THE UNIVERSITY OF MANITOBA

CONTROL OF TURBIDITY AND BETA-AMYLASE IN POTATO RINSE WATER RECYCLING

by

SONIA Y.C. LUI

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

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TO MY BROTHER

WHO SUPPORTED AND ENCOURAGED ME

THROUGHOUT MY UNIVERSITY YEARS

ABSTRACT

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by

Sonia Y.C. Lui

Stringent government regulations and guidelines on water pollution now call for a higher degree of water purification and recycling in the food industry, prior to disposal. The "total system approach" is the most ideal solution to meet these needs. Methods for enabling process waters to be used for long periods, while maintaining good quality control, are now required.

A laboratory study on potato rinse water recycling was carried out, primarily to control turbidity problems during recycling. The rinse water was recycled with intermittent carbon treatment. It was shown that a carbon dosage of 1g/l applied after every rinse was efficient in suppressing the build-up of turbidity for up to 8 hours at 22° C during reuse; while simultaneously allowing the levels of organics to accumulate to an equilibrium level. An alternate treatment of 5g/l of carbon after every fifth rinse was also found to be satisfactory in controlling the turbidity of potato rinse water, under these conditions.

Turbidity levels of the recycled potato rinse water (with intermittent carbon treatment) increased to an unacceptable level when stored

at 22°C for 16 hours. It was shown by millipore filtration that bacterial growth played a major role in causing the development of turbidity.

An increased amount of beta-amylase in recycled potato rinse water was found to support a higher population of bacterial growth, through release of glucose from starch. Carbon treatment alone was not sufficient for the adequate removal of beta-amylase. It was found that the pH of the recycled potato rinse water should be lowered to at least 4.5 with citric acid, or a combined treatment of citric acid and powdered activated carbon should be used, before the water was to be stored for more than 16 hours at 22°C, for further reuse purposes.

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INTRODUCTION

1.1 U.S. Environmental Legislation Relating to Industry Effluents

During the late 1975's and through the 1976's, several U.S. Congressional Acts were passed. Most of these Acts were the precursors to the environmental legislation established to date. The Federal Water Pollution Control Act Amendments (FWPCA) 1967 publications, series No. 3 (89) stated that by 1972, there should be removals of 68 percent of biochemical oxygen demand, 77 percent of the suspended solids and about 19 percent of the total dissolved solids in the wastes, and it was projected for 1977 that 73 percent of the biochemical oxygen demand, 82 percent of the suspended solids, and 25 percent of the total dissolved solids would be removed. On October 18, 1972, the Federal Water Pollution Control Act Amendments of 1972 were established by the Congress (89). According to the Act, by July 1, 1977, the extent of the aforementioned parameters in the effluents should be reduced through the use of "best available technology economically achievable". By July 1983, the limitations are based on application of the "best available technology economically achievable" and zero discharge. Later, in addition to the parameters included in the publication of 1972, the guidelines stated that some other components in the effluents must be considered. This included color, total phosphate, fecal coliforms, temperature, total organic compounds and total dissolved substances. In order to meet this goal, the Administrator of the Environmental Protection Agency (EPA) has established a set of

effluent guidelines and limitations. These limitations are to be based on pollutant reductions which are attainable through in-plant process changes, and wastewater treatment (74).

1.2 Canada Food Industry Effluent Guidelines (1)

Environment Canada also has developed guidelines and regulations for the food industries. The aim of the Regulations and Guidelines is to ensure that the food processing plants operating in Canada apply "best practicable process and treatment technology" in their plants. The "end-of-pipe" loadings are to be minimized through the installation of "best practicable process technology". To date, the guidelines for the effluents of the meat and fish processing industries have been established and gazetted. It is expected that the guidelines for the dairy and fruit and vegetable industries will be gazetted in the near future. The regulations and guidelines for the potato processing have already been established. The maximum daily discharge in lb/ton is set at biochemical oxygen demand (B.O.D.), 9.5, total dissolved solids (T.S.S.), 17.5 in 1977, and B.O.D., 1.6 and T.S.S., 2.7 in 1983 (8). Any plants which come on stream after the guidelines are in force, must comply with the new regulations and guidelines while existing plants will be treated in exactly the same manner as plants falling under existing Environment Canada regulations and guidelines.

1.3 U.S. Programs on Compliance with Regulations

In dealing with guidelines listed in section 1.1, a national

program was established by the U.S. Food Processing Industry and Environment Protection Agency. In 1970, the First National Symposium on Food Processing Waste was held at Oregon (90). The purpose of this Symposium was to develop a cooperative, coordinated program between industry and government, in solving water pollution problems of the food industries. The latest efforts to reduce water pollution from the food processing industry were also being reviewed. The objectives of the First Symposium included the modification of conventional methods of waste treatment; the development of industrial methods to reduce the quantity of water required for processing; the introduction of completely closed-loop systems and the development of by-products recovery.

At the Fourth Symposium in 1973, it was recommended that a "total system approach", as suggested by Gallop (91), was the most ideal solution to water and waste management in the food processing industry. The concept of the "total system" is that the entire plant is considered as a whole with the waste product ranked as equally important as the commercial products. The process water is recycled with in-plant treatment, such as physical methods, e.g. centrifugation and filtration; physicochemical methods, e.g. activated carbon adsorption. Solid wastes produced could be recovered and sold as animal feed, or coverted into activated carbon for use within the plant (90, 74).

Environment Canada is hoping that plants will not follow the conventional biological treatment approach, but will take a close look at in-plant controls and physical/chemical treatment

alternatives, in order to eventually come to a total recycle and reuse system. Many programs such as DPAT (Development and Demonstration of Pollution Abatement Technology) and UP (Unsolicited Proposal) programs are underway, with steady movement towards employment of technology that will lead to maximal by-product recovery, with water conservation and reuse.

1.4 Purpose of Project

In the case of potato processing, Gallop (32) showed that the potato plant water system can be closed by 90% or more. Most of the water can be reused to make up a cyclic system. Partial purification (i.e. removal of heat, troublesome solids, or inactivation of enzymes, or removal of organisms) is only required for recycling of effluents, unless the final effluent is required for drinking purposes. For potato processing, partial purification includes controlling:

(1) Physical factors e.g. color, turbidity, silt.

(2) Chemical factors e.g. potato enzymes, starch, sugars, proteins.

(3) Biochemical/microbiological factors e.g. enzymes, microorganisms.

(4) Aesthetic and legally significant factors.

Previous and current work in this Department, has shown the merits of using activated carbons, especially in the powdered form, for rapidly, efficiently, and cheaply purifying such recycled water, in every respect, by "subtractive" methods. But with repetitive use, the "background" levels of various factors in the water, must change quantitatively, proportionally and possibly in type too. Since these interact closely with the surface of the potato slices, they also can affect the composition beneficially or adversely. In

addition, the ability of the water to change, in every respect, during repetitive use, especially over extended times, of days, weeks and months is also dependent on the interactions between the constituents of a flow, which can occur during time. For instance, the ability of recycled water to sustain microbial flora and growth, is very much dependent on the composition of the water, as regards energy sources and micronutrients.

In this project, research was focussed on the potato rinse water produced at the slice-rinse stage, to study a problem of turbidity which developed, during repetitive use over extended times (8-72 hours), with a view to recommending a possible solution.

Beta-amylase is one of the hydrolytic enzymes in potato rinse water that causes the breakdown of starch into maltose. This molecule can easily be broken down by maltase into glucose units and utilized by bacteria as one of its essential nutrients. Beta-amylase was therefore studied in this project, with a view to investigating its ability to sustain bacterial growth in potato rinse water, and to develop possible methods for controlling its activity, if the potato rinse water is intended for repetitive use over extended time periods.

LITERATURE REVIEW

2.1 History of Activated Carbon

Historically, activated carbon was used in medicine since 1550 B.C. as mentioned in an Egyptian papyrus (15). The earliest recognition of the adsorptive phenomenon of carbon was in 1773 when Scheele (37) observed the uptake of gases by charcoal. In 1785, attention was drawn to the adsorptive effect of carbon on solutions by Lowitz (56). The evidence of the use of carbon in food industries dated back to the early 17th century when wood char was employed for purification purposes in the cane and beet sugar industries (23).

Activated carbon was first produced from wood and bone (23). During the 19th century, studies were made to produce activated carbon from various other sources, including petroleum residues, wood, coal on a laboratory scale (20, 43). Due to engineering difficulties, activated carbon could not be prepared in commercial scale until 1901, when Ostrejko (68) developed a modern method of manufacturing commercial activated carbon using steam activation. Today there are many patented thermal processes for production of carbon (37).

During the First World War, constant efforts were made to generate highly activated carbons for use in gas-mask filters. From then on, activated carbons of improved quality have been used on a commercial scale in the purification of gases and liquids. Today activated carbon is being recommended for use on a wide scale for water purification since an Environment Protection Agency survey has

reported the drinking water of over eighty cities in the United States contain small amounts of carcinogenic substances (6). A standard of not more than 150 parts per billion for chloroform and other Trihalomethane (THM) chemicals has been established. It is required that the cities should filter the water through the granulated activated carbon bed, instead of the traditional sand filtering bed, unless they can show that their supplies are not significantly polluted by industrial or agricultural chemical sources (7). The nation-wide construction costs of the required carbon filters in U.S. waterworks are estimated to be $350–450 million and annual operation costs are budgeted to be $60 million.

2.2 Elementary Aspects of Adsorption of Carbon

Adsorption is described as the phenomenon by which the molecules of gases, or dissolved substances of liquids are taken up by physical or chemical forces to the surfaces of solids or liquids with which they are in contact (37). If the adsorbent has a very porous structure, it will have a large surface area for the adsorption of molecules.

Activated carbon was described by Weber (94) as a highly porous material. Two types of pore size exist in activated carbon particles. The macropores are large, having diameters of 30–10,000 Angstrom units, they permeate the carbon particles and allow for solute diffusion, but contribute little to the surface area. The micropores are 10–30 Angstrom units in diameter. The boundary surfaces of the micropores are largely responsible for the adsorption action of the carbon. This

type of pores contributes to a surface area of approximately 400-1000 sq. m/g for an activated carbon particle.

Three kinetic steps occur consecutively in the adsorption process: transport of solute to the outer surface of the adsorbent, diffusion through the pore spaces (macropores), and the adsorption occurring at an active site on the surfaces around the inner pore space of adsorbent.

2.3 Use of Activated Carbon in Food Industries

Activated carbon was first used in the sugar industry over a hundred years ago for the removal of color and organic contaminants (37). It was observed by Owen (37) that microorganisms in cane juice could also be removed by activated carbon.

Mercer et al. (64) and Fox (30) reported the purification of brines for reuse, by using activated carbon filters to adsorb phenolics. Activated carbon is used extensively in sugar and syrup industries nowadays. Treatment with activated carbon reduces the content of protein, hydroxymethyl furfural, iron, lime and gives a stable, colorless syrup which does not darken with age (37). Schultz et al. (79) reported the use of activated carbon for adsorbing volatiles in commercial apple essence. In the fat and oil industry, activated carbon is employed for adsorbing the impurities which interfere with the complete neutralization of the fatty acids in the subsequent treatment with alkali (47). Lapter (52) reported that carbon could remove soap and other substances which had detrimental effect on the catalyst when the oil was subsequently hydrogenated. Blumenthal (18) reported carbon could be used to maintain quality standards of

alcoholic beverages. He found that cloudy wines could be improved by a 48-hours contact with small quantities of activated carbon. Ballos (12) reported that treatment of beer with activated carbon could remove the chill-sensitive protein precipitates.

2.4 Use of Activated Carbon in Industrial Waste Treatment

Since industrial wastes contain highly toxic and refractory contaminants, the use of conventional biological treatment systems for these industrial wastes will not always be effective (33). Treatment by activated carbon is an effective method of purifying industrial wastes. It has been used widely in the major areas such as food, textile, paper, chemical, petroleum and metal industries (35).

Hager in 1976 (35) conducted a laboratory adsorption study of 107 industrial wastewaters. Activated carbon effected the following dissolved organic contaminant reductions: 85% of total organic compounds in 79 of 102 samples (77%), 95% color removal in 16 of 16 samples (100%). In addition, he found that up to 99% of toxic chemicals were removed from synthetic waste in 9 out of 9 samples using the activated carbon treatment.

Carbon treatment is an effective method for the removal of organic chemicals including a wide range of organophosphorus, organochloride and polycyclic aromatic hydrocarbons compounds (4, 24). Cheremisinoff (20) reported that most of the dissolved organic toxic chemicals as cited by the Environmental Protection Agency can be removed from water by activated carbon. References for the use of activated carbon in treating industrial wastes are now very numerous.

The Water Pollution Control Federation Annual Literature Review (2) issues updated references on a variety of these applications including the industrial treatments of food, paper, textile and petroleum wastes.

2.5 Use of Activated Carbon in Municipal Waste Treatment

Activated carbon is used in municipal waste treatment. Since carbon preferentially removes the bio-resistant compounds (e.g. polycyclic organics, such as phenols, tars, oils, etc.), it can be used as a complementary partner to biological waste treatment (44).

Both laboratory and full scale field evaluations performed from 1972 to 1974, have shown that the addition of powdered activated carbon to anaerobic digestors is beneficial and can reduce sludge disposal costs. Because activated carbon is so porous, it provides sites for the anaerobic reaction to occur. By adsorbing organics such as grease and scum that can clog digestors, the efficiency of the system will be comparatively high. By the activated carbon adsorption of inhibitory substances that could be toxic to the anaerobic bacteria, the working capacity of existing digestion tanks is increased (3).

Powdered carbon systems also show promising applications in physicochemical treatment of either raw sewage or primary effluent. But it appears that it is not competitive with granular carbon systems for tertiary system (84). Suhr and Culp (84) made a study on the use of powdered carbon in the treatment of municipal wastewaters. They found that carbon contact as preceded by chemical coagulation

and sedimentation of the raw waste, the influent soluble chemical oxygen demand (COD) of 80 to 100 mg/l was reduced to 12 mg/l with 300 mg/l of powdered carbon and to 30 mg/l with 75 mg/l of carbon. The laboratory process of utilizing powdered carbon has been evaluated on a pilot scale in Albany, New York (80). The raw wastewater following comminution and grit removal, is contacted with powdered carbon, coagulated with alum, settled with a polymeric settling aid and passed through a trimedia filter. The results showed that in using doses of 600 mg/l of carbon, 200 mg/l of alum, and 2.5 mg/l of polyelectrolyte, total organic compound removal is greater than 90% and the turbidity of the settled effluent is seldom greater than one Jackson turbidity unit (J.T.U.).

2.6 Use of Activated Carbon in Treatment of Food Industrial Wastewaters

The constituents of food plant wastes are highly rich in carbohydrates, fats and proteins. The common practice of disposing waste effluents into rivers and lakes causes serious pollution problems. A report recently revealed that 45% of water pollution is caused by industry, of which 85% of the total is contributed by food processing effluents (4). Conventional biological treatment processes are mainly used to treat food processing wastewaters. However, the Legislative Guidelines in the U.S. have set standards for a higher degree of purification which cannot be achieved alone by the conventional treatment methods. In order to meet the deadlines set for 1977, 1983 and 1985, the U.S. food processing industry in 1970 began a national program of pollution control methods capable

of higher degrees of treatment. Activated carbon was recommended for use in the advanced treatment of food industrial wastewater (90).

Sugumoto et al. (83) compared the granular and ball-type activated carbon in their treatment of meat processing wastes. They found that the removal of COD using both types of activated carbon was 60%.

Berry et al. (17) investigated sand filtration and granular activated carbon treatment of chiller water and total effluent from poultry processing operation. He found that after sand filtration and activated carbon treatment, the total organic compound and suspended solid removals were 75 to 77 and 97 to 99% respectively in chiller water.

Soderquist in 1971 (81) investigated the feasibility of renovating maraschino cherry brines with granular activated carbon. The processing maraschino cherries involved bleaching with a strong sulfur dioxide before coloring and flavoring. The spent brine was difficult to handle in conventional waste treatment facilities. Preliminary results indicated that the brine could be successfully renovated by treatment with carbon.

Mercer et al. in 1970 (64) conducted an experiment on the reconditioning of spent olive storage brines in a commercial scale and evaluation of their reuse potential. Results of their experiment showed that there were substantial reductions in suspended solids and COD in the lye rinse water and transport brine after carbon treatment. These treated rinse waters were reused in commercial production with no detectable effect on the quality of the canned olives as judged by production personnel. Therefore, they suggested

that the use of carbon treatment for reconditioning liquid wastes was promising in reducing the quantity of water required as well as in reducing saline pollution.

A series of Japanese patents described waste treatment processes by electrolysis followed by activated carbon treatment (59). Adsorption of activated carbon can be used to complement electrolytic oxidation, in the treatment of cheese whey. Adsorption of lactose in cheese whey with activated carbon was shown to be comparatively weak. But the electrolytic oxidation products of lactose were readily and strongly adsorbed by carbon. These oxidation products of lactose consisted largely of carboxylic acids which were produced by the electrolytic oxidation of whey.

Hydamaka et al. (45) discussed the potential of a closed-loop recycle system at the rinse stage of potato processing as part of the "total systems" concept approach to the food industry. This laboratory-scale study found activated carbon to be an effective substance in removing polyphenolase which causes browning in potato water.

2.7 Measurement of Turbidity

The term turbidity is applied to waters containing suspended matter which interferes with the passage of light through the water or in which visual depth is restricted. Turbidity is caused by a wide variety of suspended materials, which range in size from colloidal to coarse dispersions. These materials can be organic, inorganic or microbiological in nature (78).

2.7.1 Application of Turbidity Measurements

Turbidity is an important consideration in the following three major areas.

(1) Water Treatment

Turbidity is an important consideration in public water supplies for the following reasons (78):

(i) Any turbidity in drinking water is usually associated with sewage pollution which can cause water-borne epidemics.

(ii) High turbidity will cause difficulties in filtering through sand filter and thus increase filtering costs.

(iii) Pathogenic organisms may be encased in the suspended particles and be prevented from exposure to the disinfectant.

For these reasons, the U.S. Public Health Service (92) has placed a limit of 5 units of turbidity as the maximum amount allowable in public water supplies.

In December 1974 the Safe Drinking Water Act became law in the U.S. One primary component in Protection Agency regulations for drinking water became effective in June, 1977 in the States. The regulations require that the suppliers of water for both community and non-community water systems sample water for turbidity measurement at representative entry points to the distribution system at least once a day (5).

(2) Sewage Treatment

Turbidity measurement is used to check the performance of clarifiers and to report the final effluent quality (53).

(3) Industrial Applications

The description of the quality of water for industrial uses is incomplete without some measurement of the visual clarity such as turbidity (5). In the beverage industry and plastics manufacturing industry, where clarity is desirable for aesthetic and commercial reasons, turbidity measurement provides an efficient method for quality control (61). Measurement of turbidity can be used industrially for microbiological and cell growth studies. It is also used for the detection of contaminants such as oil in water or moisture in oil (53).

Turbidity is an expression of the optical property of a sample which causes light to be scattered and absorbed rather than transmitted in straight lines through the sample (53). The standard method for the determination of turbidity has been based on the Jackson candle turbidimeter. However, the lowest turbidity value that can be measured on this instrument is 25 units. The nephelometer is one standard instrument for measurement of low turbidities. These nephelometers are relatively unaffected by small changes in design parameters. There is no basis for calibration of nephelometer units in terms of candle units since there is no direct relationship between the intensity of light scattered at 90^{0} and the Jackson candle turbidity. The units derived from the nephelometer and visual methods should be expressed as nephelometer turbidity units and Jackson turbidity units respectively. The nephelometric method is of greater precision, sensitivity, and applicability over a wide range of turbidity. The candle turbidimeter is limited in the examination of highly turbid waters since its lowest limit is 25 turbidity units.

A formazin polymer is used as the reference standard for the nephelometric method. The turbidity of a given concentration of formazin suspension is defined as 40 nephelometer units. This same suspension of formazin has an approximate turbidity of 40 Jackson units when measured on the candle turbidimeter. Therefore nephelometric turbidity units with a formazin suspension used as a reference will approximate units determined from the candle turbidimeter but will not be identical to them (9).

2.7.2 Relationship Between Bacteria and Turbidity

The occurrence of turbidity is attributed, to some extent by bacterial growth. It was shown by Kitahara et al. (50) that the bacterial strain M-25 isolated from soy-sauce mash, and M-160 from soy-koji can cause turbidity in soy-sauce.

Turbidity measurements have been used to estimate the germicidal effect of cetyltrimethyl ammonium bromide (CTAB) on yeast cells. The turbidity change observed was accounted for by the change in cell volume resulting from cell degradation (31).

Watson et al. (93) reported that the growth of a bacterial cell could be recorded by a simple inexpensive turbidity system. They stated that the system responded linearly to changes in bacterial concentration over a hundred range, up to fully grown cultures and this provided an adequate range for most purposes.

Measurement of turbidity is a common and convenient means for estimating the concentration of a bacterial population. The first systematic use of microbial populations was by Alper and Sterne in

1933 (8). Forrest and Stephen (28) also described the use of a nephelometer for the continuous recording of the turbidity of a suspension of growing microorganisms. Djurici and Emira (23) applied the turbidimetric method for counting the number of bacteria in various media.

Arthur (11) stated that in an ideal turbidimeter, the turbidity of a given dry weight of cells would be nearly independent of size, to the first approximation. In such circumstances, the turbidity was a measure of the solid concentration, but not of cell number concentration unless constant growth conditions were maintained.

2.8 Properties of Alpha- and Beta-Amylases

Alpha-amylase can be isolated from potato tuber homogenate by extraction at pH 5.0 with acetate buffer followed by glycogen precipitation and chromatography on Sephadex G75. The molecular weight is 46, 000. The optimum pH is 5.5-6.0 and the optimum temperature is 42° C (26). As early as 1938, Sukhorukov et al. (80) demonstrated the presence of a water soluble amylase inhibitor in leaves and tubers of potatoes. The physical and chemical properties were examined by Hemberg et al. (39). However, its identity remains obscure. The inhibitor repressed the activity of alpha-amylase but did not have any inhibitory effect on beta-amylase.

Beta-amylase is distributed in various species ranging from microorganisms to higher plants and animals, mainly in higher plants. Purified beta-amylases have been obtained from barley (66), wheat (65), sweet potato (13) and soybean (70). Meyer et al. (65) indicated

relationships between pH and activity, pH and stability and temperature and activity of the wheat and barley beta-amylases. It was noted that the stability dropped off very sharply at pH levels between 3 and 4. Piguet (72) indicated that neither prolonged dialysis nor treatment with chelating agents could remove any low-molecular-weight substance essential for beta-amylase activity. However, the activity was rapidly destroyed by SH reagents. Sweet potato beta-amylase crystallized from ammonium sulfate was found by Balls et al. (13) to be octahedral in shape. Fan (27) partially purified beta-amylase from fresh potato homogenate, by acetate buffer extraction, dialysis, ammonium sulfate fractionation, chromatography on Sephadex G75 and CM-cellulose. The approximate molecular weight calculated by Sephadex G150 is 122,000. The optimum pH is 5.1-5.5 and the optimum temperature is 55° C. The enzyme was found to be highly sensitive to acidic conditions at which pH was lower than 4.5 and basic conditions where pH was greater than 6.5.

2.9 Adsorption of Enzymes

Adsorption of enzymes can occur at air-water, water-oil, and water-solid interfaces and it can be achieved by simply bringing an enzyme solution in contact with the adsorbent (41, 96). The adsorption of enzymes onto water-insoluble supports is an exceedingly simple method operationally. The binding forces between enzyme and adsorbent in most cases are extremely weak and often causes little or no enzymatic inactivation. The activity of adsorbed enzymes can vary anywhere from nil to fairly high values (55).

The mechanism of the adsorption process is often multivariant and it is at times difficult to differentiate clearly between the various possibilities. Adsorption of enzymes onto water-insoluble matrices can be attributed to an ion-exchange mechanism, to simple physical adsorption at the external surface of a particle, or to "physicochemical bonds" created by hydrophobic interactions, Van der Waals attractive forces, etc. (62, 95). The mode of adsorption will depend greatly on the nature of the water-insoluble carrier.

The adsorbents which are either organic or inorganic in nature often require special pre-treatments in order to insure good adsorption. The adsorption of an enzyme onto a water-insoluble material is dependent on such experimental variables as pH, nature of the solvent, ionic strength, concentration of protein and adsorbent, and temperature. Good discussions of these variables have been given by Zittle (95), James and Augenstein (47), McLaren et al. (62) and Hummel and Anderson (41).

2.10 Composition of Potato Tubers

Potatoes are made up of 77.5% water with the major portion of the solid fraction existing as starch (86). The starch content comprises 65 to 80% of the total dry solids, and consists of 20% soluble amylose and 80% insoluble amylopectin (49). The composition of potato is shown as follows:

Constituent	Composition (%)
Water	77.5
Carbohydrate	19.4
Protein	2.0
Fat	0.1
Ash	1.0

Starch can be directly broken down by the enzyme diastase into the disaccharide maltose and then to the monosaccharide glucose by further hydrolysis with the enzyme maltase.

In a biological system, amylases and phosphorlyase act upon starch components to produce products more easily assimilated by microorganisms (15).

Other compounds are also present in the potato. The reducing sugar content of white potatoes constitutes 10% of the potato's dry weight (86). In regard to nitrogen, only 2% of the potato's weight is protein and accounts for one-third to one-half of the total nitrogen present. The remaining nitrogen is largely in the form of free amino-acids. Phosphorus, potassium and other elements are present in smaller quantities.

2.11 Water Reuse in Food Industry

The concluding remarks of the Fourth National Symposium on Food Processing Wastes in 1973 (91) suggested that the total system approach, as presented by Dr. Gallop, is the ideal solution to water and waste management in the industry. Hunt (42) stated that the total system concept is economically compatible, especially on a long

term basis. He also mentioned that the high water demands and high growth rate of water use in American industry, cannot continue to rely solely on traditional water sources for supply and at the same time as a depository for waste effluents. Industries should reuse water as an alternative for making in-plant pollution control possible. This approach has been employed by a sugar cane plant in Hawaii for effluent pollutant control and has proven to be successful (48). This system has also been developed in the paper industry and is considered to be the most promising answer to water pollution (82).

Esvelt and Hart (25) reported on wastewater reuse at Snokist Growers Cannery. The wastewater treatment system was monitored for performance during the 1974, 1975 and 1976 processing seasons. The results of a two-year study on the reuse of treated process wastewater showed the reclaimed water quality to be suitable for use in the following areas of the fruit cannery:

(1) Initial fruit wash and conveying
(2) Washdown of fruit peeling and conveying equipment
(3) Steam generation boiler feed
(4) Floor and gutter wash
(5) Direct contact container cooking

Reclaimed water reuse during the 1976 process season resulted in approximately 35% reduction in wastewater discharged.

McFeeters et al. (60) developed recycling procedures for brines as a means of minimizing the waste generated to tankyards in cucumber fermentation. They compared chemical and pasteurization procedures in commercial brining operations to evaluate the adequacy of the

treatment procedures. They found no difference between the cucumber obtained from pasteurized as opposed to chemically treated brine. The pasteurized treatment will allow up to 95% recovery. Data from 1975 experiments did not indicate any build-up of compounds as a result of recycling, the presence of which would have limited the application of recycling procedures.

Clise (21) demonstrated the installation of recycling water in a poultry processing line. The reclamation system consisted of aerated lagoons, followed by microstraining, flocculation, sedimentation, and filtration with two stages of chlorination. The initial study showed that the reclaimed water met U.S. Public Health Service 1962 Drinking Water Standards for chemical, microbiological and physical constituents.

Concern was shown in regards to the possibility of build up of pathogenic microorganisms, heavy metals, pesticides and toxic organic chemicals during recycling in the processing plant. Further research by Andelman and Clise (10), showed that no pathogenic bacteria were detected in the recycled water. All the coliform and fecal coliform concentrations were below the level of detection. They reported that the renovation process can deliver a safe and potable water and therefore posed no threat to human health.

Hamaza (36) reported on a case study on water reuse in poultry processing. The results showed that water used for cooling the compressors can be reused in the scalder without treatment as it is virtually free from contamination. Use of renovated water can enhance the quality of process water in these operations, besides

saving part of the energy required for cooling and heating processes.

The preparation of olives for canning creates a strong liquid which is high in both biochemical oxygen demand and sodium choride content. A pilot-scale brine reconditioning plant was constructed at four olive canneries in the Central Valley of California and was studied by Mercer et al. (64). The lye rinse water and transport brine were treated with activated carbon resulting in substantial reductions in suspended solids and chemical oxygen demand. These treated rinse waters were reused in commercial production with no detectable effect on the quality of the canned olives as judged by production personnel.

Lash et al. (54) reported on the use of a recycling system for potato processing wastewater. Water and sewer charges were directly reduced as a result of the recycling system. The tertiary treatment process demonstrated that the biochemical oxygen demand and suspended solids were essentially eliminated from the wastewater.

Advances have been made by the potato processing industry in pollution control at the slice-rinse stage, by means of recycling of wash water and recovery of solid wastes. The C-E Bauer Company reported that with the use of cyclones and a hydrasieve screen, a potato processing plant received $439 per day in solid waste products, and saved $18,000 per year in sewage charges (88). The Sweco recovery system has been installed in the Perfect Potato Chip Plant in Decatur, Illinois. A gross revenue of $10,000 through starch reclamation is expected each year (71).

Hydamaka et al. (45) studied the control of color problems

during recycling of potato waters. They reported that the combined treatment of pH and carbon proved to be beneficial. Activated carbon was found to be an essential ingredient in the control of color, chemical oxygen demand, odor, foam and bacterial build-up that resulted from continuous reuse.

2.12 Water Reuse in Potato Processing Plant

The in-plant control of potato processing effluent has been implemented by the industry in order to reduce waste loads and to recover valuable by-products (88). Water reuse and by-product recovery in each unit operation are discussed in the following sections:

(1) Fluming and Washing

Wastewater from these operations is amenable for separation and recycling since the waste stream from these operations contributes a minimal organic load. Grames (34) stated that the waste from these operations is amenable to separate treatment and recycling. Settling tanks and cyclone separators have been used for the removal of silt and sand from these waste streams, and approximately 70% of the wastewater is allowed to be reused. Fossum (29) reported that treatment efficiency of the settling tanks is improved with the addition of anionic polymer.

(2) Peeling

Kropp (51) demonstrated the in-plant screening of peel wastes through 40 mesh screens. At a low rate of 18.9 meters per hour, the suspended solids concentration was reduced from 4610 to 1760 mg/l.

At 9.6 meters per hour, it was reduced from 1850 to 800 mg/l. In both cases, approximately 60% of the suspended solids were removed by the screen. The waste potato product was reported by the Canada Department of Agriculture to be useful as cattle feed (67).

(3) Slicing

Taylor (87) recommended the use of hydrasieves and hydrocyclones for concentrating the dilute starch slurry from the slicer waste, recovering suspended solids and allowing the wastewater to be reused. Payne (69) showed the use of reverse osmosis is feasible for concentrating and recovering starch and other soluble compounds in the slicer waste. Hautala (38) reported that the concentrate could be used as cattle feed to offset treatment costs. Vacuum flash evaporation has been employed in pilot plant studies to concentrate soluble components in the recycled slicer waste (38).

(4) Blanching

The waste constituents of this unit operation are mostly soluble organic load. Attempts that have been made thus far to reduce the waste load have been proven to be unsuccessful (57).

SCOPE OF INVESTIGATION

Activated carbon has been shown to have great potential for the removal of a wide range of substances from water samples and was therefore used in this study, in an attempt to control the turbidity problem and the beta-amylase level in recycled potato rinse water. This study was divided into two sections under which the following specific areas were investigated:

Section 1

(1) Investigating the feasibility of extended recycling of potato rinse water and one of its related problems, namely, the occurrence of turbidity when the rinse water is left in storage for periods of time at room temperature.

(2) Establishment of turbidity control with activated carbon, millipore filtration and other treatment methods.

Section 2

(3) Investigation of the effect of beta-amylase on the breakdown of starch and its subsequent effect on bacterial growth.

(4) Study of build-up in beta-amylase level in multiple rinses of potato rinse water and its subsequent reduction with application of activated carbon.

Throughout the study, twenty-five J.T.U.* were arbitarily chosen as the turbidity standard based on visual judgement. Any value exceeding this limit was taken as turbid, and any values under

*Jackson turbidity unit

this limit was considered to be clear.

METHODS AND MATERIALS

3.1 MATERIALS

Source of Potatoes

Netted Gem potatoes were obtained from the Manitoba Vegetable Producer's Marketing Board, Winnipeg.

Source of Powdered Activated Carbon

The adsorbent chosen for use in this study was Purifying Darco-S-51 Charcoal obtained from Atlas Chemical Industries, Inc. Wilmington, Delaware, U.S.A.

Source of Enzymes

Beta-amylase (from sweet potato), obtained from Worthington Biochemical Corporation, Freehold, New Jersey, U.S.A. It was supplied as a suspension in 0.6 saturated ammonium sulfate at pH 3.7. Minimum specific activity is 500 units/mg protein.

Diastase (from malt), obtained from Fisher Scientific Co., Limited, Winnipeg, Manitoba.

Chemicals

All laboratory tests were performed using analytical grade chemicals. Soluble starch powder was obtained from Fisher Scientific Co., Limited, Winnipeg, Manitoba.

Centrifugation

Potato rinse waters were centrifuged on Sorvall Superspeed-RC2-B Automated Refrigerated Centrifuge for 5 minutes at 1000 r.p.m. (1200xg) at 22°C.

Bacterial cultures were centrifuged on Sorvall Superspeed-RC2-B Automated Refrigerated Centrifuge for 10 minutes (6600xg) at 22°C.

Shakers

The shaking of potato rinse waters with powdered activated carbon was performed at 300 r.p.m. for 3 minutes on a New Brunswick Scientific (model C-33) Laboratory Rotary Shaker having a 3/4 in. (1.9 cm) circular orbit stroke at 22°C.

Dialyzing Tubing

The dialyzing tubing (D1615-2), obtained from Canlab was 3.3 cm in width and 24 Angstroms in pore radius. It cuts off molecules with molecular weights ranging from 80,000 - 100,000.

Sterilization

All equipment were sterilized in a Barnstead autoclave for 15 minutes at 15 psi. Potato rinse waters were sterilized via millipore filtration using a 0.2 mu filter in conjunction with a hydrosol stainless filter holder unit.

Filtration

All carbon-treated potato rinse waters were separated from powdered activated carbon by filtering through the Whatman (GF/C) glass filter paper with pore size 1.2 mu, using vacuum filtration.

Turbidity Measurement

Turbidity measurements were performed on the DRT-100 Turbidimeter at 22°C. The minimal sample size (25 ml) was placed into the flat bottom borosilicate glass sample cuvet and ready for measurement. The meter is sensitive to a change of 0.01 Jackson turbidity unit (J.T.U.). The repeatability of the instrument is ±1% full scale.

CO_2D Determination

CO_2D is defined as the amount of oxygen demand expressed in mg/l as determined by "Precision" Aquarator. It was used to determine the amount of total organics in the potato rinse waters in this project.

Calibration of CO_2D Standard Curve

A stock solution was prepared by dissolving 2.127 gm of sodium acetate trihydrate in distilled water and diluted to one litre.

The standard solutions were made up for calibration by pipetting the following amounts of the stock solution into separate 100-ml volumetric flasks and diluted to the 100-ml mark.

Flask#	Stock Solution ml.
1	5
2	10
3	15
4	20
5	25
6	30

A calibration curve with units of peak height versus mg/litre oxygen demand was plotted for the determination of 20 microlitres of water sample. (Figure 1)

The Aquarator method is efficient in measuring oxygen demand in the range of 10 to 30 mg/litre. If the oxygen demand of the sample is greater than 300 mg/litre range of the unit, a simple dilution of the original sample is required. A detailed description of the operation of the "Precision" Aquarator was described in the Aquarator manual (73).

3.2 Chemical Analysis

Determination of Beta-Amylase (17, 27)

Definition: One unit of specific amylase activity was defined in this study as the amount of enzyme which will produce 1 mg of maltose from a 1% starch solution in one hour at pH 5.5 in sodium acetate buffer.

Method: The rate at which maltose is produced from starch was measured by its ability to reduce 3,5-dinitrosalicyclic acid.

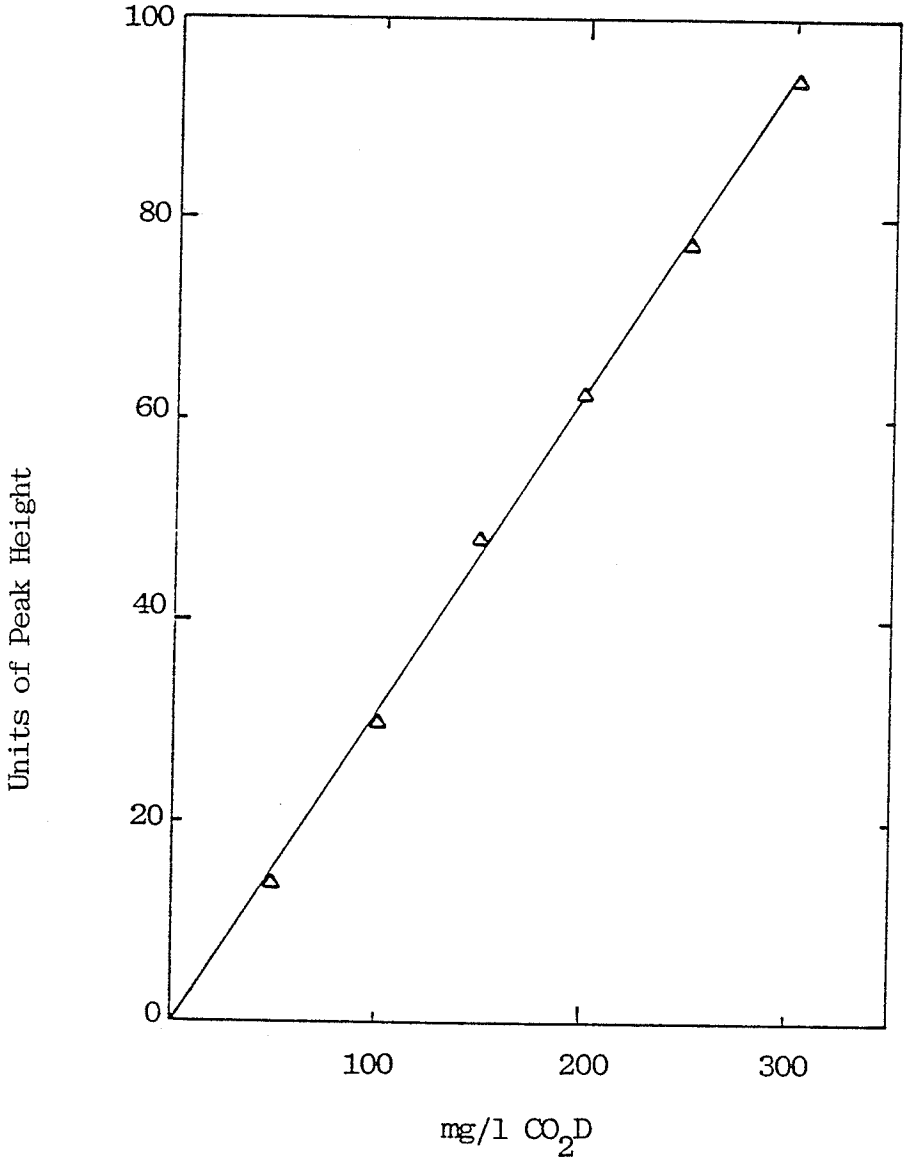


Fig. 1. CO_2D Standard Curve

Appendix Table 1

Color Reagent: 1 gm of 3,5-dinitrosalicyclic acid in 20 ml. of 2N NaOH and 50 ml of H_2O. 30 gm of potassium tartrate (Rochelle salt) was added and the solution was diluted to 100 ml.

Substrate: 1% soluble starch in 0.2M sodium acetate pH 5.5.

Calibration of Standard Curve

A stock solution was prepared by dissolving 0.5 gm of maltose in a 500-ml. volumetric flask and diluting to the mark with distilled water. Aliquots of 5, 10, 15, 20, 25, 30, 35, 40, 45, 50 ml were pipetted from the stock solution into the 50-ml volumetric flasks. The quantities of 0.1, 0.2, 0.3, 0.4, 0.5, 0.6, 0.7, 0.8, 0.9 and 1.0 mg. of maltose were obtained by pipetting 1 ml from each of the flasks to the test tube. One ml of the color reagent was added to the test tube. The tube was heated in a boiling water bath for 5 minutes and then cooled. Ten ml of distilled water was added and the sample was read using a Hitachi Perkin-Elmer Coleman 111 Spectrophotometer at 540 nm. Distilled water was used as the blank.

A calibrated curve was established with units of absorbance versus mg of maltose (Figure 2).

Procedures

An aliquot of 0.5 ml. test solution which contained beta-amylase was added to 0.5 ml. of substrate. A blank with 0.5 ml water in place of test solution was included. The test solutions were incubated at 55°C for 1 hour in a water bath. One ml of color reagent was then added. The test solution was again heated in boiling water for 5 minutes and cooled. Ten ml of distilled

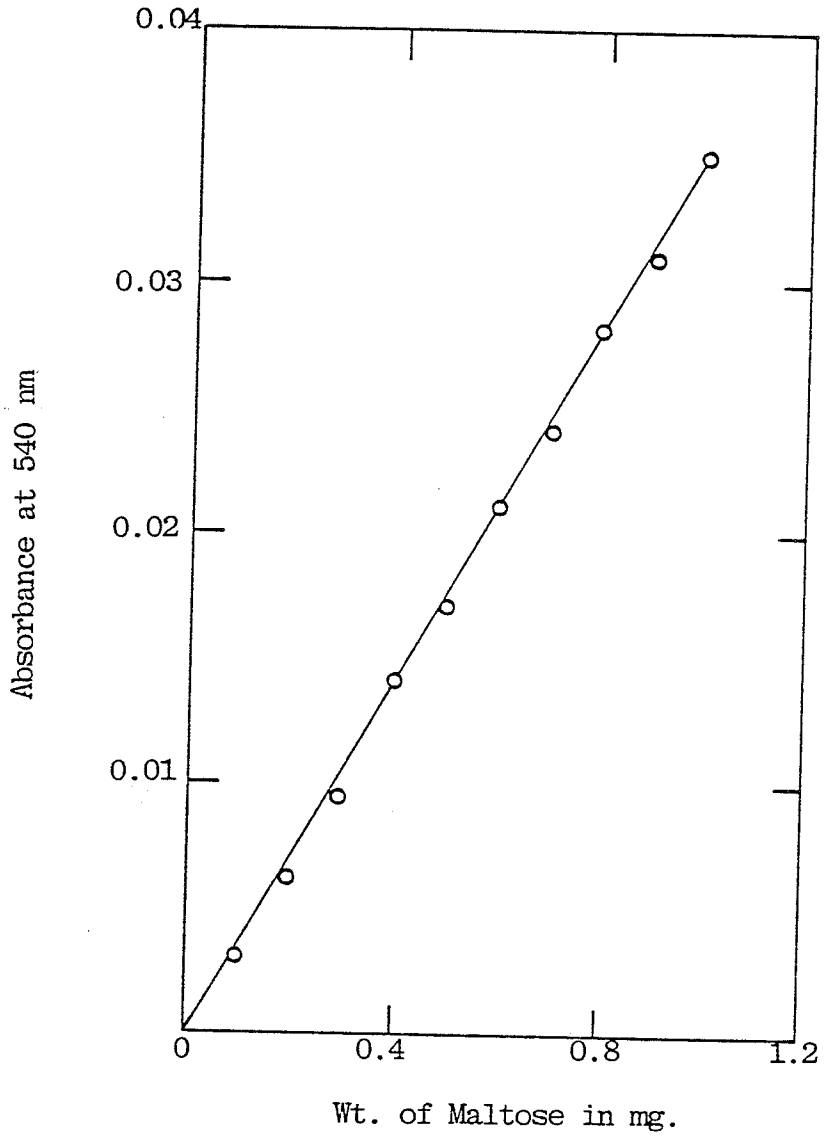


Fig. 2 Maltose Standard Curve for Determination of Beta-Amylase

Appendix Table 2

water was added and the test solution was read in the spectrophotometer at 540 mu.

Determination of Total Reducing Sugar

Assay for total reducing sugar expressed as glucose was performed according to the Shaffer-Somogyi micro method as outlined in the A.O.A.C., 14th edition (40). A standard curve with ml 0.005 N thiosulfate versus weight of glucose was plotted (Figure 3).

Determination of Nitrogen

Nitrogen determination of potato rinse water was determined using a Kjeldahl procedure, performed by the Department of Plant Science, University of Manitoba.

3.3 Bacterial Analysis

Standard Plate Count was used in this study to determine:

(1) The number of viable bacterial cells in potato rinse water before and after the combination of millipore filtration and carbon treatment.

(2) The effect of diastase on the number of viable bacterial cells in the starch based medium.

(3) The effect of beta-amylase on the number of viable bacterial cells in potato rinse water.

(4) The bacterial growth rates in different rinses of potato water.

Aliquots of 0.1, 1.0 ml of potato rinse water were pipetted and transferred to the petri plates aseptically. The liquified

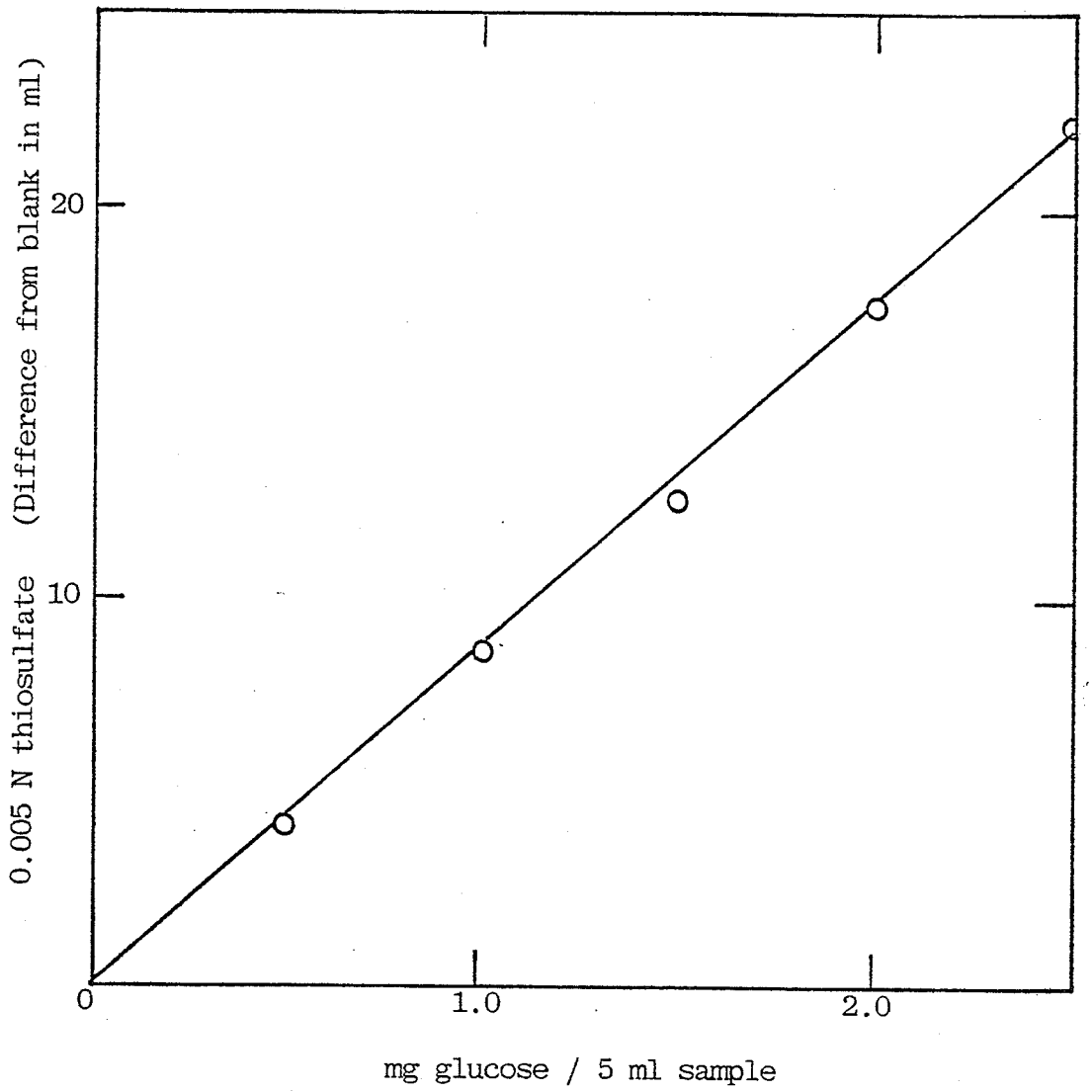


Fig. 3. Standard Curve for Determination of Total Reducing Sugar

Appendix Table 3

standard plate count agar was poured into the plates. After the agar had solidified, the plates were incubated at 32°C for 48 hours. Agar plates containing between 30-300 colonies were recorded and selected to enumerate the viable bacteria cells per ml. Where necessary, dilutions were performed by using a serial dilution blanks technique. All samples were performed in duplicate.

3.4 Preparations

3.4.1 Preparation of Potato Rinse Water

(1) First Rinse (1X)

The potato tubers were hand-peeled and washed in order to reduce the initial bacterial load and extraneous materials. The tubers were then cut into French fry slices by a potato chipper with an approximate size of 0.8cm x 0.8cm. The potato slices were rinsed using cold tap water (solid : liquid ratio of 3 : 10) over a fine mesh screen. The potato rinse water was collected and centrifuged at 1000 r.p.m. (1,200xg) for 5 minutes in a Sorvall Superspeed Refrigerated Centrifuge at 22°C to remove insoluble solids consisting mainly of starch.

(2) Multiple Rinses

Multiple rinses were prepared by rinsing the fresh batch of potato French fry slices with the first rinse of potato water (solid : liquid of 3 : 10) over a fine mesh screen. The water collected (second rinse) was reused to rinse another fresh batch of

potato French fry slices over a fine mesh screen. The above procedure was repeated until the required number of rinse water was obtained. It was then centrifuged to remove the insoluble solids. Throughout the preparation, rinsings of potato slices with water were performed in the ratio of (solid : liquid of 3 : 10).

3.4.2 Preparation of Bacterial Culture from Potato Rinse Water Culture

The first rinse of potato water was prepared as described previously. A volume of 500 ml of the potato rinse water was transferred to a one-litre Erlenmeyer flask. The flask was stoppered and shaken for 24 hours on the rotary shaker at 100 r.p.m. at 32° C.

Preparation of Inoculum

At 24 hours, the potato rinse water appeared to have bacterial growth as the water was observed to be cloudy. An aliquot of 20 ml of the incubated potato rinse water was withdrawn and transferred into the plastic centrifuge tubes. The tubes were centrifuged at 10,000 r.p.m. (6,600xg) for 10 minutes in the Sorvall Superspeed Centrifuge at 22° C. The supernatant was decanted and the bacterial pellet was washed with 0.8% saline solution. Two-hundred ml of fresh saline solution was again added to the pellet and the cells resuspended. A homogenous suspension of bacterial culture was obtained by mixing the suspension with a glass rod. A further mixing was performed with a Vortex mixer.

3.4.3 Dialysis of Potato Rinse Water

The potato rinse water was dialyzed against tap water before beta-amylase activity was determined.

An aliquot of 25 ml of potato rinse water was pipetted into the pre-cut dialysis tubing, 13 cm in length. It had been tied at one end with a string. After the rinse water was completely transferred, the open end of the tubing was tied tightly with a string. The sac was immersed in a beaker which contained 60 ml of cold tap water. A static dialysis was performed without changing of water. Continuous stirring of tap water was achieved by magnetic stirring. At the end of 24 hours, 0.5 ml of the potato rinse water contained within the dialysis sac was withdrawn and analyzed for beta-amylase activity.

3.5 Laboratory Procedures

This section deals with the appropriate experimental procedures in each of the studies conducted.

3.5.1. Potato Rinse Water Recycling Studies

The first part of the study was to collect 50 ml samples of the 5th; 10th, 15th, 20th, 25th, 30th and 35th rinses of potato water (prepared as described in section 3.4.1) and analyze for CO_2D and nitrogen contents.

The second part of the study was to study the effects of intermittent carbon treatment on turbidity and CO_2D of potato rinse water during recycling. In this study, the apparatus, equipment and glassware were sanitized with 200 mg/l chlorine water before use. Three-hundred gm of potato French fry slices were rinsed by

one litre of tap water. The rinse water was partially destarched by centrifugation. Fifty ml of the resultant supernatant was withdrawn and analyzed for CO_2D and turbidity. The remaining portion of the supernatant was collected and shaken with powdered carbon (carbon : potato rinse water ratio of 1 : 1,000) on the rotary shaker for 3 minutes at 22°C. The rinse water was filtered to remove carbon. Fifty ml of the potato rinse water filtrate was removed and analyzed for turbidity and CO_2D. The remaining portion of the potato rinse water filtrate was used to rinse successive batch of potato French fries over the fine mesh screen (solid : liquid ratio of 3 : 10). The above procedure was repeated for 10 rinses with intermittent powdered carbon treatment after every rinse. After the carbon treatment at the tenth rinse, the rinse water was held at 22°C for 16 hours and analyzed for CO_2D and turbidity.

3.5.2 Cause of Turbidity

Two samples (250 ml) of the first rinse of partially destarched potato water were passed through a millipore filter with pore size (0.2 mu) into previously sterilized 500-ml Erlenmeyer flasks. The control was transferred to the sterilized flasks without being filtered through millipore filter. All flasks were incubated at room temperature (22°C) for 72 hours. One of the millipore filtered potato rinse water samples was millipore filtered every 24 hours. An aliquot of 25 ml sample was withdrawn from each flask and determined for turbidity every 24 hours.

3.5.3 Control of Turbidity with Powdered Activated Carbon on the First and Tenth Rinses of Potato Water

Powdered activated carbon ranging from relatively low to high dosages were used to control developing turbidity in potato rinse water. Different weights of powdered carbon were placed into 250-ml Erlenmeyer flasks. The weights of carbon used were 0.00, 0.01, 0.05, 0.10, 0.15, 0.20, 0.25, 0.30 gm. Aliquots of 100 ml of potato rinse water (first rinse) prepared as described were transferred to the flasks and were shaken on the rotary shaker (300 r.p.m.) at 22°C. The carbon-treated potato rinse water was separated from the powdered activated carbon by filtration.

A similar set of experiments was set up; however, in this case, relatively high dosages of powdered activated carbon were used. Weights of 2, 5, 10, 2 x 2.5 gm (split-treatment) of powdered activated carbon were weighed and placed into the flasks. An aliquot of 100 ml of potato rinse water (first rinse) was transferred into each flask. The flasks were shaken on the rotary shaker (300 r.p.m.) for 3 minutes at 22°C. This procedure was followed by filtration to remove the carbon. The potato rinse water filtrates were incubated at 22°C.

Another set of experiments were similarly performed, but this time, the tenth rinse of potato water was used instead of the first rinse.

Split-Treatment with 2 x 25 g/l Powdered Activated Carbon

A sample of 100 ml of potato rinse water was added into the 250-ml Erlenmeyer flask, containing 2.5 gm of powdered activated

carbon. The flasks were shaken for 3 minutes at 300 r.p.m. on the rotary shaker. The mixture of carbon and potato rinse water was passed through the Whatman (GF/C) filter paper, with pore size (1.2 mu). The potato rinse water filtrate was transferred to another 250-ml Erlenmeyer flask which contained 2.5 gm of powdered activated carbon. The flask was similarly shaken on the rotary shaker, operated at 300 r.p.m. for 3 minutes at 22° C. The powdered activated carbon was removed by means of filtration. The filtrates obtained were incubated at 22° C for the specified period as required by individual experiment. In this case, the respective incubation times were 62 hours and 40 hours for the first and the tenth rinse of potato waters.

3.5.4 Effect of Powdered Activated Carbon on the Removal of Turbidity

The tenth rinse of potato water was centrifuged at 1,200xg for 5 minutes at 22°C in a Sorvall Superspeed Centrifuge to remove the insoluble starch. Powdered activated carbon with weights 0.0, 0.1, 0.3, 0.5, 1.0 gm were placed into 250-ml Erlenmeyer flasks. One hundred ml of potato rinse water was transferred into each flask and the flasks were shaken at 300 r.p.m. for 3 minutes on the rotary shaker. The carbon was removed from the potato rinse water by means of filtration. The carbon-treated potato rinse waters were measured for turbidity.

3.5.5 Control of Turbidity by the Combinations of Various Treatment Methods

This section deals with the increase and decrease in turbidity

and total organics in potato rinse water after it has been millipore filtered in comparison with the other treatment methods. Samples of partially destarched potato water (first rinse) were obtained and subjected to the following treatments:

(1) Millipore filtration

(2) Carbon Treatment (4 g/l)

(3) Refrigeration

(4) Carbon treatment (4 g/l) and millipore filtration

(5) Carbon treatment (4 g/l) and refrigeration

(6) Millipore filtration and refrigeration

(1) Millipore Filtration

An aliquot (100 ml) of the first rinse of potato water was passed through a millipore filter (0.2 mu) into a previously sterilized Erlenmeyer flask. Control sample was transferred into the sterilized flask without being millipore filtered. Samples (25 ml) were taken every 24 hours for turbidity and CO_2D analyses.

(2) Carbon Treatment

A sample of 100 ml of the first rinse of potato water was added into the 250-ml Erlenmeyer flasks which contained 0.4 gm of activated carbon. The flasks were shaken for 3 minutes at 300 r.p.m. at 22°C. The sample was filtered through the glass filter paper. The filtrates were analyzed for turbidity and CO_2D. Samplings (25 ml) were made every 24 hours for the same analysis.

(3) Refrigeration

An aliquot of 100 ml of potato rinse water was left at refrigerated temperature (4°C). Samplings (25 ml) were made every

24 hours for the analyses of turbidity and CO_2D.

(4) Carbon Treatment and Millipore Filtration

An aliquot of 100 ml of the first rinse of potato water was subjected to carbon treatment and then stored at 4°C. The procedure was the same as described in the sections under carbon treatment and refrigeration. Samples (25 ml) were taken every 24 hours for turbidity and CO_2D determinations.

(5) Carbon Treatment and Refrigeration

An aliquot of 100 ml of the first rinse of potato water was subjected to carbon treatment and then stored at 4°C. The procedure was the same as described in the sections under carbon treatment and refrigeration. Samples (25 ml) were taken every 24 hours for turbidity and CO_2D determinations.

(6) Millipore Filtration and Refrigeration

An aliquot of 100 ml of the first rinse of potato water was subjected to millipore filtration and kept under refrigerated temperature (4°C). Samples (25 ml) were taken every 24 hours for turbidity and CO_2D determinations.

Standard Plate Count (SPC) was performed at 0, 24 and 48 hour intervals on the above experiments.

3.5.6 Bacterial Growth in Different Rinses of Potato Water

The first, tenth, fifteenth and twentieth rinses of potato water (destarched) were obtained as described previously. An aliquot of 100 ml of the rinse waters was passed through a millipore filter into the 250-ml Erlenmeyer flask. The addition of 10 ml of bacterial culture (35×10^4 cells/ml) to each flask gave a total volume of

110 ml to each flask. The flasks were incubated for 144 hours at 22°C. Samples were drawn from the flasks and analyzed for total reducing sugars and SPC.

3.5.7 The Effect of Diastase on Bacterial Growth in a Starch-Based Medium

Varying levels of diastase, ranging from 0.0 to 0.2 gm were added into two 500-ml Erlenmeyer flasks containing 100 ml growth media. The growth medium was comprised of 0.35 gm of soluble starch and 0.1 gm of NH_4Cl. The growth medium and diastase were sterilized by millipore filtration using a 0.2 mu filter. An aliquot of 10 ml of a bacterial culture (57×10^4 cells/ml) was aseptically added into each flask. An additional 500-ml flask containing growth medium and bacterial inoculum was also set up. This flask served as the control. All flasks were incubated, simultaneously at room temperature (22°C) for 144 hours. Ten ml samples were withdrawn approximately every 24 hours and analyzed for total reducing sugars and SPC.

A separate experiment was performed using the same procedure, however, the time at which samples were taken for the analyses of CO_2D and turbidity was every 3 hours for 12 hours.

3.5.8 The Effect of Varying Levels of Beta-Amylase on Bacterial Growth

One hundred ml of the potato rinse water (first rinse) was destarched and added into each of the three 250-ml Erlenmeyer flasks, containing diluted pure beta-amylase with activities of 0, 1100 and 5500 mg of maltose liberated per litre. The enriched potato rinse waters were

millipore filtered and collected in the sterilized 250-ml Erlenmeyer flasks. A bacterial culture (33×10^4 cells/ml) was inoculated into each flask. All flasks were incubated, simultaneously at room temperature (22°C) for 144 hours. A sample size of 10 ml was withdrawn from each flask and analyzed for SPC and total reducing sugar content every 48 hours.

3.5.9 Adsorption of Pure Beta-Amylase with Powdered Activated Carbon

A stock solution of beta-amylase was prepared by diluting 50 microlitres of pure beta-amylase into a one-litre volumetric flask with phosphate buffer solution (0.1 M, pH 6.8). A sample size of 100 ml of the stock beta-amylase solution was withdrawn from the one-litre volumetric flask into the 250-ml Erlenmeyer flask, containing 0.05 gm of powdered activated carbon. The flasks were left on the rotary shaker and shaken for 15 minutes at 300 r.p.m. at 22°C. At 0, 1, 2, 3, 5, 7, 10, 15 minutes intervals, individual flask was taken from the rotary shaker.

The mixture of enzyme solution and powdered activated carbon was filtered through the Whatman (GF/C) glass filter paper and the enzyme filtrate was collected in a test tube, placing in the ice bath. A sample size of 0.5 ml of carbon-treated enzyme solution was pipetted from the test tube for beta-amylase determination. The experiment was repeated for beta-amylase dissolved in acetate buffer solution (0.3 M, pH 4.5).

A separate experiment was performed using the same procedure; however, 0.1 and 0.5 gm of powdered activated carbon were used instead

of 0.05 gm.

3.5.10 Effect of pH and Carbon Treatment on Beta-Amylase Activity

Samples of 100 ml of the fifth rinse of potato water were destarched and adjusted to pH 3.2, 4.5, 5.7 with 0.1 M citric acid. A control sample was unadjusted so as to obtain a neutral pH of 6.8. All samples were transferred into the 250-ml Erlenmeyer flasks, containing 0.05 gm of powdered activated carbon. The flasks were shaken on the rotary shaker, operated at 300 r.p.m. for 3 minutes at 22°C. The carbon was removed from the samples by filtering through Whatman (GF/C) glass filter paper. The potato rinse water filtrates were collected; dialyzed for 24 hours and determined for beta-amylase activity.

A separate experiment was performed using the same procedure; however, 0.1 and 0.5 gm of powdered activated carbon were used instead of 0.05 gm.

3.5.11 Effect of Powdered Activated Carbon on Quality Control Factors of Recycled Potato Rinse Water

The fifth rinse of destarched potato rinse water was shaken with powdered activated carbon (activated carbon : potato rinse water ratio of 1 : 1,000) on the rotary shaker at 300 r.p.m. for 3 minutes at 22°C. The rinse water was filtered through Whatman (GF/C) glass filter paper for carbon removal. The filtrate (carbon-treated potato rinse water) was used to rinse the fresh batch of potato French fry slices over the fine mesh screen (solid : liquid ratio of 3 : 10).

The slice-rinse procedure was repeated until the potato rinse water had been used for five times. Each time a fresh batch of potato slices was rinsed. This procedure was followed by the application of powdered activated carbon treatment (1 g/l). The filtrate was reused until the 20th carbon-treated potato rinse water was obtained, with intermittent carbon treatment after every fifth rinse.

A sample size of 50 ml was taken before and after carbon treatment at every fifth rinse stage for CO_2D, turbidity and beta-amylase determinations.

A similar experiment was carried out with the application of 5g/l of powdered activated carbon treatment at every fifth rinse stage.

RESULTS AND DISCUSSION

4.1 Potato Rinse Water Recycling Studies

In the first part of this study, different batches of potato French fry slices were rinsed by the same amount of water. Different rinses of potato water (5th, 10th, 15th, 20th, 25th, 30th and 35th) at the slice-rinse stage were obtained. Detailed procedures are described in section 3.5.1. From the analyses of CO_2D and nitrogen shown in Figures 8 & 9, the fifth rinse contained approximately 2500 mg/l CO_2D, 1.2% nitrogen which increased up to 11,000 mg/l CO_2D and 3.8% nitrogen correspondingly at the fifteenth rinse, and in further rinses small build-up of organics was observed. From the first rinse to the fifteenth rinse, every fifth rinse leached an average of approximately 5,000 mg/l CO_2D, showing that the "reuse" water still performed the function of removing 'surface' organics. However, at the twenty-fifth rinse, the water came to an equilibrium level and only permitted small quantities of organics to leach from the surface of potato French fry slices. When the rinse water reached an equilibrium level, it was very valuable for reuse as the nutrients were mainly kept inside the potato slices and only a physical cleaning of the potato slices could occur. This surface cleaning of the potato slices was the intended purpose, during potato water recycling at the slice-rinse stage. The only treatment required was to control the aesthetic factors, that is, turbidity,

foam and microbial growth.

In the second part of this study, powdered activated carbon was incorporated into the recycling process to control the turbidity and CO_2D levels. The suspended matter, colloidal particles and microorganisms contribute to the turbidity which was considered as one of the major aesthetic factors that has to be controlled. The CO_2D level in potato rinse water would serve as an index to indicate when the equilibrium level was reached during recycling.

A study was made to control the parameters of turbidity and CO_2D by using 1 g/l of powdered activated carbon was shown to render clear potato rinse water filtrate after each treatment, during preliminary investigation studies. The potato rinse water at every rinse stage was treated with 1 g/l of powdered activated carbon to ensure that turbidity would be immediately controlled without further accumulation while at the same time allowing the level of organics to increase until a CO_2D equilibrium condition was reached. The turbidity and CO_2D levels of potato rinse water after carbon treatment were reduced; however, the accumulative effects of powdered activated carbon treatment during the ten recycling washes showed a net increase (Figures 4A & 4B).

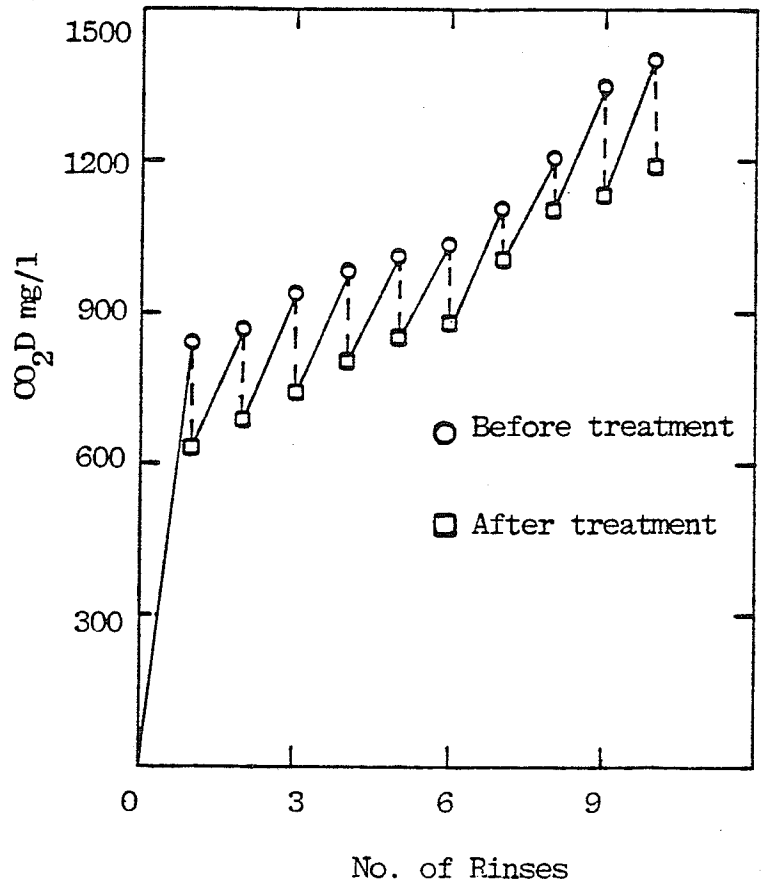
At the tenth rise, the turbidity level of the rinse water was 21 J.T.U. after carbon treatment, and it was observed to increase to 46 J.T.U. after the carbon-treated potato rinse water had been left for 16 hours at 22^{o} C (room temperature). According to visual judgement, the water was turbid and therefore unacceptable for any recycling purpose without further treatment. The feasibility of

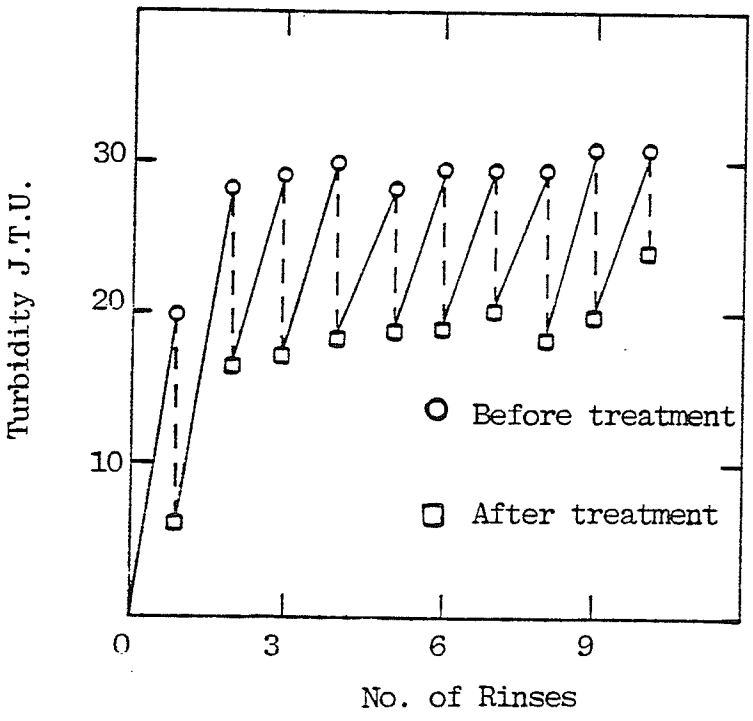
Fig. 4A The Effect of Powdered Activated Carbon (1g/l) on CO_2D Control

Appendix Table 4

Fig. 4B The Effect of Powdered Activated Carbon (1g/l) on Turbidity Control

Appendix Table 4


1500
1200
900
600
300
CO_2D mg/l
Before treatment
After treatment
0
3
6
9
No. of Rinses


30
20
10
Turbidity J.T.U.
Before treatment
After treatment
0
3
6
9
No. of Rinses

the implementation of recycling system into the food processing line has been shown in several literature sources (54, 64). Also, detailed studies of recycling and the means of controlling the physical, physicochemical,biochemical and biological problems have been reported in literature review (45).

4.2 Cause of Turbidity

Occurrence of turbidity in potato rinse water is due to the presence of suspended matter, clay, silt, organics, inorganics and microscopic organisms (78). It has also been reported from several literature sources that bacterial growth can play an important role in causing turbidity (50).

Bacterial growth was considered to be an important factor which contributed to turbidity development especially in high organic rinse waters since, in general, the more favourable the concentration of nutrients in the medium, the more rapid is the bacterial growth (75). Results of the following experiment demonstrated the partial involvement of microorganisms as a contributory factor in turbidity development.

As shown in Table 1, turbidity values of the potato rinse water samples which were millipore filtered at 0 hour and every 24 hours remained the same throughout the experiment. There was no sign of turbidity. Millipore filtration removed the microflora and colloids of size greater than 0.2 mu, leaving the filtrate essentially sterilized and any occurrence of turbidity was then mainly due to post-contamination. The control sample which had not been millipore

Table 1. Changes in Turbidity of Potato Rinse Water (1X) after Millipore Filtration Treatment

Incubation Time (hr.)	Turbidity (J.T.U.)		
	A	B	C
0	14.0	9.2	13.0
24	26.0	10.0	13.0
48	57.0	10.0	17.0
72	177.0	12.0	17.0

A. Control flask - potato rinse water was incubated at room temperature without millipore filtration.

B. Flask containing potato rinse water - millipore filtered every 24 hrs. Readings represent values prior to subsequent millipore filtration.

C. Flask containing potato rinse water - millipore filtered only at 0 hr.

filtered, was shown to have an increase in turbidity over the test period.

4.3.1 Control of Turbidity with Powdered Activated Carbon on Potato Rinse Water (First Rinse)

At 0, 13, 16, 28 hours, samples (25 ml) were taken from the flasks, containing filtrates which were treated with different dosages of powdered activated carbon, ranged from 0 to 3 g/l for turbidity measurements. Changes in turbidity with respect to time for each sample were recorded and listed in Table 2. It was shown that by the 28th hour excessive turbidity was found to occur in all samples except those which were treated with powdered activated carbon dosages higher than 2.0 g/l.

At 0, 16, 24, 40, 41, 62 hours, samples (25 ml) were taken from the 250-ml Erlenmeyer flasks, containing potato rinse water filtrates which had been treated with 20 g/l, 2 x 25 g/l (split-treatment), 50g/l, 100 g/l of powdered activated carbon for turbidity measurements. Changes in turbidity with respect to time for each sample were recorded and listed in Table 3. Although high carbon dosages were used in this case, the potato rinse water still showed signs of cloudiness after a period of time. The time at which the samples turned turbid differed and was dependent on carbon dosage. It seemed that the clarity of potato rinse water could be kept for a longer period with higher dosages of activated carbon. For example, potato rinse water which was treated with 20 g/l of activated carbon turned turbid ($>$ 25 J.T.U.) by the 40th hour while potato rinse waters

Table 2. Control of Turbidity on Potato Rinse Water (First Rinse) with "Low Dosages" of Powdered Activated Carbon

Carbon dosages g/l	Turbidity (J.T.U.)			
	0 hr.	13 hrs.	16 hrs.	28 hrs.
0.0	4.9	13.0	23.0	31.0
0.1	4.8	9.5	17.0	25.0
0.5	4.9	7.5	17.0	27.0
1.0	4.6	6.5	12.0	26.0
1.5	4.8	7.1	12.0	27.0
2.0	4.0	7.1	12.0	28.0
2.5	4.0	7.0	8.9	9.3
3.0	3.9	4.8	7.3	8.9

Table 3. Control of Turbidity on Potato Rinse Water (First Rinse) with "High Dosages" of Powdered Activated Carbon

Carbon dosages g/l	Turbidity (J.T.U.)					
	0 hr.	16 hrs.	24 hrs.	40 hrs.	41 hrs.	62 hrs.
20.0	1.8	1.6	1.9	26.0	42.0	49.0
50.0	1.7	1.9	1.9	13.0	25.0	31.0
2x25.0 (Split-treatment)	1.5	1.6	1.7	5.2	6.6	7.8
100.0	1.4	1.6	2.1	12.0	12.5	16.5

which were treated with 50 g/l, or 2 x 25 g/l (split-treatment), or 100 g/l was found to show signs of cloudiness by the 70th hour. The results also showed that by a split-treatment, the water could be kept clear for a longer period of time, for a given total carbon dosage. In summary, the data indicated that although all samples eventually showed excessive turbidity, the "holding time" was dependent on the carbon dosage used to treat the water.

4.3.2 Control of Turbidity with Powdered Activated Carbon On Potato Rinse Water (Tenth Rinse)

At 0, 12, 15, 18 hours, samples (25 ml) were taken from the 250-ml Erlenmeyer flasks, containing potato rinse water filtrates which were treated with 0.0-3.0 g/l of powdered activated carbon for turbidity measurements. Also at 0, 16, 24, 40 hours, 25 ml samples were drawn from the flasks, containing potato rinse water filtrates which were treated with 20, 50, 2 x 25 (split-treatment) and 100 g/l of powdered activated carbon for turbidity measurements. Changes in turbidity with respect to time for each sample were recorded and listed in Tables 4 & 5. As shown from the results, samples taken from the tenth rinse of potato water filtrates showed the same trend in changes of turbidity as the first rinse. The turbidity measurements in the tenth rinse of potato water filtrates increased with respect to time. However, the time required for the tenth rinse potato water filtrate to become turbid was shorter than the time required for the first rinse filtrate even though the same quantity of activated carbon was applied to both rinses. The time

Table 4. Control of Turbidity in Potato Rinse Water (Tenth Rinse) with "Low Dosages" of Powdered Activated Carbon

Carbon Dosages g/l	Turbidity (J.T.U.)			
	0 hr.	12 hrs.	15 hrs.	18 hrs.
0.0	19.0	24.0	86.0	530.0
0.1	18.0	21.0	46.0	420.0
0.5	18.0	17.0	29.0	380.0
1.0	17.0	21.0	28.0	68.0
1.5	17.0	21.0	25.0	61.0
2.0	17.0	21.0	25.0	58.0
2.5	10.0	13.0	22.0	54.0
3.0	10.0	10.0	14.0	48.0

Table 5. Control of Turbidity in Potato Rinse Water (Tenth Rinse) with "High Dosages" of Powdered Activated Carbon

Carbon Dosages g/l	Turbidity (J.T.U.)			
	0 hr.	16 hrs.	24 hrs.	40 hrs.
20.0	7.1	11.0	15.0	240.0
50.0	2.4	2.1	2.6	35.0
2x25.0 (Split-treatment)	1.7	1.7	2.2	21.0
100.0	1.6	1.7	2.1	31.5

at which excessive turbidity was detected was by 15 hours for the tenth rinse of potato water filtrate compared to 28 hours for the first rinse.

As in the previous studies, the problem of excessive turbidity still existed even when the potato rinse waters were treated with high dosages of powdered activated carbon. It was shown that potato rinse waters treated with 20 g/l, 50 g/l and 100 g/l of powdered activated carbon became turbid by the 40th hour. But for the potato rinse water that was treated with 2 x 25 g/l of powdered activated carbon, the occurrence of turbidity was obvious by the 46th hour.

4.4 Effect of Powdered Activated Carbon on the Removal of Turbidity

The turbidity of the carbon-treated potato rinse waters was expressed as the percentage of the untreated sample which was arbitrarily assigned as having 100% turbidity. The rate curve was plotted with percentage removal of turbidity as ordinate and weight of powdered activated carbon as abscissa. The rate of turbidity removal is shown in Figure 5. Results showed that the turbidity levels of the potato rinse water could be lowered with the application of powdered activated carbon. The employment of higher carbon dosages would result in a greater removal of turbidity. The curve was characterized by a rapid declining rate with the use of powdered activated carbon. A maximum removal of turbidity was reached between 3 g/l and 5 g/l of carbon dosage. Beyond these levels the curve started to level off.

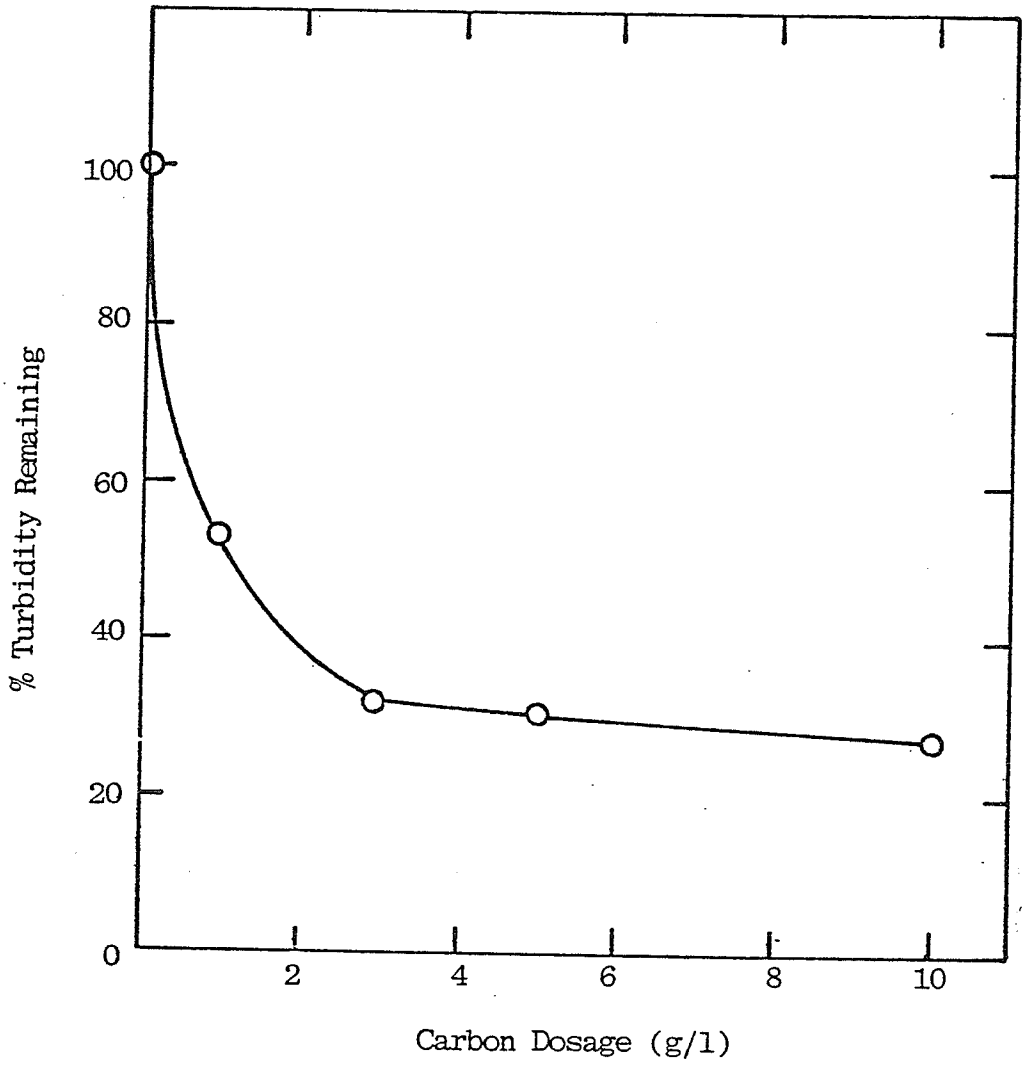


Fig. 5. Effect of Powdered Activated Carbon on the Removal of Turbidity

Appendix Table 5

4.5 Control of Turbidity on Potato Rinse Water by Various Methods

Results of comparative treatment methods for the control of turbidity such as millipore filtration, refrigeration and carbon treatment are given in Table 6. The net rate of increase in turbidity and decrease in CO_2D with respect to time are illustrated in Figures 6 & 7.

For the carbon treated potato rinse water sample (4 g/l), turbidity was found to be 28.5 J.T.U. at the 48th hour. For the sample which was kept at 4°C there was no sign of cloudiness for 48 hours. The millipore-filtered potato rinse water remained clear throughout the experiment. The turbidity reading increased by only 0.55 J.T.U. and accompanied by a slight drop in CO_2D level within 48 hours. As shown in Table 7, there was no detectable microbial growth in the potato rinse water after millipore filtration. Therefore, the potato rinse water which had been passed through millipore filter was considered to be aesthetically acceptable and fit for reuse, even after 48 hours and incubated at 22°C.

4.6 Control of Turbidity and CO_2D on Potato Rinse Water by the Combination of Various Treatment Methods

In the in-plant recycling of potato rinse water, it is important to control the microbial problem and the organic fraction that contributes undesirable factors such as excess color, foam and turbidity. Of the various treatment combinations, the most ideal would be activated carbon and millipore filtration. The millipore filtration kept the water bacteriologically clean and the activated

Table 6. Control of Turbidity and CO_2D on Potato Rinse Water by Various Treatment Methods

Time in hrs.	Room temperature		Millipore filtration		Carbon treatment		Refrigeration (4°C)	
	Turbidity (J.T.U.)	CO_2D (mg/l)	Turbidity (J.T.U.)	CO_2D (mg/l)	Turbidity (J.T.U.)	CO_2D (mg/l)	Turbidity (J.T.U.)	CO_2D (mg/l)
0	11.0	737.0	3.9	731.0	5.9	362.0	9.0	750.0
24	13.0	625.0	4.2	712.0	6.2	262.0	11.0	750.0
48	71.0	349.0	4.4	687.0	29.0	125.0	11.0	720.0

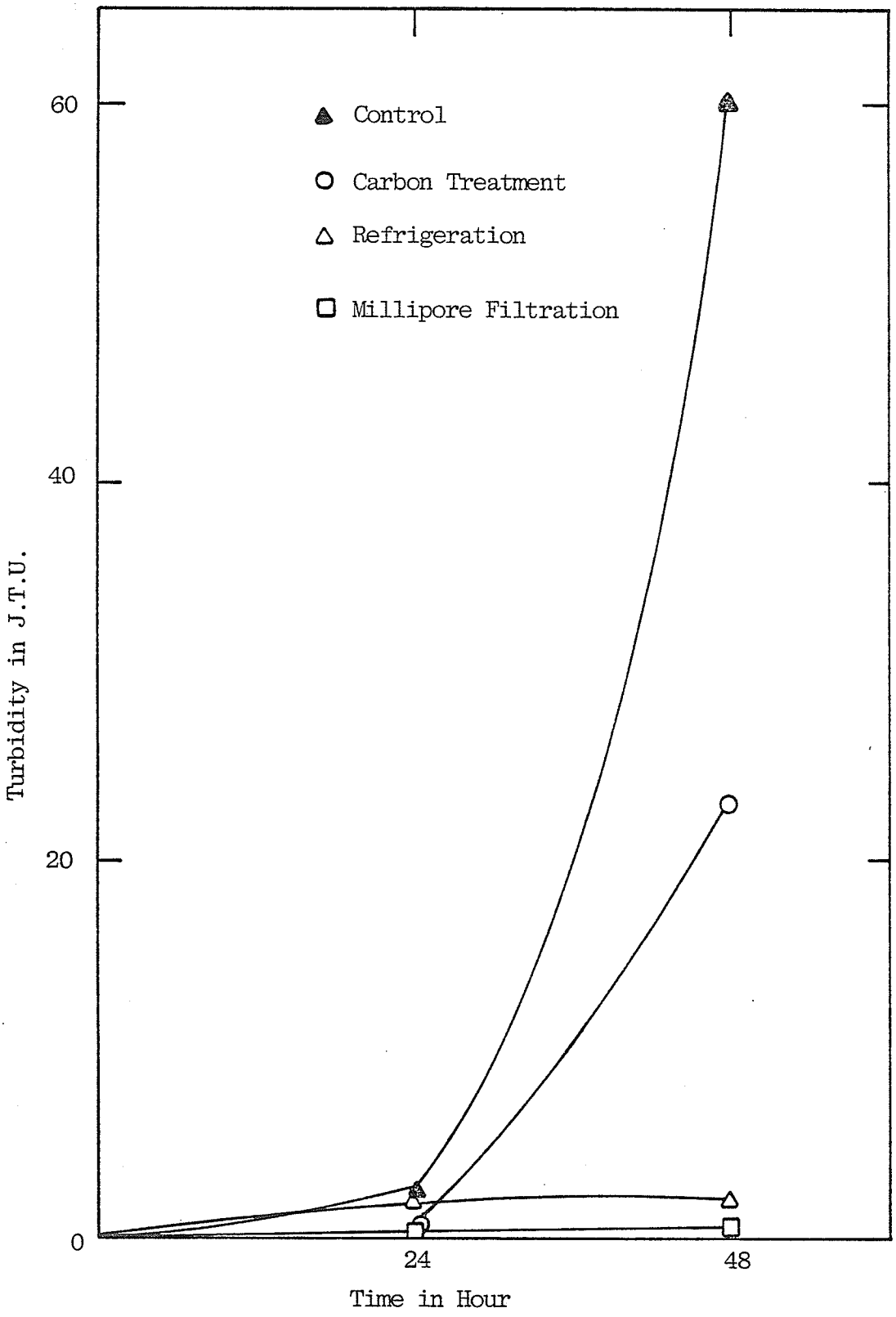


Fig. 6. Comparison of Treatment Methods on Potato Rinse Water (Net Increase in Turbidity)

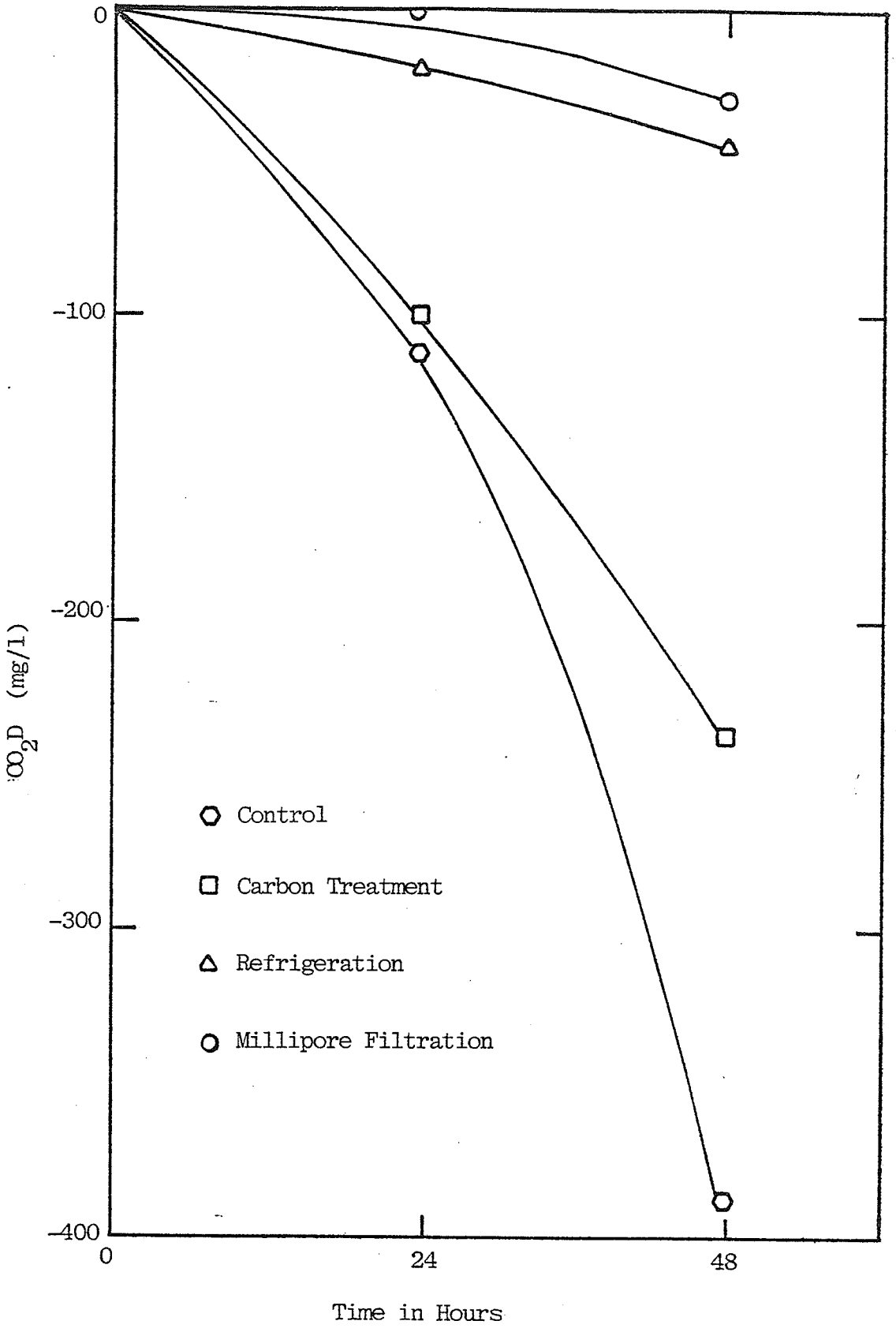


Fig. 7. Comparison of Treatment Methods on Potato Rinse Water (Net Decrease in CO_2D)

carbon removed the undesirable organics. As indicated in Table 7, the potato rinse water showed no visible bacterial growth after it had been passed through the millipore filter. There was no distinct rise or drop in turbidity and CO_2D levels throughout the entire experiment which lasted 48 hours. Experimental results on the control of turbidity and CO_2D by various combination methods are shown in Table 8.

Powdered activated carbon was the only treatment method that could control the organics. The millipore filtration and refrigeration were successful in controlling the turbidity problem but had minimal effects on the removal of organics as expected.

Control of turbidity and CO_2D levels could also be attained by means of carbon treatment and refrigeration since the initial values of CO_2D and turbidity remained relatively the same throughout the experiment (48 hours), as shown from the results in Table 8.

4.7 Bacterial Growth in Different Rinses of Potato Water

A sample size of 50 ml of potato rinse was drawn at every fifth rinse stage for the beta-amylase, % nitrogen[1] and CO_2D determinations. As shown in Figures 8,9, & 10, the amount of beta-amylase, nitrogen and CO_2D in the potato rinse water were the highest towards the end of the multiple reuse. The fifth rinse contained approximately 2,500 mg/l CO_2D, 1.2% nitrogen and 800 maltose units of beta-amylase which increased up to 12,000 mg/l CO_2D, 4.5% nitrogen and 1,500 maltose units of beta-amylase correspondingly at the twentieth rinse.

1. % Nitrogen was used as an index indicating the amount of total extractable nitrogenous compounds from the potato slices into the water during multiple reuse of potato rinse water.

Table 7. The Effect of Various Treatment Methods on Bacterial Growth in Potato Rinse Water

Time in hrs.	Standard Plate Count					
	A	B	C	D	E	F
0	13×10^1	0	56×10^0	0	56×10^0	0
24	42×10^3	0	70×10^1	0	24×10^1	0
48	96×10^5	0	84×10^3	0	87×10^1	0

A. Control flask containing potato rinse water - without treatment.

B. Flask containing potato rinse water - millipore filtered at 0 hr.

C. Flask containing potato rinse water - treated with 4g/l powdered activated carbon.

D. Flask containing potato rinse water - treated with 4g/l powdered activated carbon and millipore filtered.

E. Flask containing potato rinse water - treated with 4g/l powdered activated carbon and refrigerated.

F. Flask containing potato rinse water - millipore filtered and refrigerated.

Table 8. Control of Turbidity and CO_2D on Potato Rinse Water by the Combinations of Various Treatment Methods

Time in hrs.	Room Temperature		Carbon treatment + Millipore filtration		Carbon treatment + Refrigeration		Millipore filtration + Refrigeration	
	Turbidity (J.T.U.)	CO_2D (mg/l)	Turbidity (J.T.U.)	CO_2D (mg/l)	Turbidity (J.T.U.)	CO_2D (mg/l)	Turbidity (J.T.U.)	CO_2D (mg/l)
0	14.0	875.0	3.4	348.0	11.0	306.0	6.4	813.0
24	22.5	626.0	3.6	348.0	12.5	306.0	6.9	807.0
48	76.0	412.0	5.6	327.0	17.5	302.0	7.3	801.0

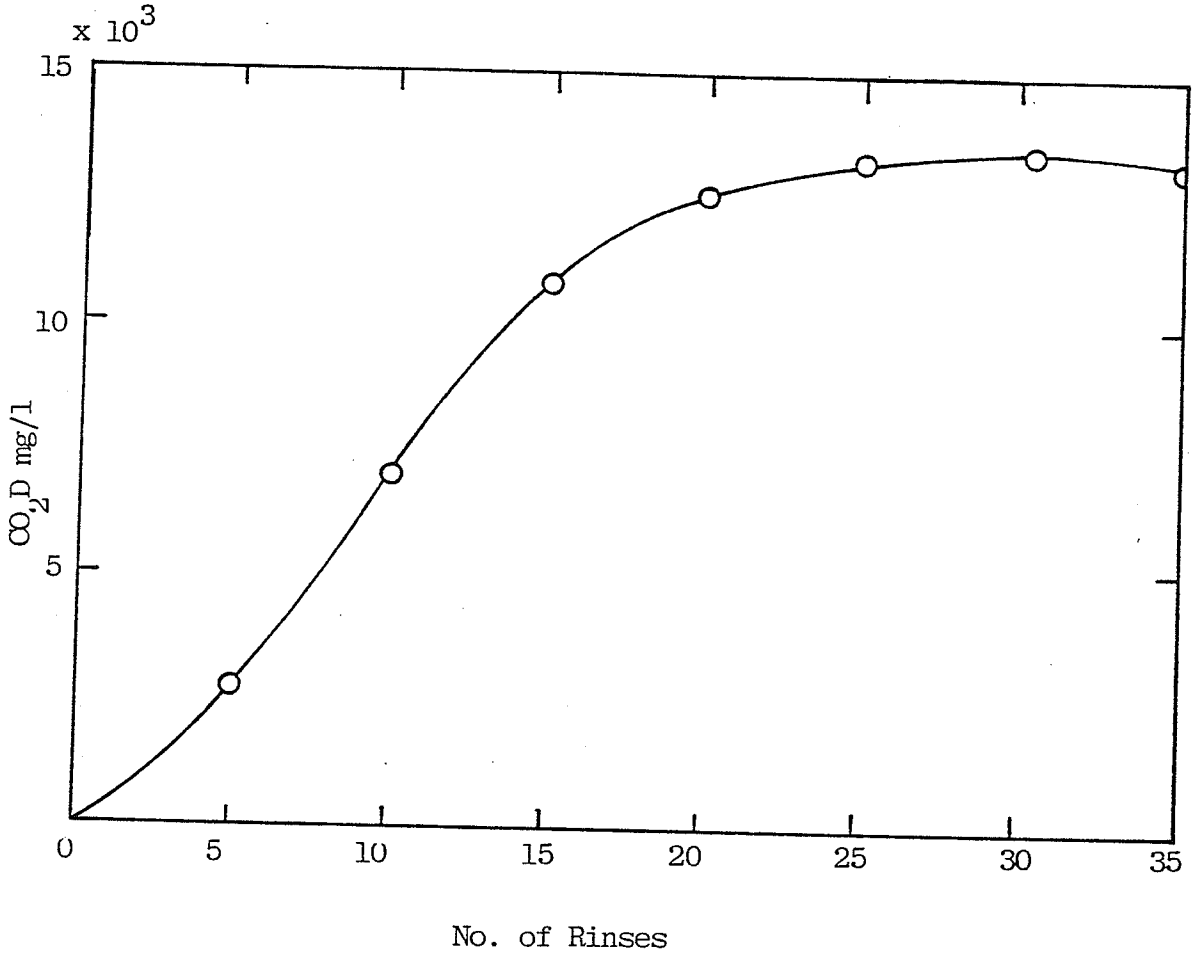


Fig. 8. Relation of CO_2D Concentration to Multiple Reuse of Potato Rinse Water.

Appendix Table 5.

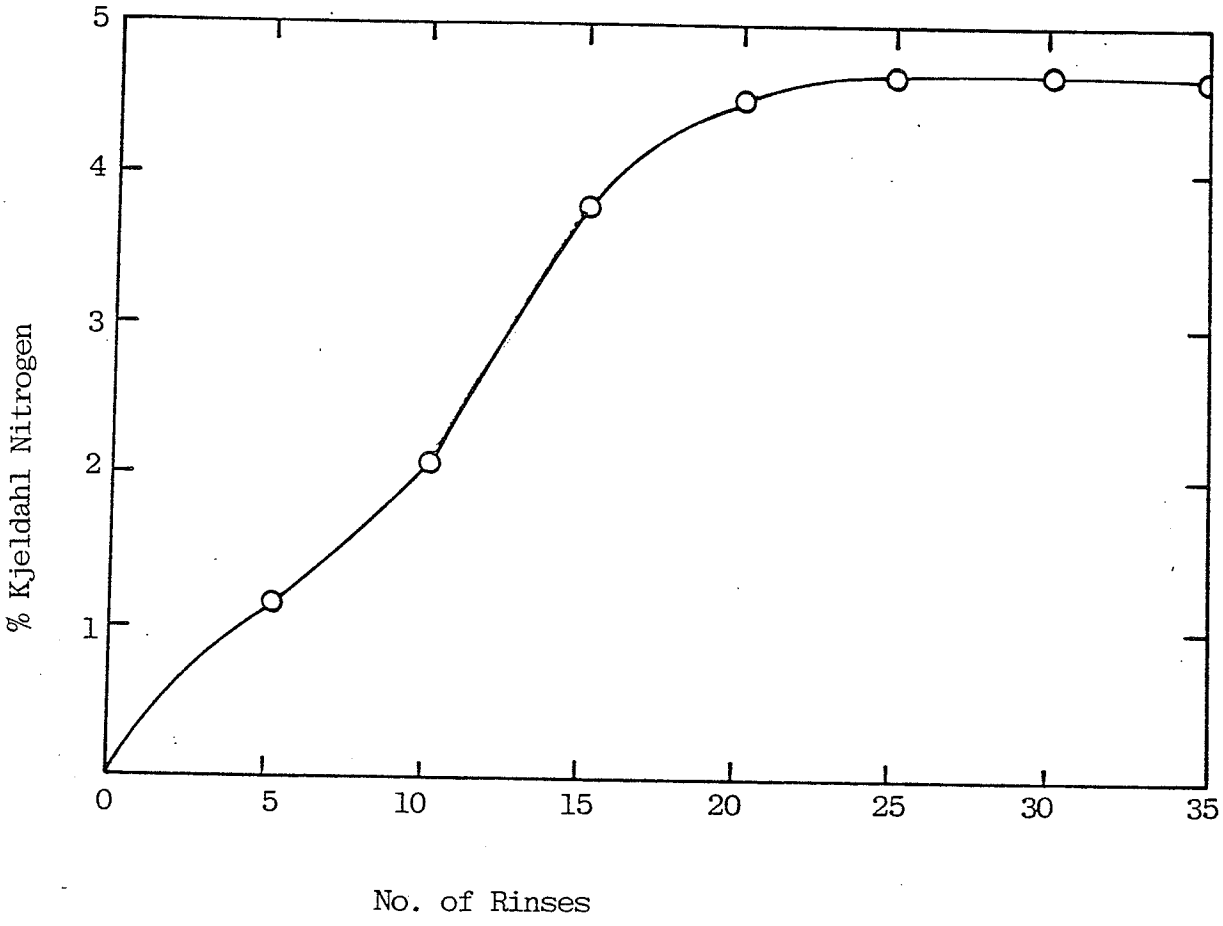


Fig. 9. Relation of Nitrogen Concentration to Multiple Reuse of Potato Rinse Water.

Appendix Table 15.

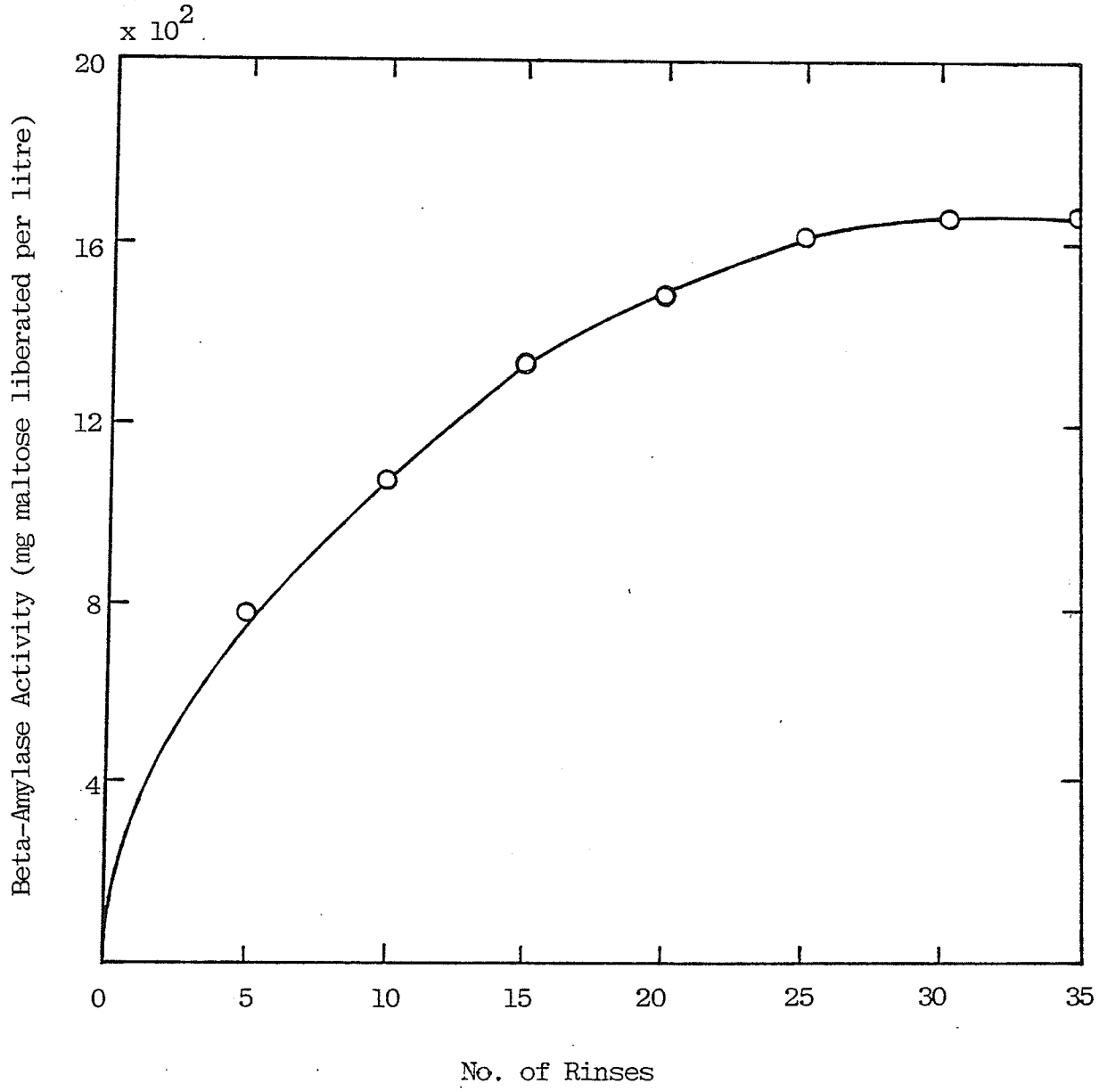


Fig. 10. Relation of Beta-Amylase Concentration to Multiple Reuse of Potato Rinse Water.

Appendix Table 15.

At 0 hour, the potato rinse waters (first, tenth, fifteenth and twentieth) were inoculated with relatively the same level (35 x 10^4 cells/ml) of viable bacteria. After 48 hours, samples were taken and analyzed for SPC. It was found that the highest bacterial population, approximately 45 x 10^8 cells/ml, was found in the twentieth rinse of potato water while the first rinse contained the least bacterial population (98 x 10^5 cells/ml). Experimental data are shown in Table 9. Results showed that a higher rinse stage would support more bacterial growth. This might be due to the accumulation of CO_2D, nitrogen and beta-amylase levels in the higher rinse stage, that provided enough nutrients for a higher bacterial population.

4.8 The Effect of Diastase on Bacterial Growth in a Starch-Based Medium

As indicated from the results in Table 10, bacterial growth was shown to increase as the amount of diastase was increased. It was observed that the growth medium which contained the highest level of diastase (0.2 g/100 ml) supported the greatest bacterial growth, which occurred within 48 hours. The bacterial growth rate in 0.2 g/ml diastase media was found to be the highest when compared with those media that contained lower dosages of diastase. The bacterial population that grew in 0.2 g/ml diastase growth medium increased by approximately 3 log cycles. The bacterial populations growing in 0.01 and 0.05 g/100 ml diastase growth medium showed growth increments of approximately 2 log cycles.

As shown in Table 11, bacterial growth in media containing varying concentrations of diastase showed no great difference. All

Table 9. Bacterial Growth in Different Rinses of Potato Water

Time in hrs.	No. of Rinses							
	1		10		15		20	
	SPC	S	SPC	S	SPC	S	SPC	S
0	44×10^4	181	43×10^4	614	25×10^4	1074	30×10^4	1667
48	98×10^5	58	38×10^8	173	50×10^8	219	45×10^8	378
96	23×10^6	55	35×10^8	88	21×10^8	150	67×10^8	154
144	53×10^5	50	69×10^6	81	15×10^8	131	20×10^8	139

SPC - Standard Plate Count. (No. of Viable Cells/ml)

S - Total Reducing Sugar in mg/l

Table 10. The Effect of Varying Levels of Diastase on Bacterial Growth in a Starch-Based Medium

Time in hrs.	Amount of Diastase (g/100 ml)					
	0.01		0.05		0.20	
	SPC	S	SPC	S	SPC	S
0	26×10^4	0	29×10^4	0	36×10^4	0
0.5	26×10^4	431	29×10^4	470	36×10^4	2723
48	46×10^6	377	63×10^6	357	42×10^7	1737
96	36×10^6	313	96×10^6	296	34×10^7	1626
144	32×10^5	292	90×10^5	260	72×10^6	1587

S - Total Reducing Sugar in mg/l

SPC - Standard Plate Count. (Viable Cells/ ml)

Table 11. The Effect of Diastase on Bacterial Growth in a Starch-Based Medium

Time in hrs.	gm/100 ml diastase 0	0.01	0.05	0.20
	Standard Plate Count (Viable Cells / ml)			
0	58×10^4	48×10^4	62×10^4	60×10^4
3	23×10^5	31×10^5	25×10^5	25×10^5
5	28×10^6	21×10^6	21×10^6	29×10^6
9	96×10^6	11×10^7	14×10^7	13×10^7
12	52×10^7	16×10^8	20×10^8	32×10^8

flasks containing diastase increased in population by approximately 4 log cycles after 12 hours of incubation. The control flask containing no diastase showed little difference in growth when compared to diastase containing media during the first five hours of incubation. After five hours of incubation, the control flask showed smaller bacterial populations when compared to diastase-containing flasks. The control flask showed an approximate increase of 3 log cycles at 12 hours of incubation. Also, the increase in bacterial growth was accompanied by a decrease in total reducing sugar level (Table 10).

4.9 Correlation of Beta-Amylase with Bacterial Growth

Results (Table 12) indicated that at 96 hours, potato rinse water enriched with the highest beta-amylase level (5500 mg maltose/litre) contained the highest bacterial growth (103 x 10^7 cells/ml) while the control potato water sample contained the least (54 x 10^6 cells/ml). Since the nutrient sources in all potato water samples were present in the same quantities, the variable factor, beta-amylase which breaks down starch to maltose appeared to be the major factor causing the high bacterial growth.

4.10 Adsorption of Pure Beta-Amylase with Powdered Activated Carbon

The rate curves for the adsorption of pure beta-amylase with different weights of carbon at pH 4.5 and 6.8 were obtained. Graphical representations are illustrated in Figures 11 & 12.

Beta-amylase was removed by powdered activated carbon to a much

Table 12. The Effect of Varying Levels of Beta-Amylase on Bacterial Growth

Time in hrs.	Beta-Amylase Activity in mg/l of Maltose					
	0		1100		5500	
	SPC	S	SPC	S	SPC	S
0	32×10^4	173.0	38×10^4	174.0	30×10^4	178.0
48	59×10^6	60.0	60×10^6	106.0	25×10^6	127.0
96	54×10^6	38.0	12×10^7	75.0	10×10^7	55.0
144	24×10^5	34.0	54×10^6	55.0	19×10^8	63.0

SPC - Standard Plate Count. (Viable cells/ml)

S - Total Reducing Sugar in mg/l

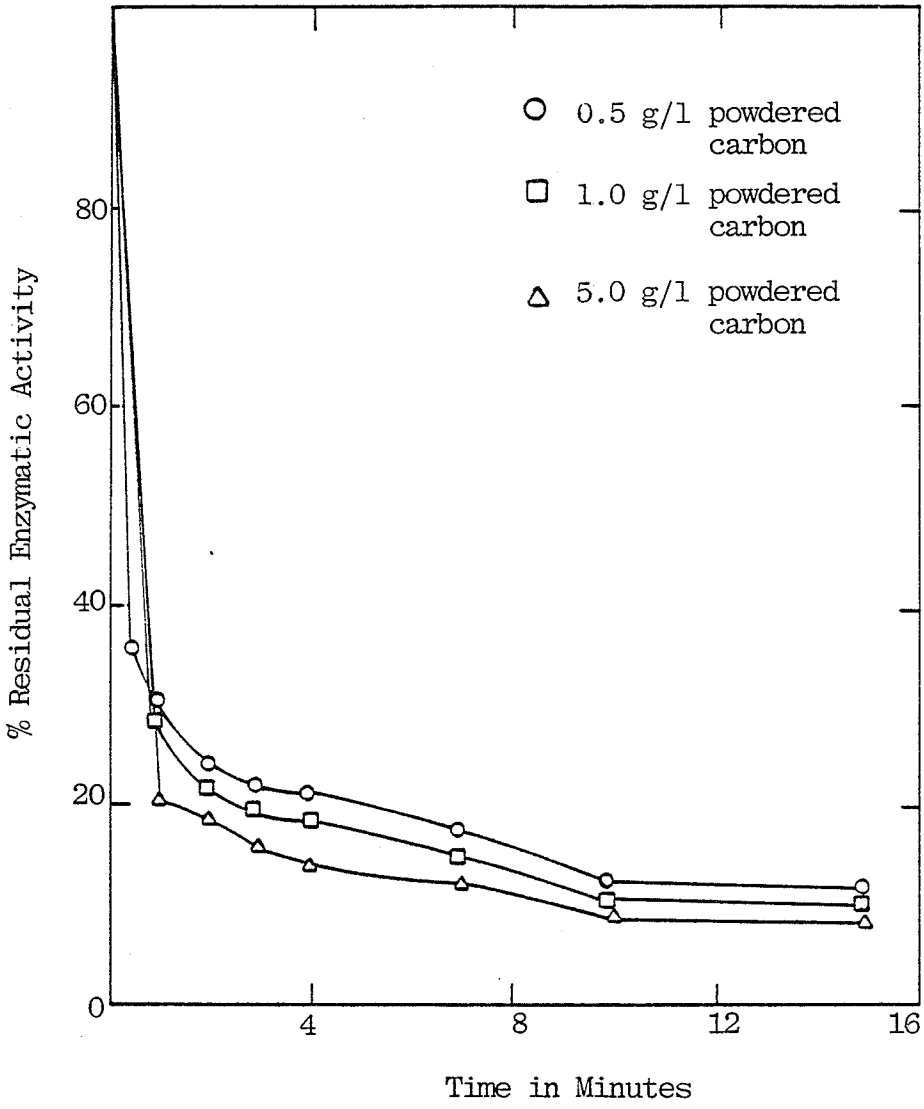


Fig. 11. Rate of the Removal of Beta-Amylase by Powdered Activated Carbon at pH 4.5

Appendix Tables 6,7 & 8

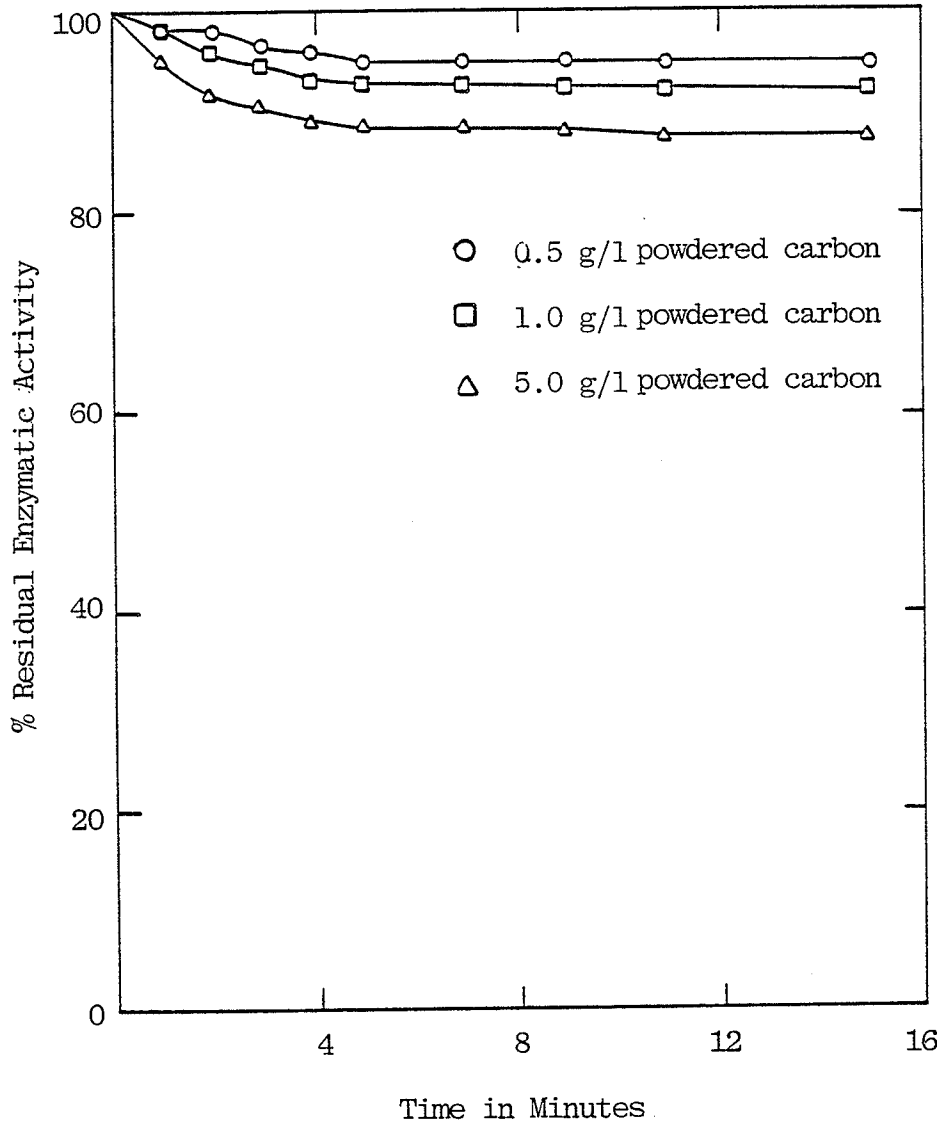


Fig. 12. Rate of the Removal of Beta-Amylase by Powdered Activated Carbon at pH6.8

Appendix Tables 9, 10 & 11

greater extent at pH 4.5 than at pH 6.8. The rate curves at pH 4.5 were characterized by a relatively rapid initial rate of removal which increased markedly after approximately 3 minutes to a gradual approach to an equilibrium condition. Results indicated that the removal of beta-amylase increased with increasing dosage of powdered activated carbon. The order of removal was 5 g/l carbon-treated sample greater than 1 g/l which in turn was greater than 0.5 g/l, though the difference was not appreciable.

At pH 6.8, beta-amylase was removed to a small extent. The maximum removal of beta-amylase was 13% even when a relatively high dosage of carbon (5 g/l) was employed. At low carbon dosage (0.5 g/l), only 5% of the beta-amylase was removed.

These results are in agreement with Sablilschka's findings (77). He reported that removal of amylases with activated carbon was poor at neutral pH. But the adsorption was enhanced with the lowering of pH to 4.5 or lower. However the enzyme would be denatured if the pH is lowered beyond 3.2. The adsorption was due to the association of the enzyme as its isoelectric point of 3.2 was approached.

4.11 Effect of pH and Carbon Treatment on Beta-Amylase Activity

Graphical representation of this study is shown in Figure 13. Residual activity of beta-amylase was expressed as percentage of the activity of potato rinse water treated with the same level of carbon at pH 6.8.

Results indicated that the use of 0.5 g/l, 1 g/l and 5 g/l of activated carbon for treatment at pH 4.5, would leave the potato rinse water with 56%, 52% and 45% residual enzymatic activity

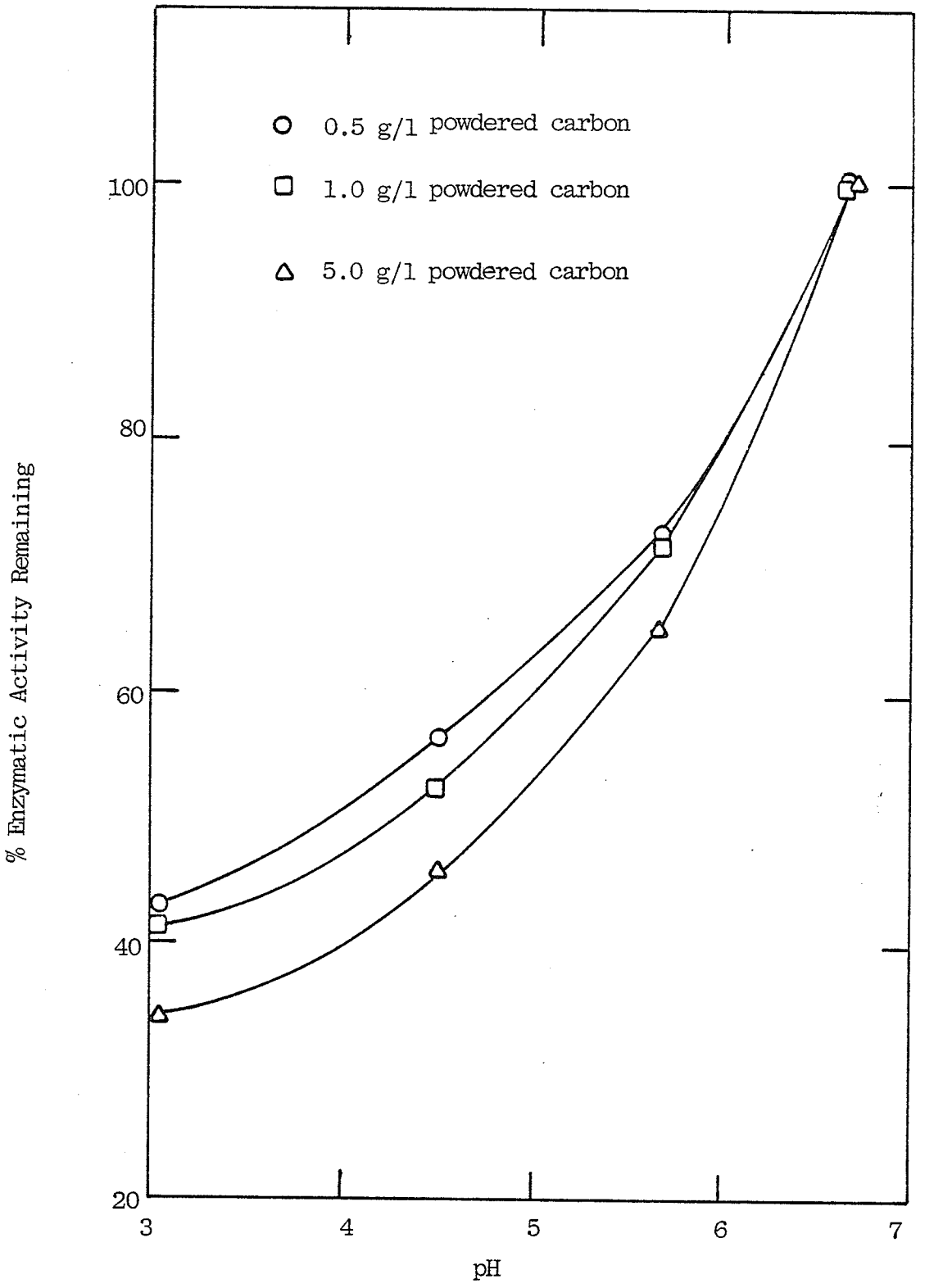


Fig. 13. Control of Beta-Amylase with 0.5, 1.0 & 5 g/l of Powdered Activated Carbon in Potato Rinse Water at Various pH's

Appendix Tables 12, 13 & 14

respectively. At neutral pH, there was no adsorption of beta-amylase, while at pH 3.2, 45,41 and 35% of beta-amylase were left after the applications of 0.5, 1, 5 g/l of powdered activated carbon. The adsorption of beta-amylase with carbon depended greatly on pH.

Citric acid was used in this experiment to lower the pH of the potato rinse water. The fact that addition of such an acid would increase the oxygen demand of potato rinse water had to be considered. It was studied by Hydamaka et al. (45). When potato rinse water was adjusted to pH levels ranging from 4.5 to 6.8, and then dosed with up to 0.2% of powdered activated carbon, samples which were lowered to a pH of either 5.0 or 5.5 had a slightly lower oxygen demand than the sample which was unadjusted. The results were explained by the fact that the additional oxygen demand of citric acid was compensated for, by the removal of slight amounts of protein which denatured upon acid addition, and then was removed in the filtration step.

In the light of their findings, beta-amylase in potato rinse water can be controlled by lowering pH with citric acid without appreciable effects on the oxygen demand.

4.12 Effect of Powdered Activated Carbon on Quality Control Factors of Recycled Potato Rinse Water

In this experiment, powdered activated carbon was used to control the aesthetic factors such as turbidity and beta-amylase that breaks down starch to simpler molecules for the growth of bacteria. Results of turbidity and beta-amylase activity are tabulated in Tables 13 & 14. Graphical representations of CO_2D are shown in Figures 14 & 15.

Table 13 . The Effect of Intermittent Carbon Treatment (1g/l) on Potato Rinse Water During Recycling

No. of rinses	Carbon Treatment	Turbidity	Beta-Amylase Activity
5	Before	28.0	875.0
	After	16.0	870.0
10	Before	29.0	1276.0
	After	23.0	1210.0
15	Before	28.0	1760.0
	After	25.0	1694.0
20	Before	34.0	1926.0
	After	26.0	1901.0

Turbidity in J.T.U.
Beta-Amylase Activity in mg/l of maltose

Table 14. The Effect of Intermittent Carbon Treatment (5g/l) on Potato Rinse Water During Recycling

No. of rinses	Carbon Treatment	Turbidity	Beta-Amylase Activity
5	Before	25.0	876.0
	After	14.0	800.0
10	Before	25.0	1058.0
	After	18.0	940.0
15	Before	26.0	1464.0
	After	21.0	1368.0
20	Before	27.0	1541.0
	After	21.0	1479.0

Turbidity in J.T.U.
Beta-Amylase Activity in mg/l of maltose

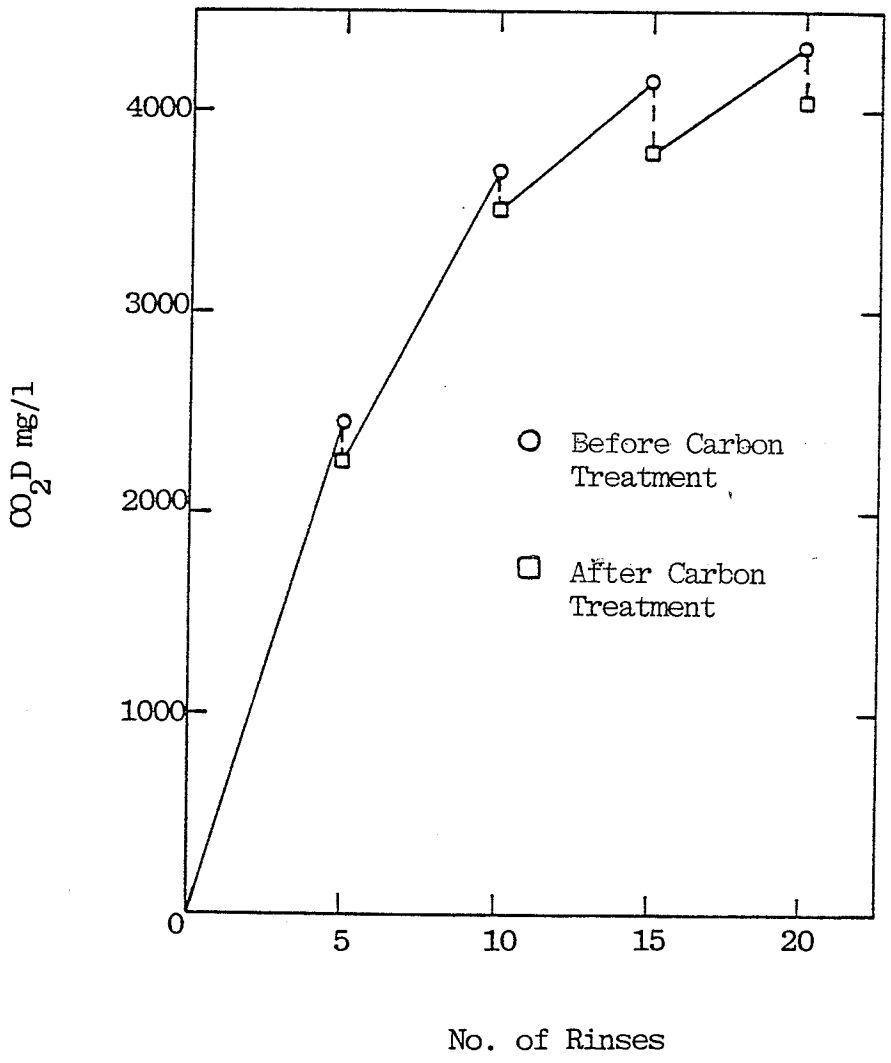


Fig. 14. Control of CO_2D with Powdered Activated Carbon (1g/l) in Potato Rinse Water

Appendix Table 17

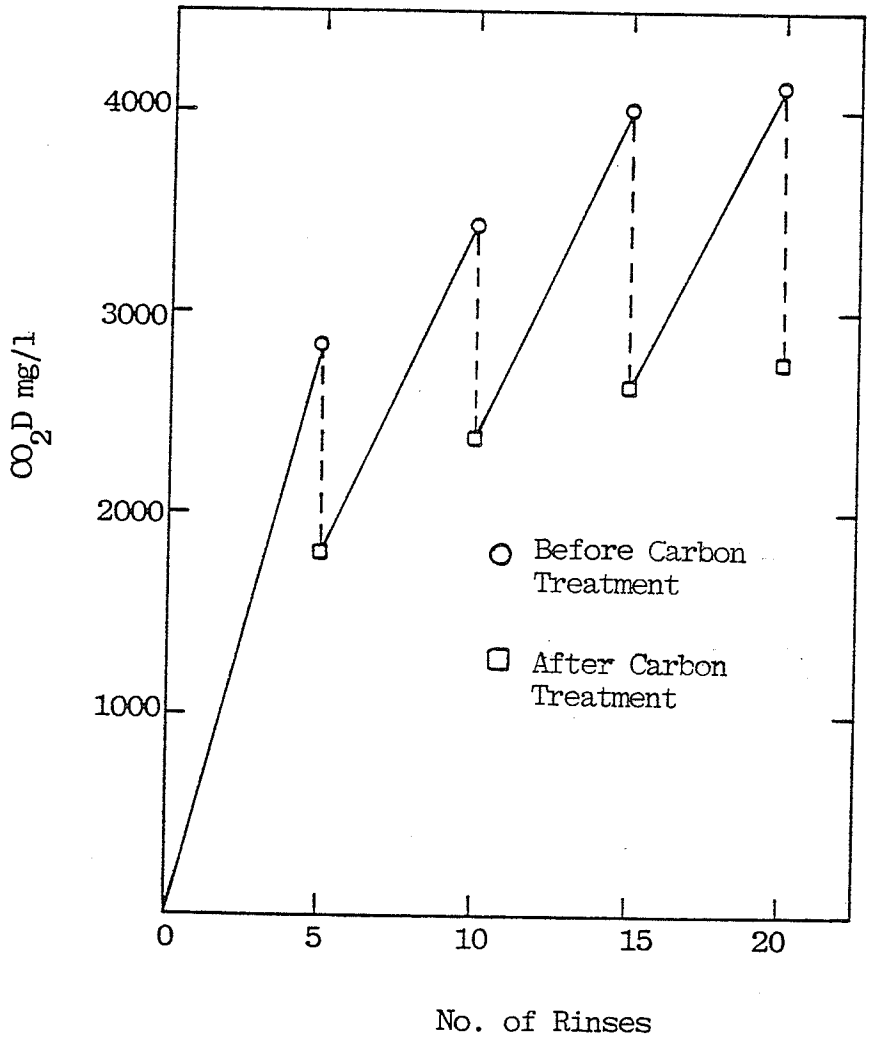


Fig. 15. Control of CO_2D with Powdered Activated Carbon (5g/l) in Potato Rinse Water

Appendix Table 18

Levels of 1, 5 g/l of powdered activated carbon were used for intermittent treatment at every fifth rinse stage. It was found that 1 g/l of carbon treatment slightly reduced the amount of total organics and the level of turbidity while the beta-amylase activity was insufficiently unremoved. At this carbon level, the potato rinse water produced was slightly brown in color, possibly due to the incomplete removal of polyphenolase. At higher rinse stages, such as the fifteenth and twentieth rinse, the CO_2D and beta-amylase levels started to build up. The water was brown and was considered "aesthetically" not acceptable for further recycling, unless treated with higher levels of powdered activated carbon.

At a higher dosage, 5 g/l of powdered carbon was found to be effective in controlling these factors. This figure was based on the minimum amount of carbon required to produce a clear potato water filtrate free from browning and turbidity after the potato rinse water had been used for five times. A dosage of 5 g/l of activated carbon could also suppress the level of total organics level. The use of this amount of carbon would allow the physical, physico-chemical, biochemical and biological factors to rise to the equilibrium levels at which stage a "saw tooth" effect would result showing suppression of leaching from the potato French fry slices, while simultaneously producing water which was desirable for the particular unit operation. However beta-amylase level accumulated as the the rinsing continued. The activity increased by 68% at the end of the twentieth rinse. As illustrated from the use of lowering pH on effective adsorption of beta-amylase, it is recommended that the pH of the potato rinse water

should be lowered to reduce the beta-amylase activity if the potato rinse is required to be stored for further recycling purposes.

CONCLUSIONS AND RECOMMENDATIONS

(1) Control of Turbidity

The first part of the project was to investigate various factors contributing to turbidity development in recycling of potato water, generated at the slice-rinse stage. The following treatment schemes were studied:

(1) Millipore filtration

(2) Carbon treatment

(3) Refrigeration

(4) Millipore filtration with powdered activated carbon treatment

(5) Millipore filtration with refrigeration

(6) Powdered activated carbon treatment with refrigeration

Millipore filtration treatment was found to permit the potato rinse water from becoming turbid for at least 72 hours. It was also observed that millipore filtration with carbon treatment, millipore filtration with refrigeration, and refrigeration alone could keep the turbidity level of the potato rinse water at a relatively constant level for 72 hours. However, millipore filtration with refrigeration is not expected to be feasible on a commercial practice.

Intermittent activated carbon treatment was used to study the feasibility of controlling the turbidity, an aesthetic factor, in the potato rinse water during multiple reuse. Carbon dosage of 1g/l applied after every rinse has been shown to be successful not only in controlling the turbidity level, but also in allowing

the soluble CO_2D to build up in the potato rinse water to an equilibrium condition thus preventing further extraction of solutes from the food product. An alternate treatment of 5 g/l of carbon after every fifth rinse has been shown to be satisfactory, specifically in controlling turbidity of potato rinse water.

However, if the recycled water is intended to be left for an extended period of time, such as overnight or weekend storage in a potato plant, a turbidity problem arises. Since government policies are stringent on the quality standard of water being used for food processing purposes, this problem has been investigated closely. As indicated in the results, turbidity is caused mainly by bacterial growth. The use of activated carbon, regardless of carbon dosage, cannot indefinitely suppress turbidity development. The time for excessive turbidity to appear was found to be dependent upon three factors:

(a) The organic strength of the potato waste.

(b) The amount of carbon used. The higher the organic strength of the potato water, the faster the water turned turbid. The greater the amount of carbon used, the longer could the water be kept from becoming turbid.

(c) The original bacterial load.

(2) Control of Beta-Amylase

Bacterial growth depends on the amount of available diffusable nutrient sources. The presence of beta-amylase in potato water causes the starch to be hydrolyzed into maltese and other sugar units which in turn are broken down by maltase, providing an additional source of utilizable glucose for bacteria. Results have shown that

increased amount of beta-amylase in recycled potato rinse water is capable of supporting a higher population of bacterial growth. This would lead to an increase in turbidity levels. Since beta-amylase is considered to be active in the breakdown of starch in potato rinse water, it is therefore essential to include it as one of the controlling factors in recycling studies. Attempts that were made to control the beta-amylase activity with 1 and 5 g/l carbon applied every fifth rinse, were unsuccessful. Beta-amylase can be controlled by lowering pH, or by the combined treatment of carbon and acidic pH. During continuous recycling, beta-amylase levels were observed to build up. However, this is not of immediate concern, since the time factor is required for the enzymatic breakdown of starch to glucose which is the essential nutrient for bacterial growth. If the water is left for long periods of time, the bacterial population increase due to enzymatic action and it is recommended that the pH of the recycled potato rinse water should be controlled by lowering the pH to at least 4.5 with citric acid or combined treatment of citric acid and powdered activated carbon, before the water is stored for further reuse purposes. Previous work in the Food Science Department has shown citric acid to be beneficial in controlling the color problem that arises during potato rinse water recycling (45).

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Appendix Table 1. CO_2D Standard Curve

CO_2D (mg/l)	Absorbance
50.0	14.2
100.0	30.4
150.0	48.0
200.0	62.5
250.0	77.6
300.0	94.5

Appendix Table 2. Maltose Standard Curve for Determination of Beta-Amylase Activity

Wt. of Maltose (mg.)	Absorbance at 540 mu
0.1	0.03
0.2	0.06
0.3	0.09
0.4	0.14
0.5	0.17
0.6	0.21
0.7	0.24
0.8	0.28
0.9	0.31
1.0	0.35

Appendix Table 3. Standard Curve for Determination of Total Reducing Sugar

mg glucose / 5 ml sample	0.005 N thiosulfate Difference from blank (ml)
0.5	4.10
1.0	8.68
1.5	12.50
2.0	17.75
2.5	22.30

Appendix Table 4. The Effect of Powdered Activated Carbon (1g/l) on Turbidity and CO_2D Control

No. of rinses	Treatment	Turbidity (J.T.U.)	CO_2D (mg/l)
1	Before	20.0	840.0
	After	6.0	630.0
2	Before	28.0	875.0
	After	16.0	687.0
3	Before	29.0	940.0
	After	17.0	740.0
4	Before	30.0	980.0
	After	18.0	800.0
5	Before	28.0	1010.0
	After	19.0	824.0
6	Before	30.0	1037.0
	After	19.0	880.0
7	Before	30.0	1102.0
	After	20.0	1002.0
8	Before	30.0	1206.0
	After	18.0	1100.0
9	Before	31.0	1354.0
	After	20.0	1128.0
10	Before	31.0	1400.0
	After	24.0	1194.0

Appendix Table 5. Effect of Powdered Activated Carbon on the Removal of Turbidity

Carbon dosage (g/l)	Turbidity (J.T.U.)	% Remaining
0.0	25.0	100.0
1.0	13.0	53.2
3.0	8.0	32.5
5.0	7.7	31.4
10.0	6.4	27.6

Appendix Table 6. Rate of The Removal of Beta-Amylase with 0.5 g/l of Powdered Activated Carbon at pH 4.5

Time in min.	pH	*Beta-Amylase Activity	Residual Activity %
0.0	4.5	7147	100.0
0.5	4.5	2548	35.7
1.0	4.5	2182	30.5
2.0	4.5	1725	24.1
3.0	4.5	1594	22.4
5.0	4.5	1450	20.3
7.0	4.5	1267	17.8
10.0	4.5	927	13.0
15.0	4.5	876	12.3

*Beta-Amylase Activity in mg/l of maltose

Appendix Table 7. Rate of the Removal of Beta-Amylase with 1.0 g/l of Powdered Activated Carbon at pH 4.5

Time in min.	pH	*Beta-Amylase Activity	Residual Activity %
0.0	4.5	7147	100.0
0.5	4.5	2359	33.0
1.0	4.5	1572	21.9
2.0	4.5	1429	19.9
3.0	4.5	1429	19.9
5.0	4.5	1215	17.0
7.0	4.5	1092	15.3
10.0	4.5	715	10.0
15.0	4.5	715	10.0

*Beta-Amylase Activity in mg/l of maltose

Appendix Table 8. Rate of the Removal of Beta-Amylase with 5.0 g/l of Powdered Activated Carbon at pH 4.5

Time in Min.	pH	*Beta-Amylase Activity	Residual Activity %
0.0	4.5	7147	100.0
0.5	4.5	2216	31.0
1.0	4.5	1429	19.9
2.0	4.5	1286	17.9
3.0	4.5	1021	14.3
5.0	4.5	1006	14.0
7.0	4.5	858	12.1
10.0	4.5	572	8.0
15.0	4.5	572	8.0

*Beta-Amylase in mg/l of maltose

Appendix Table 9. Rate of the Removal of Beta-Amylase with 0.5 g/l of Powdered Activated Carbon at pH 6.8

Time in min.	pH	*Beta-Amylase Activity	Residual Activity %
0.0	6.8	7147	100.0
1.0	6.8	7018	98.2
2.0	6.8	7004	98.0
3.0	6.8	6968	97.5
5.0	6.8	6890	96.4
7.0	6.8	6890	96.4
10.0	6.8	6853	95.6
15.0	6.8	6804	95.2

*Beta-Amylase Activity in mg/l of maltose

Appendix Table 10. Rate of the Removal of Beta-Amylase with 1.0 g/l of Powdered Activated Carbon at pH 6.8

Time in min.	pH	*Beta-Amylase Activity	Residual Activity %
0.0	6.8	7147	100.0
1.0	6.8	7004	98.0
2.0	6.8	6997	96.5
3.0	6.8	6797	95.0
5.0	6.8	6675	93.4
7.0	6.8	6675	93.4
10.0	6.8	6574	92.0
15.0	6.8	6574	92.0

*Beta-Amylase Activity in mg/l of maltose

Appendix Table 11. Rate of the Removal of Beta-Amylase with 5.0 g/l of Powdered Activated Carbon at pH 6.8

Time in min.	pH	*Beta-Amylase Activity	Residual Activity %
0.0	6.8	7147	100.0
1.0	6.8	6833	95.6
2.0	6.8	6604	92.4
3.0	6.8	6432	90.0
5.0	6.8	6397	89.5
7.0	6.8	6389	88.0
10.0	6.8	6218	87.0
15.0	6.8	6218	87.0

*Beta-Amylase Activity in mg/l of maltose

Appendix Table 12. Control of Beta-Amylase in Potato Rinse Water with 0.5g/l of Powdered Activated Carbon at Various pH

pH	*Residual Beta-Amylase Activity	Residual Activity %
6.5	2800	100.0
5.7	2030	70.5
4.5	1563	55.8
3.0	1264	45.1

*Residual Beta-Amylase Activity in mg/l of maltose

Appendix Table 13. Control of Beta-Amylase in Potato Rinse Water with 1.0g/l of Powdered Activated Carbon

pH	*Residual Beta-Amylase Activity	Residual Activity %
6.5	2800	100.0
5.7	2010	71.8
4.5	1451	51.8
3.0	1152	41.0

*Residual Beta-Amylase Activity in mg/l of maltose

Appendix Table 14. Control of Beta-Amylase in Potato Rinse Water with 5g/l of Powdered Activated Carbon at Various pH

pH	*Residual Beta-Amylase Activity	Residual Activity %
6.5	2800	100.0
5.7	1824	65.1
4.5	1273	45.5
3.0	964	34.5

*Residual Beta-Amylase Activity in mg/l of maltose

Appendix Table 15. Relation of CO_2D, Nitrogen & Beta-Amylase to Multiple Reuse of Potato Rinse Water.

No. of Rinses	CO_2D mg/l	Nitrogen %	*Beta-Amylase Activity
5	2920	1.15	800
10	5200	2.21	1096
15	11060	3.87	1355
20	12780	4.50	1480
25	13050	4.60	1605
30	13100	4.65	1620
35	13100	4.65	1620

* Beta-Amylase Activity in mg/l of maltose.

Appendix Table 16. Control of CO_2D with Powdered Activated Carbon (1 g/l) in Potato Rinse Water

No. Of Rinses	Treatment	CO_2D mg/l
5	Before	2475
	After	2250
10	Before	3720
	After	3500
15	Before	4120
	After	3800
20	Before	4340
	After	4040