THE CHARACTERIZATION OF THE AEROBIC - THERMOPHILIC DEGRADATION OF POTATO WASTES

by

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ABSTRACT

Feasibility of the biodegradation of neutralized-caustic potato peel waste by aerobic-thermophilic fermentation was examined. Fermentation temperature, pH, rate of solids utilization, dissolved oxygen and total organic nitrogen were characterized with respect to the fermentation process. Examination of these parameters showed distinct characteristic behaviour, though the parameter magnitude varied between the trials. The naturally occurring flora of thermophilic microorganisms of the potato was sufficient as an inoculum, with their propagation creating the fermentation characteristics.

The solids content of the potato waste was reduced by the thermophilic fermentation with a noticeable improvement in the nitrogen content in the remaining residue. Protein and amino acid analyses supported the feasibility of using the remaining residue as animal feed.

Increases in the nitrogen content of the substrate was assumed to be due to the conversion of atmospheric nitrogen by nitrogen-fixing microorganisms within the fermentation. Microbial identification indicated that these organisms belonged to the species Bacillus coagulans. No strains within this species have been previously reported to fix atmospheric nitrogen.

It was concluded that an aerobic-thermophilic fermentation can be utilized as a waste treatment process for caustic potato peel waste. Besides the numerous advantages of an aerobic-thermophilic fermentation, the validity for the utilization of this fermentation process in the treatment of potato peel waste is the near total disposal of the waste.

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Dedicated with love

to my late Grandmother

INTRODUCTION

Ideally, many food plant wastes should be developed as a utilizable resource, but this is not always possible due in part to unfavorable economics and to the lack of suitable technology. Generally, wastes have been treated as a management or disposal problem. Agricultural wastes constitute by far the largest volume of organic wastes, amounting to over 200 million tons in Canada in 1975 (42). Beef and dairy cattle manure accounts for the majority of the animal wastes while the largest proportion of the plant wastes are contributed by straw residues. Of the various food industries, potato processing accounts of over 450,000 tons annually, fruit and other vegetable wastes exceed one million tons and meat processing wastes are produced at levels of 150,000 tons. Other wastes of significant importance in relation to our environment are those from dairy, brewery and distillery industries (42).

In recent years, the disposal of large volumes of wastes has become a matter of serious economic concern to the processed potato industry. If the solid potato wastes are not sold directly as cattle feed, they must be treated by a conventional waste disposal method in which the cost may be economically unfavourable.

The composition of a waste stream from a potato processing plant is largely determined by the process used. Generally, the potato process involves the following steps: washing of the raw potatoes; peeling, which includes washing to remove softened tissue; trimming, to remove defective portions; shaping, washing and separation; heat treatment which is optional; final processing or preservation; and packaging. The composition of waste streams from potato processing plants is closely related to the composition of the potato itself. The components which are foreign to the potato and which may be present in the waste stream include dirt or silt, caustic soda, fat, cleaning and preserving chemicals, and other food ingredients in lesser amounts. Normally, most streams in the processing plant are combined before being discharged. The types of waste should be considered separately since they can be removed at different steps of the processing operation and, in some cases, can be treated more effectively if separated within the plant. Since the peeling operation generates more waste than all of the other potato processing operations combined, an attack on this major fraction was considered to be the most important in alleviating a pollution problem. The caustic peel waste fraction has a pH of over 12 and in this form it cannot be

used as livestock feed, and if dumped without further processing it will form a gel that is resistant to decomposition. In addition, with the use of caustic sodium hydroxide solution as a peeling agent, the high sodium content in the waste water may make the soil impermeable to water and toxic to plant growth (41).

Aerated lagoons and activated sludge systems are treatment processes commonly used in this industry. However, these processes involve a lengthy treatment before the waste is discharged. The purpose of this investigation was to determine whether exothermic aerobic thermophilic waste treatment processing can be used to degrade potato wastes. The uniqueness of the thermophilic process is provided by the temperature of the degradation. High temperatures are created by the heat energy developed metabolically by the aerobic organisms in the degradation of the substrate. This exothermal process is intensified, within limits, by higher substrate concentrations since the amount of heat energy increases proportionally to the amount of organic substances being biochemically converted (43). The increased exothermic heat of the reaction accelerates the rate of substrate degradation thus increasing the efficiency of the process over that attained by mesophilic systems. The increased reaction rates result in shorter detention

times in comparison to mesophilic systems for the same degree of purification, thus contributing to decreasing operational costs.

With this process, as applied to potato waste, an improved solids-liquid separation occurs. Water separation is important in order to reduce the volume of waste to obtain a high settleable solids concentration. In primary waste treatment, some form of concentration is normally employed. Both continuous vacuum filters and continuous centrifugal units are used commercially for this purpose. Additional dewatering by pressing is not feasible (61). The solids which result from the caustic peeling process, with its high pH, will not easily dewater on a vacuum filter (61). Thus the thermophilic process provides an additional benefit in improving solids-liquid separation of the digested sludge which must be dewatered prior to further processing or ultimate disposal. Garber (20) showed that thermophilic digested sludge had a greatly improved vacuum filter yield as compared to mesophilic sludge, together with a lower coagulant demand.

The destruction of organisms pathogenic to animals at temperatures achieved in the thermophilic waste treatment systems is of special significance (4,55). As an animal feed, utilization of the sludge from this process

is a possible asset because of its pathogen-free quality. Even if the sludge was disposed on soil, the public health aspects would be of considerably less concern than the mesophilic sludge discarded in a similar manner.

In comparison, to the mesophilic systems, the increased temperatures achieved in thermophilic fermentations is advantageous in the overall treatment of the waste especially during the cold season. The rate of decomposition accomplished at thermophilic conditions is unaffected by low ambient temperatures provided sufficient fermenter insulation is applied, whereas mesophilic reactions may be hampered by freezing environmental conditions.

The possible disadvantage of the thermophilic process is its poor process stability especially with respect to temperature. It is well known that as biological processes approach environmental extremes (pH, salinity, etc.) in the substrate, fewer species are capable of surviving and the fermentation becomes unstable or less resistant to change in the substrate composition. Larger installations, however, show greater stability and temperature maintenance for a longer duration because of their increased fermentation mass. Accordingly, adequate control and monitoring of the treatment system is an important requirement for the successful application of a thermophilic digestion operation.

The purpose of this thesis was threefold. First, to establish the composition of the substrate, namely, the caustic potato peel waste, as to its nutritional availability to microorganisms. Secondly, to construct a laboratory system of sufficient size in which the thermophilic process has enough stability and operational controllability during the digestion to enable adequate study. Thirdly, if thermophilic conditions are obtainable with this substrate in the laboratory system, to study and to characterize the thermophilic digestion of potato peel waste.

LITERATURE REVIEW

I. Thermophilic Bacteria

The thermobiosis phenomenon or the ability of biological entities to grow at elevated temperatures has been known for centuries. The initial recorded discovery of a thermophilic bacterium was credited to Miguel in 1879 (1,11, 19,21). Morrison and Tanner (40) outlined the methodology for the study of aerobic thermophilic bacteria from water. Robertson (45) presented a complete bibliography of early workers prior to 1927 in which he described both the sporulating and the non-sporulating thermophilic bacilli. Gaughran (21), and subsequently, Allen (1) presented excellent reviews on the state of the art concerning thermophilic microorganisms. Koffler (29) discussed studies involving the thermal stability of enzymes and structural proteins of thermophiles. Campbell and Pace (11) reviewed the literature on the microbial physiology of growth at elevated temperatures and more recently, Farrell and Campbell (19) published a review describing the thermophilic bacteria belonging to the genus Bacillus and their associated bacteriophages.

Many of the mentioned studies have questioned how these microorganisms grow at high temperatures, and in many instances are actually obligate to these high temperatures; that is, growth at temperatures which would normally destroy or inactivate the cellular components of most life forms. Generally, two explanations have been offered. Firstly, and the most obvious, is that the essential cellular components necessary for life of the thermophilic organisms are relatively more heat stable than their mesophilic counterparts. Secondly, the cells are capable of rapid resynthesis of the destroyed or inactivated cellular components.

Numerous studies have shown the ubiquitous nature of the thermophilic microorganisms. They have been found to occur in such diverse climates as in tropical soils, in desert sands and in fallen snow. Thermophiles have been isolated from air, from salt and fresh water, from feces of all domestic animals and man, from grains and foods of all varieties, from raw and pasteurized milk, from stored vegetables, and especially from masses of decaying plant materials (1,21). Climatic conditions apparently have no influence on the distribution of the thermophilic bacteria and there seems to be little doubt that the thermophiles are members of the naturally occurring soil inhabitants

and can be isolated from any material which has come in contact with soil. Due to their universal distribution, it is evident that proliferation of these organisms will take place whenever conditions are favourable within a natural or an artificial environment.

II. Application of the Aerobic-Thermophilic Bacteria

The aerobic-thermophilic wastewater treatment process is a recent development and is considered as a method for the treatment of concentrated organic wastewater (35). McCarty (37) showed that the thermophilic microorganisms utilized nutrients in wastewater for cell synthesis and maintenance. The efficiency of the transfer of the contained substrate energy to usable cell energy by the microorganisms was considered to be in the 20-40 per cent range, although, McCarty acknowledged that it was closer to 70 per cent. It was obvious that a significant amount of energy was lost to the surrounding environment. Rich and Andrew (44), in their discussion of McCarty's paper, noted that this escaped energy was responsible for raising the temperatures of composting solid wastes as high as 70-75°C. In contrast to composting, in systems where there is a recognizable liquid phase, as in aerobic-thermophilic

digestion of wastewater, it is more difficult to notice the heat liberated by the microorganisms since the heat is readily disseminated and lost to the system.

Kambhu and Andrew (28) showed by a computer simulation study of aerobic thermophilic digestion of sludge, that sufficient heat was generated by the destruction of organic solids to make the process self-sustaining in the thermophilic range. However, in their model study of primary and activated sludge solids, heat was supplied to raise the temperature of the reactor body. Pöpel and Ohnmacht (43) working with sewage sludges, industrial wastes and liquid manure, showed in a batch test with sludge of 10.8% solids concentration that the organic solids content was reduced by 90% over a 10.5 day period. With frequent feeding of fresh sludge, it was possible to maintain a temperature near 50°C even though the ambient temperature decreased from 20°c to 5°C. The latter authors found that there was a 75% reduction of organic material in as little as 21.5 hours. Smith et al. (50) concluded that aerobic-thermophilic digestion can accomplish the same degree of organic solids destruction as an optimally operated conventional mesophilic waste treatment system with the same amount of oxygen supplied but with a much greater loading input (4.6 X) and in less time (20% less). Also the same

reduction of organic matter could be accomplished in a tank which was one-sixth the volume normally required in mesophilic operating systems. Oxygen gas was used in place of air in order to minimize heat loss from the digester and to permit the digestion of a higher solids content sludge. Loll (36) reported that the treatment of undiluted sludge liquors by the aerobic-thermophilic process reduced the chemical oxygen demand (C.O.D.) by about 70% and the biochemical oxygen demand ($B.O.D._5$) by about 95%. In contrast with aerobic-thermophilic digestion, anaerobic thermophilic digestion reduced the $B.O.D._5$ by only 73%, whereas, the C.O.D. reduction was about the same (68%).

In a previous paper by Loll (35) it was shown, when using $B.O.D._5$ as a reference parameter, a biological reduction of between 95% and 97% was reached in batch digestion tests with yeast waste, molasses waste and pig manure. A 5 to 7-day retention time was used for all three substrates. Maximum substrate temperatures of 46°C to 50°C were reached within a 3 to 5-day period after the beginning of the experiment, during which time the ambient temperature varied between 17°C and 20°C. The temperature of the fermentation rose to a maximum at a point where the bulk of the organic substances had been degraded, and then subsequently decreased due to reduced microbial metabolic activity.

Recently, the recovery of protein in the form of bacterial cells from the thermophilic aerobic treatment of high organic strength wastewater has received considerable attention. The protein content and nutritional profile of thermophilic microorganisms may be better than that obtained from mesophilic organisms (4,55). Bellamy (4) concluded that the thermophilic protein contained relatively high concentrations of lysine, tryptophane and sulfur amino acids and should have a high nutritional value for monogastric animals as well as for ruminants.

Investigations on the application of the aerobic-thermophilic degradation process of organic wastewaters originating from the food industry are almost non-existent in comparison to the information available on animal waste management. It is evident that the potential of the thermophilic microbiological treatment of high strength wastewater should be of interest to the food processing industries.

MATERIALS AND METHODS

I. Analyses of the Caustic Soda Potato Peel Wastes

Four-gallon samples of lye-peeled potato waste were obtained directly from the caustic peelers (Figure 1) of Fraser Valley Frosted Foods Ltd., Chilliwack, B.C. The samples were brought to the laboratory, at the University of British Columbia, and stored in a refrigerated room at 5°C. These processing plant samples were subsequently analyzed and compared to freshly homogenized whole potatoes of the same variety.

1. Total Kjeldahl Nitrogen Determination

Total organic nitrogen was done in triplicate according to the rapid micro-Kjeldahl method of Concon and Soltess (16). The amount of nitrogen in each digested sample was determined using an Auto Analyser II (Technicon Instruments Corp., Tarrytown, N.Y.). All nitrogen analyses were calculated on a dry basis.

2. Total Carbon and Inorganic Carbon Determination

The samples were analyzed by using a Beckman Model 915 Total Organic Carbon Analyser connected to a

Figure 1. Caustic potato peel waste from the mechanical peeler.

Beckman Infra-Red Analyzer (Beckman Instruments Inc., Fullerton, CA). Standard solutions of potassium hydrogen phthalate (100 - 500 ppm) and sodium bicarbonate (10 - 50 ppm) were prepared according to A.O.A.C. 33.047 (2) for total carbon determination and for inorganic carbon determination, respectively. Calibration curves for total carbon and organic carbon were prepared by plotting millivolt reading versus concentration of the carbon-containing stock solutions.

The potato samples were dried at atmospheric pressure to a constant weight in a drying oven (see section on "Solids determination") and then crushed to a powder with a mortar and pestle. A 40 mg portion of the powder was placed into a 7 ml Ten-Broeck Tissue Grinder (Corning Glass Works, Corning, N.Y.) with a small amount of distilled-deionized water. The sample was homogenized until the particles were extremely fine and suspended in solution. This solution was removed from the homogenizer and made up to volume in a 100 ml volumetric flask with distilled-deionized water. Twenty microliters of this final sample solution (400 ppm in terms of dried potato solids) was injected into the Beckman Total Carbon Analyzer with a calibrated Hamilton syringe (Hamilton Co., Whittier, CA).

All of the glassware used in these experiments was thoroughly cleaned by soaking in a potassium

dichromate-concentrated sulfuric acid solution overnight. The glassware was removed from the chromic acid solution, rinsed thoroughly in distilled water and then in distilled-deionized water.

3. Total Phosphorus Determination

The method used for the preparation of the samples for phosphorus analysis was similar to those used in the Kjeldahl nitrogen determinations. Oven dried samples of the caustic potato peel waste and fresh potatoes were weighed and placed into Kjeldahl digestion flasks. After digestion, the samples were analyzed on the Technicon Auto-Analyzer. A blank, containing only the reagents, was prepared in conjunction with the samples.

A KH_2PO_4 standard solution was prepared within the estimated range of the phosphorus content of the potato samples. A dilution of this concentrated standard was applied to the autoanalyzer as a reference standard.

All glassware for this analysis was soaked overnight in a chromic acid solution. After rinsing in deionized-distilled water, the glassware was soaked in a 1 to 1 aqueous solution of hot HCl (2) and then rinsed in deionized-distilled water.

4. Total Sodium Determination

A wet ashing method (24) was used for the sodium analyses of the dried potato samples by atomic absorption spectrophotometry. Sample digestion was carried out using a 30 ml volume of a mixture of nitric-perchloric-sulfuric acids added into a long-neck Kjeldahl digestion flask. The mixture was added cold and the flask was heated slowly to encourage a smooth digestion without charring. After completion of the digestion, the flask was heated more intensely to remove excess nitric and perchloric acid and to reduce the volume of the solution. The end of the digestion was indicated by sulfuric acid fuming in the neck of the flask.

All the glassware for this analysis was soaked overnight in a solution of potassium dichromate-concentrated sulfuric acid. The glassware was rinsed in deionized-distilled water, followed by a concentrated nitric acid soak to remove all the remaining sodium ions. A final rinse in deionized-distilled water was employed to remove the nitric acid.

An overall standard curve was calibrated by atomic absorption spectrophotometry (Model 82-800, Jarrell-Ash, Division of Fisher Scientific Co., Waltham, MA) using

varing diluent concentrations of a commercial stock solution composed of sodium bicarbonate (Fisher Scientific Co., Fair Lawn, N.J.). On determining the profile of the overall standard curve, it was found that the linear portion ranged from 0 to 10 ppm. A more finite calibration was required for this linear portion of the standard curve. Standard solutions of 0 to 10 ppm were prepared for the determination of this more finite standard curve.

Linear regression analysis was applied to the data. From this analysis, a straight line equation was obtained for the finite standard curve ($y = 0.041x - 0.004$). This equation was used to calculate the concentration of the sodium ion from the readings obtained for the samples.

Caustic potato peel waste, and peelings and pulp (parenchyma and pith tissue) from fresh potato were analyzed. These samples were adjusted so the concentration of sodium would fall within the range of the finite standard curve. Duplicate analyses were run for each sample at two different concentrations (20 ppm and 200 ppm, in terms of dried potato solids). Along with the samples, a blank was prepared following the exact glassware cleaning procedure and containing the same reagents as the samples.

5. Ash Determination

A clean, preignited and desiccator-cooled porcelain crucible with cover was weighed. To this an appropriate amount of potato sample was added and then reweighed. The sample was ignited at 550°C until a constant cooled weight was obtained.

6. Solids Determination

The samples were placed into preheated and preweighed aluminum drying dishes. These were dried at atmospheric pressure to a constant weight at 80°C in a drying oven. This temperature was chosen to be representative of the thermophilic fermentation temperature. It allowed for sufficient drying without causing possible heat alteration of the samples. These dried samples were used for the previous analyses.

7. Measurement of the pH of the Plant Samples

The pH of the caustic potato peel samples obtained from the processing plant were recorded within 2 h of sampling using a pH meter (Accumet Model 230, Fisher Scientific Co., Pittsburgh, PA).

II. Construction of the Fermentation System

1. Construction of the Fermenter Cells

The exterior shell of the two fermenter cells were constructed with ½" plywood, fastened together with 1¼" steel brads and 5/8" x 5 corrugated fasteners. The size of this outer plywood shell was made to accommodate an inner polypropylene container of 56 cm x 49 cm diameter plus approximately 7 cm to 15 cm of insulation material on the side and bottom of the container, respectively. These plastic containers are utilized for domestic batch wine fermentations and were considered to be the most suitable in terms of cost and durability (Figure 2). The insulating material surrounding these plastic containers was vermiculite (Zonolite, Grace Construction, Scarborough, ON). This material was chosen since it was in a free form, inert and would not settle within the insulating space. The insulating or R value of 10.0 was determined from the package instructions. The whole unit was mounted on wheels to facilitate mobility during loading and cleaning.

2. Top Assembly for Fermenter Cells

This assembly was found to be necessary in order to decrease water evaporation and minimize substrate overflow

Figure 2. Polypropylene fermentation vessel (A) with the baffle assembly (B) inserted.

due to excessive foaming. The top assembly was considered to have a minimal effect on heat retention in comparison to the insulating effect of foam, which accumulated on the top of the fermenting liquid. This top was constructed from a circular piece of ½" plywood heavily covered with a plastic-like finish (Flecto Varathane, Flecto Co. Inc., Oakland, CA) (Figure 3). This coating protected the wood from attack by cellulosic bacteria, which might alter the nutritional profile of the substrate.

The top was attached to the fermenter body by 5 evenly spaced wing-nut bolts. The top and top edge of the plastic container was sealed with a rubber gasket to prevent leakage. Four holes (2.5 cm diameter) were bored into the top. The center hole served as the entry port for the impeller shaft assembly. Another hole (6 cm diameter) was used as a sampling port. The other holes were stoppered with tygon tubing to serve as an air vent and to act as a condenser in minimizing water loss from the system during aeration.

3. System for Substrate Agitation

The impeller was constructed of 4 pieces of 2.5 cm x 3.8 cm x 2.5 mm aluminum plate on a central aluminum coupling as shown in Figure 4. The rectangular

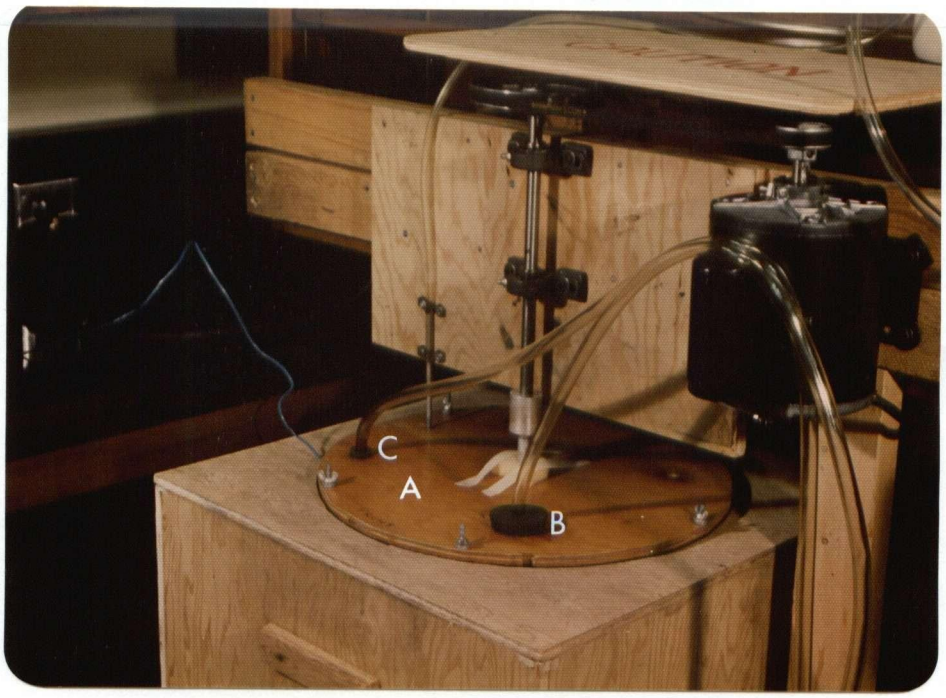

Figure 3. Top assembly (A) with sampling port (B) and vent ports (C), bolted over the fermenter vessel.

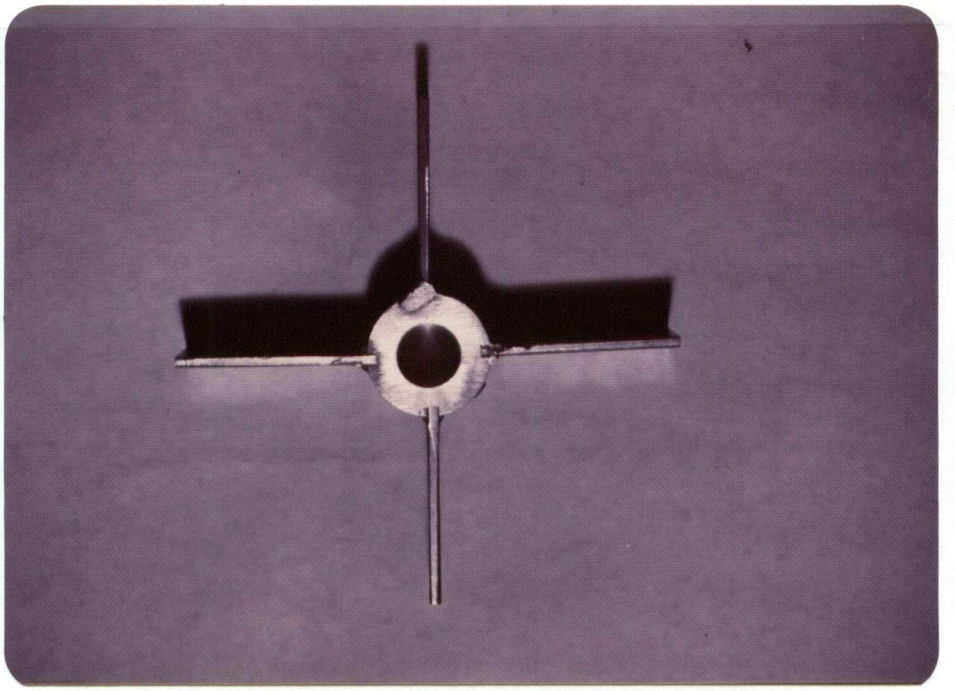

Figure 4. Assembly of the aluminum impeller.

plates were aluminum welded onto the coupling (3.2 cm O.D., 1.6 cm I.D. x 2.5 cm thick) forming 4 equal quadrants at right angles to each other. The coupling was attached to the bottom of an aluminum shaft, 1.6 cm diameter x 53.5 cm long, with Allen screws (Figure 5) to form the agitator.

Another similar impeller with aluminum plates, 2.5 cm x 5.2 cm x 2.5 mm, was attached close to the top half of the impeller shaft. The purpose of this top impeller was to mechanically break the foam which built up during the course of the fermentation (Figure 6). This impeller was positioned on the aluminum shaft so that it was slightly below the opening of the top assembly. Both impellers were placed on the aluminum shaft in such a way as to dynamically balance the entire agitation assembly. A balance system was required to prevent shaft wobble during impeller operation.

The dimensions of the agitating impeller blades were determined from preliminary experiments on the mechanical heat contribution (see pages 42 and 43). The final impeller dimensions were such that the greatest agitation would be provided with minimal frictional heat input into the system. During the mechanical heat determination experiment, the size of the blades were reduced approximately 1/8"

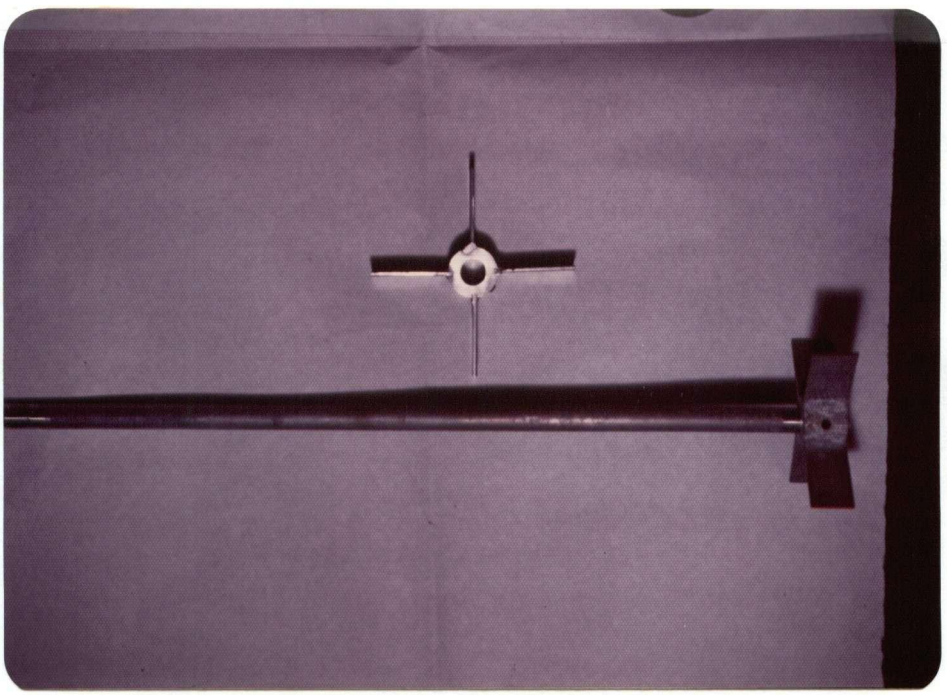

Figure 5. Agitator assembled with impeller positioned on aluminum shaft.

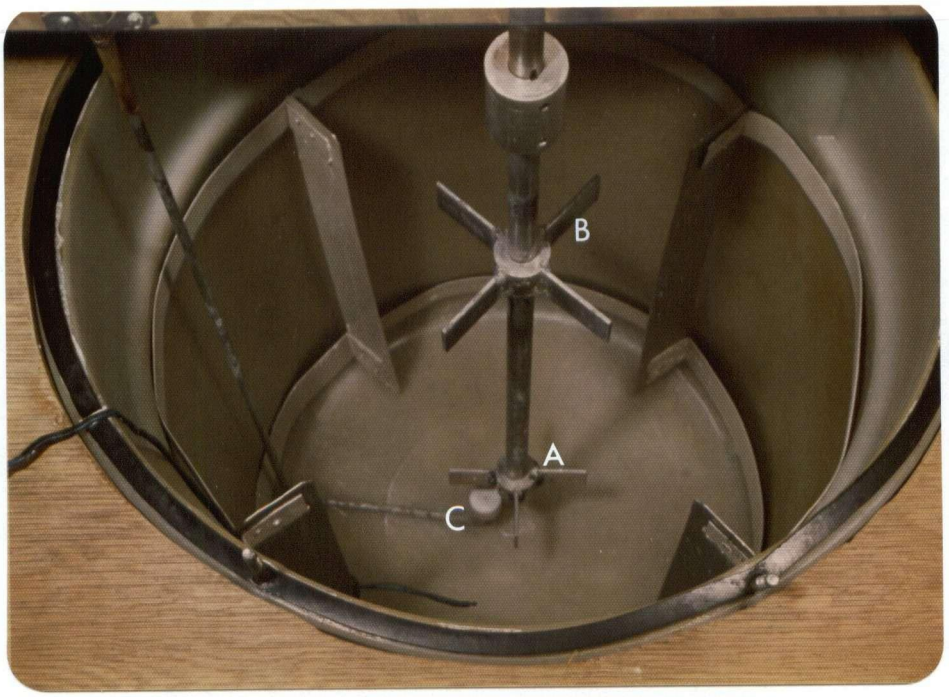

Figure 6. Impeller (A) in position, over the sparger (C), with the foam-breaking impeller (B) situated towards the top of the aluminum shaft.

at a time (on all 4 blades to maintain the balance) until the mechanical heat input into the system was reduced to the desired level. The linear distance travelled by the outside edge of each blade of the impeller was 189 m over a period of 1 min for fermenter #1 and 196.4 m/min for the second fermenter.

4. Baffles

The fins for the baffle were constructed of No. 18 gauge (Brown and Sharpe) aluminum plates 5 cm x 40.5 cm and held together by cross-membering pieces of aluminum plating of the same thickness, see Figures 2 and 6. These were joined with aluminum pop rivets.

The baffles were formed into a hoop and were snugly fitted into the inner sides of the polypropylene containers of the fermenter cells. Baffles were required to prevent vortexing of the liquid medium. If the substrate "whirlpooled", then mixing would be decreased. The baffles created a "Waring blender" effect in which the substrate at the side was deflected towards the center of the polypropylene container. The result was a more constant, vigorous mixing of the substrate.

5. The Power Train for the Agitator

Power was supplied from a ½ h.p. electric motor (Canadian General Electric Co., Peterborough, ON). The speed of this motor (1760 r.p.m.) exceeded our requirements and created excessive mechanical heat. A pulley system was devised to reduce the speed of the impeller. A 3:1 ratio (motor pulley to drive shaft pulley ratio) was used, thus reducing the r.p.m. of the agitator by approximately one third. The exact number of r.p.m. of the shaft was determined using a strobe light. The agitator speed for fermenter #1 was 584 r.p.m. and 607 r.p.m. for fermenter #2.

The drive shaft assembly was an aluminum shaft of the same diameter as the agitator shaft. This drive shaft was mounted in a fixed position juxtaposed to the motor. Two pillow blocks or collar bearings were used to accomplish this fixed position (required to decrease wobbling) and also to allow the shaft to rotate freely (Figure 7).

An aluminum coupling (3.8 cm diameter x 4.8 cm length) was used to join the agitator shaft to the drive shaft (Figures 6 and 7). It formed a sleeve over each

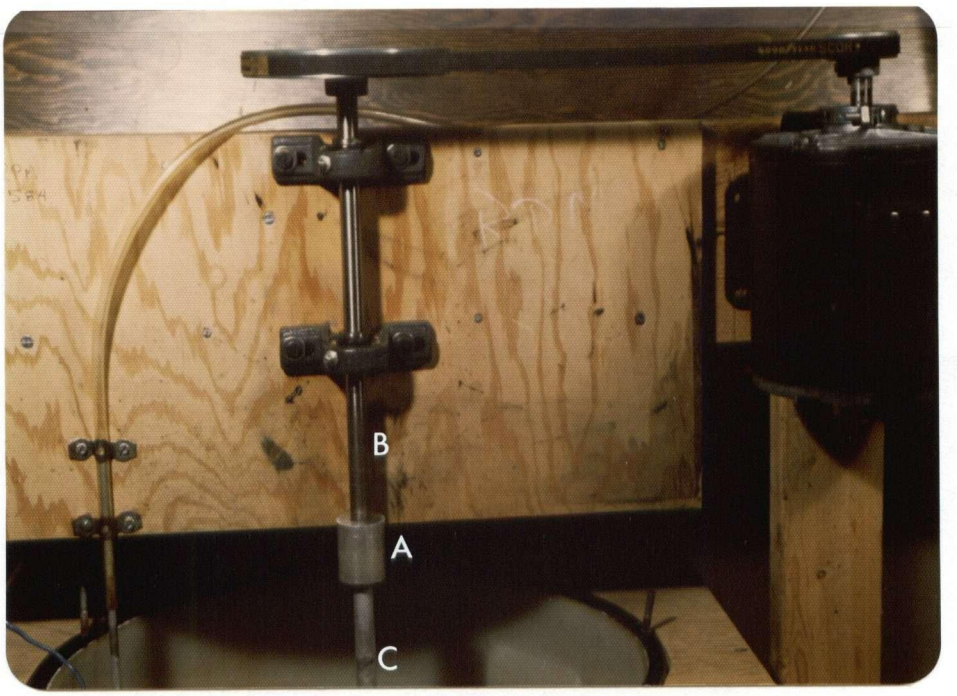

Figure 7. Drive system assembly with the aluminum coupling (A) between the drive shaft (B) and agitator shaft (C).

end of both shafts to transfer power to the impeller and to reduce the wobbling effect. The coupling facilitated setting up or dismantling the agitation system.

6. Aeration System

Air was supplied to the fermenters by an air compressor (Webster Co., London, ON). A problem was created by this reciprocal compressor. The air forced from the compressor pulsated rather than flowed evenly. This air action deterred the proper measurement of air flow through the air flow meters and also caused an excessive violent action at the sparger. An air reservoir system was utilized using a 2 liter polyvinyl bottle (Figure 8). The bottle was packed with nonabsorbent cotton. The cotton decreased the pulsation effect and also acted as a filter against particles from the air and oil droplets from the compressor. Two holes were bored into the plastic bottle, one towards the bottom and the other near the top. These holes were fitted with plastic tubing fittings, as required for tubing attachment. The bottom hole was the air intake from the compressor while the top hole was the outlet which led to the air flow meters. The bottle top was used as a regulator to control the air flow.

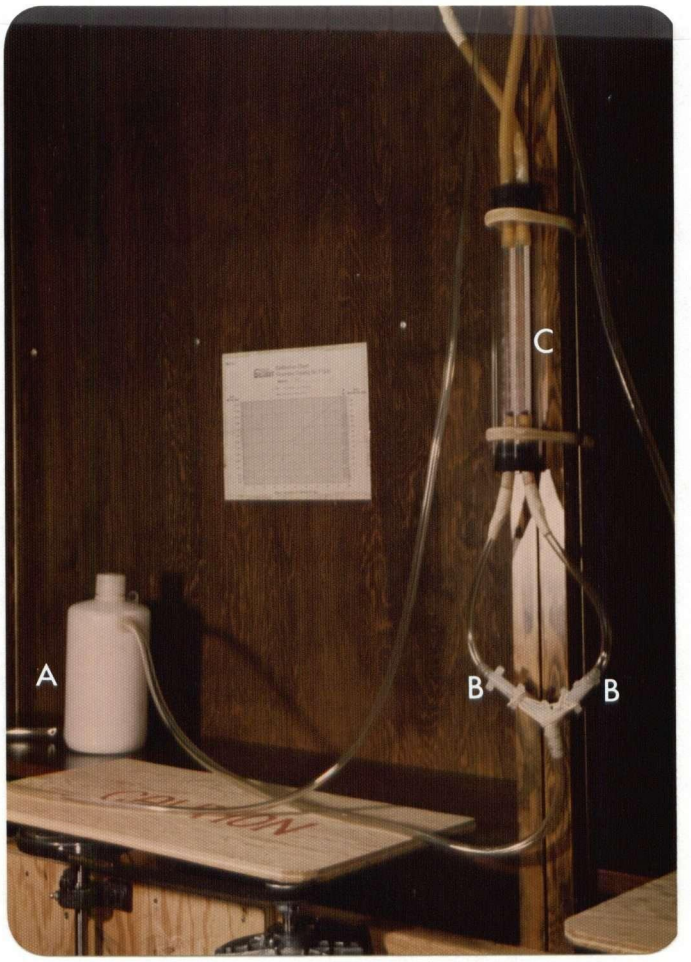

Figure 8. Aeration system with air reservoir-regulator (A), twistcock regulators (B) and air flow meters (C).

7. Flow Meters

The flow meters (model no. 3, Gilmont Instruments Inc., Great Neck, N.Y.) were required to determine the amount of air input into each fermenter. They were chosen to meet the specific capacity required in the fermentation. Each air flow meter was calibrated by Gilmont and a graph was supplied to obtain the proper readings by interpolation. Another regulator was attached preceding each of the air flow meters. Besides acting as airflow regulators, the twistcock tubing connectors were used as "cut-offs" to stop the air flow completely from going into the fermenter (Figure 8).

8. Sparger Construction

Air entered the fermenter via the sparger which was placed on the bottom of the fermenter cell (Figure 6). The sparger was composed of an aluminum tube with an aluminum or hard teflon sparger head (Figure 9). The aluminum tube, 7 mm O.D. x 5 mm I.D., was bent into a "J" configuration so that the sparger head could be situated under the impeller. This location ensured dispersion of the air bubbles in the fermenting liquor by the impeller (Figure 6).

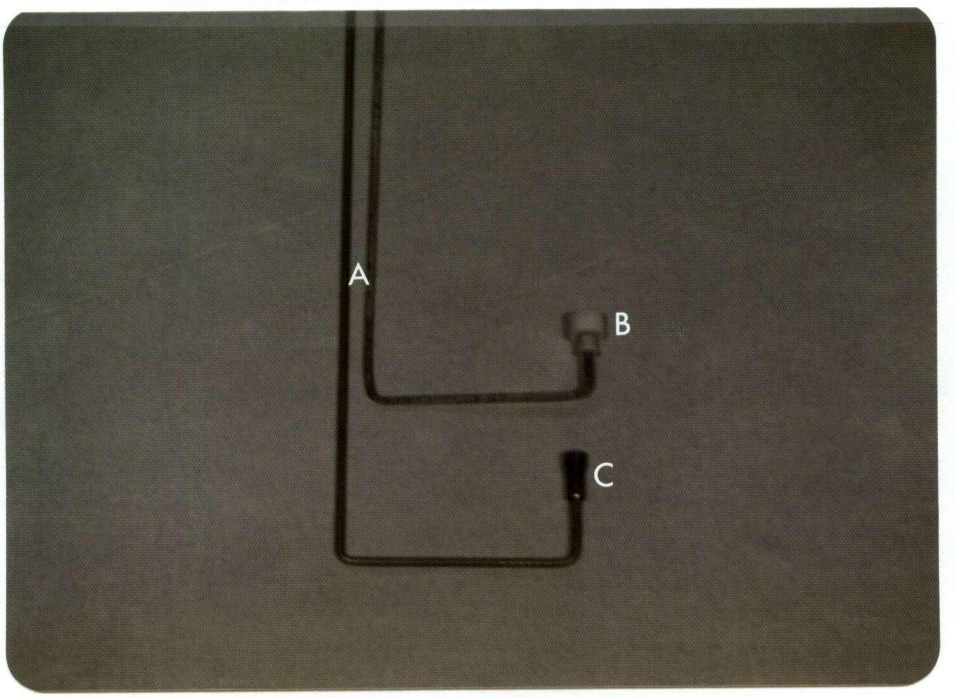

Figure 9. Sparger assemblies, with aluminum tubing (A) attached to the teflon sparger head (B) and the aluminum sparger head (C).

The aluminum sparger head was constructed of an aluminum bar of 1.3 cm diameter. The center of the bar was drilled (6 mm diameter) to provide passage for the air. Also this bore was tapped for attachment to the aluminum tube which was threaded. One end of the sparger head was countersunk (1 cm diameter x 6 mm deep) to receive a stainless steel ball. This ball bearing acted as a plug to prevent backflow of the fermentation liquor into the sparger which would interfere with the air flow. The outside of the sparger head was threaded to receive a cap which was used to retain the ball bearing in place. Essentially, the cap was used to diffuse or break up the air entering the system. Numerous holes (2 mm diameter) were drilled into this cap to provide the proper air diffusion. A hard plastic (teflon) sparger head, similar in construction to the aluminum head, was also used. Unlike aluminum which tended to decompose after several fermentations, the hard plastic remained stable (Figure 10).

9. Tubing Connections

Plastic tubing was used throughout the aeration system. When connections were made, for example, at the regulators or airflow meters, etc., these connected

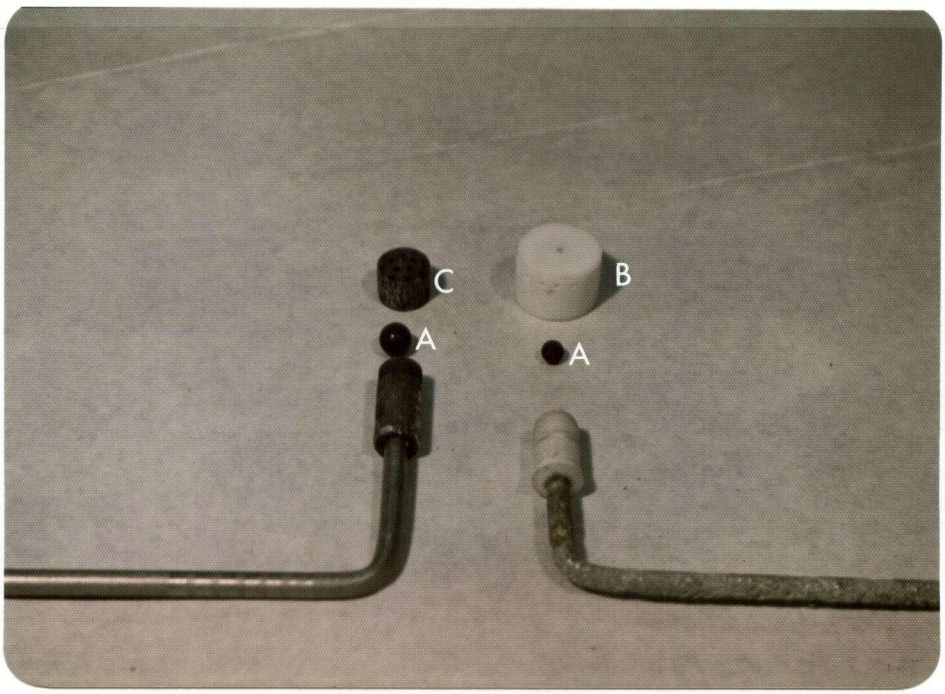

Figure 10. Sparger head assemblies consisting of the stainless steel balls (A), the teflon sparger head (B) and the aluminum sparger head (C).

joints were held on with wires and tape to prevent "blow offs" due to the pressure exerted by the compressor.

10. Monitoring (see also section on "Measurements Taken from the Fermentation Trials")

A copper-constantan thermocouple was utilized to monitor the temperature of the system. It was attached to the baffle on one of the aluminum plates and protruded into the fermenter. It led through a notch placed into the rubber gasket on the top of the fermenter (Figure 11).

An electronic thermocouple ice point (Omega Engineering, Inc., Stamford, CT) was used as reference. This was attached to a data recorder (Digitec, United Systems Corp., Dayton, OH) on which a "print out" was obtained in millivolt (m.v.) readings (Figure 12). These m.v. readings were translated into temperature readings (°C) using a conversion table.

11. Calibration of Thermocouples

The thermocouples used were calibrated with their respective electronic ice points and channel positions on the Digitec recorder. The ends of the thermocouples were immersed in an ice bath along with a

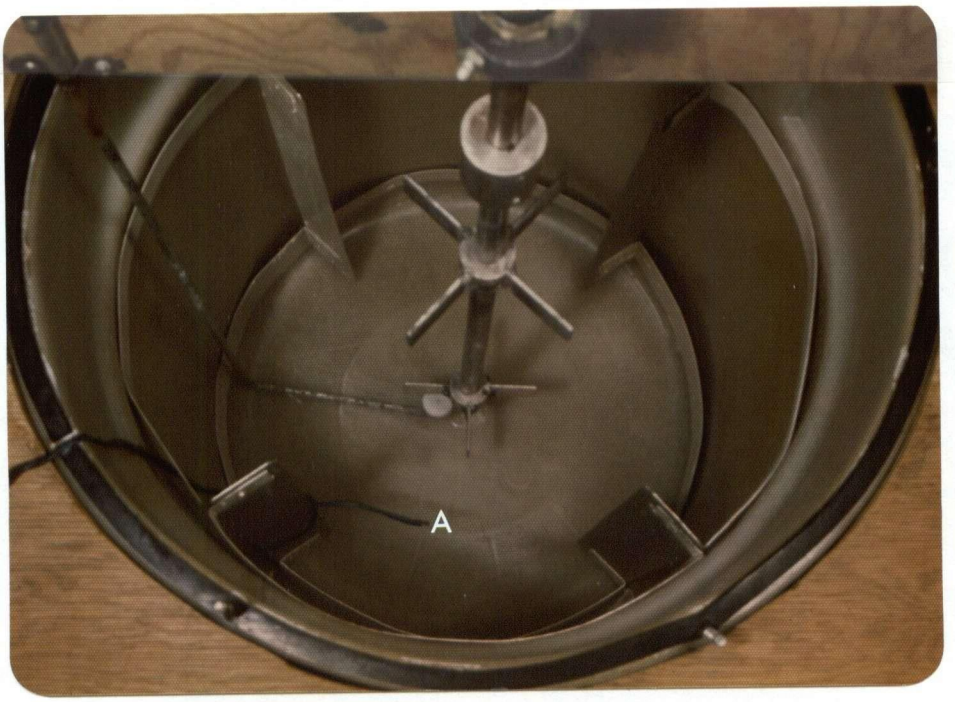

Figure 11. Thermocouples (A) positioned within the fermenter vessel.

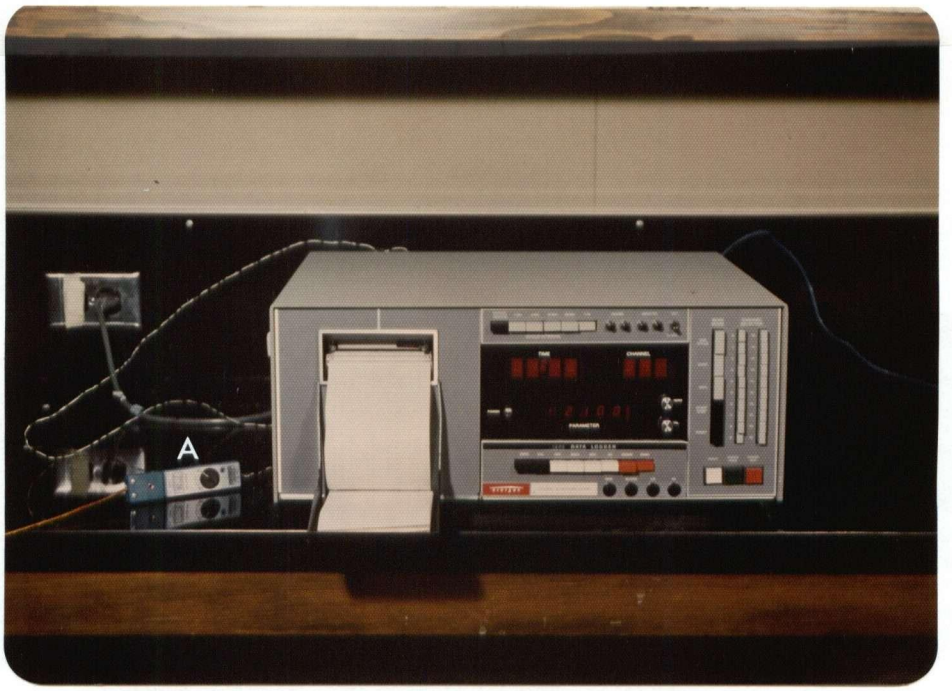

Figure 12. Data recorder used to monitor the fermentation temperatures along with the electronic thermocouple ice point (A).

thermometer. Agitation was provided to both to prevent "hot spots". The thermometer reading was recorded simultaneously with the m.v. readings from the Digitec. The millivolt readings were converted to °C using the conversion table and both results were compared. Heat was gradually applied to the ice bath and the readings were recorded and compared until the temperature reached approximately 80°C.

12. Location of the System

The entire fermenter assembly was located within a well insulated incubator room (Figure 13). However, this particular room had poor air circulation. The result of this was a build up of ambient heat caused by the motors and the air compressor during the operation of the fermenter. To control this heat increase, a large fan and humidifier was used. This not only decreased the temperature in the room but the humidifier also decreased the water loss from the fermentation. The fan was directed at the motor and the drive system to dissipate the heat on the system before it was conducted into the fermenter.

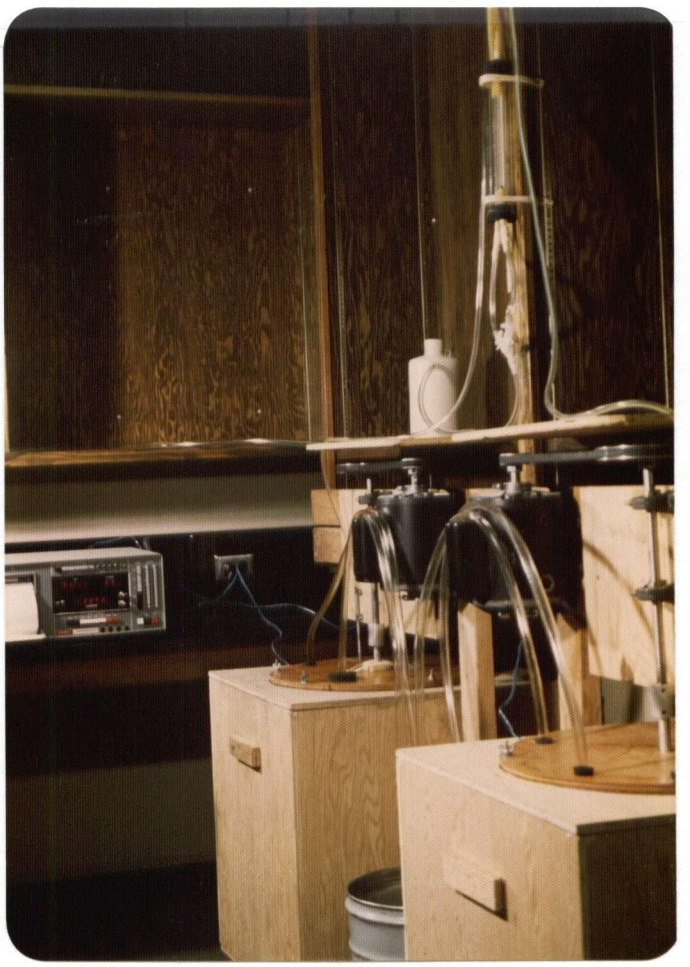

Figure 13. Fermenters assembled within the insulated room.

III. Measurements of the System Constructed

These tests were required since heat input into the system by sources other than from the thermophilic bacteria would invalidate the entire project. The total mechanical heat input into the fermenter was kept well below the 50°C mark which was considered to be the starting temperature for thermophilic bacterial growth.

1. Mechanical Heat Input Determination

Fifty litres of cold tap water along with a small amount of bleach was placed into the fermenter cell. The bleach was used to stop or impede the growth of biological flora, e.g., algae, bacteria, etc. in the water which may affect the determination. The entire system was assembled (with a set air flow, 900 ml/min) and was operated until the temperature increased to a plateau. The maximum temperature reached was 41.4°C. This was considered acceptable since it was well below 50°C. Adjustments were made to the system accordingly to obtain the above temperature.

2. Mechanical Heat Input Determination in the Absence air sparging

Medium cooling caused by heat removal through aeration was considered to be an important heat-loss

factor. In this evaluation, a tap water system previously described was used. The operation of the agitator in the absence of the air sparging was measured. The system was operated until a maximum temperature was reached. This value was compared to the value obtained from the mechanical heat input determination with aeration.

3. Adjustments

The mechanical heat was decreased by: decreasing the rate of rotation (speed of the impeller); linear distance travelled by the impeller (i.e., r.p.m. x πd); (see section on "System for Substrate Agitation") and the insulation in the fermenter cell.

4. Mechanical Heat Determination in the Presence of Potato Solids

Since it could be argued that the water system utilized in the above determination was not indicative of an actual fermentation, a medium containing 12.3 g % potato solids was used. It was thought that additional heat would be created by the impeller working in the "puree-like" potato medium. Sodium hypochlorite solution was added to the potato substrate to deter the growth of the microorganisms

during this test. No aeration was applied to the system. This determination was continued until a maximum temperature was obtained.

5. Heat Lost from the Fermenter Cell

The heat lost from the fermenter cell was determined in order to evaluate the effectiveness of the surrounding insulation. Fifty liters of heated water, 79.5°C, was poured into the fermenter and the fermenter top installed together with four exhaust tubings. The aeration system was not activated. The temperature decrease within the fermenter was recorded hourly as heat was lost from the system.

6. Heat Loss Determination in the Presence of Surface Foam

As a comparison to the previous determination, the insulating effect of a foam layer on heat retention was determined. During agitation of the aqueous potato medium a heavy layer of foam is formed. In order to imitate the normal fermentation system, a hot water medium (69.6°C) was used containing a foam-forming commercial

dishwashing detergent. Agitation and aeration was turned on to create the heavy foam. Throughout the determination, the system was continually replenished with the detergent to maintain a constant foam level.

IV. Analyses of the Thermophilic Fermentations

1. The Potato Substrate

Potatoes were obtained from a local supermarket usually in 22.7 kg (50 lb) boxes. The g % total solids of the potatoes was determined for each box prior to the fermentation trials. A random sampling of 5-6 medium size potatoes were obtained from throughout the box. Each potato was ground and a portion of the macerate placed into a preweighed drying dish and oven-dried at 80°C to a constant weight at atmospheric pressure.

Knowing the g % total solids of the potatoes, a specific amount of potato was weighed for the fermentation. These potatoes were macerated and mixed with tap water within the fermenter. Maceration was accomplished with a Hobart food mill (Kitchen Aid, Model K5-A, Hobart Mfg. Co., Troy, OH) with a no. 3 slicing attachment. The

potato slices were passed through the slicer several times until small pieces were obtained. The final working volume of the fermenter for all the fermentation trials was 50 l.

2. Sanitation of the Fermenter Vessel and Accessories Prior to Operation

After each fermentation trial, the fermenters were emptied and thoroughly washed with commercial detergent. Each of the other components were dismantled and also washed. The entire system was then soaked or washed with approximately 0.5% hypochlorite solution. The whole floor area and the other affected areas (i.e., walls and shelves) of the incubator room were washed with hypochlorite. The fermenter and its components were rinsed free of hypochlorite with tap water before use.

3. Measurements Taken from the Fermentation Trials

i) Temperatures

Temperature readings for the fermentation were obtained through a thermocouple-recorder system as previously described. A second thermocouple was used to record the ambient room temperature of the area surrounding the fermenter.

ii) Dissolved Oxygen and pH

Samples were removed from the fermenter at approximately 12 h intervals. The hydrogen ion activity was recorded using a pH meter. A dissolved oxygen (D.O.) reading was also taken by immersing the D.O. probe directly into the fermentation liquor within the fermenter. The YSI model 57 Oxygen meter and YSI model 5739 Oxygen probe (Yellow Springs Instruments Co., Inc., Yellow Springs, OH) was calibrated up to 45°C by the supplier. Further calibrations were required to match the temperatures expected from the fermentation to that of oxygen saturation of the medium. This was done by, first, bubbling air into a water bath at 21°C until saturation was reached. This reading was recorded. The temperature was then increased in increments to a maximum of 72°C with air continuously bubbling through the water bath. Both temperature and dissolved oxygen readings were recorded accordingly (Figure 14).

iii) Total Solids Remaining in the Fermenter and Total Kjeldahl Nitrogen

Approximately every 12 h during the course of the fermentation, a sample (250 ml) was removed from the fermenter. This sample was frozen and stored at -20°C prior to subsequent analysis. Per cent total solids was

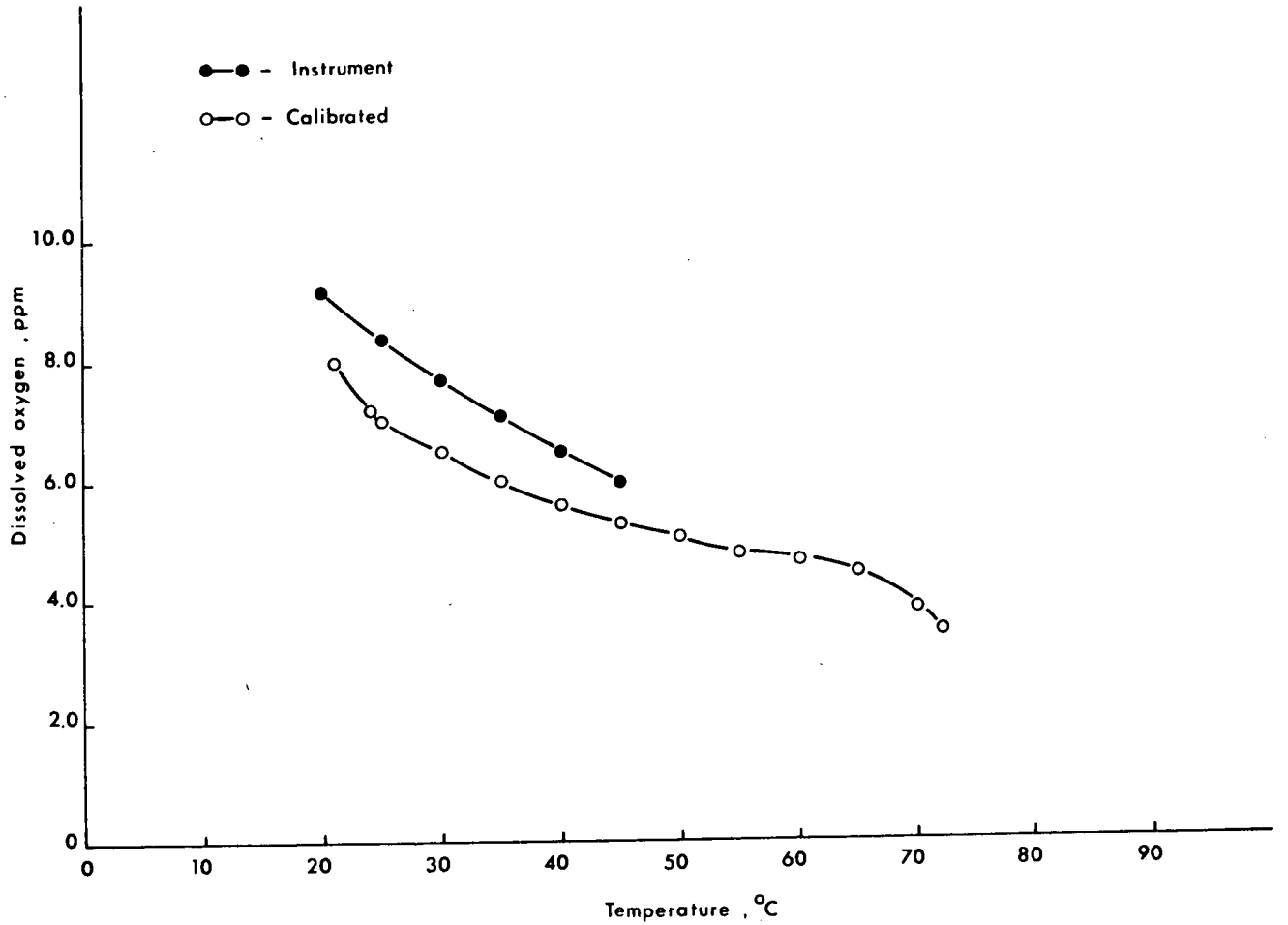

Figure 14. Dissolved oxygen probe calibration curve for air saturated water at high temperatures.

determined by drying the sample in an oven at 80°C to a constant weight.

The same dried sample was digested using the method of Concon and Soltess (16) prior to the measurement of total Kjeldahl nitrogen with the Technicon Auto Analyzer.

V. "True" Protein Nitrogen Using Trichloroacetic Acid (TCA) Precipitation

Samples of fermented potato and fresh potatoes were ground individually in a waring blender. To 5 ml of sample, a 5 ml aliquot of a 10% (W/V) TCA was added. The preparation was mixed and then centrifuged (Ivan Sorvall, Norwalk, CT) at 7900 x g for 30 min. The resulting precipitate was discarded and a further 1 ml of 10% TCA solution added to ensure completeness in precipitation. The preparation was re-centrifuged at 7900 x g for another 30 min and the supernatant analyzed for non-protein nitrogen. The difference between this value and total nitrogen was considered to represent "True protein" nitrogen.

VI. Amino Acid Analysis

Analyses were performed on potato samples removed prior to the fermentation and on the fermented residue. Sample particle size was reduced using a Waring blender. The samples were boiled to the point of starch gelatinization, and lyophilized.

Amino acid analysis of the samples was carried out in two stages. A selective alkylation of the cystine and cysteine residue into 5-β-(4-pyridylethyl)-L-cysteine was done according to the method of Cavins et al. (12). These authors found that alkylation was required to stabilize the above residues under acid hydrolysis conditions.

Hydrolysis of the alkylated samples followed. This was accomplished using p-toluene-sulfonic acid as suggested by the method of Liu and Chang (34). This digested mixture was then filtered through an ultra-fine sintered glass filter (Appendix A).

The filtered samples were analyzed on an amino acid analyzer (Phoenix Precision Instruments Co., Philadelphia, PA) utilizing a single column elution system (Durrum Chemical Corporation, Palo Alto, CA).

VII. Microbial Identification

1. Preparation of Isolation Media for N-Fixing Bacteria

i) Yeast Carbon Base (YCB) Medium

This nitrogen deficient media (Difco Laboratories, Detroit, MI) contains low amounts of nitrogen in the form of essential nutrients and vitamins required for microbial growth. The medium was filter sterilized through a Type HA, 0.45 µm Millipore membrane filter (Millipore Filter Corp., Bedofrd, MA), mixed with sterile agar at 45°C and poured into sterile petri dishes.

ii) Nitrogen Fixation Medium for Non-Symbiotic Soil Microorganisms

This mineral medium was adapted from "A Practical Manual of Soil Microbiology Laboratory Methods" (56) and is used for the isolation of Azotobacter species. A hard agar is required for incubation of the cultures at 55°C; modification of this medium consisted of using 2.5% (W/V) agar. The media was autoclaved at 15 psi (121°C) for 15 min, cooled to 45°C and poured in 100 mm x 15 mm sterile plastic petri dishes.

iii) Nitrogen-Fixing Medium for Genera Azotobacter and Beijerinckia in Soil

This mineral medium was adapted from Meiklejohn (38). It is composed of two solutions, A and B, in which 2.5% agar was used in solution A. Both solutions were autoclaved separately at 15 psi (121°C) for 15 min. The solutions were cooled to 50°C, and 10 ml of solution B were added to A, mixed, and poured into 100 mm x 15 mm sterile petri dishes.

2. Isolation of the Microorganism

Aliquots of the fermentation liquor were obtained from the fermenter subsequent to reaching the thermophilic temperatures. The samples were streaked onto the 3 different plated media and incubated at 55°C in plastic bags to prevent dehydration. Incubation periods ranged from 24-48 h. During this period, visually distinct colonies were isolated and re-inoculated on new media. This isolation procedure was repeated 6 times to ensure a pure culture whose growth was dependent on the nutrients supplied by the media and not by the carry-over of the fermenter liquor.

3. Visual Appearance of the Microbial Colonies

Isolated microbial colonies from the 3 different media were grown on BBL Trypticase Soy Agar (Becton, Dickinson and Co., Cockeysville, MD) plates. After 24 h of growth, the colonies were examined for morphology and cultural characteristics according to Mitruka and Bonner (39). See Appendix B.

4. Gram Reaction

Colonies from the 3 media were removed and grown on Trypticase Soy Agar (TSA). The cultures were Gram stained after 24 h and 48 h or when visible growth occurred. The Gram reaction, agglomeration (groupings), and morphology of the cells were noted.

5. Spore Staining (Wirtz-Conklin)

Three to five-day old cultures grown on TSA at 55°C were suspended in a sterile water droplet and observed under phase microscopy. The location and nature of the sporangia was noted. Spores were confirmed by staining with a 5% solution of malachite green and counterstained

with safranin. The spores were observed to stain green against red cytoplasm under oil immersion microscopy (33).

6. Lipid Staining (Burdon's Method)

Three-day old colonies grown on TSA at 55°C were stained for the presence of lipid bodies. Slides were heat fixed and flooded with a Sudan Black B solution (Fisher Scientific Co., Fair Lawn, N.J.) for 10 min and xylol cleared. They were counterstained with Ziehl-Neelsen's Carbol-Fuchsin (1:10 dilution, see Appendix C) for approximately 3 sec and then rinsed. The cells were stained red and the fat globules were blue-black in colour (48).

7. Motility

A colony was suspended in a sterile water droplet. Under phase microscopy with oil immersion, the cells were observed for movement in excess of Brownian movement.

A 0.2% TSA gel in tubes was also used to observe cell motility. Using this method, cultures were stabbed into the agar and incubated at 55°C. Positive results were indicated visually by diffusion of microbial cells through the soft agar (15).

8. Catalase Reaction

Colonies from the 3 different culture media were grown on TSA slants at 55°C. After sufficient growth was obtained, a 3% hydrogen peroxide solution was added to the colonies . An immediate formation of bubbles indicated a positive reaction (33).

9. Anaerobic Test

Stab cultures were prepared using tubes of TSA. After incubation for approximately 2 d, the location of growth was observed visually. Growth on the top of the agar indicated an aerobic culture whereas growth along the length of the stab indicated degrees of anaerobism.

10. Obligative Anaerobic Test

As a confirmation to the above test, a further determination was required to establish whether the organism was a facultative or obligative anaerobe. Anaerobic jars were used with a BBL Gas Pak and catalyst system. A 1% methylene blue solution was included in the jar as an indicator of anaerobic conditions. TSA plates were inoculated and placed into anaerobic jars before the Gas Pak envelopes were activated. Incubation was at 55°C.

11. Voges-Proskauer Test (V-P)

The Voges-Proskauer test is used to determine the presence of acetylmethylcarbinol (acetoin). Acetoin is oxidised by the reagent to diacetyl which produces a red colour with a constituent of the peptone medium (15). Five milliliters of V-P media of Gordon et al (22) were placed into test tubes, capped and autoclaved at 15 psi (121°C) for 15 min.

The cultures were prepared from colonies isolated from 3 different media. Each of the cultures were inoculated in triplicate and incubated for 3, 5 and 7 d. At the end of these time periods, the presence of acetoin was tested for by adding 3 ml of a 40% (W/V) NaOH solution to the culture along with approximately 0.75 mg of 90% creatine (Eastman Organic Chemicals, Rochester, N.Y.). A red colour after 30 to 60 min at room temperature indicates the presence of acetoin.

12. pH in Voges-Proskauer Broth

Before the cultures were tested for acetoin, the pH of the 7 d old cultures were measured by using a pH meter. The initial pH of the V-P broth after sterilization was also measured to serve as a control comparison.

13. Methyl Red Test

Voges-Proskauer media was used for the methyl red acidity test (see Voges-Proskauer test). Ten milliliters of the V-P broth in duplicate tubes were incubated for 6 days. At the end of this time interval, 5 ml from each culture was pipetted into a separate empty tube and 5 drops of methyl red solution were added and the tube shaken. The methyl red solution was composed of 0.1 g of methyl red in 300 ml ethanol (95%) plus 200 ml distilled water. A red coloration indicates a positive test and yellow a negative test.

14. Temperature Range Determination

Triplicate tubes of cultures in trypticase soy broth were incubated at a constant temperature in water baths at varying temperatures. Culture tubes showing growth at temperatures above 55°C were recorded after a three-day period. Cultures incubating between room temperature (21°C) to 30°C were incubated up to 2 weeks.

Determination of the cultures maximum growth temperature involved the growth of the different cultures at each of the following temperatures: 60, 65, 70 and 75°C. At these higher temperatures, an ethylene glycol

base (Gulf Oil, Canada Ltd., Toronto, ON) was used in the constant temperature water bath to decrease evaporation. The low temperature range determinations involved growth of the cultures at 55, 50, 45, 40, 35, 30, 25°C and at room temperature (21°C).

15. Growth in Lysozyme

The lysozyme solution was prepared by adding 0.1 g of lysozyme (B grade, Calbiochem, Los Angeles, CA) in approximately 60 ml of sterile 0.01 N HCl in a 100 ml volumetric flask. This mixture was carefully boiled for 20 min. After boiling, the flask was plugged with sterilized non-absorbant cotton and allowed to cool to room temperature. The volume was made up to 100 ml with sterile 0.01 N HCl. From this lysozyme solution, 1 ml was removed and mixed with 99 ml of sterile nutrient broth. This was dispensed in 2.5 ml aliquots into sterile plugged test tubes and inoculated with cultures previously grown in nutrient broth. Also tubes of 0.001% lysozyme and nutrient broth were inoculated as control comparisons. Growth was recorded at 7 and 14 d.

16. Growth in NaCl

Tubes containing nutrient broth were inoculated with colonies from the 3 isolation media and incubated for 48 h. The nutrient broth cultures then served as an inoculum for nutrient broth tubes containing 0, 5, 7 and 10% (w/v) of sodium chloride. Parafilm was used to seal the caps of the test tubes to decrease evaporation. The NaCl-broth was incubated at 55°C for 7 and 14 d.

17. Growth in Sodium Azide

Azide dextrose broth (Fisher Gram-Pac, Fisher Scientific Co., Pittsburg, PA) was prepared as directed by the manufacturer.

Duplicate tubes were inoculated with cultures previously grown on nutrient broth. The caps of the test tubes were wrapped with parafilm to prevent dehydration. The tubes were incubated at 55°C and observed for growth at 7 and 14 d.

18. pH Range Test

BBL trypticase soy broth (Becton, Dickinson and Co., Cockeysville, MD) was prepared and the pH adjusted (initial pH 7.3) with 1N HCl or 1N NaOH to yield a series

of media with pH ranging from 3 to 11 in 1 unit increments. A 10 ml portion of each of these media was filter sterilized through a Type HA, 0.45 µm Millipore membrane system (Millipore Filter Corp., Bedford, MA) into sterile test tubes. Duplicate tubes were prepared and inoculated with a culture previously grown in nutrient broth from the 3 isolation media. The caps of the test tubes were wrapped with parafilm and the tubes were incubated at 55°C. Control media tubes with and without inoculum were used for each of the pH ranges tested. The culture tubes were observed over a 7 d period for the presence of growth.

Following the determination of the pH at which the various cultures showed growth inhibition, a more finite range of 0.1 pH increments were used to redetermine these growth sensitive areas.

19. Acid Formation from Carbohydrate Substrates

A modified Ayers, Rupp and Johnson (3) basal media with 2.5% (w/v) agar was prepared. Fifteen milliliters of a 0.04% (w/v) solution of bromocresol purple (Eastman Organic Chemicals, Rochester, N.Y.) were added and the medium sterilized at 15 psi (121°C) for 15 min.

Ten per cent (w/v) aqueous solutions of D(+)-glucose, L(+)-arabinose, D(+)-xylose and D(-)-mannitol were sterilized by autoclaving at 15 psi (121°C) for 15 min. Prior to solidification of the basal medium, the test carbohydrate was added to yield a final concentration of 0.5% (w/v) and poured into 100 mm x 15 mm sterile plastic petri plates.

The culture growth and the production of acid on each of the carbohydrates was noted after 7 d and 14 d periods.

20. Starch Hydrolysis

Starch agar was prepared according to Blair, Lennette and Truant (5). The medium was autoclaved at 15 psi (121°C) for 30 min and dispensed into 100 mm x 15 mm sterile plastic petri dishes.

Colonies from the 3 isolation media were streaked onto the plates and incubated at 55°C. When good growth was obtained, starch hydrolysis was tested by flooding a small portion of the plate with Gram's iodine. The absence of starch hydrolysis was indicated by a dark blue color in the immediate vicinity of the culture; a partial or complete hydrolysis of starch was indicated by a reddish color or by no visible color change in the medium.

21. Utilization of Sodium Citrate and Sodium Propionate

Utilization of sodium citrate and sodium propionate as carbon sources was measured by using a modification of Koser's citrate agar (30). The medium is composed of 0.2% (w/v), sodium citrate or sodium propionate; 0.1% (w/v), NaCl; 0.02% (w/v), $MgSO_4 \cdot 7H_2O$; 0.05% (w/v), $(NH_4)_2 HPO_4$ and 2.5% (w/v), agar. Also, 2.0% (v/v) of a 0.04% (w/v) solution of phenol red was added, with the pH of the medium adjusted to 6.8 prior to autoclaving.

The medium was heated to dissolve the agar and dispensed into test tubes. It was autoclaved at 15 psi (121°C) for 15 min and solidified as slants. Cultures grown on nutrient broth from the 3 isolation media were streaked onto the slants. A parafilm wax wrap was used around the caps to prevent dehydration during incubation. The utilization of these two organic acids was established if the alkaline color of phenol red appeared after a 2 week incubation period, viz., cultures show yellow at pH 6.8 and turn red at pH 8.2.

22. Reduction of Nitrate to Nitrite

Cultures were grown in a nitrate broth prepared according to Gordon, Haynes and Pang (22). Ten milliliters

of medium was dispensed into test tubes and autoclaved at 15 psi (121°C) for 10 min. The incubation period was for 3, 7 and 14 d; three sets of triplicate tubes for each culture from the 3 isolation media were made. Parafilm wraps were used for the caps to prevent evaporation with incubation at 55°C. At the end of each incubation period, the cultures were tested by mixing 1 ml of culture with 3 drops from each of the following 2 test solutions: a) sulfanilic acid, 0.8 g (Mallinckrodt, St. Louis, MO) dissolved in 100 ml of 5N acetic acid (glacial acetic acid and distilled water in 1:2.5); b) dimethylnapthylamine, 0.6 g (Sigma Chemical Co., St. Louis, MO) dissolved in 100 ml of 5N acetic acid. Either a red or yellow color development respectively indicated the presence of nitrite. If after the fourteenth day, the cultures did not react positive, 4 to 5 mg of zinc dust was added to the tubes which previously were tested for nitrite. This was to demonstrate the absence of reduction or that nitrate was still present in the media. A red coloration would indicate the presence of NO_3. This residual nitrate test is required after 14 days because some organisms can reduce the nitrate beyond the nitrite very rapidly so that the presence of nitrite may not be detected after the third day of incubation.

23. Dihydroxyactone Production

A glycerol agar was prepared using the procedure of Gordon, Haynes and Pang (22). This medium was autoclaved at 15 psi (121°C) for 15 min, cooled and poured into sterilized plastic petri plates. The inoculum was streaked once across the plate and incubated for 10 d. After incubation, the plates were flooded with Fehling's solution. Fehling's solution A was composed of $CuSO_4 \cdot 5H_2O$, 34.66 g and distilled water, 500 ml. Fehling's solution B was composed of $KNaC_4H_4O_6 \cdot 4H_2O$ (sodium potassium tartrate or Rochelle salt), 173 g; NaOH, 50 g and distilled water, 500 ml. Both solution A and B were kept refrigerated until needed, and a mixture of A and B in a 1:1 ratio was used. Approximately 2 h later, the plates were examined for the presence of a red halo around the colonies which indicated the production of dihydroxyacetone.

24. Production of Indole

Cultures from the 3 isolation media were inoculated into sterile tubes containing 10 ml of 1% (w/v) of tryptone broth (Difco) and a 1% (w/v) of trypticase soy broth (BBL). Triplicate tubes were made for each culture and incubated at 55°C for 14 d. The caps were

wrapped with parafilm wax. After incubation, 2 ml of a test solution was added to each of the cultures and shaken. This test solution consists of paradimethyl-aminobenzaldehyde (Eastman Organic Chemicals, Rochester, N.Y.), 5 g, iso-amyl alcohol (J.T. Baker Chemical Co., Phillipsburg, N.J.), 75 ml and concentrated HCl, 25 ml. The presence of indole was indicated when the alcohol layer turned pink to red (22).

25. Deamination of Phenylalanine

Slants of phenylalanine agar were prepared according to the method of Gordon, Haynes and Pang (22).

Duplicate tubes were made for each of the cultures from the 3 isolation media. Incubation was for 7 and 21 d at 55°C, with all caps wrapped in parafilm wax. A test solution of 10% (w/v) $FeCl_3$ was used to indicate the formation of phenylpyruvic acid from phenylalanine. Four or five drops of this solution were pipetted over the growth on the slants. A green coloration of the agar beneath the colonies indicated the formation of phenylpyruvic acid.

26. Decomposition of Casein

Ten grams of Bacto-skim milk powder (Difco) dissolved in 100 ml of distilled water and 2.5 g of agar in 100 ml of distilled water were autoclaved separately at 15 psi (121°C) for 15 min, cooled to about 45°C, mixed together, and poured into 60 mm x 15 mm sterile plastic petri dishes. The plates were kept at room temperature for 3 d to dry the surface of the agar before inoculation. Duplicate plates from each of the 3 isolated cultures were made. Inoculation was by a simple streak and the plates were incubated for 7 and 14 d. After the incubation periods, the plates were examined for clearing around and beneath the colonies, indicating casein decomposition (22).

27. Decomposition of Tyrosine

One gram of L-tyrosine (Eastman Organic Chemicals, Rochester, N.Y.) was suspended in 20 ml of distilled water. Similarly, 4.6 g of nutrient agar (Difco) with an additional 3 g of agar was prepared in 200 ml of distilled water. These were autoclaved separately at 15 psi (121°C) for 15 min, cooled to about 45°C and thoroughly

mixed together. The resulting media was poured into 60 mm x 15 mm sterile plastic petri dishes ensuring that there was an even distribution of L-tyrosine crystals throughout the solidified agar. The plates were kept at room temperature for 3 d to dry the agar surface and then inoculated by a single streak. Duplicate plates were made for each isolated culture. After 7 and 14 d of incubation, the plates were examined for clearing beneath the growth (22).

28. Reaction in Litmus Milk

Litmus milk was prepared by dissolving 100 g of Bacto-skim milk powder and 0.75 g of litmus powder (J.T. Baker Chemical Co., Phillipsburg, N.J.) into 1 l of distilled water. Fifteen milliliters were dispensed into tubes and autoclaved at 15 psi (121°C) for 15 min. The tubes were inoculated, with 6 replicates for each culture. Three of the tubes were covered with paraffin oil (Fisher Scientific Co., Fair Lawn, N.J.) for litmus reduction determination. Following incubation for 7 and 14 d, the cultures were observed for color and reduction of the indicator, formation of curd, gas and digestion of casein (22).

EXPERIMENTAL RESULTS AND DISCUSSION

I. Composition of the Caustic Soda Potato Peel Wastes

Analyses of the processing plant samples were required to obtain information on the composition of the waste material. This was required to determine the suitability of the caustic potato peel waste as a substrate for the thermophilic fermentation. Four waste samples were removed from the potato peeler during the course of a lye peeling process. It was considered that the samples were fairly representative of the waste that was discharged from this particular peeling process.

1. Carbon-Nitrogen Ratio, Carbon-Phosphorus Ratio and Moisture Content Requirements

Utilization of potato waste alone was questionable. As a sole fermentation substrate, it was considered deficient in some essential nutrient requirements for growth and maintenance of the microorganisms. There was definitely a question concerning its nitrogen availability. Since the available nitrogen in the waste was

low (as compared to carbon, Table 1), it was thought an insufficient amount was present to maintain a prolonged fermentation. The loss of the element in the form of ammonia or some other gaseous by-product during the course of the fermentation was a possibility. Since the growth and maintenance of the organisms depends on a carbon to nitrogen ratio (C/N) ranging from 9.7:1 to 18.3:1 (8); it was thought that this requirement had to be met or the attainment and/or maintenance of a thermophilic fermentation would be difficult. Even a C/N ratio greater than 7.5 was considered nitrogen limiting (8, 13). Surucu et al (55), developed a chemically defined medium containing glucose as the carbon source and ammonium chloride as the nitrogen source for the culturing of a mixed population of thermophilic organisms. They found the mixed culture, obtained from soil, stream water, raw wastewater and silage, to be comprised of a mixture of organisms having different and more demanding nutritional requirements for growth. Formulation of their medium constitutes a C/N ratio of 3.45 for the mixed thermophilic culture. Toth (57) stated that the nitrogen content and the C/N ratio of the organics will largely determine the rate of decomposition and that extra nitrogen must be added to ensure decomposition. Almost all organic waste that

Table 1. Composition of caustic potato peel waste and fresh potato.

	Caustic Potato Peel Waste, % Dry Weight	Unpeeled Fresh Potato, % Dry Weight
Total Kjeldahl Nitrogen	1.7 - 2.0	1.8
Carbon Analyses		
Total Carbon	45.8	43.6
Inorganic Carbon	1.56	1.73
Organic Carbon	44.24	41.87
Total Phosphorous	0.031	0.028 (0.021)[a]
Total Sodium	5.66	0.04 (0.03)[a]
Ash	19.9	9.9 (5.6)[a]
Moisture Content	88.9	79.2
Dry Matter Content	11.1	20.8
pH	12.5	-

[a] Results obtained for fresh, peeled potatoes.

contains 1.5 to 2.5% total nitrogen requires the addition of extra nitrogen. Therefore, analyses were required for the amounts of carbon and nitrogen present in the sample. Total Kjeldahl nitrogen of the four plant samples tested varied from 1.7 to 2.0%, on a dry weight basis, or an average of 1.9% or an average crude protein content (N x 6.25) of 11.9%. The store bought or fresh potatoes yielded an average value for total Kjeldahl nitrogen of 1.8%, on a dry weight basis, see Table 1. These values were similar to reported literature values of 1.8% - 2.0% (47, 51).

Total carbon analysis of the plant samples gave an average value of 45.80%, on a dry weight basis. Fresh store bought potatoes had a 43.60% total carbon content. Average values for inorganic carbon for the plant samples and fresh potatoes were 1.56% and 1.73%, respectively. The difference of the above two determined values (total carbon - inorganic carbon) gives an average organic carbon value of 44.24% for the plant samples and 41.87% for the fresh store bought potato.

Therefore, considering the amount of available Kjeldahl (organic) nitrogen present in the plant sample and the amount of carbon present, the ratio of carbon to nitrogen represents 45.8:1.9 or 24.1:1 (total organic

C to total organic N represents 44.24:1.9 or 23.3:1). It was thought that addition of a nitrogen source was required to decrease this ratio. The nutrient considered was urea since it was readily available and fairly inexpensive.

An initial fermentation trial (Figure 15) was performed with freshly macerated potatoes. Since the C/N ratio was similar to the processing plant samples, no additional nitrogen was added to the substrate. This initial trial had a threefold objective: 1) to determine whether thermophilic conditions were obtainable, and, if conditions were reached, what would be the duration of the thermophilic period? And thus, determine the amount of nitrogen needed to prolong and intensify the fermentation; 2) to correct and improve the mechanical components of the system and 3) to utilize the fresh potato fermentation as a control for the eventual fermentation trial with the caustic soda-treated potato. Surprisingly, even in the presence of low nitrogen content, thermophilic conditions were reached and were reasonably prolonged. This was considered extremely important and advantageous, since additional costs of a nitrogen source to treat this waste product would not be required. Further discussion on this is continued in a later section.

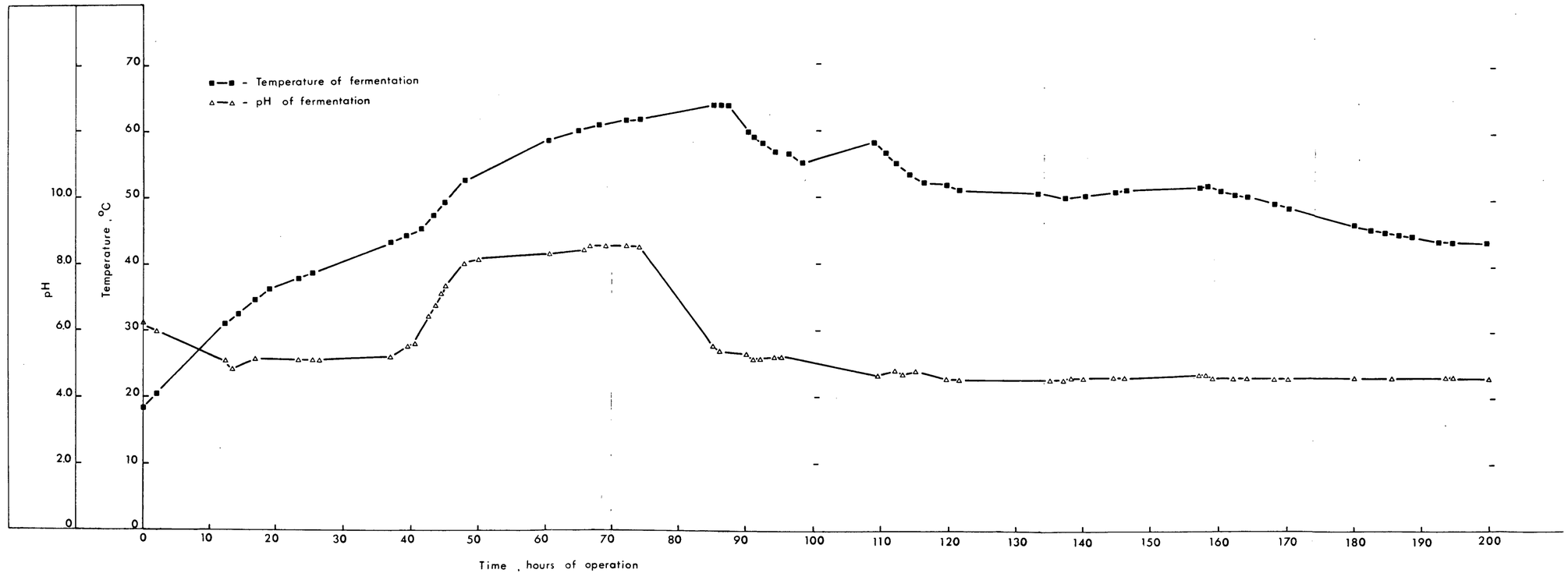

Figure 15. Thermophilic fermentation of a potato waste substrate; initial trial.

The other element considered essential for proper bacterial metabolism is phosphorus. Since nitrogen and phosphorus are required for synthesis of cellular material, these substances are needed in amounts related to the net cellular growth within the capacity of the fermentation system. Total phosphorus determination showed the caustic potato peel waste from the processing plant to be 0.031%, as compared to 0.028% phosphorus from fresh, unpeeled potato (and 0.021% for fresh, peeled potato). Literature (51) has cited values of 0.093% phosphorus in whole starch granules of potatoes. These values are dependent on numerous factors such as varietal characteristics, maturity, storage and phosphorus content of the soil in which the potatoes were grown.

Busch (8) quoted ratios of 51.4:1 to 85.7:1 of carbon to phosphorus as the range for phosphorus requirement. Converting the values obtained for the caustic potato waste samples yielded a total carbon to total phosphorus ratio of 1477.4:1, a value which was far below the minimum phosphorus requirements. However, as previously stated, thermophilic conditions were obtained in the initial fermentation with the existing nutrient profile.

An essential requirement, in conjunction with the metabolites required for bacterial metabolism, is the availability of water for the biochemical systems to react. Water content is important in this particular fermentation in consideration of the physical nature of the substrate. The moisture content of the substrate plays an important role in decomposition rates of the solids. Analyses of the four processing plant samples showed an average dry matter content of 11.1 g %, or a moisture content of 88.9%. The solid matter content of fresh potato averaged 20.8% or a moisture content of 79.2%. The reference literature (51) quotes average percent values for potatoes to be 22.5 g % total solids with the water content of 77.5%. The higher moisture content of the processing plant samples comes from the water added during processing.

2. Other Factors Affecting the Fermentation of the Substrate

The caustic potato peel waste samples obtained from the discharge of the mechanical scrubber (Figure 1) had a pH of 12.5. This pH value was within the range

(11.9 - 12.7) reported in the literature (6). In order to maintain this alkalinity, about 2 pounds of sodium hydroxide were used per 1000 pounds of potatoes for the peeling process, subsequently forming approximately 125 pounds of spent caustic peel waste (6). Due to the high concentration of lye within the peel waste, problems are encountered in finding a suitable method of disposal. For example, the high alkalinity of the waste inhibits bacterial initiation and thus the fermentation or degradation process does not start promptly.

The high concentration of sodium hydroxide will also inhibit the initiation of a fermentation by thermophilic organisms. Neutralization of the substrate was required, but it was thought that even at neutrality the high sodium content may still have a detrimental effect on microbial growth. The amount of sodium present in the samples was determined, not only for its effect on thermophilic microbial growth but also for the possible utilization of the fermented residue as livestock feed. In the latter case, the detrimental effect of a high sodium content in the fermented residue can be diminished with the addition of silage.

Analyses by atomic absorption spectrophotometry showed that the processing plant sample contained

an average of 5.66% sodium, as compared to 0.04% for fresh, unpeeled potato and 0.03% for freshly peeled potato. This value was also important in that fermentations were eventually conducted duplicating this amount of sodium in the laboratory experiments by adding sodium hydroxide into freshly macerated potato. This, of course, was to simulate the processing plant waste. The results of this fermentation will be discussed in a later section.

Preliminary analyses of the composition of caustic potato peel wastes and the fresh store bought potato compared favourably (Table 1). Variations occurred in the sodium content and the ash content of the samples. The values obtained for the ash determination were 19.9% for the caustic potato peel waste versus 9.9% for fresh, unpeeled potato and 5.6% for fresh, peeled potato. The higher ash content of the caustic potato peel waste can be accounted for by the presence of the caustic soda.

From the preliminary analyses of the potato wastes, a general idea can be obtained as to whether a fermentation can be initiated with the potato peel waste in its existing form, or, whether the waste requires a possible pre-treatment in the form of the addition or the deletion of certain elements. The results of

the above analyses are summarized in Table 1. Fresh, pH adjusted, potato macerate was used to simulate processing plant samples on the basis of the above results. This was required since seasonal manufacturing of potato chips by the processing plant restricted the availability of the caustic peel waste samples.

II. Assembly and Measurements of the Thermophilic Fermentation System

1. Construction of the Fermentation System

Development of this particular thermophilic fermentation system to its present state, as stated in the Materials and Methods, was by numerous trial and error experiments using water and potato systems. Various components were altered or incorporated to increase the efficiency of the system in which undesirable mechanical breakdowns were negated. These changes were adapted also to improve the operational stability and the controllability of the fermentation during the course of an extended trial.

For example, the top assembly was incorporated after the initial potato fermentation trial. It was used to prevent solids being lost due to foaming. Substrate foaming was the major problem encountered during the initial stages of the fermentation of potato waste. Solids lost from the fermenter will result in a higher solids degradation value than what was actually decomposed by the thermophiles. The problem was resolved by sealing the foam within the fermenter cell, thus confining the solids. The incorporation of a rubber seal around the fermenter cell formed a sufficient seal to prevent foam from escaping. The utilization of a mechanical foam breaker in the form of an impeller, situated just below the opening in the top assembly, alleviated the foam accumulating beneath the lid by physically destroying the excessive foam. Finally, a sponge soaked with anti-foam was used as a seal around the impellor shaft opening in the fermentor top, thus eliminating any foam from escaping.

The aeration system was modified to include the incorporation of a stainless steel ball (Figure 10) into the sparger head in order to prevent the infiltration of the substrate into the sparger lines. The stainless

steel ball acted as a plug to prevent the entrance of potato solids into the sparger lines during line pressure fluctuations. The entrance of the potato substrate into the sparger lines not only interfered with the air flow but also increased the back-pressure in the aeration lines, resulting in "blow offs" at tubing connections.

Besides the two examples provided above, other factors affecting the fermentation were alleviated or decreased as much as possible (see Materials and Methods). Those variables which still affected the outcome of the fermentation were determined and measured.

2. Measurements of Heat Input from the System Constructed

Mechanical heat produced by friction within the system was an important consideration involved in determining the thermal energy released by microbial metabolism. The growth of the microflora initially found on macerated potato was established in this study to release sufficient energy to raise the substrate through the mesophilic ambient temperatures up into the thermophilic range. If excessive mechanical heat was produced

by the system it would complicate the interpretation given to the role of the heat producing organisms. That is, if mechanical heat input resulted in creating a fermenting medium temperature greater than 50°C, it could be argued that the heat produced was not due to the contribution of the thermophilic orgamisms. Also, it was realized that excessive mechanical heat may progressively select orgamisms from the initial mixed microflora based on their growth rates and their ability to compete at temperatures attained during the course of the fermentation.

Artificial media systems were used to determine the mechanical heat input into the substrate. Fifty liters of cold tap water were placed into the fermenter and agitated along with an input of 900 ml/min of air. This volume of air input in 50 l of water was determined to yield 5.5 ppm of dissolved oxygen, Figure 14, at the equilibrium mechanical heat temperature of 41.4°C. Excessive aeration can lower the temperature of the medium substantially (28) and also cause excessive foaming. After numerous dimensional adjustments to the impeller, decreasing the rate of agitator rotation and adjusting the insulation, the maximum resultant temperature reached by the entire, assembled system was 41.4°C; well

below the 50°C thermophilic mark (Figure 16). It was possible to further lower the mechanical heat input by reducing the impeller size and the r.p.m. of the agitator. Under these circumstances, however, agitation of the substrate was considered to be insufficient, resulting in the settling of the heavier potato solids. A compromise was made as to the optimum between the best possible agitation with the lowest possible mechanical heat input temperature.

The extent of medium cooling caused by the removal of heat through aeration in relation to mechanical heat input was considered. Using the water system as before, but without aeration, it was determined that aeration, at 900 ml/min had minimal effect. As shown in Figure 16, the equilibrium temperature for the non-aerated system was 41.8°C, an increase of 0.4°C over the aerated water system.

It could be argued that the water system was not indicative of the potato waste substrate. Frictional heat created by the impeller against the potato solids may be considered a contributing heat factor. In order to determine the effect of potato solids, the fermenter was operated using 12.3 g % of potato solids (similar

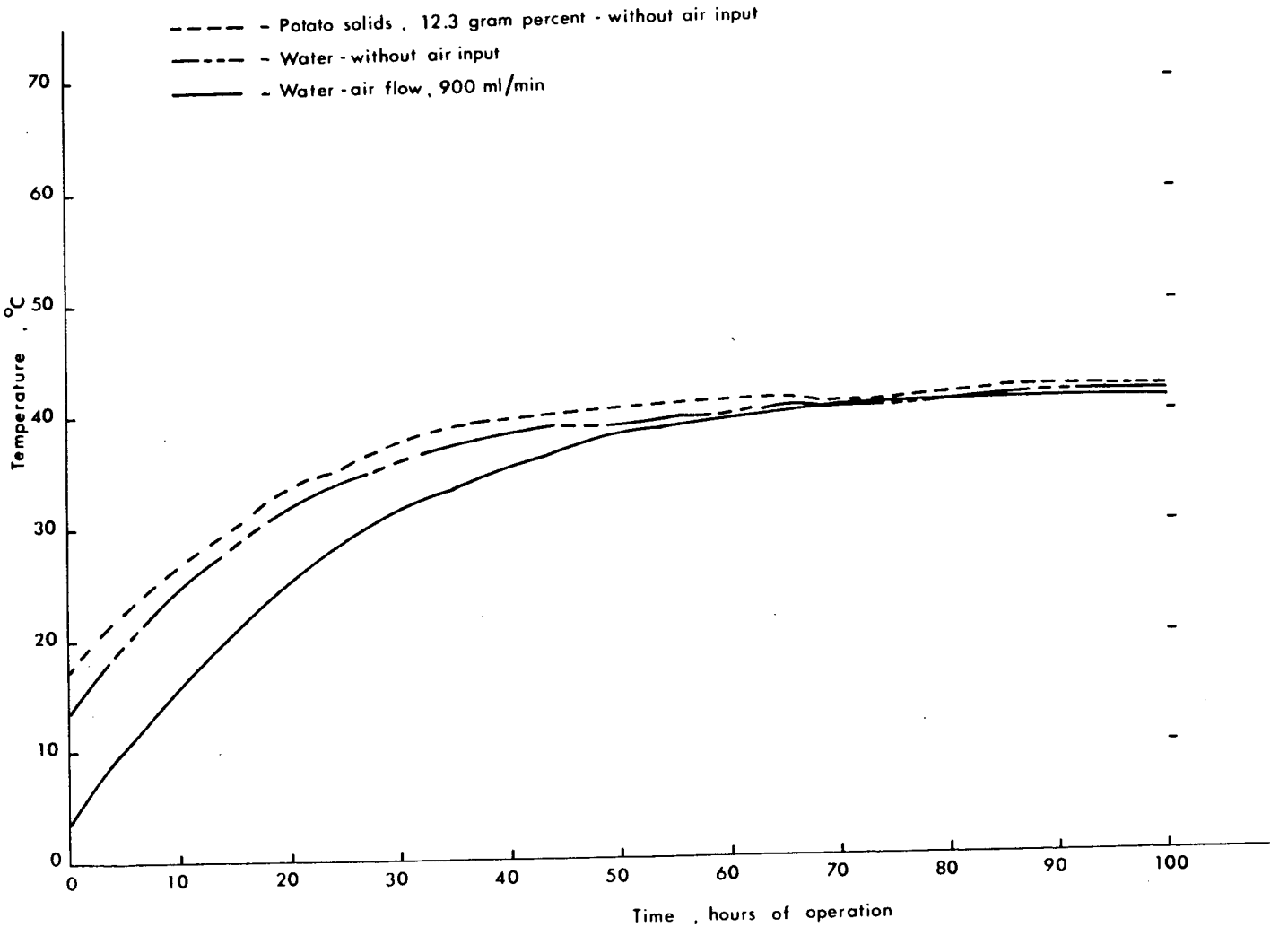

Figure 16. Mechanical heat input.

to actual fermentation trials), with sodium hypochlorite solution added to inhibit microbial growth. To deter aerobic growth, no air was supplied, There was insufficient foam created during agitation to cause significant heat retention. The maximum temperature obtained with 12.3 g % of potato solids was 42.1°C (Figure 16) or a temperature increase of 0.7°C over that of the water system. This value was considered to be satisfactory since aeration would also slightly decrease the temperature.

3. Measurements of Heat Loss from the System Constructed

The effectiveness of the insulation (R of 10 for the amount of vermiculite used) placed in the fermenter cell was evaluated in regards to the heat lost from the system. Information was required on the operational behaviour to ensure that heat was not retained nor the rate of heat loss too slow. Heat build up in the fermenter at the initial stages of the fermentation could induce or select the growth of thermophiles. Figure 17 shows a curvilinear relationship between temperature decrease in the fermenter with time. The result yielded an average

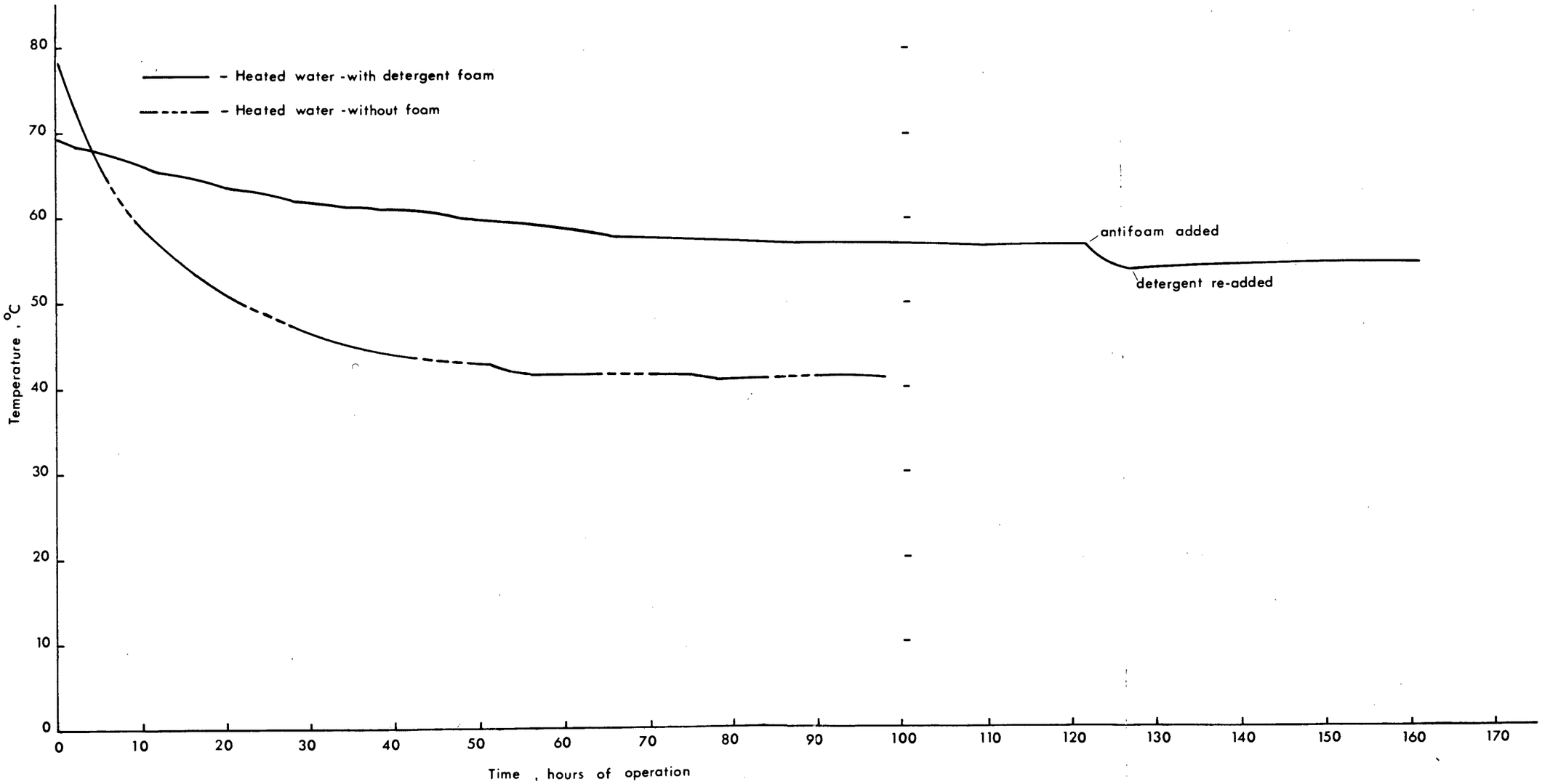

Figure 17. Heat loss determination from heated aqueous systems; with and without a heavy surface foam.

temperature decrease of 0.49°C/h; from 79.5°C to mechanical heat input temperature (41.4°C) in 77 h.

The heat retention characteristic of surface foam was determined with the use of a high foaming, commercial dishwashing detergent. As a comparison to the previous experiment, Figure 17 shows the difference between the two systems and the insulating effect of a heavy surface foam. The average rate of temperature decrease was 0.16°C/h for this determination over the same 77 h period. Over the same time period, this average rate would probably be slightly higher if the starting temperature of the heated water was the same as the above experiment. Since this was also a curvilinear relationship, the average rate of temperature decrease would probably be less than the value quoted when determined over a more extended time period. Even though the starting temperatures were different between the two experiments, the average rate of temperature decrease was definitely greater in the foamless system. A depression in the heat loss curve, at 122 h, was created by the addition of the antifoaming agent. A rapid temperature drop occurred once the foam was removed from the system. On re-addition of more detergent, the recreated foam retained the heat within the system.

In summary, this particular fermentation system developed for this project was sufficient to yield data that can be utilized for the characterization of aerobic-thermophilic potato waste fermentations. Mechanical breakdowns which occurred during the course of the fermentation were alleviated. Factors which affected the results of the fermentation were controlled or stabilized to a degree where it was not considered an important variable, e.g., foaming, aeration, ambient heat build up and continuous temperature recording. Mechanical heat was adjusted to where its input was not considered a serious factor in the maintenance or the encouragement of an aerobic-thermophilic fermentation to occur with the potato waste substrate. Countering the input of mechanical heat, heat dissipation was sufficient within the fermenter cell so that temperature maintenance during the thermophilic conditions were not due to the insulation but rather due to the metabolic activity of thermophilic organisms.

III. Results of the Aerobic-Thermophilic Fermentations of Potato Wastes

To fully characterize and examine the behaviour of the aerobic-thermophilic fermentation of potato wastes,

as previously stated, mechanical problems and equipment assemblies had to be solved first. The first couple of trials, using macerated potatoes, were to alleviate extensive foaming problems and temperature monitoring problems; but surprising results were noticed from these trials. Analyses of the composition of potato showed that as a substrate, potato was nitrogen and phosphorus limiting. In all probability, aerobic-thermophilic temperatures were not possible without supplementing this waste medium with an additional nitrogen source. Nevertheless, thermophilic temperatures were obtained, and were of a reasonably prolonged duration. This was extremely important since thermophilic conditions were obtainable without further addition of a nutrient source. An advantage is that it means a possible cost reduction in the treatment of this waste product.

In comparison to later trials, the duration and intensity of the thermophilic temperatures of these initial runs were relatively short and heat production was less intense (Figure 15). This was due mainly to the continual loss of potato solids from the substrate because of foaming. After the incorporation of the top assembly onto the system, the duration and intensity

of the thermophilic temperatures noticeably increased. It was obvious that the key to attaining higher temperatures and a longer thermophilic fermentation was greater nutrient availability. The analogy drawn by Pöpel and Ohnmacht (43) was that "to make the fire hotter, then an ample supply of fuel must be supplied". It was also possible that temperatures obtained from these initial trails were lower due to manual temperature readings. Extended periods of readings were not taken due to the time factor. During these overnight periods, it was possible for high temperatures to occur without being noticed. There was a possibility that crucial readings at specific apexes of the temperature curve were also missing. A continuous thermocouple-data recorder monitoring system was eventually utilized for the following trials.

Upon attainment of thermophilic conditions with this substrate, several possible ramifications of nutrient limitations were realized. 1) The previously quoted maximum and minimum requirements for nitrogen and phosphorus are guidelines and need not always judiciously apply (8). These are the basis for maximum synthesis requirements and they differ markedly from amounts

of nutrients required in continuous reaction at less than maximum synthesis. 2) Almost any amount of nitrogen and phosphorus will eventually permit the completion of the conversion of soluble carbon (potato starch), although the reaction rate of the fermentation would reflect the release of these nutrients from lysed cells for reuse. Therefore, the failure to meet these quantitative nutrient requirements would produce a rate limitation on the conversion of the potato starch. Since the rate of cell lysis determines the rate of nutrient availability and thus, the rate of carbon (potato starch) removal, it will eventually determine the detention time required to decompose the potato starch within the substrate.

From the standpoint of the actual, practical application of this system, one has to consider the cost of adding nutrients to shorten the detention time or consider the cost of a prolonged detention, in the sense of energy supplied for mixing and aeration and the time factor and availability of treatment space. In practice, the best solution is the balance between the cost of nutrient addition against the cost of an increased detention time.

For this project, further additions of nutrients were not utilized. It was obvious that nutrient addition

would create a thermophilic fermentation, since it was realized that thermophilic organisms existed as part of the external flora of potatoes. It was of interest to characterize the aerobic-thermophilic fermentation of potato waste alone (without additions) since it was now realized that this nutrient-limiting substrate was capable of reaching thermophilic temperatures.

1. Control Trials of Aerobic-Thermophilic Potato Fermentations

The behaviour of the thermophilic fermentation of fresh potato had to be established for comparison with sodium hydroxide-treated potatoes. These were control trials used to characterize the trends of the fermentation.

From Figure 18 (Control trial 3), it can be observed that a maximum temperature of 75°C was obtained. The thermophilic temperature (50°C) was reached after 64 h (2.7 d) from the start of the fermentation and lasted for 391 h (16.3 d). An initial total solids content of 8.32 g % was placed into the fermenter with a working volume of 50 liters. At the end of the fermentation trial, the final solids content of the residue was 2.05 g % or a decrease of 6.27 g % of the total solids over a 500 h

(20.8 d) detention time, a reduction of 75.4% of the initial total solids. More than 50% (50.6%) of the solids were degraded after 278 h (11.6 d) from the beginning of the fermentation or 214 h (8.9 d) after the thermophilic temperatures was reached. The overall average rate of solids utilization for this particular fermentation was 0.125 g/l/h (Table 2). This solids degradation rate, of course, will vary depending on the temperature of the fermentation and the bacterial population within the fermentation.

This trial served to determine: the effect of ambient temperature on the fermentation; the pH of the thermophilic fermentation; the rate of solids utilization by the thermophiles; the total organic nitrogen within the substrate at various stages of the fermentation and, of course, the temperature of the thermophilic fermentation. Each of these determinations is described in the following discussion.

Ambient temperatures were monitored throughout the duration of the fermentation to determine if the temperature within the fermenter was affected by the surrounding external temperature. The various fluctuations in the ambient temperature curve were due to heat build up in the incubator room and cooling due to opening

Table 2. Comparison of the various aerobic-thermophilic potato fermentations.

Parameters	Fermentation trials		
	Control trial 3	Control trial 4	With 5.66% Na
Maximum temperature, °C	75.0	68.9	63.2
Time to reach 50°C, h (d)	64 (2.7)	110 (4.6)	82 (3.4)
Duration above 50°C, h (d)	391 (16.3)	255 (10.6)	483 (20.1)
Detention time, h (d)	500 (20.8)	455 (18.9)	860 (35.8)
Total Solids Utilization			
Initial solids content, g %	8.32	10.67	16.56
Residual solids content, g %	2.05	8.34	6.66
Amount of solids lost, g %	6.27	2.33	9.90
Total solids reduction, %	75.4	21.8	59.8
Overall average rate, g/l/h	0.125	0.051	0.115

of the door. It can be observed from these ambient temperature fluctuations that there was no effect on the internal temperature of the fermenter. The system was sufficiently insulated so that the fermentation was completely isolated from external influences. One of the most important advantages of a thermophilic fermentation is its independence from ambient temperature, so that the fermentation proceeds unaffected even when external temperatures are close to freezing (43). This is due to the process, since it is thermally self-supporting.

The temperature curves obtained for these aerobic-thermophilic fermentations showed numerous depressions rather than the expected smooth, even curve. These depressions, i.e., temperature decreases, were interpreted as population changes of thermophilic bacteria within the medium and/or the decrease of thermophilic bacteria numbers. An analogy can be drawn between the temperature curve to that of a growth curve. Temperature increases and decreases represent the mixed thermophilic bacteria population in transition from exponential phase to the death phase, back through the lag phase, etc.

Temperature decreases due to decreasing mixed thermophilic bacteria numbers are the result of nutrient limitations. Deficient elements such as phosphorus and

nitrogen (viz., nitrogen that was available and not necessarily all the nitrogen present in the substrate) which was fully utilized, caused the thermophilic population to stop growing. As a result, the temperature stopped increasing. During the death phase, the thermophilic population decreased in numbers resulting in a temperature decrease. Cell lysis returns required nutrients back into the substrate causing the next increase in the thermophilic population.

Temperature decreases due to population changes may also occur with the emergence of a single variety of thermophilic bacteria within the mixed population of thermophiles. This may occur when certain conditions are suitable for the dominance of a particular single variety of bacteria. The suitable conditions may not necessarily be nutrient related but rather other factors, such as oxygen availability, acidity or alkalinity of the medium or by-product inhibition are possible. The emergence of a single variety over the mixed thermophilic population results in the competitive inhibition of the other thermophiles. These population changes were suggested by the different odors being emitted into the surroundings during different times of the fermentation. The feasibility of competitive inhibition occurs when suitable conditions

accentuate the growth rate for one thermophile causing it to dominate over the others. This may cause either a decrease or stoppage in growth and/or the death of the other bacteria. (This may also be due to the varying conditions described above). As a result, a decrease in temperature occurs due to the inhibition effect. The dominance of this other variety of thermophiles results in a temperature increase of a greater magnitude during the next temperature cycle.

Increases in the magnitude and duration of the temperature curve was also due to the further availability of nutrient from the potato substrate. Mechanical agitation aids in the reduction of the particle sizes of the macerated potato pieces. In addition to this, the initial temperature increase into the thermophilic range also caused the disruption of the potato starch granules. As the temperature duration and intensity increased, potato starch granules gelatinized and ruptured, releasing further nutrients into the medium for the thermophiles. The dry matter of the granule consists of 99.5% starch, 0.3% ash, 0.01% nitrogen (from protein debris) and as much as one-third of the total phosphorus of the potato is combined with the starch (31,46,47). This described process represented the degradation of the potato solids

during the thermophilic fermentation. In comparison, it would be more difficult for mesophilic bacteria to utilize the starch without a more complete heat treatment (gelatinization) of the potato waste.

The pH curve produced by this particular fermentation trial correlated with the temperature curve. Corresponding increasing and decreasing cycles of both curves exhibited a surprising synchrony. Two possible hypotheses for the fluctuation of the pH curve correspond with the hypotheses for the fluctuations of the temperature curve. As previously mentioned, growth conditions within the fermenting substrate will affect the thermophilic bacteria population with the possible dominance of one variety of thermophiles, and inhibition of others thus leading to fluctuations of the temperature curve. It is seen in Figure 18, that increased pH will eventually result in a decrease in temperature. The pH around neutrality or slightly alkaline appeared to cause inhibition of the thermophiles particular to the potato flora. The growth of other thermophiles more suitable to these higher pH conditions (also with the availability of further nutrients) decreases the pH due to the organic acid production. A decreasing pH would select for the regrowth

of the previous thermophiles or the emergence of another variety of thermophilic bacteria. The increasing population of these more acidophilic thermophiles resulted in the temperature increase. This cycle appeared to be repeated three consecutive times during the first half of the fermentation. Towards the last half of the fermentation, the pH value tends to move into the alkaline range (about pH 9) as the temperature decreased. This agreed with aerobic-thermophilic degradation of other substrates, such as yeast waste, molasses wastes, pig manure and sludge liquors (35,36). Termination of the thermophilic fermentation may be due to: 1) growth conditions too extreme for recovery, due to increased alkalinity and by-products excreted into the menstruum, and/or 2) insufficient nutrients available (2.05 gm % total solids remaining) and 3) the increased temperatures (after the second cycle) may have killed the acidophilic thermophiles required to reduce the pH.

The other reason, and perhaps the most accurate one for pH fluctuations, is related to availability of nutrients. Thermophilic fermentations tend to move into the alkaline pH range. However, with more nutrients at the initial stages, in this case larger amounts of starches than other nutrients (viz., proteins) a rapid

fermentation of the carbohydrates occurs. The starch fermentation increases organic acid production, thus reducing the pH. Increases in pH were due to the increase in substrate nitrogen (see Figure 18, Total organic nitrogen curve). This may be due to the incorporation of nitrogen into the system via aeration, an important characteristic which will be discussed later. Also, additional ammonia will be derived from cell lysis. Ammonification will involve the hydrolysis of cellular proteins and nucleic acids and as a result amino acids and nucleic acids are liberated into the menstruum. These simpler nitrogenous compounds are fermented resulting in the liberation of ammonia into the menstruum. Solubilization of this ammonia into the water phase will result in a basic pH response.

The last half of this fermentation trial did not fluctuate in either temperature or pH but rather the temperature was maintained for approximately 100 h (300 h to 400 h after the start) above 70^{o}C and the pH during this period increased gradually from neutrality to 8.2. Accepting the latter of the above reasons for pH fluctuations, the reason for this duration was mainly due to a nutritionally balanced substrate during this stage of the fermentation. The first half of the fermentation had

depleted sufficient amounts of the carbon source to a point where the C/N ratio had fallen within the requirement range. Also it was seen that the amount of organic nitrogen in the substrate was increasing, resulting in further lowering of the C/N ratio. This improved nutritional profile resulted in a maintained thermophilic temperature due to continual growth (and death) of thermophiles (i.e., stationary phase) within the substrate.

One of the most important findings of this project was the discovery of the increasing amounts of total organic nitrogen during the course of the fermentation. As previously stated, thermophilic fermentation of the substrate should not have occurred with such intensity or magnitude. It was realized that cannibalism of lysed cellular components was occurring to maintain the fermentation. And also, it was thought that the total organic nitrogen initially present in the system would be fully utilized, and as a result, remain constant within the system, but most likely decrease as ammonification occurs during the fermentation. Kjeldahl analyses were decided upon to show the rate of protein nitrogen lost from the system, as ammonia, into the atmosphere. However, the course of the fermentation showed both

decreasing, and surprisingly, increasing total organic nitrogen within the system (Figure 18). This increase in total organic nitrogen was the reason the fermentation worked effectively. It can be seen from Figure 18, that decreasing organic nitrogen occurred correspondingly with increasing pH and increasing temperature and vice versa. Correlation of decreasing total organic nitrogen to increasing pH can be due to ammonia incorporation from protein breakdown into the menstruum. The decreasing amounts of total organic nitrogen was due to its utilization by the increasing thermophilic bacterial population and thusly, increasing the temperature.

It can be asked, what caused the total organic nitrogen to fluctuate during the fermentation? The decreasing portions of the curve were understandable, but what causes the increasing portions? Since there was no further input of nutrients into the system, the overall total organic nitrogen should have been fairly constant. The only possible source of nitrogen input into the system is derived from the air. Nitrogen gas incorporation via aeration would have saturated the system and the solubility of nitrogen into the substrate would make it readily available for the organisms to use. However, the nitrogen constituent in the substrate as

determined by the micro-Kjeldahl method was in a more complex form than just the soluble nitrogen gas form. It was believed that nitrogen incorporation into the thermophilic fermentation could be due to bacterial conversion of the solubilized nitrogen gas into proteins. The presence of thermophilic nitrogen fixing organisms in this system was highly probable. Allen (1) cited literature of E. de Kruijff who observed growth of thermophilic bacteria in nitrogen-free media but was not able to obtain sufficient nitrogen fixation for an increase in nitrogen content to be detectable by a Kjeldahl analysis. Also cited was a report by H. Pringsheim, who detected nitrogen fixation by crude cultures of thermophilic bacteria. It was noticed that 3 to 6 mg N was fixed per gram of sugar utilized. This amount was appreciably less than that fixed by Azotobacter spp. at mesophilic temperatures. No attempts were made to isolate the causative organisms in pure culture nor was any description given. Allen (1) concluded that thermophiles carrying out transformation of nitrogenous compounds are rare and that the conditions for obtaining their development are not understood.

For this trial (Figure 18), the total organic nitrogen within the system, from beginning to end

increased from 1.42% to 4.02%, respectively, up 2.60% or a 2.8 fold increase. The amount increased within the thermophilic range was up to 2.80%, about a 2 fold increase. A distinct increase of the nitrogenous compounds was noticed towards the end (485 h) of the thermophilic fermentation. Therefore, it was reasonable that this particular fermentation with potato waste worked viz., because of nitrogen maintenance and/or incorporation into the system. Also, it was interesting to note the survival of this particular organism within the thermophilic temperatures and conditions and its growth within the mesophilic temperatures. Isolation and identification, from the fermenting substrate, were carried out for this causative organism(s) as verification of the existence of thermophilic organism(s) which maintain and/or incorporate nitrogen within the system. The results of these microbial analyses are discussed in a later section.

The last parameter measured for this control trial was the rate of solids utilization, in g/l/h, during the course of the fermentation. It appears that the greatest breakdown of potato solids occurred during the first half of the fermentation. Figure 18 indicated that the peaks of the curve occur at 114 h, 205.5 h and

241 h after the start of the experiment. This corresponded with increasing temperatures and decreasing total organic nitrogen content of the substrate. This was sensible since increasing bacteria numbers, thus increasing temperatures, resulted in a greater amount of solids degraded and utilized by the organisms. Because of increasing bacterial numbers, the available nitrogen within the system was utilized, and nitrogen emitted as ammonia with the air passing through the system. These rates were of greater magnitude at the first half of the fermentation due to the presence of large amounts of starch. More than 50% of the solids content was utilized after the third peak. Towards the latter half of the fermentation, the rate of utilization stabilized along with more stable temperatures. It appeared that the maintenance of high thermophilic temperatures does not require as high a solids breakdown rate as compared to the increasing bacterial population growth (increasing temperatures).

Another control trial (control trial 4) was performed in order to provide a comparison to the previous trial. The characteristics of this fermentation are shown in Figure 19. The measurements determined were: the pH of the fermentation; the dissolved oxygen (D.O.)

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in the substrate; the total organic nitrogen and the temperature of the fermentation. A maximum temperature of 68.9°C was reached with this trial. Thermophilic temperature (50°C) was obtained after 110 h (4.6 d) from the start of the fermentation, i.e., approximately 2 days later than the previous trial. The duration of the thermophilic temperature was 255 h (10.6 d), 5.7 days shorter than the previous trial. The initial total solids content placed into the fermenter was 10.67 g % and the final residue yielded a solids content of 8.34 g %. This was a 2.33 g % reduction in total solids over a 455 h detention time, or a reduction of 21.8% of the total solids initially present within the fermenter. This yielded an overall average rate of solids utilization of 0.051 g/l/h, far less than the previous trial (Table 2). It was obvious that this particular fermentation was not identical to the previous control trial. This was likely due to the variation in the bacterial flora between the two batches of potato substrate. As a result, the dominance of different thermophiles created lower temperatures and of shorter duration. In the first trial the temperature maximums and prolonged high temperature detentions degraded the bulk of the organic substances. This was not the case

for the latter trial, where lower temperatures dominated as a result of lower microbial metabolic activity. It appears that these potato fermentations are not exactly reproducible. Without further controls; temperature, pH and solids degradation rates are dependent on the existing flora of the potatoes and the dominance of these individual thermophiles during the course of the fermentation.

The fermentation characteristics exhibited by this second trial were similar to the first; the magnitude and intensity of the thermophilic fermentation varied only slightly. Again, 3 cycles were noted, with the last cycle not as distinct as the first two. Each cycle consisted of the corresponding temperature and pH increases with the total organic nitrogen decreasing. The aspects of these traits were discussed for the previous trial and would apply for this fermentation. The amount of total organic nitrogen incorporated into the final residue for this trial yielded a value of 1.91% from the initial input of 1.58% nitrogen up 0.33%, or a 1.2 fold increase.

An additional parameter measured in this trial was dissolved oxygen (D.O.) in the substrate. The readings obtained were compared with the calibration curve for the D.O. probe in Figure 13. Available oxygen for an aerobic-thermophilic fermentation was monitored

to ensure anaerobic conditions did not occur. Information on the bacterial population was also provided with the D.O. readings. The D.O. curve fitted within the established cycle with the other parameters. Decreasing D.O. within the substrate correlated with increasing temperature (decreasing total organic nitrogen and increasing pH), and vice versa. Obviously, increasing aerobic-thermophilic bacteria growth would put a demand on the oxygen supply within the substrate. With increasing temperatures, due to increasing thermophilic bacterial numbers, the D.O. content of the substrate would decrease. Limiting oxygen availability would inhibit growth, and with increasing temperatures, the amount of oxygen soluble in the liquid substrate would decrease; see Figure 19. Therefore, inhibition of bacterial growth (viz., further temperature increase) could be due to two factors: 1) increasing bacterial numbers would deplete the amount of oxygen available for further population increase, and/or 2) with increasing substrate temperature, the solubility of oxygen in the liquid decreases thus limiting further aerobic-thermophilic bacterial growth.

Aeration of the medium was not started at the beginning of the fermentation but was intentionally delayed until the mechanical heat temperature was reached by the

potato medium. This occurred at 87 h after the start. With agitation and aeration, a heavy starch foam was created at mesophilic temperatures. This foam production was difficult to control. To negate this initial solids loss due to excessive foaming, aeration was started within the mechanical heat temperatures, prior to attainment of thermophilic temperatures. This was important since in the trial with the high sodium hydroxide-treated potato, a more extensive "soapy-starchy"foam was created.

2. Aerobic-thermophilic Fermentation of Sodium Hydroxide-treated Potato

To simulate the caustic soda peeled potato waste, a calculated amount of sodium hydroxide was added to 50 l of macerated potato, yielding a sodium content of 5.66%. This basic mixture was neutralized with sulfuric acid to a pH of approximately 6. Sulfuric acid was used instead of hydrochloric acid since high concentrations of NaCl could hinder growth of the thermophiles (Table 9). However, in practice, if the residue is to be utilized as animal feed, sulfuric acid should be partly replaced with HCl for neutralization since a large amount of sulfate in the feed may not be desirable (6). Initial neutralization of the

caustic potato substrate was required before micro-organisms can propagate on the potato substrate. This could constitute an additional cost in treatment of the waste. However, if a continuous fermentation was utilized, after the initial neutralization, the caustic potato waste could be added to the fermentation during the high organic acid production stages of the fermentation.

This fermentation was performed in order to determine if the thermophilic temperatures were achievable with a high sodium content substrate. The results of the fermentation are indicated in Figure 20. The maximum temperature obtained was 63.2^{o}C. Thermophilic temperatures were reached after 82 h (3.4 d) from the start of the fermentation with a detention time in thermophilic range of 483 h (20.1 d). An initial solids content of 16.56 g % (10.90 g % potato solids and 5.66% sodium solids) was placed into the fermenter resulting in a residue of 6.66 g % (1.00 g % potato residue and 5.66% sodium solids), a decrease of 9.90 g % or a total solids reduction of 59.8% (90.8% reduction of total potato solids). The overall average rate of solids utilization was 0.115 g/l/h, which compared favorably with the control trial 3, Table 2. Since this calculated rate

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of solids utilization was based on detention time, the rate for this particular experiment was higher than control trial 3. A longer detention time (295 h vs. 30 h) after the temperature had dropped below 50°C, was required to bring the fermentation to mechanical heat temperatures. This extra time below the thermophilic temperature reduced the value of the calculated rate for solid utilization.

It was possible to obtain thermophilic conditions with sodium hydroxide treated potato macerate. However, the magnitude and intensity of the obtained values were again different from those of the previous trials. This may be due to the difference of the flora present in the potato substrate. Treating the macerated potato with sodium hydroxide and then with sulfuric acid may have selected out organisms which were incapable of surviving under these extreme conditions. The remaining flora were shown to consist of thermophiles capable of surviving the lye treatment process. The characteristics of the fermentation produced by these remaining thermophiles resemble the control trials though the curves were more level (Figure 20). One other factor which may have affected this fermentation was the stabilization of

the pH of the substrate. There appeared to be a buffering action created in the substrate due to the neutralization of the sodium hydroxide with sulfuric acid. The pH held constant at 5.1 throughout the fermentation. This may have further selected out the growth of high temperature producing thermophiles. Large temperature fluctuations appeared to be missing from this fermentation when compared to the control trials. However, characteristics resembling those of the control runs were still present, that is, increasing temperatures corresponding to decreasing D.O. and total organic nitrogen, but the cycles were not as distinct. Likewise, the total organic nitrogen increased from 1.68% to 2.07% up 0.39%, or a 1.2 fold increase.

It is seen that an aerobic-thermophilic fermentation was possible with neutralized caustic soda treated potato substrate. However, it is likely that the fermentation can be improved in terms of greater rate of solids utilization, higher temperature production and shorter detention times by the addition of further nutrients and probably, the addition of an inoculum consisting of thermophilic bacteria from established thermophilic fermentations of manure or potato.

IV. Results of the Analyses of the Fermented Residue

The upgrading of the potato waste by aerobic-thermophilic fermentation was due to the increase of nitrogen in the residue. Nitrogen incorporation, as previously stated, by the thermophilic organisms into the menstruum showed increases of 20.9% to as high as 183.1%, Table 3, for the fermentation trials conducted. The "quality" of this incorporated nitrogen into the substrate by aerobic-thermophilic fermentation was determined as to whether it was converted into protein or remained in the substrate in simpler forms such as free amino acid or peptides. Trichloroacetic acid precipitation was used to determine the "true" amount of protein nitrogen within the fermented residue. This "true" protein nitrogen was determined by taking the difference between total Kjeldahl nitrogen and the non-protein Kjeldahl nitrogen. The latter was obtained by TCA deproteinization of the fermented residue in which the (anionic) TCA forms an insoluble salt with (cationic) proteins. Kjeldahl determination was performed on the resultant supernatant to give the non-protein nitrogen value. The percent "true" protein obtained for the control trials and with 5.66%

Table 3. Amount of nitrogen in the fermented residue as compared to the initial potato used.

Fermentation trial	Total Kjeldahl nitrogen,%		Amount increased, %
	At start	In residue	
Control trial 3	1.42	4.02	183.1
Control trial 4	1.58	1.91	20.9
With 5.66% Na	1.68	2.07	23.2

sodium are shown in Table 4. These fermented residues showed a marked increase in "true" protein content over that of the fresh potato sample of 68.3%. The data presented in Table 4 were values obtained by Kjeldahl analyses of 5 ml aliquot samples. Values from the assay for total Kjeldahl nitrogen indicated that the fresh potato sample contains a higher nitrogen level (in ppm) than the fermented substrate. This was due to the higher solids content within the 5 ml aliquot used in the fresh potato analysis as compared to the solids within the fermented samples. These values were comparable to the total solids utilization values of Table 2 in which residual solids content increased from control trial 3, experiment with 5.66% Na to control trial 4, respectively. The non-protein values (Table 4) followed the same trend as the residual solids values. The conversion of nitrogenous compounds into "true" protein increased with increasing nitrogen incorporation (Table 3).

The protein nitrogen determined in the fermented residues probably contain a greater portion of bacterial protein than potato protein. This was considered to be a benefit in that the total waste product would be fully utilized, i.e., the residue which was left

Table 4. Kjeldahl nitrogen determination (wet basis) of the "true" protein nitrogen in the fermented potato using trichloroacetic acid precipitation.

Sample	Total Kjeldahl nitrogen, ppm	Non-protein Kjeldahl nitrogen, ppm	"True" protein nitrogen, ppm	"True" protein, %[a]
Fresh potato	85.3	27.0	58.3	68.3
Control trial 3	32.5	6.0	26.5	81.5
Control trial 4	73.7	18.0	55.7	75.8
With 5.66% Na	58.0	12.0	46.0	79.9

[a] As percent of "true" protein nitrogen (ppm) to total Kjeldahl nitrogen (ppm).

over from the fermentation could serve as a single cell protein (SCP) source. Thus, the advantage of utilizing the aerobic-thermophilic process as the treatment system for potato waste was not only with respect to reducing the solids, but also, for the conversion of the carbohydrate waste into SCP. The formation of SCP presents a possible utilization of the fermented residue for livestock feed. The increased nitrogen content is an improvement over the initial levels in terms of serving as an animal feed. For monogastrics, the amino acid content of the fermented residue, as shown in the profile in Table 5, is important for nutrition. The variation observed in the amino acid composition of the SCP could be the result of a number of different factors, e.g., method of culture, time of harvest, the substrate utilized and strains of bacteria (4). The amino acids of the three fermented substrates which occurred in increased quantities over that of potato were: glutamic acid, alanine, valine and methionine. Those which showed decreased values for the three fermented substrate were: aspartic acid, threonine, serine, proline, leucine and cystine. The remainder of the amino acids varied between the trials. A comparison of the essential amino acid requirement for chickens to that found in the fermented residue indicated that the fermented residue would be

Table 5. Amino acid composition of the fermented residue compared with the potato used in the fermentation.

	Control trial 3		Control trial 4		With 5.66% Na	
	Potato %	Residue %	Potato %	Residue %	Potato %	Residue %
Aspartic acid	11.36	10.11	11.31	10.53	11.40	10.10
Threonine	6.36	5.40	6.43	5.92	6.28	5.95
Serine	5.13	4.41	5.27	4.60	5.35	4.78
Glutamic acid	10.01	11.97	10.04	11.73	10.04	11.03
Proline	3.90	2.68	4.58	4.32	3.56	2.68
Glycine	4.80	5.74	5.09	4.81	4.75	4.96
Alanine	5.11	7.12	5.08	6.35	5.09	6.28
Valine	5.01	5.43	5.06	6.13	5.23	5.54
Methionine	2.30	2.89	2.26	2.45	2.32	3.22
Isoleucine	5.11	6.02	4.64	6.09	5.78	4.92
Leucine	9.01	8.25	9.09	8.58	8.39	6.91
Tyrosine	3.53	4.70	4.09	3.91	3.37	4.53
Phenylalanine	5.41	5.73	5.51	4.71	4.58	3.95
Lysine	6.96	6.62	6.89	6.31	7.17	8.35
Histidine	1.90	1.80	1.98	2.44	2.02	2.35
Tryptophan	2.81	trace	2.31	trace	3.04	3.83
Arginine	4.51	4.47	3.51	4.44	4.65	3.89
Pyridylethyl-L-cystine	1.76	1.60	1.85	1.66	1.96	1.73
Total percent	94.98	94.98	94.98	94.98	94.98	95.00

favourably accepted (Table 6). For the control trials, the limiting amino acid appeared to be tryptophan. However, for the high sodium content trial, the tryptophan value can be considered to be nutritionally adequate. Using the residue as a feed supplement, the proportions can be adjusted accordingly to the amounts shown in Table 6. However, in order to fully answer the question of whether the SCP would serve as an adequate animal feed; further studies are required on culture stability in production of SCP in large scale operations, animal acceptance, animal toxicity and the quality of the animal products. An obvious advantage of thermophilic SCP production is the destruction of pathogens during the process, negating the need for pasteurization of the product. Also, the initial sterilization of the fermentation substrate is not required for the thermophilic SCP culturing.

A possible potential problem concerning utilization of the fermented residue as feed is that the sodium content is high. The toxic levels of various sodium compounds for poultry is shown in Table 7. For immature turkeys, it can be seen that a ten-fold higher level of sodium, as NaCl, can be consumed with the feed as compared to its consumption within water. A level of 40,000 ppm NaCl or 4% (w/w) was tolerated within the feed

Table 6. Amino acid requirements of chickens compared to the values of the fermented residues.

Amino Acid	Broilers		Replacement pullets (egg or meat type)			Laying and breeding hens	Fermented residues	
	0-6 wks %	6-9 wks %	0-6 wks %	6-14 wks %	14-20 wks %	%	Controls[a] %	With Na %
Arginine	1.4	1.2	1.2	0.95	0.72	0.8	4.44	3.89
Glycine	1.15	1.0	1.0	0.8	0.6	?	4.81	4.96
Histidine	0.46	0.4	0.4	0.32	0.24	?	1.80	2.35
Isoleucine	0.86	0.75	0.75	0.6	0.45	0.5	6.02	4.92
Leucine	1.6	1.4	1.4	1.1	0.84	1.2	8.25	6.91
Lysine	1.25	1.1	1.1	0.9	0.66	0.5	6.31	8.35
Methionine	0.86	0.75	0.75	0.6	0.45	0.53	2.45	3.22
Phenylalanine	1.5	1.3	1.3	1.05	0.78	?	4.71	3.95
Threonine	0.8	0.7	0.7	0.55	0.42	0.4	5.40	5.95
Trypotphan	0.23	0.2	0.2	0.16	0.12	0.11	trace	3.83
Valine	1.0	0.85	0.85	0.7	0.5	?	5.43	5.54

From: Nutrient Requirements of Domestic Animals,"Nutrient Requirements of Poultry", 6th Edition, 1971, Subcommittee on Poultry Nutrition, Committee on Animal Nutrition, Agricultural Board, National Research Council, National Academcy of Sciences, Washington, D.C.

[a] Lowest values obtained for the two control fermentations.

Table 7. Toxic sodium levels for poultry.

Species	Age	Compound	Toxic level, ppm	Physiological Effect
Chicken	Immature	Na glutamate	8,900[a]	Reduced growth
Chicken	Laying hen	Na_2SO_4	12,000[b]	Reduced egg production
Chicken	Immature	NaCl	7,000[b]	Reduced growth; mortality
Chicken	Laying hen	NaCl	10,000[b]	Reduced egg production
Turkey	Immature	NaCl	4,000[b]	Reduced body weight; mortality
Turkey	Immature	NaCl	40,000[c]	Reduced growth; pendulous crop
Turkey	Mature	NaCl	60,000[c]	Reduced growth
Duck	Immature	NaCl	4,000[b]	Reduced body weight

From: Nutrient Requirements of Domestic Animals, "Nutrient Requirements of Poultry", 6th Edition, 1971, Subcommittee on Poultry Nutrition, Committee on Animal Nutrition, Agricultural Board, National Research Council, National Academy of Sciences, Washington, D.C.

[a] Diet low in Cl^- ion.

[b] In water.

[c] In feed.

for immature turkeys, whereas mature turkeys can consume up to 60,000 ppm or 6% (w/w). Since the fermented residue would contain approximately 5-6% (w/w) it is quite feasible that its utilization as feed would not be detrimental. Also, when used as a supplement, a dilution effect would be provided as it is incorporated along with other feed material.

Thus, as an added advantage, besides the treatment of the potato waste in order to decrease the total organic solids load by the aerobic-thermophilic process, a by-product was produced by this fermentation which is a valuable protein source for animal feed. This would mean that it would appear possible to have the total utilization (or disposal) of the entire waste. However, one disadvantage would be the cost of dewatering the final fermented residue. A possible solution in-part could involve the harvesting of the heat produced by the thermophiles for the drying of the fermented residue. The use of this fermented residue as animal feed should aid in reducing the overall cost of the waste treatment process.

V. Identification of the Aerobic-Thermophilic Bacteria Present in the Thermophilic Fermentation of Potato Wastes

As verification of the existence of aerobic-thermophilic bacteria that incorporated nitrogen within the potato substrate, isolation and identification of the organism(s) responsible for fixing atmospheric nitrogen were conducted. This would serve as added definitive proof that atmospheric nitrogen was incorporated into the fermentation and subsequently converted into protein. It was realized from the literature that the more commonly known nitrogen-fixing organisms were unable to survive under the aerobic-thermophilic conditions. The more commonly known nitrogen-fixing organisms belong to the Azotobacteraceae family, consisting of the genera Azotobacter and Beijerinckia and the lesser known Azomonas and Derxia. Bergey's manual (7) stated an optimum growth temperature of between 20 and 30°C for the first three genera with no growth occurring at temperatures higher than 37°C. Derxia has an optimum growth temperature at 25-35°C and no growth at 50°C or above. Also, certain species of the genera Pseudomonas, Clostridium, Klebsiella and Bacillus are known to fix nitrogen (7,22,49,52). These are summarized in Table 8 along with their optimum

Table 8. Atmospheric nitrogen-fixing organisms as stated in Bergey's Manual of Determinative Bacteriology.

Species	Optimum growth temperature, °C	Comments
Clostridium		
C. pasteurïanum	37	limited fixation of atmospheric nitrogen
C. butyricum	25 - 37	
C. acetobutylicum	37	
Klebsiella		
K. pneumoniae	35 - 37	from V.B.D. Skerman (48)
Pseudomonas		
P. methanitrificans (Methylomonas methanitrificans)	20 - 35	
Bacillus		
B. polymyxa	35 - 45	under anaerobic conditions by majority of strains
B. macerans	40 - 50	under anaerobic conditions by majority of strains

growth temperatures. However, only certain strains of these species fix nitrogen. The other well known organisms which can fix molecular nitrogen belong to the genus Rhizobium. However, these organisms require a symbiotic relationship with roots of leguminous plants. This mutualistic partnership is required since neither the plant nor the bacteria can fix nitrogen individually. Organisms of this particular genus were unlikely to have occurred within the fermentation. The possible organism(s) fixing nitrogen within the substrate would have to be a non-symbiotic or free-living, high temperature, facultative aerobic or aerobic organism. This would appear to rule out the species stated in Table 8.

The organism(s) responsible for nitrogen fixation were isolated from the fermentation on three different nitrogen-free selective media. One of the media was a commercially prepared medium, Difco Yeast Carbon Base (YCB), used for testing nitrogen assimilation by yeasts. This basal medium was filter-sterilized and incorporated into sterile agar to form the selective medium. The other two media were synthetic media specific for the isolation of non-symbiotic, nitrogen-fixing, soil organisms. One medium (abbreviated as Azo) was specific for Azotobacter species and the other

(abbreviated as Mei), adapted from Meiklejohn (38), was specific for organisms of both the Azotobacter and Beijerinckia genera. The composition of these synthetic media were very similar. The most important requirement being molybdenum (7), which was present in all three media in the form of sodium molybdate. Since these bacteria do not require organic substances, sucrose was suitable as the carbon source. The media were fairly simple and well buffered with phosphate and potassium salts. Kjeldahl nitrogen analyses showed the absence of any detectable nitrogen in any of the three media.

A sample of the substrate was removed from the fermenter once the fermentation was well into the thermophilic temperature range. The sample was streaked onto each medium, and after incubation at 55°C, visually distinct colonies were isolated and restreaked onto fresh plates. After six transfers, it was assumed that these surviving colonies growing at thermophilic temperatures on nitrogen-free media were utilizing atmospheric nitrogen as a source of nitrogen. These six consecutive transfers were required to ensure adequate dilution of the fermenter nitrogen and thus ensure that the new colony formations obtained the nitrogen required for growth only from the air. Growth on the later transfers were slow on all

three media; with colonies growing on the synthetic media more slowly than on YCB. Increased growth could be due to the presence of vitamins and amino acids in the YCB composition.

After selective isolation, the pure cultures were classified according to their Gram reaction and cell morphology. Microscopic observations showed the organisms to be Gram variable, long rods, occurring singly or in chains (Figure 21). Endospore formation was noted under phase contrast microscopy along with motility (as opposed to Brownian movement). These visual observations suggested the organisms to belong to the Bacillaceae family. Further differential characteristics were determined in order to identify the genus in which these particular organisms belong within this family. Stab cultures into tubed Trypticase Soy Agar showed growth on top of the agar and along the entire stab for all the organisms isolated. Growth also occurred under anaerobic conditions at 55°C, indicating the bacteria to be facultative anaerobes. These organisms were also catalase positive. From the above properties it was concluded that all the isolated cultures from the selective media belong to the genus Bacillus. Species of the genus Clostridium resemble the above description, however,

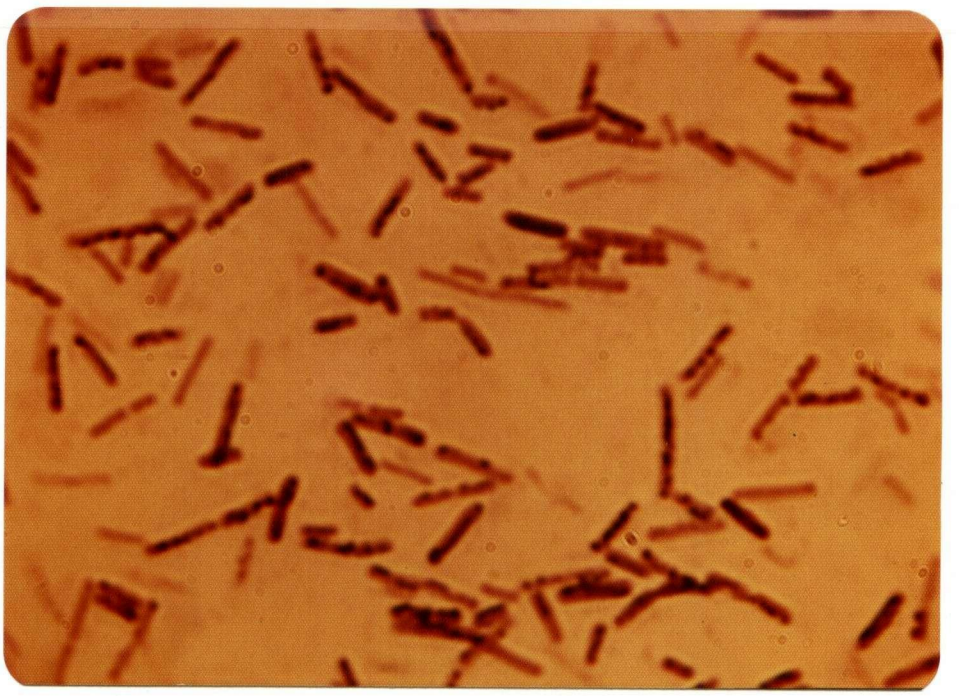

Figure 21. Gram reaction and cellular morphology of the isolated organism.

they are strict anaerobes and usually catalase negative. The other genera of this family are either strict anaerobes (Desulfotomaculum), strict aerobes (Sporosarcina) or microaerophilic, catalase negative (Sporolactobacillus) (7). Further differential characteristics and biochemical profiles of the isolated organisms are shown in Table 9. Evaluation of this profile places the species within the genus Bacillus. The biochemical profile was compared to the characteristic provided by Bergey's manual (7) and Gordon et al. (22). The organisms isolated from the control trial 4 and from the potato fermentation with 5.66% sodium, on all three selective media had similar characteristics (Table 9). These particular organisms were characterized on the differential characteristics, as described by Bergey's manual (7) and Gordon et al. (22), and concluded to belong to the species Bacillus coagulans. This conclusion was reached by the following reasons: 1) One of the most definitive tests to indicate that the organisms are of this species is the growth temperature. The maximum temperature obtained was 60°C ± 5°C. Thermophiles of this genus capable of surviving at this temperature maximum are B. stearothermophilus (65 - 75°C), B. coagulans (55 - 60°C) and B. brevis (40 - 60°C) and possibly, B. licheniformis

Table 9. Characteristics of the organisms isolated on nitrogen-free media; Yeast Carbon Base (YCB), modified Meiklejohn's (Mei) and Azotobacter specific medium (Azo).

Characteristics	Control trial 4			With 5.66% Na		
	YCB	Mei	Azo	YCB	Mei	Azo
Appearance of colonies (TSA):						
form	circular	circular	circular	circular	circular	circular
elevation	umbonate	umbonate	umbonate	umbonate	umbonate	umbonate
margin	entire	entire	entire	entire	entire	entire
Agar stroke (TSA):	filiform	filiform	filiform	filiform	filiform	filiform
Growth in broth (TSB):	suspension	suspension	suspension	suspension	suspension	suspension
Microscopic appearance:						
shape	rods	rods	rods	rods	rods	rods
arrangement	chains	chains	chains	chains	chains	chains
size: width, μ	0.9-1.3	0.9-1.3	0.9-1.3	0.9-1.3	0.9-1.3	0.9-1.3
length, μ	2.2-8.7	2.2-8.7	2.2-8.7	2.2-8.7	2.2-8.7	2.2-8.7
Gram reaction:	variable	variable	variable	variable	variable	variable
Spore:						
shape	cylindrical	cylindrical	cylindrical	cylindrical	cylindrical	cylindrical
position	terminal	terminal	terminal	terminal	terminal	terminal
sporangia	swollen	swollen	swollen	swollen	swollen	swollen

cont'd

Table 9 (cont'd)

Characteristics	Control trial 4			With 5.66% Na		
	YCB	Mei	Azo	YCB	Mei	Azo
Motility:	+	+	+	+	+	+
Catalase:	+	+	+	+	+	+
Anaerobic growth:	+	+	+	+	+	+
VP reaction:	weak +	+	weak +	+	+	+
Methyl red test:	-	-	-	-	-	-
pH in VP broth:	4.70	4.65	4.75	4.85	4.90	4.85
pH range for growth:	4.4-7.8	4.4-7.8	4.4-7.8	4.5-8.0	4.5-8.0	4.4-8.0
Temperature of growth:						
Maximum, °C	55-60	60-65	60-65	60-65	60-65	60-65
Minimum, °C	25-30	25-30	25-30	25-30	25-30	25-30

cont'd

Table 9. cont'd

Characteristics	Control trial 4			With 5.66% Na		
	YCB	Mei	Azo	YCB	Mei	Azo
Growth in:						
0.001% lysozyme (14 d)	-	-	-	-	-	-
Na Azide (0.02%)	+	+	+	+	+	+
NaCl (0%)	+	+	+	+	+	+
NaCl (2%)	+	+	+	+	+	+
NaCl (5%)	-	-	-	-	-	-
Nacl (7%)	-	-	-	-	-	-
NaCl (10%)	-	-	-	-	-	-
Acid from carbohydrate (14 d)						
Glucose	+	+	+	+	+	+
Arabinose	+	+	+	+	+	+
Xylose	±	-	-	-	-	-
Mannitol	-	-	-	-	-	-
Hydrolysis of starch:	+	+	+	+	+	+
Use of citrate (14 d)	-	-	-	± (pink)	-	± (pink)
Use of propionate (14 d)	-	-	± (pink)	-	-	-
NO_2 from NO_3	-	±	+	-	-	-

cont'd

Table 9. cont'd

Characteristics	Control trial 4			With 5.66% Na		
	YCB	Mei	Azo	YCB	Mei	Azo
Dihydroxyacetone:	± (reddish purple)	± (")	± (")	± (")	± (")	± (")
Indole:	-	-	-	-	-	-
Deamination of phenylalanine: (1 wk.)	-	-	-	-	-	-
Decomposition of:						
Casein (14 d)	-	-	-	-	-	-
Tyrosine (14 d)	-	-	-	-	-	-
Litmus milk:	Acid	Acid	Acid	Acid	Acid	Acid
reduction of litmus	+	+	+	+	+	+
curd formation	+	-	+	-	-	-

+ = growth

- = no growth

± = character inconsistent

(50 - 55°C) and B. subtilis (45-55°C) (7,22). 2) Of the thermophiles just mentioned, the only one capable of growth in 0.02% sodium azide is B. coagulans. 3) Acid production in Voges-Proskauer broth by B. coagulans is between pH 4.2 - 4.8, the others are not high acid producers. and 4) In comparison, both B. stearothermophilus and B. brevis fail to grow anaerobically. The properties of 22 strains of B. coagulans as summarized by Gordon et al (22) matched the properties of the isolated organisms (Appendix D). Thus, due to selective isolation of this particular species on nitrogen-free media and growth of this organism on all three media, it was quite possible that a strain of B. coagulans fixes atmospheric nitrogen. No information is provided in Bergey's manual of Manual of Determinative Bacteriology (7), Gordon et al (22) or recent literature, on any B. coagulans strains which fix atmospheric nitrogen. It is believed that this could be an unrecorded strain of B. coagulans.

The existence of B. coagulans within the fermenter may be questioned on the basis that the maximum growth temperature of 60°C ± 5°C was exceeded in the fermentation. Since thermophilic temperatures within the fermentation exceeded this growth temperature the vegetative form of the bacteria would have been dormant

or destroyed. The organism may have survived as spores within the fermentation. It has been shown by Degryse (17) that B. coagulans spores can tolerate 100°C. However, the presence of spores within the medium would not account for the incorporation of atmospheric nitrogen into the fermentation. Therefore, it was hypothesized that the vegetative form of the organism could exist within the foam layer where temperatures were lower and the atmospheric nitrogen and nutrients would be in abundance. Growth and death of this organism may occur within this foam layer. With the perpetual breakage and reformation of the foam from the substrate, the organism would be continually incorporated into the substrate, and on cellular lysis, provide nitrogenous compounds to the thermophiles existing in the substrate.

Additional information provided from the identification of the organism indicated that growth of B. coagulans within a totally hydrochloric acid neutralized-caustic soda treated potato waste fermentation will not be possible (Table 9). A 5-6% sodium content is present in the caustic peel waste and on HCl neutralization the amount of NaCl formed would be too large. From growth studies with varing NaCl concentrations in nutrient broth, the isolated organisms showed no growth at 5% (w/v) NaCl

(Table 9). Therefore, it would probably be necessary for partial sulfuric acid - hydrochloric acid neutralization; rather than just sulfuric acid since large sulfate content may also be objectionable as livestock feed (6).

GENERAL DISCUSSION

The characteristics of the aerobic-thermophilic fermentation of potato were determined within laboratory scale fermentors. The fermentation system was developed to a stage where sufficient operational stability (from foaming, ambient temperatures, mechanical heat) and operational control (temperature monitoring, aeration and agitation) were obtained. Analyses of the caustic potato peel waste indicated this substrate to be deficient in nitrogen and phosphorus. In comparison to the total carbon content, the nitrogen and phosphorus contents were below the requirement necessary for a thermophilic fermentation at the maximum degradation rate, i.e., the C/N and C/P ratios were too high. Nevertheless, fermentations with macerated potato without further nutrient supplementation were capable of reaching thermophilic temperatures. Distinct characteristics were noticed in these trials in which the pH of the substrate increased with the temperature of the fermentation, which also results in an increased rate of solids utilization. Also, increasing temperatures were accompanied by decreasing dissolved oxygen concentrations within the substrate and decreasing total organic nitrogen content. These

cycles were common to all the fermentation trials. Fermentation of caustic soda-treated potato waste, neutralized with sulfuric acid, showed no difference from the characteristics of the control trials. Though the characteristics were similar, the magnitude of these characteristics were not as large. Alteration of the microflora of the potato by the sodium hydroxide treatment and the sulfuric acid neutralization was suspected. However, a sufficient number of thermophiles resisted the base and acid treatment to create a thermophilic fermentation.

It was discovered that the nitrogen content within the substrate increased during certain stages of the fermentation. Most of this nitrogen was determined, by trichloroacetic acid precipitation, to be in the form of protein nitrogen. It was postulated that this nitrogen incorporation into the substrate maintained the fermentation within a favourable C/N ratio, especially during the latter half of the fermentation. This nitrogen increase was shown to be beneficial in the treatment of potato waste since an inexpensive carbon source was converted to bacterial protein (SCP). This fermented residue, containing mostly SCP and some potato protein, could be used as a supplement for animal feed, which

can offset the cost of the waste treatment. As a result, the total waste could be disposed of through degradation by aerobic-thermophilic fermentation, and the remaining residue utilized as feed.

Microbial analyses for the organism responsible for incorporating atmospheric nitrogen within the substrate suggested that it belonged to the B. coagulans species. This forms the first report that a B. coagulans strain fixed atmospheric nitrogen.

It appears that the aerobic-thermophilic fermentation of potato conducted in this study can be adapted to treat caustic potato peel wastes emitted from food processing plants. This sytem would be suitable for the smaller food plants in which space for waste treatment and cost are limiting factors. A continuous fermentation system would also appear to be feasible. With seasonal processing of potatoes by smaller food plants, the thermophilic system can also be used to treat vegetable wastes, other than potatoes, emitted from the plant.

The advantages of using the aerobic-thermophilic process for waste treatment are: increased degradation rates with respect to the destruction of organic solids;

elimination of pathogenic organisms; improved solid-liquid separation for dewatering of the waste; conversion of the waste into SCP for ultimate disposal, and continual digestion during cold ambient temperatures. It is evident that this process has potential as a food processing plant waste treatment system, but it is conceivable that the utility of any process depends upon economic considerations.

CONCLUSIONS

The analyses of the caustic soda potato peel waste from a commercial potato processing plant showed it to be limiting in nitrogen and phosphorus. As a substrate for an aerobic-thermophilic fermentation the C/N ratio of 24.1:1 and C/P ratio of 1477.4:1 of potatoes are not within the nutrient requirement range for maximum solids degradation of the waste.

Using laboratory scale fermenters, fermentation of macerated potatoes reached thermophilic temperatures, with the existing microflora of the potato as an inoculum. Repeated fermentation trials exhibited similar distinct characteristics of temperature, pH, dissolved oxygen and total nitrogen content, though the magnitudes reached by these parameters were different among the trials. Simulating the processing plant potato peel waste, fermentation of sodium hydroxide treated, sulfuric acid neutralized, macerated potato behaved similarly to the control trials. The presence of a high sodium content in the potato substrate did not interfere with the growth of thermophiles.

The total organic nitrogen content of the substrate increased in the overall fermentation to as much

as 2.8 times the initial nitrogen content. This increase in the nitrogen content was common to all the trials. "True" protein determination showed that this nitrogen was in the form of protein nitrogen. It was hypothesized that atmospheric nitrogen was converted into protein nitrogen.

Microbial analyses of the organism responsible for the fixation of atmospheric nitrogen into the substrate indicated the species to be Bacillus coagulans. No strains of this species have been reported previously to fix atmospheric nitrogen.

This study showed that an aerobic-thermophilic fermentation can be obtained with potato as a substrate. With the numerous advantages of the aerobic-thermophilic process, application of this process for the treatment of the caustic potato peel waste is feasible.

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APPENDICES

Appendix A.

Washing of the Ultra-fine Sintered Glass Filters:

The ultra-fine sintered glass filter was reverse flushed with a 1N NaOH solution using a vacuum aspirator. This was followed by a distilled water rinse and neutralization with 1N HCl. The filter was rinsed finally with distilled water and dried with acetone.

Appendix B.

Description of the Visual Appearance of the Colonies:

Colony forms can be described as punctiform, circular, filamentous, irregular, rhizoid or spindle. Colony elevation (observed from the side) are described as flat, raised, convex, pulvinate or umbonate. The morphology of the colony margins are described as entire, undulate, lobate, erase, filamentous or curled.

The growth form on an agar stroke are differentiated as: filiform, echinulate, beaded, effuse, arborescent or rhizoid.

Surface growth on nutrient broth are described as flocculant, ring, pellicle or membranous (39).

Appendix C.

Ziehl-Neelsen's Carbol-Fuchsin was composed of:

Solution A

Basic Fuschin (90% dye content)	0.3 g
Ethyl alcohol (95%)	10 ml

Solution B

Phenol crystals	5 g
Distilled water	95 ml

Solution A and B were mixed when required and filtered through coarse filter paper (32).

Appendix D.

Summary of Properties by Gordon et al. (22)
of 22 Strains of B. coagulans:

Rods
- Generally 0.6 μ - 1.0 μ in width by 2.5 μ to 5.0 μ in length.
- Gram positive.
- Motile.

Spores
- Ellipsoidal or cylindrical in shape.
- Generally subterminal or terminal, sometimes central in position.
- On the line dividing Group 1 (sporangia not appreciably swollen by oval or cylindrical spores) and Group 2 (sporangia swollen by oval spores).

Growth temperatures
- Maximum, 55 to 60°C.
- Minimum, 15 to 25°C.

Positive reactions
- Catalase; VP; acid in V-P broth (pH 4.2-4.8); anaerobic growth; acid from glucose; hydrolysis

of starch; growth in 0.02% azide; growth at pH 5.7; litmus milk shows acid and reduction of the indicator.

Negative reactions

- Growth in 7% NaCl; utilization of propionate; resistance to lysozyme; production of dihydroxyacetone and indole; deamination of phenylalanine; and decomposition of tyrosine.

Variable reactions

- Formation of acid from arabinose, xylose and manitol; utilization of citrate; reduction of nitrate to nitrite; decomposition of casein; and curdling of litmus milk.

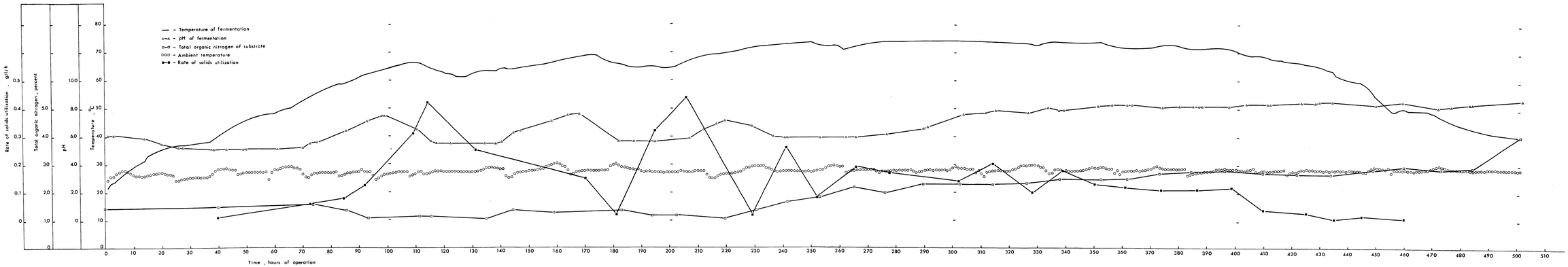

Figure 18. Thermophilic fermentation of a potato waste substrate; no.3.

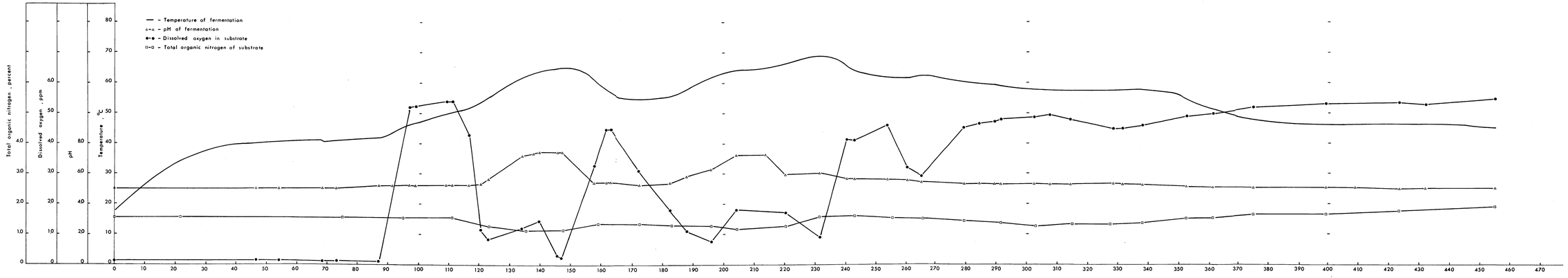

Figure 19. Thermophilic fermentation of a potato waste substrate ; no. 4 .

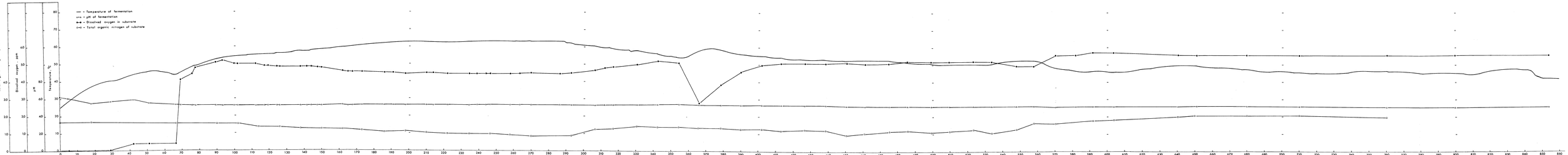

Figure 20. Thermophilic fermentation of a potato waste substrate with 5.66 percent sodium content.