DEVELOPMENT OF PROTOCOLS FOR ASSESSING THE EFFECT OF PHENOL ON THE DRY ANAEROBIC DIGESTION PROCESS

by

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ABSTRACT

The study was conducted to develop protocols for assessing the effect of phenol on the dry anaerobic digestion process. The experiment was carried out in two phases. Phase I was designed to observe the effect of increasing phenol concentrations on the process and to assess phenol monitoring program. The first eight-week period covered the operation of two bench scale semi-continuous reactors and the measurement of system performance parameters such as: gas production, gas composition, pH, volatile fatty acids, volatile solids removal efficiency. After eight weeks phenol was added to one reactor in increasing concentrations from 50 mg kg^{-1} to 379.65 mg kg^{-1}. The second reactor was operated as a control. The performance parameters were measured at the same time. Phenol addition from 50 mg kg^{-1} to 168.75 mg kg^{-1} through three additions did not have a deleterious effect on the fermentation process since the system had a stable performance. The effect of phenol concentrations from 168.75 mg kg^{-1} to 379.65 mg kg^{-1} could not be determined since both reactors failed. It was possible that changing in feeding schedule and feedstock composition had deleterious effect on the reactors performance. This resulted in high accumulation of volatile fatty acids and ammonia. There was a high potential for free ammonia inhibition in the systems.

Phenol monitoring program covered specific issues such as: sampling from heterogenous compost material at different stages of the digestion process, phenol quantification using extraction technique, and high-performance liquid chromatography measurement. Phenol was determined by drawing ten samples and extracting them with deionized water on a vortex mixer. There was a high experimental uncertainty caused by

sampling method and poor mixing.

Phase II was designed to develop a standard operating procedure for phenol analysis and extraction from the organic fraction of municipal solid waste. Alternatives of sample handling and high-performance liquid chromatography settings were tested. Phenol extraction was performed using five different solvents: deionized water, alkaline water at pH = 11.5, tap water, deionized water at 50°C, and a solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v. Samples were extracted 24 hours after incubation. Alkaline water and the solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile were the best extractants. Sample filtering, placing a PRP-1 guard column in front of a PRP-1 reverse phase column and using a mobile phase of 88% of 0.1 N sodium phosphate and 12% acetonitrile with a flow rate of 1 mL min^{-1} seemed to be the optimal conditions for high performance liquid chromatography measurements. The reproducibility of results was comparable with the reproducibilities found by others researchers in extraction of organics from environmental solids. Based on the study, the procedure can be used as a screening method for quantifying phenol in feedstock, however, more work needs to be done to validate the method through interlaboratory studies and practical application.

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NOMENCLATURE AND ABBREVIATIONS

ANOVA	-	analysis of variance
A.P.H.A	-	American Public Health Association
AW, pH=11.5	-	alkaline water at pH of 11.5
BUFFER	-	solution of 95% sodium phosphate and 5% acetonitrile
CI	-	confidence interval
COD	-	chemical oxygen demand
DAD	-	dry anaerobic digestion
DW	-	deionized water
DWH	-	hot deionized water
Deg	-	degree
E	-	multiply by 10 to the power (ex. 3.7 E3 = $3.7x10^3$)
g	-	gram
g L^{-1}	-	gram per litre
Δ Go	-	free energy change at defined conditions, (kJ mol^{-1})
HPLC	-	high-performance liquid chromatography
H_2SO_4 -	-	sulfuric acid
hydrophilic	-	having high affinity to water
hydrophobic	-	having low affinity to water
L	-	litre
μL	-	micro litre, (E^{-6} litre)
mg g^{-1} -	-	milligram per gram
mg kg^{-1}	-	milligram per kilogram
mg L^{-1}	-	milligram per litre
mL	-	millilitre (E^{-3})
mL min^{-1}	-	millilitre per minute
mL s-1	-	millilitre per secund
mm	-	millimeter (E^{-3} meter)

μm	-	micro meter (E^{-6} mole)
MRT	-	mass retention time
MSW	-	municipal solid waste
N	-	one normal
NaCL	-	sodium chloride
nm	-	nano meter (E^{-9})
N_3PO_4 $12H_2O$	-	trisodium phosphate
OFMSW	-	organic fraction of municipal solid waste
pKa	-	ionization constant ($-\log_{10}[H^+]$)
P_{oct}	-	octanol-water partition coefficient
POTW	-	publicly owned treatment works
R1	-	reactor without phenol
R2	-	reactor supplemented with phenol
rpm	-	rotations per minute
TKN	-	total Kjeldhal nitrogen
TW	-	tap water
UAN	-	unionized
US-EPA	-	United States Environmental Protection Agency
UV	-	ultra-violet
VFA	-	volatile fatty acids
VS	-	volatile solids
v/v	-	volume by unit volume

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CHAPTER 1

INTRODUCTION

Phenol is among the fifty chemicals produced in the greatest quantities in the USA, estimated to be 1.2 million tonnes in 1983 (Webber, 1984). It can be brought into the environment deliberately, as in pest control, or accidentally as in seepage from toxic waste disposal sites and spills (Boyd, 1982; Boyd et al. 1983; Environment Canada, 1985). Phenol can be present in wastewater and thus accumulate in sludge (Bandyopadhyay et al. 1995) and also through spills and accidental seepage from toxic waste disposal can enter the municipal solid waste stream. Phenol is a semivolatile compound and moderately soluble in water. It can also dissolve in vegetable and mineral fats and oils that are sludge components. It can also be sorbed and concentrated on the primary and secondary solids of the mixed sludge. Fricke et al. (1985) presented the results of an US-EPA study in 1982 on 'Fate of priority pollutants in Publicly Owned Treatment Works (POTWs) which showed that phenol was detected in sludge in 39 out of 44 surveyed POTWs and in 189 out of 256 POTWs from other surveys and studies. Phenol is a hazardous chemical that has been found toxic to human, aquatic life, and invertebrates at very low concentrations (Environment Canada, 1985). Since phenol can be found in solid waste and biosolids, there is a concern with its presence in composting feedstock. As a result, the accumulated phenol toxicant can enter and affect the composting system.

One of the composting processes is a fermentation process of the Organic Fraction of Municipal Solid Waste (OFMSW) that has become a promising means of waste transformation (Rich et al. 1994). The process also called dry anaerobic digestion or

anaerobic composting, digests the refuse in sealed, controlled, anaerobic fermenters and provides energy recovery in the form of biogas. The refuse used in the process can include the components of the organic fraction of Municipal Solid Waste (MSW) such as: biosolids, paper, and food waste. Since the process is a part of biosolids or sludge treatment system, it can be exposed to the sudden appearance of a toxic compound in sludge or feedstock. Phenol, a priority pollutant, which often finds its way in wastewater can affect the fermentation process.

Much of the research on solid waste digestion has focused on changes in the operational parameters and efficiency of the process (Cecchi et al.1988; Kasali, 1986; Kasali and Senior, 1989; Oleszkiewicz and Poggi-Varaldo, 1993; Kayhanian et al. 1991), and the physical and chemical parameters of the final products (Kayhanian et al. 1991; Yu, 1992). The literature has no reports on the effect or transformations of phenol in the process. There are, however, reports on the effect of phenol on anaerobic wastewater fermentation processes. The effect and transformations of phenol under methanogenic conditions were investigated by Healy and Young (1978); Young and Rivera (1985); Wang et al. (1989); Fedorak and Hrudey (1984); Knoll and Winter (1987); Létourneau et al. (1995); Bisaillon et al. (1991); Béchard et al. (1990); Bisaillon et al. (1993); Ejlertsson et al. (1996). Phenol was reported to affect the performance of anaerobic waste transformation systems such as: anaerobic sludge digestion (Owen and Young, 1989; Fedorak and Hrudey, 1989; Wang et al. 1991), an anaerobic hybrid reactor treating landfill leachate (Britz et al. 1992), and landfill refuse decomposition (Watson-Craik and Senior, 1989a, 1989b, 1990). The effect of phenol was shown by variations in methane production rate, COD removal, lower pH, and variations in

volatile fatty acids (VFA) concentrations.

Although dry anaerobic digestion (DAD) is a relatively new technology, it has a high potential to become a future waste management practice. When more organic pollutants are present in the environment which can enter and affect the process, there is a need for research on their effect and quantification. So far, no reports have been found on the effect or transformations of phenol in the process. Also, the techniques of phenol quantification in refuse found in literature are not specific to DAD process. They include phenol analysis in landfill leachate (Watson-Craik and Senior, 1989a, 1989b, 1990; Ejlertsson et al. 1996) and in refuse sorption tests (Knox and Newton, 1976).

The purpose of the study was the development of protocols for assessing the effect of phenol on the dry anaerobic digestion process. The specific objectives were:

1. Observation the effect of increasing phenol concentrations on DAD performance,
2. Assessment of phenol monitoring program at different stages of the digestion process,
3. Development of a standard operating procedure for phenol extraction and analysis from the Organic Fraction of Municipal Solid Waste (OFMSW).

CHAPTER 2

LITERATURE REVIEW

2.1 INTRODUCTION

The purpose of the study was the development of protocols for assessing the effect of phenol on the dry anaerobic digestion process. The specific objectives were: observation of the effect of increasing phenol concentration on DAD performance, assessment of phenol monitoring program, phenol quantification during the process using extraction and HPLC analysis, development of a standard operating procedure for phenol extraction and analysis from the Organic Fraction of Municipal Solid Waste (OFMSW).

The impact and transformation of an organic chemical released in the environment depends on its interactions with the different components of the environment such as: air, water, solid phases and microorganisms. As a consequence the chemical can undergo various physical, chemical and biological processes. The physical processes do not alter the molecular structure of the compound and they include solubilization in bodies of water, sorption, transfer between bodies of water and the atmosphere (Schwarzenbach et al. 1993).The impact of the compound in the environment is in close relation with these processes. It has been found that the same compound behaves differently in solubilized and sorbed form.

There are also processes that change the structure of the compound such as: chemical, photochemical and biological transformations. Various chemical processes include oxidation and reduction reactions. During photochemical transformation reactions the compound is transformed after direct adsorption of light. Biological processes involve

microorganisms that transform the compound (Schwarzenbach et al. 1993).

The presence of different phases (solid, liquid, gas) of the environment made the transformations of the chemical very complex. All the transformation processes can take place simultaneously and can have affect on each other.

There are various aspects that have to be considered in predicting the transformations of the compound in the environment. The knowledge of physical and chemical properties of the compound is important to assess its environmental behaviour. For instance structural characteristics define whether the compound 'likes' or 'dislikes' water and hence its solubility. Octanol-water partition coefficient describes the tendency of the chemical to sorb while vapour pressure is a measure of volatility or the evaporative loss of pure compound (Verschueren, 1983). Environmental factors such as: moisture content, temperature, pH, natural organic matter content, microbial activity are also significant. Microbial activity relates to biological transformations of the compound. Temperature, moisture content, pH are the vital factors that affect microbial activity. The natural organic matter content describes the extend of sorption.

Quantification and monitoring of the compound is one of the most important and most challenging steps in determining its transformations in any environment including compost. Quantification and monitoring should include a design of a proper sampling plan and economic considerations. Number of samples, sampling frequency, length of study, etc should be defined. Very often the complex matrix of solid samples requires sample extraction, clean up before analysis. The fact that the compound can undergo various processes such biodegradation, volatilization, and sorption makes the analysis even more

difficult (Baker, 1994). The widely used analytical methods for quantification involve gas chromatography, mass spectrometry, and high-performance liquid chromatography.

2.2 PHENOL PRESENCE IN THE ENVIRONMENT

Phenol is an industrial chemical used primarily as an intermediate in a wide variety of chemical processes such as production of phenolic resins, pharmaceutics, germicides, fungicides, herbicides, dyes, and a variety of industrial important acids (US-EPA, 1980). It is also a transformation product of pesticide degradation (Boyd, 1982).

Phenol is produced during the coking of coal, distillation of wood, operation of gas works, and oil refineries, as normal constituent of human and animal wastes, and microbial decomposition of organic matter (US-EPA, 1980).

Phenol has been found as a natural intermediate in landfill leachate as a degradation product of solid waste. It is produced as an intermediate in degradation of proteins, lipids, carbohydrates. The levels of phenol in leachate depend on leachate age and are higher in younger leachate (Artiola - Fortuny and Fuller, 1982).

2.3 THE PHYSICAL AND CHEMICAL CHARACTERISTICS OF PHENOL

Phenol sometimes referred to as "carbolic acid" is a mono hydroxybenzene. It is a clear, colourless (light pink when impurities are present), hygroscopic, crystalline solid at 25 °C (Environment Canada, 1985). The chemical structure of phenol is shown in Figure 2-1.

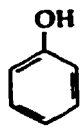

Figure 2-1. Chemical structure of phenol (Callewaert, 1980).

Phenol is a polar compound and it forms hydrogen bonding between its molecules (Loudon, 1984). When interacting with water, phenol also forms hydrogen bonds as its phenyl group pulls an electron away from - OH group and transfers a proton to the water molecule (Figure 2-2). As a result the OH group becomes weakly acidic.

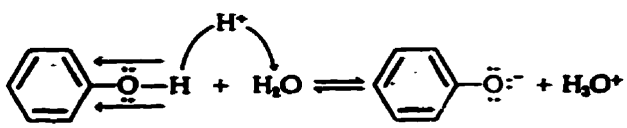

Figure 2-2. Protonation of phenol with water molecule (Callewaert, 1980).

Phenol solubility in water is due to the hydrogen bond formation (Wolfe, 1986). With its solubility of 8.2 g per 100g (8.2%) at 20°C phenol is referred to as moderately soluble (Loudon, 1984). Based on its solubility characteristics and octanol/water partitioning coefficient, phenol is also referred to as a hydrophilic compound or a compound with high affinity to water. Literature reports that a hydrophilic contaminant has a solubility in water at 25 ° C greater than 10 g L^{-1}, while a hydrophobic compound has an

octanol/water partition coefficient (P_{oct}) greater than 100 (Evangelista et al.1990). Phenol has solubility in water of 84 g L^{-1} and a octanol/water partition coefficient of 29 what means that it is hydrophilic.

The relatively high boiling point of 182°C of phenol is a consequence of significant attractive forces between its molecules in the liquid state mostly due to its polarity and hydrogen bonding formation. In general, the attraction between molecules in phenol results from its molecular weight, molecular shape, polarity, and hydrogen bonding within the molecule (Loudon,1984). Other physical and chemical properties of phenol relevant to the study are listed in Table 2-1.

Phenol as a contaminant has been recognized for its high human and aquatic toxicity. It is toxic to aquatic life, microorganisms, and invertebrates at very low concentrations (Environment Canada, 1985). Bactericidal property of phenol was reported by Karabit et al. (1985) who showed that phenol is more effective in killing bacteria at pH values below its pKa. This property makes phenol more toxic to microorganisms at lower pH values.

Table 2-1. Summary of phenol characteristics relevant to the study

Property	Description
chemical formula	C_6H_5OH
appearance	colourless to white to pink solid crystals (Environment Canada, 1985).
density	1.132 g cm^{-3} (25°C) (Environment Canada, 1985).
molecular weight of pure substance	94.11 (Verschueren, 1983)
specific gravity	1.07 (Verschueren, 1983)
melting point	41 ° C (Verschueren, 1983)
boiling point	182 ° C (Verschueren, 1983)
vapour pressure	0.2 mm at 20°C 1 mm at 40° C (Environment Canada, 1985)
pKa	9.82 (Schwarzenbach at al. 1993)
logP_{oct}	1.46 (Verschueren, 1983)
solubility in water	8.2 g per 100mL (20°C) (Verschueren, 1983) 6.7 g per 100 mL (16°C) (Environment Canada, 1985). soluble in all proportion above 63.5°C (Environment Canada, 1985)
in other common materials	soluble in acetone, hot benzene, ethanol, carbon disulphide, carbon tetrachloride, and chloroform (Environment Canada, 1985)

2.4 DRY ANAEROBIC DIGESTION PROCESS

Dry anaerobic digestion (DAD) or high - solid anaerobic digestion is a biotransformation process of solid waste at a total solids concentration of more than 22 percent in the absence of oxygen (Kayhanian et al. 1991). The digestion process can be operated under mesophilic (35°C) or thermophilic conditions (55°C) (Kayhanian et al. 1991). The process is also sometimes referred to as anaerobic composting. The feedstock used in the digestion process may include the organic fraction of Municipal Solid Waste (MSW) and various sludges such as raw primary sludge, thickened activated sludge and digested sludge (Kayhanian et al. 1991).

During the process, the waste is metabolized by different microbial species which break the complex organic matter in three stages hydrolysis (polymers breakdown), acetogenesis (acid production), and methanogenesis (methane formation). Final products of the process include a mixture of gases such as methane, carbon dioxide, and small amounts of hydrogen sulfide, hydrogen, nitrogen, and also low molecular weight hydrocarbons (Kayhanian et al. 1991). The humus material produced from the fermentation process can be used as a soil amendment or as a fuel source after aerobic biodrying.

The transformations of an organic compound in the process of dry anaerobic digestion can be discussed by analogy to other solid phase systems such as soil, sediment, or the organic fraction of humic substances. For instance soil has been described as a dynamic system because of instability and variability of different phases such as: clay, organic matter, various metal oxides and hydroxides, and microorganisms (Morill et al.

1982). The anaerobic compost system can also be referred to as a dynamic system of changing phases such as: solid phase, liquid, gas, and microorganisms.

An organic compound can be removed from a system with solid phase by three major processes: biodegradation, volatilization, and sorption (Baker, 1994). Biodegradation is a biologically catalysed process where microorganisms change the structure of an organic compound to intermediate products that can be finally transformed to final products (Alexander, 1994). Volatilization is of great concern in case of volatile organic compounds (Baker, 1994). Sorption is a process of binding a chemical to solid phase. It greatly affects the transformations of a compound, since it changes mobility and biodegradability of the compound. (Alexander, 1994; Schwarzenbach et al. 1993).

Since a compost system is a solid phase systems, the three processes can affect the transformations of phenol in compost. Phenol can be either biodegraded by microorganisms present in the system, sorbed on the solid phase, dissolved in the liquids or volatilized. Since phenol is a semivolatile compound, its volatilization will be of less concern. The middle range of phenol vapour pressure relative to other phenols, significant ability to sorb onto solids and high affinity to water make phenol volatilization less likely (Verschueren, 1983). Phenol volatilization has been found to depend on moisture loss in sludge drying studies (Bandyopadhyay et al. 1995). At high temperatures of 150, 200, and 250°C, more phenol was volatilized until removed completely as moisture was totally removed. It can be concluded that phenol volatilization in dry anaerobic digestion will be of little importance due to not very high temperature of 55°C and constant production of moisture in the system.

As in the case of soil and other solids the presence of solid surfaces in compost system can have a tremendous effect on the activity of microorganisms. The surfaces can change the accessibility of organic compounds, alter pH levels, preserve microorganisms, alleviate toxic effect of inhibitors (Alexander, 1994). Finally, the solid surfaces can act as a sorption medium for an organic compound. The sorption process will result in building sorption zones that are different from the surrounding solution (Alexander, 1994).

Quantification of phenol in a compost system is one of the most important step in biodegradation studies. Several issues should be considered in phenol determination. They include statistical certainty required of the analytical results, number of replicate samples, sampling frequency, and the choice of analytical method.

2.4.1 Feedstock

The main components of the feedstock for DAD include: the organic fraction of MSW and different sludges such as: raw primary sludge, thickened activated sludge, and digested sludge. The primary sludge for instance has a total solids content from 2 to 8 percent and is composed mainly of grease, fats, and proteins (Metcalf & Eddy, 1991).

The organic fraction of MSW consists of materials such as: food waste, paper, corrugated cardboard, plastics, textiles, wood, yard wastes (Tchobanoglous, 1993). The chemical components found in the Organic Fraction of Municipal Solid Waste (OFMSW) can include: lignocelluloses, polysaccharides, fat-containing organic molecules, and proteins (Senior, 1990).

Lignocellulose consists of three major polymers: lignin, hemicellulose and cellulose. Lignin, one of most abundant polymers, constitutes 18 to 30 percent of the dry weight of dry wood tissue. Its polymerized structure made of aromatic alcohols and many different chemical bonds results in a 'much-branched polymer' with molecular weight from a thousand to million. Hemicelluloses are polysaccharides found in hardwood, softwoods and grasses. The cellulose polymer is a major constituents of most woody tissue (34 to 45 percent of dry weight). Polysaccharides are mostly found in stem, potatoes, seeds, and roots. Fat-containing organic molecules are present in plant and microbial cells. They are characterized by low polarity and consist mainly of fats, oils, and waxes. Proteins are mainly distributed in plant. Because of their four basic structural levels, protein molecules are coiled and folded and make a complex, rigid structure (Senior, 1990).

2.4.2 Microbial biotransformations

Biotransformation processes are accomplished by microorganisms to change the structure of an organic compound and to remove the compound from the environment. They are very important, since they greatly affect the removal of the chemical from the environment (Schwarzenbach et al. 1993). Biotransformation results in production of intermediate organic compounds that finally can be transformed to final products.

There are many reports in the literature about microbial degradation of phenol in the absence of oxygen (Wang et al. 1993; Béchard et al. 1990; Bisaillon et al. 1991 Bisaillon et al. 1993; Létourneau et al. 1995; Watson-Craik and Senior, 1989a, 1989b, 1990; Ejlertsson et al. 1996). Still, no data was found on phenol degradation in the DAD

process. However, the biotransformation in different anaerobic systems can be related to the fermentation process of solid waste because of the similarity of conditions and bacterial species (Zinder et al. 1984; Metcalf and Eddy, 1991; Senior, 1990).

An example of solid phase resembling dry anaerobic digestion process is landfill system. A landfill is defined as a designed refuse disposal site which is usually isolated from the environment by covers and layers of impermeable material. There are similarities between landfill ecosystem and anaerobic digester system, although landfill systems are characterized by higher heterogeneity of the refuse (Senior, 1990). Preceded by aerobic conditions at the beginning the landfill operation, the environment is mostly anaerobic. Like in the process of dry anaerobic digestion, the catabolic transformations of organic compounds are accomplished by mixed bacterial populations in steps such as: hydrolysis and fermentation, propionogenesis, acetogenesis, and methanogenesis (Senior, 1990).

There are two phenol degradation pathways under anaerobic conditions that have been reported in the literature. First pathway, called reductive pathway cited by Evans (1977) includes the formation of intermediates such as: cyclohexanol, cyclohexanone and adipic acid that are transformed to final products carbon dioxide and methane. There have been, however, a few reports about the intermediates found for this pathway (Williams and Evans, 1975; Grbić-Galić and Vogel, 1987; Grbić-Galić and Young, 1985) and some workers have failed to detect the intermediates (Kobayashi et al. 1989, Béchard et al. 1990). Second pathway via carboxylation and formation of benzoic acid under methanogenic conditions has been found by other workers (Kobayashi et al. 1989; Knoll and Winter, 1987; Béchard et al. 1990; Bisaillon et al. 1993). In this pathway, the

intermediate benzoic acid was easily detected. Based on the most recent literature, the pathway through carboxylation is the observed phenol degradation pathway.

The biotransformation of organics under anaerobic conditions requires cooperation of different bacterial species (Alexander, 1994). A single bacterial species perform only some part of the processes required in total biodegradation of the compound. In the case of phenol there have been reported at least three bacterial species responsible for its biodegradation (Dwyer et al.1986; Wang et al. 1989; Fedorak and Hrudey, 1984; Létourneau et al. 1995; Bisaillon et al. 1991).

Several factors affect biotransformation processes. They include acclimation period or lag period, presence of toxins, and environmental factors such as: temperature, pH, moisture level (Alexander, 1994).

The rate of biotransformation can be affected by three main mechanisms. They include: delivery of substrate molecules to microorganisms, enzyme's ability to perform the initial transformation of the chemical, and growth of a population of organisms in the environment with the chemical of concern (Schwarzenbach et al. 1993). The delivery of substrate molecules or mass transport appeared to be very significant in a system with solid phase and microbia consortia (Schwarzenbach et al. 1993). It has been reported that many chemicals persists in the environment containing degrading microorganisms because the microorganisms do not have access to them (Alexander, 1994).

In solid phase systems such as in soil and sediments, the mass transport of compounds can be affected by a partition of a chemical between different phases such as solid, gas, and liquid. The partitioning will result in phenomena such as sorption, volatility,

and solubility (Schwarzenbach et al. 1993). The effect of sorption on biotransformation of an organic chemical can be very strong, since sorbed chemical is not available for microbial transformation. The extend of sorption will be determined by the chemical solubility characteristics. There will be higher bioavailability of hydrophilic compounds because the metabolism takes place in an aqueous phase (Schwarzenbach et al. 1993; Reinhart et al.1991).

Substrate delivery of phenol in DAD will also play a significant role since there is a structural analogy of the digestion system to soil or sediment. As in soil there are also various physical phases in DAD. Dry anaerobic digestion system is a complex refuse matrix composed of solid phase, gases, water, and microorganisms (Barlaz et al. 1989). As a consequence the mass transfer of phenol will be affected by a partition of phenol between different phases in the digestion process and various processes such as sorption, volatility, and solubility.

Since the refuse system includes different types of solid phases characterized by different solid-water partitioning coefficients such as: paper, food wastes, it is expected that the extend of sorption of phenol will vary depending on the solid type. The amount of each solid type will also change throughout digestion process and any variations in feedstock composition.

2.4.3 Sorption

Sorption is defined as a process of binding a chemical on a solid phase. The solubility of a compound mostly determines its tendency for sorption. Hydrophilic compounds with high affinity to water tend to be less sorbable whereas hydrophobic compounds with low affinity to water are more likely to be sorbed.

Sorption is a very important process since it also have an effect on transformations and impact of a chemical in the environment (Schwarzenbach et al. 1993). Compounds in solutions are more chemically mobile than compounds sorbed, thus bacterial decomposition of sorbed compounds is slower than dissolved particles. The sorbed chemical becomes less available or completely unavailable to microbial degradation. When the microbial degradation includes the action of intracellular enzymes (the metabolism of low-molecular-weight compounds), the immobilized chemical on solid phase is not free to go through the outer surface and it cannot enter the microbial cell. When the microbial metabolism involves the action of extracellular enzymes (metabolism of some low-molecular-weight and high-molecular-weight molecules), the enzymes can also be sorbed and may lose their enzymatic activity. (Alexander, 1994).

Sorption of an organic compound on solid phase can be affected by type and quantity of clay minerals, the organic matter content, pH, temperature and characteristics of the chemical of interest (Alexander, 1994). The organic matter content in solid phase have been found a chief factor in sorption of organic compounds (Isaacson and Frink, 1984; Bishop et al. 1989; O'Neill et al. 1993; Schwarzenbach et al. 1993). The characteristics of organic matter that are important in sorption include high surface area,

porous structure and ability for ion exchange (Schwarzenbach et al. 1993; Alexander, 1994). In some cases, an organic compound can interact to form stable linkages that are not referred to as sorption. These linkages include complexes formation through covalent bonds.

Sorption of an organic chemical in DAD systems seems to be important due to the significant quantity of solids with high organic content in the digesting refuse. The feedstock to the process is composed of sludge and organic fraction of municipal solid waste that makes the solids rich in organic matter.

2.5 THE EFFECT AND BIOTRANSFORMATION OF PHENOL DURING ANAEROBIC BIOLOGICAL WASTE PROCESSES

At the time of writing this paper no reports have been found about the effect and biotransformation of phenol in high solids anaerobic digestion of OFMSW and sludge. The available literature covered the effect and biotransformations of phenol in the anaerobic/methanogenic sludge digester processes and landfill refuse systems, and phenol effect on anaerobic digestion of leachate.

2.5.1 Anaerobic sludge digestion

Anaerobic sludge digestion is a process of degradation of a complex organic material by different microbial populations to carbon dioxide and methane in the absence of oxygen. The process resembles the system of dry anaerobic digestion due to similar anaerobic transformations and microbial populations involved in the process.

Many workers have investigated the effect and biotransformations of phenol in sludge digestion systems. The effect of phenol on methanogenic bacteria was examined by Wang et al. (1989, 1991, 1993), Fedorak and Hrudey (1989), Owen and Young (1989) whereas studies on phenol degradation were conducted by Knoll and Winter (1987), Kobayashi et al. (1989), Young and Rivera (1985). Phenol effect on the digestion process was shown by variations in the rate of gas production, and gas composition. Toxic effect of increasing phenol concentrations caused a decrease in methane production (Wang et al. 1991). Phenol degradation (disappearance of phenol) caused the recovery of phenol in the form of biogas in the phenol uninhibited digesters. It was reported that both the toxic effect and phenol

degradation depended on phenol concentration. Some workers pointed out that microbial acclimation to phenol can make the process more robust to increasing phenol concentration. It can decrease the lag period, shorten degradation time, increase the range of phenol concentration transformed, and improve gas production (Healy and Young, 1978; Wang et al. 1989, 1993; Kobayashi et al. 1989).

As the degradation pathway of phenol was described, it involved several steps including ring saturation, ring fission and production of various aromatic intermediate products, volatile fatty acids, and hydrogen. Volatile fatty acids and hydrogen were used by methanogenic bacteria to produce the final products: carbon dioxide and methane (Young and Rivera, 1985; Kobayashi et al. 1989).

Phenol conversion to carbon dioxide and methane in the absence of oxygen is accomplished by non-methanogenic and methanogenic populations of bacteria. At least three bacterial species involved in phenol degradation have been recognized: 'a phenol - metaboliser, an hydrogen-utilizing methanogen, and an acetotrophic methanogen' (Sheridan et al. 1985; Dwyer et al. 1986; Bisaillon et al. 1991; Lètourneau et al. 1995). The overall rate of phenol conversion to methane could be affected by the activity of the three bacterial species responsible for its degradation (Wang et al.1989; Sheridan et al. 1985). Some workers reported that methanogenic bacteria is less susceptible to inhibition by high phenol concentrations than phenol-degraders (Fedorak and Hrudey, 1984; Wang et al. 1989).

The effect of various phenol concentrations on methane production in batch cultures inoculated with fresh sewage sludge was examined by Fedorak and Hrudey (1989). The cultures received various phenol concentrations from 500 to 3000 mg L^{-1}. The cultures with

phenol concentration of 500 mg L^{-1} started to produce more methane than the controls after 15 days with simultaneous decrease in phenol concentrations. However, the cultures with phenol concentrations of 1200, 2000, and 3000 mg L^{-1} showed greater inhibition since the methane concentrations and production were less than those in the controls.

Owen and Young (1989) in his experiment on toxicity and biodegradability of phenol in anaerobic digester unacclimated sludge showed that phenol was not toxic to methanogenesis at concentrations 100 and 200 mg L^{-1} and was completely mineralized at concentrations from 20 to 200 mg L^{-1}. The cumulative volume of methane produced from cultures containing phenol concentrations 20, 100, and 200 mg L^{-1} exceeded the one of the control. There was no lag period observed and the maximum of methanogenesis was established after 25 days. However, phenol concentration of 300 mg L^{-1} was toxic to methanogenesis since the volume of methane was less than volume for the control.

Similar results of batch anaerobic toxicity test on phenol effect and degradation on acetate methanogenesis in digested municipal sludge were reported by Wang et al. (1991). Phenol concentrations of 100 and 200 mg L^{-1} did not inhibit methane production at the end of 77 days incubation. As the methane production in these cultures was higher than in the controls, there was also a indication of phenol biodegradation to methane. Higher phenol concentrations did not result in significant degradation as methane production was lower relative to the controls. It was also reported that methanogenic activity expressed as a ratio of volume of methane produced relative to control decreased with increasing phenol concentration. Fifty percent inhibition of acetate methanogenesis took place at phenol concentration of 1250 mg L^{-1}.

The stoichiometric reactions of phenol degradation to methane suggested by Sheridan et al. (1985) are presented in equations 2-1 to 2-4. The equations demonstrate that phenol degradation results in production of acetic acid and hydrogen that are transformed to methane and carbon dioxide. The stoichiometric analysis of the equations shows that acetate accounts for approximately 86 percent of methane produced during phenol degradation.

$$C_6H_6O+5H_2O \rightarrow 3CH_3COOH+2H_2 \qquad 2\text{-}1$$

$$2H_2+0.5CO_2 \rightarrow 0.5CH_4+H_2O \qquad 2\text{-}2$$

$$3CH_3COOH \rightarrow 3CH_4+3CO_2 \qquad 2\text{-}3$$

Overall reaction:

$$C_6H_6O+4H_2O \rightarrow 3.5CH_4+2.5CO_2 \qquad 2\text{-}4$$

The steps of phenol transformations during anaerobic digestion of sewage sludge were also described by Knoll and Winter (1987). The experiments with degradation of radiolabelled phenol in their study showed that acetate was an intermediate product in phenol conversion since it accumulated first. Subsequently, it was converted to methane and carbon dioxide. It was also pointed out that phenol cannot be degraded at elevated H_2 and acetate concentrations since reduced phenol degradation rates were observed in experiment with elevated H_2 and acetate concentrations. This can be explained as a direct result of free

energies values produced during phenol degradation according to equation 2-5 (Fedorak and Hrudey, 1989).

$$C_6H_5OH+5H_2 \rightarrow 3CH_3COOH+2H_2 \qquad \Delta G_o = +6.6\,kJ/mol \qquad 2\text{-}5$$

$$C_6H_5OH+4H_2 \rightarrow 3.5CH_4+2.5CO_2 \qquad \Delta G_o = -166\,kJ/mol \qquad 2\text{-}6$$

A more detailed study with respect to intermediate products in phenol degradation pathway during anaerobic batch sludge digestion was conducted by Young and Rivera (1985). He reported that phenol transformation included ring saturation, ring fission, production of organic acids which were used as a precursors for methane production. The metabolic pathway of phenol under anaerobic, methanogenic conditions based on the findings of other workers was presented (Evans, 1977; Balba and Evans, 1980; Sulfita et al. 1982; Boyd et al. 1983; Boyd and Shelton, 1984). The intermediates of the degradation pathway are presented in Figure 2-3.

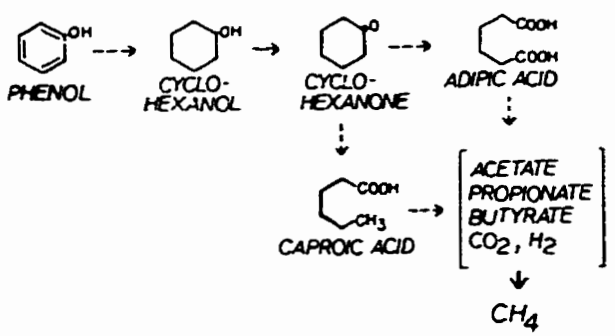

Figure 2-3. Phenol degradation pathway in anaerobic sludge digestion (Young and Rivera, 1985).

There have been a few reports since 1985 where the intermediates of the pathway by Young and Rivera et al. 1985 were found. They include work by Grbić-Galić and Vogel (1987) and Grbić-Galić and Young (1985). Other workers have added cyclohexanol or cyclohexanone to phenol-degrading anaerobic cultures and did not find the utilization of these intermediates (Tschech and Fuchs, 1987; Kobayashi et al. 1989; Béchard et al. 1990)

Some workers reported another phenol degradation pathway via carboxylation with benzoic acid, as an intermediate. Knoll and Winter (1987) have suggested the presence of benzoate as a one of the product of phenol transformation in anaerobic sludge digestion. Similar conclusion was drawn by Kobayashi et al. (1989) who examined phenol degradation in an anaerobic phenol-acclimated culture. They have found that benzoic acid was the first intermediate of the pathway during phenol degradation. The proposed pathway of anaerobic phenol degradation unlike the one proposed by Young and Rivera (1985) is presented in Figure 2-4.

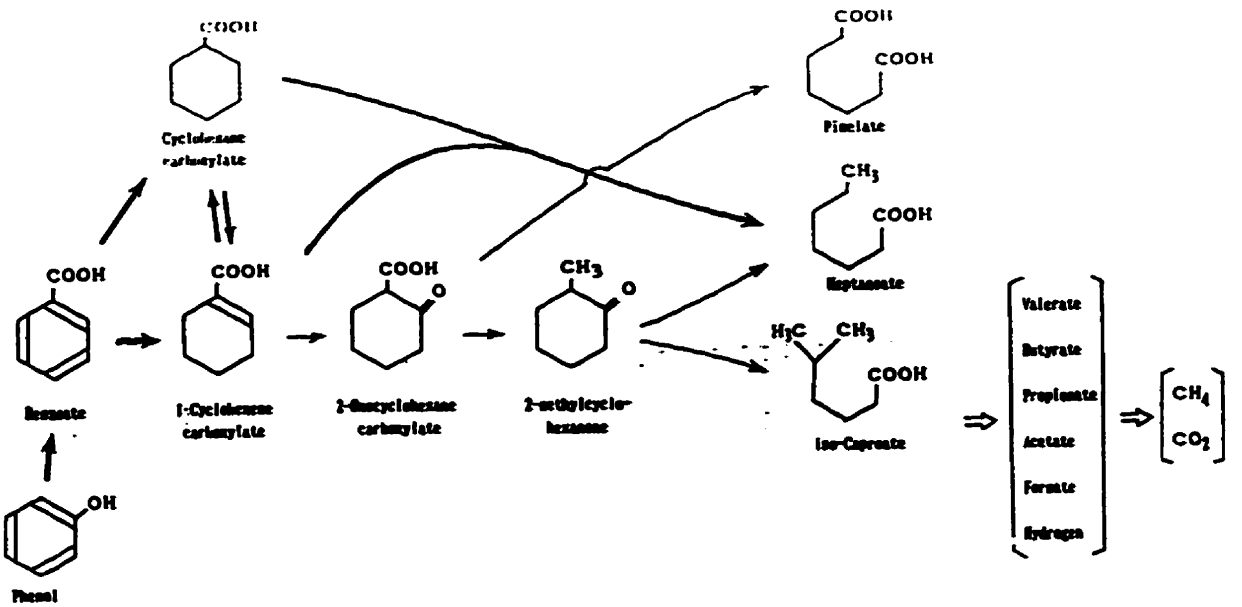

Figure 2-4. Phenol degradation pathway in anaerobic phenol acclimated sludge. Bold lines represent the proposed pathway (Kobayashi et al. 1989).

The evidence of phenol degradation pathway with benzoic acid as an intermediate was also reported by Bisaillon et al.1991; Létorneau et al. 1995; Béchard et al. 1990; Bisaillon et al. 1993. Béchard et al. 1990 found that phenol was transformed to benzoate under methanogenic conditions. They also reported that two intermediates characteristic to reductive pathway such as cyclohexanol and cyclohexanone were not accumulated. Bisaillon et al. 1991 reported methanogenic phenol degradation via carboxylation. The observed intermediate products of phenol degradation were: benzoic acid, 1-cyclohexene carboxylate, cyclohexane carboxylate, and heptanoate. Phenol degradation via carboxylation was also reported by Bisaillon et al.1993.

As reported in some studies, system acclimation to phenol can make the process insensitive to phenol loading, a decrease in the lag period, or an increase the range of phenol concentrations transformed to final products.

Healy and Young (1978) in their experiment on phenol degradation in methane digester culture showed that the system acclimation could result in shortening the degradation time and the lag period. It has been reported that the initial degradation of phenol at concentration of 300 mg L^{-1} started after 2.5 weeks of acclimation or lag period. Phenol degradation was complete after 14 days. The second addition of phenol resulted in complete phenol disappearance in 10 days with a very short lag period. Phenol disappearance and increased gas production at the same time indicated phenol transformation to biogas. The total amount of biogas and methane measured relative to the theoretical value from complete phenol degradation accounted for 79 and 89 percent, respectively.

Another study on the effect of different phenol concentrations on phenol-acclimated batch methanogenic cultures was carried out by Wang et al. (1989). Phenol degradation and methane production were measured at ten incremental phenol concentrations between 28 and 1430 mg L^{-1}. It has been shown that phenol was transformed to methane at all concentrations and methane produced accounted for 78 to 109 percent of the theoretical methane production. Phenol concentration of 1000 mg L^{-1} was degraded to methane after 44 days incubation.

Similar results on the effect of bacterial acclimation on the range of phenol concentrations degraded were presented by Kobayashi et al. (1989). They reported that degradation of phenol at a concentration of 1000 mg L^{-1} in acclimated sludges for six months

took only five to seven days.

As for phenol inhibition, some workers pointed out that phenol can be more inhibitory to phenol-degrading microorganisms than to methanogens. It has been reported by Fedorak and Hrudey (1984) that phenol can be more inhibitory to phenol-degraders than to methanogens.

Similar conclusions have been drawn by Wang et al. (1989) who showed that phenol degradation in phenol-enriched methanogenic culture was affected by various phenol concentrations. Bacterial cultures with the initial phenol concentrations of 28 to 220 mg L^{-1} exhibited a constant phenol utilization rate. As the phenol concentration increased, the degradation rate decreased and the reduced methane production rate was observed. It has been concluded that the high phenol concentrations inhibited the phenol-degrading microorganisms not the methanogens, since the degradation rate was higher at lower concentrations. At the same time, no significant amount of acetate, an intermediate product of phenol degradation was observed. It was suggested that phenol degraders are more susceptible to inhibition by phenol than are methanogens.

2.5.2 Landfill refuse system.

The effect of phenol on landfill refuse decomposition was investigated by Watson-Craik and Senior (1989a, 1989b). The experiment involved the treatment of two settings of small-scale landfill refuse columns: single-elution column and column operated with leachate recycle with phenol concentration of 188 mg L^{-1}. The experiment was performed on 'fresh' (one month) and ' active' (four months) refuse and involved the monitoring of pH in

leachate, phenol, organic acids, and methane headspace concentration. In general, phenol applied to the column without leachate recycle showed deleterious effect on refuse catabolism as the pH and volatile fatty acids concentrations were lower than the values in a control column between days 8 and 42. The headspace methane concentrations were also lower in the column without recycling between days 24 and 91. On the contrary, the column operated with leachate recycle did not show phenol inhibition since pH, volatile fatty acids, and methane concentrations were comparable with a control water-perfused column.

Studies on phenol biodegradation in the same experiment were also performed by measuring phenol in leachate effluent. It has been shown that phenol concentration of 188 mg L^{-1} was continuously removed from the system with recirculation for 'fresh' and 'active' refuse, whereas only 31 percent and 47.55 percent of phenol were removed using a single - elution column from 'fresh' and 'active' refuse, respectively. It was also reported that phenol removal can be affected by a refuse age, since phenol was removed faster from the column packed with older refuse. Further, it was pointed out that leachate pH and refuse buffer capacity might have effect on the process as phenol is added. Low pH and phenol supplementation could cause higher phenol bacterial inhibition since phenol becomes stronger disinfectant at lower pH.

In an experiment with a multistage refuse column (eight columns connected in series) Watson-Craik and Senior (1990) examined the effect of organic loading on the attenuation of phenolic wastewater in domestic refuse. Different phenol concentrations were perfused through columns filled with landfill refuse. The residue phenol concentration and pH were analysed in the effluent leachate stream. They reported that the influent phenol concentration

2 to 470 mg L^{-1} with a dilution rate of 0.007 h^{-1} was mineralized. Like in the experiment with single-elution column, an increase in influent phenol concentration from 188 to 470 mg L^{-1} caused temporary pH decrease from 6.55 to 5.7 within ten days without elevated VFA concentrations. The initial increase in phenol concentrations in different columns was followed by period where the phenol concentration declined and phenol was not detected in the effluent by day 101. At the same time an increase in leachate pH from 5.7 to 6.4 was observed.

With the influent phenol concentration of 564 mg L^{-1} a lower dilution rate of 0.004 h^{-1} was required in order to have phenol degradation. As phenol concentration was increased to 752 mg L^{-1} , the rate of methane production decreased and phenol was detected in the effluent. It was reported that this condition was attributed to toxic and inhibitory effects of phenol on the degradation process.

A study of phenol interactions with landfill refuse by Knox and Newton (1976) also provides some insight on phenol degradation in refuse systems. In the study, various phenol solutions were mixed with refuse using shake tests. It was reported that phenol concentrations up to 200 mg L^{-1} was degraded in aged refuse (four years old). No apparent phenol degradation was reported for fresh refuse (8 weeks old). The source reports; however, that the conditions in the experimental vessels might not be strictly anaerobic. So, there was no strong evidence of anaerobic biodegradation of phenol.

The most recent study on phenol degradation under methanogenic conditions in a laboratory scale landfill reactor was carried out by Ejlertsson et al. 1996. They reported that phenol concentration up to 50 mg L^{-1} was completely degraded to carbon dioxide and

methane without a lag phase during the incubation period of 100 days.

2.5.3 Anaerobic hybrid reactor for leachate treatment.

The effect of phenol additions on the efficiency of a 'phenol-unconditioned' anaerobic hybrid digester treating landfill leachate was examined by Britz et al. (1992). Phenol was added stepwise in increasing concentrations from 2 to 60 mg L^{-1} to a digester at 35°C and hydraulic retention time of one day. Phenol concentrations were increased stepwise after five weeks of operation. It has been reported that each phenol addition was followed by a decrease in COD removal, volatile fatty acids removal, and biogas production within 24 hours. At the same time pH drop was observed and the accumulation of organic acids, especially propionic. In a contrary, the methane content in the biogas increased immediately after phenol was added. Therefore, it has been suggested that phenol could be more inhibitory to acidogenic microorganisms than to methanogens. It was also pointed out that the operation of unacclimated anaerobic system for leachate treatment under phenol loading can result in failure unless bacterial acclimation or bacterial species selection is provided.

2.5.4 The effect of surface attachment on phenol inhibition

The advantages of cells attachment to surface or immobilization have been recognized in biological waste processes. The immobilization of cells plays a significant role especially when toxic compounds are present in the substrate. The system with immobilized cells offers the protection of cells from the effects of inhibitory substances, better substrate mineralization due to the retention of intermediate product (Dwyer et al. 1986), and longer retention time of biomass (Hanaki et al. 1994). Longer retention time of microbial cells provides microbial acclimation to toxic compound (Parkin et al.1983).

Activated carbon has been found to be an effective solid support medium against phenol shock loads since it provides the surface area for attached growth and phenol sorption. Khan et al. (1981), who has examined the effects of increasing phenol concentration in a continuously operated system in an activated carbon-packed up flow system suggested that the presence of an activated carbon could protect bacteria against shock load.

Cell immobilization on other support mediums such as agar have also been found effective in reduction phenol shock load. Dwyer et al. (1986) studied kinetics of phenol degradation in phenol-degrading methanogenic culture immobilized on agar. He reported that the immobilization protected cells from toxic effect of phenol. The native cells were completely inhibited at phenol concentration of 2000 $\mu g\ L^{-1}$ whereas the immobilized cells remained at one-half of their phenol degrading activity. The immobilized cells showed a decrease in lag period as compared to the native cells.

In biological systems of solid waste, the microorganisms can also be attached to the surface of solid phase. Barlaz et al. (1989) reported that microorganisms in the refuse ecosystem may be attached to fibrous material such as cellulose or solids and may be present in a liquid phase of the refuse system. Other workers also recognized cell attachment to cellulosic substrates in the ruminal habitat and showed it to be applicable to refuse ecosystems (Leedle and Hespell, 1980; Dehority and Grubb, 1980). Reinhard et al. (1991) pointed out that the landfill environment promotes cell attachment and sorption of the compound because of large surface area of complex refuse matrix. It was reported that the cell attachment results in an extended retention time and thus in microbial acclimation.

2.6 PHENOL IN SOLID PHASE SYSTEMS. SORPTION

2.6.1 Introduction

Sorption is defined as a process in which compound becomes bound on a solid phase (Schwarzenbach et al. 1993). It is a very important mechanism that determines the transformations of an organic compound in solid phase system. In soil systems sorption was reported to have an impact on the compound's bioavailability, persistence, mobility, and volatility (Boyd et al. 1982). In general, the sorbed chemical is resistant to microbial degradation.

Some workers, however, report that in some cases compounds can be utilized by microorganisms even in sorbed state (Aronstein and Alexander, 1992; Manilal and Alexander, 1991). In some instances phenol was biologically utilized when sorbed on sediments and granular activated carbon (Shimp and Young, 1988; Speitel et al. 1989). Still the mechanisms of utilizing sorbed chemicals is not clear.

The recent theory assumes that the sorbed chemical can be available to microorganisms after it enters liquid phase. At equilibrium when the chemical is sorbed on solid some partion is retained in liquid phase. As the microorganisms utilize the compound in solution, the equilibrium is shifted and more of the compound is desorbed and enters the liquid phase. In the liquid phase the chemical is taken up by microorganisms (Alexander, 1994).

Besides affecting the bioavailability of the compound sorption can also affect microbial degradation and activity in other ways. It can reduce the growth rate of microorganisms as the inorganic nutrients become sorbed. On the other hand the

concentration of nutrients at the surface of the sorbent may enhance microbial biodegradation. The zones near the surfaces that act as sorption media are characterized by lower pH that is less favourable for microbial growth. The negatively charged surfaces attract and concentrate H^+ from solution. Finally, the microorganisms can be sorbed mostly on particulate organics, so the microorganisms become more attached to solid then to liquid phase. Cell attachment may results in changes in their physiological and metabolic activity as compared to the cells free in solution and in bacterial acclimation (Alexander, 1994).

Several factors affect sorption of an organic compound on solid phase such as: the type and quantity of clay minerals, the amount of organic matter, pH, the specific surface area of the sorbent, temperature (Alexander, 1994). The importance of organic matter content in sorption has been reported by several workers (Alexander, 1994, Isaacson and Frink, 1984; Schwarzenbach et al. 1993). One of the most important characteristics of organic matter regarding sorption is its large surface area of 20-80 $m^2 g^{-1}$ (Alexander, 1994). The organic matter has been found responsible in sorption of many compound, especially in sorption of non-polar, hydrophobic compounds. Sorption of hydrophobic compounds on organic matter refers to as hydrophobic sorption where the compound diffuses and is retained in the matrix of the organic matter (Isaacson and Frink, 1984). In the case of hydrophilic compounds sorption may involve the entrapment in the matrix of organic matter (hydrophobic sorption), H-bonding, ion exchange, and complex formation (Schwarzenbach et al 1993; Alexander, 1994).

There are several mechanisms that are involved in sorption processes. They include van der Waals forces, hydrogen bonding, ionic interactions (Schwarzenbach et al. 1993). In

ionic interactions the ionizable organic compound is electrostatically attracted to the opposite charged surfaces. The prediction of any sorption mechanisms is based on the physical and structural characteristics of the compound and solid (Schwarzenbach et al. 1993).

Sorption of phenol on solid surfaces can include various sorption mechanisms. As reported by Bishop et al. (1990) phenol can be sorbed to charged surfaces through H-bonding and/or complexes and also through hydrophobic sorption characteristic for neutral compounds. Isaacson and Frink (1984) pointed out that phenol sorption on sediments involved hydrogen bond formation and hydrophobic sorption. The sorptive behaviour of phenol was in close relation to its solubility and the organic matter content.

2.6.2 Role of the natural organic matter in a solid phase in a sorption

The natural organic matter includes biopolymers such as proteins, lignin, cellulose, and also residues from degradation products and organics remaining from organisms (Schwarzenbach et al. 1993). It is mostly made of carbon (40 to 50 percent by weight).

The are several properties of natural organic matter that are relevant to sorption. They include: lower polarity as compared to water and ability for H - bonds formation at few sites such as: carboxy, phenoxy, hydroxy, and carbonyl substituents (Schwarzenbach et al. 1993).

Structural characteristics of organic matter are very important in sorption of non-polar compounds or hydrophobic sorption. The porous nature of the organic materials consists predominantly of 'organic chains coiled into globular units, much like globular

proteins'(Schwarzenbach et al. 1993). As a consequence, the organic matter exhibits a relatively nonpolar environment which causes the nonpolar compounds to go into the structure of the organic matter during sorption (Schwarzenbach et al. 1993). In the case of sorption of polar organic compounds such as phenol, besides entrapment into porous structure of organic matter, hydrogen bond formation shall be considered as an important mechanism of interactions because of phenol polarity and the ability of organic matter for H - bond formation (Isaacson and Frink, 1984).

The natural organic matter can undergo charge build up as a consequence of pH changes. This includes ionization of carboxyl groups (-COOH), ionization of phenolic groups (aromatic ring-OH), and ionization of carbonates (Schwarzenbach et al. 1993). The charged groups of organic matter can participate in ionic bond formation with an organic chemical.

2.6.3 Sorption of charged phenol species on a solid phase

Phenol and other organic acids can undergo proton transfer reactions and produce charged compounds (Schwarzenbach et al. 1993). The protonation reaction for phenol can be described according to equation 2-7 (Solomon, 1987):

$$C_6H_7OH \rightleftharpoons C_6H_7OH^- + H_3O^+ \quad at \quad pK_a = 9.82 \qquad 2\text{-}7$$

According to equation 2-7 at pH equal pKa = 9.82 there are same amounts of neutral and ionized phenol species. As the pH increases in pH range higher than pKa the proportion of ionized form increases. At pH less than 9.82 more than 50% phenol remains in its neutral form.

The charged compounds exhibit different characteristics than the neutral species also in regard to sorption processes. Sorption of the ionized form occurs mainly in two processes such as: the electrostatic interactions of charged molecules with charged sites on the sorbent, and exchange reactions with ligands already bound to the solids. The extent of sorption of ionized species depends on the concentration of the charged sorbate which also varies with pH. At different pHs, there will be various proportion of sorption of neutral and ionized phenol species (Schwarzenbach et al. 1993). According to Schwarzenbach et al. 1993 the ionized, deprotonated phenol compound is more water soluble than the neutral form and less sorbed on organic matter.

2.6.4 Phenol sorption in soil and sediments.

Sorption of organic compounds onto soil and sediments depends on the sorbent properties such as organic matter content, type and content of clay, pH, cation exchange capacity, and physico-chemical characteristics of the compound such as water solubility (Rajput et al. 1994). A factor that mostly determines soil sorption of a contaminant is its affinity to solid and to solvent. Hydrophilic compounds tend to remain in liquid solution and are less likely to be sorbed on soil.

Isaacson and Frink (1984) in their study on sorption of various phenol species on sediments reported that besides organic matter content that affects phenol sorption there are other factors such as: pH, moisture, and the solubility of the compound. Sediment fraction after treatment to remove organic matter sorbed less phenol than the untreated one. The sorption of phenol was reported to strongly depend on pH. There was, however, a limited

explanation of the role of pH in sorption mechanism especially in sediment organic matter interactions. The sediment with higher solid content or low moisture content sorbed more phenol.

Bishop et al. (1990) studied behaviour of phenol on various sorbents such as: solid waste materials from coal combustion, oil-shale restoring, soils, and soil components. Soil sorbents included: silica sand, iron oxide containing soil, and high organic content soil. It was reported that the amount of phenol sorbed depended upon phenol concentration and the sorbent. The linear sorption isotherms showed that as phenol concentration increased sorption also increased. The sorbents with highest sand content (silica and high temperature spend oils shale) had the lowest sorption for phenol whereas kaolinite, iron containing soil and high organic content soil showed significant sorption of phenol. It was concluded that phenol sorption depends on both organic and mineral components of a sorbent. No significant correlation has been found between sorption and pH of the sorbent. A desorption study showed that phenol desorption was slower process than sorption in most cases. Despite phenol's high affinity to water it was reported that a fraction of phenol was irreversibly held on solid phase. In a conclusion to his study, Bishop described the sorption of phenol on all sorbents as low. Since significant amounts of phenol were extracted with methanol, it was concluded that the mobility of phenol can be affected by the presence of alcohols or other organic solvents in waste materials.

2.6.5 Phenol sorption in refuse systems

There are no reports in the literature on sorption of phenol in a continuous digesting system of solid waste. However, there are limited data on phenol sorption on solid waste. Sorption of organic compounds on solid waste in landfill system was discussed by Reinhart et al. (1991). It was reported that the large surface area of the refuse made the suitable conditions for sorption. The presence of physical processes besides microbial degradation that could effect the retention time of phenol in landfill refuse was also suggested by Senior (1990). The interactions of phenol with refuse have been referred mainly to as physico-chemical process of adhesion of phenol from a liquid to solid surfaces or as a immobilization process of phenol in a refuse system (Senior, 1990).

A more detailed experiment was performed by Knox and Newton (1976) on phenol interaction with domestic refuse. The experiment was carried in shake tests where containers with 1 kg refuse and 5 litres of a solution of phenol and two other phenols were shaken by hand. Five initial concentrations of phenol were analysed: 200, 500, 1000, 1500, and 2000 mg L^{-1}. Two types of landfill refuse were used: fresh (8 weeks old) with moisture content of 30% and decomposed (4 years old) with moisture content of 49%. Liquid samples were taken at different times intervals up to 60 days. The results showed a rapid decrease in phenol concentration within six hours following the contact of the solution with refuse. It was concluded that the initial decrease in phenol concentration could be caused by chemical reaction or sorption. The results of phenol concentrations measured during 36 hours after contact showed phenol sorption onto refuse. Desorption tests were carried out by a comparison of phenol mean concentration in the period 1-26 hour with the mean

concentration measured after 14 days and longer. The desorption tests were run with water.

The following conclusions were drawn from their study:

1. phenol was sorbed more on fresh than on a aged refuse

2. phenol sorption depended on moisture content, at higher moisture content phenol sorption was smaller

3. phenol sorption depended on phenol concentration, for fresh refuse more phenol was sorbed at the lower concentrations, for aged refuse less phenol was sorbed at lower concentrations

4. there was not much of phenol desorption from fresh and aged refuse, the increase in phenol concentrations for aged and fresh refuse was from 5 to 7% and from 4 to 5%, respectively.

They also pointed out that since phenol sorption decreases at lower concentrations for aged refuse, low phenol concentrations less than 50 mg L^{-1} will not be effectively sorbed on refuse. In case of fresh refuse, however, phenol sorbed effectively at low concentrations can be released into solution after decomposition of the refuse or it can be degraded in the sorbed form.

2.6.6 Summary

Based on the presented literature, sorption of phenol on solid phase depends on organic and mineral components of solid phase, moisture, pH, and phenol concentration. More phenol is sorbed on solid phase with higher organic matter content. Solid phase with lower moisture sorbs more phenol. At various pH's, there are different amounts of neutral

and ionized phenol species that exhibit different sorption characteristics. Ionized, deprotonated phenol is more water soluble than neutral form and less sorbed. Phenol sorption on soil increases with phenol concentration. In the case of solid waste, more phenol is sorbed at lower concentrations on fresh refuse and less phenol is sorbed at lower concentrations on aged refuse. Sorption could affect bioavailability of phenol and make it more resistant to microbial degradation.

2.7 PHENOL EXTRACTION AND ANALYSIS.

2.7.1 Introduction

Extraction is the first step in the biological and environmental analysis process. In most cases, the matrix of the environmental samples makes them unsuitable for direct analysis using chromatography or other methods. In case of high performance liquid chromatography measurements, solid samples are too complex and incompatible to be directly injected.

Extraction is a separation of an analyte of interest based on the analyte affinity for different phases. The mechanism of a compound separation from solid samples using extraction includes different affinity of the compound to solid and liquid phases.

Solvent extraction is one of the extraction techniques which can be applied to solid samples including compost. It involves the solubilization of an analyte by contacting the sample with liquid followed by solid solvent separation. When the liquid used in extraction is water or water in solution with acidic, basic compounds, and surfactants, the extraction is referred to as water washing (Raghavan et al. 1990).

The equipment used during solvents extraction is simple and usually includes: shaker, homogenizer, and Soxhlet extractor (Poole, 1991). In extraction using a homogenizer the bulky samples are dried, cut, ground before extraction to improve the efficiency of extraction.

The procedure using Soxhlet extractor includes continuous extraction at room temperature or extraction at boiling point of the solvent. The solvent is vaporized, condensed, and percolated through the solid sample. Soxhlet extractor is best suitable to

samples which can be processed to a powder and for a compounds with high solubility in extracting agents.

In extraction using shaker samples are mechanically shaken with the solvent for a desired period of time. Extraction using shaker give the best results when the analyte is very soluble in extracting solvent and is most suitable for porous solid samples (Poole, 1991).

Solvent extraction is simple, however, phase separation appeared to be very important step in the procedure. The most common means of phase separation include: phase separation through filter papers and centrifugation (Poole, 1991).

The results of solvent extraction depend on the affinity of the analyte to the extracting solvent and the number of extractions. In some instances the efficiency of extraction can be increased by heating the solvent (Poole, 1991).

The selection of the solvent suitable for extraction is based on the chemical structure of the contaminant, solid phase type, equilibrium characteristics, chemical stability, and also on its toxicity to man, and environment, ease of use, and cost. Hydrophilic compounds, for example, can be effectively extracted using water washing alone (Raghavan et al. 1990).

2.7.2 Phenol extraction from soil.

The property of being a hydrophilic compound or water liking makes phenol suitable for removal by washing in aqueous solution or water washing from contaminated soil (Evangelista et al. 1990; Rajput, 1988; Raghavan et al. 1990).

Literature reports several extracting agents that have been effectively used in phenol extraction from soil. They include: deionized water at various pH's and temperatures,

deionized water containing surfactants, aqueous solution of sodium hydroxide, hydrogen peroxide (Rajput, 1988; O'Neill et al. 1993; Evangelista et al. 1990; Raghavan et al. 1990). Hot water as a phenol extractant has been also used to facilitate the efficiency of extraction since phenol solubility increases with the increase of temperature (Evangelista et al. 1990).

The effectiveness of extraction from soil has been found to depend on soil type or the organic carbon content in soil. In general, the extraction is easier to perform from sandy soil than from loamy and clay-like soils (Rulksen and Assink, 1983).

In case of sandy soil, it is easier to separate sand particles from extracting agent due to high settling velocities of sand particles. Sorption of a contaminant on sand particles is also relatively low since the sand particles have a relatively small surface area.

Extraction is more difficult to perform from loamy and clay-like soils for two main reasons. First, is that small clay particles tend to form rather stable suspension with extracting liquid especially at high pH's. This make the separation of the contaminant from soil even more difficult. Second, is that loam and clay particles tend to adsorb the contaminant and make the removal more difficult (Rulksen and Assink, 1983). The same extraction limitations mentioned for loamy and clay-like apply to peaty soils and highly heterogeneous soils. If soil has a high organic matter content such as plants remains, humus compounds, the organic matter material tends to dissolve in the extraction liquid and form a suspension.

In order to improve the extraction process some chemicals can be added to the water. They include: acids - hydrochloric acid (HCL), sulphuric acid (H_2SO_4), nitric acid (HNO_3) to dissolve impurities, bases - sodium bicarbonate (Na_2CO_3) and sodium hydroxide (NaOH)

to dissolve impurities or to disperse insoluble impurities in extracting agent (Rulksen and Assink, 1983).

Rajput (1988) carried out a study on treatment of hazardous waste contaminated soils using extraction/washing experiment. He showed effective extraction of phenol from sandy loam soil using deionized water and solution of surfactant. He achieved 97.8% phenol removal after one wash with surfactants followed by three rinses with water in extraction experiments using a shaker table-centrifugation method. In the same experiment, the reported phenol removal after four washes using only deionized water was 99.2%. The results showed that water alone was very effective in phenol extraction. It was concluded that the effectiveness of water as a phenol extractant was caused by a high affinity of phenol for water.

In extraction experiments using a bench scale type washing system where soil with extracting agent were mixed at 800-1000 rpm for 5 minutes high phenol removal efficiencies using water alone were also reported (Rajput, 1988). Phenol removal efficiencies after four washes using deionized water alone were 99.1 and 98.7% whereas the efficiency using 2% solution of surfactant and water was 96.6%.

Evangelista et al. (1990) also conducted phenol extraction from phenol-contaminated soil using washing technique. He used several solvents as phenol extractant such as tap water, water at various pH's 9.3, 10.5, 11.5, hot water at 50°C, and water with surfactant. The extraction procedure involved mixing 10 g of contaminated soil with 200 mL of extractant and agitating on a automatic shaker for 10 minutes, centrifuging for 20 minutes, and analysing phenol in the supernatant. The results were presented as relative to phenol

extraction using US-EPA method number 420.1 (US-EPA, 1979) performed together with soil washing. Alkaline water at pH 11.5 and hot water were the most efficient extractant with 100% relative recovery. The relative phenol extraction efficiency using solution of water and surfactant and tap water ranged from 72 to 97% and from 82 to 95% respectively. The tap water was the preferred extractant because of its high extraction efficiency, and simplicity.

O'Neill et al. (1992) investigated phenol extraction from a slightly organic, loamy soil using the successive reverse isotherm (SRI) method. Three extracting agents were used: deionized water, hydrogen peroxide at varying concentrations, and aqueous solution of sodium hydroxide at varying pHs. First, soil was contaminated with phenol by mixing a six-gram soil sample with 5 mL phenol solution for 24 hours on a shaking table. The slurry was then centrifuged at 2000 rpm and 2.5 mL of supernatant decanted to determine phenol remaining in aliquot. Phenol extraction involved adding 2.5 mL of extraction agent, hand agitation for 5 minutes, centrifuging, and phenol analysis in supernatant. The procedure was repeated until approximately 15 mL cumulative volume of extracting solution was used. The reported extraction efficiencies were: 30 percent for deionized water, 42 and 45% for hydrogen peroxide at concentrations 200 mg L^{-1} and 500 mg L^{-1} respectively, and 40 and 70 percent for NaOH at pH 8 and 10 respectively.

As mentioned in the introduction the efficiency of extraction depends on the number of extractions from the same sample. The efficiency of phenol removal from contaminated soils has been found to increase with the number of water washes and the cumulative volume of extracting agent used in soil washing and SRI method, respectively. Rajput (1988) who conducted shaker table experiments in phenol removal from soil using deionized water found

that phenol removal efficiency increased with the number of washes. However, the highest phenol removal occurred during the first wash. For 90% phenol extracted using total of four washes, 70% was extracted during the first wash.

2.7.3 Phenol analysis in leachate, sludge, domestic and industrial wastes

The following section will provide an overview on phenol analysis in some anaerobic biological waste processes and domestic and industrial wastes. The procedures for phenol analysis were taken from analytical methods section of various studies presented in section 2.5.1 and from US-EPA method 420.1 (US-EPA, 1979). In most instances, phenol determination in sludge included filtering sludge samples and analysis using chromatography. Some procedures involved solvent extraction using organic solvent.

Boyd et al. (1983) analysed phenol in anaerobic digester sludge by taking 2 mL samples that were frozen, thawed, filtered by vacuum through 0.45 μm cellulose acetate filters, and analysed using HPLC. Similar procedure was used by Young and Rivera (1985) in phenol determination in anaerobic batch studies. Samples were centrifuged or filtered and analysed using spectrophotometer or HPLC. Kobayashi et al. (1989) measured phenol in methanogenic sludges by filtering samples through 0.45 μm Millipore filter (Millex-HV) and analysing with HPLC.

Wang et al. (1986) determined phenol in an expanded - bed anaerobic reactor by sample filtering, acidification to pH of 2, extraction using ethyl ether, and analysing solvent phase using gas chromatography. The same procedure was used by Wang et al. (1989) in phenol analysis in batch methanogenic cultures.

Based on available literature, phenol determination in leachate from solid waste was limited only to studies conducted by Watson-Craik and Senior (1989a, 1989b, 1990) and Knox and Newton (1976). Watson-Craik and Senior analysed phenol in leachate by direct injection leachate samples into gas chromatograph. Knox and Newton (1976) analysed phenol during shake tests by taking 2 mL samples from slurry of solid waste and a liquid phenol solution, filtering through Whatman GF/C filter, and analysing using gas- liquid chromatography.

US-EPA method 420.1 spectrophotometric with distillation (US-EPA, 1979) can be applied to phenol determination in domestic and industrial wastes and also in drinking, surface and saline waters. In the method, phenol reacts with 4-aminoantipurine in the presence of potassium ferricyanide at pH of 10 to from reddish-brown colored antipyrine dye. The procedure involves distillation of liquid samples, addition of aminoantipyrine and potassium ferricyanide and reading the absorbance of the samples and standards. The method does not describe the distillation process of solid waste.

2.7.4. Summary

Sections 2.1 to 2.7.3 provide an overview of processes affecting transformations of phenol in a solid phase system, physical characteristics relevant to predict its transformations and impact, and also its effect and biotransformations in anaerobic biotransformation processes, and methods of phenol extraction and analysis in various waste systems. Because the literature lacks any data about phenol in dry anaerobic digestion process the overview was limited to anaerobic waste transformation processes in different solid phase systems.

The presented literature review was important to the study since the various processes that affect phenol in other systems could be related to dry anaerobic digestion system. The processes affecting phenol in solid phase system such as sorption, solubillization, and volatilization determine its transformations and impact also in DAD due to the structural analogy of DAD to solid phase system. The review of phenol effect and biotransformation in anaerobic waste biotransformations processes was important to set the range of phenol concentration used in the study and to refer our conclusions to the findings of other workers. The methods of extraction and analysis from soil and solid waste tests could be adapted to the dry anaerobic digestion process.

2.8 ASSESSMENT OF LABORATORY AND ANALYTICAL METHOD. STATISTICAL ANALYSIS. SAMPLING.

The reliability criteria of a laboratory method include (Sachs, 1984):

1. Specificity. This includes a characterization of a chemical substance to be analysed and is focused mainly on qualitative description.

2. Accuracy. This includes the determination of the exact amount of the chemical present in the material under study. The accuracy can be determined if the true value of the chemical present is known according to equation 2-8:

$$accuracy=\frac{(\bar{x}-\mu)}{\mu} \qquad 2\text{-}8$$

Where: μ is known true value.

$\bar{x}$ is sample mean

The accuracy can be checked using one of the following procedures:

Comparison tests can be run which include the comparison of the results with results obtained by another standard method, or with results of numbers of interlaboratory comparisons.

3.Precision or reproducibility. The errors in the method caused by changing laboratory conditions such as reagents, different days, temperature could be assessed using the standard deviations and the coefficient of variation. Coefficient of variation is a dimensionless relative measure of dispersion and is defined by the ratio of the standard deviation to the mean value.

4. Practical long-range considerations. This includes the amount of time required, costs, difficulties in carrying the experiments, equipment costs etc. associated with presented laboratory method.

Accuracy and precision are the most important considerations in assessing the reliability of a method.

The overall error in measurements consists of random error and systematic error and can be expressed as:

$$G = \text{random error} + \text{systematic error} \qquad 2\text{-}9$$

Where: G is the overall error

Systematic errors can be caused by contaminated reagents, unreliable equipment, poor calibration, and errors in data handling (Sachs, 1984; Kratochvil and Taylor, 1981). The errors can be minimized by proper using of standard, blanks and reference samples. However, it is not possible to eliminate them. In evaluation of solid waste the overall standard deviation for random errors is related to standard deviation for sampling operation and standard deviation for analytical measurements according to equation 2-10 (Kratochvil and Taylor, 1981):

$$(s_o)^2 = (s_a)^2 + (s_b)^2 \qquad 2\text{-}10$$

Where: s_o is overall standard deviation

s_a is standard deviation from analytical operations

s_s is standard deviation from sampling operation

In other words the overall uncertainty in laboratory measurements is caused by sampling uncertainty and analytical uncertainty (Kratochvil and Taylor, 1981).

The literature reports that sampling is a major source of errors and sampling uncertainty accounts mostly for overall uncertainty (Kratochvil and Taylor, 1981; Woodbury and Breslin, 1992). As a result the sampling uncertainty adds to the uncertainty from analytical measurements and tremendously reduce the reliability of results.

In order to assess the uncertainty during an experiment, the evaluation of variance for analytical operation and for sampling operation should be made. The estimation of both variances can be done by analysing series of replicate analytical measurements and replicate samples.

Sampling errors originate from the nature of the matter being sampled (material heterogeneity), the sampling operation, sampling errors made by a sampler, the treatment of the sample after collection or sampling device and its proper use (Kratochvil et. al. 1984). Gy (1994) points out the significance of distribution heterogeneity in causing sampling error so the overall sampling variance is proportional to the distribution heterogeneity. As consequence mixing of the material have also been found as a significant source of error besides sampling in some compost studies (Woodbury and Breslin, 1992).

The overall uncertainty can be minimized by the reduction of analytical uncertainty or variance from analytical operations. Literature reports that the reduction of analytical uncertainty to a third or less of the sampling uncertainty is sufficient. If the sampling uncertainty is very large, a rapid, approximate analytical method can be used and further improvements in measurements are of little importance (Kratochvil and Taylor, 1981).

Because of the improvement of the analytical methods in the last decades, the control of the sampling error has become very important. Methodology of sampling from compost material has become critical since there is a need to assess compost maturity, heavy metals levels or to gain data on fate of various contaminants during composting process (Woodbury and Breslin, 1992). The significance of proper sampling applies not only to compost system but also to any environmental sampling including solid waste, soil, sediments etc.

Since sampling error is much higher than analytical error, it became a limiting factor in overall experimental uncertainty (Woodbury and Breslin, 1992). In order to minimize sampling error a sampling plan is required. The plan should consists of statistical design, guidelines for sample collection, conservation, and storage, trained personnel in sampling.

The US-EPA report on Test methods for evaluating solid waste gives important characteristics regarding a proper sampling from solid waste (US-EPA, 1982). They include collection of representative samples, variability of the waste and statistical characteristics such as: a mean concentration, standard deviation, standard error, and confidence interval within the true value of the chemical probably occur. A representative sample is defined as a sample which exhibit average properties of the waste where each part of the waste has the chance to be sampled. According to the report collection of representative samples is related to sampling accuracy whereas variability of the waste to sampling variability or sampling precision (US-EPA, 1982).

Based on the report, sampling accuracy describes the closeness of a sample value to its true value and is usually accomplished by random sampling (US-EPA, 1982). Random sampling defines sampling where every unit in solid has theoretically equal chances to be sampled. This can be achieved by dividing the solid waste by imaginary grid, assigning consecutive numbers to each segment of the grid, and chose segment number to be sampled using a random numbers table (US-EPA, 1982).

Sampling precision or variability of the waste describes the closeness of repeated sample values. It is defined by a sufficient number of samples (not less than four) collected over period of time. The desired sampling precision can be achieved by a sufficient number of samples and the sample size. An increase in the number of samples and the physical size of the samples leads to increase in the sampling precision. However, in most cases the desired precision can be achieved only by taking an appropriate number of samples (US-EPA, 1982). More detailed information regarding the assessment of sample size can be found in the report 'Sampling for chemical analysis' by Kratochvil and Taylor (1981).

The three major steps in making a proper sampling plan can be described as follows (Kratochvil and Taylor, 1981; US-EPA, 1982):

1. collection of a small number of samples that are representative as much as possible
2. calculation of mean value, standard deviation for single observation, standard error for sample mean, and confidence interval where the true value can be found, according to equations 2-11, 2-12, 2-13, 2-14, respectively.

$$\bar{x}=\frac{\sum_{i=1}^{n} x_i}{n} \qquad 2\text{-}11$$

$$s=\sqrt{\frac{\sum (x_i-\bar{x})^2}{n-1}} \qquad 2\text{-}12$$

$$s_{\bar{x}}=\frac{s}{\sqrt{n}} \qquad 2\text{-}13$$

$$CI=\bar{x}\pm t^{*}s_{\bar{x}} \qquad 2\text{-}14$$

Where: x_i is single analytical measurement

$\bar{x}$ is average of analytical measurement

n is number of analytical measurements

s is standard deviation for single measurement

$s_{\bar{x}}$ is standard error of mean

$t^{*}$is critical point from t-list with n-1 degrees of freedom

CI is confidence interval for true value

$t^{*}s_{\bar{x}}$ is margin of error

3. calculation the required number of samples at desired margin of error according to equation 2-15

$$n_d = \left(\frac{t^* s}{\Delta} \right)^2 \qquad 2\text{-}15$$

Where: n_d is required number of samples at desired margin of error

t^* is critical point from t-list with n-1 degrees of freedom

Δ is desired margin of error

Usually the steps performed on one or two sampling sets are sufficient to define parameters to determine required number of samples with a high level of confidence.

As reported by US-EPA the results of solid waste evaluation usually exhibit normal distribution regardless the sampling strategy used (US-EPA, 1982). In same cases however, the results can deviate from normal distribution, since the number of samples is usually small. Even if the distribution is abnormal, it does not affect the mean which is most important (US-EPA, 1982). As for confidence interval (CI), US-EPA recommends CI of 90% to be used to for all practical purposes to evaluate solid waste (US-EPA, 1982), although some workers use 95% CI for compost studies (Woodbury and Breslin, 1992).

2.8.1 Summary

Section 2.8 presents the reliability criteria in assessment of a laboratory method. It also stresses the importance of sampling in analysis of solid waste.

The reliability criteria by Sachs (1984) were used to assess the laboratory method in the

study. The most important criteria were accuracy and precision of the method. Accuracy and precision were discussed according to definitions for solid waste analysis from US-EPA report (US-EPA, 1982).

Since the study involved analysis of heterogenous solid waste a sampling was significant. The sampling in the study was discussed based on characteristics and steps of a proper sampling given in US-EPA report (US-EPA, 1982).

CHAPTER 3

RESEARCH OBJECTIVES AND EXPERIMENTAL METHODS

The objective of the study was the development of protocols for assessing the effect of phenol on the dry anaerobic digestion process. The study was conducted in two phases. Phase I was designed to observe the effect of phenol on the DAD process by adding increasing concentrations to a thermophilic digester treating the organic fraction of municipal solid waste (OFMSW) operated at mass retention time (MRT) of 21 days and to assess phenol monitoring program. Phase II was designed to develop a standard operating procedure to analyse phenol in feedstock.

3.1 PHASE I - OBJECTIVES AND METHODS.

The objective of Phase I was to observe the effect of increasing phenol concentrations on the process of DAD and to assess phenol monitoring process during the digestion process. The effect was evaluated by measuring the following performance characteristics of the digestion system: gas production, gas composition, volatile solids (VS) removal efficiency, volatile fatty acids (VFA), and ammonia. The initial phenol concentration added to the system was 50 mg kg^{-1} and consecutive additions were incrementally increased by 50 percent up to a maximum of 379.65 mg kg^{-1}. Phenol monitoring program included: sampling from heterogenous compost material at different stages of the digestion process, extraction and analysis of phenol using HPLC.

3.1.1 Experimental methods

Two identical bench-scale DAD reactors systems were operated. Each system consisted of a 10 litre digester reactor and a gas collection system. Both reactors were operated in the thermophilic temperature range at a temperature of 55°C, an MRTof 21 days, using a semi-continuous feeding system.

The experiment involved two phases. The first phase was from day one to day 47. Both reactors R1 and R2 were fed identical feedstock containing a mixture of paper, food waste and sludge. The second phase was from day 48 to day 99. The reactor R2 was fed with phenol while R1 without phenol was operated as the control.

The start up of the anaerobic digestion process took place in a slurry form. Each reactor was filled with a mixture of 925 g of anaerobic digester sludge, 925 g cow manure, 925 g digestate from a previous dry anaerobic digestion study and 925 g of feedstock. Cow manure was sampled from a University of Manitoba (UM) Animal Science Department. Digestate from the previous dry anaerobic digestion study was frozen and kept in the laboratory. The feedstock consisted of food waste, office paper and primary sludge. The food waste was collected from UM cafeteria and contained mainly food from breakfast and lunch preparation. The determined solid content in the food waste was 10.2 percent. The paper consisted of white office paper from UM administration building. The primary sludge was taken from North End Water Pollution Control Centre (NEWPCC) which contained 5.5 percent solids.

The paper was shredded twice. First, it was cut in stripes using an office shredder. Then, the stripes were cut diagonally into smaller pieces 0.5 x 3 cm using the same shredder. The food waste was blended using a kitchen blender. Food and paper were mixed in a 1: 1 ratio based

on wet weight. The primary sludge was then added to the mixture in a ratio 1: 1 based on wet weight. The mixture containing paper, food and sludge was blended using a Hobart Bread Mixer for about 15 minutes until the blended mass reached a constant colour and become well mixed.

Approximately 400 gram of prepared feedstock was packed into separate plastic bags. Enough feedstock to last for one month of feeding was prepared and stored in a freezer at minus 20°C.

Two plastic reactors with total volumes of 10 L and active volumes of 4.5 to 5.0 L were used during the experiment. Each reactor was connected with two 20 L plastic containers. The containers held liquid displacement solutions consisting of a saturated solution of NaCl and 5% H_2SO_4. The set-up of the reactors is shown schematically in Figure 3-1. The reactors were flushed with nitrogen for five minutes, covered with rubber stoppers and sealed with metal clamps. Reactors with substrate were weighted and then transferred to a thermophilic chamber (55°C).

Since there was no gas production in both reactors during first days of the experiment, the reactors were reseeded. The content of each reactor was replaced with cow rumen. The rumen was sampled from a fistulated hay-fed cow at the university Animal Science Department and immediately transported to the laboratory in containers in warm water bath.

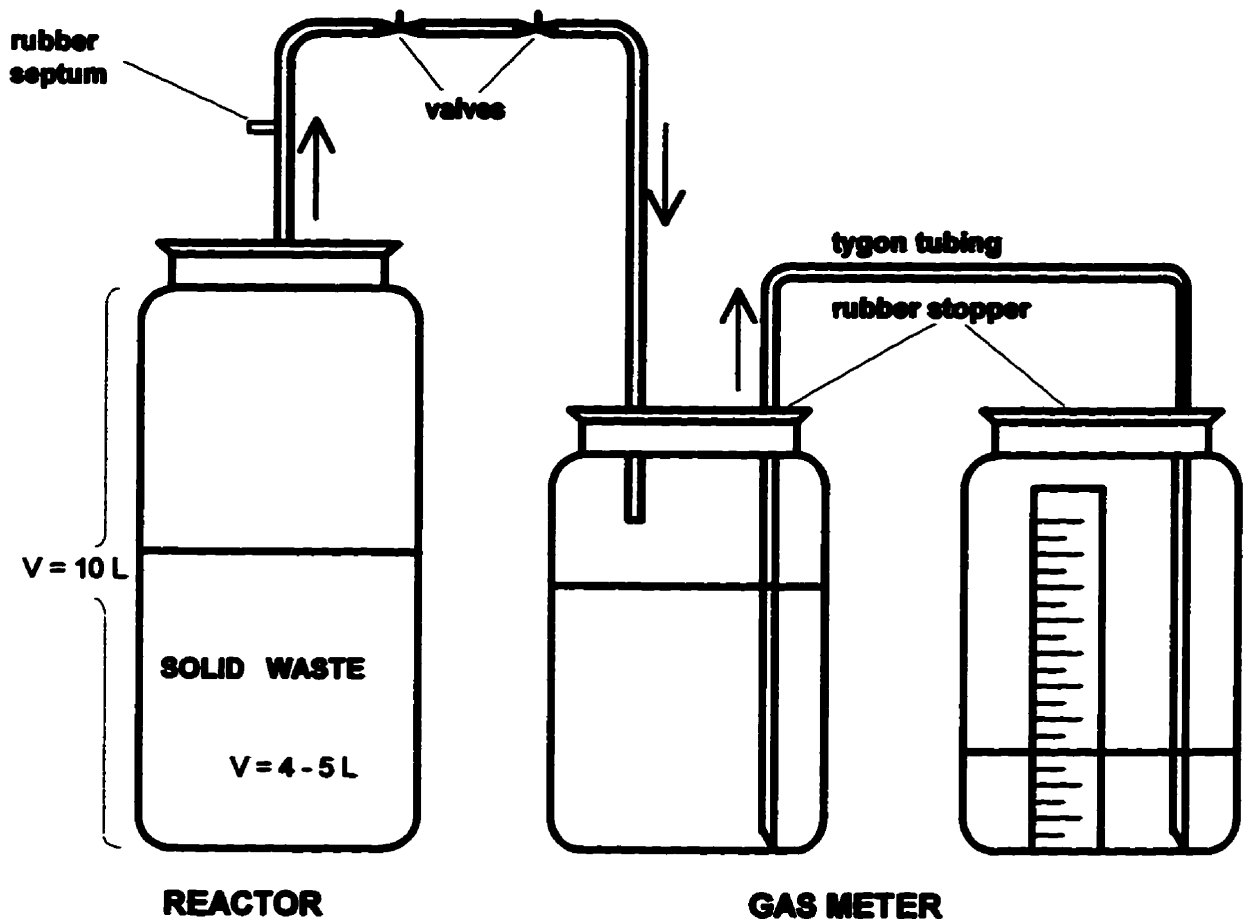

Figure 3-1. Phase I - DAD reactor set-up.

The rumen fluid was added immediately in amounts 1423 g and 1373 g to R1 and R2, respectively. The corresponding amounts of digestate were removed so that each reactor contained a constant mass 3800 g of the digestate. At this point, the contents of the reactor were as follows: R1 - 1423 g of cow rumen and 2377 g of a mixture of anaerobic digester sludge, cow manure, feedstock, and digestate from previous DAD study, R2 - 1373 g cow manure and 2427 g of the mixture. The total solids in the reactors was 14.5%.

The amount of feedstock to be added to the reactors was calculated according to equation 3-1.

$$M_f = \frac{3800}{MRT} T \qquad 3\text{-}1$$

Where: M_f is mass of feedstock added to reactor, (g)

3800 is constant mass of reactor content, (g)

MRT is mass retention time, (days)

T is time preceding the next draw and feel, (days)

The amount of digestate to be drawn from the reactors was calculated according to equation 3-2.

$$M_d = M_b - M_e + M_f - 3800 \qquad 3\text{-}2$$

Where: M_d is the mass of digestate to be drawn, (g)

M_b is the mass of reactor before feeding, (g)

M_e is the mass of empty reactor, (g)

M_f is mass of feedstock added to reactor, (g)

Removing digestate from the reactors and addition of feedstock took place in a glove box flushed with nitrogen gas. After feeding was completed, the solid waste in each reactor was mixed for four minutes using a Black & Decker drill (type 1, variable speed and reversing) with a machined attachment constructed in our laboratory. The reactors were then sealed, weighed and returned to the chamber.

From day 1 to day 57, the reactors were fed twice a week. Starting on the experimental 58th day, the feeding schedule was changed and the reactors were fed once a week. The change in feeding schedule was done to perform more efficient phenol monitoring in longer periods between feedings. Less frequent feeding operations would allow more frequent phenol determination. The amount of feedstock supplied to the reactors during twice a week feeding schedule on Tuesday and Friday was 542.9 g and 723.81 g, respectively. The amount of feed supplied to the reactors during once a week feeding schedule was 1266.67 g.

The total solids content in each reactor was increased gradually by adding drier feedstock during draw and fill. Before each feeding operation, a 5-gram sample of the feedstock prepared on that day was used for moisture determination using CSC Moisture Balance. The reading on the Moisture Balance was shown in % moisture. Since the mass of the sample originally included the mass of moisture and the mass of solids, the solid content was calculated by substraction according to equation 3-3.

$$\%solids = 100\% - \%moisture \qquad 3\text{-}3$$

In order to have a drier feedstock, the feedstock was kept in a muffle oven at 90°C for about 20 minutes. At the same time the moisture or solid content was determined periodically using the Moisture Balance until the solids content of about 35 percent was reached.

To maintain the optimal range of alkalinity in the reactors sodium bicarbonate was added at each feeding in ratio sodium bicarbonate/substrate solids = 0.06 based on solids content in the feedstock determined using the Moisture Balance. The ratio bicarbonate to substrate solids was proposed by Ten Brummeler and Koster (1989). During periods of increased acid quantities, the amount of sodium bicarbonate was increased by 50 percent of the calculated amount.

Starting on day 48, phenol was added to R2. The subsequent additions took place on days: 51, 65, 72, 79 and 83. Each time the amount of phenol added was 50 percent higher than the dosage from the previous addition. The increasing amounts of phenol were added

to R2 from 64 g L^{-1} phenol stock solution prepared from reagent grade phenol. Phenol was spiked during feeding operation using a 10 ml plastic syringe. The amounts of phenol added are listed in Table 3-1:

Table 3-1. Phase I - Amount of phenol added to R2.

Day of the experiment	Phenol added (mg kg_R^{-1})	Volume of stock solution 64 g L^{-1} (mL)
48	50.00	2.97
51	75.00	4.45
65	112.50	6.68
72	168.75	10.02
79	253.10	15.03
83	379.65	22.50

NOTE: kg_R is kg of reactor content

Digestate samples for phenol quantification were drawn from R2 at different stages of the digesting process and occasionally from R1. The samples from R2 were drawn during feeding from removed digestate, reactor content after mixing with feedstock and between feedings. Sampling from reactor content after mixing with feedstock and between feedings included drawing small portions of digestate from different places of R2 for a total of about 15 grams. The schematic of sampling in R2 is shown in Figure 3-2. The samples from R1 were drawn during feeding from removed digestate.

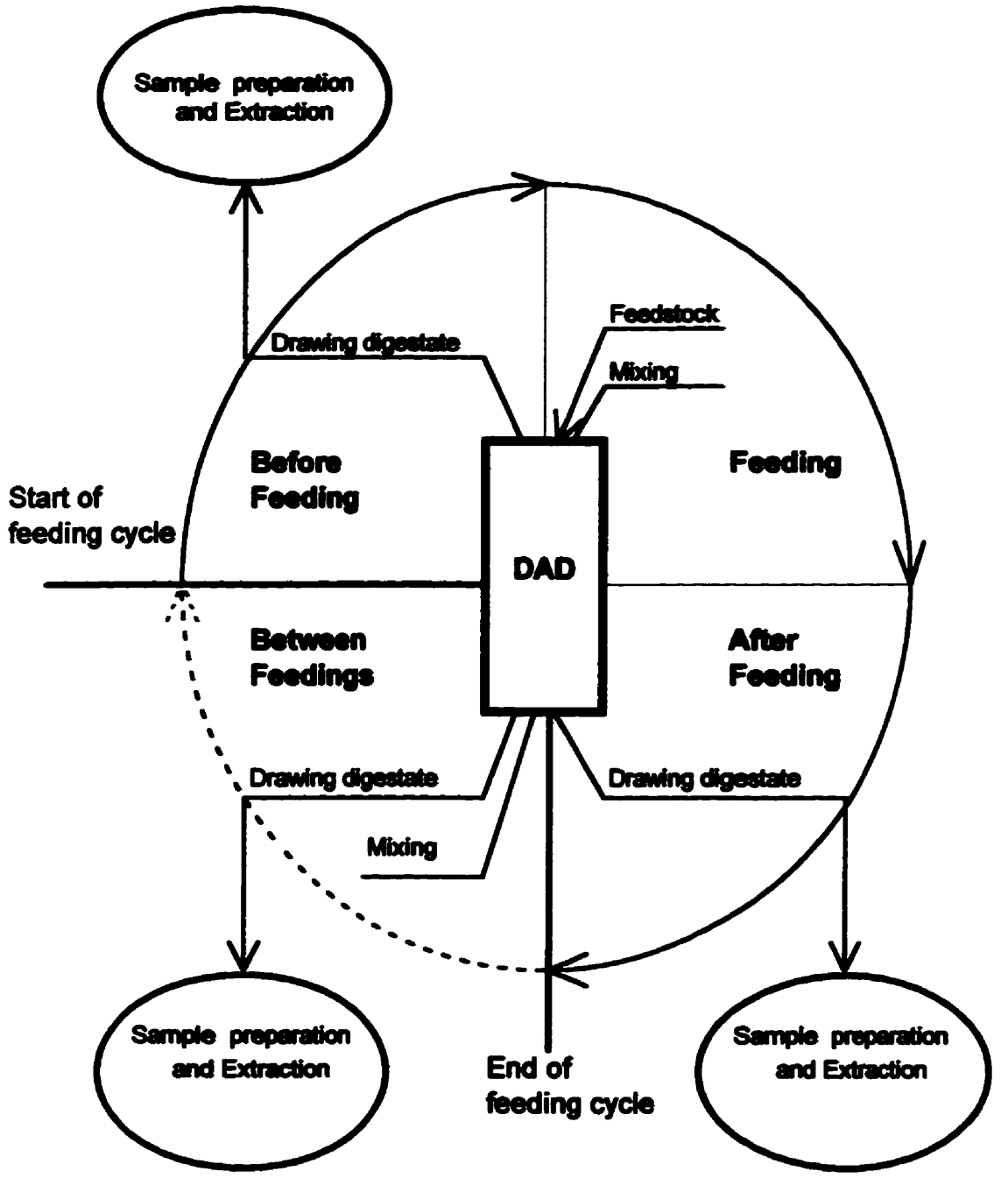

Figure 3-2. Phase I - Schematic of sampling.

Ten and two tubes of one gram were prepared from drawn digestate for R2 and R1 respectively. The samples were then extracted with deionized water. Five millilitres of deionized water was added to each tube and the slurry was shaken using S/P Vortex Mixer cat. S8223-1 for two minutes. Following the shaking, the slurry from each tube was transferred into two plastic vials of 1.5 mL which were centrifuged for 10 minutes at 10000 rpm using a high speed centrifuge. The clear supernatant from two vials, representing one sampling tube was transferred to an empty vial for phenol determination. The analysis was performed on the same day or the samples were frozen and analysed within seven days.

Before phenol extraction from R2 started, few extractions were performed on sterilized digestate samples in order to estimate the range of phenol recovery. Three solvents were used: methanol, deionized water, and a solution of methanol and water in a proportion 75:25 (v/v). Phenol recoveries using methanol and the solution of methanol and water were low and ranged from 10 to 40%. The HPLC peaks of these solvents were not well separated and in a flat shape. Deionized water gave the best results with recovery at 80% and well separated peaks. Deionized water was then chosen to be used during the phenol extraction study.

3.1.2 Analytical methods

The performance of the reactors was monitored by analysing gas production, gas composition, pH, TKN, ammonia, volatile fatty acids (VFA), and percentage of volatile solids removal. Alkalinity, pH, TKN, and VFA were also determined in the feedstock added to the reactors. All the analyses, were made in duplicates for each reactor, except gas samples, for

gas composition analysis, which were taken in triplicates.

Biogas production was recorded twice daily. The volume of gas produced was measured using a liquid displacement system which included two 20 litre tanks with saturated NaCl and 5% H_2SO_4 solution. The recorded gas volume was converted to volume at standard temperature and pressure.

Biogas composition was determined using a Gow Mac 550 gas chromatograph with a thermal conductivity detector and column Poropak Q. A helium carrier gas was used at a flow rate of 10 ml 10 sec^{-1}. The column, detector and injector temperatures were 55°C, 110°C, and 95°C, respectively. Initially prepared calibration standards covered the entire range of values. The standard deviation of the readings for the above calibrations setting was ±2.0%. Biogas composition analysis covered the determination of gasses methane and carbon dioxide.

Alkalinity, pH and VFA were determined according to procedures used by Yu (1992). Fifty millilitres of deionized water was added to 10 g of digestate. The mixture was vigorously stirred for 20 seconds and allowed to stand for 10 minutes in a cool chamber. The pH measurement was taken using a Fisher model 230 pH/ion meter.

Alkalinity was determined on a slurry used for pH measurement. The slurry was filtered using 1.8 mm meshfilter funnel. The filtrate was centrifuged for 20 minutes at 3000 rpm using a centrifuge. Twenty millilitres of centrate sample was then taken for alkalinity determination using 0.2 N H_2SO_4 (method 2320 B, A. P. H. A, 1992).

One and a half millilitres of filtrate were centrifuged for 10 minutes at 10000 rpm in high speed centrifuge for VFA determination. The clear supernatant was directly analysed in

GC or stored at 4°C after acidification with phosphoric acid for less than seven days before analysis. Volatile fatty acid analyses were performed using a Hewlett Packard 5890 GC with FID detection with hydrogen as a carrier gas. The used column was HP-FFAP (cross-linked), 10 m x 0.53 m x 1.0 μm film for volatile acids, with a glass guard column insert.
The temperature program in the chromatograph was oven initial temperature 85°C; oven initial time 1.5 min; oven initial rate-20 Deg/min; final temperature 123°C; final time 1.00 min; rate A 30 Deg/min; final temperature A-153. Six volatile fatty acids were analysed: acetic, propionic, isobutyric, n-butyric, isovaleric, n-valeric.

Clean, porcelain dishes ignited at 550° C for at least one hour in a muffle oven, cooled and stored in a desiccator until weighted, were used for total solids and volatile solids determination according to A. P. H. A (1992) methods 2540 B and 2540 F, respectively. Approximately 10 g sample was placed into a weighted porcelain dish and dried over night at 103 °C. The total solids content was determined from the weight loss upon drying. Following total solids determination the dish with the sample was placed in the muffle oven at 550°C. Volatile solid content was calculated from the weight loss upon ignition.

Ammonia-nitrogen was measured using distillation followed by a titration in Kjeltec Auto 1030 Analyser based on titration method for ammonia 4500-NH_3 E, A. P. H. A (1992). Hydrochloric acid 0. 01 M was used as a titrant. The method was based on the method used by Rich et al. (1994). One hundred millilitres of deionized water was added to a small amount of a sample (four to six g wet) in a distillation tube. The slurry was stirred using a glass rod for a few seconds. The samples were then analysed using the distillation apparatus. Ammonia in mg g^{-1} was calculated according to equation 3-4.

$$NH_3 mg g^{-1} = \frac{(A-B) \times 0.01 \times 14.1}{C} \qquad 3\text{-}4$$

Where: A is volume of HCL titrated for sample, (mL)

B is volume of HCL titrated for blank, (mL)

C is wet weight of sample, (g)

Kjeldhal nitrogen in the digestate and feedstock was determined using Total Kjeldhal Nitrogen Digestion Method using Tecator DS 20-1015 digester. A small amount of a sample (approximately 0.5 g wet) was placed into a digestion tube. The addition of fifty millilitres of deionized water and two kjeltabs was followed by transfer of fifty millilitres of concentrated sulfuric acid to the tube. The prepared tubes were placed into digester which has the digestion tube holder in place. The samples were digested with the autostep controller program set according to digester manual except the third step in step 2 of the program was set at 45. After completion of the digestion, cooling and addition of fifty millilitres of deionized water to each tube, the samples were distillated using Kjeltec Auto 1030 Analyser. Total Kjeldhal Nitrogen in mg g^{-1} was calculated using equation 3-4.

Phenol was analysed using High Performance Liquid Chromatography equipped with Waters 600E Waters pump, Reverse Phase column, Rheodyne injector with 20 μL loop, Waters 740 Data Module integrator, and UV detector at wavelength 254 nm. The flow of mobile phase was 2 mL min^{-1}. The mobile phase consisted of 0.1 N trisodium phosphate buffer solution and 1:1 acetonitrile solution with water that were mixed in a proportion: 5

percent acetonitrile and 95 percent trisodium phosphate buffer. The components of the mobile phase were degassed and filtered prior to use using the millipore vacuum filtration system with 0.45 μm membrane filters. The steps in the preparation of the mobile phase are outlined in Table 3-2.

Table 3-2. Phase I - Protocol to prepare the HPLC mobile phase

Description
I Prepare trisodium phosphate 1. Weight 25.4 g of powdered Na_3PO_4 $12H_2O$ into a plastic dish 2. Dissolve the weighted mass in 2 L deionized water in a flask 3. Mix the solution using a magnetic stirrer for few minutes
II Prepare acetonitrile solution 1:1 1. Measure known volume of acetonitrile liquid 2. Add exactly the same amount of deionized water and mix 3. Filter the solution using the millipore vacuum filtration system with 0.45 μm membrane filters 4. Stir rapidly when filtering
III Prepare mobile phase 1. Set the prepared solutions to be used by 600 E Waters pump 2. Set the desired proportion of mobile phase components for the pump

3.2 PHASE II - OBJECTIVES AND METHODS

The objective of Phase II was to develop a standard operating procedure for phenol extraction and analysis from compost feedstock. Alternatives of sample handling and HPLC settings, and different extractants were optimized and the least error procedure presented. The experiment in Phase II included two stages. Stage A was designed to optimize a method

of sample handling and phenol analysis and to compare various extractants. Stage B was designed to examine the effect of shaking time on phenol recovery using different extractants.

3.2.1 Experimental methods

The feedstock was prepared according to feedstock preparation procedure outlined in section 3.1.1 and consisted of paper, food waste and sludge. The feedstock was analysed for total and volatile solids, and pH using procedures outlined in section 3.1.2. The analyses were performed during preparation of sampling sets. The feedstock was characterized by 35% total solids, 70% volatile solids, and pH of 7.0 based on an average of eight analyses.

The extraction from feedstock was performed at room temperature and covered nine sampling sets. Two hundred fifty samples were prepared and analysed during the experiment. Autoclavable vials (85x20 mm) with screw bakelite caps were used to prepare one gram feedstock samples which were autoclaved preceding extraction. Table 3-3 summarizes the steps during sample preparation. The known amount of phenol from phenol stock solution 64 g L^{-1} was added to defrosted and autoclaved feedstock samples to give a phenol concentration of 32 mg g^{-1}. Following phenol addition the samples were left for 24 hours at 25°C before extraction. A vortex at a speed control of 6 manufactured by American Scientific catalogue number 8223 or a shaker manufactured by Thermolyne model type Maxi-Mix 6580 at 1600 rpm were used to agitate the samples during extraction. A centra-M-Centrifuge operating at 10000 rpm was used to separate the solid phase from the solvent following extraction.

Table 3-3. Phase II - Protocol to prepare samples for extraction

Step	Description
I	Defrost the feedstock in a cool chamber at 4°C one day before sample preparation
II	Mix the defrosted feedstock using a plastic rod to homogenize and to break up big particles
III	Prepare the desired number of samples by weighing a one gram of the feedstock into a bottle
IV	Autoclave

Stage A involved extraction and analysis of five sampling sets numbered A-1 to A-5. Each sampling set consisted of thirty samples. The samples were prepared according to steps outlined in Table 3-3. Twenty five samples were spiked with phenol and five samples were used as blanks. Five extractants were used in each sampling set: deionized water (DW), solution of 95% of 0.1 N sodium phosphate and 5% of 1:1 acetonitrile (BUFFER), hot deionized water (DWH), alkaline water by addition of 0.2 N NaOH to achieve pH=11.5 (AW, pH=11.5) and tap water (TW). Each extractant was used to extract phenol from five samples. The blanks were extracted using DW and DWH in sets A-1 to A-2 and A-3 to A-5, respectively

The extraction procedure was performed as follows: one gram of a sample was mixed with 5 ml of extractant and agitated in a vial using either the vortex mixer or shaker. The vortex mixer was used for sampling sets A-1 to A-2, whereas the shaker was used for sampling sets A-3 to A-5. The shaker was used to increase the number of samples extracted simultaneously. Samples of sampling sets A-1 to A-3 were agitated for 2 minutes whereas

samples of sets A-4 to A-5 were agitated for 5 minutes. Following shaking, the slurry was transferred to 2.5 mL plastic vials and centrifuged using a high speed microcentrifuge at 10000 rpm for four minutes. The supernatant was separated from the solids and frozen for 24 to 72 hours until the day of HPLC analysis.

Stage B involved extraction and analysis of four sampling sets numbered B-1 to B-4. Sample preparation, phenol addition, and the extraction procedure were performed according to the procedures in phase A except samples were extracted after shaking times of 1, 2, 10, and 30 minutes. Two samples were extracted at each time. The samples were agitated using a shaker at 1600 rpm.

Set B-1 consisted of 30 samples. Ten each were extracted using DW and AW, pH=11.5, while ten had no phenol addition and were used as blanks. The blanks were extracted using DWH.

Set B-2 consisted of 30 samples. Ten each were extracted using TW and BUFFER, while ten had no phenol addition and were used as blanks. The blanks were extracted using BUFFER.

Set A-3 consisted of 20 samples. Ten were extracted using DWH, while ten had no phenol addition and were used as blanks. The blanks were extracted using AW, pH=11.5

Set A-4 consisted of 20 samples. Ten samples were extracted using DWH, while ten had no phenol addition and were used as blanks. The blanks were extracted using DWH.

During extraction the temperature and pH of each extractant were recorded. The pH of each extractant was as follows: 5.5 for DW (based on six measurements); 11.5 for AW, pH=11.5; 5.4 for DWH (based on one measurement); 11.9 for BUFFER (based on six

measurements); 7 for TW (based on eight measurements). Occasionally, the pH of a slurry of sample and extractant was also measured. The steps taken during phenol addition to feedstock, sample handling and extraction in phases A and B are outlined in Table 3-4.

Table 3-4. Phase II - Steps in phenol addition to feedstock, sample handling and extraction

Step	Description	
	Stage A	Stage B
I	Add 5μL from 64 mg L^{-1} phenol stock solution to 25 samples	Add 5μL from 64 mg L^{-1} phenol stock solution to to assigned samples
II	Transfer the samples to an incubator and incubate for 24 hours at 25°C	Transfer the samples to an incubator and incubate for 24 hours at 25°C
III	Perform extraction after 24 hour incubation 1. take ten samples and add 5 mL of different extractant to five samples 2. shake the samples for desired length of time 3. transfer the slurry from each sample to two 2.5 mL plastics vials and centrifuge them using high speed microcentrifuge for four minutes 4. collect a clear supernatant from two vials representing one sample into a clean 2.5 mL plastics vial 5. freeze the vial until the day of HPLC analysis 6. take next 10 bottles and perform steps listed above	Perform extraction after 24 hour incubation 1. take ten samples and add 5 mL of extractant to each sample. 2. shake each two samples for a desired length of time 3. transfer the slurry from each sample to two 2.5 mL plastics vials and centrifuge them using high speed microcentrifuge for four minutes 4. collect a clear supernatant from two vials representing one bottle into a clean 2.5 mL plastics vial 5. freeze the vial until the day of HPLC analysis 6. take next 10 bottles and perform steps listed above

3.2.2 Analytical methods

Phenol was analysed using two HPLC assemblies:

1. Stage A and Stage B (sampling set B-1). HPLC equipped with Waters 600E Waters pump, Reverse Phase column, Rheodyne injector with 20 μL loop, Waters 740 data module integrator, and UV detector Lambda-Max Model 482 at wavelength 254 nm .

2. Stage B (sampling sets B-2 to B-4). HPLC assembly the same as this in Stage A except Shimadzu variable UV detector SPD-10A Module and Hewlett-Packard integrator were used.

The HPLCs conditions during the experiment are summarized in Table 3-5.

Table 3-5. Phase II - The high-performance liquid chromatography conditions used during the experiment

	Sets A-1 to A-5	Sets A-5 and B-1 to B-4
calibration	2 point	2 point
mode	isocratic	isocratic
mobile phase	95% of 0.1 N sodium phosphate and 5% acetonitrile solution (50% v/v in water)	88% of 0.1 N sodium phosphate and 12% acetonitrile solution (50% v/v in water)
flow (mL min^{-1})	2	1
wavelength (nm)	254	254
column (10μm, 150 x 4.1 mm)	reverse phase polymeric PRP -1	guard PRP -1 column installed in front of the reversed PRP -1 column

Various sample handling procedures during HPLC measurements were tested. Sample handling procedure for set A-1 included: defrosting at room temperature, and direct measurement by single HPLC analysis. Sample handling procedure of sampling sets A-2 to A-5 included defrosting at room temperature, manual shaking, centrifuging at 10000 rpm for one minute, and HPLC analysis. The samples of sampling set A-2 were analysed twice. First analysis (assigned A-2a) involved sample handling technique as in set A-1. The second analysis on the next day included a different handling technique using manual shaking and centrifuging (assigned A-2b). Starting with set A-4 at least two injections were made for each sample. Sample filtering was introduced in the handling procedure in set A-5. The sample handling procedure with filtering included: defrosting at 4°C, manual shaking, filtering of approximately one millitre of a sample using phenex, nylon 13 mm diameter, 0.45 μm pore filter into a plastic vial, manual shaking, and HPLC analysis. The filtrate was kept at 4°C between measurements.

CHAPTER 4

EXPERIMENTAL RESULTS AND DISCUSSION

The study was conducted to develop protocols for assessing the effect of phenol on the dry anaerobic digestion process. The study was designed in two phases. Phase I was designed to observe the effect of increasing phenol concentrations on the process and to assess phenol monitoring program. The response of DAD system to phenol was determined by measuring the process performance parameters such as: gas production and composition, volatile solids removal efficiency, pH, and volatile fatty acids. Phenol monitoring program included sampling from heterogenous compost material at different stages of the digestion process, and phenol quantification using extraction and HPLC. Phase II was designed to develop a standard operating procedure for phenol extraction and analysis from compost feedstock. The procedure was optimized for sample handling technique, HPLC setting, the best extractant, and extraction time.

4.1 PHASE I - EXPERIMENTAL RESULTS AND DISCUSSION

Phase I included the observation the effect of increasing phenol concentrations and assessment of phenol monitoring program during dry anaerobic digestion. Phenol monitoring program included sampling from heterogenous compost material, extraction and HPLC analysis. The results of Phase I are presented in Appendix A and Figures 4-1 to 4-4 and include general performance characteristics, performance parameters of the digestion process, and assessment of sampling method. General performance characteristics for both reactors are outlined in Table A-1 in Appendix A. Table A-1 contains data of biogas volume produced

during each feeding period, gas production rate per litre of working reactor volume, organic loading rate, and volatile solids (VS) removal efficiency. Figures 4-1 to 4-4 summarize performance parameters such as: pH, VS removal efficiency, volatile fatty acids, changes in methane production versus unionized ammonia concentrations in the reactors.

The experimental run from day one to 48 was mainly focused on the overall performance of reactors. It was important to have the reactors at more or less the same operating conditions before phenol addition. Although the systems were maintained at identical operating parameters such as mass retention time, temperature, feeding schedule, feedstock composition, their performances were different. In general, the performance of R1 was less stable what was shown by lower pH, higher tendency for volatile acids accumulations, lower biogas production and lower methane content in the biogas. The addition of sodium bicarbonate for pH adjustment was more frequent in R1 than R2. The average gas production rate was 1.5 and 1.9 litre of gas per litre of working reactor volume per day in R1 and R2, respectively. The average methane content in biogas was 32 and 36 percent in R1 and R2 respectively.

From day 48 to the end of the experimental run, R2 was fed with phenol and feedstock. The phenol additions took place on day 48, 51, 65, 72, 79, and 83.

The pH data for the reactors is shown in Figure 4-1. In general, the pH in the reactors had a tendency to increase which could partially be caused by more frequent addition of higher sodium bicarbonate dosages.

As can be seen from Figure 4-1 phenol addition to R2 did not cause a major change in pH values. The pH in R2 was above a normal range for anaerobic digestion and was higher than pH in the control reactor. The normal pH range for high solids anaerobic digestion by Kayhanian et al. (1991) ranges from 6.8 to 7.2. However, starting on day 72 until the end of the experiment pH in R2 was slightly lower than in R1.

PH values in the reactors were characterized by high fluctuations (Figure 4-1). There were high fluctuations in pH in R1 and R2 for the period before phenol addition and for the period starting on day 48 when phenol was added to R2. For example, pH in R2 ranged from 6.5 to 8.6 before phenol addition and from 7.5 to 8.7 after phenol addition (Figure 4-1). The range of pH values in R1 were from 6.5 to 8.15 from day 7 to day 44 and from 6.7 to 8.7 starting on day 48 until the end of the experiment. Because any pH changes in R2 after phenol addition were within the range of fluctuations before the addition period, no relationship could be found between phenol and pH in the reactor.

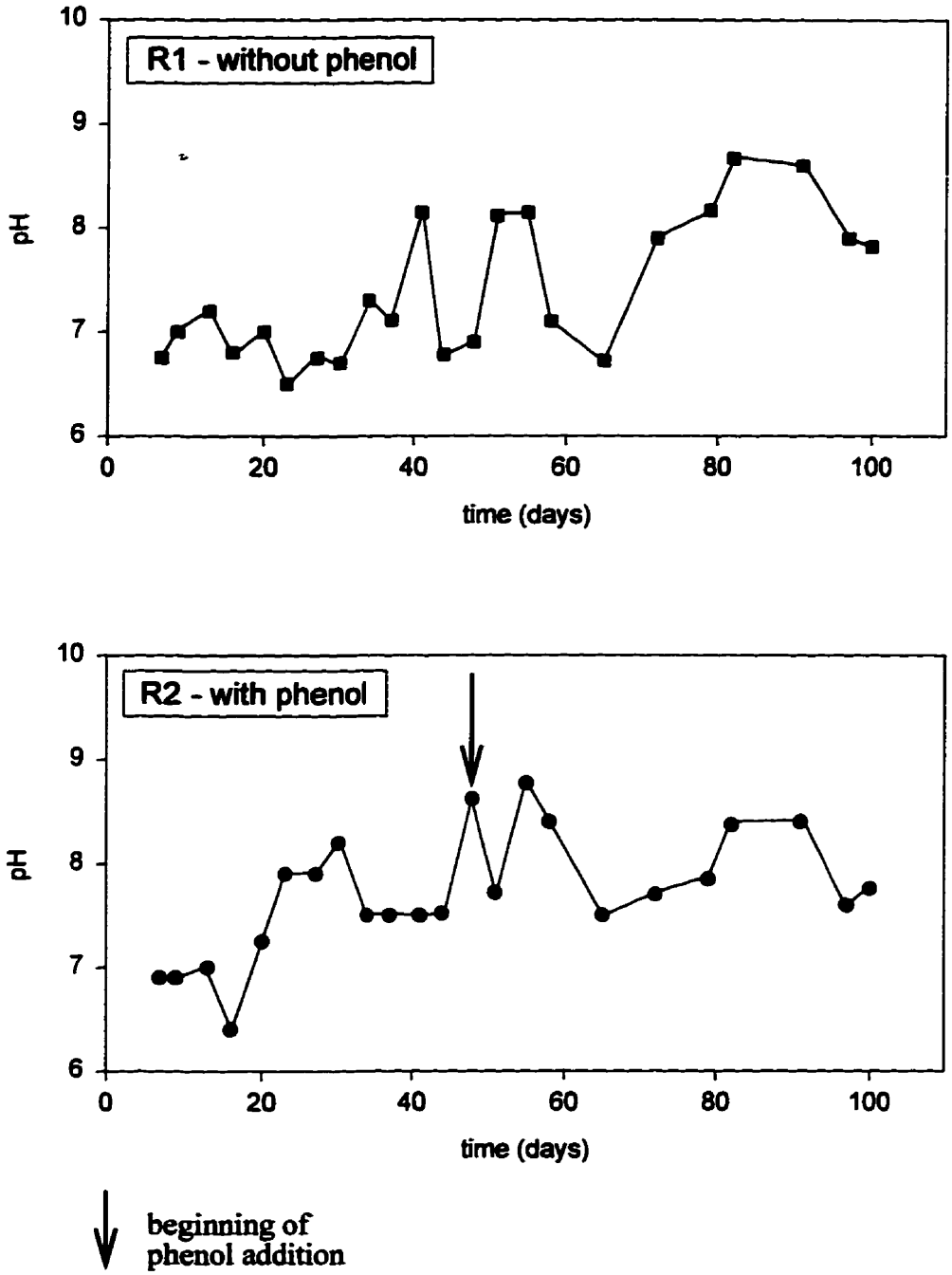

Figure 4-1. Phase I - pH in the reactors.

The volatile solids removal efficiency was calculated for each period between feedings using equation 4-1:

$$rem.efficiency(\%) = \frac{VSr_b Mr_b + \sum VS_f m_f - \sum VS_r m_r - VSr_a Mr_a}{\sum VS_f m_f} \qquad 4\text{-}1$$

Where: Mr_b is mass of reactor contents before draw and fill, (g),

Mr_a is mass of reactor contents after draw and fill, (g),

VSr_b is volatile solids of compost before draw and fill, (fractional),

VS_r is volatile solids of compost product drawn from reactor, (fractional),

VS_f is volatile solids of feedstock, (fractional),

VSr_a is volatile solids of compost after draw and fill, (fractional),

m_f is mass of feedstock going into the reactor, (g),

m_r is mass of compost drawn from the reactor, (g),

Figure 4-2 shows volatile solids removal efficiencies in reactors during the experimental run. The graph does not show removal efficiency values in both reactors between days 34 to 36 inclusive, and in R1 for periods between days 48 to 50 inclusive, 55 to 57 inclusive, and 79 to 82 inclusive since the calculated efficiencies were either too low or too high for the experimental conditions. This could be caused by errors made at weight measurements. Each time, the reactor was placed on the balance with tygon tubing resting on the floor. This caused variation in the balance reading and errors in volatile solids removal efficiency which was calculated based on weight.

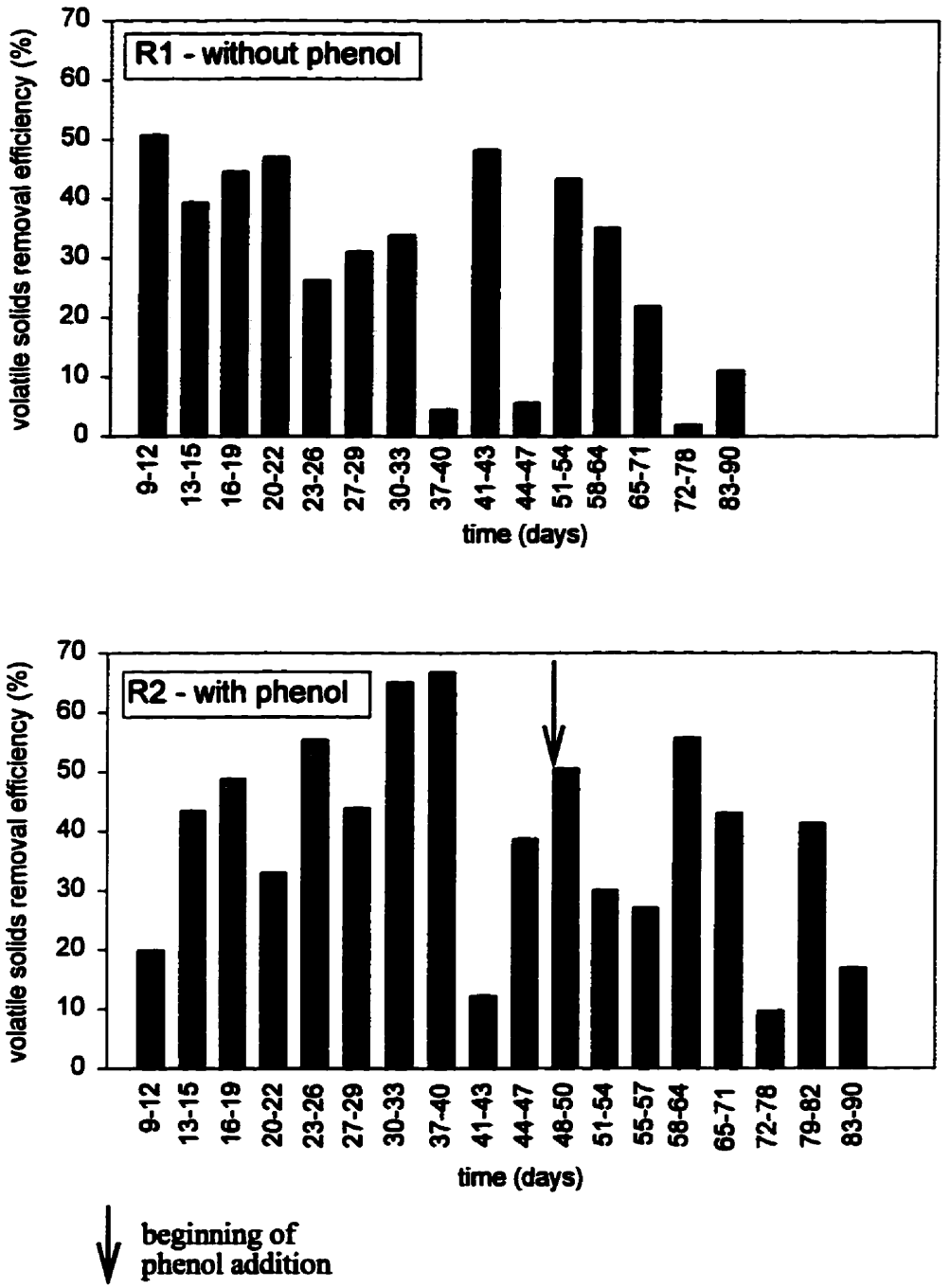

Figure 4-2. Phase I - Volatile solids removal efficiency in the reactors.

There were also high fluctuations in volatile solids (VS) removal efficiency in the reactors during the experimental run (Figure 4-2). The VS removal efficience in R1 ranged from 1.9 to 50.7%. The VS removal efficiencies in R2 fluctuated from 12.2 to 66.8% before and from 9.6 to 55.7% after phenol addition. In general, the average VS removal efficiencies in R1 were lower than those in R2 for the periods before and after phenol addition. It shows that R2 was performing better throughout the experiment. In view of high fluctuations in removal efficiencies in R2 before and after addition and the fact that R2 was performing better, no relationship could be found between phenol additions and VS removal efficiencies.

Volatile fatty acids (VFA) concentrations in the reactors throughout the experimental run are shown in Figure 4-3. In general, the performance of the reactors was characterized by accumulation of volatile fatty acids (Figure 4-3). It could result from the organic overloading of the systems especially after day 48. The normal range of organic loading rate (OLR) for DAD operated at MRT of 26 days is 14 gVS kg_r^{-1} according to Kayhanian et al. (1991). In the experiment, the reactors operated at MRT of 21 days had OLR at about 14 gVS kg_r^{-1} frequently and higher after day 48. It shows that the reactors with MRT shorter than 26 days were operated in the normal range of OLR typical for a system with MRT of 26 days. It means that the reactors in the experiment were operated in their high range of OLR. This resulted in organic overloading in the reactors. The organic overloading was caused by high protein food waste in feedstock, specially at the end of the experiment.

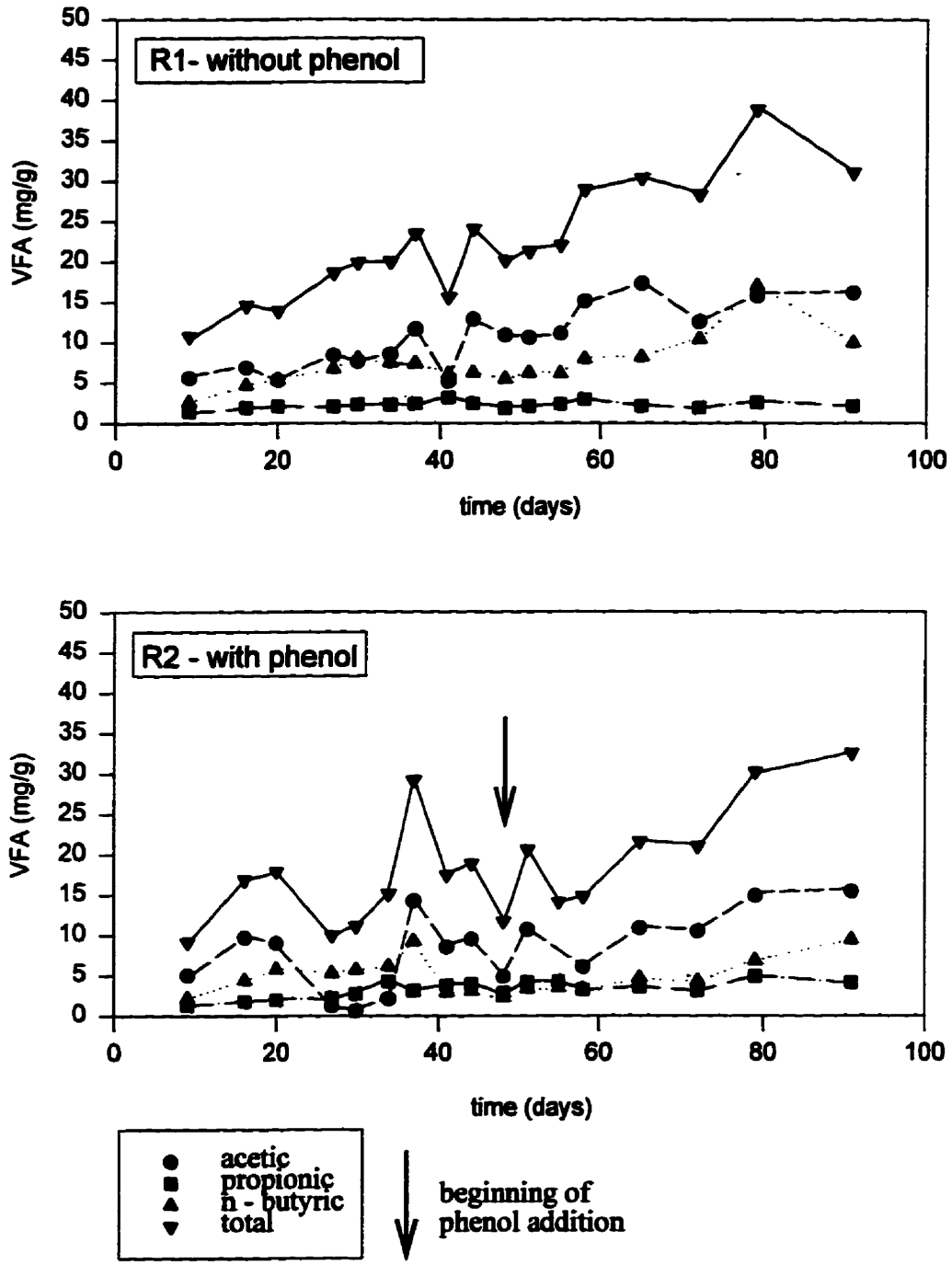

Figure 4-3. Phase I - Volatile fatty acids in the reactors.

Although the first phenol addition to R2 on day 48 was followed by an increase in VFA levels, the subsequent addition on day 51 was not accompanied with elevated acids concentrations (Figure 4-3). The reactor exhibited a stable performance between days 48 and 64 what was shown by relatively low acids levels as compared to the control.

Biogas production and composition data is shown in Appendix A. The volume of biogas produced was converted to gas volume at standard temperature and pressure using a correction factor for thermal expansion and water vapour content (Kayhanian et al. 1991). The correction factor for the biogas at temperature 55° C is 0.7024. Because of a few gas leakages in gas collection system specially at the end of the experiment, gas data is not so reliable as expected. The very low concentrations of methane and carbon dioxide on day 71 in R2 and on day 75 in R1 were due to a leakage in gas collection system since very high amounts of nitrogen were detected at the same time. In general, R2 was producing more methane than R1 for periods before and during phenol addition. Before phenol addition to R2, the average methane production was 2.1 and 3.1 L d^{-1} in R1 and R2, respectively. During the period of phenol addition, the average methane production was 1.5 and 3.0 L d^{-1} in R1 and R2, respectively.

The periods from day 72 in both reactors until the end of the experiment were characterized by poor performance. A decrease in methane production and a rapid accumulation of VFAs was observed (Table A-1, Appendix A and Figure 4-3). It seemed that both reactors were exposed to the same type of stress. A plausible explanation is the fact that the change in the feeding schedule on day 58 in the reactors might have a deleterious effect on both systems. Starting on day 58 to day 78 inclusive, the reactors were fed once a week

what means that every week 1266.7 g of feedstock was added and more than 1000 g of digestate was removed from each reactor. As the fresh feedstock was added, it could have caused a rapid grow of acid forming bacteria that resulted in high accumulations of volatile fatty acids. It is also possible that more oxygen from feedstock could have entered the systems as higher amount of feedstock was added to each reactor every week. Feedstock supplied to the reactors was not degassed using nitrogen to remove any oxygen within solid waste particles. Since methanogenic bacteria are obligate anaerobes, any additional amount of oxygen could have killed their metabolic activity.

Poor performance of the reactors at the end of the experiment could also be associated with high concentrations of ammonia. Both reactors exhibited similar patterns of ammonia concentrations during the experiment (Appendix A). The initial time of higher ammonia content between days 13 and 23 was followed by a period of lower concentrations between days 30 and 60 (Appendix A). The lowest ammonia concentrations were 110 mg L^{-1} in R1 on day 44 and 39.2 mg L^{-1} in R2 on day 34. Starting on day 65, the concentrations of total ammonia increased significantly in both reactors and reached values of 2.22 mg g^{-1} (3025 mg L^{-1}) and 2.37 mg g^{-1} (2470 mg L^{-1}) on day 80 in R1 and R2, respectively. According to Kayhanian et al. 1991, anaerobic digestion process is less stable at total ammonia concentrations higher than 1500 mg L^{-1}. The optimum concentration for stable performance is 750 mg L^{-1}. In the end of the experiment, the ammonia concentrations in the reactors were much higher than the optimum values so ammonia could be the factor that was inhibitory to DAD. It was also possible, that the elevated pH present in the reactors from day 72 could cause an increase in the concentration of free ammonia at the same time. Ammonium ions

(NH^{4+}) exist in equilibrium with ammonia (NH_3) according to equation 4-2.

$$NH_3 + H_2O \rightleftharpoons NH_4^+ + OH^- \qquad 4\text{-}2$$

At pH above seven, the equilibrium is shifted what favours the presence of free or unionized ammonia that is inhibitory to anaerobic microorganisms. According to Kayhanian et al. 1991 the concentration of 50 mg L^{-1} of free ammonia is inhibitory to anaerobic microorganisms. The amount of free ammonia was calculated using equation 4-3 according to Kayhanian et al. (1991).

$$NH_3 = \frac{TAN \times \frac{K_a}{H}}{\frac{K_a}{H} + 1} \qquad 4\text{-}3$$

Where: NH_3 is free ammonia concentration, (mg L^{-1}),

TAN is total ammonia concentration, (mg L^{-1}),

Ka is temperature dependant dissociation constant (3.77×10^{-9} at 55°C),

H is hydrogen ion concentration equal 10^{-pH},

Methane production versus various levels of unionized ammonia in the reactors is presented in Figure 4-4. Figure 4-4 shows that decreased methane production in R1 after day 71 and in R2 after day 72 were accompanied by an increase in concentrations of free ammonia. For example, free ammonia concentrations in R2 on days 66 and 80 were 21 and 525 mg L^{-1}, respectively. The concentrations of free ammonia in the reactors on day 80 were much higher than the inhibitory threshold concentration of 50 mg L^{-1}. It shows that the fermentation process could have been inhibited by unionized ammonia.

The high ammonia levels in the reactors could have been caused by changes in feedstock composition. Each time, feedstock was prepared for only three week period, so there were variations in its composition every three weeks. It was also noted that as the food waste was collected from cafeteria there was a significant amount of ham in the waste at the end of the experiment. It caused an increase in protein content in the feedstock going into the reactors. Ratio C/N for feedstock was also calculated. As the ratio decreased from 329 on day 27 to 105 on day 66 and 85 on day 80, it showed an increase in nitrogen in feedstock. Higher content of nitrogen in feedstock resulted in the increased levels of ammonia.

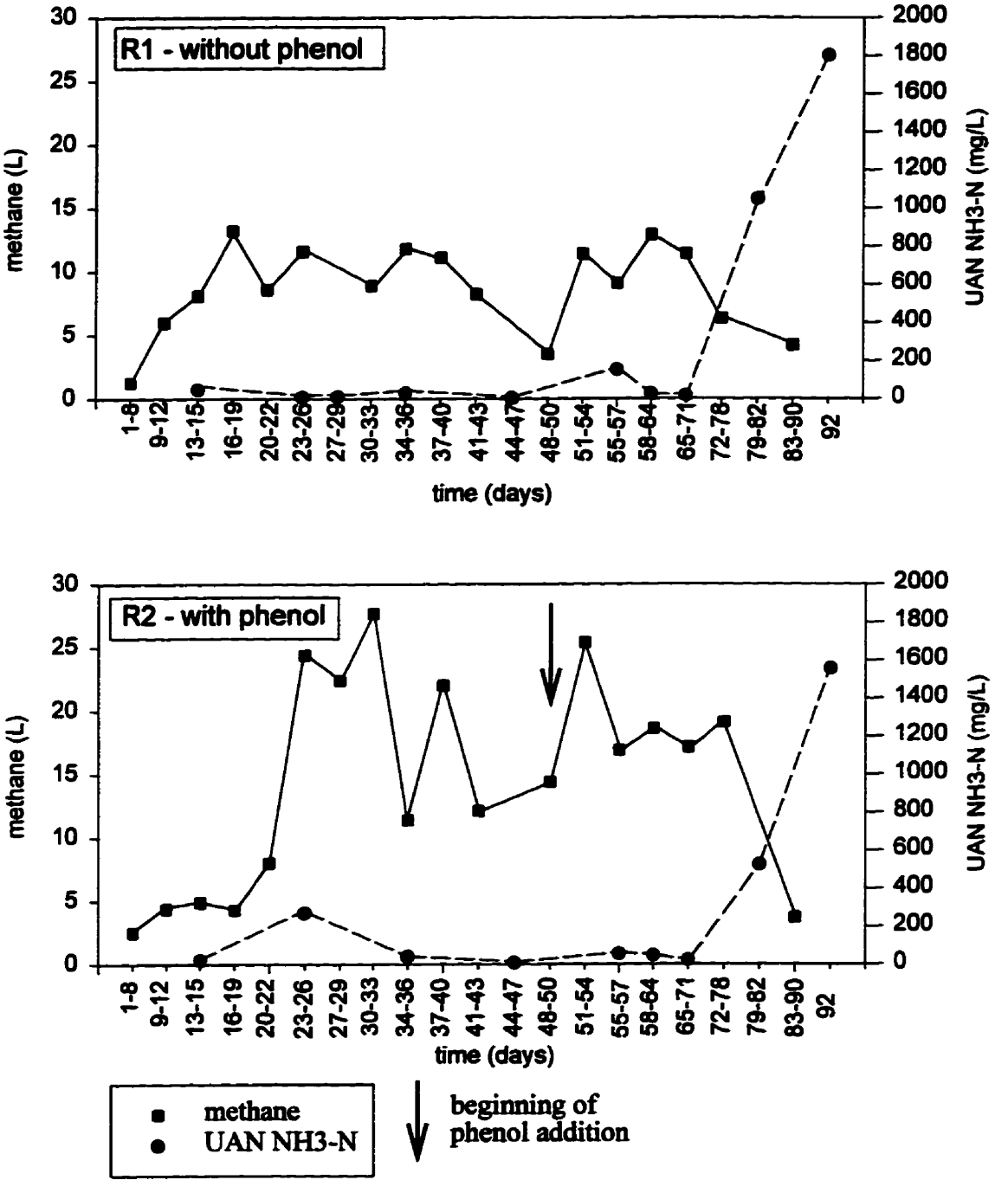

Figure 4-4. Phase I - Changes in methane production and unionized ammonia-nitrogen concentration in the reactors.

In general, the effect of phenol on the fermentation process could be discussed between days 48 to 72. The period starting on day 73 was critical for both reactors since the systems were exposed to some kind of stress which could not be easily determined. It is suggested that the change in feeding schedule and the increase in free ammonia concentrations could have most deleterious affect on the fermentation performance.

Phenol addition from 50 mg kg^{-1} (65 mg L^{-1}) to 168.75 mg kg^{-1} (224 mg L^{-1}) from day 48 to 72 did not have deleterious effect on methane production in R2. As those additions were made, the system in R2 was producing methane (Figure 4-4) and methane concentration in biogas was from 32.4 to 49.4% (Appendix A) that is the normal range for this fermentation process. The results showed also that microorganisms could be acclimated to phenol. Phenol extraction data in Appendix A shows that phenol was detected in the control reactor. The recorded phenol concentrations were as follows: on day 55 - 3 and 8 mg kg^{-1}, on day 58 - 1 and 6 mg kg^{-1}, on day 65 - 2 mg kg^{-1}. It shows that phenol could be present in sludge which was a component of feedstock. Also, the extraction performed on the feedstock during Phase II showed phenol presence frequently at concentration of about 12 mg kg^{-1}. It means that the microorganisms could be acclimated to phenol since the experiment started and any addition of the compound did not have a deleterious effect on their metabolism. Also, the presence of solid surfaces could alleviate toxic effect of phenol by changing the accessibility of phenol, phenol sorption on solid waste and cell immobilization. The decreased effect of phenol shock sorbed on solid surfaces against methanogenic bacteria has been reported by Dwyer et al. (1986), Khan et al. (1981). Since the microorganisms can be present in immobilized form as attached to solids in DAD system, they can provide better resistance to phenol loading.

The effect of phenol additions higher than 168.75 mg kg^{-1} was not determined since both reactors exhibited poor performance that was already discussed.

The results show that it is difficult to conduct a good experiment on phenol effect when phenol is originally present in feedstock and could cause microbial acclimation. In this case, it is hard to determine the minimum phenol concentration affecting the process. To conduct an experiment on phenol effect, it is recommended to quantify phenol in feedstock or sludge. Should there be phenol in sludge, a new source of biosolids without phenol should be found. However, this is not always simple as the liquid waste stream is polluted with organics.

During Phase I, phenol was quantified in R2 at different stages of the digesting process and occasionally in R1. Samples from R2 were drawn during feeding from removed digestate, reactor content after mixing with feedstock, between feedings and from R1 during feeding from removed digestate. The samples were then extracted and analysed using HPLC.

The results of extraction in R2 are as shown in Table 4-1. From day 48 to 65 the extraction was performed only on digestate drawn during feeding operations which were done on days 51, 55, 58, 65. From day 70 to 91 inclusive the extraction was performed on digestate drawn and also on the reactor content after mixing with feedstock during feeding and occasionally on reactor content between feedings. Phenol concentrations after mixing with feedstock were calculated for days 48 to 65 inclusive and determined from extraction for days 72 to 91 inclusive (Table 4-1). Phenol concentration was calculated using equation 4-4.

$$C_a = \frac{(M_b - m_r)\ C_b + C_d \times 3.8)}{M_a} \qquad 4\text{-}4$$

Where: C_a is phenol concentration after feeding, (mg kg^{-1})

M_b is mass of reactor content before feeding, (kg)

m_r is mass of digestate removed during feeding, (kg)

C_b is phenol concentration from extraction before feeding, (kg)

C_d is concentration of phenol added on that day, (mg kg^{-1})

3.8 is constant mass of digestate in reactor, (kg)

M_a is mass of reactor content after feeding, (kg)

In Table 4-1, the days with assigned two phenol concentrations represent days where feeding operation took place and different phenol concentrations were recorded before and after feeding. When no phenol was added to R2, the concentration after feeding was lower than the concentration before feeding on days 55, 58 and 91 (Table 4-1). On these days, "dilution" took place as after drawing digestate feedstock with almost zero phenol was added. When phenol was added to the reactor, the concentration after feeding was higher than concentration before feeding on days 51, 65, 72, 79, and 83 (Table 4-1).

Table 4-1. Phase I-The estimated phenol concentrations in R2.

Day	Phenol addition	Phenol concentration before feeding	Phenol concentration after feeding (after mixing with feedstock)		Phenol concentration between feedings
		from extraction	calculated	from extraction	from extraction
	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$	$mg\ kg^{-1}$
48^F	50.00	NA	50.00	NA	NA
51^F	75.00	26.00	96.00	NA	NA
55^F		80.00	69.00	NA	NA
58^F		49.00	33.00	NA	NA
65^F	112.00	30.00	132.00	NA	NA
70		NA	N/A	NA	124.00
71		NA	N/A	NA	129.00
72^F	168.75	69.00	214.00	202.00	NA
76		NA	NA	NA	167.00
79^F	253.10	152.00	399.00	357.00	NA
83^F	379.65	320.00	649.00	487.00	NA
91^F		478.00	338.00	311.00	NA

NOTE: F is feeding day.
NA is not analysed

To answer a question how reliable the number representing phenol concentrations in R2 are a comparison of phenol concentration after feeding with respect to the amount of phenol added on days 51, 65, 72, 79 and 83 was made (Table 4-1). In other words phenol concentration after feeding minus phenol concentration added should equal phenol concentration before feeding. The comparison involved only the values after feeding

obtained from extraction on days 72, 79, and 83, since the extraction method was most important in phenol determination. The difference between phenol concentration after feeding and before feeding accounts for 79, 81, and 44 percent of phenol added on days 72, 79, and 83, respectively. The very low value of 44 percent on day 83 probably resulted from poor mixing that took place at the end of the experiment. It caused nonuniform phenol distribution in solid waste that lowered the result of phenol determination. The other two values are satisfactory for the experimental conditions especially when there were sampling errors. Also they encountered phenol sorption that could take place on the fresh feedstock added. On the other hand, the values represent only two days of extraction so there is also not enough data to make a strong conclusion about the measured phenol concentrations in the reactor.

Since phenol was determined in a system of solid waste system and microorganisms, its recovery could have been affected by various processes such as: sorption and biodegradation. The analysis of recovered phenol concentration in R2 with respect to such processes will be made in the following section. There will be, however, some limitations in the analysis that could affect the recovered phenol concentrations. They include operational problems and sampling method that are discussed later in this section. Especially the sampling method could affect the recovered phenol concentrations, should be taken into account.

It was difficult to determine the exact amount of phenol that could be degraded. Calculations of the differences in the amount of gas produced from the whole system with the respect to the potential amount of gas produced from phenol degradation were meaningless because the theoretical volume of biogas produced from phenol degradation were too small as compared to the total volume of gas produced and was in the range of errors made at gas volume readings. For example, the total amount of biogas produced from 253 mg of phenol would be 0.36 L. It is much smaller than the average daily amount of biogas from the rest of the fermentation process that was about 12 L. The study did not include measurements of intermediate products of phenol degradation so the possible degradation pathway could not be determined.

Based on the results of phenol determination, there is a little evidence about phenol sorption. The decrease in phenol concentration after each feeding addition could result from phenol sorption on digesting solid waste. For instance the calculated phenol concentration after phenol addition on day 65 was 132 mg kg^{-1} (Table 4-1). Phenol concentrations from extraction on days 70, 71 and 72 were lower and equal 124, 129 and 69 mg kg^{-1}, respectively. Similarly, day 72 with phenol concentration of 202 mg kg^{-1} after phenol addition was followed with lower phenol concentrations of 167 and 152 mg kg^{-1} on days 76 and 79, respectively (Table 4-1).

The experimental program did not allow determination all transformations of phenol in the process. The complexity of phenol transformations during fermentation process was due to different processes taking place simultaneously such as: phenol sorption, solubility, and microbial degradation. Also the extend of each process was related to changing

conditions in the reactor such as: pH, moisture content, and microbial activity. It was also hard to depict the transformations of phenol because of the results of performance data were not reliable as expected and the possible intermediates products of phenol degradation were not determined. It is recommended for future studies that transformations of phenol be best described when phenol quantification be made with simultaneous accurate measurements of performance characteristics of the system. This would include gas production, gas composition, VFA, VS removal efficiency, and pH. The combined results would make it easier to draw reasonable conclusions about phenol transformations during the process. There would also be a need to measure concentration of phenol in the control reactor more frequently in order to determine any changes in phenol content more accurately. This would involve changes from variations of phenol content originally present in feedstock and changes of phenol from eventual production from solid waste. It would be crucial to determine phenol content in feedstock as precisely as possible.

The assessment of the phenol determination from digesting refuse was done based on the reliability criteria according to Sachs (1984) outlined in section 2-8.

The specificity of the method came from the fact that the method involved phenol determination from solid waste that was composed of paper, food waste and sludge. Because of the heterogeneity of the solid waste, mixing and sampling appeared to be very important. The waste has also high content of organic matter so the samples had to be cleaned up before analysis.

The accuracy of the method was discussed based on the definition given in US-EPA (1982). According to the report accuracy in solid waste determination is accomplished by

taking representative samples. Representative sampling is achieved by random sampling of the solid waste so that each unit of the waste has equal chances to be sampled. In the study, there was no random sampling and sampling operation did not produce representative samples. Samples were taken as a grab samples. Literature reports that grab sampling or non-probabilistic sampling is the most uncertain form of sampling since it can cause high systematic error or bias (Gy, 1994). Because of nonrandom sampling it is assumed that there was low accuracy during the experiment.

The precision of the method was discussed based on the definition given in US-EPA (1982). According to US-EPA (1982), sampling precision is defined by closeness of several samples (US-EPA, 1992). In the study, phenol was determined as an average value from ten samples. Precision or variability of the samples was determined using standard deviation or coefficient of variation. Standard deviation and coefficient of variation were calculated according to equations 2-12 and 4-5, respectively:

$$c = \frac{s}{\bar{x}} \qquad 4\text{-}5$$

Where: s is standard deviation

$\bar{x}$ is average value

c is coefficient of variation

The range of coefficients of variation was from 0.1 to 1.06 which is significantly higher than the guideline value of 0.05 given in literature (Sachs, 1984). Since the coefficient of variation serves as a general measure of dispersion of the sample, it was obvious that the range of coefficients of variation in the study showed a high dispersion of the results. It means that there was low precision or reproducibility in the experiment.

The extraction is a simple procedure. The solvents used are inexpensive and relatively nonhazardous. However, the procedure is time consuming, because of long sample preparations and analysis. The HPLC equipment is necessary to perform the final phenol analysis.

The results of extraction, that are presented in Appendix A, are characterized by high standard deviation and standard error of mean value. It shows high variability of the results and high overall experimental uncertainty. Based on the literature the overall experimental uncertainty includes analytical and sampling uncertainty (Kratochvil and Taylor, 1981). In the experiment the analytical uncertainty was represented by standard deviation on the HPLC column. The analytical standard deviation was determined by injections of a 100 mg L^{-1} phenol standard and was equal 2% of mean value. It means that the overall uncertainty was mostly caused by errors from sample variability, sampling, and extraction. These errors made up the overall sampling error in the experiment. Many workers have pointed out sampling error a major component of overall error in analysis of solid phase (Kratochvil and Taylor, 1981; Gy, 1994; Woodbury and Breslin, 1992). They reported that the sampling error can be reduced by reducing the heterogeneity of waste or sample variability and by performing random sampling (improving sampling operation).

In the study, there were two main factors that contributed to sampling error. First, there was no random sampling. Samples were taken as grab samples. According to the literature grab sample can be biased up to 50% (Gy, 1994). Second, there was a high heterogeneity of solid waste in reactor. The waste was characterized by constitution and distribution heterogeneity. Constitution heterogeneity originated from different phases as the waste was made of paper, food waste and biosolids. Distribution heterogeneity was caused by poor mixing due to failing equipment. The mixing ceased several times as the mixing part of the drill become stuck in digesting waste and frequently fell off. Literature reports that sampling error results from constitution and distribution heterogeneity (Gy, 1994).

Although Phase I was characterized by sampling error, a calculation was done to determine the required number of samples for the desired lower margin of error. In Phase I where there have been sampling errors, further attempts to improve reliability by increasing number of samples is of little importance. The following discussion, however, can serve as an example how the number of samples relates to the level of uncertainty.

During the experiment in Phase I ten grab samples from 3.8 kg digesting solid waste were used in phenol determination. The average value or mean from ten samples was used as an estimate of phenol concentration present at the time of sampling in the reactor. For each mean, a margin of error and 95% confidence interval (CI) were calculated according to equation 2-14 (Table 4-2). The data in Table 4-2 shows that by taking ten grab samples the margin of error can be as high as 76 percent that means a high level of uncertainty.

In order to reduce the level of uncertainty, the required number of samples at desired margin of error or lower level of uncertainty was calculated for each mean value. The

objective was: how many samples would be required for a desired margin of error of 10%. It was assumed that the values have normal distribution as the results of solid waste evaluation usually exhibit normal distribution regardless of sampling strategy (US-EPA, 1982). The required number of samples was calculated using equation 2-15. The results are outlined in Table 4-2.

Table 4-2. Phase I - Estimated required number of samples in assessing phenol concentration at a desired margin of error for mean.

Mean from ten samples	Standard deviation for single observation	Margin of error	95% C.I for mean	Estimated required number of samples @desired margin of error 10%
26	15	14	26±54%	199
80	85	61	80±76%	578
49	8	6	49±12%	14
30	4	3	30±10%	9
124	15	11	124±9%	8
129	16	11	129±8.5%	8
69	15	11	69±16%	24
202	100	72	202±36%	125
167	28	20	167±12%	14
152	24	17	152±11%	13
357	103	74	357±21%	43
320	31	22	320±7%	5
487	145	104	487±21%	45
478	225	161	478±34%	113
311	95	68	311±22%	48

NOTE: CI - confidence interval

The number of samples to obtain 10 percent uncertainty or margin of error ranges from five to 578. Equation 2-15 shows that the number of samples increases for higher standards deviation and lower level of uncertainty. When the standard deviation is high or the precision is low, a lot of samples is required to lower the level of uncertainty. A question can arrive whether it is economically feasible to have so many samples. In the experiment, the estimated concentrations with low standard deviations required fewer samples to achieve the desired level of uncertainty. Low standard deviations of results were observed on days 65, 70 and 71 when there was good mixing of the solid waste (Appendix A). It provided better distribution of phenol in the reactor. In this case low range of 95% confidence interval (CI) was observed. However, most of the estimated phenol concentrations were characterized by high standard deviation. The ranges of CI and number of required samples for lower uncertainty were higher. It is concluded that improper mixing caused by failing equipment resulted in poor distribution of phenol and high variability of results.

4.1.1 Phase I - conclusions

From the results of the study on phenol effect and determination in dry anaerobic digestion in a system of two semi-continuously operated reactors at MRT equal 21 days the following conclusions can be made:

1. There was a high variability of the experimental data in both reactors.
2. Reactor supplemented with phenol was performing better than the control reactor throughout the experiment. Phenol additions up to 168.75 mg kg^{-1} (224 mg L^{-1}) did not have a deleterious effect on methanogenesis in R2.

3. From day 72 to 92 both reactors failed, so phenol was not the variable that affected the performance of R2. Changing feeding schedule was the factor that most likely had deleterious affect. This resulted in high accumulations of VFA in the systems.

4. There was a high potential for ammonia inhibition in both reactors at the end of the experiment. High levels of ammonia were caused by the increased protein content in food waste. This was shown by the decrease in the ratio C/N in the feedstock. There was also a high potential for free ammonia inhibition in the systems.

5. There was a high experimental uncertainty caused by poor mixing and the sampling method. This could have an effect on the determined concentrations of phenol in solid waste.

4.2 PHASE II - EXPERIMENTAL RESULTS AND DISCUSSION

As there was a high dispersity of results in phenol monitoring program in Phase I, it was decided to conduct experimental Phase II which would be focused only on the analytical part of phenol determination. The objective of the study during Phase II was to develop a standard operating procedure for phenol extraction and analysis from compost feedstock. Various sample handling procedures, HPLC settings, and extractants were tested. The study was conducted in two stages. Stage A was designed to optimize a method of sample handling and phenol analysis and as a screening method to identify the best extractant. Stage B was designed to investigate the effect of shaking time on phenol recovery.

The selection of the phenol extractants was based upon several factors such as: extractants used successfully in previous phenol extraction studies (Evangelista et al. 1990; O'Neill et al. 1993, Rajput et al. 1994), hydrophilic characteristics of phenol, low toxicity, and ease of use (Rulksen and Assink, 1983). Five extractants were chosen: deionized water (DW), alkaline water at pH of 11.5 (AW, pH=11.5), hot deionized water at 50°C (DWH), tap water (TW), and a solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v (BUFFER). The percent recovery of phenol was calculated according to the following relationship:

$$\% \text{ recovery} = \left[\frac{(\text{spiked feedstock concentration} - \text{unspiked feedstock concentration})}{\text{spike level}}\right] \times 100 \qquad 4\text{-}6$$

Stage A

Stage A was designed to optimize a method of sample handling and phenol analysis and to compare the extractants. Various sample handling procedures and HPLC settings were investigated in order to improve the precision of results and to eliminate sensitivity and resolution losses on the column. Five extractants were investigated for optimum phenol recovery.

The samples of sampling set A-1 were analysed by direct, single injection into HPLC. The handling procedure of samples from sets A-2 to A-5 included manual shaking, centrifuging and HPLC analysis. Sample filtering was introduced in analysis of samples from set A-5. Due to appearance of an additional peak which was poorly separated at flow of 2 ml min^{-1}, it was necessary to change the mobile phase composition and the flow rate. The mobile phase with 88 percent of 0.1 N sodium phosphate and 12 percent acetonitrile in solution with deionized water 1:1 v/v at 1 ml min^{-1} significantly improved peak separation. The placement of the guard column was done to reduce the deposition of substances from the sample that decreased the sensitivity of the reversed phase column. The operation without the guard column required more frequent cleaning and maintenance.

The samples of sampling set A-2 were analysed twice (method section 3.2.2). First analysis (assigned A-2a) involved direct injection into the HPLC while the second analysis included a different handling technique using manual shaking and centrifuging (assigned A-2b). The results of the first analysis showed that consecutive injections from a sample gave different concentrations with the lowest value representing the top of the sample (Table 4-3). The numbers assigned top represent first injection whereas the numbers assigned

bottom represent the second injection of a sample. The results show that almost all phenol concentrations representing the top part of the sample are lower than those representing the bottom part. To compare statistically the results of these injections a paired t-test was used. The parameter t statistic of 2.087 indicated that the populations of bottom and top concentrations were different (P(t>2.087) = between 0.2 and 0.1) . The more precise hypothesis for these two populations was not proven since there was not enough experimental data for both concentrations.

Table 4-3. Stage A - Phenol concentrations from consecutive injections in analysis of set A-2a.

Phenol concentration ($mg\ L^{-1}$)		Extractant
Top	Bottom	
28	35	DW
21	24	DW
20	26	AW, pH=11.5
22	21	AW, pH=11.5

As we noticed in the first analysis of set A-2 that the sample handling including direct injection could lower the recoveries, the procedure was changed to manual shaking and centrifuging before injection into the HPLC. After withdrawing the top portion of the sample during the first analysis, the remaining portion had higher phenol content and was analysed for the second time. The second analysis resulted in much higher recoveries as compared to sets A-1 and A-3 (Table 4-4). The results also showed that the recoveries in sampling set

A-1 were the lowest (Table 4-4). It could be caused by the sample handling technique that included a single measurements of the top portion of the sample with lower phenol content. The change in sample handling procedure to manual shaking and centrifuging was made to improve the precision of results. Table 4-5 summarizes the precision of the results of two sampling techniques: direct consecutive injections in set A-2a and manual shaking and centrifuging in set A-3. The relative standard deviations obtained from the results of the first handling technique are much higher than those of the second handling technique. It shows that the technique with manual shaking and centrifuging has higher precision.

Table 4-4. Stage A- Phenol recovery and relative standard deviation in extraction using different extractants- recovery % (%RSD)

Sampling set	Extractant				
	DW	AW pH=11.5	TW	BUFFER	DWH
A-1	61[b](27)	61[c](4)	59[a](8)	56[a](4)	67[a](6)
A-2b	70[d](40)	72[a](5)	67[a](35)	61[a](5)	77[a](3)
A-3[a]	56(5)	61(5)	61(9)	61(7)	69(6)
A-4[f]	55(6)	56(9)	55(6)	53(8)	58(4)
A-5[f]	53(10)	47(15)	52(23)	58(10)	58(10)

NOTE: *a* - each sample analysed by a single measurement
b - two samples out of five analysed by double measurement
c - one sample out of five analysed by double measurement
d - one sample out of five analysed by triple measurement
f - each sample analysed by two and more measurements
samples of sampling sets A-1 to A-3 agitated at shaking time 2 minutes
samples of sampling sets A-4 to A-5 agitated at shaking time 5 minutes

Table 4-5. Stage A - Precision comparison of two sample handling techniques in set A-2a and A-3.

Sampling set A-2a		Sampling set A-3	
DW	AW, pH=11.5	DW	AW, pH=11.5
28	20	37	39
35	26	40	43
21	22	43	44
24	21	40	44
$\bar{x}$ = 27	$\bar{x}$ = 22	$\bar{x}$ = 40	$\bar{x}$ = 43
s = 5.91	s = 2.63	s = 2.45	s = 1.89
%RSD = 22	%RSD = 12	%RSD = 6.1	%RSD = 4.4

NOTE: $\bar{x}$ is average
s is standard deviation
%RSD is relative standard deviation

Sample filtering was introduced for better solids cleanup in the samples in order to eliminate sensitivity and resolution losses on the column. From the beginning of the experiment, there was a need for frequent column washing and maintenance. Finally, it was decided to use a PRP-1 guard column and change the flow to 1 ml min^{-1} using a mobile phase composition of 88% of 0.1 N sodium phosphate and 12% acetonitrile in a solution with deionized water 1:1 v/v. This significantly improved the HPLC performance. The guard column helped prevent particulates and highly adsorptive compounds from reaching the analytical column. After all the final changes were made, sensitivity and resolution losses were greatly minimized.

Examples of chromatographs are shown in Figure 4-5. Chromatographs A, B, and C show phenol peaks for phenol standard 10 mg L^{-1}, a sample spiked with phenol, and unspiked sample, respectively. The retention time for phenol was at 2.5 minutes.

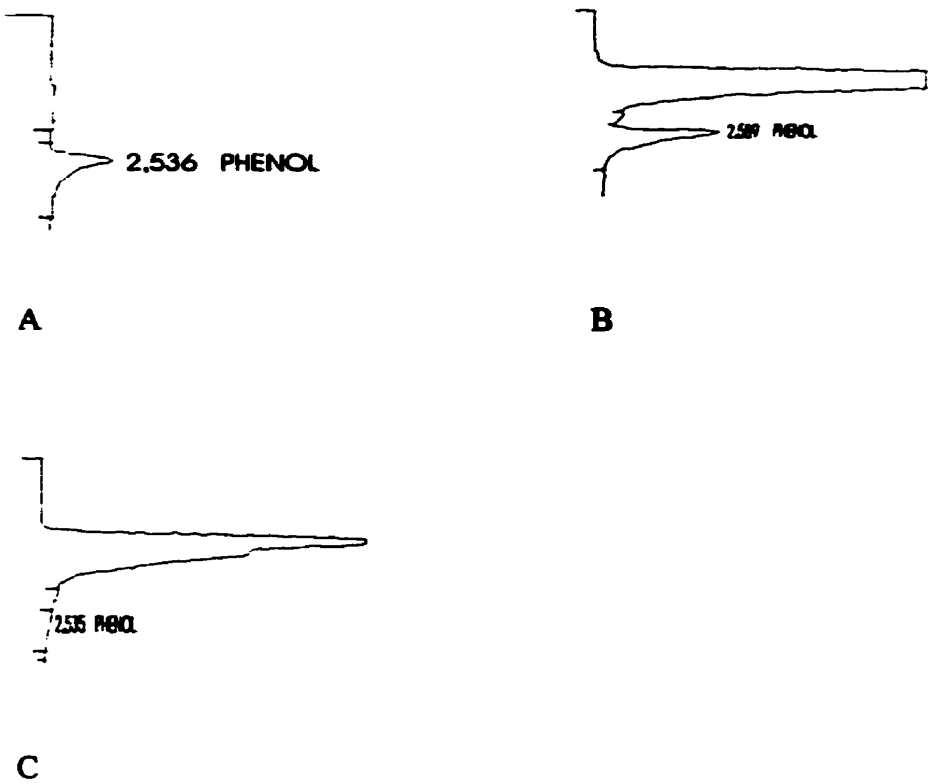

Figure 4-5. Phase II - HPLC chromatographs in phenol extraction.
HPLC conditions: flow of 1 mL min^{-1}, mobile phase composition of 88% of 0.1 N sodium phosphate and 12% acetonitrile in a solution with deionized water 1:1 v/v, and a Hamilton PRP-1 column and a PRP-1 guard column.
A - phenol standard 10 mg L^{-1}, phenol retention time - 2.536 min,
B - spiked sample extracted with BUFFER, phenol retention time - 2.589 min,
C - unspiked sample extracted with BUFFER, phenol retention time - 2.535 min.

The optimal sample handling procedure and HPLC conditions in the experiment were:

- defrosting samples in a refrigerator at 4°C one day before HPLC analysis
- manual shaking and filtering about 1mL of a sample into plastic vial
- manual shaking of the filtrate followed by injection to HPLC
- storage of the filtrate in the refrigerator at 4°C between injections
- analysing the samples with mobile phase of 88% of 0.1 N sodium phosphate and 12% acetonitrile in a solution with deionized water 1:1 v/v at 1 mL min^{-1} using a reverse PRP-1 column and guard column.

The method detection limit was 1 mg L^{-1}. It was very important to check any loss of column sensitivity by injecting a phenol standard every four to five samples.

Stage A was also designed as an extraction screening method to compare different extractants. Table 4-4 summarizes phenol recoveries for different extractants. Since the sampling handling procedure was changed during the experiment, it was hard to make a meaningful comparison of the extractants. However, some conclusions can be drawn in selection of the best extractant.

Based on the recoveries, hot, deionized water was the best extractant with phenol recoveries from 67 to 77% after shaking for 2 minutes (Table 4-4). Alkaline water at pH = 11.5 was the second best extractant with recoveries from 61 to 72%. The highest phenol recoveries using hot water at 50°C were due to the increased phenol solubility. Phenol solubility increases with temperature up to it maximum solubility at 63.5 °C (Environment Canada, 1985).

The decreased recoveries at the longer extraction times in sets A-4 and A-5 (Table 4-4) were not ease to explain. In addition all the recoveries were comparable. The decreased performance of the DWH extractant was probably due to heat loss during shaking. The shaking was done at room temperature, so the phenol equilibrium favoured the solid phase as the temperature of the samples decreased. Unfortunately, the sample temperatures were not measured during shaking so this hypothesis was not confirmed. Similarly, the poorer performance of the pH=11.5 extractant could have been due to temporal changes in pH, but this too was not confirmed. In set A-4 two solvents: hot deionized water (DWH) and AW at pH = 11.5 appeared to be the best extractants. In set A-5 BUFFER and DWH were the best.

Stage B

Stage B was designed to examine the effect of shaking time on phenol recovery. The effect of shaking time on phenol recoveries is shown in Figures 4-6, 4-7 and Table 4-6. Figure 4-6 shows the effect of shaking time on phenol recovery using TW and DW. Figure 4-7 shows the effect of shaking time on phenol recovery using BUFFER; AW, pH=11.5, and DWH. As shown in Figures 4-6 and 4-7 the extraction efficiency for almost all extractants increased with time. After shaking of 5 and 10 minutes, alkaline water at pH=11.5 and BUFFER were the first and second best extractants, respectively (Table 4-6). BUFFER and alkaline water at pH=11.5 gave the highest phenol recoveries of 84 and 83 percent, respectively after shaking of 30 minutes (Table 4-6).

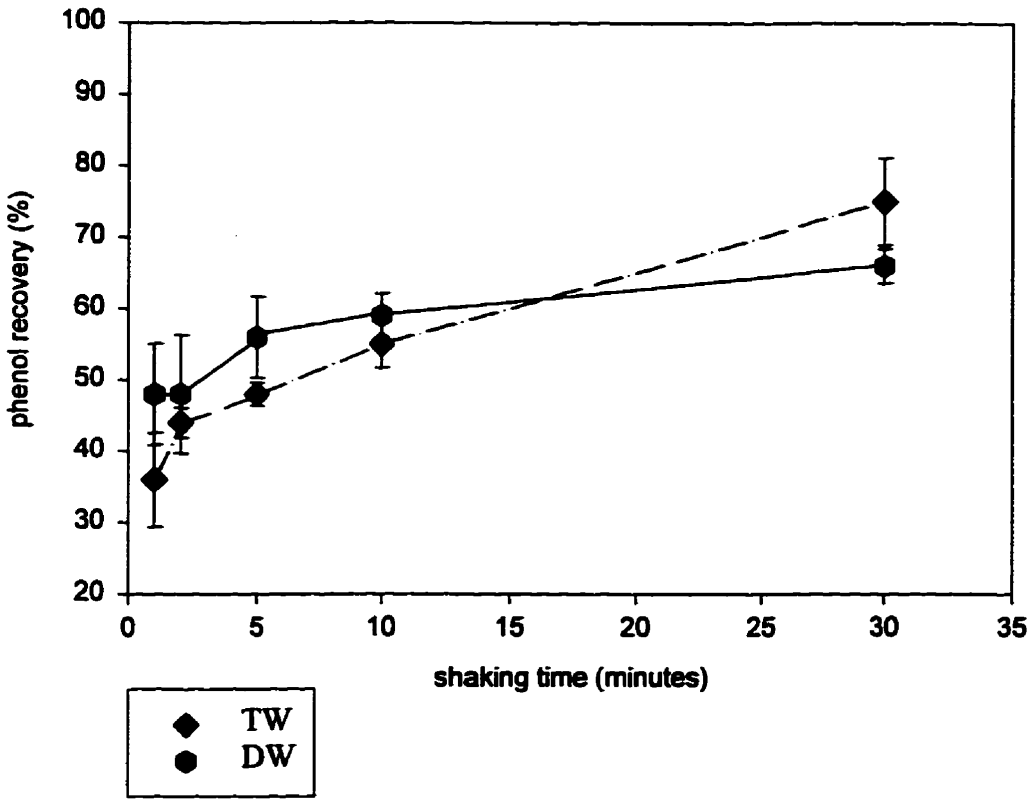

Figure 4-6. Phase II - Effect of shaking time on phenol recovery using TW and DW. Each point represents average from two samples analysed twice (four results). Error bars represent standard deviation for a single observation.

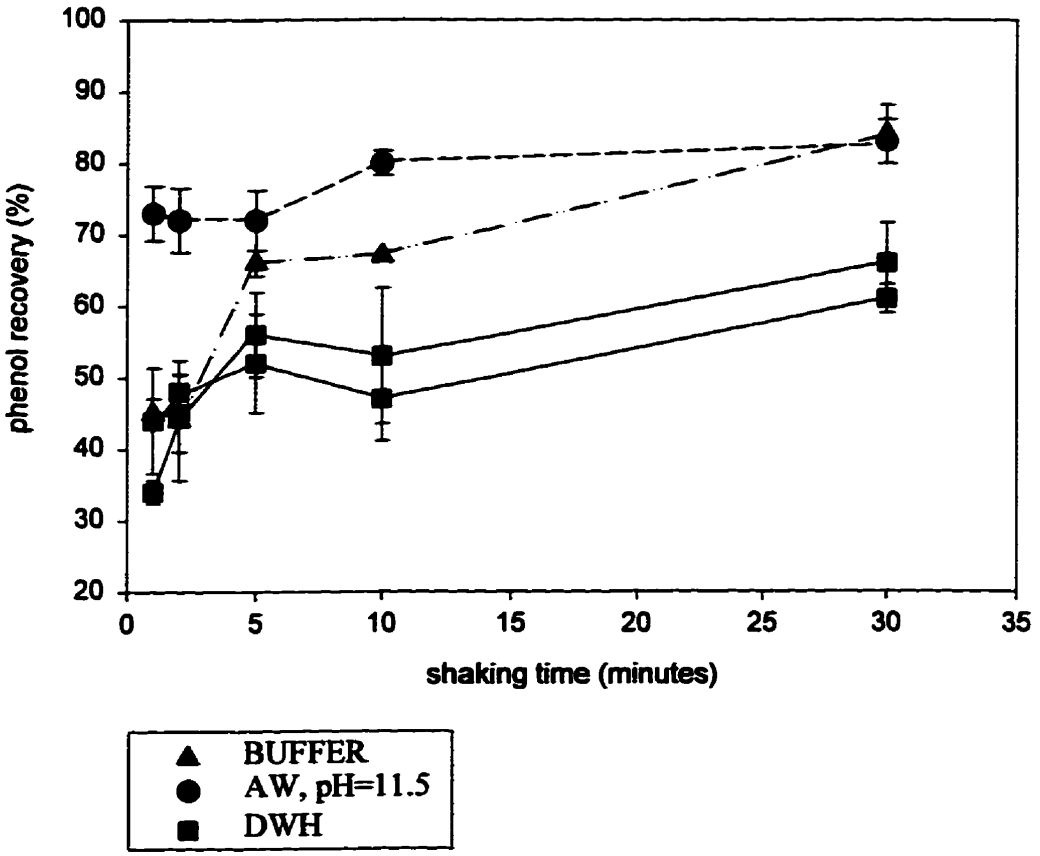

Figure 4-7. Phase II - Effect of shaking time on phenol recovery using BUFFER; AW, pH=11.5; and DWH. Each point represents average from two samples analysed twice (four results). Error bars represent standard deviation for a single observation.

After shaking time of 5 and 30 minutes, the recoveries using hot deionized water were comparable to those using deionized water whereas after shaking time of 10 minutes they were lower than those using deionized water (Table 4-6). The decreased performance of the DWH was probably due to heat loss during shaking.

Based on our results, we recommend alkaline water at pH=11.5 (AW, pH=11.5) and the solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v (BUFFER) to be used as the best extractants for phenol at an extraction time of 30 minutes. The recoveries at longer shaking times were not determined. However, based on Figures 4-6 and 4-7, it can be concluded that the recoveries would be higher after longer shaking times for example 40 minutes.

Similar results were reported by Evangelista et al. (1990) who performed soil washing tests using various solvents such as: tap water, water at pH of 9.3, 10.5, and, 11.5, and hot water at 50°C. He found that alkaline water at pH of 11.5 and heated water gave the highest recoveries, although he did not report whether the water was constantly heated during extraction.

Since the extractant used in phenol extraction had different pH's, the effect of pH on phenol recovery was also investigated. The results are presented in Figure 4-8 that shows phenol recovery after shaking time of 30 minutes versus pH. Various pH's of 5.4; 5.5; 7; 11.5; 11.9 correspond to hot, deionized water (DWH), deionized water (DW); tap water (TW); alkaline water at pH=11.5; and 95% of 0.1 N sodium phosphate and 5% acetonitrile (BUFFER) (Figure 4-8). Figure 4-8 shows that phenol extraction efficiency increases with pH. Corresponding relationship between pH and extraction efficiency was

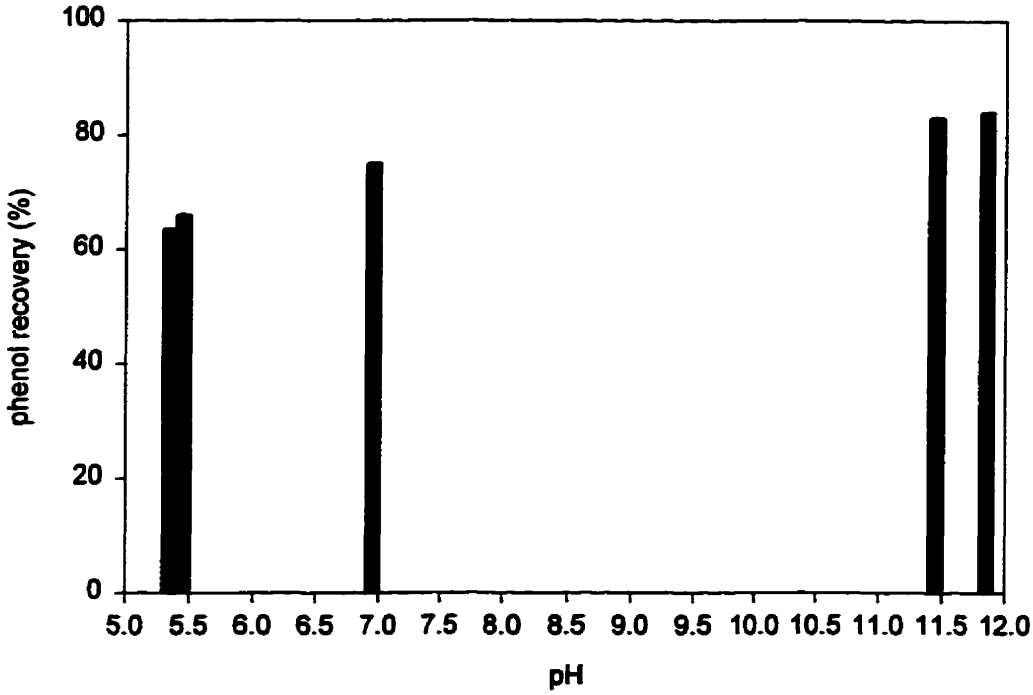

Figure 4-8. Phase II - Phenol recovery versus pH.

found in the study by Evangelista et al. (1990). In their experiment, there was also an increase in phenol removal from soil as the pH of various extractants increased. The consecutive increased phenol removal efficiency were recorded for the following extractants: plain water, water at pH=9.3, water at pH=10.5, and water at pH=11.5.

Table 4-6. Stage B - Phenol recovery and relative standard deviation in extraction versus shaking time - recovery % (%RSD)

Set	Extractant	Shaking time (min)				
		1 min	2 min	5 min	10 min	30 min
B-1	AW, pH=11.5	73(5)	72(6)	72(6)	80(2)	83(4)
B-2	BUFFER	45(4)	44(15)	66(2)	67(1)	84(4)
B-1	DW	48(14)	48(16)	56(10)	59(5)	66(4)
B-2	TW	36(14)	44(4)	48(3)	55(5)	75(7)
B-3	DWH	34(3)	45(9)	56(8)	53(14)	66(7)
B-4	DWH	44(13)	48(4)	52(11)	47(10)	61(3)

NOTE: % recovery at a given shake time determined from two samples measured twice
% RSD determined from two averages representing two samples

Precision and variability of extraction.

The results of precision of extraction in Stages A and B are shown in Tables 4-4 and 4-6, respectively. The Tables show recovery (%) and relative standard deviation (%RSD) for feedstock spiked with phenol at 0.32 mg wet g^{-1}.

Standard deviations were calculated from results of five and two samples in Table

4-4 and 4-6, respectively. The presented relative standard deviations vary from 1 to 40 percent. Fifty eight percent of the values are higher than the target relative standard deviation of 5% (Sachs, 1984). The results show an improvement in precision throughout the experiment. Overall, the precision in Stage A was lower than the precision in Stage B. During Stage A, the experimental conditions were changed frequently thus causing variations in standard deviations. This included changing sample handling procedures and analysing various number of replicates between sampling sets and samples among extractants (private communication, Dennis Murphy, Statistical Advisory Service, UM). The relative standard deviation in set A-2 (Stage A) was as high as 40%. The relative standard deviations in phase B were lower with maximum of 16%.

It is suggested that the high standard deviations were mainly caused by nonhomogeneous nature of the feedstock rather than by the laboratory analytical precision (Tables 4-4 and 4-6). The heterogeneity of the feedstock samples was due to presence of different types of solids such as paper, food waste, biosolids, variations in ratios between the phases, and differences in moisture content. As a result, phenol spiked on the feedstock was distributed between these phases and underwent different partitioning or sorption mechanisms on each phase. Thus, it resulted in different recoveries of spiked phenol for different replicates. The heterogeneity of the samples could cause the variations in phenol recovery for a given shake time and for a single extractant. The similar, high variations due to the non-homogenous nature of samples were also reported by Davis et. al. (1993) who extracted various organic pollutants from sediments samples by analysing duplicate samples. The reported relative standard deviations in recovery of various compounds from

homogenized sediments samples were also high and ranged from 13.8 to 129%. The relative standard deviation for phenol extraction was 18.5%.

A two-way ANOVA with interactions with two sources of variability was used to assess the analytical and the sampling variability in the total variability of the extraction method. The sampling variability (variance component of sampling variability) included the variability between two replicates shaken at the same shaking time. It included the variability due to sampling, sample preparation, and extraction since the replicates were sampled independently. The analytical variability (variance component due to analytical variability) included the variability between two measurements from replicate sample after a given shaking time. Only the results of Stage B were used for ANOVA analysis because the overall laboratory conditions were the same through the experiment. It included shaking time, sample handling, HPLC settings, number of replicates for each shaking time. Since Stage A included many changes in sample handling techniques, number of replicates analysed, HPLC settings as the method was optimized, the results were not used in ANOVA analysis. Table 4-7 shows the results of ANOVA analysis for sets B-1 to B-4.

Table 4-7. Stage B - Results of ANOVA analysis - analytical (between two measurements from replicate sample) and sampling (from sampling, sample preparation, and extraction) variability as a percentage of total experimental variability.

Sampling set	Variance component due to analytical variability	Variance component due to sampling variability
B-1	82.9	17.1
B-2	44.7	55.3
B-3	4.7	95.3
B-4	7.8	92.2

The results of ANOVA analysis show that the analytical variability accounted for a great part of the total experimental variability in sets B-1 and B-2 (Table 4-7). The analytical variability significantly decreased in sets B-3 and B-4 with variance components of 4.7 and 7.8%, respectively. It is difficult to explain that high analytical variability in sets B-1 and B-2 since the literature reports that the sampling variability is the major source of overall variability (Kratochvil and Taylor, 1981). The results for sets B-3 and B-4 show much lower analytical variability (Table 4-7). In these sets the sampling variability was the major source of overall variability. The high variability could be caused by heterogeneity of the samples and also by nonrandom sampling of the feedstock to prepare the samples (private communication, Dennis Murphy, Statistical Advisory Service, UM). Our results show that analytical variability decreased throughout the experiment. The literature reports that analytical variability should account for a one third or less of sampling variability (Kratochvil and Taylor, 1981). At the end of our experiment in sets B-3 and B-4 the analytical variability was much less than one third of sampling variability.

Comparison with other methods and techniques.

Since the literature lacks methods of phenol extraction from feedstock or solid phase resembling the organic fraction of MSW, the direct comparison cannot be made with respect to recoveries. The precision in the method presented here, however, can be discussed in relation to other extraction methods. The precisions obtained in our experiment are comparable with those in other methods of extraction of various organics from environmental samples (Davis et al. 1993, Hawthorne and Miller, 1994, Lopez-Avila et al.1983). A study on Soxhlet and supercritical CO_2 extraction of different organics from various environmental solids such as: soils and soot reports the relative standard deviations that are similar or somewhat higher than the relative standard deviations obtained in our study (Hawthorne and Miller, 1994). The relative standard deviations for chlorinated phenols ranged from 12 to 41% and from 11 to 152% for Soxhlet and supercritical CO_2 extraction, respectively. The results from another study of extraction of various organic compounds from homogenized standard reference sediment show similar range of precision (Lopez-Avila et al.1983). For example, the reported relative standard deviation for phenol extraction was 21%.

4.2.1 Phase II - conclusions

The objective of this study was to develop a standard operating procedure for phenol extraction from the organic fraction of MSW used as a composting feedstock. The method was optimized for sample handling technique, HPLC setting, the best extractant, and extraction time. Based on the results the following conclusions can be made:

1. Different techniques experimented throughout the study resulted in the improvement of the method precision, and HPLC measurement. ANOVA analysis also showed a decrease in analytical variability that demonstrates an improvement in the analytical part of the method.

2. Taking into account the complexity of the feedstock matrix, the precision of the results was comparable with the precision found by others researches in extraction of organics from environmental solids.

3. Solvent washing technique originally applied to contaminated soil can be used for phenol extraction in a much more complex organic matrix of organic fraction of MSW. However, sample cleaning through placing a guard column before HPLC appeared to be the most critical step in the whole procedure. The optimal HPLC settings were: mobile phase of 88% of 0.1 N sodium phosphate and 12% acetonitrile in a solution with deionized water 1:1 v/v with a flow rate of 1mL min^{-1}, using a Hamilton PRP-1 column and guard column.

4. Alkaline water and BUFFER were the best extractants for phenol.

5. Since the samples contain various organic compounds originating from the feedstock, they can affect the sensitivity of the column. Therefore, it is very important to

check the column sensitivity by analysing phenol standard every four to five samples.

6. Based on our experience the presented procedure can be used as a rapid screening method for quantifying phenol in feedstock. The extraction procedure is simple. The time of 40 minutes (extraction and filtering) required to prepare a sample for HPLC analysis is relatively short. The solvents used in the extraction are inexpensive and nonhazardous. It is suggested, however, that more work to be done to validate this method. This would include recoveries from interlaboratory comparison and practical applications of the method.

CHAPTER 5

RESEARCH OVERVIEW

The following section will summarize the objectives, observations, and engineering significance of the study. The purpose of the study was the development of protocols for assessing the effect of phenol on the dry anaerobic digestion process. The study was conducted in two phases. Phase I involved the examination of phenol effect on the process of dry anaerobic digestion and the assessment of phenol monitoring program during the digestion process. Phase II involved the development of a standard operating procedure for phenol extraction and analysis from compost feedstock.

The primary significance of the study was that it was probably the first since the recent literature lacks any experimental data. No literature reports have been found either on the effect of phenol on DAD or phenol extraction from the organic fraction of MSW. When phenol is present in the environment and can enter the process, there is a need for research on its effect, and its quantification during the digestion process and in feedstock. The quantification of phenol can ultimately be used to determine its transformations during the process.

The secondary significance of the study was that some findings can be adapted also to other composting technologies as well. These would include sampling from bulk compost, phenol extraction technique, sample handling during HPLC analysis, and statistical analysis of the results.

5.1 PHASE I - SUMMARY

The objective of Phase I was to examine the effect of increasing phenol concentrations on the process of dry anaerobic digestion and to assess phenol monitoring program in the reactor supplemented with phenol. Two DAD reactors of co-mingled paper, food industry waste, and sewage sludge at 21 MRT were operated. Phenol was added to one reactor in concentrations from 50 mg kg^{-1} to 379.65 mg kg^{-1} over five week period. The second reactor was operated as a control. The effect of phenol on the system was examined by measuring performance parameters such as: biogas production and composition, pH, volatile solids removal efficiency, and volatile fatty acids. In general, the reactor receiving phenol was performing better than the control throughout the experiment. The results showed that phenol addition from 50 mg kg^{-1} to 168.75 mg kg^{-1} did not have a deleterious effect on the fermentation process as a stable performance of the system was observed. At the same time the methane content in biogas ranged at 45 percent. The effect of phenol higher concentrations was not determined since both reactors showed unbalanced performance that was characterized by high levels of volatile fatty acids, ammonia and decrease in methane production. At the end of Phase I both reactors failed, so phenol was not the variable that affected the performance of the process. Changing in feeding schedule and feedstock composition that caused high accumulation of VFA and ammonia in the system were the factors that most likely had deleterious effect.

Phenol monitoring program included phenol quantification at different stages of the fermentation process. Phenol quantification was performed for preliminary determination what processes could have affected the retention of phenol in DAD. Phenol was

determined by drawing ten samples and extracting them with deionized water using a vortex mixer. There was a high experimental uncertainty caused by sampling method and poor mixing. This could have an effect on the determined concentrations of phenol in solid waste. It was difficult to determine the exact amount of phenol that could be degraded, since the potential volume of gas produced from phenol degradation was too small as compared to the total amount of gas produced from the rest of the fermentation system.

5.2 PHASE II - SUMMARY

The objective of Phase II was to develop a standard operating procedure of phenol extraction and analysis from compost feedstock. The procedure was optimized for sample handling, HPLC setting, the best extractant, and extraction time. The results showed that sample cleaning through filtering and placing in HPLC a guard column were the most important steps in the procedure. The optimal HPLC settings were: mobile phase of 88% of 0.1 N sodium phosphate and 12% acetonitrile in a solution with deionized water 1:1 v/v with a flow rate of 1 mL min^{-1}. Alkaline water at pH of 11.5 and a solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v were the best extractants for phenol.

5.3 ENGINEERING SIGNIFICANCE AND RECOMMENDATIONS.

The study was significant since it was the first experiment on phenol effect on the dry anaerobic digestion process and on phenol extraction from organic fraction of municipal solid waste. This preliminary work will be of great value to future studies.

Phase I contains principal information which can be used in other DAD studies such as: detailed data of reactors settings and operation. It emphasizes the difficulty of conducting a good experiment on adding phenol to the DAD process, because phenol can be present in the original sludge used for feedstock preparation. In this case, it is hard to establish the minimum phenol concentration affecting the process. The results of the experiment showed that phenol addition from 50 mg kg^{-1} through three additions over three weeks until 168.75 mg kg^{-1} did not have a detectable effect on DAD with a MRT of 21 days. It is suggested that phenol detected in the control reactor and feedstock caused microbial acclimation.

The study during Phase I was also very significant because it covered important issues such as: sampling from heterogenous compost material at different stages of the digestion process, phenol quantification using extraction technique, and HPLC measurement. The presented procedure of phenol quantification during DAD was probably the first since no reports had been found in the recent literature. The procedures for sampling, extraction, and HPLC measurement could be adapted for future studies including aerobic composting.

Statistical analysis and a good sampling plan is often not considered in a reactor experiment, although they are very important to obtain reliable results. So the statistical

analysis regarding sampling presented in the study are of great significance to any future research. The results of extraction in the study showed high variability that evidenced errors in sampling and mixing. The recommendation for future studies is that good mixing equipment be used and a sampling plan be developed.

The experiment during Phase II was very important since it was the first study on method development from organic fraction of municipal solid waste. The presented procedure for phenol analysis in the organic fraction of MSW would be beneficial for future DAD studies as a valid screening method of phenol presence in the feedstock. The procedure can also be adapted for aerobic composting. Phase II covered specific issues such as: extraction procedure, sample handling, and HPLC measurements. It is recommended, however, that more work be done to validate this method. This would include peer interlaboratory studies of extraction and practical applications of the method.

REFERENCES

Alexander, M. Biodegradation and bioremediation. 1994. Academic Press, USA.

American Public Health Association. 1992. Standard methods for the examination of water and wastewater, 18th eddition, A. P. H. A. Washington, DC, USA.

Aronstein, B. N., and Alexander, M. 1992. Surfactants at low concentrations stimulate biodegradation of sorbed hydrocarbons in samples of aquifer sands and soil slurries. Environmental Toxicology and Chemistry 11: 1227-1233.

Artiola-Fortuny, J., and Fuller, W. H. 1982. Phenols in municipal solid waste leachates and their attenuation by clay soils. Soil Science, 133(4): 218 - 226.

Bak, F., and Widdel, F. 1986. Anaerobic degradation of phenol and phenol derivatives by *Desulfobacterium phenolicum* sp. Archives of Microbiology 146: 177-180.

Baker, K. H., Herson, D. S. 1994. Bioremediation. McGraw-Hill, Inc., USA.

Balba, M. T., and Evans W.C. 1980. The anaerobic dissimilation of benzoate by *Pseudomonas aeroginosa* coupled with *Desulfovibrio vulgaris*, with sulphate as a terminal electron acceptor. Biochemical Society Translations 8: 624-625.

Bandyopadhyay, S., Sanjoy, K., Bhattacharya, K., Farell, J. B. 1995. Fate of semivolatile organic compounds during heat drying of mixed primary and waste-activated sludge. Water Environment Research, 67: 57-64.

Barlaz, M.A., Schaefer, D. M., and Ham, R. K. 1989. Effects of prechilling and sequential washing on numeration of microorganisms from refuse. Applied and Environmental Microbiology, 55(1): 50 - 54.

Béchard, G., Bisaillon, J.-G., Beaudet, R., and Sylvestre, M. 1990. Degradation of phenol by a bacterial consortium under methanogenic conditions. Canadian Journal of Microbiology, 36: 573-578.

Bisaillon, J.-G., Lépine, F., and Beaudet, R. 1991. Study on the methanogenic degradation of phenol via carboxylation to benzoate. Canadian Journal of Microbiology, 37: 573-576.

Bisaillon, J.-G., Lépine, F., Beaudet, R., and Sylvestre, M. 1993. Potential for carboxylation-dehydroxylation of phenolic compounds by a methanogenic consortium. Canadian Journal of Microbiology, 39: 642-648.

Bishop, D.J., Knezovich, J.P., and Harrison, F.L. 1990. Behavior of phenol and aniline on

selected sorbents and energy-related solid wastes. Water, Air, and Soil Pollution, 49: 93 - 106.

Boyd, S.A. 1982. Adsorption of substituted phenols by soil. Soil Science, 134(5): 337-343.

Boyd, S. A and Shelton, D. R. 1984. Anaerobic biodegradation of chlorophenols in fresh and acclimated sludge. Applied and Environmental Microbiology, 47: 272-277.

Boyd, S.A., Shelton, D.R., Berry, D., and Tiedje, J.M. 1983. Anaerobic biodegradation of phenolic compounds in digested sludge. Applied and Environmental Microbiology, 46(1): 50-54.

Britz, T. J., Van Der Merwe, M., and Riedel, K-H. J. 1992. Influence of phenol additions on the efficiency of an anaerobic hybrid digester treating landfill leachate. Biotechnology Letters, 14(4): 323-328.

Callewaert, D. M., Genyea, J. 1980. Basic chemistry. General, organic, biological. Worth Publishers, Inc., New York, NY.

Cecchi, F., Traverso, P. G., Perin, G., Vallini, G. 1988. Comparison of co-digested performance of two differently collected organic fractions of municipal solid waste with sewage sludges. Environmental Technology Letters, 9: 391 - 400.

Craik, S. A., Fedorak, P. M., Hrudey, S. E., and Gray, M. R. 1992. Kinetics of methanogenic degradation of phenol by activated-carbon-supported and granular biomass. Biotechnology and Bioengineering, 40: 777-786.

Davis, W. M., Coates, J. A., Garcia, K. L., Signorella, L. L., and Delfino, J. J. 1993. Efficient screening method for determining base/neutral and acidic semi-volatile organic priority pollutants in sediments. Journal of Chromatography, 643: 341-350.

Dehority, B. A., and Grubb, J. A. 1980. Effect of short-term chilling of rumen contents on viable bacterial numbers. Applied and Environmental Microbiology, 39(2): 376-381.

Dwyer, D.F., Krumme, M.L., Boyd, S.A., and Tiedje, J.M. 1986. Kinetics of phenol biodegradation by an immobilized methanogenic consortium. Applied and Environmental Microbiology, 52(2): 345-351.

Ejlertsson, J., Johansson, E., Karlsson, A., Meyerson, U., and Svensson, B. H. 1996. Anaerobic degradation of xenobiotic by microorganisms from municipal solid waste under landfilling conditions. Antonie van Leeuwenhoek International Journal of General and Molecular Microbiology, 69: 67-74.

Environment Canada.1985. Environmental and technical information for problems spills.

Environmental Protection service. Ottawa, Ontario.

Environmental Protection Agency. 1979. Methods for chemical analysis of water and wastes. Project summary, EPA-600/4-82-057, USA.

Environmental Protection Agency. 1980. Ambient water quality criteria for phenol. Office of Water Regulations and Standards Criteria and Standards Division, Washington, D.C.

Environmental Protection Agency. 1982. Test methods for evaluating solid waste. Physical/Chemical Methods, SW-846, USA.

Evangelista, R.A., Allen, H. L., Mandel, R. M. 1990. Treatment of phenol and cresol contaminated soil. Journal of Hazardous Materials, 25: 343 - 360.

Evans, W. C. 1977. Biochemistry of the bacterial catabolism of aromatic compounds in anaerobic environments. Nature, 270: 17 - 22.

Fedorak, P.M., and Hrudey, S. E. 1984. The effects of phenol and some alkyl derivatives on batch anaerobic methanogenesis. Water Research, 18(3): 361-367.

Fedorak, P.M., and Hrudey, S.E. 1987. Inhibition of anaerobic degradation of phenolics and methanogenesis by coal coking wastewater. Water Science and Technology, 19: 219 - 228.

Fedorak, P.M., and Hrudey, S. E. 1989. Proceeding of the fifth general meeting of BIOMINET. Energy, Mines, and Resources Canada: CANMET report (Canada Centre for Mineral and Energy Technology), Canada.

Fricke, C., and Clarkson, C., Lomnitz, E., and O'Farell, T. 1985. Comparison priority pollutants in municipal sludges. BioCycle, 26(1): 35-37.

Grbić-Galić, D., and Young, L.Y. 1985. Methane fermentation of ferulate and benzoate: anaerobic degradation pathways. Applied and Environmental Microbiology, 50, 292-297.

Grbić-Galić, D., and Vogel, T. M. 1987. Transformation of toluene and benzene by mixed methanogenic cultures. Applied and Environmental Microbiology, 53: 254-260.

Grob, R. L. 1993a. Environmental analyses utilizing gas and liquid chromatography. Journal of Liquid Chromatography, 16(8): 1783-1802.

Grob, R. L. 1983b. Chromatographic analysis of the environment. Marcel Dekker, Inc., N. J.

Gy, P. M. 1994. Sampling or gambling. Process Control and Quality, 6: 97-102.

Hall, D. W., Sandrin, J. A., and McBride, R. E. 1990. An overview of solvent extraction treatment technologies. Environmental Progress, 9: 98-105.

Hanaki, K., Hirunmasuwan, S., and Matsuo,T. 1994. Protection of methanogenic bacteria from low pH and toxic materials by immobilization using polyvinyl alcohol. Water Research, 28(4): 877-885.

Hawthorne, S. B., and Miller, D. 1994. Direct comparison of Soxhlet and low- and high-temperature supercritical CO_2 extraction efficiencies of organics from environmental solids. Analytical Chemistry, 66: 4005-4012.

Healy, J.B., and Young, L.Y. 1978. Catechol and phenol degradation by methanogenic population of bacteria. Applied and Environmental Microbiology, 35(1): 216-218.

Healy, J.B., and Young, L.Y. 1979. Anaerobic biodegradation of eleven aromatic compounds to methane. Applied and Environmental Microbiology, 38(1): 84-89.

Healy, J. B., Young, L. Y., and Reinhard, M. 1980. Biodegradation of the aromatic compound ferulic acid to methane. Applied and Environmental Microbiology, 39: 436-444.

Isaacson, P. J., and Frink, C. R. 1984. Nonreversible sorption of phenolic compounds by sediment fractions: the role of sediment organic matter. Environmental Science and Technology, 18(1): 43-48.

Jianmin, W., Gu Guowei. , and Chonghua, Z. 1993. Anaerobic biodegradation of phenol: bacterial acclimation and system performance. Water Science and Technology, 28(7): 17-22.

Johnson, D., Young, J.C. 1983. Inhibition of anaerobic digestion by organic priority pollutants. Journal of Water Pollution Control Federation (WPCF), 55(12): 1441- 1449.

Kasali, G.B. 1986. Optimisation and control of methanogenesis in refuse fractions. Ph.D Thesis, University of Strathelyde, Glasgow, UK.

Karabit, M. S., Juneskans, O. T., and Lundgren, P. 1985. Studies on evaluation of preservative efficacy. The determination of antimicrobial characteristics of phenol. Acta Pharmaceutica Suecica, 22: 281 - 290.

Kasali, G.B., and Senior, E. 1989. Effects of temperature and moisture on the anaerobic digestion of refuse. Journal of Chemical Technology and Biotechnology, 44: 31 - 41.

Kayhanian, M., Lindenauer, K., Hardy, S., Tchobanoglous, G. 1991. The recovery of energy and production of compost from the biodegradable organic fraction of MSW using the high-solids anaerobic digestion/aerobic biodrying process. A report prepared for the

Prison Industry Authority, State of California. Department of Civil and Environmental Engineering, University of California, Davis, California, USA.

Khan K. A., Suidan, M. T., Cross, W.H. 1981. Anaerobic activated carbon filter for the treatment of phenol-bearing wastewater. Journal of Water Pollution Control Federation (WPCF), 53(10): 1519-1532.

Knoll, G., and Winter, J. 1987. Anaerobic degradation of phenol in sewage sludge. Applied Microbioogy andl Biotechnology, 25: 384-391.

Knox, J., and Newton, J. R. 1976. Study of landfill disposal of acid tars and phenol-bearing lime sludges.I. Characterization of water-soluble constituents of wastes and preliminary study of interactions of phenolics solutions with domestic refuse. Report of an investigation for the Department of the Environment. Water Research Centre, Stevenge Laboratory, Stevenge, Hertfordshire.

Kobayashi, T., Hashinaga, T., Mikami. E., and Suzuki. 1989. Methanogenic degradation of phenol and benzoate in acclimated sludges. Water Science and Technology, 21: 55-56.

Kratochvil, B., and Taylor, J. K. 1981. Sampling for chemical analysis. Analytical Chemistry, 53(8): 924A-932A.

Kratochvil, B., Wallance, D., Taylor, J. K. 1984. Sampling for chemical analysis. Analytical Chemistry, 56: 113R-129R.

Leedle, J. A. and Hespell, R. B. 1980. Differential carbohydrate media and anaerobic replica plating techniques in delineating carbohydrate-utilizing subgroups in rumen bacterial populations. Applied and Environmental Microbiology, 39(4): 709-719.

Létourneau, L., Bisaillon, J.-G., Lépine, F., and Beaudet, R. 1995. Spore-forming bacteria that carboxylate phenol to benzoic acid under anaerobic conditions. Canadian Journal of Microbiology, 41: 266-272.

Londry, K. L., and Fedorak, O. M. 1992. Benzoic acids intermediates in the anaerobic degradation of phenols. Canadian Journal of Microbiology, 38: 1-11.

Lopez-Avila,V., Northcutt, R., Onstot, J., and Wickham, M. 1983. Determination of 51 priority organic compounds after extraction from standard reference materials. Analytical Chemistry, 55: 881-889.

Loudon, G., M. 1984. Organic chemistry. Addison-Wesley Publishing Company, Inc., USA.

Manilal, V. B., and Alexander, M. 1991. Factors affecting the microbial degradation of phenanthrene in soil. Applied Microbiology and Biotechnology 35(3): 401-405.

Metcalf & Eddy, Inc. 1991. Wastewater Engineering. Treatment, Disposal, and Reuse. McGraw-Hill, Inc., USA.

Morill, L. G., Mahilum, B. C., Mohiuddin, S. H. 1982. Organic compounds in soils: sorption, degradation and persistence. Ann Arbor Science Publishers, Inc. USA.

Oleszkiewicz, J.A., and Poggi-Varaldo, H.M. 1993. Anaerobic digestion of municipal solid waste, sludge and industrial waste. First Biomass Conference of the Americas, Burlington, VT.

O'Neill, M.W., Symons, J.M., Lazaridou, M.E., and Park, J.B. 1993. In situ treatment of soil for the extraction of organic contaminants. Environmental Progress, 12(1), 12 -23.

Owen, A. O., and Young, L. Y. 1989. Toxicity and anaerobic biodegradability of substituted phenols under methanogenic conditions, Environmental Toxiciology and Chemistry, 8: 853 - 862.

Parkin, G. F., Speece, R. E., Yang, C.H.J., Kocher, W.M. 1983. Response of methane fermentation systems to industrial toxicants. Journal of Water Pollution Control Federation (WPCF), 55(1):44-53.

Poggi-Varaldo, H. M., and Oleszkiewicz, J. A. 1992. Anaerobic co-composting of municipal solid waste and waste sludge at high total solids levels. Environmental Technology, 13: 409-421.

Poole, C. F., and Poole, S. K. 1991. Chromatography today. Elsevier Science Publishers B.V., N.Y.

Raghavn, R., Coles, E., Dietz, D. 1990. Cleaning excavated soil usiing exraction agents: a state of the art review. Project summary. EPA, EP1-89/2: 600/S2-89/034, USA.

Rajput, V. S. 1988. Treatment of hazardous waste contaminated soils by extraction and washing. Ph.D Thesis, The State University of New Jersey, New Jersey.

Rajput, V. S., Higgins, A. J., Singley, M. E. 1994. Cleaning of excavated soil contaminated with hazardous organic compounds by washing. Water Environment Research, 66(6): 819-827.

Reinhart, D. R., Pohland, F. G., Gould, J. P., Cross. 1991. The fate of selected organic pollutants codisposed with municipal refuse. Journal of Water Pollution Control Federation (WPCF), 63(5): 780-788.

Rich, D., Kayhanian, M., Hardy. S., Tchobanoglus, G. 1994. Co-management of municipal solid waste and wastewater treatment plant sludges using an anaerobic composting process. A report prepared for the California Integrated Waste Management Board. Department of Civil and Environmental Engineering, University of California, California, USA.

Rulksen, W. H., and Assink, J. W. 1983. Extraction as a method for cleaning contaminated soil: possibilites, problems and research. Fifth conf. Manage. Uncontrolled hazard.waste sites. Washington, D.C.

Sachs, L. 1984. Applied statistics. Springer-Verlag New York Inc.

Senior, E. 1990. Microbiology of landfill sites. CRC Press, Inc., Florida.

Sheridan, W. G., Jones, W. J., Wolfe, R. S., and Suidan, M. T. 1985. Fundamentals associated with biodegradation of phenols and polycyclic N-armatic compounds. Report to US-EPA, Cooperative Agreement CR806819, 1985.

Shimp, R. J., and Young, R. L. 1988. Availability of organic chemicals for biodegradation in settled bottom sediments. Ecotoxicology and Environmental Safety 15(1): 31-45.

Shwarzenbach, R., Gschwend, P., Imboden, D. 1993. Environmental organic chemistry. John Wiley & Sons, New York.

Snyder, L. R., Glajch, J. L., Kirkland, J. J. 1988. Practical HPLC method development. Wiley-Interscience publication, USA.

Solomon, S.1987. Introduction, general, organic, and biological chemistry. Mc.Graw-Hill Book Company.

Speitel, G. E. Jr., Lu, C.-J., and Zhu. X,-J. 1989. Biodegradation of trace concentrations of substituted phenols in granular activated carbon. Environmental Science and Technology, 23: 68-74.

Sulfita, J. M., Horowitz, A., Shelton, D., and Tiedje, J. M. 1982. Dehalogenation: a new pathway for the anaerobic degradation of haloaromatic compounds. Science, 218: 1115-1117.

Tchobanoglous, G. 1993. Integrated solid waste management. Engineering principles and management issues. McGraw-Hill, Inc., USA.

Ten Brummeler, E., and Koster, I. W. 1989. The effect of several pH control chemicals on the dry batch digestion of the organic fraction of municipal solid waste. Resources, Conservation and Recycling, 3: 19-32.

Tschech, A., and Fuchs, G. 1987. Anaerobic degradation of phenol by pure of newly isolated denitrifying pseudomonads. Archives of Microbiology 148: 213-217.

Young, L.Y., and Rivera, M.D. 1985. Methanogenic degradation of four phenolic compounds. Water Research, 19(10): 1325-1332.

Verscheuren, K. 1983. Handbook of environmental data on organic chemicals. Van Nostrand Reinhold, New York.

Wang, J., Guowei, G., and Chonghua. 1993. Anaerobic biodegradation of phenol: bacterial acclimation and system prformance. Water Science and Technology, 28(7): 17-22.

Wang, Y. T., Gabbard, H. D., and Pai, P. C. 1991. Inhibition of acetate methanogenesis by phenols. Journal of Environmental Engineering, 117(4): 487-500.

Wang, Y.T., Suidan, M.T., Pfeffer, J. T., Najam, I. 1989. The effect of concentration of phenols on their batch methanogenesis. Biotechnology and Bioengineering, 33: 1353 - 1357.

Wang, Y.T., Suidan, M.T., Rittman, B.E. 1986. Anaerobic treatment of phenol by an expanded-bed reactor. Journal of Water Pollution Control Federation (WPCF), 58(3): 227-232.

Watson-Craik, I.A., and Senior, E. 1989a. Treatment of phenolic wastewaters by co-disposal with refuse. Water Research, 23(10): 1293-1303.

Watson-Craik, I.A., and Senior, E. 1989b. Effects of phenol wastewater co-disposal on the attenuation of the refuse leachate molecule hexanoic acid. Letters in Applied Microbiology, 9: 227-232.

Watson-Craik, I.A., and Senior, E. 1989c. Landfill co-disposal: hydraulic loading rate consideration. Journal of Chemical Technology and Biotechnology, 45: 203 - 212.

Watson-Craik, I.A., and Senior, E. 1990. Landfill co-disposal of phenol-bearing wastewaters: organic loading considerations. Journal of Chemical Technology and Biotechnology, 47: 219-233.

Wolfe, D. H. 1986. General, organic, and biological chemistry. McGraw-Hill Book Company, USA.

Woodbury, P. B., and Breslin, V. T. 1992. Assuring compost quality: suggestions for facility managers, regulators, and researches. Biomass and Bioenergy, 3: 213-225.

Young, L.Y., and Rivera, M.D. 1985. Methanogenic degradation of four phenolic compounds. Water Research, 19(10): 1325-1332.

Yu, C. L. A. 1992. Two stage anaerobic digestion of municipal solid waste. M. Sc. Thesis, University of Manitoba, Canada.

Webber, D. 1984. Top fifty chemical products. Chemical and Engineering News, 62: 8-10.

Zinder, S. H., Cardwell, S. C., Anguish, M. L., and Koch, M. 1984. Methanogenesis in a thermophilic (58°C) anaerobic digestor: *Methanothrix* sp. as an important aceticlastic methanogen. Applied and Environmental Microbiology, 47(4): 796-807.

APPENDIX A - PHASE I RAW DATA

Table A-1. Performance data

day of experiment	reactor	GP @ STP	CH4	Active reactor volume	Feed added	VS added	GPR	OLR	VS removal efficiency
		L	L	L	G	G	L/Lr x d	gVS/kgr x d	%
1-8	R1	9.1	1.26	4.0	NA	NA	0.3	N/A	N/A
	R2	14.4	2.50	4.0	NA	NA	0.4	N/A	N/A
9-12	R1	17.6	6.00	4.1	250.1	94.6	1.1	6.26	50.7
	R2	9.3	4.40	4.1	250.1	94.6	0.6	6.27	19.9
13-15	R1	20.2	8.10	4.1	542.86	186.96	1.6	16.57	39.3
	R2	16.0	4.90	4.3	542.86	186.96	1.2	16.44	43.4
16-19	R1	29.0	13.20	4.0	723.81	210.41	1.8	13.99	44.5
	R2	16.4	4.30	4.3	723.81	210.41	1.0	13.95	48.8
20-22	R1	21.6	8.60	3.8	542.86	154.06	1.9	13.59	46.9
	R2	23.9	8.00	3.6	542.86	154.06	2.2	13.55	33.0
23-26	R1	27.3	11.60	4.3	723.81	196.36	1.6	9.48	26.2
	R2	49.4	24.40	4.3	723.81	196.36	2.9	9.64	55.5
27-29	R1	1.3	0.50	4.5	542.86	177.41	0.1	10.32	31.0
	R2	41.3	22.40	4.7	542.86	177.41	2.9	10.30	43.9
30-33	R1	31.5	8.90	4.2	723.81	212.8	1.9	14.20	33.8
	R2	50.9	27.70	5.0	723.81	212.8	2.5	13.99	65.1
34-36	R1	29.3	11.80	4.2	542.86	140.93	2.3	12.47	N/A
	R2	31.3	11.40	5.0	542.86	140.93	2.1	12.49	N/A
37-40	R1	35.6	11.10	4.1	732.81	188.91	2.1	12.56	4.5
	R2	46.4	22.00	4.1	732.81	188.91	2.8	12.55	66.8
41-43	R1	20.9	8.20	4.3	542.86	149.4	1.6	13.26	48.2
	R2	34.4	12.10	4.4	542.86	149.4	2.6	13.20	12.2
44-47	R1	24.7	5.20	4.1	723.81	200.64	1.5	13.30	5.6
	R2	28.9	7.00	4.3	723.81	200.64	1.7	13.40	38.7
48-50	R1	21.0	3.50	4.3	542.86	173.74	1.6	15.30	N/A
	R2	34.3	14.40	4.3	542.86	173.74	2.7	15.35	50.5
51-54	R1	35.8	11.40	4.0	723.81	156.25	2.2	10.32	43.3
	R2	48.0	25.40	4.0	723.81	156.25	3.0	10.39	30.1
55-57	R1	28.0	9.10	4.3	542.86	165.71	2.2	14.60	N/A
	R2	38.0	16.90	4.4	542.86	133.83	2.9	11.87	27.1
58-64	R1	43.1	12.90	4.2	1266.67	368.67	1.5	13.90	35.0
	R2	45.7	18.60	4.2	1266.67	307.8	1.6	11.82	55.7
65-71	R1	36.5	11.40	4.5	1266.67	386.75	1.2	14.66	21.8
	R2	35.5	17.10	4.3	1266.67	386.75	1.2	14.80	43.0
72-78	R1	48.3	6.30	4.6	1266.67	372.5	1.5	14.00	1.9
	R2	59.1	19.10	4.5	1266.67	372.5	0.8	14.10	9.6
78-82	R1	25.4	3.30	4.3	723.81	220.9	1.2	11.56	80.8
	R2	31.4	9.42	4.3	723.81	220.9	1.5	11.60	41.3
82-90	R1	40.4	4.20	4.2	542.86	150.0	1.1	4.30	11.0
	R2	43.0	3.70	4.2	542.86	150.0	1.1	4.40	16.9

NOTE: kgr - kg of reactor content
OLR - organic loading rate
GP gas produced
Lr - litre of working reactor volume
NA - not added
GPR - gas production rate

Gas composition

day of experiment	R1-CH4 %	R1-CO2 %	R2-CH4 %	R2-CO2 %
5	10.0	41.4	11.9	38.7
6	13.2	32.2	16.8	37.2
8	18.2	33.5	22.9	52.3
9	32.2	49.7	39.8	57.4
12	35.8	43.0	54.2	56.3
14	39.7	65.2	31.0	59.9
15	40.6	56.4	30.5	50.3
19	45.6	50.4	25.9	51.7
21	37.4	60.6	25.9	59.1
22	42.4	59.5	41.0	58.3
26	42.4	50.0	49.3	48.8
28	39.0	58.9	54.3	55.9
30	18.6	20.9	56.3	50.2
32	37.8	40.3	52.6	51.4
35	46.6	62.4	47.1	60.0
36	33.8	37.4	25.8	29.8
37	31.2	38.5	47.4	58.3
42	39.0	56.8	35.2	49.9
49	16.9	36.4	42.0	57.4
54	31.8	31.0	53.0	35.0
56	32.4	46.6	44.5	54.3
59	24.2	50.8	32.2	62.8
62	35.6	47.6	49.4	38.2
69	28.2	57.0	48.0	47.8
70	26.4	54.2	48.3	51.1
71	38.9	34.6	4.0	4.7
75	3.6	23.5	47.7	49.4
76	13.0	54.0	25.0	26.2
78	12.9	52.9	45.2	43.9
85	19.5	56.8	8.9	49.4
86	7.9	24.4	0.6	7.8
89	7.8	25.6	10.4	32.6
90	5.9	23.6	6.6	61.2
93	18.6	58.2	11.3	47.5

pH

day of experiment	sample	pH
7	1a	6.80
	1b	6.70
	2a	6.90
	2b	6.90
9	1a	7.00
	2a	6.90
13	1a	7.20
	2a	7.00
	F1	7.30
16	1a	6.80
	2a	6.40
	F1	7.20
20	1a	7.00
	1b	7.00
	2a	7.10
	2b	7.40
	F1	7.80
23	1a	6.45
	1b	6.50
	2a	7.80
	2b	8.05
	F1	7.60
	F2	7.62
27	1a	6.80
	1b	6.70
	2a	7.85
	2b	7.95
	F1	7.60
	F2	7.60
30	1a	6.68
	1b	6.70
	2a	8.10
	2b	8.25
	F1	7.80
	F2	7.70
34	1a	7.25
	1b	7.30
	2a	7.50
	2b	7.50
	F1	7.68
	F2	7.70

Note: 1 - R1, 2 - R2, F - feedstock

pH

day of experiment	sample	pH
37	1a	7.10
	1b	7.12
	2a	7.50
	2b	7.45
	F1	7.90
	F2	8.00
41	1a	8.30
	1b	8.00
	2a	7.50
	2b	7.50
	F1	8.00
	F2	8.00
44	1a	6.75
	1b	6.80
	2a	7.35
	2b	7.70
	F1	7.50
	F2	7.60
48	1a	6.80
	1b	7.00
	2a	8.65
	2b	8.60
	F1	8.00
	F2	8.00
51	1a	8.40
	1b	7.85
	2a	7.75
	2b	7.70
	F1	7.90
	F2	8.00
55	1a	8.00
	1b	8.30
	2a	8.80
	2b	8.75
	F1	7.80
	F2	7.80
58	1a	7.00
	1b	7.20
	2a	8.40
	2b	8.40
	F1	7.45
	F2	7.50
65	1a	6.75
	1b	6.70
	2a	7.10
	2b	7.90
	F1	7.50
	F2	7.45
72	1a	7.90
	2a	7.70
	F1	7.50

Note: 1 - R1, 2 - R2, F - feedstock

pH

day of experiment	sample	pH
79	1a	8.00
	1b	8.35
	2a	7.85
	2b	7.85
	F1	7.00
	F2	7.00
82	1a	8.65
	1b	8.70
	2a	8.35
	2b	8.40
	F1	6.90
	F2	6.90
91	1a	8.60
	1b	8.60
	2a	8.40
	2b	8.40
	F1	6.70
	F2	6.70
97	1a	7.90
	1b	7.90
	2a	7.60
	2b	7.60
100	1a	7.85
	1b	7.80
	2a	7.65
	2b	7.88

Note: 1 - R1, 2 - R2, F - feedstock

SOLIDS

day of experiment	sample	dish	dish+sample	dish+sample dry	dish + sample after ignition
		g	g	g	g
1	1,2	94.1	110.0	96.4	94.5
	1,2	28.4	39.4	30.1	28.6
9	1a	66.4	83.2	68.7	66.7
	1b	66.8	78.8	68.6	67.1
	2a	73.2	83.9	74.7	73.5
	2b	67.8	82.6	69.9	68.1
	F1	99.6	107.9	103.0	100.3
	F2	66.1	77.3	70.3	66.7
13	1a	28.4	34.3	29.4	28.6
	1b	104.0	125.5	107.4	104.8
	2a	32.8	38.8	33.7	33.1
	2b	90.3	100.0	92.0	90.6
	F1	64.8	72.7	68.0	65.3
	F2	94.0	102.9	97.3	94.7
16	1a	36.7	45.5	38.3	37.1
	1b	29.7	37.3	31.0	29.9
	2a	64.8	77.4	66.9	65.2
	2b	32.7	41.8	34.3	33.1
	2c	66.9	77.9	68.7	67.2
	F1	34.2	40.2	36.3	34.5
	F2	34.3	38.2	35.6	34.5
20	1a	33.5	40.2	34.7	33.7
	1b	66.1	74.6	67.6	66.4
	2a	99.7	108.7	101.5	100.2
	2b	66.4	76.8	68.3	66.8
	F	67.8	76.4	70.6	68.2
23	1a	90.3	108.6	94.0	91.1
	1b	66.8	85.2	70.2	67.5
	2a	64.9	74.5	66.6	65.2
	2b	32.8	40.7	34.2	33.0
	F1	34.3	37.7	35.3	34.4
	F2	34.2	39.8	36.1	34.5
27	1a	36.8	44.8	38.3	37.1
	1b	66.1	81.2	69.3	66.8
	2a	66.4	78.2	68.7	66.9
	2b	67.8	79.2	70.1	68.3
	F1	33.5	39.4	35.7	33.8
	F2	99.8	109.2	103.5	100.3
30	1a	66.8	77.6	69.2	67.4
	1b	90.7	106.3	94.1	91.5
	2a	34.3	42.5	36.0	NA
	2b	64.8	81.4	68.4	65.7
	F1	90.3	98.5	93.4	90.7
	F2	34.1	38.6	35.7	34.4
34	1a	67.8	78.8	71.0	68.2
	1b	36.8	42.1	38.4	30.0
	2a	66.1	83.0	69.9	67.0
	2b	104.0	124.6	109.0	104.8
	F1	66.3	77.6	68.6	66.9
	F2	33.4	40.4	34.8	33.8

NOTE: 1 - R1, 2 - R2
F - feedstock
NA - not analysed

SOLIDS

day of experiment	sample	dish	dish+sample	dish+sample dry	dish + sample after ignition
		g	g	g	g
37	1a	64.8	70.1	66.4	65.0
	1b	90.7	97.7	92.8	91.0
	2a	90.3	103.6	93.2	91.0
	2b	34.2	44.2	36.2	34.8
	F1	66.6	78.1	69.3	67.4
	F2	34.2	44.2	36.4	34.7
41	1a	94.1	111.0	98.0	95.0
	1b	66.1	78.0	68.8	66.7
	2a	104.0	114.2	106.1	104.5
	2b	66.3	75.5	68.1	66.8
	F1	67.6	75.2	70.2	68.1
	F2	36.7	42.7	38.6	37.0
44	1a	97.6	107.1	99.9	98.4
	1b	85.3	95.4	87.6	85.7
	2a	64.9	76.8	67.7	65.5
	2b	66.8	77.2	69.0	67.4
	F1	90.3	102.5	94.4	91.0
	F2	97.5	108.2	100.9	98.0
48	1a	66.1	76.1	68.5	66.6
	1b	104.0	114.3	106.7	104.7
	2a	67.8	78.3	70.3	68.4
	2b	66.3	75.8	68.5	66.9
	F1	94.0	104.0	97.6	94.5
	F2	93.0	103.4	96.9	93.5
51	1a	97.5	108.7	100.6	98.2
	1b	66.9	76.3	69.4	67.4
	2a	85.2	96.3	87.8	85.8
	2b	90.7	1000.0	92.9	91.3
	F1	90.2	101.0	93.7	90.8
	F2	64.8	74.2	67.9	65.3
55	1a	94.1	104.0	96.7	94.6
	1b	93.0	103.0	95.6	93.5
	2a	66.3	74.2	68.2	66.7
	2b	104.0	114.2	106.4	104.6
	F1d	66.1	77.5	70.4	66.7
	F2d	34.2	37.8	35.5	34.4
	F1w	67.6	77.0	70.4	68.1
	F2w	90.3	100.1	93.1	90.7
58	1a	64.8	79.1	68.3	65.7
	1b	97.5	104.1	99.1	97.9
	2a	90.3	100.2	92.6	90.9
	2b	85.2	96.1	87.9	85.5
	F1d	66.9	75.3	69.6	67.2
	F2d	36.8	43.1	38.8	37.0
	F1w	34.3	40.2	35.9	34.5
	F2w	27.9	33.1	29.3	28.0
66	1a	67.6	79.3	70.4	68.4
	1b	94.1	105.3	96.6	94.6
	2a	66.1	76.2	68.5	66.7
	2b	66.3	75.7	68.6	66.9
	F1	93.0	102.6	96.4	93.4
	F2	104.0	114.4	107.5	104.4

NOTE: 1 - R1, 2 - R2
Fd - feedstock after drying
Fw - feedstock wet

SOLIDS

day of experiment	sample	dish	dish+sample	dish+sample dry	dish + sample after ignition
		g	g	g	g
73	1a	66.9	97.4	89.3	85.7
	1b	90.4	100.6	93.1	91.0
	2a	90.3	102.2	93.2	91.0
	2b	97.5	114.5	101.7	98.6
	F1	85.1	97.4	89.3	85.7
	F2	64.9	74.4	68.1	65.3
80	1a	66.3	77.9	69.7	67.0
	1b	73.3	83.7	76.2	73.8
	2a	90.7	100.7	93.5	91.4
	2b	66.1	77.4	69.2	66.8
	F1	27.8	33.0	29.6	28.0
	F2	104.0	113.9	107.4	104.4
83	1a	94.1	103.2	96.4	94.6
	2a	34.3	42.6	36.4	34.7
	F1	67.7	77.4	70.9	68.2
	F2	89.7	111.7	103.6	100.3
92	1a	90.3	102.8	93.6	NA
	1b	97.5	109.5	100.6	NA
	2a	85.2	96.3	88.1	NA
	2b	66.8	76.8	69.5	NA
	F1	64.9	74.7	68.0	NA
	F2	90.4	100.7	93.6	NA
					NA
96	1a	94.1	103.6	96.4	NA
	1b	66.1	78.1	69.2	NA
	2a	67.8	77.3	70.3	NA
	2b	99.8	110.2	102.3	NA

NOTE: 1 - R1, 2 - R2
F - feedstock
NA - not analysed

VFA

day of experiment	sample	sample weight g	extract mL	acetic mg/l	propionic mg/l	i sobutyric mg/l	n - butyric mg/l	isovaleric mg/l	n - valeric mg/l	total mg/l	acetic mg/g	propionic mg/g	i sobutyric mg/g	n - butyric mg/g	isovaleric mg/g	n - valeric mg/g	total mg/g
9	1a	10	61	921.5	241.8	44.8	431.3	94.4	37.1	1770.9	5.6	1.5	0.3	2.6	0.6	0.2	10.8
	2a	10	61	817.3	208.9	34.7	351.4	60.1	28.8	1501.1	5.0	1.3	0.2	2.1	0.4	0.2	9.2
16	1a	10	64	1077.1	307.8	53.8	735.3	68.6	36.0	2278.6	6.9	2.0	0.3	4.7	0.4	0.2	14.6
	2a	10	62	1559.3	287.4	53.5	708.1	81.5	34.1	2723.7	9.7	1.8	0.3	4.4	0.5	0.2	16.9
	F	10		259.3	88.1	25.1	53.9	13.4	18.7	458.4							
20	1a	10	59	912.4	371.7	76.8	948.6	81.0	40.8	2431.2	5.4	2.2	0.5	5.6	0.5	0.2	14.3
	1b	10	60	878.4	334.6	80.5	850.7	75.3	34.9	2254.5	5.3	2.0	0.5	5.1	0.5	0.2	13.5
	2a	10	66	1515.7	325.3	62.5	945.7	103.8	37.6	2990.5	10.0	2.2	0.4	6.2	0.7	0.3	19.7
	2b	10	61	1313.7	288.2	54.5	862.5	86.2	28.9	2634.0	8.0	1.8	0.3	5.3	0.5	0.2	16.1
	F	10	70	227.5	72.2	56.4	52.2	28.2	17.5	454.0	1.6	0.5	0.4	0.4	0.2	0.1	3.2
27	1a	10	60	1509.0	382.8	73.6	0.7	110.0	63.8	2159.8	9.1	2.3	0.4	0.0	0.7	0.5	13.0
	1b	10	61	1378.8	338.5	64.9	1101.2	98.0	74.3	3055.7	8.4	2.1	0.4	6.7	0.6	0.5	18.6
	2a	10	59	355.9	435.4	112.6	0.5	106.8	40.5	1051.6	2.1	2.6	0.7	0.0	0.6	0.2	6.2
	2b	10	60	195.2	363.9	95.4	874.2	94.4	35.6	1657.2	1.2	2.2	0.6	5.2	0.6	0.2	9.9
	F1	10	61	207.7	93.6	20.9	84.6	34.2	23.3	464.0	1.3	0.6	0.1	0.5	0.2	0.1	2.8
	F2	10	61	269.6	76.5	34.4	64.2	39.3	21.9	464.5	1.6	0.5	0.2	0.4	0.2	0.1	3.1
30	1a	10	60	1232.5	369.7	72.7	1275.3	123.6	109.6	3183.3	7.4	2.2	0.4	7.7	0.7	0.7	19.1
	1b	10	61	1304.0	405.4	83.1	1358.7	130.2	114.4	3395.7	8.0	2.5	0.5	8.3	0.8	0.7	20.7
	2	10	60	107.4	456.0	148.7	949.1	15.0	37.0	1851.2	0.6	2.7	0.9	5.7	0.9	0.2	11.1
34	1a	10	60	1635.5	406.3	90.6	1328.1	121.7	98.2	3680.5	9.8	2.4	0.5	8.0	0.7	0.6	22.1
	1b	10	62	1170.9	349.5	77.1	1126.1	96.9	85.9	2906.4	7.3	2.2	0.5	7.0	0.6	0.5	18.0
	2a	10	61	327.0	654.4	204.9	956.0	162.9	43.6	2348.6	2.0	4.0	1.3	5.8	1.0	0.3	14.3
	2b	10	62	357.2	722.9	230.3	1037.5	178.1	48.2	2574.2	2.2	4.5	1.4	6.4	1.1	0.3	16.0
	F1	10	63	56.3	31.7	11.3	31.3	10.0	7.2	147.7	0.4	0.2	0.1	0.2	0.1	0.0	0.9
	F2	10	59	96.3	31.4	11.0	28.4	9.1	7.5	173.8	0.6	0.2	0.1	0.2	0.1	0.1	1.1
37	1a	10	61	1909.4	398.1	106.8	1211.8	127.0	99.2	3852.3	11.7	2.4	0.7	7.4	0.8	0.6	23.5
	1b	10	60	1892.0	4009.8	110.5	0.8	127.5	101.8	2642.1	11.4	2.5	0.7	0.0	0.8	0.6	15.9
	2a	10	59	1347.6	685.4	223.5	0.7	158.0	44.5	2459.7	8.0	4.0	1.3	0.0	0.9	0.3	14.5
	2b	10	59	2412.3	526.6	144.4	1569.5	162.0	130.9	4945.6	14.2	3.1	0.9	9.3	1.0	0.8	29.2
	F1	10	58	148.8	8.8	38.8	14.9	4.4	2.3	218.0	0.9	0.1	0.2	0.1	0.0	0.0	1.3
	F2	10	59	161.5	6.9	53.1	14.9	6.1	3.4	249.9	1.0	0.0	0.3	0.1	0.0	0.0	1.5
41	1a	10	59	NP	627.6	4878.2	1250.5	138.5	99.2	2602.9	NP	3.7	2.9	7.4	0.8	0.6	15.4
	1b	10	59	876.6	1543.9	NP	1031.7	113.8	82.8	2649.1	5.2	3.2	NP	6.1	0.7	0.5	15.6
	2a	10	59	1444.7	842.7	201.5	488.4	143.3	38.5	2959.1	8.5	3.8	1.2	2.9	0.8	0.2	17.5
	2b	10	60	1388.9	660.4	210.0	NP	148.1	39.3	2446.4	8.3	4.0	1.3	NP	0.9	0.2	14.7
	F1	10	62	23.3	10.6	1.1	1.2	2.7	1.8	40.7							
	F2	10	60	15.4	9.1	NP	NP	2.6	1.9	29.0							

NOTE: Extract - volume of slurry after addition of 50 ml deionized water to sample
1 - R1, 2 - R2, F - feedstock

VFA

day of experiment	sample	sample weight g	extract mL	acetic mg/l	propionic mg/l	i sobutyric mg/l	n - butyric mg/l	isovaleric mg/l	n - valeric mg/l	total mg/l	acetic mg/g	propionic mg/g	i sobutyric mg/g	n - butyric mg/g	isovaleric mg/g	n - valeric mg/g	total mg/g
44	1a	10	60	2300.1	433.9	147.0	1112.1	143.2	96.6	4332.9	13.8	2.6	0.9	6.7	0.9	0.6	26.0
	1b	10	59	2034.4	396.2	133.3	968.9	124.3	85.2	3762.3	12.0	2.3	0.8	5.8	0.7	0.5	22.2
	2a	10	60	1559.1	640.2	197.3	513.6	144.5	38.5	3093.3	9.4	3.8	1.2	3.1	0.9	0.2	18.6
	2b	10	60	1607.6	671.3	209.9	515.6	149.2	37.3	3190.5	9.6	4.0	1.3	3.1	0.9	0.2	19.1
	F1	10	63	65.1	22.9	32.9	18.2	9.3	3.0	151.4							
	F2	10	59	53.0	25.6	21.5	12.8	7.5	NP	120.4							
48	1a	10	60	1919.1	345.7	138.8	1004.2	134.9	131.2	3673.9	11.5	2.1	0.8	6.0	0.8	0.8	22.0
	1b	10	60	1721.4	287.1	112.4	823.1	109.6	0.1	3053.7	10.3	1.7	0.7	4.9	0.7	0.0	18.3
	2a	10	60	747.0	456.5	153.5	372.5	106.8	24.8	1861.0	4.5	2.7	0.9	2.2	0.6	0.2	11.2
	2b	10	60	870.8	493.7	156.6	391.8	106.9	21.6	2043.4	5.2	3.0	0.9	2.4	0.7	0.1	12.3
	F1	10	59	122.5	39.8	7.1	21.0	9.2	4.1	203.7							
	F2	10	59	56.2	32.5	24.1	14.5	8.2	NP	135.5							
51	1a	10	60	1837.5	384.8	144.2	1107.9	149.2	115.5	3739.1	11.0	2.3	0.9	6.7	0.9	0.7	22.4
	1b	10	60	1700.7	325.8	127.6	967.3	130.7	111.9	3364.0	10.2	2.0	0.8	5.8	0.8	0.7	20.2
	2a	10	60	1786.2	642.5	202.8	529.3	149.9	29.2	3340.0	10.7	3.9	1.2	3.2	0.9	0.2	20.0
	2b	10	60	1775.1	738.9	232.5	586.0	165.1	30.1	3527.7	10.7	4.4	1.4	3.5	1.0	0.2	21.2
	F1	10	62	81.6	34.5	41.1	50.9	11.3	4.5	223.8							
	F2	10	60	66.3	41.1	61.9	22.7	9.5	5.7	207.2	0.4	0.3	0.4	0.1	0.1	0.0	1.2
55	1a	10	60	1940.1	379.4	136.5	994.1	146.7	95.6	3692.4	11.6	2.3	0.8	6.0	0.9	0.6	22.2
	1b	10	60	1766.7	412.7	149.7	1064.5	156.6	110.7	3661.4	10.6	2.5	0.9	6.4	0.9	0.7	22.0
	2a	10	60	749.6	695.4	213.2	591.9	162.0	30.6	2442.9	4.5	4.2	1.3	3.6	1.0	0.2	14.7
	2b	10	61	545.6	715.7	215.9	560.6	161.0	28.1	2226.9	3.3	4.4	1.3	3.4	1.0	0.2	13.6
	F1	10	59	103.2	65.3	68.8	29.9	15.0	6.2	288.7	0.6	0.4	0.4	0.2	0.1	0.0	1.7
	F2	10	60	109.0	47.7	59.3	21.4	9.1	4.3	250.2	0.4	0.3	0.4	0.1	0.1	0.0	1.2
55	1a	10	60	2524.6	493.3	171.9	1328.5	186.9	127.0	4832.4	15.2	3.0	1.0	8.0	1.1	0.8	29.0
	1b	10	59	1688.1	378.0	130.3	NP	145.4	99.4	2441.1	10.0	2.2	0.8	NP	0.9	0.6	14.4
	2a	10	50	1168.7	638.2	224.2	NP	178.3	26.6	2236.0	5.1	2.7	1.1	NP	0.9	0.1	11.2
	2b	10	60	1011.0	533.3	192.0	552.1	153.4	22.6	2464.4	6.1	3.2	1.2	3.3	0.9	0.1	14.8
	F1	10	58	94.0	53.5	77.6	25.5	12.2	5.1	267.8							
	F2	10	60	83.9	42.9	44.3	21.1	10.6	4.3	206.1							
65	1a	10	60	3197.6	394.2	144.9	1511.6	185.7	191.6	5626.1	19.2	2.4	0.9	9.1	1.1	1.2	33.8
	1b	10	60	2569.7	317.6	114.4	1219.5	144.7	144.9	4510.9	15.4	1.9	0.7	7.3	0.9	0.9	27.1
	2a	10	60	2272.1	569.2	191.2	660.1	169.4	63.0	4145.0	13.6	3.5	1.2	5.2	1.0	0.4	24.9
	2b	10	60	1349.7	595.3	197.4	698.0	172.9	44.4	3057.6	8.1	3.6	1.2	4.2	1.0	0.3	18.4
	F1	10	58	159.2	73.3	12.2	57.6	19.2	12.6	334.5							
	F2	10	58	140.7	71.4	11.9	46.4	15.8	10.8	300.1							

NOTE: NP - no peak detected
Extract - volume of slurry after addition 50 ml deionized water to sample
1 - R1, 2 - R2, F - feedstock

VFA

day of experiment	sample	sample weight g	extract mL	acetic mg/l	propionic mg/l	i sobutyric mg/l	n - butyric mg/l	isovaleric mg/l	n - valeric mg/l	total mg/l	acetic mg/g	propionic mg/g	i sobutyric mg/g	n - butyric mg/g	isovaleric mg/g	n - valeric mg/g	total mg/g
72	1a	10	60	2147.1	326.3	129.5	1751.2	224.6	148.9	4727.9	12.4	2.0	0.8	10.5	1.4	0.9	28.4
	1b	10	60	2123.0	321.6	146.1	1738.6	255.1	149.8	4714.3	12.7	1.9	0.9	10.4	1.5	0.9	28.3
	2a	10	60	2013.7	572.5	226.6	772.6	262.7	58.7	3906.6	12.1	3.4	1.4	4.6	1.6	0.4	23.4
	2b	10	60	1484.2	465.5	203.5	658.8	240.0	50.7	3102.7	8.9	2.8	1.2	4.0	1.4	0.3	18.6
79	1a	10	60	2878.7	466.6	170.5	3101.7	351.1	134.4	7103.1	17.3	2.8	1.0	18.6	2.1	0.8	42.6
	1b	10	60	2375.9	388.1	146.0	2547.6	287.2	113.1	5858.1	14.3	2.3	0.9	15.3	1.7	0.7	35.2
	2a	10	60	2579.5	845.8	251.9	1191.3	316.1	55.1	5239.7	15.5	5.1	1.5	7.2	1.9	0.1	31.4
	2b	10	60	2382.5	788.2	234.8	1068.7	287.3	50.0	4831.5	14.3	4.7	1.4	6.5	1.7	0.3	29.0
	F1	10	58	290.5	75.0	15.2	54.9	19.4	11.4	466.4	1.7	0.4	0.1	0.3	0.1	0.1	2.7
91	1a	10	61	2628.0	343.8	132.5	1627.4	249.5	93.1	5074.2	16.0	2.1	0.8	9.9	1.5	0.6	31.0
	1b	10	60	2789.8	359.0	136.2	1664.9	262.0	91.4	5303.2	16.7	2.2	0.8	10.0	1.6	0.6	31.2
	2a	10	58	2528.1	672.8	204.9	1491.5	331.8	29.3	5510.7	14.7	3.9	1.2	8.7	1.9	0.2	32.0
	2b	10	62	2585.6	688.3	208.8	1464.5	331.7	41.0	5339.8	16.0	4.3	1.3	9.2	2.1	0.3	33.1
	F1	10	62	649.3	69.3	15.1	53.6	19.1	11.4	817.8	4.0	0.4	0.1	0.3	0.1	0.1	5.1
	F2	10	62	730.6	79.2	16.2	59.8	23.4	13.8	925.0	4.5	0.5	0.1	0.4	0.1	0.1	5.7

NOTE: Extract - volume of slurry after addition 50 ml deionized water to sample
1 -R1, 2 - R2, F - feedstock

NH3 and TKN

NH3

day of experiment	sample	sample weight g	HCL ml	HCL concentration M	NH3 mg/g
13	1a	3.7	19.15	0.01	0.72
	1b	3.1	16.1	0.01	0.72
	2a	2.5	9.063	0.01	0.50
	2b	3.2	11.38	0.01	0.49
	BL	0.2			
23	1a	3.0	10.77	0.01	0.49
	1b	3.5	12.86	0.01	0.50
	2a	2.9	20.3	0.01	0.97
	2b	3.2	21.65	0.01	0.97
	F1	5.3	10.03	0.01	0.26
	F2	4.8	9.587	0.01	0.27
	BL		0.265		
27	1c	4.1	10.71	0.01	0.35
	1d	3.7	10.46	0.01	0.39
	2c	5.1	2.257	0.01	0.06
	2d	3.4	1.782	0.01	0.06
	F	2.8	6.772	0.01	0.33
	BL		0.265	0.01	
34	1a	4.7	11.25	0.01	0.33
	1b	4.0	8.758	0.01	0.30
	2a	3.6	1.047	0.01	0.03
	BL		0.328	0.01	
44	1a	3.2	2.259	0.01	0.09
	1b	4.2	2.656	0.01	0.08
	2a	3.5	1.532	0.01	0.05
	2b	3.3	1.72	0.01	0.06
	F1	3.8	5.591	0.01	0.20
	F2	3.2	4.488	0.01	0.19
	BL		0.243	0.01	

TKN

day of experiment	sample	sample weight g	HCL ml	HCL concentration M	TKN mg/g
13	1a	0.6	17.26	0.01	2.94
	1b	0.9	19.36	0.01	2.94
	2b	0.6	16.26	0.01	2.76
		1.0	21.69	0.01	2.97
	BL		0.477	0.01	
23	1a	1.0	20.12	0.01	2.59
	1b	0.9	21.72	0.01	3.13
	2a	1.2	23.11	0.01	2.51
	2b	0.6	12.69	0.01	2.63
	F1	0.7	10.44	0.01	1.76
	BL1		1.868	0.01	
	Bl2		1.368	0.01	
27	1c	0.5	13.85	0.01	3.46
	1d	0.5	11.26	0.01	2.70
	2c	0.7	18	0.01	3.28
	2d	0.8	18.56	0.01	2.97
	F3	0.6	0.391	0.01	1.81
44	1a	0.9	12.36	0.01	1.83
	1b	0.7	11.04	0.01	2.09
	2a	0.7	10.32	0.01	1.94
	2b	0.6	9.165	0.01	2.00
	F1	0.6	10.61	0.01	2.38
	F2	0.7	10.43	0.01	1.97
	BL1		0.68	0.01	
	BL2		0.539	0.01	
48	1a	0.7	21.92	0.01	4.27
	1b	0.4	7.624	0.01	2.48
	2a	0.6	15.91	0.01	2.68
	2b	0.6	9.43	0.01	2.08
	BL1		0.68	0.01	
	BL2		0.539	0.01	
	F1	0.7	7.515	0.01	1.38
	F2	0.6	7.687	0.01	1.65

NOTE: 1 - R1, 2 - R2
F - feedstock
BL - blank
M - mole

NH3 and TKN

NH3

day of experiment	sample	sample weight g	HCL ml	HCL concentration M	NH3 mg/g
55	1a	3.5	8.719	0.01	0.33
	1b	3.6	8.656	0.01	0.32
	2a	3.8	2.166	0.01	0.06
	2b	3.5	1.861	0.01	0.06
	F1	3.7	0.555	0.01	0.00
	F2	3.7	0.531	0.01	0.00
	BL1		0.5	0.01	
	BL2		0.414	0.01	
58	1a	3.6	11.88	0.01	0.44
	1b	3.5	10.92	0.01	0.42
	2a	3.6	2.15	0.01	0.07
	2b	3.5	2.244	0.01	0.07
	F1	3.7	6.506	0.01	0.23
	F2	3.7	6.795	0.01	0.24
	BL1		0.5	0.01	
	BL2		0.414	0.01	
66	la	2.7	12.89	0.01	0.65
	1b	2.7	12.75	0.01	0.64
	2a	2.7	3.675	0.01	0.17
	2b	2.8	3.002	0.01	0.13
	F1	2.7	8.211	0.01	0.41
	F2	2.8	7.679	0.01	0.37
80	1a	2.7	41.76	0.01	2.15
	1b	2.8	43.64	0.01	2.17
	2a	2.9	31.98	0.01	1.53
	2b	2.9	42.61	0.01	2.04
	F1	2.9	9.79	0.01	0.46
	F2	2.8	9	0.01	0.43
	BL		0.336	0.01	
92	1a	2.9	43.42	0.01	2.08
	1b	2.9	49.2	0.01	2.36
	2a	2.7	46.88	0.01	2.42
	2b	2.8	45.96	0.01	2.28
	F1	2.7	27.67	0.01	1.42

TKN

day of experiment	sample	sample weight g	HCL ml	HCL concentration M	TKN mg/g
55	1a	0.5	9.821	0.01	2.41
	1b	0.4	8.453	0.01	2.54
	2a	0.5	10.24	0.01	2.53
	2b	0.5	11.42	0.01	1.43
	F1	0.7	15.41	0.01	2.84
	F2	0.6	13.88	0.01	2.96
	BL1		1.188	0.01	
	BL2		1.219	0.01	
58	1a	0.7	14.22	0.01	2.61
	1b	0.7	12.69	0.01	2.30
	2a	0.8	16.96	0.01	2.86
	2b	0.9	18.92	0.01	3.55
	F1	0.9	16.92	0.01	2.76
	F2	0.5	8.148	0.01	1.95
	BL1		1.188	0.01	
	BL2		1.219	0.01	
66	1a	0.6	9.665	0.01	2.11
	2a	0.8	15.16	0.01	2.55
	F	0.6	23.02	0.01	5.23
	BL1		0.57	0.01	
	BL2		0.664	0.01	
80	1a	0.6	15.66	0.01	3.51
	2a	0.9	22.89	0.01	3.47
	2b	0.8	20	0.01	3.39
	F1	0.5	17.29	0.01	4.67
	F2	0.8	48.06	0.01	8.31
	BL1		0.57	0.01	
	BL2		0.664	0.01	

NOTE: 1 - R1, 2 - R2
F - feedstock
BL - blank
M - mole

DRAW AND FILL

day of experiment	reactor	Me	Mb	md	Ma	Feed	Buffer	
		g	g	g	g	g	g	
1	R1	1471.6	N/A	N/A	5158.6	3700(*)	NA	
	R2	1466.9	N/A	N/A	5154.9	3700(*)	NA	
6	R1	1471.6	5138.0	N/A	N/A	NA	NA	
	R2	1466.9	5142.0	N/A	N/A	NA	NA	
7	R1	1471.6	5140.2	1294.0	5240.3	1422.8	NA	rumen
	R2	1466.9	5138.5	1244.0	5241.0	1373.1	NA	addition
9	R1	1471.6	5218.8	197.3	5276.6	250.1	5.55	
	R2	1466.9	5229.0	212.2	5259.0	250.1	5.55	
12	R1	1471.6	5256.8	N/A	N/A	NA	NA	
	R2	1466.9	5240.9	N/A	N/A	NA	NA	
13	R1	1471.6	5233.7	505.0	5261.5	542.9	10.42	
	R2	1466.9	5212.0	488.0	5270.7	542.9	10.42	
16	R1	1471.6	5209.1	661.31	5268.4	723.81	11.29	
	R2	1466.9	5236.4	692.91	5254.0	723.81	11.29	

NOTE: Me- mass of empty reactor
Mb - mass of reactor with digestate befor feeding
md - mass of digestate drawn from reactor during feeding
Ma - mass of reactor after feeding
Feed - mass of the feedstock going into reactor
Buffer - sodium bicarbonate
(*) - initial mass of reactor content composed of anaerobic digester sludge, cow manure, digestate from previous DAD study and feedstock
NA - not added

DRAW AND FILL

day of experiment	reactor	Me	Mb	md	Ma	Feed	Buffer
		g	g	g	g	g	g
20	R1	1471.6	5202.3	473.56	5271.3	542.86	11.29
	R2	1466.9	5227.8	503.76	5276.8	542.86	15.0
23	R1	1471.6	5223.8	676.01	5271.2	723.81	20.08
	R2	1466.9	5239.9	696.81	5264.7	723.81	14.33
27	R1	1471.6	5208.5	479.71	5264.9	542.86	12.83
	R2	1466.9	5135.7	411.61	5267.1	542.86	12.83
30	R1	1471.6	5213.9	666.11	5218.6	723.81	25.5
	R2	1466.9	5156.9	613.81	5269.0	723.81	17.73
34	R1	1471.6	5140.4	411.61	5274.5	542.81	14.33
	R2	1466.9	5152.2	428.11	5268.3	542.81	14.33
37	R1	1471.6	5211.8	664.01	5273.0	723.81	13.46
	R2	1466.9	5201.4	658.31	5269.9	723.81	13.46
41	R1	1471.6	5192.8	464.06	5265.5	542.86	11.4
	R2	1466.9	5192.1	468.06	5259.0	542.86	11.4
44	R1	1471.6	5187.0	639.21	5268.9	723.81	12.59
	R2	1466.9	5201.1	658.01	5268.8	723.81	12.59
48 phenol addition to R2	R1	1471.6	5212.4	483.66	5276.1	542.86	15.15
	R2	1466.9	5170.1	446.06	5272.1	542.86	15.15

NOTE: Me- mass of empty reactor
Mb - mass of reactor with digestate befor feeding
md - mass of digestate drawn from reactor during feeding
Ma - mass of reactor after feeding
Buffer - sodium bicarbonate
Feed - mass of the feedstock going into reactor

DRAW AND FILL

day of experiment	reactor	Me	Mb	md	digestate drawn between feedings	Ma	Feed	digestate drawn after feeding	Buffer	digestate mass drawn for check
		g	g	g	g		g	g	g	g
51	R1	1471.6	5248.0	700.21		5263.6	723.81		19.54	
	R2	1466.9	5206.4	663.21		5276.0	723.81		13.03	
55	R1	1471.6	5222.6	493.86		5268.0	542.86		9.45	
	R2	1466.9	5179.0	454.96		5271.0	542.86		11.4	
58	R1	1471.6	5217.1	1212.17		5270.8	1266.67		24.32	
	R2	1466.9	5180.9	1180.67		5269.0	1266.67		24.32	
65	R1	1471.6	5209.8	1204.87		5281.9	1266.67		25.08	8.6 (m. d)
	R2	1466.9	5106.4	1106.17		5277.2	1266.67		25.08	9.3 (m.d)
70	R1	1471.6	N/A	N/A	15.8	N/A	NA		12.5	
	R2	1466.9	N/A	N/A		N/A	NA		12.5	
71	R2	1466.9	N/A	N/A	13.6	N/A	NA			
72	R1	1471.6	5209.2	1204.3		5297.7	723.81		39.9	15.8 (pH)
	R2	1466.9	5120.4	1060.0		5296.5	723.81	12.7	39.9	24.1 (pH)
76	R2	1466.9	N/A	N/A	14.3	N/A	NA			
79	R1	1471.6	5246.2	698.41		5297.6	723.81		29.6	
	R2	1466.9	5170.4	613.01		5297.2	723.81	14.1	29.6	
83	R1	1471.6	5286.4	557.7		5290.1	524.86		10.7	
	R2	1466.9	5252.6	528.6		5296.9	524.86	13.8	10.7	
91 rumen addition	R1	1471.6	5273.1	1050.1		5247.3	666.0			
	R2	1466.9	5264.0	1047.2		5257.8	666.0	14.6		

NOTE: Me- mass of empty reactor
Mb - mass of reactor with digestate befor feeding
md - mass of digestate drawn from reactor during feeding
Ma - mass of reactor after feeding; Buffer - sodium bicarbonate
m.d - moisture determination, pH - pH check, NA - not added

alkalinity

day of experiment	sample	mass	extract	H2SO4	alkalinity	alkalinity
		g	ml	ml	mg/l	mg/g
7	1	10	62	15.4	7700	47.7
	2	10	62	5.6	2800	17.4
13	1	10	62	16.3	8100	50.2
	2	10	61	15.7	7850	47.9
16	1	10	64	21.8	10900	69.8
	2	10	62	22.9	11450	71.0
23	1a	10	61	26.6	13300	81.1
	1b	10	59	27.4	13700	80.8
	2a	10	62	26.5	13250	82.2
	2b	10	61	26.7	13350	82.8
27	1a	10	60	28.0	14000	84.0
	1b	10	61	28.3	14150	86.3
	2a	10	59	21.2	10600	62.5
	2b	10	60	22.0	11000	66.0
30	1a	10	60	28.9	14450	86.7
	1b	10	61	28.0	14000	85.4
	2a	10	60	24.2	12100	72.6
	2b	10	60	24.5	12250	73.5
34	1a	10	60	34.4	17200	103.2
	1b	10	62	37.2	18600	115.3
	2a	10	61	33.5	16750	102.2
	2b	10	62	33.6	16800	104.2
37	1a	10	61	12.2	6100	37.2
	1b	10	60	6.1	3050	18.3
	2a	10	59	5.4	2700	15.9
	2b	10	59	6.2	3100	18.3
41	1a	10	59	5.9	2925	17.3
	1b	10	59	5.7	2850	16.8
	2a	10	59	4.8	2400	14.2
	2b	10	60	5.0	2500	14.8

NOTE: 1-R1, 2-R2

alkalinity

day of experiment	sample	mass g	extract ml	H2SO4 ml	alkalinity mg/l	alkalinity mg/g
44	1a	10	60	5.60	2800	16.8
	1b	10	59	5.60	2800	16.5
	2a	10	60	5.05	2525	15.2
	2b	10	59	4.40	2200	13.0
48	1a	10	60	7.50	3750	22.5
	1b	10	60	6.00	3000	18.0
	2a	10	60	5.30	2650	15.9
	2b	10	60	5.55	2775	16.7
51	1a	10	60	7.05	3525	21.2
	1b	10	60	6.35	3175	19.1
	2a	10	60	6.30	3150	18.9
	2b	10	60	5.90	2950	17.7
55	1a	10	60	7.00	3500	21.0
	1b	10	60	6.40	3200	19.2
	2a	10	60	6.60	3300	19.8
	2b	10	61	6.50	3250	19.8
58	1a	10	60	7.40	3500	21.0
	1b	10	59	5.40	2700	15.9
	2a	10	59	5.20	2600	15.3
	2b	10	60	4.50	2250	13.5
65	1a	10	60	7.10	3550	21.3
	1b	10	60	7.10	3550	21.3
	2a	10	60	7.10	3550	21.3
	2b	10	60	5.70	2850	17.1
72	1	10	60	8.10	4050	24.3
	2	10	60	8.20	4100	24.6
79	1a	10	60	8.40	4200	25.2
	1b	10	60	7.80	3900	23.4

NOTE: 1-R1, 2-R2

alkalinity

day of experiment	sample	mass	extract	H2SO4	alkalinity	alkalinity
		g	ml	ml	mg/l	mg/g
79	2a	10	60	8.20	4100	24.60
	2b	10	60	8.00	4000	24.00
86	1a	10	60	7.40	3700	22.20
	1b	10	61	7.70	3850	23.49
	2a	10	60	8.00	4000	24.00
	2b	10	60	8.00	4000	24.00
90	1	10	60	7.80	3900	23.40
	2a	10	58	7.10	3550	20.59
	2b	10	62	7.60	3800	23.56
97	1a	10	60	5.95	2975	17.85
	1b	10	60	5.85	2925	17.55
	2a	10	61	6.30	3150	19.22
	2b	10	60	6.30	3150	18.90
100	1a	10	60	4.64	2325	13.95
	1b	10	60	4.90	2450	14.70
	2a	10	60	5.35	2675	16.05
	2b	10	60	5.50	2750	16.50

NOTE: 1-R1, 2-R2

HPLC calibration

day of analysis	phenol standard 10 mg/l		phenol standard 100 mg/l	
	HPLC reading		HPLC reading	
	RT	H	RT	H
49	2.121	5817	2.051	58164
51	2.154	6322	NR	55167
58	2.096	6519	2.096	61733
61	2.097	7181	2.247	65421
70	2.143	4449	2.158	41081
70	2.136	4025	2.094	40104
71	1.967	5426	1.964	50973
77	1.979	4895	1.964	46148
83	2.365	4488	2.221	44376
98	1.771	4283	1.762	42738

Background phenol in R1

day of experiment	day of analysis	sample no	extractant type	added volume	HPLC reading	phenol analysed
				mL	H	mg/l
51	51	1	DW	5	5246	8.2
51	51	2	DW	5	2196	3.2
51	51	3	DW	5	7799	12.5

NOTE: RT - retention time
H - hight
NR - not recorded

Sterilized digestate samples

sample	digestate	phenol solution conc.	phenol solution added	phenol added	extractant type	extractant volume	phenol conc. initial	HPLC reading	phenol analysed
no	g	g/l	mL	mg		mL	mg/l	H	mg/l
1	1	64	0.0039	0.25	methanol	5	50	5129	10.7
2	1	64	0.0039	0.25	methanol	5	50	4110	8.6
3	1	64	0.0039	0.25	methanol	5	50	3903	8.2
4	1	64	0.0039	0.25	DW	5	50	20508	40.6
5	1	64	0.0039	0.25	DW	5	50	20214	40.0
6	1	64	0.0039	0.25	DW	5	50	21195	41.9
BLD1	1	N/A	N/A	N/A	DW	5	50	525	1.0
BLD2	1	N/A	N/A	N/A	DW	5	50	485	0.9
BLD3	1	N/A	N/A	N/A	DW	5	50	ND	ND
BLD4	1	N/A	N/A	N/A	methanol	5	50	ND	ND
BLD5	1	N/A	N/A	N/A	methanol	5	50	ND	ND
1	1	64	0.0039	0.25	DW	5	50	25302	42.9
2	1	64	0.0039	0.25	DW	5	50	44641	75.3
3	1	64	0.0039	0.25	DW	5	50	49828	84.0
4	1	64	0.0039	0.25	DW+methanol	5	50	13421	22.9
5	1	64	0.0039	0.25	DW+methanol	5	50	6352	10.9
BLD1	1	N/A	N/A	N/A	DW	5	50	1100	1.9
BLD2	1	N/A	N/A	N/A	DW	5	50	1250	2.1
BLD3	1	N/A	N/A	N/A	DW	5	50	690	1.2
BLD4	1	N/A	N/A	N/A	DW+methanol	5	50	ND	ND
BLPS1	N/A	64	0.0039	0.25	DW	5	50	29168	49.4
BLPS2	N/A	64	0.0039	0.25	DW	5	50	25654	43.5
BLPS2	N/A	64	0.0039	0.25	DW	5	50	27695	46.9

NOTE:
BLD - blank digestate
DW - deionized water
BLPS - blank phenol solution
ND - not detected
H - hight

RESULTS OF EXTRACTION

day of experiment	day of analysis	sample	sampling	HPLC	reading	phenol	phenol average	standard deviation	standard error of mean
				H	mg/l	mg/kg	mg/kg		
51	58	1	before	5344	8.2	41			
		2	feeding	5612	9.2	46			
		3		NA	NA	NA			
		4		5842	8.9	22			
		5		8092	12.5	31			
		6		2096	3.1	8	sample 4-8- 2g digestate used		
		7		1763	2.6	7			
		8		6479	9.9	25	26	15.0	5
55	58	1	before	37814	60.5	303			
		2	feeding	7617	11.7	59			
		3		5316	8.1	41			
		4		4012	6.1	30			
		5		450	0.6	3			
		6		1453	2.2	11			
		7		11562	18.0	90			
		8		11587	18.0	90			
		9		10219	15.8	79			
		10		12372	19.1	95	80	85	27
		1a		1149	0.6	3			
		1b			1.7	8			
58	61	1	before	8429	11.8	59			
		2	feeding	8004	11.2	56			
		3		6061	8.4	42			
		4		6789	9.4	47			
		5		7953	11.1	56			
		6		4946	6.8	34			
		7		4044	5.5	55	(10 ml deionized water used)		
		8		6686	9.3	46			
		9		7706	10.7	54			
		10		6521	9.0	45	49	8	3
		1a		191	0.2	1			
		1b		547	1.3	6			
		F1		ND					
		F2		ND					

NOTE: NA - not analysed

RESULTS OF EXTRACTION

day of experiment	day of analysis	sample	sampling	HPLC	reading	phenol	phenol average	standard deviation	standard error of mean
				H	mg/l	mg/kg	mg/kg		
65	70	1	before	3028	6.8	34			
		2	feeding	2351	5.3	26			
		3		2728	6.1	31			
		4		2986	6.7	34			
		5		2124	4.8	24			
		6		2650	6.0	30			
		7		2848	6.4	32			
		8		2712	6.1	31			
		9		2170	4.9	24			
		10		2716	6.1	31			
		1a		151	0.3	2	30	4	1
		1b		ND					
		F1		ND					
		F2		ND					
70	70	1	between	11617	28.9	145			
		2	fedings	10194	25.4	127			
		3		10606	26.4	132			
		4		10485	26.1	130			
		5		10844	27.0	135			
		6		9859	24.5	123			
		7		10330	25.7	129			
		8		9460	23.5	118			
		9		7947	19.8	99			
		10		8066	20.1	100	124	15	5
71	71	1	between	16405	31.2	156			
		2	feedings	15232	28.9	144			
		3		13548	25.6	128			
		4		14825	28.1	141			
		5		14551	27.6	138			
		6		12954	24.5	122			
		7		11490	21.6	108			
		8		13346	25.2	126			
		9		12731	24.0	120			
		10		10998	20.7	103	129	16	5

RESULTS OF EXTRACTION

day of experiment	day of analysis	sample	sampling	HPLC	reading	phenol	phenol average	standard deviation	standard error of mean
				H	mg/l	mg/kg	mg/kg		
72	77	1	before	9560	19.9	99			
		2	feeding	4720	9.6	48			
		3		7649	15.8	79			
		4		6699	13.8	69			
		5		6263	12.9	64			
		6		6885	14.2	71			
		7		5227	10.7	54			
		8		7226	14.9	75			
		9		7535	15.6	78			
		10		5257	10.8	54	69	15	5
		1a		2221	4.4	22			
		1b		3284	6.6	33			
		F1		3022	6.1	31			
		F2		2735	5.5	28			
72	77	1	after	13813	29.0	145			
		2	feeding	17271	36.5	182			
		3		12133	25.4	127			
		4		12556	26.3	131			
		5		14453	30.4	152			
		6		21863	46.5	232			
		7		11786	24.6	123			
		8		29928	64.1	321			
		9		39928	86.2	431			
		10		16925	35.7	179	202	100	32
76	77	1	between	12864	27.0	135			
		2	feedings	12572	26.3	132			
		3		14876	31.3	156			
		4		18207	38.5	193			
		5		14083	29.6	148			
		6		17009	35.9	180			
		7		13454	28.2	141			
		8		17462	36.9	184			
		9		17436	36.8	184			
		10		20191	42.8	214	167	28	9

RESULTS OF EXTRACTION

day of experiment	day of analysis	sample	sampling	HPLC	reading	phenol	phenol average	standard deviation	standard error of mean
				H	mg/l	mg/kg	mg/kg		
79	83	1	before	13282	29.8	149			
		2	feeding	15095	33.8	169			
		3		12826	28.7	144			
		4		12842	28.8	144			
		5		16408	36.8	184			
		6		11728	26.3	131			
		7		17741	39.8	199			
		8		12154	27.2	136			
		9		12029	26.9	135			
		10		11594	26.0	130	152.0	24	8
		1a		3769	8.4	42			
		F1		3444	7.7	38			
79	83	1	after	24480	55.0	275			
		2	feeding	33683	75.8	379			
		3		37214	83.8	419			
		4		33321	75.0	375			
		5		14361	32.2	161			
		6		31912	71.8	359			
		7		23385	52.5	263			
		8		39802	89.6	448			
		9		33085	74.4	372			
		10		46431	104.7	523	357	103	33
83	84	1	before	28717	64.6	323			
		2	feeding	27637	62.1	311			
		3		26095	58.6	293			
		4		32740	73.7	368			
		5		27821	62.5	313			
		6		28052	63.1	315			
		7		30849	69.4	347			
		8		318.75	71.7	359			
		9		23707	53.3	266			
		10		27036	60.8	304	320	31	10
		1a		4363	9.7	49			
		1b		4904	10.9	55			
		F1		2394	5.3	27			
		F2		1677	3.7	19			

RESULTS OF EXTRACTION

day of experiment	day of analysis	sample	dilution	sampling	HPLC H	reading mg/l	phenol mg/kg	phenol average mg/kg	standard deviation	standard error of mean
83	84	1		after	36430	82.0	410			
		2		feeding	48687	109.8	549			
		3			32319	72.7	364			
		4			49728	112.1	561			
		5	1:1		22014	49.4	494			
		6	1:1		24803	55.7	557			
		7	1:1		24892	55.9	559			
		8	1:1		34300	77.2	772			
		9	1:1		13744	30.7	307			
		10	1:1		13321	29.8	298	487	145	46
91	98	1	1:1	before	30944	36.2	362			
		2	1:1	feeding	19824	23.2	232			
		3	1:1		36841	43.1	431			
		4	1:4		14757	17.2	345			
		5	1:1		25159	29.1	292			
		6	1:4		9915	11.6	232			
		7	1:4		32496	38.0	760			
		8	1:4		34246	40.1	801			
		9	1:4		24729	28.9	578			
		10	1:4		32014	37.4	749	478	225	71
		1a	1:1		3681	4.3	43			
		1b			2975	3.5	17			
		F1			445	0.5	3			
		F2			168	0.2	1			
91	98	1	1:1	after feeding	29630	34.6	346			
		2	1:1		21395	25.0	250			
		3	1:1		14593	17.1	171			
		4	1:1		31419	36.7	368			
		5	1:1		23386	27.3	273			
		6	1:1		34597	40.5	405			
		7	1:1		17665	20.6	207			
		8	1:1		27902	32.6	326			
		9	1:1		41476	48.5	485			
		10	1:1		23761	27.8	278	311	95	30

NOTE: ND - not detected
1a,1b - samples from R1
F1, F2 - feedstock
NA - not analysed

APPENDIX B - PHASE II RAW DATA

Sampling set A-1

incubation at ambient temperature of 24C
vortex mixer
shake time - 2min
centrifuging time - 4 min.

1 date	2 sample no	3 feedstock g	4 phenol conc. mg/l	5 solution added mL	6 phenol added mg	7 extractant type	8 extractant volume mL	9 phenol conc. initial mg/l	10 HPLC reading H	11 phenol conc.anal. mg/l	12 phenol conc.aver. mg/l	13 standard deviation
01-Jun-95	1	1	64000	0.005	0.32	DW	5	64	18177	35		
01-Jun-95	2	1	64000	0.005	0.32	DW	5	64	15642	30		
01-Jun-95	2	1	64000	0.005	0.32	DW	5	64	17751	34		
01-Jun-95	3	1	64000	0.005	0.32	DW	5	64	19557	37		
01-Jun-95	4	1	64000	0.005	0.32	DW	5	64	18888	36		
01-Jun-95	5	1	64000	0.005	0.32	DW	5	64	29219	56		
01-Jun-95	5	1	64000	0.005	0.32	DW	5	64	29364	57	41	10.98
01-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	21558	41		
01-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	21656	42		
01-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	22922	44		
01-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	20532	39		
01-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	20403	39		
01-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	21041	40	41	1.60
01-Jun-95	11	1	64000	0.005	0.32	TW	5	64	17913	34		
01-Jun-95	12	1	64000	0.005	0.32	TW	5	64	21247	41		
01-Jun-95	13	1	64000	0.005	0.32	TW	5	64	22181	43		
01-Jun-95	14	1	64000	0.005	0.32	TW	5	64	21532	41		
01-Jun-95	15	1	64000	0.005	0.32	TW	5	64	20788	40	40	3.21
01-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	18348	35		
01-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	19932	38		
01-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	19681	38		
01-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	19439	37		
01-Jun-95	20	1	64000	0.005	0.32	BUFFER	5	64	20701	40	38	1.66
01-Jun-95	21	1	64000	0.005	0.32	DWH	5	64	21474	41		
01-Jun-95	22	1	64000	0.005	0.32	DWH	5	64	22870	44		
01-Jun-95	23	1	64000	0.005	0.32	DWH	5	64	22859	44		
01-Jun-95	24	1	64000	0.005	0.32	DWH	5	64	24665	48		
01-Jun-95	25	1	64000	0.005	0.32	DWH	5	64	24470	47	45	2.67
01-Jun-95	BLF 26	1	N/A	N/A	N/A	DW	5	N/A	954	2		
01-Jun-95	BLF 27	1	N/A	N/A	N/A	DW	5	N/A	1207	2		
01-Jun-95	BLF 28	1	N/A	N/A	N/A	DW	5	N/A	1209	2		
01-Jun-95	BLF 29	1	N/A	N/A	N/A	DW	5	N/A	982	2		
01-Jun-95	BLF 30	1	N/A	N/A	N/A	DW	5	N/A	1161	2	2	0.23

NOTE: DW - deionized water; AW, pH=11.5 - alkaline water @pH=11.5
TW - tap water; BUFFER -solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v
DWH - deionized water hot at temperature 50 C; BLF -blank feedstock (unspiked)
H - hight

Sampling set A - 2a

incubation @ 25 C
vortex mixer
shaking time - 2 min.
centrifuging time - 4 min.

1 date	2 sample	3 feedstock	4 phenol solution conc.	5 added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l	
08-Jun-95	1	1	64000	0.005	0.32	DW	5	64	24265	30		
08-Jun-95	2	1	64000	0.005	0.32	DW	5	64	24145	29		
08-Jun-95	3	1	64000	0.005	0.32	DW	5	64	26020	32		
08-Jun-95	4	1	64000	0.005	0.32	DW	5	64	23260	28		
08-Jun-95	4	1	64000	0.005	0.32	DW	5	64	26656	33		
08-Jun-95	4	1	64000	0.005	0.32	DW	5	64	28345	35		
08-Jun-95	5	1	64000	0.005	0.32	DW	5	64	17948	21		
08-Jun-95	5	1	64000	0.005	0.32	DW	5	64	20615	24	29	4.60
08-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	17227	20		
08-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	21436	26		
08-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	21924	26		
08-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	18965	22		
08-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	18206	21		
08-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	18892	22		
08-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	22335	27		
08-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	21376	26	24	2.68
08-Jun-95	11	1	64000	0.005	0.32	TW	5	64	17734	21		
08-Jun-95	11	1	64000	0.005	0.32	TW	5	64	17967	21		
08-Jun-95	12	1	64000	0.005	0.32	TW	5	64	19729	24		
08-Jun-95	13	1	64000	0.005	0.32	TW	5	64	32028	45		
08-Jun-95	14	1	64000	0.005	0.32	TW	5	64	26817	37		
08-Jun-95	15	1	64000	0.005	0.32	TW	5	64	29697	41	32	10.86
08-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	28540	40		
08-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	31117	43		
08-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	31047	43		
08-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	6180	8		
08-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	5147	7		
08-Jun-95	20	1	64000	0.005	0.32	BUFFER	5	64	31166	44	31	16.81
08-Jun-95	21	1	64000	0.005	0.32	DWH	5	64	28570	40		

Sampling set A - 2a (cont'd)

1 date anal.	2 sample	3 feedstock	4 phenol solution conc.	5 added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l		
08-Jun-95	22	1	64000	0.005	0.32	DWH	5	64	28429	40		
08-Jun-95	23	1	64000	0.005	0.32	DWH	5	64	27805	39		
08-Jun-95	24	1	64000	0.005	0.32	DWH	5	64	28740	40		
08-Jun-95	25	1	64000	0.005	0.32	DWH	5	64	25334	35	39	1.99
08-Jun-95	BLF 26	1	N/A	N/A	N/A	DW	5	N/A	0	0.00		0.0
08-Jun-95	BLF27	1	N/A	N/A	N/A	DW	5	N/A	0	0.00		0.0
08-Jun-95	BLF28	1	N/A	N/A	N/A	DW	5	N/A	0	0.00		0.0
08-Jun-95	BLF29	1	N/A	N/A	N/A	DW	5	N/A	0	0.00		0.0
08-Jun-95	BLF30	1	N/A	N/A	N/A	DW	5	N/A	0	0.00		0.0

NOTE: DW - deionized water; AW, pH=11.5 - alkaline water @ pH=11.5
TW - tap water; BUFFER -solution of 95% sodium phosphate and 5% acetonitrile
DWH - deionized water hot at temperature 50 C; BLF -blank feedstock (unspiked)
H - hight

Sampling set A - 2b

incubation @ 25 C
vortex mixer
shaking time - 2 min.
centrifuging time - 4 min.

1 date	2 sample	3 feedstock	4 phenol solution conc.	5 added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l	
09-Jun-95	1	1	64000	0.005	0.32	DW	5	64	27205	44		
09-Jun-95	2	1	64000	0.005	0.32	DW	5	64	NA	NA		
09-Jun-95	3	1	64000	0.005	0.32	DW	5	64	19178	30		
09-Jun-95	4	1	64000	0.005	0.32	DW	5	64	14924	23		
09-Jun-95	4	1	64000	0.005	0.32	DW	5	64	29187	47		
09-Jun-95	4	1	64000	0.005	0.32	DW	5	64	46028	75		
09-Jun-95	5	1	64000	0.005	0.32	DW	5	64	32986	53	46	18.29
09-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	30358	49		
09-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	28906	47		
09-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	31359	51		
09-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	28441	46		
09-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	27859	45	47	2.40
09-Jun-95	11	1	64000	0.005	0.32	TW	5	64	35175	57		
09-Jun-95	12	1	64000	0.005	0.32	TW	5	64	29145	47		
09-Jun-95	13	1	64000	0.005	0.32	TW	5	64	11126	17		
09-Jun-95	14	1	64000	0.005	0.32	TW	5	64	29794	48		
09-Jun-95	15	1	64000	0.005	0.32	TW	5	64	30409	49	44	15.26
09-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	23575	38		
09-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	25009	40		
09-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	25581	41		
09-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	26661	43	40	2.13
09-Jun-95	20	1	64000	0.005	0.32	DWH	5	64	31810	51		
09-Jun-95	21	1	64000	0.005	0.32	DWH	5	64	31330	51		
09-Jun-95	22	1	64000	0.005	0.32	DWH	5	64	29399	47		
09-Jun-95	23	1	64000	0.005	0.32	DWH	5	64	30326	49		
09-Jun-95	24	1	64000	0.005	0.32	DWH	5	64	31131	50	50	1.59
09-Jun-95	BLF 25	1	N/A	N/A	N/A	DW	5	N/A	497	1		
09-Jun-95	BLF 26	1	N/A	N/A	N/A	DW	5	N/A	441	1		
09-Jun-95	BLF 27	1	N/A	N/A	N/A	DW	5	N/A	639	1		
09-Jun-95	BLF 28	1	N/A	N/A	N/A	DW	5	N/A	884	1	1	0.29

TW - tap water; BUFFER -solution of 95% of 0,1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v
DWH - deionized water hot at temperature 50 C; BLF -blank feedstock (unspiked0
H - hight
NA - not analysed

Sampling set A - 3

incubation @ 25 C
shaker, speed - 1600
shaking time - 2 min.
centrifuging time - 4 min.

1 date	2 sample no	3 feedstock g	4 phenol solution conc. mg/l	5 added mL	6 phenol added mg	7 extractant type	8 extractant volume mL	9 phenol conc. initial mg/l	10 HPLC reading H	11 phenol conc.anal. mg/l	12 phenol conc. average mg/l	13 standard deviation
15-Jun-95	1	1	64000	0.005	0.32	DW	5	64	18861	37		
15-Jun-95	2	1	64000	0.005	0.32	DW	5	64	20350	40		
15-Jun-95	3	1	64000	0.005	0.32	DW	5	64	21700	43		
15-Jun-95	4	1	64000	0.005	0.32	DW	5	64	2009	40		
15-Jun-95	5	1	64000	0.005	0.32	DW	5	64	20960	42	40	2.15
15-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	19869	39		
15-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	21584	43		
15-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	22341	44		
15-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	22335	44		
15-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	22622	45	43	2.26
15-Jun-95	11	1	64000	0.005	0.32	TW	5	64	18534	37		
15-Jun-95	12	1	64000	0.005	0.32	TW	5	64	20535	41		
15-Jun-95	13	1	64000	0.005	0.32	TW	5	64	23034	46		
15-Jun-95	14	1	64000	0.005	0.32	TW	5	64	22989	46		
15-Jun-95	15	1	64000	0.005	0.32	TW	5	64	22136	44	43	3.87
15-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	18189	39		
15-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	18534	40		
15-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	20984	45		
15-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	19951	43		
15-Jun-95	20	1	64000	0.005	0.32	BUFFER	5	64	21110	46	43	2.96
15-Jun-95	21	1	64000	0.005	0.32	DWH	5	64	19963	43		
15-Jun-95	22	1	64000	0.005	0.32	DWH	5	64	23018	50		
15-Jun-95	23	1	64000	0.005	0.32	DWH	5	64	22793	49		
15-Jun-95	24	1	64000	0.005	0.32	DWH	5	64	23392	51		
15-Jun-95	25	1	64000	0.005	0.32	DWH	5	64	21580	47	48	3.06
15-Jun-95	BLF 26	1	N/A	N/A	N/A	DWH	5	N/A	2134	4		
15-Jun-95	BLF 27	1	N/A	N/A	N/A	DWH	5	N/A	1803	4		
15-Jun-95	BLF 28	1	N/A	N/A	N/A	DWH	5	N/A	1834	4		
15-Jun-95	BLF 29	1	N/A	N/A	N/A	DWH	5	N/A	1824	4		
15-Jun-95	BLF 30	1	N/A	N/A	N/A	DWH	5	N/A	1887	4	4	0.29

NOTE: DW - deionized water; AW, pH=11.5 - alkaline water @pH=11.5
TW - tap water; BUFFER -solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v
DWH - deionized water hot at temperature 50 C; BLF -blank feedstock (uspiked)
H - hight

Sampling set A-4.

incubation @ 25C
shaker,speed - 1600
shaking time - 5 min.
centrifuging time - 4 min.

1 date	2 sample no	3 feedstock g	4 phenol conc. mg/l	5 solution added mL	6 phenol added mg	7 extractant type	8 extractant volume mL	9 phenol conc.initial mg/l	10 HPLC reading H	11 phenol conc.anal. mg/l	12 phenol conc. aver. mg/l	13 standard deviation
21-Jun-95	1	1	64000	0.005	0.32	DW	5	64	17123	35		
21-Jun-95	1	1	64000	0.005	0.32	DW	5	64	16537	33		
21-Jun-95	2	1	64000	0.005	0.32	DW	5	64	19072	39		
21-Jun-95	2	1	64000	0.005	0.32	DW	5	64	18458	37		
21-Jun-95	3	1	64000	0.005	0.32	DW	5	64	17944	36		
21-Jun-95	3	1	64000	0.005	0.32	DW	5	64	18418	37		
21-Jun-95	4	1	64000	0.005	0.32	DW	5	64	19712	40		
21-Jun-95	4	1	64000	0.005	0.32	DW	5	64	19939	40		
21-Jun-95	5	1	64000	0.005	0.32	DW	5	64	19389	39		
21-Jun-95	5	1	64000	0.005	0.32	DW	5	64	18219	37	37	2.25
21-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	18828	43		
21-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	19405	44		
21-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	17508	40		
21-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	17246	39		
21-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	18230	37		
21-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	17515	35		
21-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	17425	35		
21-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	18882	38		
21-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	18252	37		
21-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	18335	37	38	3.26
21-Jun-95	10	1	64000	0.005	0.32	TW	5	64	16442	41		
22-Jun-95	11	1	64000	0.005	0.32	TW	5	64	15341	38		
22-Jun-95	11	1	64000	0.005	0.32	TW	5	64	15365	38		
22-Jun-95	12	1	64000	0.005	0.32	TW	5	64	15059	37		
22-Jun-95	13	1	64000	0.005	0.32	TW	5	64	14771	37		
22-Jun-95	13	1	64000	0.005	0.32	TW	5	64	15097	38		
22-Jun-95	14	1	64000	0.005	0.32	TW	5	64	13939	35		
22-Jun-95	14	1	64000	0.005	0.32	TW	5	64	13375	33		

Sampling set A-4 (cont'd)

1	2	3	4	5	6	7	8	9	10	11	12	13
date	sample	feedstock	phenol conc.	solution added	phenol added	extractant type	extractant volume	phenol conc.initial	HPLC reading	phenol conc.anal.	phenol con. aver.	standard deviation
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l	
22-Jun-95	15	1	64000	0.005	0.32	TW	5	64	15689	39		
22-Jun-95	15	1	64000	0.005	0.32	TW	5	64	15212	38	37	2.17
22-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	13854	34		
22-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	13468	33		
22-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	14752	37		
22-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	14872	37		
22-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	12894	32		
22-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	13273	33		
22-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	15728	39		
22-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	16437	41		
22-Jun-95	20	1	64000	0.005	0.32	BUFFER	5	64	14893	37		
22-Jun-95	20	1	64000	0.005	0.32	BUFFER	5	64	15774	39	36	2.99
21-Jun-95	21	1	64000	0.005	0.32	DWH	5	64	19215	39		
21-Jun-95	21	1	64000	0.005	0.32	DWH	5	64	18629	37		
21-Jun-95	22	1	64000	0.005	0.32	DWH	5	64	19602	39		
21-Jun-95	22	1	64000	0.005	0.32	DWH	5	64	19588	39		
21-Jun-95	23	1	64000	0.005	0.32	DWH	5	64	18504	37		
21-Jun-95	23	1	64000	0.005	0.32	DWH	5	64	18207	36		
21-Jun-95	24	1	64000	0.005	0.32	DWH	5	64	20292	41		
21-Jun-95	24	1	64000	0.005	0.32	DWH	5	64	19920	40	39	1.50
21-Jun-95	BLF 26	1	N/A	N/A	N/A	DWH	5	N/A	1118	2		
21-Jun-95	BLF 26	1	N/A	N/A	N/A	DWH	5	N/A	1033	2		
21-Jun-95	BLF 27	1	N/A	N/A	N/A	DWH	5	N/A	982	2		
21-Jun-95	BLF 27	1	N/A	N/A	N/A	DWH	5	N/A	925	2		
21-Jun-95	BLF 28	1	N/A	N/A	N/A	DWH	5	N/A	1165	2		
21-Jun-95	BLF 28	1	N/A	N/A	N/A	DWH	5	N/A	1149	2		
21-Jun-95	BLF 29	1	N/A	N/A	N/A	DWH	5	N/A	1022	2		
21-Jun-95	BLF29	1	N/A	N/A	N/A	DWH	5	N/A	1091	2		
21-Jun-95	BLF 30	1	N/A	N/A	N/A	DWH	5	N/A	834	2		
21-Jun-95	BLF 30	1	N/A	N/A	N/A	DWH	5	N/A	616	1	2	0.31

NOTE: DW - deionized water; AW, pH=11.5 - alkaline water @pH=11.5
TW - tap water; BUFFER - solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v
DWH - deionized water hot at temperature 50 C; BLF -blank feedstock (unspiked0
H - hight

Sampling set A - 5

incubation @ 25 oC
shaker, speed - 1600
shaking time - 5 min.
centrifuging time - 4 min.

1 date	2 sample	3 feedstock	4 phenol conc.	5 solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc. initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc. average	13 standard deviation
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l	
28-Jun-95	1	1	64000	0.005	0.32	DW	5	64	17567	27		
28-Jun-95	1	1	64000	0.005	0.32	DW	5	64	22377	35		
28-Jun-95	2	1	64000	0.005	0.32	DW	5	64	24697	39		
28-Jun-95	2	1	64000	0.005	0.32	DW	5	64	23898	38		
28-Jun-95	3	1	64000	0.005	0.32	DW	5	64	24300	38		
28-Jun-95	3	1	64000	0.005	0.32	DW	5	64	23855	37		
28-Jun-95	4	1	64000	0.005	0.32	DW	5	64	24888	39		
28-Jun-95	4	1	64000	0.005	0.32	DW	5	64	23775	37		
28-Jun-95	5	1	64000	0.005	0.32	DW	5	64	25142	40		
28-Jun-95	5	1	64000	0.005	0.32	DW	5	64	24616	39	37	3.57
28-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	24168	38		
28-Jun-95	6	1	64000	0.005	0.32	AW,pH=11.5	5	64	23812	37		
28-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	23397	37		
28-Jun-95	7	1	64000	0.005	0.32	AW,pH=11.5	5	64	22781	36		
28-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	14805	30		
28-Jun-95	8	1	64000	0.005	0.32	AW,pH=11.5	5	64	14486	29		
29-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	20621	39		
29-Jun-95	9	1	64000	0.005	0.32	AW,pH=11.5	5	64	17079	32		
29-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	14045	27		
29-Jun-95	10	1	64000	0.005	0.32	AW,pH=11.5	5	64	13980	26	33	4.89
	Analysis on filtered samples											
29-Jun-95	11	1	64000	0.005	0.32	TW	5	64	38101	36		
29-Jun-95	11	1	64000	0.005	0.32	TW	5	64	35994	34		
29-Jun-95	12	1	64000	0.005	0.32	TW	5	64	36210	34		
29-Jun-95	12	1	64000	0.005	0.32	TW	5	64	29751	28		
29-Jun-95	12	1	64000	0.005	0.32	TW	5	64	31408	29		
29-Jun-95	12	1	64000	0.005	0.32	TW	5	64	37786	35		
29-Jun-95	12	1	64000	0.005	0.32	TW	5	64	34067	32		
29-Jun-95	13	1	64000	0.005	0.32	TW	5	64	31900	30		

Sampling set A - 5 (cont'd)

1 date	2 sample	3 feedstock	4 phenol conc.	5 solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc. average	13 standard deviation
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l	
	Analysis on fittered samples using quard column.											
30-Jun-95	14	1	64000	0.005	0.32	TW	5	64	38931	51		
30-Jun-95	14	1	64000	0.005	0.32	TW	5	64	38496	51	36	8.43
30-Jun-95	15	1	64000	0.005	0.32	BUFFER	5	64	31965	42		
30-Jun-95	15	1	64000	0.005	0.32	BUFFER	5	64	28543	38		
30-Jun-95	15	1	64000	0.005	0.32	BUFFER	5	64	27922	37		
30-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	29245	38		
30-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	25846	34		
30-Jun-95	16	1	64000	0.005	0.32	BUFFER	5	64	25292	33		
30-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	35765	47		
30-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	31439	41		
30-Jun-95	17	1	64000	0.005	0.32	BUFFER	5	64	33130	44		
30-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	30398	40		
30-Jun-95	18	1	64000	0.005	0.32	BUFFER	5	64	28816	38		
30-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	33215	44		
30-Jun-95	19	1	64000	0.005	0.32	BUFFER	5	64	31997	42	40	4.02
11-Jul-95	20	1	64000	0.005	0.32	DWH	5	64	32697	44		
11-Jul-95	20	1	64000	0.005	0.32	DWH	5	64	28480	38		
12-Jul-95	21	1	64000	0.005	0.32	DWH	5	64	32254	41		
12-Jul-95	21	1	64000	0.005	0.32	DWH	5	64	30049	38		
12-Jul-95	21	1	64000	0.005	0.32	DWH	5	64	27916	35		
12-Jul-95	22	1	64000	0.005	0.32	DWH	5	64	36606	46		
12-Jul-95	22	1	64000	0.005	0.32	DWH	5	64	31106	39		
12-Jul-95	22	1	64000	0.005	0.32	DWH	5	64	30309	38		
12-Jul-95	23	1	64000	0.005	0.32	DWH	5	64	34537	44		
12-Jul-95	23	1	64000	0.005	0.32	DWH	5	64	33098	42		
12-Jul-95	23	1	64000	0.005	0.32	DWH	5	64	27934	35		
12-Jul-95	24	1	64000	0.005	0.32	DWH	5	64	36430	46		
12-Jul-95	24	1	64000	0.005	0.32	DWH	5	64	30829	39		
12-Jul-95	24	1	64000	0.005	0.32	DWH	5	64	27619	35	40	3.9
30-Jun-95	BLF 25	1	N/A	N/A	N/A	DWH	5	N/A	N/A	N/A		
30-Jun-95	BLF 25	1	N/A	N/A	N/A	DWH	5	N/A	N/A	N/A		
12-Jul-95	BLF 26	1	N/A	N/A	N/A	DWH	5	N/A	0.00	0.0		
12-Jul-95	BLF 26	1	N/A	N/A	N/A	DWH	5	N/A	0.00	0.0		
12-Jul-95	BLF 27	1	N/A	N/A	N/A	DWH	5	N/A	0.00	0.0		
12-Jul-95	BLF 27	1	N/A	N/A	N/A	DWH	5	N/A	0.00	0.0		

Sampling set A - 5 (cont'd)

1 date	2 sample	3 feedstock	4 phenol conc.	5 solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc. average	13 standard deviation
	no	g	*mg/l*	*mL*	*mg*		*mL*	*mg/l*	H	*mg/l*	*mg/l*	
12-Jul-95	BLF 28	1	N/A	N/A	N/A	DWH	5	N/A	2023.00	2.5		
12-Jul-95	BLF 29	1	N/A	N/A	N/A	DWH	5	N/A	2880.00	3.5	3	1.28
		1										

NOTE: DW - deionized water; AW, pH=11.5 - alkaline water @pH=11.5
TW - tap water; BUFFER - solution of 95% of 0.1 N sodium phosphate and 5% acetonitrile in a solution with deionized water 1:1 v/v
DWH - deionized water hot at temperature 50 C; BLF -blank feedstock (unspiked)
H - hight

Sampling set B - 1

incubation @ 25 C
shaker, speed - 1600
shaking time - 1,2,5,10, 30 min.
centrifuging time - 4 min.

1 date	2 sample no	3 feedstock g	4 phenol conc. mg/l	5 solution added mL	6 phenol added mg	7 extractant type	8 extractant volume mL	9 phenol con.initial mg/l	10 HPLC reading H	11 phenol conc.anal. mg/l	12 phenol conc.aver. mg/l	13 standard deviation	15 shaking time min
13-Jul-95	1	1	64000	0.005	0.32	DW	5	64	18497	27			1
13-Jul-95	1	1	64000	0.005	0.32	DW	5	64	19384	29			1
13-Jul-95	2	1	64000	0.005	0.32	DW	5	64	25117	37			1
13-Jul-95	2	1	64000	0.005	0.32	DW	5	64	22892	34	32	4.57	1
13-Jul-95	3	1	64000	0.005	0.32	DW	5	64	24587	36			2
13-Jul-95	3	1	64000	0.005	0.32	DW	5	64	20981	31			2
13-Jul-95	4	1	64000	0.005	0.32	DW	5	64	22140	33			2
13-Jul-95	4	1	64000	0.005	0.32	DW	5	64	19554	29	32	5.28	2
13-Jul-95	5	1	64000	0.005	0.32	DW	5	64	27952	41			5
13-Jul-95	5	1	64000	0.005	0.32	DW	5	64	23957	35			5
13-Jul-95	6	1	64000	0.005	0.32	DW	5	64	25686	38			5
13-Jul-95	6	1	64000	0.005	0.32	DW	5	64	22229	33	37	3.63	5
13-Jul-95	7	1	64000	0.005	0.32	DW	5	64	28370	42			10
13-Jul-95	7	1	64000	0.005	0.32	DW	5	64	25566	38			10
13-Jul-95	8	1	64000	0.005	0.32	DW	5	64	25774	38			10
13-Jul-95	8	1	64000	0.005	0.32	DW	5	64	27412	40	39	2.00	10
13-Jul-95	9	1	64000	0.005	0.32	DW	5	64	30714	45			30
13-Jul-95	9	1	64000	0.005	0.32	DW	5	64	28810	43			30
13-Jul-95	10	1	64000	0.005	0.32	DW	5	64	29949	44			30
13-Jul-95	10	1	64000	0.005	0.32	DW	5	64	28430	42	43	1.56	30
14-Jul-95	11	1	64000	0.005	0.32	AW,pH=11.5	5	64	32907	51			1
14-Jul-95	11	1	64000	0.005	0.32	AW,pH=11.5	5	64	29357	45			1
14-Jul-95	12	1	64000	0.005	0.32	AW,pH=11.5	5	64	32331	50			1
14-Jul-95	12	1	64000	0.005	0.32	AW,pH=11.5	5	64	30994	48	48	2.43	1
14-Jul-95	13	1	64000	0.005	0.32	AW,pH=11.5	5	64	31218	48			2
14-Jul-95	13	1	64000	0.005	0.32	AW,pH=11.5	5	64	30013	46			2
14-Jul-95	14	1	64000	0.005	0.32	AW,pH=11.5	5	64	32549	50			2
14-Jul-95	14	1	64000	0.005	0.32	AW,pH=11.5	5	64	28154	43	47	2.88	2
14-Jul-95	15	1	64000	0.005	0.32	AW,pH=11.5	5	64	30557	47			5
14-Jul-95	15	1	64000	0.005	0.32	AW,pH=11.5	5	64	33103	51			5

Sampling set B - 1 (cont'd)

1 date	2 sample	3 feedstock	4 phenol conc.	5 solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation	15 shaking time
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l		min
14-Jul-95	16	1	64000	0.005	0.32	AW,pH=11.5	5	64	30079	46			5
14-Jul-95	16	1	64000	0.005	0.32	AW,pH=11.5	5	64	28991	45	47	2.68	5
14-Jul-95	17	1	64000	0.005	0.32	AW,pH=11.5	5	64	34543	53			10
14-Jul-95	17	1	64000	0.005	0.32	AW,pH=11.5	5	64	32905	51			10
14-Jul-95	18	1	64000	0.005	0.32	AW,pH=11.5	5	64	33411	51			10
14-Jul-95	18	1	64000	0.005	0.32	AW,pH=11.5	5	64	33495	52	52	1.06	10
14-Jul-95	19	1	64000	0.005	0.32	AW,pH=11.5	5	64	36651	56			30
14-Jul-95	19	1	64000	0.005	0.32	AW,pH=11.5	5	64	35385	54			30
14-Jul-95	20	1	64000	0.005	0.32	AW,pH=11.5	5	64	34843	54			30
14-Jul-95	20	1	64000	0.005	0.32	AW,pH=11.5	5	64	33559	52	54	1.97	30
17-Jul-95	BLF 21	1	N/A	N/A	N/A	DWH	5	N/A	ND				1
17-Jul-95	BLF 21	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			1
17-Jul-95	BLF22	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			1
17-Jul-95	BLF 22	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			1
17-Jul-95	BLF 23	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			2
17-Jul-95	BLF 23	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			2
17-Jul-95	BLF 24	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			2
17-Jul-95	BLF24	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			2
17-Jul-95	BLF 25	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			5
17-Jul-95	BLF 25	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			5
17-Jul-95	BLF 26	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			5
17-Jul-95	BLF 26	1	N/A	N/A	N/A	DWH	5	N/A	ND	ND			5
17-Jul-95	BLF 27	1	N/A	N/A	N/A	DWH	5	N/A	291	0			10
18-Jul-95	BLF 27	1	N/A	N/A	N/A	DWH	5	N/A	289	0			10
18-Jul-95	BLF 28	1	N/A	N/A	N/A	DWH	5	N/A	429	1			10
18-Jul-95	BLF 28	1	N/A	N/A	N/A	DWH	5	N/A	388	1	1	0.17	10
18-Jul-95	BLF 29	1	N/A	N/A	N/A	DWH	5	N/A	403	1			30
18-Jul-95	BLF29	1	N/A	N/A	N/A	DWH	5	N/A	334	1			30
18-Jul-95	BLF 30	1	N/A	N/A	N/A	DWH	5	N/A	451	1			30
18-Jul-95	BLF 30	1	N/A	N/A	N/A	DWH	5	N/A	477	1	1	0.12	30

NOTE - DW - deionized water; AW,pH=11.5 - alkaline water @ pH=11.5
DWH - deionized water hot @ 50C; BLF - blank feedstock (unspiked)
H- hight, ND - not detected

Sampling set B-2

incubation @ 25 C
shaker, speed - 1600
shaking time - 1,2,5,10, 30 min.
centrifuging time - 4 min.

1 date	2 sample	3 feedstock	4 phenol conc.	5 solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol con.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation	15 shaking time
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l		min
21-Aug-95	1	1	64000	0.005	0.32	TW	5	64	236323	30			1
21-Aug-95	1	1	64000	0.005	0.32	TW	5	64	227813	24			1
21-Aug-95	2	1	64000	0.005	0.32	TW	5	64	259531	33			1
21-Aug-95	2	1	64000	0.005	0.32	TW	5	64	255190	33	30	4.22	1
21-Aug-95	3	1	64000	0.005	0.32	TW	5	64	262049	34			2
21-Aug-95	3	1	64000	0.005	0.32	TW	5	64	270634	35			2
21-Aug-95	4	1	64000	0.005	0.32	TW	5	64	283570	37			2
21-Aug-95	4	1	64000	0.005	0.32	TW	5	64	287338	36	35	1.35	2
22-Aug-95	5	1	64000	0.005	0.32	TW	5	64	298536	38			5
22-Aug-95	5	1	64000	0.005	0.32	TW	5	64	311685	40			5
22-Aug-95	6	1	64000	0.005	0.32	TW	5	64	296733	38			5
22-Aug-95	6	1	64000	0.005	0.32	TW	5	64	289496	37	38	1.02	5
22-Aug-95	7	1	64000	0.005	0.32	TW	5	64	330528	42			10
22-Aug-95	7	1	64000	0.005	0.32	TW	5	64	348039	44			10
22-Aug-95	8	1	64000	0.005	0.32	TW	5	64	307932	39			10
22-Aug-95	8	1	64000	0.005	0.32	TW	5	64	324521	41	42	2.11	10
22-Aug-95	9	1	64000	0.005	0.32	TW	5	64	453868	58			30
22-Aug-95	9	1	64000	0.005	0.32	TW	5	64	392269	50			30
22-Aug-95	10	1	64000	0.005	0.32	TW	5	64	430170	55			30
22-Aug-95	10	1	64000	0.005	0.32	TW	5	64	458910	58	55	3.88	30
21-Aug-95	11	1	64000	0.005	0.32	BUFFER	5	64	271888	35			1
21-Aug-95	11	1	64000	0.005	0.32	BUFFER	5	64	272605	35			1
21-Aug-95	12	1	64000	0.005	0.32	BUFFER	5	64	280603	36			1
21-Aug-95	12	1	64000	0.005	0.32	BUFFER	5	64	293470	38	36	1.34	1
21-Aug-95	13	1	64000	0.005	0.32	BUFFER	5	64	303949	39			2
21-Aug-95	13	1	64000	0.005	0.32	BUFFER	5	64	311682	40			2
21-Aug-95	14	1	64000	0.005	0.32	BUFFER	5	64	238946	31			2
21-Aug-95	14	1	64000	0.005	0.32	BUFFER	5	64	238005	31	35	5.36	2
21-Aug-95	15	1	64000	0.005	0.32	BUFFER	5	64	373818	49			5
21-Aug-95	15	1	64000	0.005	0.32	BUFFER	5	64	365124	48			5
21-Aug-95	16	1	64000	0.005	0.32	BUFFER	5	64	376808	49			5
21-Aug-95	16	1	64000	0.005	0.32	BUFFER	5	64	385860	50	49	1.14	5

Sampling set B-2(cont'd)

1 date	2 sample	3 feedstock	4 phenol conc.	5 solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol conc.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation	15 shaking time
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l		min
21-Aug-95	17	1	64000	0.005	0.32	BUFFER	5	64	386542	50			10
21-Aug-95	17	1	64000	0.005	0.32	BUFFER	5	64	386276	50			10
21-Aug-95	18	1	64000	0.005	0.32	BUFFER	5	64	379247	49			10
21-Aug-95	18	1	64000	0.005	0.32	BUFFER	5	64	376439	49	50	0.41	10
21-Aug-95	19	1	64000	0.005	0.32	BUFFER	5	64	480927	63			30
21-Aug-95	19	1	64000	0.005	0.32	BUFFER	5	64	439044	57			30
21-Aug-95	20	1	64000	0.005	0.32	BUFFER	5	64	477426	63			30
21-Aug-95	20	1	64000	0.005	0.32	BUFFER	5	64	455899	60	61	2.62	30
21-Aug-95	BLF 21	1	N/A	N/A	N/A	BUFFER	N/A	N/A	36036	4			1
21-Aug-95	BLF21	1	N/A	N/A	N/A	BUFFER	N/A	N/A	33198	4			1
21-Aug-95	BLF22	1	N/A	N/A	N/A	BUFFER	N/A	N/A	42827	5			1
21-Aug-95	BLF 22	1	N/A	N/A	N/A	BUFFER	N/A	N/A	40794	5	5	0.53	1
21-Aug-95	BLF23	1	N/A	N/A	N/A	BUFFER	N/A	N/A	23755	3			2
21-Aug-95	BLF23	1	N/A	N/A	N/A	BUFFER	N/A	N/A	33682	4			2
21-Aug-95	BLF24	1	N/A	N/A	N/A	BUFFER	N/A	N/A	58833	7			2
21-Aug-95	BLF24	1	N/A	N/A	N/A	BUFFER	N/A	N/A	52645	6	5	1.95	2
21-Aug-95	BLF25	1	N/A	N/A	N/A	BUFFER	N/A	N/A	42363	5			5
21-Aug-95	BLF25	1	N/A	N/A	N/A	BUFFER	N/A	N/A	26445	3			5
21-Aug-95	BLF26	1	N/A	N/A	N/A	BUFFER	N/A	N/A	45967	5			5
21-Aug-95	BLF26	1	N/A	N/A	N/A	BUFFER	N/A	N/A	40770	5	5	0.98	5
21-Aug-95	BLF 27	1	N/A	N/A	N/A	BUFFER	N/A	N/A	61684	7			10
21-Aug-95	BLF27	1	N/A	N/A	N/A	BUFFER	N/A	N/A	57735	7			10
21-Aug-95	BLF28	1	N/A	N/A	N/A	BUFFER	N/A	N/A	45880	5			10
21-Aug-95	BLF28	1	N/A	N/A	N/A	BUFFER	N/A	N/A	34643	4	6	1.47	10
21-Aug-95	BLF29	1	N/A	N/A	N/A	BUFFER	N/A	N/A	63028	8			30
21-Aug-95	BLF29	1	N/A	N/A	N/A	BUFFER	N/A	N/A	64106	8			30
21-Aug-95	BLF30	1	N/A	N/A	N/A	BUFFER	N/A	N/A	65749	8			30
21-Aug-95	BLF30	1	N/A	N/A	N/A	BUFFER	N/A	N/A	56115	7	7	0.51	30

NOTE:TW - tap water; BUFFER - solution of 95% of 0.1 N sodium phosphate and 5%acetonitrile in a solution with deionized water 1:1 v/v ;
H - hight, BLF - blank feedstock (unspiked)

Sampling set B-3

incubation @ 25 C
shaker, speed - 1600
shaking time - 1,2,5,10, 30 min.
centrifuging time - 4 min.

1 date	2 sample	3 feedstock	4 phenol solution conc.	5 added	6 phenol added	7 extractant type	8 extractant volume	9 phenol con.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation	15 shaking time
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l		min
24-Aug-95	1	1	64000	0.005	0.32	DWH	5	64	260286	32			1
24-Aug-95	1	1	64000	0.005	0.32	DWH	5	64	247902	30			1
24-Aug-95	2	1	64000	0.005	0.32	DWH	5	64	267358	33			1
24-Aug-95	2	1	64000	0.005	0.32	DWH	5	64	262862	32	32	0.99	1
24-Aug-95	3	1	64000	0.005	0.32	DWH	5	64	348928	42			2
24-Aug-95	3	1	64000	0.005	0.32	DWH	5	64	340597	41			2
24-Aug-95	4	1	64000	0.005	0.32	DWH	5	64	287847	35			2
24-Aug-95	4	1	64000	0.005	0.32	DWH	5	64	306090	37	39	3.44	2
24-Aug-95	5	1	64000	0.005	0.32	DWH	5	64	410174	50			5
24-Aug-95	5	1	64000	0.005	0.32	DWH	5	64	401376	49			5
24-Aug-95	6	1	64000	0.005	0.32	DWH	5	64	346496	42			5
24-Aug-95	6	1	64000	0.005	0.32	DWH	5	64	358117	43	46	3.75	5
24-Aug-95	7	1	64000	0.005	0.32	DWH	5	64	412237	50			10
24-Aug-95	7	1	64000	0.005	0.32	DWH	5	64	400723	48			10
24-Aug-95	8	1	64000	0.005	0.32	DWH	5	64	311511	38			10
24-Aug-95	8	1	64000	0.005	0.32	DWH	5	64	313481	38	44	6.08	10
24-Aug-95	9	1	64000	0.005	0.32	DWH	5	64	445110	54			30
24-Aug-95	9	1	64000	0.005	0.32	DWH	5	64	459232	55			30
24-Aug-95	10	1	64000	0.005	0.32	DWH	5	64	411060	50			30
24-Aug-95	10	1	64000	0.005	0.32	DWH	5	64	394492	48	52	3.57	30
24-Aug-95	BLF21	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	NA	NA			1
24-Aug-95	BLF21	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	NA	NA			1
24-Aug-95	BLF22	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	53711	7			1
24-Aug-95	BLF22	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	55826	7	7	0.19	1
24-Aug-95	BLF23	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	56571	7			2
24-Aug-95	BLF23	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	59955	8			2
24-Aug-95	BLF24	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	44711	6			2
24-Aug-95	BLF24	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	45129	6	7	1.00	2
25-Aug-95	BLF25	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	59837	6			5
25-Aug-95	BLF25	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	52842	6			5
25-Aug-95	BLF26	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	87080	9			5
25-Aug-95	BLF26	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	83469	9	7	1.78	5

Sampling set B-3 (cont' d)

1 date	2 sample no	3 feedstock g	4 phenol solution conc. mg/l	5 added mL	6 phenol added mg	7 extractant type	8 extractant volume mL	9 phenol conc.initial mg/l	10 HPLC reading H	11 phenol conc.anal. mg/l	12 phenol conc.aver. mg/l	13 standard deviation	15 shaking time min
25-Aug-95	BLF27	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	85444	9			10
25-Aug-95	BLF27	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	80429	8			10
25-Aug-95	BLF28	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	93077	10			10
25-Aug-95	BLF28	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	78510	8	9	0.59	10
25-Aug-95	BLF29	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	86277	9			30
25-Aug-95	BLF29	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	88227	9			30
25-Aug-95	BLF30	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	111478	12			30
25-Aug-95	BLF30	1	N/A	N/A	N/A	AW,pH=11.5	N/A	N/A	105725	11	10	1.26	30

NOTE: DWH - deionized hot water @ 50 C; AW,pH=11.5 -alkaline water at pH=11.5.
BLF - blank feedstock (unspiked)
NA - not analysed

Sampling set B-4

incubation @ 25 C
shaker, speed - 1600
shaking time - 1,2,5,10, 30 min.
centrifuging time - 4 min.

1 date	2 sample	3 feedstock	4 phenol conc.	5 solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol con.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation	15 shaking time
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l		min
28-Aug-95	1	1	64000	0.005	0.32	DWH	5	64	419421	42			1
28-Aug-95	1	1	64000	0.005	0.32	DWH	5	64	388503	39			1
28-Aug-95	2	1	64000	0.005	0.32	DWH	5	64	336213	33			1
28-Aug-95	2	1	64000	0.005	0.32	DWH	5	64	316446	31	36	4.75	1
28-Aug-95	3	1	64000	0.005	0.32	DWH	5	64	395315	39			2
28-Aug-95	3	1	64000	0.005	0.32	DWH	5	64	407732	41			2
28-Aug-95	4	1	64000	0.005	0.32	DWH	5	64	376578	37			2
28-Aug-95	4	1	64000	0.005	0.32	DWH	5	64	375104	37	39	1.58	2
28-Aug-95	5	1	64000	0.005	0.32	DWH	5	64	449346	45			5
28-Aug-95	5	1	64000	0.005	0.32	DWH	5	64	452909	45			5
28-Aug-95	6	1	64000	0.005	0.32	DWH	5	64	372277	37			5
28-Aug-95	6	1	64000	0.005	0.32	DWH	5	64	378937	38	41	4.39	5
28-Aug-95	7	1	64000	0.005	0.32	DWH	5	64	344093	34			10
28-Aug-95	7	1	64000	0.005	0.32	DWH	5	64	362189	36			10
28-Aug-95	8	1	64000	0.005	0.32	DWH	5	64	405924	40			10
28-Aug-95	8	1	64000	0.005	0.32	DWH	5	64	424464	42	38	3.75	10
28-Aug-95	9	1	64000	0.005	0.32	DWH	5	64	473623	47			30
28-Aug-95	9	1	64000	0.005	0.32	DWH	5	64	460087	46			30
28-Aug-95	10	1	64000	0.005	0.32	DWH	5	64	491275	49			30
28-Aug-95	10	1	64000	0.005	0.32	DWH	5	64	475155	47	47	1.28	30
28-Aug-95	BLF11	1	N/A	N/A	N/A	DWH	5	N/A	47651	5			1
28-Aug-95	BLF11	1	N/A	N/A	N/A	DWH	5	N/A	53559	5			1
28-Aug-95	BLF12	1	N/A	N/A	N/A	DWH	5	N/A	28186	3			1
28-Aug-95	BLF12	1	N/A	N/A	N/A	DWH	5	N/A	28306	3	4	1.27	1
28-Aug-95	BLF13	1	N/A	N/A	N/A	DWH	5	N/A	64080	6			2
28-Aug-95	BLF13	1	N/A	N/A	N/A	DWH	5	N/A	59157	6			2
28-Aug-95	BLF14	1	N/A	N/A	N/A	DWH	5	N/A	41514	4			2
28-Aug-95	BLF14	1	N/A	N/A	N/A	DWH	5	N/A	40407	4	5	1.17	2
28-Aug-95	BLF15	1	N/A	N/A	N/A	DWH	5	N/A	92250	9			5
28-Aug-95	BLF15	1	N/A	N/A	N/A	DWH	5	N/A	86176	8			5
28-Aug-95	BLF16	1	N/A	N/A	N/A	DWH	5	N/A	77458	7			5
28-Aug-95	BLF16	1	N/A	N/A	N/A	DWH	5	N/A	69420	7	8	0.96	5

Sampling set B-4 (cont'd)

1 date	2 sample	3 feedstock	4 phenol solution conc.	5 phenol solution added	6 phenol added	7 extractant type	8 extractant volume	9 phenol con.initial	10 HPLC reading	11 phenol conc.anal.	12 phenol conc.aver.	13 standard deviation	15 shaking time
	no	g	mg/l	mL	mg		mL	mg/l	H	mg/l	mg/l		min
28-Aug-95	BLF17	1	N/A	N/A	N/A	DWH	5	N/A	68815	7			10
28-Aug-95	BLF17	1	N/A	N/A	N/A	DWH	5	N/A	67854	7			10
28-Aug-95	BLF18	1	N/A	N/A	N/A	DWH	5	N/A	78690	8			10
28-Aug-95	BLF18	1	N/A	N/A	N/A	DWH	5	N/A	75223	7	7	0.47	10
28-Aug-95	BLF19	1	N/A	N/A	N/A	DWH	5	N/A	87789	8			30
28-Aug-95	BLF19	1	N/A	N/A	N/A	DWH	5	N/A	89776	9			30
28-Aug-95	BLF20	1	N/A	N/A	N/A	DWH	5	N/A	84512	8			30
28-Aug-95	BLF20	1	N/A	N/A	N/A	DWH	5	N/A	83620	8	8	0.29	30

NOTE: DWH - deionized ,hot water @ 50C; BLF - blank feedstock.
H - hight, BLF - blank feedstock (unspiked)

ANOVA

```
data set6;
  infile '/home/u1/dmurphy/trybula/set6.dat';
  input sample test extract $ shaktime phenol;
run;

proc print;
run;

proc glm;
 class extract shaktime sample;
 model phenol = extract|shaktime sample(extract*shaktime);
run;

data set8;
  infile '/home/u1/dmurphy/trybula/set8.dat';
  input sample test extract $ shaktime phenol;
run;

proc print;
run;

proc glm;
 class extract shaktime sample;
 model phenol = extract|shaktime sample(extract*shaktime);
run;

data set9;
  infile '/home/u1/dmurphy/trybula/set9.dat';
  input sample test extract $ shaktime phenol;
run;

proc print;
run;

proc glm;
 class extract shaktime sample;
 model phenol = extract|shaktime sample(extract*shaktime);
run;

data set10;
  infile '/home/u1/dmurphy/trybula/set10.dat';
  input sample test extract $ shaktime phenol;
run;

proc print;
run;

proc glm;
 class shaktime sample;
 model phenol = shaktime sample(shaktime);
run;
```

General Linear Models Procedure

Dependent Variable: PHENOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	2318.19278750	122.01014671	18.03	0.0001
Error	20	135.31165000	6.76558250		
Corrected Total	39	2453.50443750			

R-Square	C.V.	Root MSE	PHENOL Mean
0.944850	6.028155	2.60107334	43.14875000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
EXTRACT	1	1665.51930250	1665.51930250	246.18	0.0001
SHAKTIME	4	496.45045000	124.11261250	18.34	0.0001
EXTRACT*SHAKTIME	4	60.68591000	15.17147750	2.24	0.1007
SAMPL(EXTRAC*SHAKTI)	10	95.53712500	9.55371250	1.41	0.2448

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXTRACT	1	1665.51930250	1665.51930250	246.18	0.0001
SHAKTIME	4	496.45045000	124.11261250	18.34	0.0001
EXTRACT*SHAKTIME	4	60.68591000	15.17147750	2.24	0.1007
SAMPL(EXTRAC*SHAKTI)	10	95.53712500	9.55371250	1.41	0.2448

SAMPLING SET B-1

variance components due to sampling error (observed)- 9.5537
variance components due to analytical error (observed)- 6.7656

$$9.5537 = \sigma_\tau^2 + 2\,\sigma_e^2 \quad (*)$$

where: σ_τ^2 - variance component due to analytical error
σ_e^2 - variance component due to sampling error
2 - number of tests per sample

The estimate of sampling error variance component comes from solving equation (*):

$\sigma_\tau^2 = 6.766$

$\sigma_e^2 = (9.5537 - 6.766) \times 0.5 = 1.394$

The total variation is the sum of the estimated variance components

$\sigma_t^2 = \sigma_\tau^2 + \sigma_e^2 = 6.766 + 1.394 = 8.16$ where: σ_t -total variation

From the total variations, the proportion associated with analytical error:

$\sigma_\tau^2 / (\sigma_\tau^2 + \sigma_e^2) = 6.766/8.16 = 0.829$

From the total variations, the proportion associated with sampling error:

$\sigma_e^2 / (\sigma_\tau^2 + \sigma_e^2) = 1.394/8.16 = 0.171$

General Linear Models Procedure

Dependent Variable: PHENOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	3718.59535000	195.71554474	44.92	0.0001
Error	20	87.13220000	4.35661000		
Corrected Total	39	3805.72755000			

R-Square	C.V.	Root MSE	PHENOL Mean
0.977105	4.840840	2.08724939	43.11750000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
EXTRACT	1	368.20624000	368.20624000	84.52	0.0001
SHAKTIME	4	3063.86130000	765.96532500	175.82	0.0001
EXTRACT*SHAKTIME	4	135.29341000	33.82335250	7.76	0.0006
SAMPL(EXTRAC*SHAKTI)	10	151.23440000	15.12344000	3.47	0.0086

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXTRACT	1	368.20624000	368.20624000	84.52	0.0001
SHAKTIME	4	3063.86130000	765.96532500	175.82	0.0001
EXTRACT*SHAKTIME	4	135.29341000	33.82335250	7.76	0.0006
SAMPL(EXTRAC*SHAKTI)	10	151.23440000	15.12344000	3.47	0.0086

SAMPLING SET B-2

variance component due to sampling error (observed) - 15.1234
variance component due to analytical error (observed) - 4.3566

$$15.1234 = \sigma_\tau^2 + 2\,\sigma_e^2 \quad (*)$$

where: σ_τ^2 - variance component due to analytical error
σ_e^2 - variance component due to sampling error
2 - number of tests per sample

The estimate of sampling error variance component comes from solving equation (*):

$\sigma_\tau^2 = 4.3566$

$\sigma_e^2 = (15.1234 - 4.3566) \times 0.5 = 5.384$

The total variation is the sum of the estimated variance components

$\sigma t^2 = \sigma_\tau^2 + \sigma_e^2 = 4.3566 + 5.384 = 9.7406$ where: σ_t -total variation

From the total variations, the proportion associated with analytical error:

$\sigma_\tau^2 / (\sigma_\tau^2 + \sigma_e^2) = 4.3566/9.7406 = 0.447$

From the total variations, the proportion associated with sampling error:

$\sigma_e^2 / (\sigma_\tau^2 + \sigma_e^2) = 5.384/9.7406 = 0.553$

General Linear Models Procedure

Dependent Variable: PHENOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	19	2871.80904750	151.14784461	189.04	0.0001
Error	20	15.99115000	0.79955750		
Corrected Total	39	2887.80019750			

R-Square	C.V.	Root MSE	PHENOL Mean
0.994463	2.072968	0.89417979	43.13525000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
EXTRACT	1	26.29262250	26.29262250	32.88	0.0001
SHAKTIME	4	2405.88573500	601.47143375	752.26	0.0001
EXTRACT*SHAKTIME	4	110.60976500	27.65244125	34.58	0.0001
SAMPL(EXTRAC*SHAKTI)	10	329.02092500	32.90209250	41.15	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
EXTRACT	1	26.29262250	26.29262250	32.88	0.0001
SHAKTIME	4	2405.88573500	601.47143375	752.26	0.0001
EXTRACT*SHAKTIME	4	110.60976500	27.65244125	34.58	0.0001
SAMPL(EXTRAC*SHAKTI)	10	329.02092500	32.90209250	41.15	0.0001

SAMPLING SET B-3

variance component due to sampling error (observed) - 32.9021
variance component due to analytical error (observed) - 0.7996

$$32.9021 = \sigma_\tau^2 + 2\,\sigma_e^2 \quad (*)$$

where: σ_τ^2 - variance component due to analytical error
σ_e^2 - variance component due to sampling error
2 - number of tests per sample

The estimate of sampling error variance component comes from solving equation (*):

$\sigma_\tau^2 = 0.70996$

$\sigma_e^2 = (32.9021 - 0.7996) \times 0.5 = 16.0512$

The total variation is the sum of the estimated variance components

$\sigma t^2 = \sigma_\tau^2 + \sigma_e^2 = 0.7996 + 16.0512 = 16.851$ where: σ_t -total variation

From the total variations, the proportion associated with analytical error:

$\sigma_\tau^2 / (\sigma_\tau^2 + \sigma_e^2) = 0.7996/16.851 = 0.047$

From the total variations, the proportion associated with sampling error:

$\sigma_e^2 / (\sigma_\tau^2 + \sigma_e^2) = 16.05120/16.851 = 0.953$

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General Linear Models Procedure

SAMPLING SET B-4

Dependent Variable: PHENOL

Source	DF	Sum of Squares	Mean Square	F Value	Pr > F
Model	9	459.88690500	51.09854500	37.96	0.0001
Error	10	13.45975000	1.34597500		
Corrected Total	19	473.34665500			

R-Square	C.V.	Root MSE	PHENOL Mean
0.971565	2.880135	1.16016163	40.28150000

Source	DF	Type I SS	Mean Square	F Value	Pr > F
SHAKTIME	4	293.23108000	73.30777000	54.46	0.0001
SAMPLE(SHAKTIME)	5	166.65582500	33.33116500	24.76	0.0001

Source	DF	Type III SS	Mean Square	F Value	Pr > F
SHAKTIME	4	293.23108000	73.30777000	54.46	0.0001
SAMPLE(SHAKTIME)	5	166.65582500	33.33116500	24.76	0.0001

variance component due to sampling error (observed) - 33.3312
variance component due to analytical error (observed) - 1.346

$33.3312 = \sigma_\tau^2 + 2\,\sigma_e^2$ (*)

where: σ_τ^2 - variance component due to analytical error
σ_e^2 - variance component due to sampling error
2 - number of tests per sample

The estimate of sampling error variance component comes from solving equation (*):

$\sigma_\tau^2 = 1.346$

$\sigma_e^2 = (33.3312 - 1.346) \times 0.5 = 15.993$

The total variation is the sum of the estimated variance components

$\sigma t^2 = \sigma_\tau^2 + \sigma_e^2 = 1.346 + 15.993 = 17.339$ where: σ_t -total variation

From the total variations, the proportion associated with analytical error:

$\sigma_\tau^2 / (\sigma_\tau^2 + \sigma_e^2) = 1.346/17.339 = 0.0776$

From the total variations, the proportion associated with sampling error:

$\sigma_e^2 / (\sigma_\tau^2 + \sigma_e^2) = 15.993/17.339 = 0.922$