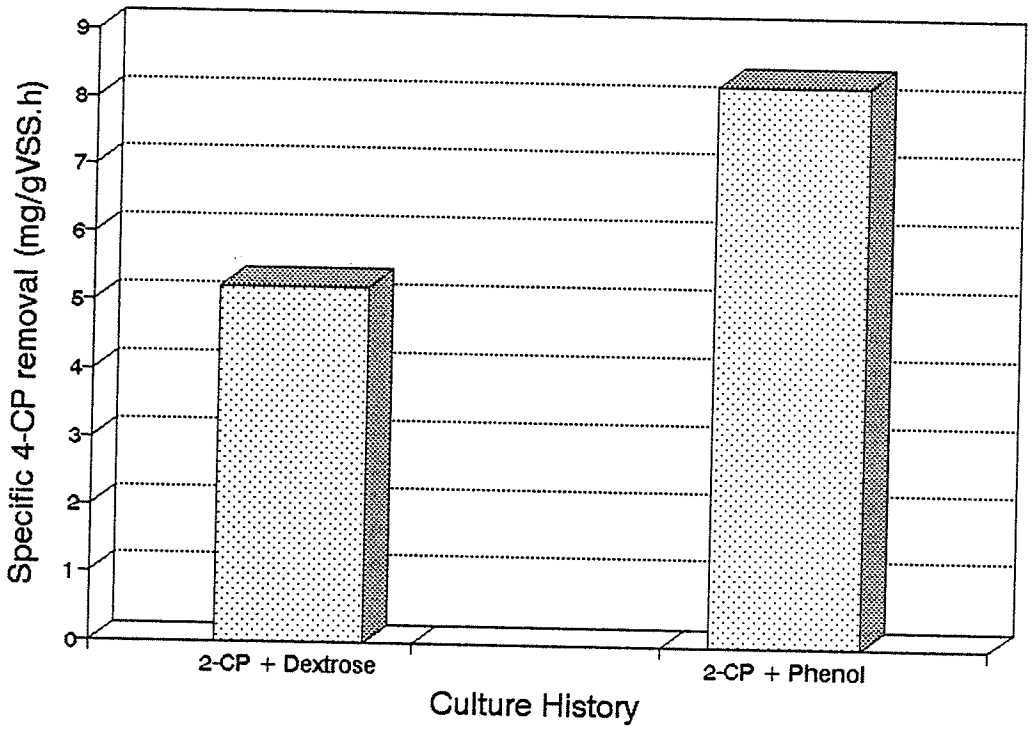

TABLE A-16

Degradation of 4-CP by 2-CP adapted cultures (Aerobic Expts)

Sample No.	Elapsed Time (minutes)	#1 Adapted to 2-CP and :- Dextrose 4-CP (mg/L)	#1 Adapted to 2-CP and :- Phenol 4-CP (mg/L)	#2 Adapted to 2-CP and :- Dextrose 4-CP (mg/L)	#2 Adapted to 2-CP and :- Phenol 4-CP (mg/L)	Average Dextrose 4-CP (mg/L)	Average Phenol 4-CP (mg/L)	Dextrose Syst 4-CP % Remaining	Phen Sys 4-CP % Remaining
1	0	30.35	30.12	29.88	31.35	30.12	30.74	100.00	100.00
2	20	28.72	24.41	27.32	26.61	28.02	25.51	93.03	82.99
3	40	25.15	19.88	24.86	21.73	25.01	20.81	83.02	67.68
4	50	22.97	17.72	21.39	18.57	22.18	18.15	73.64	59.03
5	60	21.39	14.37	19.88	15.44	20.64	14.91	68.51	48.49
6	70	18.64	11.28	17.24	12.16	17.94	11.72	59.56	38.13
7	80	17.06	6.64	16.17	8.10	16.62	7.37	55.16	23.98
8	100	15.31	3.47	14.91	4.54	15.11	4.01	50.17	13.03
10	110	13.14	0.00	11.56	0.00	12.35	0.00	41.00	0.00
11	120	11.97		10.06		11.02	0.00	36.57	0.00
12	140	8.17		7.78		7.98	0.00	26.48	0.00
13	150	4.32		5.14		4.73	0.00	15.70	0.00
14	160	1.95		2.33		2.14	0.00	7.10	0.00
15	170	0.00		0.00		0.00	0.00	0.00	0.00
Avg. VSS (mg/L)		2030	1975	1965	1990				

TABLE A-17

Un-adapted digested sludge (50% vol/vol) exposed to 30 mg/L 2,4,6-TCP; 2,4-DCP and 4-CP (Preliminary experiment to check HPLC program gradient)

Day	Ref	TCP	DCP	4CP	Phenol	2-CP
0	TCP-1	27.65	0.00	0.00	0.00	0.00
6		20.20	3.50	0.85	0.00	0.00
10		15.03	6.80	4.27	0.60	0.00
14		6.48	2.90	14.10	0.50	0.00
18		0.00	0.00	12.02	0.00	0.00
0	TCP-2	29.35	0.00	0.00	0.00	0.00
6		18.64	5.20	0.60	0.00	0.00
10		11.59	7.81	5.55	0.00	0.00
14		4.37	3.12	15.67	0.00	0.00
18		0.00	0.00	13.87	0.00	0.00
0	DCP-1	NA	30.11	0.00	0.00	0.00
6		NA	10.48	6.07	0.00	0.00
10		NA	3.20	15.64	0.00	0.00
14		NA	0.00	14.63	0.00	0.00
18		NA	0.00	13.11	0.00	0.00
0	DCP-2	NA	29.67	0.00	0.00	0.00
6		NA	9.21	6.88	0.00	0.00
10		NA	2.87	14.37	0.00	0.00
14		NA	0.00	15.28	0.00	0.00
18		NA	0.00	14.64	0.00	0.00
0	4CP-1	NA	NA	28.35	0.00	NA
6		NA	NA	26.48	0.00	NA
10		NA	NA	26.16	0.00	NA
14		NA	NA	24.42	0.00	NA
18		NA	NA	24.45	0.00	NA
25		NA	NA	22.37	0.00	NA
30		NA	NA	22.89	0.00	NA
0	4CP-2	NA	NA	30.35	0.00	NA
6		NA	NA	28.69	0.00	NA
10		NA	NA	27.33	0.00	NA
14		NA	NA	27.45	0.00	NA
18		NA	NA	25.68	0.00	NA
25		NA	NA	26.37	0.00	NA
30		NA	NA	24.91	0.00	NA

All concentrations in mg/L of respective compounds

BATCH EXPERIMENT # 1: Comparison of 2-CP degradation under methanogenic and sulfidogenic conditions

TABLE A - 18

BE#1(1) Methanogenic Cultures (70% vol/vol inocula) under CO2 + N2 headspace
No Added Sulphate (Background SO4 = 0.8 mM)

Day	2-CP (mg/L) M 1	M 2	M 3	PHENOL (mg/L) M 1	M 2	M 3	Benz(mg/L) M1	M2	M3	AVERAGE 2-CP	Phenol	% OCP M
0	47.76	48.88	51.07	0.00	0.00	0.00	ND	ND	ND	49.24	0.00	100.00
8	41.84	42.34	45.12	0.00	0.54	0.81	ND	ND	ND	43.10	0.45	87.53
10	30.32	33.02	35.69	3.85	4.61	5.86	ND	ND	ND	33.01	4.77	67.04
12	18.23	17.62	20.22	10.22	11.64	8.16	ND	ND	ND	18.69	10.01	37.96
15	10.54	8.87	12.32	13.06	14.12	12.61	4.56	3.87	4.25	10.58	13.26	21.48
17	2.65	3.01	4.44	13.13	14.66	14.58	2.15	1.83	2.76	3.37	14.12	6.84
19	0.00	0.00	0.00	5.92	3.78	4.32	ND	ND	ND	0.00	4.67	0.00
21	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	0.00	0.00	0.00

BE#1(2) Sulfidogenic Cultures (70% vol/vol inocula) under CO2 + N2 headspace
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.8 mM)

DAY	2-CP S 1	S 2	S 3	PHENOL S 1	S 2	S 3	Benz(mg/L) S1	S2	S3	SO4(mg/L) S 1	S 2	S 3	AVERAGE 2-CP	Phenol	SO4	% 2-CP	% SO4
0	49.81	46.54	48.87	0.00	0.00	0.00	ND	ND	ND	490.32	486.88	495.65	48.41	0.00	490.95	100.00	100.00
8	44.57	42.31	44.91	1.06	0.77	0.94	ND	ND	ND	423.35	408.66	428.35	43.93	0.92	420.12	90.75	85.57
10	34.59	35.76	36.84	5.08	5.47	5.28	ND	ND	ND	NA	NA	NA	35.73	5.28	NA	73.81	NA
12	20.49	21.44	20.32	11.59	12.75	12.43	ND	ND	ND	364.21	345.80	358.25	20.75	12.26	356.09	42.87	72.53
15	11.12	10.08	9.64	15.08	15.37	14.67	3.32	2.64	1.78	328.22	310.25	316.62	10.28	15.04	318.36	21.24	64.85
17	2.76	3.76	3.44	14.58	14.89	15.67	ND	ND	ND	298.55	280.75	276.28	3.32	15.05	285.19	6.86	58.09
19	0.00	0.00	0.00	4.11	4.82	5.73	ND	ND	ND	265.45	256.38	245.04	0.00	4.89	255.62	0.00	52.07
21	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	250.88	238.26	230.18	0.00	0.00	239.77	0.00	48.84
23	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	0.00	0.00	NA	0.00	NA
25	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	0.00	0.00	NA	0.00	NA
26	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	230.71	222.48	215.69	0.00	0.00	222.98	0.00	45.41

BE#1(3) Sulfidogenic Cultures (70% vol/vol inocula) with 6 mM Fe(II) under CO2 + N2 headspace
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.8 mM)

DAY	2-CP F 1	F 2	F 3	PHENOL F 1	F 2	F 3	Benz(mg/L) F1	F2	F3	SO4 F 1	F 2	F 3	AVERAGE 2-CP	Phenol	SO4	% OCP	% SO4
0	50.25	51.79	48.81	0.00	0.00	0.00	ND	ND	ND	502.21	524.88	514.55	50.28	0.00	513.88	100.00	100.00
8	46.02	47.75	46.37	0.00	0.00	0.00	ND	ND	ND	412.65	420.10	402.17	46.71	0.00	411.64	92.91	80.10
10	41.23	40.54	40.78	2.79	4.11	4.47	ND	ND	ND	NA	NA	NA	40.84	3.79	NA	81.23	NA
12	34.47	35.25	36.83	8.54	7.81	8.93	ND	ND	ND	338.42	362.69	346.86	35.52	8.43	349.32	70.64	67.98
15	27.66	24.39	25.54	12.33	11.84	13.86	ND	ND	ND	294.53	318.66	304.35	25.86	12.61	305.85	51.44	59.52
17	15.88	16.31	14.57	15.79	16.26	17.08	1.17	2.47	2.68	275.89	282.47	265.12	15.59	16.38	274.49	31.00	53.42
19	10.33	10.14	8.89	12.28	13.32	13.27	6.78	5.14	4.22	245.37	258.25	254.06	9.79	12.96	252.56	19.48	49.15
21	5.94	5.32	4.78	7.33	6.97	8.63	ND	ND	ND	232.16	237.38	228.77	5.35	7.64	232.77	10.63	45.30
23	2.36	2.06	1.32	5.27	4.34	3.82	ND	ND	ND	NA	NA	NA	1.91	4.48	NA	3.81	NA
25	0.00	0.00	0.00	1.69	1.82	1.06	ND	ND	ND	NA	NA	NA	0.00	1.52	NA	0.00	NA
26	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	210.75	222.38	212.33	0.00	0.00	215.15	0.00	41.87

BE#1(4) Sterile Controls (70% vol/vol inocula) under CO2 + N2 headspace
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.8 mM)

DAY	2-CP S 1	S 2	S 3	PHENOL S 1	S 2	S 3	Benz(mg/L) S 1	S 2	S 3	SO4 S 1	S 2	S 3	AVERAGE 2-CP	PHENOL	SO4	% 2-CP	% SO4
0	47.45	49.22	48.39	0.00	0.00	0.00	ND	ND	ND	508.68	517.65	482.27	48.35	0.00	502.87	100.00	100.00
8	47.06	48.10	48.21	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	47.79	0.00	NA	98.84	NA
10	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
12	46.68	48.00	NA	0.00	0.00	0.00	ND	ND	ND	512.74	NA	480.47	47.34	0.00	496.61	97.91	98.75
15	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	46.50	NA	47.42	0.00	0.00	0.00	ND	ND	ND	500.89	510.27	480.28	46.96	0.00	497.15	97.13	98.88
19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	46.28	47.58	47.36	0.00	0.00	0.00	ND	ND	ND	504.62	511.90	481.60	47.07	0.00	499.34	97.36	99.30
23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	46.18	47.24	47.15	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	46.68	0.00	NA	96.91	NA
26	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
28	45.29	46.06	45.88	0.00	0.00	0.00	ND	ND	ND	506.2	512.69	480.48	45.74	0.00	500.48	94.61	99.52

BE# 1 Head-Space CH4 composition (Average of three samples)

Day	M	S	F	S-Control
0	0.00	0.00	0.00	0.00
8	15.00	14.80	12.45	0.00
10	NA	NA	NA	NA
12	NA	NA	NA	NA
15	27.90	27.40	20.30	0.00
17	NA	NA	NA	NA
19	33.90	32.10	27.60	0.00
21	NA	NA	NA	NA
23	NA	NA	NA	NA
25	NA	NA	NA	NA
26	37.80	36.50	32.74	0.00
28	NA	NA	NA	NA

NA = Not Analyzed

ND = Not Detected

BATCH EXPERIMENT # 2: Comparison of 2-CP degradation under various methanogenic and sulfidogenic conditions.

TABLE A-19

BE#2(1) Methanogenic Cultures (70% vol/vol inocula) under CO2 + N2 headspace
No Added Sulphate (Background SO4 = 0.8 mM)

(a)

DAY	2-CP (mg/L) M 1	M 2	M 3	PHENOL (mg/L) M 1	M 2	M 3	Benz(mg/L) M 1	M 2	M 3	AVERAGE 2-CP	PHENOL	% 2-CP M
0	48.27	55.10	52.91	0.00	0.00	0.00	ND	ND	ND	51.43	0.00	100.00
7	42.26	47.71	46.34	0.00	0.96	0.00	ND	ND	ND	45.44	0.32	88.35
9	33.76	36.21	38.96	2.47	6.61	4.23	ND	ND	ND	36.31	4.44	70.59
12	20.23	23.52	21.87	8.85	10.62	7.85	3.21	ND	4.56	21.87	9.11	42.53
15	12.85	15.64	13.36	11.34	14.39	11.37	ND	ND	ND	13.95	12.37	27.12
17	4.23	6.79	5.18	15.67	16.89	15.26	1.87	2.72	6.35	5.40	15.94	10.50
19	0.00	0.00	0.00	7.86	8.81	6.54	ND	ND	ND	0.00	7.74	0.00
21	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	0.00	0.00	0.00

BE#2(2) Sulfidogenic Cultures (70% vol/vol inocula) under CO2 + N2 headspace
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.8 mM)

(b)

DAY	2-CP S 1	S 2	S 3	PHENOL S 1	S 2	S 3	Benz(mg/L) S 1	S 2	S 3	SO4 S 1	S 2	S 3	AVERAGE 2-CP	PHENOL	SO4	% 2-CP	% SO4
0	51.88	47.08	49.48	0.00	0.00	0.00	ND	ND	ND	522.34	497.62	516.66	49.48	0.00	512.21	100.00	100.00
7	45.87	43.91	43.62	0.55	0.00	1.22	ND	ND	ND	428.89	401.05	407.47	44.47	0.59	412.47	89.87	80.53
9	37.88	33.95	35.79	6.46	7.42	6.89	ND	ND	ND	409.58	375.64	370.28	35.87	6.92	385.17	72.50	75.20
12	25.64	22.18	23.69	14.36	17.49	15.54	ND	ND	ND	387.54	341.97	358.71	23.84	15.80	362.74	46.17	70.82
15	15.64	12.71	10.93	16.37	18.91	16.72	ND	ND	ND	345.61	318.44	325.06	13.09	17.33	329.70	26.46	64.37
17	3.68	6.12	5.08	10.29	12.99	12.21	5.66	7.99	4.11	300.17	280.55	293.61	4.96	11.83	291.44	10.02	56.90
19	0.00	0.00	0.00	3.42	4.14	3.86	ND	ND	ND	NA	NA	NA	0.00	3.81	NA	0.00	NA
21	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	272.45	254.16	265.44	0.00	0.00	264.02	0.00	51.54
23	NA	NA	NA	NA	NA	NA	ND	ND	ND	265.34	241.06	254.32	NA	NA	253.57		49.51
25	NA	NA	NA	NA	NA	NA	ND	ND	ND	NA	NA	NA	NA	NA	NA		NA
27	NA	NA	NA	NA	NA	NA	ND	ND	ND	260.59	247.26	248.85	NA	NA	252.23		49.24

BE#2(3) Methanogenic Cultures (70% vol/vol inocula) with 6 mM Fe(II) under CO2 + N2 headspace
No Added Sulphate (Background SO4 = 0.8 mM)

(c)

DAY	2-CP (mg/L) MF 1	MF 2	MF 3	PHENOL (mg/L) MF 1	MF 2	MF 3	Benz(mg/L) MF 1	MF 2	MF 3	AVERAGE 2-CP	PHENOL	% 2-CP M
0	48.74	46.32	51.34	0.00	0.00	0.00	ND	ND	ND	48.80	0.00	100.00
7	45.02	44.70	47.34	0.00	0.00	0.54	ND	ND	ND	45.69	0.18	93.62
9	40.23	38.54	37.64	1.79	3.21	4.47	ND	ND	ND	38.80	3.16	79.52
12	32.17	30.25	29.83	7.22	6.87	7.67	ND	ND	ND	30.75	7.25	63.01
15	23.94	21.49	20.36	12.13	11.54	12.94	ND	ND	ND	21.93	12.20	44.94
17	15.88	16.31	14.57	16.49	17.26	18.66	ND	ND	ND	15.59	17.47	31.94
19	8.33	9.14	7.89	11.28	12.37	11.12	6.87	7.93	8.22	8.45	11.59	17.32
21	4.94	5.48	4.67	7.03	7.42	6.68	3.42	2.65	ND	5.03	7.04	10.31
23	0.00	0.00	0.00	5.21	4.64	2.91	ND	ND	ND	0.00	4.25	0.00
25	0.00	0.00	0.00	2.69	2.82	0.00	ND	ND	ND	0.00	1.84	0.00
27	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	0.00	0.00	0.00

BE#2(4) Sulfidogenic Cultures (70% vol/vol inocula) with 6 mM Fe(II) under CO2 + N2 headspace
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.8 mM)

(d)

DAY	2-CP F 1	F 2	F 3	PHENOL F 1	F 2	F 3	Benz(mg/L) F 1	F 2	F 3	SO4 F 1	F 2	F 3	AVERAGE 2-CP	PHENOL	SO4	% 2-CP	% SO4
0	46.67	48.82	47.61	0.00	0.00	0.00	ND	ND	ND	525.67	505.75	493.78	47.70	0.00	508.40	100.00	100.00
7	43.06	44.04	43.06	0.00	0.00	0.00	ND	ND	ND	412.81	420.57	405.26	43.39	0.00	412.88	86.29	81.21
9	38.24	36.82	38.53	1.48	3.12	2.96	ND	ND	ND	380.67	389.21	365.88	37.86	2.52	378.59	75.30	74.47
12	32.25	30.71	33.22	6.35	7.46	5.13	ND	ND	ND	340.27	358.71	342.38	32.06	6.31	347.12	63.76	68.28
15	27.66	23.67	24.82	10.81	9.67	10.02	ND	ND	ND	306.41	325.04	302.53	25.38	10.17	311.99	50.48	61.37
17	18.89	17.35	15.49	12.29	13.14	12.87	ND	ND	ND	280.74	305.10	278.65	17.24	12.77	288.16	34.29	56.68
19	12.66	10.97	9.09	12.99	13.01	12.68	5.87	3.64	4.43	NA	NA	NA	10.91	12.89	NA	21.69	NA
21	5.27	4.91	3.65	7.61	7.42	6.28	ND	ND	ND	248.35	260.39	238.56	4.61	7.10	249.10	9.17	49.00
23	0.00	0.00	0.00	1.15	2.03	0.97	ND	ND	ND	232.23	250.52	225.80	0.00	1.38	236.18	0.00	46.46
25	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	0.00	0.00	NA	0.00	NA
27	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	220.23	230.11	198.8	0.00	0.00	216.38	0.00	42.56

NA = Not Analyzed

ND = Not Detected

TABLE A-19 (.... contd)

BE#2(5) Background cultures under methanogenic and sulfidogenic

(e)

	SO4 (mg/L)					2-CP(mg/L)				Phenol or Benz(mg/L)			
	BM-1	BM-2	BS-1	BS-2	Avg(SO4)	BM-1	BM-2	BS-1	BS-2	BM-1	BM-2	BS-1	BS-2
0	62.40	70.60	516.45	505.37	510.91	ND	ND	ND	ND	ND	ND	ND	ND
7	54.34	60.33	438.36	411.77	425.07	ND	ND	NA	ND	ND	ND	NA	ND
9	47.62	52.79	404.15	394.82	399.49	NA	NA	NA	NA	NA	NA	NA	NA
12	NA	NA	NA	NA	NA	NA	ND	ND	NA	NA	ND	ND	NA
15	38.54	40.79	351.74	338.12	344.93	ND	ND	ND	ND	ND	ND	ND	ND
17	32.36	38.84	305.65	297.91	301.78	ND	NA	ND	NA	ND	NA	ND	NA
19	NA	NA	NA	NA	NA	ND	NA	ND	NA	ND	NA	ND	NA
21	28.82	38.56	285.64	271.17	278.41	ND	ND	ND	ND	ND	ND	ND	ND
23	28.64	38.54	278.64	261.54	270.09	NA	NA	NA	NA	NA	NA	NA	NA
25	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
27	28.18	38.87	260.22	254.37	257.30	NA	NA	NA	NA	NA	NA	NA	NA

NA = Not Analyzed
ND = Not Detected

BE# 2 Head-Space CH4 composition (Average)

(f)

Day	M	S	MF	F	B/G
0	0.00	0.00	0.00	0.00	0.00
7	NA	NA	NA	NA	
9	20.20	18.86	16.50	15.10	22.00
12	NA	NA	NA	NA	
15	31.20	29.33	25.10	25.80	28.18
17	NA	NA	NA	NA	
19	38.35	37.71	32.22	30.20	36.75
21	NA	NA	NA	NA	
23	40.47	40.05	34.60	32.80	38.95

TABLE A-20

BE#3(1) Methanogenic Cultures (70% vol/vol inocula) under CO2 + N2 headspace
No Added Sulphate (Background SO4 = 0.75 mM)

(a)

DAY	2-CP (mg/L) M 1	2-CP (mg/L) M 2	2-CP (mg/L) M 3	PHENOL (mg/L) M 1	PHENOL (mg/L) M 2	PHENOL (mg/L) M 3	Benz(mg/L) M 1	Benz(mg/L) M 2	Benz(mg/L) M 3	AVERAGE 2-CP	AVERAGE PHENOL	% 2-CP M
0	51.66	47.91	46.89	0.00	0.00	0.00	ND	ND	ND	48.82	0.00	100.00
8	43.76	40.84	38.59	1.07	1.18	1.62	ND	ND	ND	41.06	1.29	84.11
10	34.76	32.84	31.74	5.63	4.82	5.19	ND	ND	ND	33.11	5.21	67.83
12	23.53	25.22	22.73	8.06	9.67	7.38	ND	ND	ND	23.83	8.37	48.81
14	14.67	15.37	13.79	13.06	14.00	12.88	1.27	3.31	3.65	14.61	13.31	29.93
16	3.78	5.93	4.82	13.13	13.81	14.66	ND	ND	ND	4.84	13.87	9.92
18	0.00	0.00	0.00	4.48	3.87	5.63	ND	ND	ND	0.00	4.66	0.00
20	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	0.00	0.00	0.00

BE#3(2) Sulfidogenic Cultures (70% vol/vol inocula) under CO2 + N2 headspace
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.75 mM)

(b)

DAY	2-CP S 1	2-CP S 2	2-CP S 3	PHENOL S 1	PHENOL S 2	PHENOL S 3	Benz(mg/L) S 1	Benz(mg/L) S 2	Benz(mg/L) S 3	SO4 S 1	SO4 S 2	SO4 S 3	AVERAGE 2-CP	AVERAGE PHENOL	AVERAGE SO4	% 2-CP	% SO4
0	46.94	47.76	50.86	0.00	0.00	0.00	ND	ND	ND	504.48	474.62	487.47	49.19	0.00	488.86	100.00	100.00
8	43.28	44.89	45.19	1.27	0.76	0.82	ND	ND	ND	447.38	424.22	418.67	44.45	0.95	430.09	90.37	87.98
10	36.73	33.25	37.15	5.21	7.42	6.89	ND	ND	ND	410.66	398.20	385.03	35.71	6.51	397.96	72.60	81.41
12	26.24	25.49	23.84	14.36	17.49	15.54	ND	ND	ND	376.28	360.14	355.49	25.19	15.80	363.97	51.21	74.45
14	15.32	13.81	12.55	16.37	18.91	16.72	ND	ND	ND	NA	NA	NA	13.89	17.33	NA	28.24	NA
16	4.95	4.47	5.12	10.29	12.99	12.21	ND	ND	ND	346.06	326.22	335.97	4.85	11.83	336.08	9.85	68.75
18	0.00	0.00	0.00	3.42	4.14	3.86	1.23	2.34	2.55	NA	NA	NA	0.00	3.81	NA	0.00	NA
20	0.00	0.00	0.00	0.00	0.00	0.00	ND	ND	ND	326.65	306.29	316.44	0.00	0.00	316.46	0.00	64.73
23	NA	NA	NA	NA	NA	NA	ND	ND	ND	311.88	290.47	300.02	NA	NA	300.79	NA	61.53
25	NA	NA	NA	NA	NA	NA	ND	ND	ND	304.19	281.42	292.55	NA	NA	292.72	NA	59.88

BE#3(3) Methanogenic Cultures (70% vol/vol inocula) with 10 mM BESA under CO2 + N2 headspace
No Added Sulphate (Background SO4 = 0.7 mM)

(c)

DAY	2-CP (mg/L) BESA 1	2-CP (mg/L) BESA 2	2-CP (mg/L) BESA 3	PHENOL (mg/L) BESA 1	PHENOL (mg/L) BESA 2	PHENOL (mg/L) BESA 3	Benz(mg/L) BESA 1	Benz(mg/L) BESA 2	Benz(mg/L) BESA 3	AVERAGE 2-CP	AVERAGE PHENOL	% 2-CP M	% 2-CP mM
0	47.83	46.61	48.72	0.00	0.00	0.00	ND	ND	ND	47.72	0.00	100.00	0.371
8	45.91	44.32	45.87	0.82	0.72	0.55	ND	ND	ND	45.37	0.70	95.07	0.353
10	42.85	41.71	42.21	2.86	2.30	2.28	ND	ND	ND	42.26	2.48	88.55	0.329
12	38.28	37.51	37.85	4.87	5.12	4.46	ND	ND	ND	37.88	4.82	79.38	0.295
14	32.66	30.11	29.95	6.88	7.25	7.03	ND	ND	ND	30.91	7.05	64.77	0.241
16	26.11	25.12	25.66	10.14	11.32	10.76	ND	ND	ND	25.63	10.74	53.71	0.199
18	22.73	20.54	21.81	13.91	13.74	12.81	ND	ND	ND	21.69	13.49	45.46	0.169
20	18.54	14.73	14.92	16.64	17.18	17.43	ND	ND	ND	16.06	17.08	33.66	0.125
23	13.77	10.88	11.32	18.89	19.12	19.37	ND	ND	ND	11.99	19.13	25.13	0.093
25	8.77	6.84	6.97	21.05	20.96	21.58	3.45	4.76	3.93	7.53	21.20	15.77	0.059
27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	5.32	4.26	4.48	24.97	23.06	24.46	3.23	3.72	2.49	4.69	24.16	9.82	0.036
32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
34	3.81	3.11	3.26	25.48	25.01	24.85	ND	ND	ND	3.39	25.11	7.11	0.026
36	3.38	3.18	3.12	24.89	23.36	24.33	ND	ND	ND	3.23	24.19	6.76	0.025
38	3.25	3.4	3.11	24.17	22.34	23.96	ND	ND	ND	3.25	23.49	6.82	0.025
40	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

TABLE A-20C... contd)

BE#3(4) Sulfidogenic Cultures (70% vol/vol inocula) with 20 mM Molybdate under CO2 + N2 headspace
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.75 mM)

	2-CP			PHENOL			Benz(mg/L)			SO4			AVERAGE				
DAY	Moly 1	Moly 2	Moly 3	Moly 1	Moly 2	Moly 3	Moly 1	Moly 2	Moly 3	Moly 1	Moly 2	Moly 3	2-CP	PHENOL	SO4	% 2-CP	% SO4
0	49.74	51.49	47.95	0.00	0.00	0.00	ND	ND	ND	485.66	492.27	472.74	49.73	0.00	483.56	100.00	100.00
8	48.39	49.43	47.12	0.00	0.00	0.00	ND	ND	ND	483.88	490.65	474.69	48.31	0.00	483.07	97.15	99.90
10	48.06	49.24	48.32	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	48.54	0.00	NA	97.61	NA
12	NA	NA	NA	NA	NA	NA	NA	NA	NA	485.71	489.87	468.76	NA	NA	481.45	NA	99.56
14	47.19	48.86	45.48	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	47.18	0.00	NA	94.87	NA
16	NA	NA	NA	NA	NA	NA	NA	NA	NA	480.66	491.22	470.36	NA	NA	480.75	NA	99.42
18	46.72	49.23	43.06	0.00	0.00	0.00	ND	ND	ND	NA	NA	NA	46.34	0.00	NA	93.18	NA
20	NA	NA	NA	NA	NA	NA	NA	NA	NA	482.15	486.38	465.10	NA	NA	477.88	NA	98.82
23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	46.37	47.95	44.16	0.00	0.00	0.00	ND	ND	ND	478.76	488.15	467.51	46.16	0.00	478.14	92.82	98.88
27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
34	45.02	46.75	43.11	0.00	0.00	0.00	ND	ND	ND	474.02	482.49	460.66	44.96	0.00	472.39	90.41	97.69
36	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
38	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
40	45.31	46.00	41.89	0.00	0.00	0.00	ND	ND	ND	476.25	480.19	465.34	44.40	0.00	473.93	89.28	98.01

(d)

BE#3(5) Methanogenic Cultures (70% vol/vol inocula) with 20 mM Molybdate under CO2 + N2 headspace
No Added Sulphate (Background SO4 = 0.75 mM)

	2-CP			PHENOL			Benz(mg/L)			AVERAGE		
DAY	M-Moly 1	M-Moly 2	M-Moly 3	M-Moly 1	M-Moly 2	M-Moly 3	M-Moly 1	M-Moly 2	M-Moly 3	2-CP	PHENOL	% 2-CP
0	50.56	48.25	47.95	0.00	0.00	0.00	ND	ND	ND	48.92	0.00	100.00
8	49.14	48.13	47.12	0.00	0.00	0.00	ND	ND	ND	48.13	0.00	96.78
10	48.56	48.27	48.32	0.00	0.00	0.00	ND	ND	ND	48.38	0.00	97.29
12	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
14	48.16	47.85	47.58	0.00	0.00	0.00	ND	ND	ND	47.86	0.00	96.25
16	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
18	47.62	47.91	47.14	0.00	0.00	0.00	ND	ND	ND	47.56	0.00	95.63
20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	47.31	47.45	46.86	0.00	0.00	0.00	ND	ND	ND	47.21	0.00	94.93
27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
32	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
34	45.78	47.23	46.26	NA	NA	NA	NA	NA	NA	46.42	NA	93.35
36	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
38	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
40	45.02	46.75	46.03	0.00	0.00	0.00	ND	ND	ND	45.93	0.00	92.37

(e)

TABLE A-20 (... contd)

CH4 Data (Based on average of three samples)

DAY	M	S	BESA	MOLY	M-MOLY
0	0	0	0	0	0
8	24	22	6	18	20
12	34	32	10	31	33
16	38	37	12	34	35
20	40	39	15	36	37
23	41	40	17	37	38
30			20	39	39
34			22	41	40
38			23	42	41
40			23	42	43

(f)

Sterile Controls in Batch 3

Day		2-CP		Average (mg/L)	mM
0	49.35	47.87	50.35	49.19	0.383
8	48.82	47.72	49.21	48.58	0.378
10	NA	NA	NA	NA	NA
12	47.71	47.06	48.91	47.89	0.373
14	NA	NA	NA	NA	NA
16	48.55	47.52	47.65	47.91	0.373
18	NA	NA	NA	NA	NA
20	NA	NA	NA	NA	NA
23	46.81	46.95	47.42	47.06	0.366
25	NA	NA	NA	NA	NA
27	46.55	47.17	47.08	46.93	0.365
30	NA	NA	NA	NA	NA
32	46.25	47.08	46.85	46.73	0.364
34	NA	NA	NA	NA	NA
36	45.98	47.23	46.38	46.53	0.362
38	NA	NA	NA	NA	NA
40	46.37	47.81	46.41	46.86	0.365

(g)

TABLE A-21

BATCH EXPERIMENT # 4 : Effect of BESA and Molybdate on cultures pre-exposed to 2-CP

Methanogenic Cultures (70% vol/vol) inocula under CO2 + N2 headspace
No Added Sulphate (Background SO4 = 0.65 mM)

(a)

Day	Cum Day	# 1 2-CP	# 2 2-CP	# 3 2-CP	# 4 2-CP	# 5 2-CP	# 6 2-CP	# 1 Phenol	# 2 Phenol	# 3 Phenol	# 4 Phenol	# 5 Phenol	# 6 Phenol	AVERAGE 2-CP	AVERAGE Phenol	% 2-CP
0	0	48.27	51.27	50.23	47.66	48.78	49.04	0.00	0.00	0.00	0.00	0.00	0.00	48.88	0.00	100.00
7	7	38.33	NA	40.63	NA	NA	41.57	0.81	NA	1.25	NA	NA	1.07	40.24	1.04	82.33
10	10	NA	31.07	NA	28.49	32.81	30.28	NA	5.85	NA	7.94	6.42	5.27	30.66	6.37	62.73
12	12	18.23	20.95	20.22	17.62	21.07	18.63	7.95	8.95	9.41	10.62	11.27	9.88	19.45	9.68	39.80
14	14	7.05	10.13	NA	6.94	8.36	NA	12.67	13.59	NA	13.16	14.39	NA	8.12	13.50	16.61
16	16	0.00	0.00	0.00	0.00	0.00	0.00	4.73	6.35	5.88	NA	5.71	6.08	0.00	5.75	0.00
18	18	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
0	19	49.37	47.83	48.94	52.25	50.22	48.45	0.00	0.00	0.00	0.00	0.00	0.00	49.51	0.00	100.00
4	23	NA	NA	41.23	44.18	40.01	38.76	NA	NA	4.38	5.20	6.31	6.17	41.05	0.00	82.90
7	26	22.55	20.97	21.46	NA	18.62	24.49	13.11	14.71	11.88	NA	12.57	13.71	21.62	11.00	43.66
9	28	11.17	9.76	10.33	10.21	8.79	NA	15.26	18.31	15.91	17.37	20.87	NA	10.05	13.20	20.30
11	30	5.47	NA	5.47	5.12	NA	3.34	6.31	NA	8.16	7.87	NA	9.22	4.85	7.89	9.80
13	32	0.00	0.00	0.00	0.00	0.00	0.00	0.87	1.22	1.76	1.03	2.49	2.18	0.00	1.59	0.00
15	34	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

A29

These bottles were divided into three groups containing duplicate samples as M, BESA and Moly. Sample M 1 and M 2 continued to receive 2-CP and yeast extract medium as usual while samples B 1 and B 2 received 10 mM BESA, and samples Moly 1 and Moly 2 received 20 mM Molybdate respectively on day 38 besides 2-CP and yeast extract medium.

(b)

Day	Cum Day	M 1 2-CP	M 2 2-CP	B 1 2-CP	B 2 2-CP	MOLY 1 2-CP	MOLY 2 2-CP	M 1 Phenol	M 2 Phenol	BESA 1 Phenol	BESA 2 Phenol	MOLY 1 Phenol	MOLY 2 Phenol
0	35	48.11	51.32	48.86	46.79	51.58	47.19	0.00	0.00	0.00	0.00	0.00	0.00
4	39	34.12	35.76	43.66	42.81	NA	NA	6.01	6.87	0.65	0.65	NA	NA
7	42	10.76	12.19	37.08	38.76	49.43	46.37	18.44	20.06	5.22	3.38	0.00	0.00
9	44	2.38	3.64	30.34	33.21	47.28	45.24	21.94	23.76	7.17	5.91	0.00	0.00
11	46	0.00	0.00	23.75	25.61	NA	NA	6.20	7.37	12.32	10.25	NA	NA
13	48	0.00	0.00	16.67	18.46	46.74	45.09	0.00	0.00	15.14	13.38	0.00	0.00
18	53			13.31	15.56	NA	NA			18.23	16.84	NA	NA
20	55			10.03	11.91	44.33	46.17			20.28	20.42	0.00	0.00
22	57			7.87	9.56	NA	NA			22.74	20.97	NA	NA
24	59			4.03	6.12	46.25	46.77			23.12	22.31	0.00	0.00
26	61			3.18	4.26	NA	NA			23.87	22.79	NA	NA
31	66			3.27	4.18	46.42	45.19			21.91	20.45	0.00	0.00
40	75			3.30	4.20	46.81	46.36			20.88	20.07	0.00	0.00

AVERAGE VALUES

Sample M 2-CP	Sample M Phenol	Sample B 2-CP	Sample B Phenol	Sample MOLY 2-CP	Sample MOLY Phenol
49.72	0.00	47.83	0.00	49.39	0.00
34.94	6.44	43.24	0.75	NA	NA
11.48	19.25	37.92	4.30	47.90	0.00
3.01	22.85	31.78	6.54	46.26	0.00
0.00	6.79	24.68	11.29	NA	NA
0.00	0.00	17.67	14.26	45.92	0.00
		14.44	17.54	NA	NA
		10.97	20.35	45.25	0.00
		8.72	21.86	NA	NA
		5.08	22.72	46.51	0.00
		3.72	23.33	NA	NA
		3.73	21.18	45.81	0.00
		3.75	20.48	46.59	0.00

% 2-CP Remaining

M	BESA	Moly
100.00	100.00	100.00
70.27	90.39	NA
23.08	79.28	96.98
6.05	66.43	93.66
0.00	51.60	NA
0.00	36.93	92.96
	30.18	NA
	22.94	91.62
	16.22	NA
	10.61	94.17
	7.76	NA
	7.79	92.74
	7.84	94.32

BATCH EXPERIMENT # 5

TABLE A-22

METHANOGENIC SAMPLES

BE# 5(1) Methanogenic Cultures (70% vol/vol) under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.7 2 mM)

	CH4 (%)				Acetate (mg/L)				Propionate (mg/L)				Total VFA (mg/L)			
DAY	M 1	M 2	M 3	AVG	M 1	M 2	M 3	AVG	M 1	M 2	M 3	AVG	M 1	M 2	M 3	AVG
0	0	0	0	0.00	8.64	12.31	10.21	10.39	3.20	4.85	3.76	3.94	11.84	17.16	13.97	14.32
4	24	27	28	26.33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	28	30	31	29.67	12.88	14.32	15.6	14.27	0.00	0.00	0.00	0.00	15.52	16.13	17.22	16.29
8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	34	36	38	36.00	8.37	NA	10.71	9.54	1.12	NA	0.85	0.99	10.37	NA	12.55	11.46
13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	38	40	43	40.33	5.13	6.26	NA	5.70	0.00	0.00	0.00	0.00	6.67	7.42	NA	7.05
17	40	39	41	40.00	6.93	5.85	4.63	5.80	0.51	1.03	2.21	1.25	8.28	7.64	8.86	8.26
19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(a)

BATCH EXPERIMENT # 5

BE# 5(2) Methanogenic Cultures (70% vol/vol) with 1 mM BESA under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.7 2 mM)

		CH4 (%)				Acetate (mg/L)				Propionate (mg/L)				Total VFA (mg/L)			
DATE	DAY	BESA 1A	BESA 1B	BESA 1C	AVG	BESA 1A	BESA 1B	BESA 1C	AVG	BESA 1A	BESA 1B	BESA 1C	AVG	BESA 1A	BESA 1B	BESA 1C	AVG
	0	0	0	0	0.00	7.14	9.39	11.76	9.43	1.20	2.98	3.15	2.44	8.38	11.94	12.21	10.84
	4	19	21	23	21.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	6	24	26	28	26.00	109.80	88.95	96.24	98.33	0.64	1.36	1.81	1.27	117.36	101.55	105.89	108.27
	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	11	27	28	31	28.67	297.22	254.10	NA	275.66	7.59	3.86	NA	5.73	308.71	364.58	NA	336.65
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	15	34	33	35	34.00	354.31	376.69	382.83	371.28	10.54	4.66	6.57	7.26	376.24	390.05	397.48	387.92
	17	37	37	38	37.33	445.30	NA	454.10	449.70	3.06	NA	1.78	2.42	467.73	NA	474.18	470.96
	19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	21	38	37	39	38.00	630.90	588.17	610.77	609.54	2.82	3.47	1.94	2.74	660.90	611.62	632.35	634.96
	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	25	38	39	40	39.00	691.98	678.69	NA	685.34	4.74	2.27	NA	3.51	724.98	706.57	NA	715.78

(b)

BATCH EXPERIMENT # 5

BE# 5(3) Methanogenic Cultures (70% vol/vol) with 5 mM BESA under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.7 2 mM)

		CH4 (%)				Acetate (mg/L)				Propionate (mg/L)				Total VFA (mg/L)			
DATE	DAY	BESA 5A	BESA 5B	BESA 5C	AVG	BESA 5A	BESA 5B	BESA 5C	AVG	BESA 5A	BESA 5B	BESA 5C	AVG	BESA 5A	BESA 5B	BESA 5C	AVG
	0	0	0	0	0.00	12.17	NA	9.64	10.91	3.22	NA	2.78	3.00	17.71	NA	14.65	16.18
	4	16	14	13	14.33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	6	21	20	17	19.33	323.38	311.61	NA	317.50	6.85	8.97	NA	7.91	350.71	338.19	NA	344.45
	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	11	24	22	20	22.00	896.10	947.24	NA	921.67	5.18	4.36	NA	4.77	978.02	1062.91	NA	NA
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	15	26	23	22	23.67	1031.80	NA	1050.40	1041.10	4.20	NA	5.30	4.75	1089.90	NA	1107.50	1098.70
	17	28	24	24	25.33	NA	1185.20	1166.90	1176.05	NA	4.62	4.84	4.73	NA	1251.60	1236.62	1244.11
	19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	21	28	26	25	26.33	1338.10	1355.75	1330.56	1341.47	6.16	6.52	5.31	6.00	1395.28	1406.37	1380.11	1393.92
	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	25	29	28	27	28.00	1422.49	NA	1438.63	1430.56	3.94	NA	5.68	4.81	1480.17	NA	1496.75	1488.46
	28	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	30	NA	NA	NA	NA	1454.88	1479.37	NA	1467.13	3.28	3.42	NA	3.35	1510.60	1528.91	NA	1519.76
	33	28	30	NA	29.00	1489.05	1501.53	1512.84	1501.14	4.55	2.57	2.66	3.26	1549.27	1572.41	1585.06	1568.91
	35	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

(c)

BATCH EXPERIMENT # 5 TABLE A-22 (....Contd)

BE# 5(4) Methanogenic Cultures (70% vol/vol) with 10 mM BESA under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.72 mM)

	CH4 (%)				Acetate (mg/L)				Propionate (mg/L)				Total VFA (mg/L)			
DAY	BESA 10 A	BESA 10B	BESA 10C	AVG	BESA 10 A	BESA 10B	BESA 10C	AVG	BESA 10 A	BESA 10B	BESA 10C	AVG	BESA 10 A	BESA 10B	BESA 10C	AVG
0	0	0	0	0.00	8.82	9.61	NA	9.22	2.77	3.29	NA	3.03	13.01	13.60	NA	13.31
4	8	6	NA	7.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
6	10	8	7	8.33	586.55	604.47	NA	595.51	26.17	32.49	NA	29.33	642.16	668.35	NA	655.26
8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
11	12	12	11	11.67	1036.20	NA	1015.40	1025.80	78.81	NA	92.43	85.62	1140.27	NA	1163.28	NA
13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
15	15	14	13	14.00	1063.77	1065.50	1088.79	1072.69	53.87	67.48	43.24	54.86	1213.51	1225.26	1205.64	1209.58
17	16	16	15	15.67	1259.38	NA	1326.40	1292.89	12.24	NA	18.47	NA	1286.26	NA	1359.18	NA
19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	18	18	18	18.00	1469.37	1475.21	1493.57	1479.38	8.19	10.77	13.02	10.66	1487.65	1496.38	1513.11	1499.05
23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	20	19	NA	19.50	1537.00	1576.85	NA	1556.93	6.16	5.15	NA	5.66	1558.88	1573.13	NA	1566.01
28	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
30	21	20	20	20.50	1557.52	NA	1568.19	1562.86	9.96	NA	7.26	8.61	1580.93	NA	1590.16	1585.55
33	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
35	NA	NA	NA	NA	1564.35	NA	1571.63	1567.99	6.33	NA	4.94	5.64	1583.46	NA	1596.27	1589.87
37	22	21	22	21.67	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
39	23	22	21	22.00	1570.38	1584.39	1577.55	1577.44	4.82	4.15	5.65	4.87	1588.07	1596.91	1606.17	1597.05

(d)

BATCH EXPERIMENT # 5

TABLE A-23

METHANOGENIC SAMPLES

BE# 5(Methanogenic Cultures (70% vol/vol) under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.7 2 mM)

DAY	2-CP (mg/L) M 1	M 2	M 3	AVG	Phenol (mg/L) M 1	M 2	M 3	AVG	% 2-CP
0	48.85	47.97	50.97	49.26	0.00	0.00	0.00	0.00	100.00
4	46.22	46.19	49.34	47.25	0.00	0.00	0.00	0.00	95.92
6	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	43.91	43.27	45.58	44.25	1.32	1.48	1.87	1.56	89.84
11	31.65	30.08	32.49	31.41	3.37	4.21	5.37	4.32	63.76
13	15.27	16.13	17.81	16.40	10.91	12.28	12.92	12.04	33.30
15	4.64	4.77	5.33	4.91	7.65	8.47	10.15	8.76	9.97
17	0.00	0.00	0.00	0.00	0.85	1.02	1.35	1.07	0.00
19	NA	NA	NA	NA	0.00	0.00	0.00	0.00	NA

(a)

BATCH EXPERIMENT # 5

BE# 5(Methanogenic Cultures (70% vol/vol) with 1 mM BESA under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.7 2 mM)

DAY	2-CP (mg/L) BESA 1A	BESA 1B	BESA 1C	AVG	Phenol (mg/L) BESA 1A	BESA 1B	BESA 1C	AVG	% 2-CP
0	47.72	52.57	49.14	49.81	0.00	0.00	0.00	0.00	100.00
4	46.88	51.35	47.74	48.66	0.00	0.00	0.00	0.00	97.68
6	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	44.16	50.07	46.12	46.78	0.95	0.52	0.00	0.49	93.92
11	39.11	42.76	40.73	40.87	3.88	3.44	3.36	3.56	82.05
13	33.56	34.89	34.28	34.24	6.35	5.48	5.42	5.75	68.75
15	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	23.56	26.18	25.02	24.92	11.63	12.42	10.42	11.49	50.03
19	8.94	10.12	12.63	10.56	14.19	16.63	13.59	14.80	21.21
21	3.43	4.25	4.90	4.19	8.35	8.61	7.27	8.08	8.42
23	0.00	0.00	0.00	0.00	1.86	2.29	1.38	1.84	0.00
25	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00

(b)

BATCH EXPERIMENT # 5

TABLE A-24

BE# 5(Methanogenic Cultures (70% vol/vol) with 5 mM BESA under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.7 2 mM)

	2-CP (mg/L)				Phenol (mg/L)				%
DAY	BESA 5A	BESA 5B	BESA 5C	AVG	BESA 5A	BESA 5B	BESA 5C	AVG	2-CP
0	49.36	50.89	51.18	50.48	0.00	0.00	0.00	0.00	100.00
4	48.65	48.92	50.27	49.28	0.00	0.00	0.00	0.00	97.62
6	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	45.14	46.71	NA	45.93	1.00	0.68	NA	0.84	90.98
11	41.95	43.68	45.11	43.58	2.66	2.63	1.87	2.39	86.33
13	37.26	NA	39.08	38.17	5.53	NA	4.31	4.92	75.61
15	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	29.97	31.54	32.08	31.20	8.64	7.39	8.16	8.06	61.80
19	20.60	22.64	21.29	21.51	11.52	12.67	11.97	12.05	42.61
21	14.79	NA	14.58	14.69	13.06	NA	14.68	13.87	29.09
23	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	7.40	7.86	NA	7.63	14.74	15.16	NA	14.95	15.11
28	4.79	4.65	4.30	4.58	15.12	15.69	15.88	15.56	9.07
30	NA	NA	NA	NA	NA	NA	NA	NA	NA
33	0.88	1.02	1.18	1.03	15.32	16.18	16.84	16.11	2.03
35	0.00	0.00	0.00	0.00	15.68	16.73	17.15	16.52	0.00

(a)

BATCH EXPERIMENT # 5

BE# 5(Methanogenic Cultures (70% vol/vol) with 10 mM BESA under CO2 + N2 Head-space
No added Sulphate (Background SO4 = 0.7 2 mM)

	2-CP (mg/L)				Phenol (mg/L)				%
DAY	BESA 10 A	BESA 10B	BESA 10C	AVG	BESA 10 A	BESA 10B	BESA 10C	AVG	2-CP
0	46.74	48.47	50.54	48.58	0.00	0.00	0.00	0.00	100.00
4	45.75	48.06	49.65	47.82	0.00	0.00	0.00	0.00	98.44
6	NA	NA	NA	NA	NA	NA	NA	NA	NA
8	45.22	47.84	NA	46.53	0.00	0.00	0.00	0.00	95.78
11	43.36	44.13	46.20	44.56	1.12	1.26	1.37	1.25	91.73
13	40.53	39.24	42.67	41.60	3.58	4.75	4.68	4.13	85.63
15	NA	NA	NA	NA	NA	NA	NA	NA	NA
17	33.81	34.64	35.10	34.52	6.87	6.24	7.08	6.73	71.05
19	25.64	26.26	NA	25.95	8.89	10.27	NA	9.58	53.42
21	19.73	NA	21.58	20.66	12.60	NA	13.12	12.86	42.52
23	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	NA	14.66	16.28	15.47	NA	15.34	14.97	15.16	31.84
28	10.25	NA	7.96	9.11	17.89	18.65	18.31	18.28	18.74
30	7.28	5.99	4.85	6.64	20.28	21.55	20.63	20.82	13.66
33	NA	NA	NA	NA	NA	NA	NA	NA	NA
35	4.18	4.23	4.12	4.18	21.44	22.81	23.05	22.43	8.60
37	NA	NA	NA	NA	NA	NA	NA	NA	NA
39	4.31	4.17	4.10	4.19	21.04	22.65	22.75	22.15	8.63

(b)

BATCH EXPERIMENT # 5

SULFIDOGENIC SAMPLES

TABLE A-25

BE# 5(5) Sulfidogenic Cultures (70% vol/vol) under CO2 + N2 Head-space
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.7 2 mM)

DATE	DAY	CH4 (%)				Acetate (mg/L)				Propionate (mg/L)				Total VFA (mg/L)			
		S 1	S 2	S 3	AVG	S 1	S 2	S 3	AVG	S 1	S 2	S 3	AVG	S 1	S 2	S 3	AVG
	0	0	0	0	0.00	10.85	NA	8.64	9.75	6.81	NA	4.22	5.52	17.66	NA	12.86	15.26
	4	22	24	NA	23.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	6	25	28	27	26.67	14.54	13.06	NA	13.80	2.80	1.46	NA	2.13	19.68	18.31	NA	19.00
(a)	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	11	34	35	35	34.67	8.19	4.86	5.62	6.22	0.00	0.83	0.63	0.49	9.36	7.18	8.47	8.34
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	15	37	38	40	38.33	6.12	NA	4.18	5.15	1.95	NA	2.32	2.14	8.27	NA	7.02	7.65
	17	39	40	42	40.33	3.27	2.78	1.89	2.65	0.00	0.00	0.00	0.00	3.84	2.96	2.21	3.00
	19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
		NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA

BATCH EXPERIMENT # 5

BE# 5(6) Sulfidogenic Cultures (70% vol/vol) with 1 mM Molybdate under CO2 + N2 Head-space
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.7 2 mM)

DATE	DAY	CH4 (%)				Acetate (mg/L)				Propionate (mg/L)				Total VFA (mg/L)			
		Moly 1A	Moly 1B	Moly 1C	AVG	Moly 1A	Moly 1B	Moly 1C	AVG	Moly 1A	Moly 1B	Moly 1C	AVG	Moly 1A	Moly 1B	Moly 1C	AVG
	0	0	0	0	0.00	NA	11.22	10.75	10.99	NA	4.10	4.05	4.08	NA	15.65	15.16	15.41
	4	21	23	NA	22.00	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	6	24	27	26	25.67	8.63	5.88	7.96	7.49	12.88	13.76	12.42	13.02	23.22	20.70	21.71	21.88
	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	11	29	34	33	32.00	4.73	6.01	5.34	5.36	0.00	0.00	0.00	0.00	5.88	6.73	6.41	6.34
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	15	35	38	38	37.00	3.07	4.31	2.79	3.39	0.00	0.00	0.00	0.00	4.35	6.11	3.94	4.80
(b)	17	38	NA	40	39.00	NA	2.59	NA	2.59	NA	0.00	NA	0.00	NA	4.41	NA	4.41
	19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	21	40	40	NA	40.00	3.03	NA	2.59	2.81	1.33	NA	1.69	1.51	5.79	NA	5.66	5.73
	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	25	41	NA	42	41.50	2.37	1.89	2.44	2.23	0.00	0.00	0.00	0.00	2.88	2.64	3.02	2.85
	27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	29	42	42	NA	42.00	0.87	1.12	1.49	1.16	0.95	1.52	1.34	1.27	1.97	2.84	3.21	2.67

BATCH EXPERIMENT # 5

BE# 5(8) Sulfidogenic Cultures (70% vol/vol) with 10 mM Molybdate under CO2 + N2 Head-space
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.7 2 mM)

DATE	DAY	CH4 (%)				Acetate (mg/L)				Propionate (mg/L)				Total VFA (mg/L)			
		Moly 10A	Moly 10B	Moly 10C	AVG	Moly 10A	Moly 10B	Moly 10C	AVG	Moly 10A	Moly 10B	Moly 10C	AVG	Moly 10A	Moly 10B	Moly 10C	AVG
	0	0	0	0	0.00	8.21	6.72	NA	7.47	5.71	5.22	NA	5.47	14.86	15.17	NA	15.02
	4	17	18	NA	17.50	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	6	22	24	24	23.33	8.84	8.33	7.12	8.10	65.68	71.76	60.29	65.91	77.14	84.06	71.30	77.50
	8	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	11	27	30	28	28.33	10.02	8.67	10.13	9.61	64.41	56.48	68.92	63.27	75.91	66.34	80.18	74.14
	13	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
(e)	15	34	36	NA	35.00	5.12	4.96	4.79	4.96	66.88	60.98	70.47	66.11	73.69	68.10	76.60	72.80
	17	36	38	41	38.33	4.36	3.62	5.08	3.99	69.23	66.65	63.2	66.36	74.20	71.38	69.87	71.82
	19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	21	38	38	40	38.67	6.19	NA	3.62	4.91	66.90	NA	63.21	65.06	75.50	NA	69.30	72.40
	23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	25	40	40	NA	40.00	6.12	6.15	6.02	6.10	75.16	68.82	64.90	69.63	83.40	76.48	72.38	77.42
	27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
	29	39	40	40	39.67	3.97	NA	4.12	4.05	55.86	NA	61.35	58.61	75.83	NA	70.36	73.10

BATCH EXPERIMENT # 5

TABLE A-25(.... contd)

SULFIDOGENIC SAMPLES

BE# 5(5) Sulfidogenic Cultures (70% vol/vol) under CO2 + N2 Head-space
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.7 2 mM)

DAY	2-CP (mg/L) S 1	S 2	S 3	AVG	Phenol (mg/L) S 1	S 2	S 3	AVG	Sulphate (mg/L) S 1	S 2	S 3	AVG	% 2-CP	% SO4
0	51.27	50.44	48.44	50.05	0.00	0.00	0.00	0.00	529.12	487.61	504.76	507.16	100.00	100.00
4	50.11	48.87	47.31	48.76	0.00	0.00	0.00	0.00	NA	NA	NA	NA	97.43	NA
6	NA	NA	NA	NA	NA	NA	NA	NA	435.47	420.28	430.17	428.64	NA	84.52
8	42.35	45.14	41.85	43.11	2.17	2.09	2.10	2.12	NA	NA	NA	NA	86.14	NA
11	34.56	33.86	30.57	33.00	4.02	3.78	NA	3.90	382.64	351.08	376.39	370.04	65.93	72.96
13	15.08	13.69	12.75	13.84	11.43	9.79	11.38	10.87	345.33	NA	330.69	338.01	27.65	66.65
15	3.44	3.86	2.86	3.39	14.58	14.89	13.25	14.24	300.71	285.76	296.59	294.35	6.77	58.04
17	0.00	0.00	0.00	0.00	4.82	4.11	3.53	4.15	280.08	269.27	277.52	275.62	0.00	54.35
19	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	265.12	252.14	NA	258.63	0.00	51.00
21	NA	NA	NA	NA	NA	NA	NA	NA	249.16	231.27	240.79	240.41	NA	47.40

(d)

BATCH EXPERIMENT # 5

BE# 5(6) Sulfidogenic Cultures (70% vol/vol) with 1 mM Molybdate under CO2 + N2 Head-space
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.7 2 mM)

DAY	2-CP (mg/L) Moly 1A	Moly 1B	Moly 1C	AVG	Phenol (mg/L) Moly 1A	Moly 1B	Moly 1C	AVG	Sulphate (mg/L) Moly 1A	Moly 1B	Moly 1C	AVG	% 2-CP	% SO4
0	50.73	51.01	48.89	50.21	0.00	0.00	0.00	0.00	482.19	510.69	489.67	494.18	100.00	100.00
4	49.93	50.77	48.24	49.65	0.00	0.00	0.00	0.00	NA	NA	NA	NA	98.88	NA
6	NA	NA	NA	NA	NA	NA	NA	NA	424.84	428.59	417.64	423.69	NA	85.74
8	47.93	45.74	44.68	46.12	0.00	0.00	0.00	0.00	NA	NA	NA	NA	91.85	NA
11	42.24	41.20	NA	41.72	2.10	2.02	NA	2.06	400.53	412.17	390.49	401.06	83.09	81.16
13	34.63	35.14	32.31	34.03	3.41	2.85	3.06	3.11	NA	382.38	376.72	379.55	67.77	76.80
15	24.80	26.59	25.78	25.72	3.87	3.54	3.66	3.69	367.16	360.10	348.17	358.48	51.23	72.54
17	18.49	20.05	NA	19.27	4.12	3.96	NA	4.04	352.82	347.39	NA	350.11	38.38	70.85
19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	12.43	13.71	14.46	13.53	4.75	4.57	5.88	5.07	342.13	340.08	344.82	342.34	26.95	69.28
23	6.32	5.24	6.85	6.14	3.35	3.17	3.80	3.44	NA	NA	NA	NA	12.22	NA
25	NA	0.80	1.80	1.30	NA	2.51	2.28	2.40	340.25	342.19	338.10	340.18	2.59	68.84
27	0.00	0.00	0.00	0.00	1.11	1.42	1.30	1.28	NA	NA	NA	NA	0.00	NA
29	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	344.54	341.38	340.52	342.15	0.00	69.24

(e)

BATCH EXPERIMENT # 5

BE# 5(8) Sulfidogenic Cultures (70% vol/vol) with 10 mM Molybdate under CO2 + N2 Head-space
Target Initial Sulphate Conc. = 5 mM (Background SO4 = 0.7 2 mM)

DAY	2-CP (mg/L) Moly 10A	Moly 10B	Moly 10C	AVG	Phenol (mg/L) Moly 10A	Moly 10B	Moly 10C	AVG	Sulphate (mg/L) Moly 10A	Moly 10B	Moly 10C	AVG	% 2-CP	% SO4
0	49.43	48.46	50.64	49.51	0.00	0.00	0.00	0.00	500.45	520.17	480.63	500.42	100.00	100.00
4	48.02	NA	49.64	48.83	0.00	0.00	0.00	0.00	NA	NA	NA	NA	98.63	NA
6	NA	NA	NA	NA	NA	NA	NA	NA	505.41	515.27	478.15	499.61	NA	99.84
8	48.41	48.16	49.97	48.85	0.00	0.00	0.00	0.00	NA	NA	NA	NA	98.66	NA
11	47.46	47.71	47.84	47.67	0.00	0.00	0.00	0.00	NA	NA	NA	NA	96.28	NA
13	NA	NA	NA	NA	NA	NA	NA	NA	495.49	510.72	482.50	496.24	NA	99.16
15	47.66	46.38	46.58	46.87	0.00	0.00	0.00	0.00	NA	NA	NA	NA	94.67	NA
17	47.51	46.08	45.50	46.36	0.00	0.00	0.00	0.00	NA	NA	NA	NA	93.64	NA
19	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
21	46.35	45.73	45.58	45.89	0.00	0.00	0.00	0.00	491.94	525.80	477.38	498.37	92.68	99.59
23	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
25	45.77	44.87	44.55	45.06	0.00	0.00	0.00	0.00	490.12	522.67	475.72	496.17	91.02	99.15
27	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
29	45.46	45.82	46.02	45.77	0.00	0.00	0.00	0.00	490.71	512.71	470.86	491.43	92.44	98.29

(f)

APPENDIX B

EFFECT OF DIFFERENT DEXTROSE CONCENTRATION ON 2-CP DEGRADATION

TABLE B-1

Degradation of 2-CP in absence of supplmentary substrates

Sample No.	Time (minutes)	# 1 2-CP mg/L	# 2 2-CP mg/L	# 3 2-CP mg/L	# 4 2-CP mg/L	# 5 2-CP mg/L	# 6 2-CP mg/L	Average 2-CP mg/L
1	0	29.05	29.75	28.7	29.33	30.08	29.15	29.34
2	20	28.42	27.3	27.04	27.81	27.4	28.01	27.66
3	40	28.96	25.87	26.47	28.12	25.32	26.85	26.27
4	60	22.01	21.8	23.8	22.79	21.96	22.48	22.47
5	80	20.04	18.9	22.1	19.32	18.96	19.23	19.76
6	100	18.55	17.8	19.1	16.84	16.34	17.17	17.63
7	120	14.15	14.1	16.8	14.48	13.79	14.42	14.62
8	140	10.6	9.9	13.1	12.65	10.58	11.21	11.34
9	160	6.2	5.7	6.3	6.49	5.83	6.65	6.20
10	180	3.49	3.6	4.2	3.27	3.41	3.89	3.64
11	200	1.18	1.05	2.1	1.49	1.27	1.54	1.44
12	220	0.00	0.00	0.00	0.00	0.00	0.00	0.00
								AVERAGE
MLVSS		2025	2040	2030	2010	2015	1994	2019
2-CP removal (mg/L.hr)		7.92	8.11	7.83	8.00	8.20	7.95	8.00
2-CP removal (mg/g VSS.hr)		3.91	3.98	3.86	3.98	4.07	3.99	3.96

TABLE B-2

Degradation of 2-CP in presence of 20 mg/L (as SOC) Dextrose

Sample No.	Time (minutes)	# 1 SOC (mg/L)	# 1 2-CP	# 2 SOC (mg/L)	# 2 2-CP	# 3 SOC (mg/L)	# 3 2-CP	# 4 SOC (mg/L)	# 4 2-CP	Average 2-CP	Average SOC (mg/L)
1	0	40.24	30.80	41.16	30.48	42.88	31.20	44.39	30.30	30.70	42.17
2	20	21.23	27.65	23.97	27.95	20.24	28.50	22.51	28.10	28.05	21.99
3	40	17.59	21.50	19.22	21.73	18.68	24.90	20.26	21.85	22.50	18.94
4	60	17.10	20.50	17.76	18.52	17.37	18.84	18.85	19.80	19.42	17.77
5	80	16.24	17.80	16.24	15.87	16.59	16.25	17.30	16.81	16.68	16.59
6	100	14.24	12.20	15.38	14.12	15.22	14.55	16.07	13.26	13.53	15.23
7	120	13.10	9.10	14.47	10.32	14.02	10.68	14.76	9.80	9.98	14.09
8	140	12.86	3.95	13.65	4.37	13.43	5.68	13.19	3.50	4.38	13.28
9	160	12.22	1.68	12.81	1.67	12.28	2.60	12.23	0.80	1.68	12.39
10	180	11.85	0.00	12.32	0.00	11.14	0.00	11.34	0.00	0.00	11.66
11	200	11.68	0.00	12.40	0.00	11.21	0.00	11.32	0.00	0.00	11.65
										AVERAGE	
MLVSS			1960		2010		2005		1975	1988	
2-CP removal (mg/L.hr)			10.27		10.16		10.40		10.10	10.23	
2-CP removal (mg/g VSS.hr)			5.24		5.05		5.19		5.11	5.15	

TABLE B-3

Degradation of 2-CP in presence of 40 mg/L (as SOC) Dextrose

Sample No.	Time	#1		#2		#3		#4		#5		#6		Average	
	(minutes)	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	2-CP	SOC (mg/L)
1	0	59.60	32.50	58.30	30.10	56.80	31.80	60.20	31.40	57.30	35.30	59.10	30.85	31.33	58.45
2	20	27.40	26.55	37.20	27.30	34.70	27.40	39.70	28.30	31.90	31.10	35.40	26.69	27.25	33.68
3	40	36.50	24.72	34.10	22.80	31.10	21.90	35.20	21.30	28.50	29.80	30.80	20.62	22.27	33.13
4	60	24.30	16.41	28.70	18.30	26.30	16.80	27.90	17.40	27.60	23.06	25.20	15.90	16.96	26.13
5	80	19.80	10.13	20.30	12.20	20.80	11.65	22.30	10.80	23.20	16.89	21.90	9.65	10.89	20.70
6	100	16.20	4.56	17.80	3.90	16.90	3.50	20.10	3.60	21.09	10.63	18.40	2.89	3.69	17.33
7	120	15.10	0.00	15.30	0.00	14.80	0.00	16.40	0.00	18.20	3.95	16.90	0.00	0.00	15.53
8	140	14.30	0.00	13.50	0.00	13.70	0.00	13.80	0.00	16.12	0.00	13.10	0.00	0.00	13.65
9	160	13.80	0.00	14.10	0.00	13.20	0.00	12.70	0.00	14.10	0.00	12.80	0.00	0.00	13.48
10	180	13.50	0.00	13.90	0.00	13.10	0.00	12.90	0.00	13.80	0.00	13.00	0.00	0.00	13.38

							Average
MLVSS	1950	1975	1950	1995	1965	1940	1967
2-CP removal (mg/L.hr)	16.25	15.05	15.90	15.70	15.13	15.43	15.51
2-CP removal (mg/g VSS.hr)	8.33	7.62	8.15	7.87	7.70	7.95	7.94

B2

TABLE B-4

Degradation of 2-CP in presence of 60 mg/L (as SOC) Dextrose

Sample No.	Time	#1		#2		#3		#4		Average	
	(minutes)	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	SOC (mg/L)	2-CP	2-CP	SOC (mg/L)
1	0	80.10	31.30	82.60	31.30	81.50	32.80	84.67	30.46	31.47	81.40
2	20	29.70	27.10	32.50	27.55	31.60	28.21	35.51	27.92	27.70	31.27
3	40	24.80	21.20	25.60	21.30	25.40	22.68	28.26	22.07	21.81	25.27
4	60	23.20	18.40	24.30	17.80	22.30	18.62	26.23	19.58	18.60	23.27
5	80	20.80	14.20	19.70	13.90	20.10	14.28	23.05	14.80	14.30	20.20
6	100	16.90	7.40	17.80	6.20	18.50	9.20	20.10	7.74	7.64	17.73
7	120	15.50	3.20	15.90	2.90	16.30	3.19	17.32	3.50	3.20	15.90
8	140	14.60	1.40	14.40	1.38	14.70	1.60	15.44	1.20	1.40	14.57
9	160	12.50	0.00	13.20	0.00	13.00	0.00	14.10	0.00	0.00	12.90
10	180	12.80	0.00	13.00	0.00	12.80	0.00	13.85	0.00		12.87

					Average
MLVSS	1990	2020	1995	1982	1997
2-CP removal (mg/L.hr)	11.74	11.74	12.30	11.42	11.80
2-CP removal (mg/g VSS.hr)	5.90	5.81	6.17	5.76	5.91

TABLE B-5

Degradation of 2-CP in presence of 80 mg/L (as SOC) Dextrose

Sample No.	Time	#1		#2		#3		#4		Average	
		SOC	2-CP	SOC	2-CP	SOC	2-CP	SOC	2-CP	2-CP	SOC
	(minutes)	(mg/L)		(mg/L)		(mg/L)		(mg/L)		(mg/L)	
1	0	104.40	30.85	98.90	31.30	100.60	30.80	103.80	32.30	31.31	101.93
2	20	53.60	28.55	55.60	28.10	49.50	27.40	45.90	28.30	27.59	51.15
3	40	23.40	25.18	25.20	25.80	29.70	25.50	28.90	26.10	25.65	26.80
4	60	21.90	23.07	23.80	24.35	27.10	23.80	26.80	24.20	23.86	24.90
5	80	19.30	18.28	20.10	19.76	23.40	17.50	22.40	19.10	18.66	21.30
6	100	16.80	16.33	17.90	14.23	20.90	15.60	20.60	15.90	15.52	19.05
7	120	15.80	13.09	16.50	12.25	18.90	12.80	17.10	12.75	12.72	17.08
8	140	14.90	8.39	15.20	7.47	17.60	8.60	15.50	7.60	8.02	15.80
9	160	13.70	4.38	14.10	3.29	15.30	3.80	14.30	4.20	3.92	14.35
10	180	12.80	0.00	13.00	0.00	12.20	0.00	13.10	0.00	0.00	12.78
11	200	12.70	0.00	12.90	0.00	12.50	0.00	12.80	0.00	0.00	12.73

					Average
MLVSS	1955	2005	1955	2010	1981
2-CP removal (mg/L.hr)	10.28	10.43	10.27	10.77	10.49
2-CP removal (mg/g VSS.hr)	5.26	5.20	5.25	5.36	5.27

TABLE B-6

Degradation of 2-CP in presence of 200 mg/L (as SOC) Dextrose

Sample No.	Time	#1		#2		#3		#4		Average	
		SOC	2-CP	SOC	2-CP	SOC	2-CP	SOC	2-CP	2-CP	SOC
	(minutes)	(mg/L)		(mg/L)		(mg/L)				(mg/L)	
1	0	224.56	29.24	218.18	30.20	215.50	29.94	221.68	30.38	29.94	219.98
2	20	142.25	27.10	150.72	28.10	125.58	28.90	137.46	28.14	28.06	139.00
3	40	81.80	25.35	65.40	26.64	67.23	26.21	72.65	25.82	26.01	71.77
4	60	29.71	20.22	27.15	21.80	28.27	22.62	28.10	21.45	21.52	28.31
5	80	27.42	18.34	26.07	18.95	26.69	20.32	26.22	18.72	19.08	26.60
6	100	24.65	15.38	23.40	16.82	22.87	17.30	24.08	16.23	16.43	23.75
7	120	22.90	12.85	20.90	14.10	19.14	13.67	22.17	13.25	13.47	21.28
8	140	18.14	9.71	17.37	9.25	17.58	11.06	19.21	10.18	10.05	18.08
9	160	14.32	5.42	14.46	4.83	15.26	6.12	16.55	5.47	5.46	15.15
10	180	13.13	3.17	13.75	2.75	13.17	3.41	14.17	3.05	3.10	13.58
11	200	12.25	0.00	12.18	0.00	11.85	0.00	12.45	0.00	0.00	12.18
12	220	12.17	0.00	12.10	0.00	11.72	0.00	12.32	0.00	0.00	12.08

					Average
MLVSS	1996	2025	2037	2015	2018
2-CP removal (mg/L.hr)	8.77	9.06	8.98	9.11	8.98
2-CP removal (mg/g VSS.hr)	4.39	4.47	4.41	4.52	4.45

EFFECT OF DIFFERENT PHENOL CONCENTRATION ON 2-CP DEGRADATION

TABLE B-7

Degradation of 2-CP in presence of 20 mg/L (as SOC) Phenol

Sample No.	Time (minutes)	# 1 Phenol (mg/L)	# 1 2-CP (mg/L)	# 2 Phenol (mg/L)	# 2 2-CP (mg/L)	# 3 Phenol (mg/L)	# 3 2-CP (mg/L)	# 4 Phenol (mg/L)	# 4 2-CP (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	25.90	31.30	26.80	32.50	26.60	32.10	24.82	31.96	31.97	26.03
2	20	0.80	22.80	1.90	23.57	1.50	24.12	2.68	23.61	23.53	1.72
3	40	0.00	15.50	0.00	18.72		19.06	0.00	19.23	18.13	0.00
4	60		11.24		12.71		12.45		11.92	12.08	0.00
5	80		5.37		6.64		6.36		5.82	6.05	0.00
6	100		1.23		2.36		2.06		2.25	1.98	0.00
7	120		0.00		0.00		0.00		0.00	0.00	0.00

	# 1	# 2	# 3	# 4	Average
MLVSS	1940	1950	1956	1964	1953
2-CP removal (mg/L.hr)	15.65	16.25	16.05	15.98	15.98
2-CP removal (mg/g VSS.hr)	8.07	8.33	8.21	8.14	8.19

TABLE B-8

Degradation of 2-CP in presence of 40 mg/L (as SOC) Phenol

Sample No.	Time (minutes)	# 1 Phenol (mg/L)	# 1 2-CP (mg/L)	# 2 Phenol (mg/L)	# 2 2-CP (mg/L)	# 3 Phenol (mg/L)	# 3 2-CP (mg/L)	# 4 Phenol (mg/L)	# 4 2-CP (mg/L)	# 5 Phenol (mg/L)	# 5 2-CP (mg/L)	# 6 Phenol (mg/L)	# 6 2-CP (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	51.10	30.60	53.40	29.90	52.07	31.20	50.90	30.10	48.60	31.25	53.61	30.06	30.52	51.61
2	20	4.10	21.10	5.20	19.96	7.47	20.68	6.60	18.75	3.20	20.72	5.49	19.81	20.17	5.34
3	40	0.00	14.96	0.00	12.91	0.00	13.40	0.00	12.79	0.00	14.12	0.00	12.52	13.45	0.00
4	60		6.35		3.87		4.50		5.05		5.36		4.63	4.96	0.00
5	80		0.00		0.00		0.00		0.00		0.00		0.00	0.00	0.00
6	100		0.00		0.00		0.00		0.00		0.00		0.00	0.00	0.00

	# 1	# 2	# 3	# 4	# 5	# 6	Average
MLVSS	2015	1980	1950	2005	1956	1996	1984
2-CP removal (mg/L.hr)	22.95	22.43	23.40	22.58	23.44	22.55	22.89
2-CP removal (mg/g VSS.hr)	11.39	11.33	12.00	11.26	11.96	11.30	11.54

TABLE B-9

Degradation of 2-CP in presence of 60 mg/L (as SOC) Phenol

Sample No.	Time (minutes)	#1 Phenol (mg/L)	#1 2-CP (mg/L)	#2 Phenol (mg/L)	#2 2-CP (mg/L)	#3 Phenol (mg/L)	#3 2-CP (mg/L)	#4 Phenol (mg/L)	#4 2-CP (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	74.34	30.06	76.70	29.85	77.92	30.06	79.40	30.20	30.04	77.09
2	20	8.90	10.35	12.30	11.30	11.85	9.80	10.40	9.27	10.18	10.86
3	40	0.00	2.80	0.00	4.10	0.00	3.52	0.00	3.29	3.43	0.00
4	60		0.00		0.00		0.00		0.00	0.00	0.00
5	80		0.00		0.00		0.00		0.00	0.00	0.00

	#1	#2	#3	#4	Average
MLVSS	2017	1995	2008	2025	2011
2-CP removal (mg/L.hr)	30.06	29.85	30.06	30.20	30.04
2-CP removal (mg/g VSS.hr)	14.90	14.96	14.97	14.91	14.94

TABLE B-10

Degradation of 2-CP in presence of 80 mg/L (as SOC) Phenol

Sample No.	Time (minutes)	#1 Phenol (mg/L)	#1 2-CP (mg/L)	#2 Phenol (mg/L)	#2 2-CP (mg/L)	#3 Phenol (mg/L)	#3 2-CP (mg/L)	#4 Phenol (mg/L)	#4 2-CP (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
No.	Time	98.90	29.12	102.80	29.89	101.50	30.10	103.60	30.26	29.84	101.70
1	0	23.60	12.21	16.54	13.40	22.30	12.30	19.70	10.92	12.21	20.54
2	20	0.00	3.16	0.00	5.36	0.00	3.90	0.00	4.70	4.28	0.00
3	40		0.00		0.00		0.00		0.00	0.00	0.00
4	60		0.00		0.00		0.00		0.00	0.00	0.00

	#1	#2	#3	#4	Average
MLVSS	2018	1995	2005	2030	2012
2-CP removal (mg/L.hr)	29.12	29.89	30.10	30.26	29.84
2-CP removal (mg/g VSS.hr)	14.43	14.98	15.01	14.91	14.83

TABLE B-11

Degradation of 2-CP in presence of 200 mg/L (as SOC) Phenol

Sample No.	Time (minutes)	#1 Phenol (mg/L)	#1 2-CP (mg/L)	#2 Phenol (mg/L)	#2 2-CP (mg/L)	#3 Phenol (mg/L)	#3 2-CP (mg/L)	#4 Phenol (mg/L)	#4 2-CP (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	262.16	29.82	265.80	29.75	256.80	30.30	237.85	30.56	30.11	255.65
2	20	176.61	27.64	191.30	26.80	185.90	26.89	156.23	28.32	27.41	177.51
3	40	82.29	23.55	82.40	22.60	75.30	22.65	56.32	24.11	23.23	74.08
4	60	35.45	16.82	30.60	14.50	25.60	15.95	12.30	15.63	15.73	25.99
5	80	0.00	10.23	0.00	8.20	0.00	9.65	0.00	11.80	9.97	0.00
6	100	0.00	3.23		2.90		3.12		4.10	3.34	
7	120		0.00		0.00		0.00		0.00	0.00	

	#1	#2	#3	#4	Average
MLVSS	2005	1995	2010	1955	1991
2-CP removal (mg/L.hr)	14.91	14.88	15.15	15.28	15.05
2-CP removal (mg/g VSS.hr)	7.44	7.46	7.54	7.82	7.56

TABLE B-12

Effect of various concentrations of dextrose on 2-CP removal

Time (minutes)	0 mg SOC/L		20 mg SOC/L		40 mg SOC/L		60 mg SOC/L		80 mg SOC/L		200 mg SOC/L	
	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining
0	29.34	100.00	30.70	100.00	31.33	100.00	31.47	100.00	31.31	100.00	29.94	100.00
20	27.66	94.27	28.05	91.37	27.25	86.97	27.70	88.02	27.59	88.10	28.06	93.72
40	26.27	89.54	22.50	73.29	22.27	71.08	21.81	69.30	25.65	81.90	26.01	86.87
60	22.47	76.58	19.42	63.26	16.96	54.14	18.60	59.10	23.86	76.18	21.52	71.88
80	19.76	67.35	16.68	54.33	10.89	34.75	14.30	45.44	18.66	59.59	19.08	63.73
100	17.63	60.09	13.53	44.07	3.69	11.78	7.64	24.28	15.52	49.55	16.43	54.88
120	14.62	49.83	9.98	32.51	0.00	0.00	3.20	10.17	12.72	40.63	13.47	44.99
140	11.34	38.65	4.38	14.26			1.40	4.45	8.02	25.60	10.05	33.57
160	6.20	21.13	1.68	5.47			0.00	0.00	3.92	12.51	5.46	18.24
180	3.64	12.41	0.00	0.00					0.00	0.00	3.10	10.35
200	1.44	4.91									0.00	0.00
220	0.00	0.00										

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TABLE B-13

Effect of various concentrations of Phenol on 2-CP removal

Time (minutes)	0 mg SOC/L		20 mg SOC/L		40 mg SOC/L		60 mg SOC/L		80 mg SOC/L		200 mg SOC/L	
	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining	2-CP mg/L	% Remaining
0	29.34	100.00	31.97	100.00	30.52	100.00	30.04	100.00	29.84	100.00	30.11	100.00
20	27.66	94.27	23.53	73.60	20.17	66.09	10.18	33.89	12.21	40.91	27.41	91.04
40	26.27	89.54	18.13	56.71	13.45	44.07	3.43	11.42	4.28	14.34	23.23	77.14
60	22.47	76.58	12.08	37.79	4.96	16.25	0.00	0.00	0.00	0.00	15.73	52.23
80	19.76	67.35	6.05	18.92	0.00	0.00					9.97	33.11
100	17.63	60.09	1.98	6.19							3.34	11.08
120	14.62	49.83	0.00	0.00							0.00	0.00
140	11.34	38.65										
160	6.20	21.13										
180	3.64	12.41										
200	1.44	4.91										
220	0.00	0.00										

This experiment examines the effect of elliminating either dextrose or phenol from a cycle

TABLE B-14

Data showing 2-CP removal in presence or absence of 40 mg SOC/L Dextrose for a single cycle

Sample No.	Time (minutes)	# 1 + Dextrose 2-CP (mg/L)	# 1 - Dextrose 2-CP (mg/L)	# 2 + Dextrose 2-CP (mg/L)	# 2 - Dextrose 2-CP (mg/L)	# 3 + Dextrose 2-CP (mg/L)	# 3 - Dextrose 2-CP (mg/L)	Average + Dextrose 2-CP (mg/L)	Average - Dextrose 2-CP (mg/L)	% Remaining + Dextrose 2-CP	% Remaining - Dextrose 2-CP
1	0	30.41	29.55	31.36	29.91	29.19	33.42	30.32	30.96	100.00	100.00
2	20	26.81	25.24	27.92	25.87	26.38	27.98	27.04	26.36	89.17	85.15
3	40	21.81	20.65	22.69	22.94	21.05	22.19	21.85	21.93	72.06	70.82
4	60	16.01	13.83	16.54	14.55	15.89	16.16	16.15	14.85	53.25	47.95
5	80	10.87	8.67	11.17	9.18	9.76	9.15	10.60	9.00	34.96	29.07
6	100	4.93	3.96	5.04	3.79	4.48	3.27	4.82	3.67	15.89	11.86
7	120	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Average VSS (mg/L) ==		2055	2035	1940	1955	1985	1975				

TABLE B-15

Presence or Absence of Phenol

Sample No.	Time (minutes)	# 1 + Phenol Phenol (mg/L)	# 1 + Phenol 2-CP (mg/L)	# 1 - Phenol 2-CP (mg/L)	# 2 + Phenol Phenol (mg/L)	# 2 + Phenol 2-CP (mg/L)	# 2 - Phenol 2-CP Only (mg/L)	# 3 + Phenol Phenol (mg/L)	# 3 + Phenol 2-CP (mg/L)	# 3 - Phenol 2-CP Only (mg/L)	Average + Phenol 2-CP (mg/L)	Average - Phenol 2-CP (mg/L)	% Remaining + Phenol 2-CP	% Remaining - Phenol 2-CP
1	0	50.37	30.56	29.88	54.81	30.20	30.13	52.36	29.76	30.83	30.17	30.28	100.00	100.00
2	10	19.82	22.49	27.56	22.81	22.75	28.05	6.18	21.72	28.75	22.32	28.12	73.98	92.87
3	20	2.68	19.57	22.76	7.42	20.17	24.89	0.00	18.87	25.18	19.54	24.28	64.76	80.17
4	30	0.00	12.78	19.23	0.00	13.68	20.87		13.64	21.13	13.37	20.41	44.30	67.40
5	40		6.44	16.24		7.94	17.91		7.18	19.23	7.19	17.79	23.82	58.76
6	50		1.42	12.43		3.25	13.18		2.55	13.48	2.41	13.03	7.98	43.03
7	60		0.00	7.39		0.00	8.48		0.00	8.21	0.00	8.03	0.00	26.51
8	70			2.51			3.57			3.82		3.30	0.00	10.90
9	80			0.00			0.00			0.00		0.00	0.00	0.00
Average VSS (mg/L)			1990	2105		2040	2030		2015	2010				

TABLE B-16

Cultures Adapted to 40 mg SOC/L Phenol and exposed to 2-CP only

Sample #	Elapsed Time (minutes)	Profile 1 2-CP (mg/L)	Profile 2 2-CP (mg/L)	Profile 3 2-CP (mg/L)	Profile 4 2-CP (mg/L)	Average 2-CP (mg/L)	% Remain 2-CP
1	0	29.84	30.11	29.02	30.68	29.91	100.00
2	20	24.99	26.69	25.05	26.85	25.90	86.58
3	40	19.89	20.62	20.82	21.09	20.61	68.89
4	60	15.81	17.54	16.64	17.34	16.83	56.28
5	80	11.08	13.61	11.79	12.77	12.31	41.17
6	100	7.76	9.11	7.25	8.12	8.06	26.95
8	120	3.83	5.53	3.63	4.79	4.45	14.86
10	140	0.00	0.00	0.00	0.00	0.00	0.00

TABLE B-17

Cultures Adapted to 40 mg SOC/L Dextrose and exposed to 2-CP only

Sample #	Elapsed Time (minutes)	Profile 1 2-CP (mg/L)	Profile 2 2-CP (mg/L)	Profile 3 2-CP (mg/L)	Profile 4 2-CP (mg/L)	Average 2-CP (mg/L)	% Remain 2-CP
1	0	30.23	28.94	29.55	30.15	29.72	100.00
2	20	30.16	28.83	29.50	30.20	29.67	99.84
3	40	30.18	28.16	29.43	29.86	29.41	98.95
4	60	30.20	28.32	29.38	29.78	29.42	98.99
5	80	30.16	27.99	29.12	29.65	29.23	98.35
6	120	29.94	28.06	29.03	29.53	29.14	98.05
8	160	29.93	27.79	28.85	29.62	29.05	97.74
10	180	29.71	27.72	28.78	29.17	28.85	97.06
11	200	29.80	27.60	29.85	28.68	28.98	97.52
12	240	29.81	27.61	29.62	28.76	28.95	97.41

Effect of SRT On 2-CP removal in SBR'S in presence of dextrose

a) **Degradation of 2-CP at SRT = 4 days in presence of 40 mg SOC/L Dextrose** (TABLE B-18)

Time (minutes)	# 1 2-CP mg/L	# 2 2-CP mg/L	# 3 2-CP mg/L	Average 2-CP mg/L	% Remain
0	30.2	30.8	28.4	29.80	100.00
20	29.10	28.5	25.08	27.56	92.48
40	26.20	25.3	24.57	25.36	85.09
60	23.15	23.6	22.63	23.13	77.60
80	21.40	20.45	19.55	20.47	68.68
100	18.05	17.65	16.18	17.29	58.03
120	14.10	14.8	13.93	14.28	47.91
140	10.22	10.95	9.4	10.19	34.19
160	6.15	7.15	5.2	6.17	20.69
180	3.08	3.37	1.8	2.75	9.23
200	0.00	0.00	0.00	0.00	0.00
Vol. Removal	9.06	9.24	8.52	8.94	
VSS (mg/L)	760	738	752	750	
Specific Remov.	11.92	12.52	11.33	11.92	

b) **Degradation of 2-CP at SRT = 8 days in presence of 40 mg SOC/L Dextrose**

Time (minutes)	# 1 2-CP mg/L	# 2 2-CP mg/L	# 3 2-CP mg/L	Average 2-CP mg/L	% Remain
0	30.45	30.1	29.8	30.12	100.00
20	27.10	28.05	26.75	27.30	90.64
40	24.80	25.66	23.20	24.55	81.52
60	21.05	22.19	19.54	20.93	69.48
80	16.33	17.42	14.45	16.07	53.34
100	11.40	12.20	10.10	11.23	37.30
120	6.20	5.60	6.80	6.20	20.58
140	1.94	2.34	2.65	2.31	7.67
160	0.00	0.00	0.00	0.00	0.00
Vol. Removal	11.42	11.29	11.18	11.3	
VSS (mg/L)	1035	1020	1041	1032	
Specific Remov.	11.03	11.07	10.73	10.94	

c) **Degradation of 2-CP at SRT = 12 days in presence of 40 mg SOC/L Dextrose**

Time (minutes)	# 1 2-CP mg/L	# 2 2-CP mg/L	# 3 2-CP mg/L	Average 2-CP mg/L	% Remain
0	29.54	31.55	27.27	29.45	100.00
20	25.3	27.5	23.54	25.45	86.41
40	21.25	22.9	17.99	20.71	70.33
60	17.61	16.45	15.38	16.48	55.96
80	12.36	11.68	10.29	11.44	38.86
100	7.15	6.95	5.29	6.46	21.95
120	3.44	3.12	1.45	2.67	9.07
140	0.00	0.00	0.00	0.00	0.00
Vol. Removal	12.66	13.52	11.69	12.62	
VSS (mg/L)	1424	1416	1390	1410	
Specific Remov.	8.89	9.55	8.42	8.95	

(d) **Degradation of 2-CP at SRT = 16 days in presence of 40 mg SOC/L Dextrose**

Time (minutes)	# 1 2-CP mg/L	# 2 2-CP mg/L	# 3 2-CP mg/L	Average 2-CP mg/L	% Remain
0	28.75	29.76	30.28	29.60	100
20	25.1	26.42	27.33	26.28	88.80
40	20.31	24.37	23.02	22.57	76.24
60	14.75	16.66	18.1	16.50	55.75
80	8.42	10.05	11.65	10.04	33.92
100	2.3	4.65	3.96	3.64	12.29
120	0.00	0.00	0.00	0.00	0.00
Vol. Removal	14.38	14.88	15.14	14.80	
VSS (mg/L)	1771	1758	1766	1765	
Specific Remov.	8.12	8.46	8.57	8.38	

(e) **Degradation of 2-CP at SRT = 20 days in presence of 40 mg SOC/L Dextrose**

Time (minutes)	# 1 2-CP mg/L	# 2 2-CP mg/L	# 3 2-CP mg/L	Average 2-CP mg/L	% Remain
0	29.6	31.3	30.6	30.50	100
20	26.6	28.3	27.1	27.33	89.62
40	22.72	21.97	22.55	22.41	73.49
60	16.13	17.12	16.85	16.70	54.75
80	10.35	10.8	9.65	10.27	33.66
100	4.56	3.65	2.89	3.70	12.13
120	0.00	0.00	0.00	0.00	0.00
Vol. Removal	14.80	15.65	15.3	15.25	
VSS (mg/L)	1960	1940	1965	1955	
Specific Remov.	7.55	8.07	7.79	7.80	

Effect of SRT On 2-CP removal in SBR'S in presence of phenol.

(a) **Degradation of 2-CP at SRT = 4 days in presence of 40 mg SOC/L Phenol** TABLE B-19

Time (minutes)	# 1 2-CP (mg/L)	# 1 Phenol (mg/L)	# 2 2-CP (mg/L)	# 2 Phenol (mg/L)	# 3 2-CP (mg/L)	# 3 Phenol (mg/L)	Average 2-CP mg/L	Average Phenol mg/L	% Remaining
0	30.10	54.69	29.25	49.17	31.14	56.36	30.16	53.41	100.00
20	28.51	46.88	27.43	37.02	29.62	44.04	28.52	42.65	94.56
40	26.41	27.93	25.10	23.79	27.23	22.83	26.25	24.85	87.02
60	24.87	16.66	24.02	12.82	24.54	9.83	24.48	13.10	81.16
80	21.02	4.04	20.81	1.35	22.12	2.12	21.32	2.50	70.68
100	19.16	0.00	18.10	0.00	20.47	0.00	19.24	0.00	63.80
120	16.38		15.87		18.31		16.85		55.88
140	12.34		12.77		14.72		13.28		44.02
160	9.68		8.75		10.94		9.79		32.46
180	6.75		5.23		7.84		6.61		21.91
200	3.95		3.54		4.30		3.93		13.03
220	1.68		0.92		2.51		1.70		5.65
240	0.00		0.00		0.00		0.00		0.00
Vol. Removal	7.53		7.31		7.79		7.54		
VSS (mg/L)	489		498		482		490		
Specific Remov.	15.39		14.68		16.15		15.41		

(b) **Degradation of 2-CP at SRT = 8 days in presence of 40 mg SOC/L Phenol**

Time (minutes)	# 1 2-CP (mg/L)	# 1 Phenol (mg/L)	# 2 2-CP (mg/L)	# 2 Phenol (mg/L)	# 3 2-CP (mg/L)	# 3 Phenol (mg/L)	Average 2-CP mg/L	Average Phenol mg/L	% Remaining
0	31.9	52.76	31.6	56.66	31.45	50.75	31.65	53.39	100.00
20	28.78	30.17	30.05	34.44	29.36	32.47	29.40	32.36	92.88
40	25.83	18.61	26.78	14.81	26.17	15.37	26.26	16.26	82.97
60	24.01	2.85	25.23	0.85	24.72	1.68	24.65	1.79	77.89
80	21.45	0.00	20.88	0.00	21.66	0.00	21.33	0.00	67.39
100	19.81		18.64		19.30		19.25		60.82
120	16.47		15.6		16.2		16.09		50.84
140	12.35		11.08		11.78		11.74		37.08
160	7.62		6.14		7.06		6.94		21.93
180	4.02		3.2		4.2		3.81		12.03
200	0.00		0.00		0.00		0.00		0.00
Vol. Removal	9.57		9.48		9.44		9.50		
VSS (mg/L)	694		678		668		680		
Specific Remov.	13.79		13.98		14.12		13.97		

(c) **Degradation of 2-CP at SRT = 12 days in presence of 40 mg SOC/L Phenol**

Time (minutes)	# 1 2-CP (mg/L)	# 1 Phenol (mg/L)	# 2 2-CP (mg/L)	# 2 Phenol (mg/L)	# 3 2-CP (mg/L)	# 3 Phenol (mg/L)	Average 2-CP mg/L	Average Phenol mg/L	% Remaining
0	31.42	54.53	30.65	51.91	29.37	52.95	30.48	53.13	100.00
20	25.82	28.07	23.76	22.89	24.41	24.69	24.66	25.22	80.92
40	21.35	6.28	19.58	4.34	20.88	5.06	20.60	5.23	67.60
60	17.64	0.00	14.29	0.00	16.68	0.00	16.20	0.00	53.16
80	12.89		9.84		11.41		11.38		37.34
100	7.75		5.67		7.32		6.91		22.68
120	3.57		2.45		3.08		3.03		9.95
140	0.00		0.00		0.00		0.00		0.00
Vol. Removal	13.47		13.14		12.59		13.06		
VSS (mg/L)	1172		1156		1166		1165		
Specific Remov.	11.49		11.36		10.80		11.22		

(d) **Degradation of 2-CP at SRT = 16 days in presence of 40 mg SOC/L Phenol**

Time (minutes)	# 1		# 2		# 3		Average		% Remaining
	2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	
	(mg/L)		(mg/L)		(mg/L)		mg/L		
0	32.48	54.75	29.82	50.94	30.72	53.61	31.01	53.10	100.00
20	22.73	16.94	23.48	14.42	24.56	11.39	23.59	14.25	76.07
40	16.85	0.00	17.29	0.00	18.39	0.00	17.51	0.00	56.47
60	9.62		10.19		11.40		10.40		33.55
80	5.41		5.97		6.68		6.02		19.41
100	1.97		2.64		3.21		2.61		8.41
120	0.00		0.00		0.00		0.00		0.00
Vol. Removal	16.24		14.91		15.36		15.50		
VSS (mg/L)	1498		1469		1488		1485		
Specific Remov.	10.84		10.15		10.32		10.44		

(e) **Degradation of 2-CP at SRT = 20 days in presence of 40 mg SOC/L Phenol**

Time (minutes)	# 1		# 2		# 3		Average		%Remain
	2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	2-CP	Phenol	
	(mg/L)		(mg/L)		(mg/L)		mg/L		
0	29.30	54.35	31.50	49.80	30.70	58.75	30.50	54.30	100.00
20	22.73	6.48	23.48	3.59	24.56	7.75	23.59	5.94	77.34
40	16.85	0.00	17.29	0.00	18.39	0.00	17.51	0.00	57.41
60	9.62		10.19		11.40		10.40		34.11
80	2.97		3.64		4.21		3.61		11.83
100	0.00		0.00		0.00		0.00		0.00
Vol. Removal	17.58		18.90		18.42		18.30		
VSS (mg/L)	1822		1795		1813		1810		
Specific Remov.	9.65		10.53		10.16		10.11		

Fig B-1

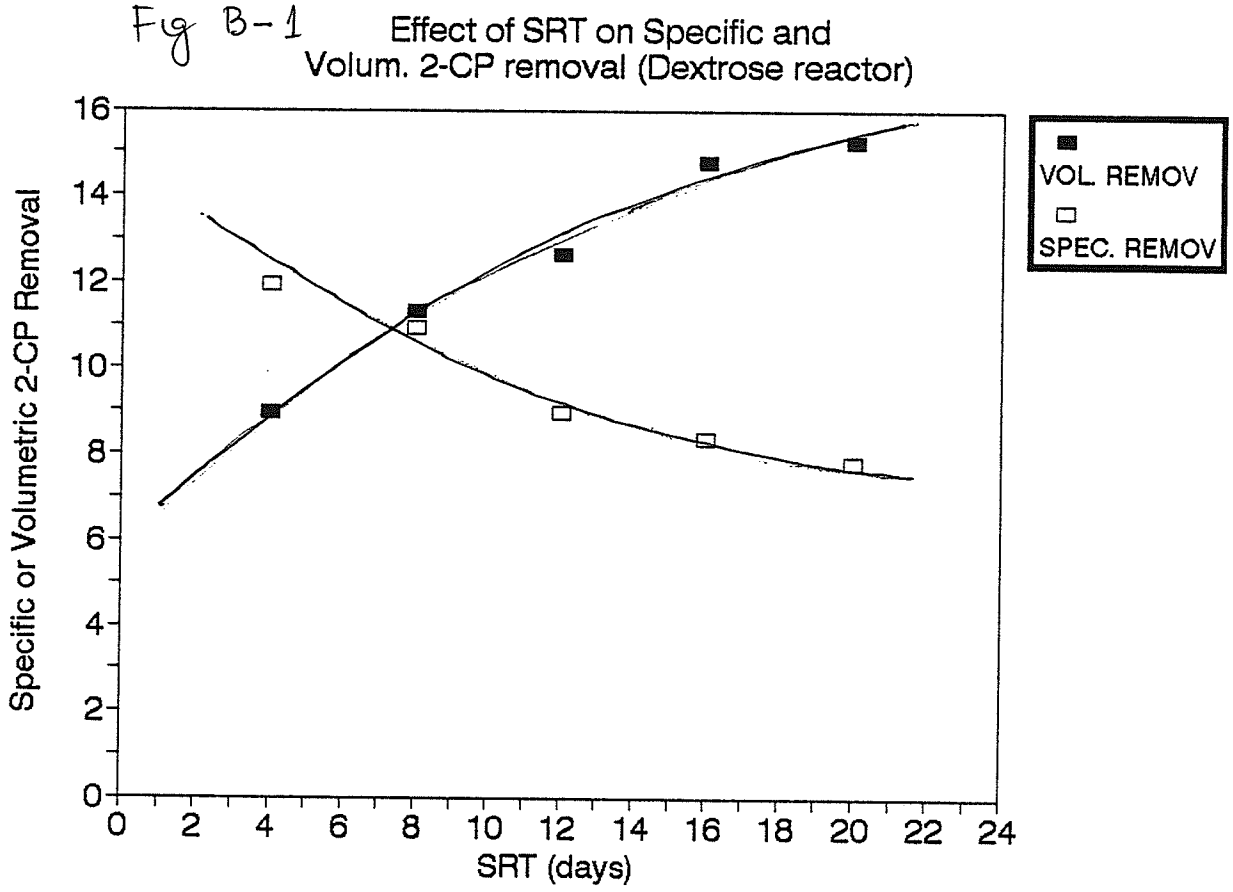
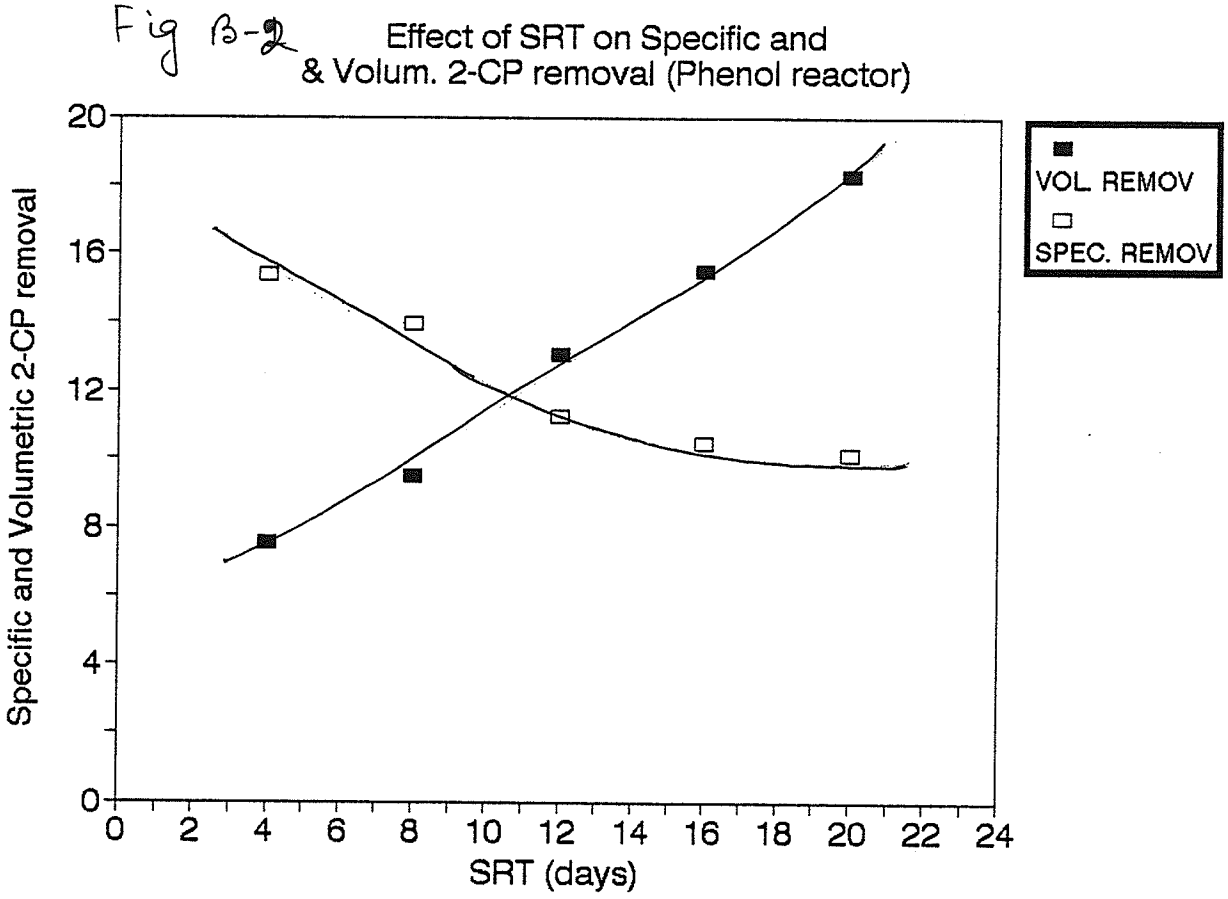

Effect of SRT on Specific and
Volum. 2-CP removal (Dextrose reactor)
Specific or Volumetric 2-CP Removal
SRT (days)
VOL. REMOV
SPEC. REMOV
0
2
4
6
8
10
12
14
16
18
20
22
24

Fig B-2


Effect of SRT on Specific and
& Volum. 2-CP removal (Phenol reactor)
Specific and Volumetric 2-CP removal
SRT (days)
VOL. REMOV
SPEC. REMOV
0
4
8
12
16
20
0
2
4
6
8
10
12
14
16
18
20
22
24

EFFECT OF SRT-CALCULATION OF Y(mg VSS/mg SOC removed)

TABLE B-20

mgSOC/mgvSS.day

		DEXTROSE			PHENOL			SPEC SOC REMOV	
SRT	1/SRT	VSS	SOC Remov	TIME	VSS	SOC Remov	TIME	Ud	Up
4	0.25	750	52.97	200	490	53.86	240	0.509	0.660
8	0.13	1032	50.1	160	680	52.1	200	0.437	0.552
12	0.08	1410	52.09	140	1165	53.4	140	0.380	0.471
16	0.06	1765	51.7	120	1485	52.4	120	0.352	0.423
20	0.05	1955	54.9	120	1810	51.25	100	0.337	0.408

DEXTROSE OUTPUT

Regression Output:

Constant	Regression Output:	-0.3390635
Std Err of Y Est		0.02004748
R Squared		0.95416541
No. of Observations		5
Degrees of Freedom		3
Degrees of Freedom		2
X Coefficient(s)	1.125254	
Std Err of Coef.	0.142388	
Std Err of Coef.	0.118083	

PHENOL OUTPUT

Regression Output:

Constant	Regression Output:	-0.269127
Std Err of Y Est		0.0196672
R Squared		0.9558879
No. of Observations		5
Degrees of Freedom		3
Degrees of Freedom		2
X Coefficient(s)	0.762382	
Std Err of Coef.	0.094556	
Std Err of Coef.	0.107092	

As per regression

	DEXTROSE				Phenol		
srt	1/srt actual	U-predict	U-actual	1/srt predict	U-predict	U-actual	1/srt predict
	NA		0.6	0.336		0.7	0.265
4	0.250	0.523	0.509	0.234	0.681	0.668	0.240
8	0.125	0.412	0.437	0.153	0.517	0.552	0.152
12	0.083	0.375	0.38	0.089	0.462	0.471	0.090
16	0.063	0.357	0.352	0.057	0.435	0.423	0.053
20	0.050	0.346	0.337	0.040	0.419	0.408	0.042
	NA		0	-0.339		0.000	-0.269

Fig B-3

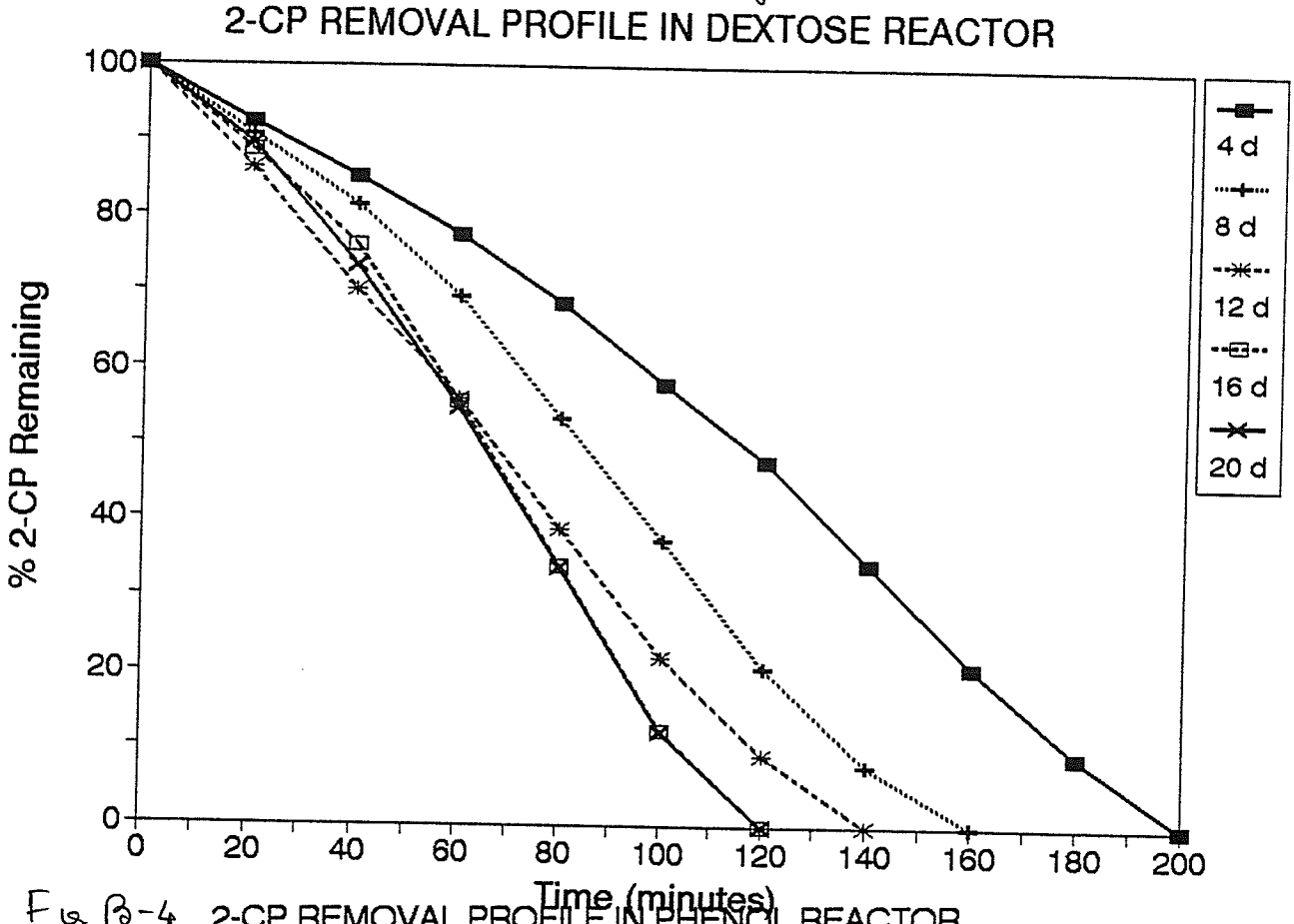
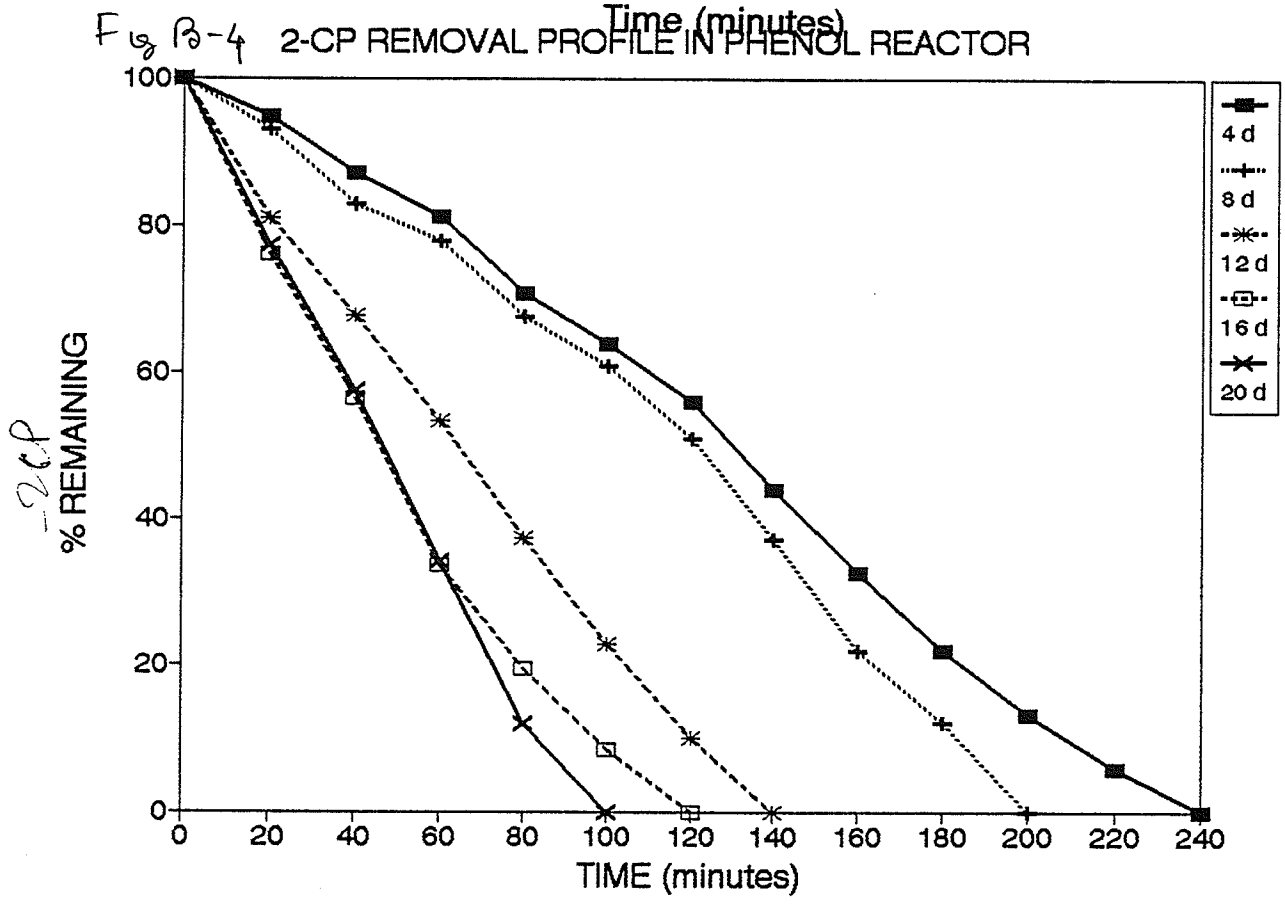

2-CP REMOVAL PROFILE IN DEXTOSE REACTOR
% 2-CP Remaining
Time (minutes)
4 d
8 d
12 d
16 d
20 d

Fig B-4


2-CP REMOVAL PROFILE IN PHENOL REACTOR
% REMAINING
TIME (minutes)
4 d
8 d
12 d
16 d
20 d

EFFECT OF BATCH ANOXIC PERIODS ON 2-CP REMOVAL BY AEROBICALLY ADAPTED MIXED CULTURES

Effect of 1 hour pre-anoxic period on subsequent aerobic removal of 2-CP in presence of 40 mg SOC/L of dextrose
Anoxic period data are shown shaded

TABLE B-21

Sample No.	Time	# 1		# 2		# 3		# 4		Average		% 2-CP Remaining
		SOC	2-CP	SOC	2-CP	SOC	2-CP	SOC	2-CP	SOC	2-CP	
	minutes	mg/L		mg/L		mg/L		mg/L				
1	0	60.08	30.92	62.32	29.79	58.15	30.31	57.89	30.72	59.61	30.44	
2	60	58.62	30.48	60.02	29.45	59.60	29.62	56.30	30.04	58.74	29.90	100.00
3	80	NA	27.34	NA	26.65	NA	26.92	NA	27.15	NA	27.02	90.35
4	100	NA	21.67	NA	21.52	NA	21.29	NA	20.83	NA	21.33	71.33
5	120	NA	14.67	NA	17.25	NA	16.47	NA	15.21	NA	15.90	53.18
6	140	NA	5.18	NA	7.59	NA	7.08	NA	6.75	NA	6.65	22.24
7	160	NA	0.00	NA	0.00	NA	0.00	NA	0.00	NA	0.00	0.00
8	180	10.81	0.00	11.57	0.00	11.31	0.00	11.90	0.00	11.40	0.00	0.00
Biomass Conc. (mg VSS/L)		1960		1990		2010		1980				

TABLE B-22

Effect of 2 hour pre-anoxic period on subsequent aerobic removal of 2-CP in presence of 40 mg SOC/L of dextrose
Anoxic period data are shown shaded

Sample No.	Time	# 1		# 2		# 3		# 4		Average		% 2-CP Remaining
		SOC	2-CP	SOC	2-CP	SOC	2-CP	SOC	2-CP	SOC	2-CP	
	minutes	mg/L		mg/L		mg/L		mg/L		mg/L		
1	0	59.36	30.30	58.23	29.94	64.51	30.53	60.40	31.64	60.63	30.60	
2	60	58.10	29.65	56.30	28.29	62.20	30.05	57.94	30.87	58.64	29.72	
3	120	56.02	29.46	55.37	28.08	60.06	29.74	56.11	30.25	56.89	29.38	100.00
4	140	NA	23.21	NA	23.55	NA	24.47	NA	24.68	NA	23.98	81.61
5	160	NA	19.26	NA	17.52	NA	18.96	NA	18.14	NA	18.47	62.87
6	180	NA	11.58	NA	13.36	NA	12.16	NA	13.21	NA	12.58	42.81
7	200	NA	5.08	NA	6.94	NA	4.21	NA	5.49	NA	5.43	18.48
8	220	NA	0.00	NA	0.00	NA	0.00	NA	0.00	NA	0.00	0.00
9	240	11.15	0.00	12.34	0.00	10.71	0.00	10.87	0.00	11.27	0.00	0.00
Biomass Conc. (mg VSS/L)		2015		1980		1995		2010				

Effect of 3 hour pre-anoxic period on subsequent aerobic removal of 2-CP in presence of 40 mg SOC/L of dextrose
Anoxic period data are shown shaded

TABLE B-23

Sample No.	Time minutes	#1 SOC	#1 2-CP	#2 SOC	#2 2-CP	#3 SOC	#3 2-CP	#4 SOC	#4 2-CP	Average SOC	Average 2-CP
		mg/L		mg/L		mg/L		mg/L		mg/L	
1	0	55.79	30.28	60.71	30.29	58.88	29.29	58.53	30.56	58.48	30.11
2	60	55.02	29.65	58.92	29.81	57.71	28.69	57.62	30.11	57.32	29.57
3	120	53.48	29.36	56.38	29.13	55.64	28.15	57.10	29.92	55.65	29.14
4	180	52.17	29.20	56.22	29.10	54.90	28.05	55.27	29.69	54.64	29.01
5	200	NA	22.18	NA	20.97	NA	21.52	NA	22.75	NA	21.86
6	220	NA	14.67	NA	15.08	NA	14.73	NA	16.21	NA	15.17
7	240	NA	5.24	NA	5.52	NA	4.45	NA	6.14	NA	5.34
8	260	NA	0.00	NA	0.00	NA	0.00	NA	0.00	NA	0.00
9	280	12.06	0.00	11.28	0.00	11.67	0.00	12.17	0.00	11.80	0.00
Biomass Conc. (mg VSS/L)		1990		2020		1985		2005			

TABLE B-24

Effect of 4 hour pre-anoxic period on subsequent aerobic removal of 2-CP in presence of 40 mg SOC/L of dextrose
Anoxic period data are shown shaded

Sample No.	Time minutes	#1 SOC	#1 2-CP	#2 SOC	#2 2-CP	#3 SOC	#3 2-CP	#4 SOC	#4 2-CP	Average SOC	Average 2-CP
		mg/L		mg/L		mg/L		mg/L		mg/L	
1	0	62.70	29.65	61.62	30.14	59.79	30.31	56.37	30.82	60.17	30.23
2	60	61.22	30.12	61.39	29.82	59.40	29.87	54.22	30.66	59.06	30.12
3	120	59.79	28.97	60.14	29.57	58.87	29.37	54.03	30.36	58.21	29.57
4	180	59.83	28.63	56.22	29.01	54.90	29.30	55.27	29.58	56.56	29.13
5	240	59.71	28.60	56.38	28.92	54.16	29.18	55.30	29.67	56.39	29.09
6	260	NA	21.94	NA	21.65	NA	21.37	NA	22.35	NA	21.83
7	280	NA	18.18	NA	18.43	NA	17.82	NA	19.58	NA	18.50
8	300	NA	7.47	NA	8.55	NA	8.17	NA	8.76	NA	8.24
9	320	NA	0.00	NA	0.00	NA	0.00	NA	0.00	NA	0.00
10	340	12.21	0.00	11.49	0.00	10.78	0.00	11.21	0.00	11.42	0.00
Biomass Conc. (mg VSS/L)		1970		1985		2005		1995			

Composite Results of Aerobic 2-CP removal following various Pre-Anoxic periods

TABLE B-25

TIME	0 hr Anoxic		1 hr Anoxic		2 hr Anoxic		3 hr Anoxic		4 hr Anoxic	
	2-CP	Removal	2-CP	Removal	2-CP	Removal	2-CP	Removal	2-CP	Removal
(minutes)	(mg/L)	(%)	(mg/L)	(%)	(mg/L)	(%)	(mg/L)	(%)	(mg/L)	(%)
0	30.22	0.00	29.90	0.00	29.38	0.00	29.01	0.00	29.09	0.00
20	26.96	10.79	27.02	9.63	23.98	18.38	21.86	24.65	21.83	24.83
40	21.48	28.92	21.33	28.66	18.47	37.13	15.17	47.71	18.50	36.29
60	15.27	49.47	15.90	46.82	12.58	57.18	5.34	81.59	8.24	71.63
80	8.96	70.35	6.65	77.76	5.43	81.52	0.00	100.00	0.00	100.00
100	2.26	92.52	0.00	100.00	0.00	100.00	NA	NA	NA	NA
120	0.00	100.00	NA	NA	NA	NA	NA	NA	NA	NA

Results of Regression on 2-CP removal data from Table PA 5

Output for 0 hour ANOX

Regression Output:

Constant	0
Std Err of Y Est	4.6664874
R Squared	0.9858041
No. of Observations	7
Degrees of Freedom	6
X Coefficient(s)	0.85772103
Std Err of Coef.	0.02445904

Output for 1 hour ANOX

Regression Output:

Constant	0
Std Err of Y Est	7.6657402
R Squared	0.9615212
No. of Observations	6
Degrees of Freedom	5
X Coefficient(s)	0.92587413
Std Err of Coef.	0.05168241

Output for 2 hour ANOX

Regression Output:

Constant	0
Std Err of Y Est	1.9678546
R Squared	0.9973126
No. of Observations	6
Degrees of Freedom	5
X Coefficient(s)	0.99115044
Std Err of Coef.	0.01326727

Output for 3 hour ANOX

Regression Output:

Constant	0
Std Err of Y Est	3.2210535
R Squared	0.9937527
No. of Observations	5
Degrees of Freedom	4
X Coefficient(s)	1.27473285
Std Err of Coef.	0.02940406

Output for 4 hour ANOX

Regression Output:

Constant	0
Std Err of Y Est	6.1615428
R Squared	0.9756231
No. of Observations	5
Degrees of Freedom	4
X Coefficient(s)	1.18715565
Std Err of Coef.	0.05624693

PREDICTED % 2-CP REMOVAL

TIME	0 HR ANX	1 HR ANX	2 HR ANX	3 HR ANX	4 HR ANX
0	0.00	0.00	0.00	0.00	0.00
20	17.15	18.52	19.82	25.49	23.74
40	34.31	37.03	39.65	50.99	47.49
60	51.46	55.55	59.47	76.48	71.23
80	68.62	74.07	79.29	101.98	94.97
100	85.77	92.59	99.12		
120	102.93				

Effect of 1 hour pre-anoxic period on subsequent aerobic removal of 2-CP in presence of 40 mg SOC/L of Phenol
Anoxic period data are shown shaded

TABLE B-26

Sample N	Time minutes	# 1 Phenol mg/L	# 1 2-CP mg/L	# 2 Phenol mg/L	# 2 2-CP mg/L		Average Phenol	Average 2-CP
1	0	47.51	28.95	50.37	30.29		48.94	29.62
2	60	45.25	28.17	48.99	29.64	No further experiments could be done due to	47.14	28.91
3	80	23.10	22.83	18.61	21.03	washout of the phenol sludge by a timer failure	20.86	21.93
4	100	0.00	12.20	0.00	15.62		0.00	13.91
5	120	0.85	0.00	0.00	0.00		0.00	0.00
Biomass Conc. (mg VSS/L)		2010		1975				
No Anoxic period (parallel experiment)								
1	0	50.02	31.08	55.14	30.78		52.58	30.93
2	20	5.90	24.62	8.67	21.32		7.29	22.97
3	40	0.00	11.49	0.00	14.67		0.00	13.08
4	60		5.21		6.15		0.00	5.68
5	80		0.00		0.00		0.00	0.00
Biomass Conc. (mg VSS/L)		1970		1990				

Fresh actvated sludge exposed to 30 mg/L 2-CP

Time (hour)	# 1 mg/L	# 2 mg/L	Avg.
0	29.11	30.28	29.70
1	28.78	30.06	29.42
2	28.82	29.91	29.37
3	27.75	29.87	28.81
4	27.56	29.81	28.69
5	27.43	29.65	28.54
6	27.48	29.74	28.61
7	28.11	28.82	28.47
VSS	2010	1985	

2-CP removal with 3 h pre-anoxic period under different cultural conditions in presence of 40 mg SOC/L of dextrose
Anoxic period data are shown shaded

TABLE B-27

	Sample No.	Time minutes	I SOC (mg/L)	I 2-CP (mg/L)	II SOC (mg/L)	II 2-CP (mg/L)	III SOC (mg/L)	III NO3-N (mg/L)	III 2-CP (mg/L)	IV SOC mg/L	IV 2-CP mg/L
	1	0	60.13	30.70	59.19	31.92	56.67	24.55	31.29	60.66	31.26
	2	20	NA	27.05	NA	NA	NA	NA	NA	NA	NA
	3	40	NA	22.54	NA	NA	NA	NA	NA	NA	NA
	4	60	NA	16.54	58.84	31.33	56.88	22.87	31.05	58.72	30.69
	5	80	NA	10.06	NA	NA	NA	NA	NA	NA	NA
	6	100	NA	5.65	NA	NA	NA	NA	NA	NA	NA
Expt # 1	7	120	15.61	0.00	57.38	30.76	54.89	22.4	30.97	57.81	30.62
	8	180	12.84	0.00	56.69	30.15	55.46	21.6	30.54	57.70	30.25
	9	200			NA	23.08	NA		21.36	NA	20.54
	10	220			NA	16.28	NA		16.28	NA	12.59
	11	240			NA	4.32	NA		8.92	NA	4.87
	12	260			NA	0.00	NA		0.00	NA	0.00
	13	280			13.14	0.00	12.54		0.00	11.49	0.00
	Biomass Conc. (mg VSS/L)			1975		1985			1995		1965

	Sample No.	Time minutes	I SOC (mg/L)	I 2-CP (mg/L)	II SOC (mg/L)	II 2-CP (mg/L)	III SOC (mg/L)	III NO3-N (mg/L)	III 2-CP (mg/L)	IV SOC mg/L	IV 2-CP mg/L
	1	0	56.85	31.36	58.51	30.79	59.71	26.8	30.65	60.74	31.12
	2	20	NA	27.9	NA	NA	NA	NA	NA	NA	NA
	3	40	NA	23.69	NA	NA	NA	NA	NA	NA	NA
	4	60	NA	15.37	57.75	30.65	59.38	25.6	30.22	60.32	30.90
	5	80	NA	8.26	NA	NA	NA	NA	NA	NA	NA
	6	100	NA	3.67	NA	NA	NA	NA	NA	NA	NA
Expt # 2	7	120	14.17	0.00	57.86	30.68	59.42	24.9	29.87	59.87	30.68
	8	180	11.92	0.00	56.32	30.42	58.64	23.6	29.65	57.93	30.76
	9	200			NA	22.19	NA		22.97	NA	19.25
	10	220			NA	14.06	NA		15.25	NA	12.92
	11	240			NA	3.28	NA		8.75	NA	5.69
	12	260			NA	0.00	NA		0.00	NA	0.00
	13	280			13.14	0.00	12.54		0.00	11.49	0.00
	Biomass Conc. (mg VSS/L)			1960		1950			1970		1980

I :- Fully Aerobic conditions (0 h Preanoxic)
II :- 3 h Preanoxic conditions
III :- 3 H preanoxic conditions with nitrate in feed
IV :- 3 h preanoxic with nitrogen free feed

comparison of removal under different cultural conditions

Time minutes	Fully aerobic				preanoxic with standard feed				No3				Nitrogen free			
0	31.36	30.70	31.03	0.00	30.15	30.42	30.29	0.00	29.65	30.54	30.10	0.00	30.76	30.26	30.51	0.00
20	27.9	27.05	27.475	11.46	23.08	22.19	22.64	25.27	22.97	21.36	22.17	26.36	19.25	20.54	19.90	34.79
40	23.69	22.54	23.115	25.51	16.28	14.06	15.17	49.92	15.25	16.28	15.77	47.62	12.92	12.59	12.76	58.18
60	15.37	16.54	15.955	48.58	4.32	3.28	3.80	87.45	8.75	8.92	8.84	70.65	5.69	4.87	5.28	82.69
80	8.26	10.06	9.16	70.48	0.00	0.00	0.00	100.00	0.00	0.00	0.00	100.00	0.00	0.00	0.00	100.00
100	3.67	5.65	4.66	84.98												
120	0.00	0.00	0	100.00												

I Fully Aerobic

Regression Output:

Constant	0
Std Err of Y Est	4.2353786
R Squared	0.9876558
No. of Observations	7
Degrees of Freedom	6
X Coefficient(s)	0.83244503
Std Err of Coef.	0.02219942

II : Standard Pre-Anoxic

Regression Output:

Constant	0
Std Err of Y Est	5.2056552
R Squared	0.9844758
No. of Observations	5
Degrees of Freedom	4
X Coefficient(s)	1.31245185
Std Err of Coef.	0.04752091

Predicted concentrations of 2-CP

Time	Fully Aerob	Preanox Std	No3	N free
0	0	0	0	0
20	16.6489	26.2	24.4	26.6
40	33.2978	52.4	48.8	53.2
60	49.9467	78.6	73.2	79.8
80	66.5956	104.8	97.6	106.4
100	83.2445			
120	99.8934			

III Preanoxcl with No3

Regression Output:

Constant	0.00
Std Err of Y Est	2.09
R Squared	1.00
No. of Observations	5.00
Degrees of Freedom	4.00
X Coefficient(s)	1.22
Std Err of Coef.	0.02

IV Preanoxic with Nitrogen free medium

Regression Output:

Constant	0
Std Err of Y Est	5.944123
R Squared	0.9773344
No. of Observations	5
Degrees of Freedom	4
X Coefficient(s)	1.33210423
Std Err of Coef.	0.05426217

TABLE B-28

TABLE B-29

Analysis of Temp data (Arrhenius Plot)

temp	K (dext)	K(phenol)	temp(kelv	ln(kd)	ln(Kp)	1/T	1000 * 1/T
5	1.5	3.36	278	0.405	1.212	0.003597	3.6
10	4.3	3.83	283	1.459	1.343	0.003534	3.53
15	5.23	5.77	288	1.654	1.753	0.003472	3.47
24	7.78	11.54	297	2.052	2.446	0.003367	3.37

Dextrose data (Arrhenius Plot) with 5 C

Regression Output:

Constant	24.72748
Std Err of Y Est	0.324335
R Squared	0.857997
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-6.68145
Std Err of Coef.	1.922039

Phenol data (Arrhenius Plot) with 5 C

Regression Output:

Constant	21.11716
Std Err of Y Est	0.146633
R Squared	0.953472
No. of Observations	4
Degrees of Freedom	2
X Coefficient(s)	-5.56302
Std Err of Coef.	0.868959

Dextrose data (Arrhenius Plot) without 5 C

Regression Output:

Constant	14.6252
Std Err of Y Est	0.021461
R Squared	0.997477
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	-3.73298
Std Err of Coef.	0.187745

Phenol data (Arrhenius Plot) without 5 C

Regression Output:

Constant	176.9701
Std Err of Y Est	0.768726
R Squared	0.981629
No. of Observations	3
Degrees of Freedom	1
X Coefficient(s)	-49.1582
Std Err of Coef.	6.724952

TABLE B-30

2-CP degradation at 10 degree Celcius in presence of 40 mgSOC/L Dextrose

Sample No.	Time (minutes)	#1 2-CP (mg/L)	#1 SOC (mg/L)	#2 2-CP (mg/L)	#2 SOC (mg/L)	#3 2-CP (mg/L)	#3 SOC (mg/L)	#4 2-CP (mg/L)	#4 SOC (mg/L)	Average 2-CP (mg/L)
1	0	30.64	60.28	31.6	58.69	30.72	57.73	30.12	61.3	30.77
2	20	28.15	NA	28.94	NA	28.36	NA	27.94	NA	28.35
3	40	26.37	NA	26.12	NA	25.57	NA	25.82	NA	25.97
4	60	23.67	NA	24.12	NA	22.52	NA	23.64	NA	23.49
5	80	20.74	NA	20.92	NA	21.94	NA	21.22	NA	21.21
6	100	18.86	NA	18.33	NA	18.59	NA	18.56	NA	18.59
7	120	16.01	NA	16.25	NA	15.29	NA	15.73	NA	15.82
8	140	12.61	NA	12.31	NA	12.57	NA	11.91	NA	12.35
9	160	11.06	NA	10.30	NA	9.86	NA	9.38	NA	10.15
10	180	8.12	NA	9.06	NA	7.03	NA	6.86	NA	7.77
11	200	5.35	NA	5.21	NA	4.06	NA	4.24	NA	4.72
12	220	0.00	NA	0.00	NA	0.00	NA	0.00	NA	0.00
13	240	0.00	11.44	0.00	10.94	0.00	11.25	0.00	10.58	0.00
MLVSS (mg/L)		1955		1990		2005		1970		1980
2-CP removal(mg/L.hr)		8.36		8.62		8.38		8.21		8.39
2-CP removal(mg/gVSS.hr)		4.27		4.33		4.18		4.17		4.24

(a)

2-CP degradation at 15 degree Celcius in presence of 40 mgSOC/L Dextrose

Sample No.	Time (minutes)	#1 2-CP (mg/L)	#1 SOC (mg/L)	#2 2-CP (mg/L)	#2 SOC (mg/L)	#3 2-CP (mg/L)	#3 SOC (mg/L)	#4 2-CP (mg/L)	#4 SOC (mg/L)	Average 2-CP (mg/L)
1	0	30.15	55.62	31.58	58.19	30.67	60.68	30.62	56.29	30.76
2	20	27.60	NA	26.75	NA	25.31	NA	25.89	NA	26.39
3	40	25.59	NA	23.82	NA	22.14	NA	24.05	NA	23.90
4	60	21.68	NA	20.29	NA	20.47	NA	21.18	NA	20.91
5	80	17.19	NA	17.78	NA	16.32	NA	16.95	NA	17.06
6	100	12.53	NA	14.79	NA	12.90	NA	13.69	NA	13.48
7	120	9.79	NA	10.28	NA	9.60	NA	10.12	NA	9.95
8	140	6.09	NA	6.68	NA	5.58	NA	5.96	NA	6.08
9	160	2.09	NA	2.85	NA	1.65	NA	2.71	NA	2.33
10	180	0.00	NA	0.00	NA	0.00	NA	0.00	NA	0.00
11	200	0.00	11.64	0.00	10.83	0.00	10.49	0.00	11.01	0.00
MLVSS (mg/l)		1960		1975		1955		1970		1965
2-CP removal(mg/L.hr)		10.05		10.53		10.22		10.21		10.25
2-CP removal(mg/gVSS.hr)		5.13		5.33		5.23		5.18		5.22

(b)

2-CP degradation at 24 degree Celcius in presence of 40 mgSOC/L Dextrose

Sample No.	Time (minutes)	#1 2-CP (mg/L)	#1 SOC (mg/L)	#2 2-CP (mg/L)	#2 SOC (mg/L)	#3 2-CP (mg/L)	#3 SOC (mg/L)	#4 2-CP (mg/L)	#4 SOC (mg/L)	Average 2-CP (mg/L)
1	0	31.14	61.55	30.48	60.28	29.96	58.62	30.75	60.76	30.58
2	20	26.97	NA	27.28	NA	26.62	NA	27.08	NA	26.99
3	40	20.89	NA	21.27	NA	22.88	NA	21.39	NA	21.61
4	60	14.44	NA	17.24	NA	15.12	NA	15.87	NA	15.67
5	80	8.17	NA	10.73	NA	8.35	NA	10.73	NA	9.50
6	100	3.34	NA	4.15	NA	3.85	NA	4.29	NA	3.91
7	120	0.00	NA	0.00	NA	0.00	NA	0.00	NA	0.00
8	140	0.00	12.85	0.00	12.05	0.00	11.51	0.00	10.85	0.00
MLVSS (mg/l)		1955		1960		1970		1980		1966
2-CP removal(mg/L.hr)		15.57		15.24		14.98		15.38		15.29
2-CP removal(mg/gVSS.hr)		7.96		7.78		7.60		7.77		7.78

(c)

Effect of Temperature on 2-CP degradation in presence of 40 mgSOC/L Dextrose

TABLE B-31

) 2-CP degradation at 5 degree Celcius in presence of 40 mgSOC/L Phenol

Sample No.	Time (minutes)	#1 2-CP (mg/L)	#1 Phenol (mg/L)	#2 2-CP (mg/L)	#2 Phenol (mg/L)	#3 2-CP (mg/L)	#3 Phenol (mg/L)	#4 2-CP (mg/L)	#4 Phenol (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	33.28	57.08	30.14	52.19	30.53	52.84	29.75	53.63	30.92	53.94
2	20	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
3	40	31.05	37.93	28.32	34.52	29.09	32.07	27.48	35.61	28.99	35.03
4	60	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
5	80	28.48	23.99	26.17	20.87	27.42	25.26	25.82	23.28	26.97	23.35
6	100	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
7	120	26.25	14.72	23.79	12.08	24.18	15.29	23.16	15.59	24.35	14.42
8	140	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA
9	160	20.72	4.88	19.12	2.33	21.26	5.32	20.11	6.17	20.30	4.68
10	180	NA	0.00	NA	0.00	NA	NA	NA	NA	NA	NA
11	200	14.49		14.81		16.14	0.00	15.93	0.00	15.34	0.00
12	220	9.79		10.94		11.38		10.65		10.69	
13	240	6.39		6.26		7.45		6.72		6.71	
14	260	2.37		2.88		3.29		2.79		2.83	
15	280	0.00		0.00		0.00		0.00		0.00	
MLVSS (mg/l)		1975		1990		1955		1965		1971	
2-CP removal(mg/L.hr)		7.13		6.46		6.54		6.38		6.63	
2-CP removal(mg/gVSS.hr)		3.61		3.25		3.35		3.24		3.36	

(a)

2-CP degradation at 10 degree Celcius in presence of 40 mgSOC/L Phenol

Sample No.	Time (minutes)	#1 2-CP (mg/L)	#1 Phenol (mg/L)	#2 2-CP (mg/L)	#2 Phenol (mg/L)	#3 2-CP (mg/L)	#3 Phenol (mg/L)	#4 2-CP (mg/L)	#4 Phenol (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	28.88	50.93	28.72	54.69	30.67	56.19	30.25	52.48	30.13	53.57
2	20	27.86	45.87	27.18	46.27	28.38	49.36	28.14	43.88	27.89	46.35
3	40	26.33	37.02	25.09	34.17	26.13	39.83	26.08	33.78	25.91	36.20
4	60	24.73	30.45	23.93	28.86	24.09	33.54	24.19	29.32	24.24	30.54
5	80	22.03	26.10	22.06	22.95	22.18	30.34	21.88	24.61	22.04	26.00
6	100	20.06	19.10	20.98	16.10	20.86	21.44	19.97	16.65	20.47	18.32
7	120	18.66	12.68	19.43	10.23	19.79	14.05	18.46	11.54	19.09	12.13
8	140	17.01	5.77	17.48	4.17	18.06	6.68	16.59	4.48	17.29	5.28
9	160	15.92	0.00	16.17	0.00	15.11	0.00	15.02	0.00	15.56	0.00
10	180	12.33		11.89		10.76		12.27		11.81	
11	200	7.62		6.72		5.88		6.64		6.72	
12	220	3.06		2.89		1.97		2.51		2.61	
13	240	0.00		0.00		0.00		0.00		0.00	
MLVSS (mg/l)		1970		1980		1955		1965		1968	
2-CP removal(mg/L.hr)		7.47		7.43		7.67		7.56		7.53	
2-CP removal(mg/gVSS.hr)		3.79		3.75		3.92		3.85		3.83	

(b)

-CP degradation at 15 degree Celcius in presence of 40 mgSOC/L Phenol

Sample No.	Time (minutes)	#1 2-CP (mg/L)	#1 Phenol (mg/L)	#2 2-CP (mg/L)	#2 Phenol (mg/L)	#3 2-CP (mg/L)	#3 Phenol (mg/L)	#4 2-CP (mg/L)	#4 Phenol (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	30.42	50.59	31.19	52.56	29.74	51.95	30.17	55.22	30.38	52.58
2	20	28.39	35.76	27.81	37.38	27.18	39.73	28.02	40.18	27.85	38.26
3	40	25.14	28.12	24.78	29.04	25.43	28.87	26.61	31.76	25.49	29.45
4	60	23.19	20.74	22.76	16.71	22.14	20.05	23.35	18.24	22.86	18.94
5	80	20.24	8.52	18.63	7.82	19.82	10.17	20.07	10.97	19.69	9.37
6	100	14.68	1.76	13.64	1.21	14.38	3.25	15.91	2.67	14.70	2.22
7	120	10.37	0.00	9.89	0.00	10.88	0.00	10.77	0.00	10.48	0.00
8	140	5.54		4.48		5.72		5.94		5.42	
9	160	0.00		0.00		0.00		0.00		0.00	
MLVSS (mg/l)		1960		1970		1970		1960		1970	
2-CP removal(mg/L.hr)		11.70		11.31		11.15		11.31		11.37	
2-CP removal(mg/gVSS.hr)		5.91		5.74		5.66		5.77		5.77	

(c)

2-CP degradation at 24 degree Celcius in presence of 40 mgSOC/L Phenol

Sample No.	Time (minutes)	#1 2-CP (mg/L)	#1 Phenol (mg/L)	#2 2-CP (mg/L)	#2 Phenol (mg/L)	#3 2-CP (mg/L)	#3 Phenol (mg/L)	#4 2-CP (mg/L)	#4 Phenol (mg/L)	Average 2-CP (mg/L)	Average Phenol (mg/L)
1	0	30.60	54.66	30.29	53.4	29.98	51.10	30.3	52.9	30.29	53.02
2	20	21.10	4.79	20.34	5.20	19.98	4.10	18.80	6.81	20.05	5.23
3	40	15.80	0.00	12.16	0.00	12.60	0.00	11.20	0.00	12.94	0.00
4	60	6.35		4.69		3.87		5.10		5.00	
5	80	0.00		0.00		0.00		0.00		0.00	
MLVSS (g/l)		1970		1965		1950		1990		1969	
2-CP removal(mg/L.hr)		22.96		22.72		22.47		22.73		22.72	
2-CP removal(mg/gVSS.hr)		11.65		11.56		11.52		11.42		11.52	

(d)

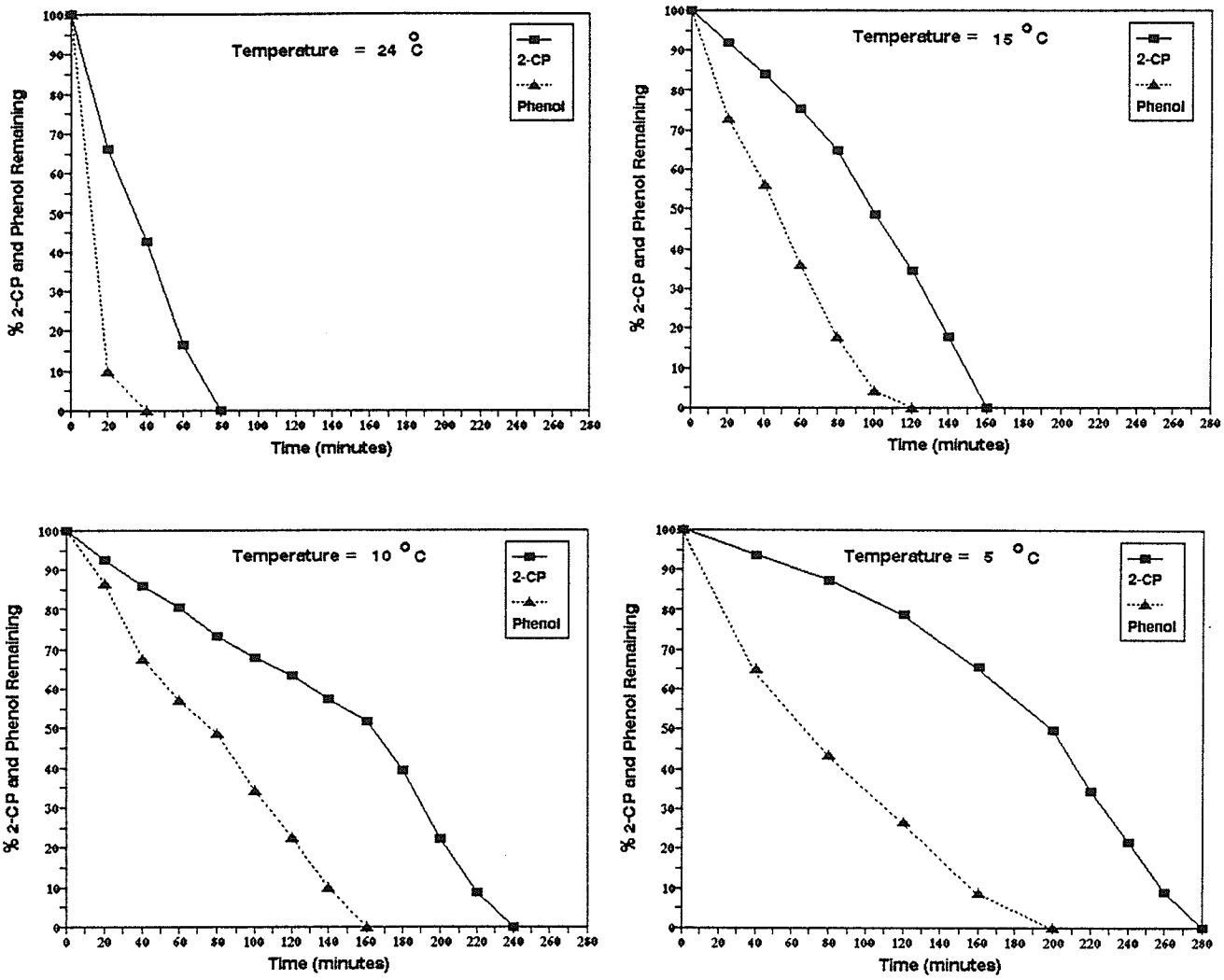


Fig B-5 Effect of temperature on removal of 2-CP and Phenol.

APPENDIX C

TABLE C-1

Performance of Anoxic-Aerobic SBR treating 2-CP in presence of 40 mgSOC/L of dextrose

Phase	Days	Total Days	pH		SOC (mg/L)			SOC(mg/L)
			Influent	Effluent	Influent	Effluent(Anox)	% Removal	Effluent(Aerob)
I	0	0	6.80	7.10	51.20	48.80	4.69	22.90
	7	7	6.90	7.30	58.40	46.20	20.89	22.70
	12	12	7.00	7.40	55.30	34.70	37.25	21.90
	18	18	7.00	7.30	53.91	30.20	43.98	20.80
	25	25	6.85	7.20	50.50	28.70	43.17	20.20
	32	32	6.90	7.10	53.60	27.20	49.25	16.10
	40	40	6.80	7.20	55.80	26.80	51.97	14.80
	48	48	6.90	7.40	59.40	22.30	62.46	13.30
	53	53	7.00	7.30	50.70	18.90	62.72	12.10
	60	60	6.80	7.20	52.30	19.70	62.33	12.80
	65	65	6.90	7.20	56.70	22.40	60.49	13.20
II	0	66	6.90	7.60	52.71	16.30	69.08	12.00
	10	76	7.10	7.30	40.70	15.10	62.90	11.40
	15	81	6.80	7.40	54.80	18.20	66.79	12.10
	20	86	7.00	7.40	58.30	18.70	67.92	11.80
	25	91	6.90	7.30	53.60	16.40	69.40	11.10
	30	96	6.95	7.30	56.30	16.80	70.16	10.80
III	0	98	6.80	7.40	50.91	17.20	66.21	12.80
	7	105	7.00	7.20	56.70	19.20	66.14	13.20
	15	113	6.90	7.20	60.30	14.60	75.79	10.40
	24	122	6.90	7.10	58.30	15.20	73.93	11.10
	36	134	6.85	7.20	57.70	16.50	71.40	12.20

TABLE C-2

Performance of Anoxic-Aerobic SBR treating 2-CP in presence of 40 mgSOC/L of PHENOL

Phase	Days	Total Days	pH		SOC (mg/L)			SOC(mg/L)
			Influent	Effluent	Influent	Effluent(Anox)	% Removal	Effluent(Aerob)
I	0	0	6.90	7.30	55.60	48.10	13.49	11.80
	7	7	7.00	7.20	59.40	45.40	23.57	13.20
	12	12	7.00	7.40	52.30	41.40	20.84	14.10
	18	18	6.90	7.30	60.10	36.10	39.93	12.70
	25	25	6.85	7.40	57.60	27.10	52.95	12.30
	32	32	6.85	7.40	54.80	30.70	43.98	12.60
	40	40	6.80	7.30	57.20	29.30	48.78	11.50
	48	48	7.00	7.50	58.70	30.80	47.53	11.70
	53	53	6.90	7.20	56.80	29.10	48.77	12.10
	60	60	6.80	7.40	60.80	28.50	53.13	14.20
	65	65	6.80	7.10	54.50	26.90	50.64	12.60
II	0	66	6.80	7.20	54.60	21.30	60.99	13.10
	10	76	7.00	7.30	58.90	22.30	62.14	13.20
	15	81	6.90	7.40	50.70	24.80	51.08	12.50
	20	86	6.80	7.20	53.60	23.40	56.34	10.80
	25	91	6.90	7.10	51.50	21.70	57.86	11.30
	30	96	6.80	7.40	55.40	20.80	62.45	12.10
III	0	98	6.90	7.40	59.70	18.90	68.34	13.10
	7	105	6.80	7.20	60.20	20.20	66.45	11.70
	15	113	6.80	7.30	51.30	17.30	66.28	12.40
	24	122	7.00	7.10	52.80	16.90	67.99	12.30
	36	134	6.80	7.20	54.70	17.40	68.19	11.90

Phase I : 2 hours Anoxic followed by 4 hours Aerobic
Phase II : 3 hours Anoxic followed by 3 hours Aerobic
Phase III : 4 hours Anoxic followed by 2 hours Aerobic

TABLE C-3

Performance of Anoxic-Aerobic SBR treating 2-CP in presence of 40 mgSOC/L of dextrose

Phase	Days	Total Days	2-CP (mg/L) Influent	2-CP (mg/L) Effluent(Anox)	% Removal	2-CP (mg/L) Effluent(Aerob)	NO3-N (mg/L) Influent	NO3-N (mg/L) Effluent(Anoxic)	% Removal
I	0	0	15.20	14.10	7.24	13.96	21.70	18.20	16.13
	7	7	14.80	14.60	1.35	14.10	27.30	24.10	11.72
	12	12	14.60	14.00	4.11	13.35	38.40	35.20	8.33
	18	18	14.90	14.70	1.34	12.63	24.70	22.30	9.72
	25	25	15.20	14.80	2.63	9.77	30.30	28.70	5.28
	32	32	15.00	14.52	3.20	8.15	36.20	30.80	14.92
	40	40	14.78	14.11	4.53	2.27	30.30	26.10	13.86
	48	48	14.81	12.52	15.46	0.00	36.70	32.50	11.44
	53	53	15.08	11.85	21.42	0.00	38.10	31.70	16.80
	60	60	15.30	12.28	19.74	0.00	34.40	28.00	18.60
	65	65	15.10	11.90	21.19	0.00	37.67	31.50	16.38
II	0	66	14.69	8.70	40.78	0.00	34.60	26.40	23.70
	10	76	9.69	2.83	70.79	0.00	32.70	25.85	20.95
	15	81	14.30	8.30	41.96	0.00	30.10	21.10	29.90
	20	86	15.10	7.70	49.01	0.00	35.50	24.50	30.99
	25	91	14.80	7.50	49.32	0.00	38.10	28.10	26.25
	30	96	14.75	8.00	45.76	0.00	31.70	21.90	30.91
III	0	98	15.20	8.00	47.37	0.00	22.10	12.10	45.25
	7	105	14.71	10.30	29.98	0.00	36.70	24.90	32.15
	15	113	14.90	6.00	59.73	0.00	32.10	20.20	37.07
	24	122	14.60	6.20	57.53	0.00	31.10	18.80	39.55
	36	134	15.10	6.80	54.97	0.00	35.30	22.60	35.98

TABLE C-4

Performance of Anoxic-Aerobic SBR treating 2-CP in presence of 40 mgSOC/L of PHENOL

Phase	Days	Total Days	2-CP (mg/L) Influent	2-CP (mg/L) Effluent(Anox)	% Removal	2-CP (mg/L) Effluent(Aerob)	Phenol (mg/L) Influent	Phenol (mg/L) Effluent(Anox)	% Removal	Phenol(mg/L) Effluent(Aerob)	NO3-N (mg/L) Influent	NO3-N (mg/L) Effluent(Anoxic)	% Removal
I	0	0	14.70	14.20	3.40	13.50	48.80	45.30	7.17	24.30	22.40	20.10	10.27
	7	7	15.10	13.70	9.27	13.00	53.10	46.80	11.86	20.10	30.40	26.80	11.84
	12	12	15.30	13.80	9.80	12.40	50.80	41.70	17.91	16.40	35.10	30.70	12.54
	18	18	15.07	12.10	19.71	4.60	52.70	36.70	30.36	6.30	30.30	27.30	9.90
	25	25	14.70	10.40	29.25	1.20	56.10	34.80	37.97	0.00	34.70	29.60	14.70
	32	32	14.20	8.20	42.25	0.00	49.80	32.10	35.54	0.00	36.20	30.70	15.19
	40	40	15.20	7.80	48.68	0.00	50.70	30.70	39.45	0.00	35.60	29.10	18.26
	48	48	15.10	7.20	52.32	0.00	53.20	31.20	41.35	0.00	39.30	31.70	19.34
	53	53	15.08	8.10	46.29	0.00	56.20	30.40	45.91	0.00	37.10	27.10	26.95
	60	60	14.70	7.70	47.62	0.00	50.10	29.30	41.52	0.00	31.40	21.50	31.53
	65	65	14.60	7.90	45.89	0.00	52.70	28.20	46.49	0.00	36.80	26.70	27.45
II	0	66	14.10	8.10	42.55	0.00	54.30	19.20	64.64	0.00	36.70	22.50	38.69
	10	76	14.70	7.10	51.70	0.00	50.10	21.70	56.69	0.00	31.20	18.40	41.03
	15	81	14.80	6.90	53.38	0.00	49.30	24.10	51.12	0.00	34.70	22.30	35.73
	20	86	14.60	5.80	60.27	0.00	52.70	23.70	55.03	0.00	30.80	17.80	42.21
	25	91	14.90	6.10	59.06	0.00	53.20	22.30	58.08	0.00	38.20	25.60	32.98
	30	96	15.10	6.40	57.62	0.00	50.80	20.68	59.29	0.00	34.50	21.10	38.84
III	0	98	15.30	6.00	60.78	0.00	56.60	19.10	66.25	0.00	36.60	21.90	40.16
	7	105	14.50	5.90	59.31	0.00	51.30	19.90	61.21	0.00	34.80	20.50	41.09
	15	113	14.60	4.70	67.81	0.00	48.90	16.60	66.05	0.00	35.10	19.30	45.01
	24	122	14.85	4.90	67.00	0.00	52.70	17.10	67.55	0.00	32.30	16.30	49.54
	36	134	15.09	5.10	66.20	0.00	51.40	18.60	63.81	0.00	37.20	21.15	43.15

Phase I : 2 hours Anoxic followed by 4 hours Aerobic
Phase II : 3 hours Anoxic followed by 3 hours Aerobic
Phase III : 4 hours Anoxic followed by 2 hours Aerobic

TABLE C-5

TIME min	SOC mg C/L	2-CP mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	NO3-N mg/L		Average SOC mg C/L	Average 2-CP mg/L	Average NO3-N mg/L
0	50.71	15.08	38.10	52.33	15.30	34.46	56.72	15.10	37.70		53.25	15.18	36.75
20	NA	14.97	NA	NA	14.82	NA	48.55	14.66	NA		NA	14.82	NA
40	34.37	14.00	35.68	36.20	14.24	31.91	36.30	14.32	35.30		35.62	14.19	34.30
60	NA	13.42	NA	NA	13.65	NA	30.82	13.85	NA		NA	13.64	NA
80	27.83	12.96	32.80	28.95	13.10	28.96	26.45	13.41	32.40		27.08	13.16	31.39
100	NA	12.25	NA	NA	12.83	NA	23.86	12.47	NA		NA	12.52	NA
120	18.96	11.85	31.72	19.77	12.28	28.19	22.41	11.93	31.50	**Anoxic**	20.38	12.02	30.47
140	16.93	8.65		17.80	7.30		18.50	9.22		**Aerobic**	17.74	8.39	
160	NA	6.85		NA	5.47		NA	5.15			NA	5.76	
170	NA	3.25		NA	2.85		NA	3.26			NA	3.12	
180	12.10	0.00		12.83	0.00		13.22	0.00			12.72	0.00	
VSS (mg/L)			1965			1995			2005				1988
Anox removl: mg 2-CP/g VSS. hr			0.82			0.76			0.79				0.79
Anox removl: mg 2-CP/L.hr			1.62			1.51			1.585				1.57

(A)

PHASE - II PROFILE (DEXTROSE): 3 Hours Anoxic + 3 Hours Aerobic

TIME min	SOC mg C/L	2-CP mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	NO3-N mg/L		Average SOC mg C/L	Average 2-CP mg/L	Average NO3-N mg/L
0	58.32	15.19	35.55	53.61	14.80	34.73	56.85	14.75	31.73		56.26	14.91	34.00
20	NA	14.20	NA	NA	14.12	NA	NA	13.80	NA		NA	14.04	NA
40	33.84	13.05	32.14	35.22	13.45	32.10	34.71	12.62	29.28		34.59	13.04	31.17
60	NA	12.75	NA	NA	12.84	NA	NA	11.91	NA		NA	12.50	NA
80	25.10	11.54	29.40	26.44	11.27	30.27	26.11	11.05	28.14		25.88	11.29	28.60
100	NA	10.47	NA	NA	10.65	NA	NA	10.45	NA		NA	10.52	NA
120	21.46	9.62	27.92	22.45	9.83	28.17	22.74	9.82	24.43		22.22	9.76	26.84
140	NA	8.85	NA	NA	8.65	NA	NA	9.01	NA		NA	8.84	NA
160	NA	8.10	NA	NA	7.91	NA	NA	8.53	NA		NA	8.18	NA
180	18.70	7.76	24.55	18.43	7.55	25.17	18.30	8.00	21.94	**Anoxic**	17.81	7.77	23.89
190	16.32	5.13		15.22	4.35		16.86	5.80		**Aerobic**	16.13	5.09	
200	NA	3.10		NA	2.82		NA	3.40			NA	3.11	
210	11.81	0.00		11.12	0.00		10.80	0.00			11.24	0.00	
VSS (mg/L)			1975			2020			1990				1995
Anox removl: mg 2-CP/g VSS. hr			1.25			1.20			1.13				1.19
Anox removl: mg 2-CP/L.hr			2.48			2.42			2.25				2.38

(B)

PHASE - III PROFILE (DEXTROSE): 4 Hours Anoxic + 2 Hours Aerobic

TIME min	SOC mg C/L	2-CP mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	NO3-N mg/L		Average SOC mg C/L	Average 2-CP mg/L	Average NO3-N mg/L
0	60.32	14.90	32.11	56.31	15.22	31.11	57.77	15.10	35.34		58.80	15.07	32.85
20	NA	14.10	NA	NA	14.81	30.02	NA	14.63	NA		NA	14.51	NA
40	36.12	13.80	29.74	32.77	14.08	29.10	21.73	14.00	32.81		30.21	13.96	30.55
60	NA	13.25	NA	NA	13.54	27.15	NA	13.08	NA		NA	13.28	NA
80	28.94	12.38	27.93	28.11	12.75	28.19	26.50	12.33	30.34		27.85	12.72	28.79
100	NA	11.89	NA	NA	11.87	25.42	NA	11.18	NA		NA	12.03	NA
120	24.73	11.37	26.75	24.45	10.89	24.63	23.18	10.33	28.62		24.12	10.86	26.67
140	NA	10.26	NA	NA	10.42	23.87	NA	9.43	NA		NA	10.62	NA
160	20.63	9.14	24.88	21.74	9.64	22.54	21.55	8.25	26.50		21.31	9.70	24.64
180	NA	8.36	NA	NA	8.49	21.46	NA	7.64	NA		NA	8.76	NA
200	18.37	7.19	22.15	19.33	7.26	20.82	19.87	7.02	24.86		19.19	7.57	22.61
220	NA	6.54	NA	NA	6.73	19.36	NA	6.80	NA		NA	6.97	NA
240	14.60	6.02	20.26	15.25	6.25	18.81	16.55	6.35	22.64	**Anoxic**	15.47	6.21	20.57
250	13.71	3.30		14.83	4.73		14.54	3.92		**Aerobic**	14.36	3.98	
260	NA	1.80		NA	2.40		NA	1.75			NA	1.98	
270	10.44	0.00		11.11	0.00		12.27	0.00			11.27	0.00	
VSS (mg/L)			2020			1995			1955				1990
Anox removl: mg 2-CP/g VSS. hr			1.10			1.12			1.12				1.11
Anox removl: mg 2-CP/L.hr			2.22			2.24			2.19				2.22

(C)

PHASE - I PROFILE (PHENOL): 2 Hours Anoxic + 4 Hours Aerobic TABLE C-6

TIME min	SOC mg C/L	2-CP mg/L	Phenol mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	Phenol mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	Phenol mg/L	NO3-N mg/L		Average SOC mg C/L	Average 2-CP mg/L	Average Phenol mg/L	Average NO3-N mg/L
0	54.52	14.62	52.73	36.87	60.84	14.71	50.12	31.41	56.86	15.08	56.21	37.11		57.41	14.80	53.02	35.13
20	NA	12.91	47.14	NA	NA	13.45	43.83	29.73	NA	13.80	52.33	35.44		NA	13.39	47.77	NA
40	NA	11.83	42.85	31.12	NA	12.22	38.51	27.19	NA	12.42	47.14	33.12		NA	12.16	42.83	30.48
60	38.87	10.42	39.54	NA	40.60	11.15	34.15	25.86	41.36	11.13	41.76	31.71		40.28	10.90	38.48	NA
80	NA	9.21	36.22	28.64	NA	9.93	32.63	23.10	NA	10.20	36.42	29.93		NA	9.78	35.09	27.22
100	NA	8.74	32.75	NA	NA	8.24	30.80	22.83	NA	9.55	32.35	28.23		NA	8.84	31.97	NA
120	26.91	7.93	28.24	26.77	28.54	7.76	29.30	21.51	29.11	8.14	30.42	27.12	Anoxic	28.19	7.94	29.32	25.13
140	16.85	3.11	0.80		19.63	2.86	1.22		15.93	3.71	0.50		Aerobic	17.47	3.23	0.84	
160	12.63	0.00	0.00		14.23	0.00	0.00		12.16	0.00	0.00			13.01	0.00	0.00	
VSS (mg/L)			1960				1950				1945					1952	
Anox removl: mg 2-CP/g VSS. hr			1.71				1.78				1.78					1.76	
Anox removl: mg 2-CP/L.hr			3.35				3.48				3.47					3.43	

(a)

PHASE - II PROFILE (PHENOL): 3 Hours Anoxic + 3 Hours AEROBIC

TIME min	SOC mg C/L	2-CP mg/L	PHENOL mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	PHENOL mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	PHENOL mg/L	NO3-N mg/L		Average SOC mg C/L	Average 2-CP mg/L	Average Phenol mg/L	Average NO3-N mg/L
0	53.52	14.85	52.74	30.84	51.52	14.90	53.22	38.22	55.40	15.10	50.81	34.51		53.48	14.95	52.26	34.52
20	NA	13.70	48.35	NA	NA	13.72	51.10	NA	NA	13.55	47.22	NA		NA	13.66	48.89	NA
40	NA	12.34	44.16	26.10	NA	12.66	47.66	34.71	NA	12.32	44.81	31.73		NA	12.44	45.54	30.85
60	42.62	11.05	40.84	NA	41.36	11.45	44.32	NA	44.30	11.78	39.73	NA		42.76	11.43	41.63	NA
80	NA	10.28	37.35	23.86	NA	10.20	40.82	31.92	NA	10.45	35.41	27.73		NA	10.31	37.86	27.84
100	NA	8.95	32.11	NA	NA	9.25	34.76	NA	NA	9.05	31.22	NA		NA	9.08	32.70	NA
120	32.34	8.14	29.93	20.95	29.22	8.46	30.15	29.62	32.60	8.12	28.93	24.91		31.45	8.24	29.67	25.16
140	NA	7.35	26.50	NA	NA	7.73	28.32	NA	NA	7.06	26.31	NA		NA	7.39	26.38	NA
160	NA	6.58	24.32	NA	NA	6.91	24.55	NA	NA	6.48	24.54	NA		NA	6.65	24.47	NA
180	23.43	5.63	23.74	17.82	21.74	6.05	22.31	25.64	20.60	5.97	20.67	21.12	Anoxic	21.99	5.88	22.24	21.53
190	17.91	3.80	5.57		16.75	2.70	4.15		18.10	4.30	3.68		Aerobic	17.59	3.60	4.47	
200	10.82	0.00	0.00		11.32	0.00	0.00		12.10	0.00	0.00			11.41	0.00	0.00	
VSS (mg/L)			1950				1990				1965					1968	
Anox removl: mg 2-CP/g VSS. hr			1.58				1.48				1.55					1.54	
Anox removl: mg 2-CP/L.hr			3.07				2.95				3.04					3.02	

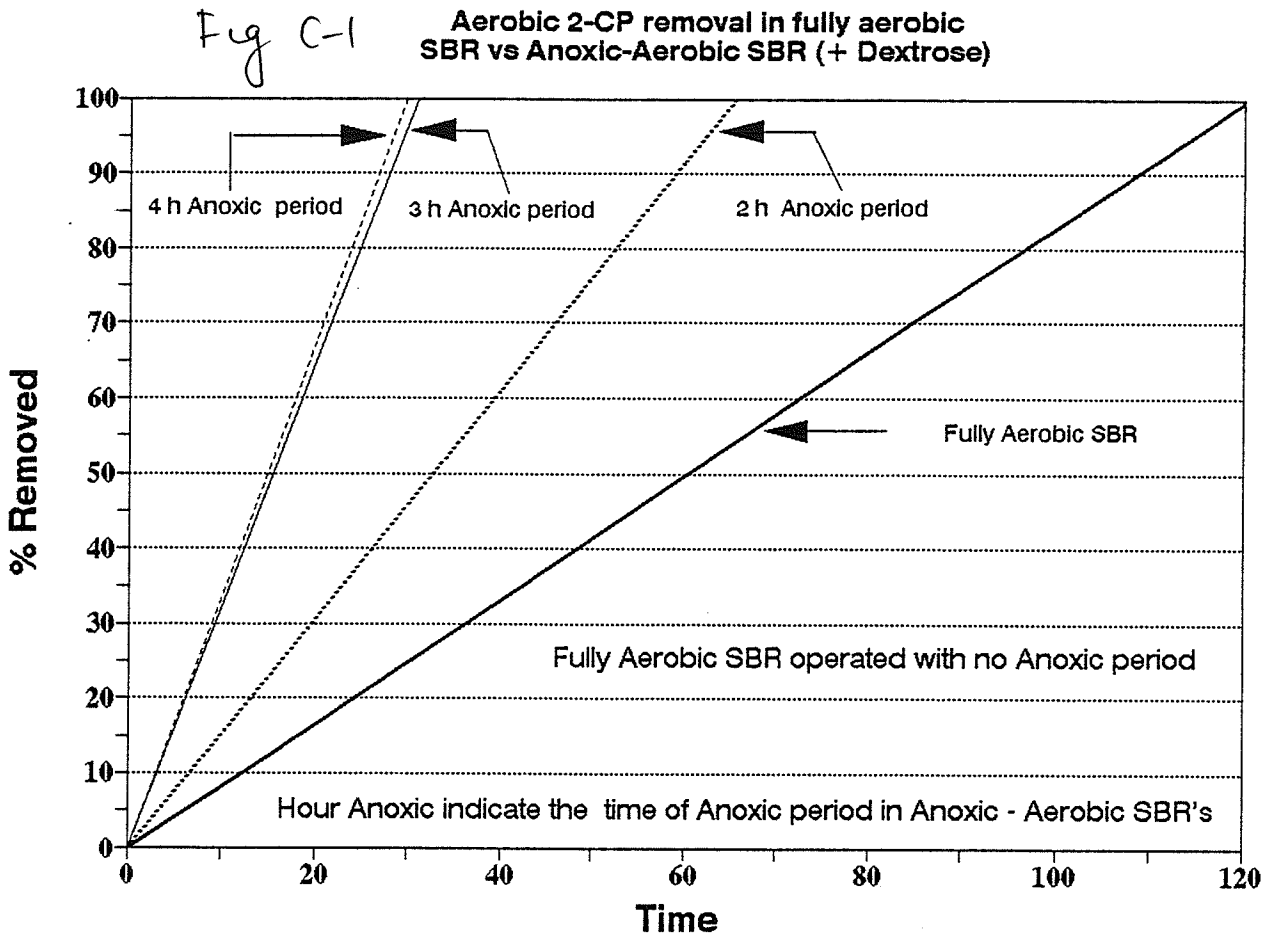
(b)

PHASE - III PROFILE (PHENOL): 4 Hours Anoxic + 2 Hours Aerobic

TIME min	SOC mg C/L	2-CP mg/L	PHENOL mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	PHENOL mg/L	NO3-N mg/L	SOC mg C/L	2-CP mg/L	PHENOL mg/L	NO3-N mg/L		Average SOC mg C/L	Average 2-CP mg/L	Average Phenol mg/L	Average NO3-N mg/L
0	52.80	14.85	52.70	32.30	54.70	15.09	51.40	37.20	53.65	15.22	53.65	39.87		53.72	15.05	52.58	36.46
20	NA	13.76	50.40	NA	NA	13.45	48.70	NA	NA	13.16	47.79	NA		NA	13.46	48.96	NA
40	NA	12.62	45.70	28.40	NA	12.65	46.30	34.20	NA	12.31	46.58	36.44		NA	12.59	46.19	33.01
60	44.10	12.01	40.10	NA	41.80	11.89	42.80	NA	40.86	11.07	43.25	NA		42.25	11.66	42.05	NA
80	NA	10.70	38.50	25.10	NA	10.78	39.90	31.40	NA	10.12	37.69	32.19		NA	10.53	38.70	29.56
100	NA	9.10	36.20	NA	NA	9.28	35.70	NA	NA	8.86	35.38	NA		NA	9.08	35.76	NA
120	34.30	8.20	32.60	22.60	34.80	8.65	31.80	27.10	31.97	8.08	32.05	29.10		33.69	8.31	32.15	26.27
140	NA	7.42	28.40	NA	NA	7.45	27.60	NA	NA	7.69	28.11	NA		NA	7.52	28.04	NA
160	NA	6.80	26.10	20.40	NA	7.05	25.10	25.40	NA	6.93	26.00	26.32		NA	6.93	25.73	24.04
180	24.80	6.10	24.60	NA	26.70	6.55	23.20	NA	25.75	6.18	24.37	NA		25.75	6.28	24.06	NA
200	NA	5.70	21.80	18.70	NA	5.78	21.90	23.10	NA	5.94	22.41	24.16		NA	5.81	22.04	21.99
220	NA	5.30	19.20	NA	NA	4.76	19.10	NA	NA	5.32	20.13	NA		NA	5.13	19.48	NA
240	16.90	4.90	17.10	16.30	17.40	4.10	18.60	21.50	17.06	4.68	18.79	20.09	Anoxic	17.12	4.56	18.16	19.30
250	14.80	1.98	0.00		14.10	1.14	0.00		13.92	1.37	0.00		Aerobic	14.27	1.49	0.00	
260	12.30	0.00	0.00		11.90	0.00	0.00		11.69	0.00	0.00			11.96	0.00	0.00	
VSS (mg/L)			1965				1980				1965					1970	
Anox removl: mg 2-CP/g VSS. hr			1.27				1.40				1.33					1.33	
Anox removl: mg 2-CP/L.hr			2.488				2.748				2.635					2.62	

(c)

Fig C-1


Aerobic 2-CP removal in fully aerobic
SBR vs Anoxic-Aerobic SBR (+ Dextrose)
% Removed
Time
4 h Anoxic period
3 h Anoxic period
2 h Anoxic period
Fully Aerobic SBR
Fully Aerobic SBR operated with no Anoxic period
Hour Anoxic indicate the time of Anoxic period in Anoxic - Aerobic SBR's
0
10
20
30
40
50
60
70
80
90
100
0
20
40
60
80
100
120