SEA GRANT
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BIOCONVERSION OF SEAWEED TO METHANE

IN A THREE-PHASE FLUIDIZED BED

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ABSTRACT

The goal of bioconversion is to decompose renewable organic wastes into products useable for consumption, fuel, or pharmaceuticals. The success of bioconversion depends on the use of cheap and abundant organic wastes which are easily decomposed by bacteria. Seaweed has been chosen for this study becuase of its abundance in local ocean waters.

The ultimate goal of this project is to study the conversion of seaweed into methane in a three-phase fluidized bioreactor. Three phase bioreactors are extremely efficient for the transport of mass and energy between phases. This will result in the optimal production of methane.

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1. INTRODUCTION

Methane is produced by anaerobic fermentation. This is a process that has been going on for millions of years. This process of methane production is found in nature where plants die and decompose under water. The water acts as a cover to prevent oxygen from entering and therefore promotes the growth of anaerobic organisms which are needed for methane production.

Methane is formed by the decomposition of cellulose. Microorganisms in regular mud cause the formation of methane from cellulose. The decomposition process involves at least three groups of bacteria. These include fermentative bacteria, acetogenic bacteria, and methanogenic bacteria. First fermentative bacteria convert the substrates to intermediates and transform them to acetate, higher acids, hydrogen, carbon dioxide and lower molecular weight compounds. Next the acetogenic bacteria produce more acetate, hydrogen, and carbon dioxide. Finally, the methanogenic bacteria yields methane and carbon dioxide from acetate, and methane and water from hydrogen and carbon dioxide.

The process of methane formation can be pursued using several reaction techniques. These include batch, semi-continuous and continuous, and two phase. Batch processes were first used, however, it was found that steady-state conditions cannot be achieved because of the continuous variation in the concentration of different species. As a result reaching a high methane yield is not possible. In a semi-continuous or continuous process, conditions of steady state can be obtained because the organisms grow at a maximum rate balanced by

the inflow of substrate and nutrients.

In an attempt to emulate the stepwise nature of the methane formation process, two stage reactors were developed and used to study the conversion process. In a two-stage type reactor, the best environment for each bacteria is maintained and as a result the methane yield is maximized. In fact, it was found that the methane production rate from the two stage was more than seven times the amount from the batch process.

In this study, we decided to take the two stage process one step further. By using fluidized bed techniques and thereby speeding up the process of the acetogens and methanogens production, it is proposed that a two phase fluidized bed process will produce the highest possible yield of methane.

The basic principles behind a fluidized bed are that mass and energy transfer are brought about as efficiently as possible. This is accomplished from the rigorous mixing that occurs within the fluidized region of the bed. Three phase fluidization refers to the interaction of solid, liquid and gaseous media. The solid particles in the bed are suspended in the fluid from drag and buoyancy forces. These forces are generated by the liquid and gas flowing up through the bed.

Another advantage of fluidized beds is the lack of temperature or concentration gradients. This allows for concise monitoring and control of the reaction in the bed.

2. BIOMETHANATION

Methane production by anaerobic fermentation (anaerobic digestion) involves the conversion of organic material (complex sugars) at modest temperatures (30- 35 C), ambient pressures and nearly neutral pH, in the absence of extraneous electron acceptors such as oxygen, nitrate and sulphate.

The gross stoichiometry of the methane fermentation of glucose can be written as:- $C_6H_{12}O_6(aq) = 3\ CH_4(g) + 3\ CO_2(g)$

The standard Gibbs free energy and enthalpy changes for this conversion under physiological conditions (pH 7, T=25 C, unit activities) per mole of glucose are about -418kJ and -131kJ. The mass and energy contents of the methane expressed as fractions of the glucose converted are about 27 and 95%. Thus the thermodynamic driving force is large and the exothermic energy loss is small. The energy is transferred at a higher density to a simple gaseous hydrocarbon that is the main component of natural gas fuel. This in turn leads to an easy separation of methane from the reacting mixture of gases, and there- fore are the primary reasons why anaerobic digestion has been used for about the past 100 years for waste stabilization and disposal and as a source of fuel particularly in the developing countries.

The basic process undergone in the fermentation of the organic material or the catabolic substrate in developing countries.

The basic process undergone in the fermentation of the organic material or the catabolic substrate is essentially three-stage:

1. Attack by fermentative and acidogenic bacteria to break down the poly mericchains(e.g. algin and mannitol polysaccharides present in giant brown kelp) into single molecules of simple sugars. The sugars are further degraded by the acidogenic bacteria- proteins to peptides and amino acids (some of which such as cysteine, help to keep the system anaerobic), triglycerides to fatty acids and glycerol. The monomeric sugars are then degraded by the enzyme pyruvate, to to acetate, higher fatty acids, carbon dioxide and hydrogen.

2. The acetogenic bacteria, now convert the alcohols and higher fatty acids produced to acetate, hydrogen and carbon dioxide.

3. Methanogenic bacteria use the acetate and hydrogen substrates to produce methane gas.

The reaction scheme can be characterized as follows:

REACTION.....DELTA G (KJ)

fermentative bacteria

$(C_6H_{10}O_5)_n + H_2O = C_6H_{12}O_6$.....-17.7

$C_6H_{12}O_6 = 3CH_3CO_2^- + 3H^+$.....-311.0

$C_6H_{12}O_6 + 2H_2O = CH_3CH_2CO_2^- + H^+ + 3CO_2 + 5H_2$.....-192.0

$C_6H_{12}O_6 = CH_3CH_2CH_2CO_2^- + H^+ + 2CO_2 + 2H_2$.....-264.0

$C_6H_{12}O_6 + 6H_2O = 6CO_2 + 12H_2$.....-25.9

acetogenic bacteria

$C_6H_{12}O_6 + 2H_2O = 2CH_3CO_2^- + 2H^+ + 2CO_2 + 4H_2$.....-216.0

$CH_3CH_2CO_2^- + H^+ + 2H_2O = CH_3CO_2^- + H^+ + CO_2 + 3H_2$.....+71.7

$CH_3CH_2CH_2CO_2^- + H^+ + 2H_2O = 2CH_3CO_2^- + 2H^+ + 2H_2$.....+48.3

$CH_3CH_2OH + H_2O = CH_3CO_2^- + H^+ + 2H_2$.....+9.7

$2CO_2 + 4H_2 = CH_3CO_2^- + H^+ + 2H_2O$.....-94.9

methanogenic bacteria

$CH_3CO_2^- + H^+ = CH_4 + CO_2$.....-35.8

$CO_2 + 4H_2 = CH_4 + 2H_2O$.....-131.0

$HCO3^- + H^+ + 4H2 = CH4 + 3H2O$.....-136.0

3. EQUIPMENT AND APPARATUS

The apparatus consists of a 27.5" long vertical Plexiglas column with an inner diameter of 2.75" connected to a 22.75" long vertical Plexiglas column with an inner diameter of 4.5". The first column is the bioreactor. It has a nitrogen feed line in the bottom of the column and a gas exit coming out the top. This gas line is important in that it supplies the reactor with a fluidization medium so that better mixing can occur and it also helps to keep the system anaerobic.

Since the fresh feed flowrate is low, a small feed pump is used to introduce the seaweed slurry into the first column.

A self priming utility pump (.5 hp) is used to recycle the seaweed slurry from the first column into the second column and then back to the first column.

The second column is used to separate the liquid phase from the gas phase. Before implementing the separation tank into the system, it was noticed that both nitrogen gas and seaweed slurry was pumped out of the first column. Unfor tunately, the pump was not capable of handling two phases. As a result, continuous accumulation of the gas phase within the pump casing blocked the pssage for the recycled liquid stream. With the addition of the separation tank, the nitrogen gas is allowed to separate from the slurry and escape through a gas exit valve at the top of the second column. The gas-free slurry is then normally pumped and circulated through the system.

All connections between the columns and pumps were brought about by using plastic tubing.

Samples were acquired by using sampling ports in the first column. These ports were made by drilling holes into the column and then placing rubber septum over the holes. This enabled sample withdrawal by use of a syringe.

Valves were placed in the gas supply line and the slurry recycle line to provide control over the flowrates and therefore, control over the amount of fluidization. Schematic diagrams of initial, intermidate, and final system designs are given in appendix I.

4. EXPERIMENTAL PROCEDURE

To operate the reactor unit in a continuous fashion flow rates of the liquid feed, recycled slurry, and feed gas are adjusted to the conditions of the bubble flow regime.

Because of limitations on the availability and source of the nitrogen gas, i.e., industrial type gas cylinders, the gas flow was maintained at the lowest possible rate.

The flowrate of slurry recycle was set near its maximum value. This was done to provide the maximum fluidization that the system was capable of delivering.

A slurry solution of dried, crushed seaweed was prepared and placed in the reactor along with a small amount of mud from the flats of Great Bay, N.H. This mud contains the bacteria needed to digest the seaweed. This was allowed to run for one week to allow the bacteria population to increase before a fresh feed of seaweed was

added.

The fresh slurry concentration is kept constant so that a uniform feed can be obtained. This aids in providing a control over the amount of reactants entering the system. The concentration of slurry feed is expressed as grams of seaweed per unit volume of water. The initial concentration was set at (10g/l).

The temperature of the reactor is another important manipulated variable. The higher the temperature, the more active the bacteria will become. One of the goals of this experiment is to determine the optimum temperature. Initially, the temperature of the bed was set at 40 C. Care must be taken so as to prevent killing the bacteria from excessive heat. This means keeping the temperature below 50 C.

Once all of the variables are set, the system is allowed to run contin uously for one week. This duration is necessary because it takes some time for the bacteria to multiply and react. During this time, readings are taken of the pH, temperature and pressure of the system. The outflowing gas must also be analyzed so as to determine if the product gas is indeed methane.

5. ANALYSIS

The gas product collected from the system is to be analyzed by using a gas chromatograph (GC). The analysis consists of injecting a gas sample into the GC and waiting for a signal to register on the chart recorder. The signal gives an indication of the residence times of the gases present.

Calibration curves of pure substances: CO_2, CO, H_2, CH_4, and C_2H_6, must first be found. These curves would make it possible to easily analyze the gas product from the bioreactor simply by comparing residence times. For example, if pure methane had a residence time of 8 minutes, then that would mean that a peak at 8 minutes for the product gas sample would indicate that methane was present.

To verify that biological decay is occurring, an elemental analysis of the seaweed slurry is performed. The analysis is made by using the Carlo Erba Nitrogen Analyzer 1500. This analyzer is capable of determining the weight percent of carbon, nitrogen and sulfur in any given sample. Initially, the fresh slurry feed is analyzed to determine the amounts of carbon, nitrogen and sulfur which naturally occur in seaweed. Then, a sample of some digested slurry is withdrawn and analyzed. The results from both tests, are then compared to calculate losses in the three elements. Such losses are a strong indication of biological decay and can be used together with the chromatograph analysis of the produced gas stream in obtaining an overall elemental balance.

Another method of determining if methane is produced is to see if the methanogenic bacteria are present. This is done by fluorescent spectroscopy. A sample of the decaying seaweed slurry is placed on a glass slide with .5% formaldehyde solution and subjected to ultraviolet light. If methanogens are present, they will show up as glowing blue-green cells under a microscope. It is important to state that methanogens are the only bacteria in nature which will give off blue-green light when subjected to ultraviolet light.

6. PROCESS CONTROL

Process control consists of computerized control of a system. The computer will be able to monitor the system and make any necessary adjustments if changes in temperature or pressure occur. In this case, process control can be used to control the flowrate of the recycle and the temperature of the bioreactor.

Appendix II shows the apparatus involved in performing process control for the bioreactor. A solenoid valve is used in the main water line to control the flow of cooling water to the temperature bath. When conditions require the temperature of the system to decrease, a computer signal is sent to the solenoid valve and the flow of cooling water is begun. Upon reaching a set temperature, the signal will shut off and the flow of cold water will cease.

If, however, the temperature of the system needs to be increased, a different signal will be sent to turn on the water heater. The heater will remain on until the system temperature is equilibrated to its predetermined set point (chosen as 40 C).

These controls are fail-safe in that the temperature of the system can never go below the lower set point (30 C) and never above the higher set point (40 C). The reason for this is that the computer can simultaneously control the two temperature variables (cooling water and heater) to turn them on when necessary. This insures that the system can maintain itself at its desired temperature.

The flow of recycle is controlled by a pneumatic valve. Since the system pressure is dependent on the amount of fluidization within the bed, it is possible to control the level of fluidization by reading the system pressure and altering it accordingly. When the system pressure drops below its set point, the computer will send a signal to the power supply so that the voltage can be adjusted accordingly. The voltage controls the resistance of a diaphragm within the pneumatic valve which affects the flow of water through the valve. If the resistance is lowered, the flow of water will increase and the pressure drop will increase. This increase in pressure will be read by the computer so that the computer will 'know' when to stop adjusting the output voltage.

7. RESULTS

The gas chromatograph was the most troublesome of the three tests. Many weeks were spent trying to obtain calibrations peaks to compare with the peaks obtained from the product gas. The major problem was the difficulty of reproducing the results of calibration peaks for both pure and mixures of known gas samples. Two possible explanations can be given for such difficulty; the first is possible leaks in the sampling ports and the second is possible damage of the operating coil. Consequently, no usable results were obtained from this analysis.

The ultra-violet test for the methanogenic bacteria was positive. This test only proves the existence of the bacteria and confirms that the system has the ability to produce methane. This test also shows that the presence of any oxygen in the system does not have an adverse effect on the anaerobic bacteria. A sketch of the bacteria present

appears in Appendix III.

The results from the nitrogen analyzer were also positive. Two samples of fresh seaweed and two samples of digested seaweed were tested. Although the weights of the digested samples were greater, the carbon and sulfur peaks were smaller than those of fresh seaweed. This shows that the relative amounts of carbon and sulfur decreased from the fresh to the digested samples. It can be concluded from this that the carbon chains in the substrate are being broken down and also that sulfur containing compounds are being produced. The printed results of these tests appear in Appendix III.

8. CONCLUSIONS

The presence of methanogenic bacteria and the evidence of sulfur and carbon compounds being produced gives a good possibility of methane production. However, without an operational gas chromatograph the relative amount of methane cannot be determined.

An operational fluidized bed now exists for the study of the biomethanation of seaweed. Considerable time was spent correcting physical problems and suggesting solutions to those problems that were not attended to. The results have shownthat the system has the ability to produce methane. In order to examine the large-scale feasibility of this process, additional experimentation should be completed on the effect of variations of temperature, pressure, pH and fluidization of the system.

9. RECOMMENDATIONS

There are a number of recommendations to be made regarding the reactor system, the monitoring procedures, and the reaction parameters.

As the development of the reactor system proceeded, periodic problems developed. Some of these problems could be corrected, others had to be accepted as permanent. First flaw discovered was that the tubing used in the recycle line was oxygen permeable. To replace this with impermeable tubing or stainless steel piping was too expensive. It was assumed that any aerobic bacteria would use the oxygen up as fast as it contaminated the system. This is a valid assumption for a laboratory scale system, however, to assure a good methane yield, the tubing used should be oxygen impermeable.

Another suspected source of oxygen contamination is the pump. A pump is capable of sucking air in through its seals. To prevent this from occurring, a diaphragm pump should be used instead. It would also be advantageous to remove any dissolved oxygen in the water by boiling and filtering through a solution that will accomplish this.

To accurately control the temperature of the reactor, a thermocouple should be placed directly in the bed. This presents some problems because the flow of the liquid across the thermocouple tip, and the noise generated from the pump cause extraneous voltage readings. To compensate for this, the thermocouple was placed in the water bath. It is suggested that the use of a thermowell in the bed may avoid this problem and allow more accurate temperature control.

Due to time constraints, it was not possible to study the effects of temperature and pressure variations on methane yield. In the future, these effects should be studied as well as variations of pH and feed concentration. Changes in the reactor configuration, residence time and seaweed types should also be studied to allow more comprehensive results.

The possibility of isolating the methanogenic bacteria in a separate stage of the reaction should be researched. This could greatly enhance the ability to produce methane as well as provide further control over the reaction.

The possibility of incorporating an in-line gas chromatograph should be investigated. This would allow for a more efficient means of monitoring the system's methane production. This would require the removal of hydrogen sulfide from the product gas stream. This gas is known to be corrosive, and may damage the coils in the chromatograph.

APPENDIX I

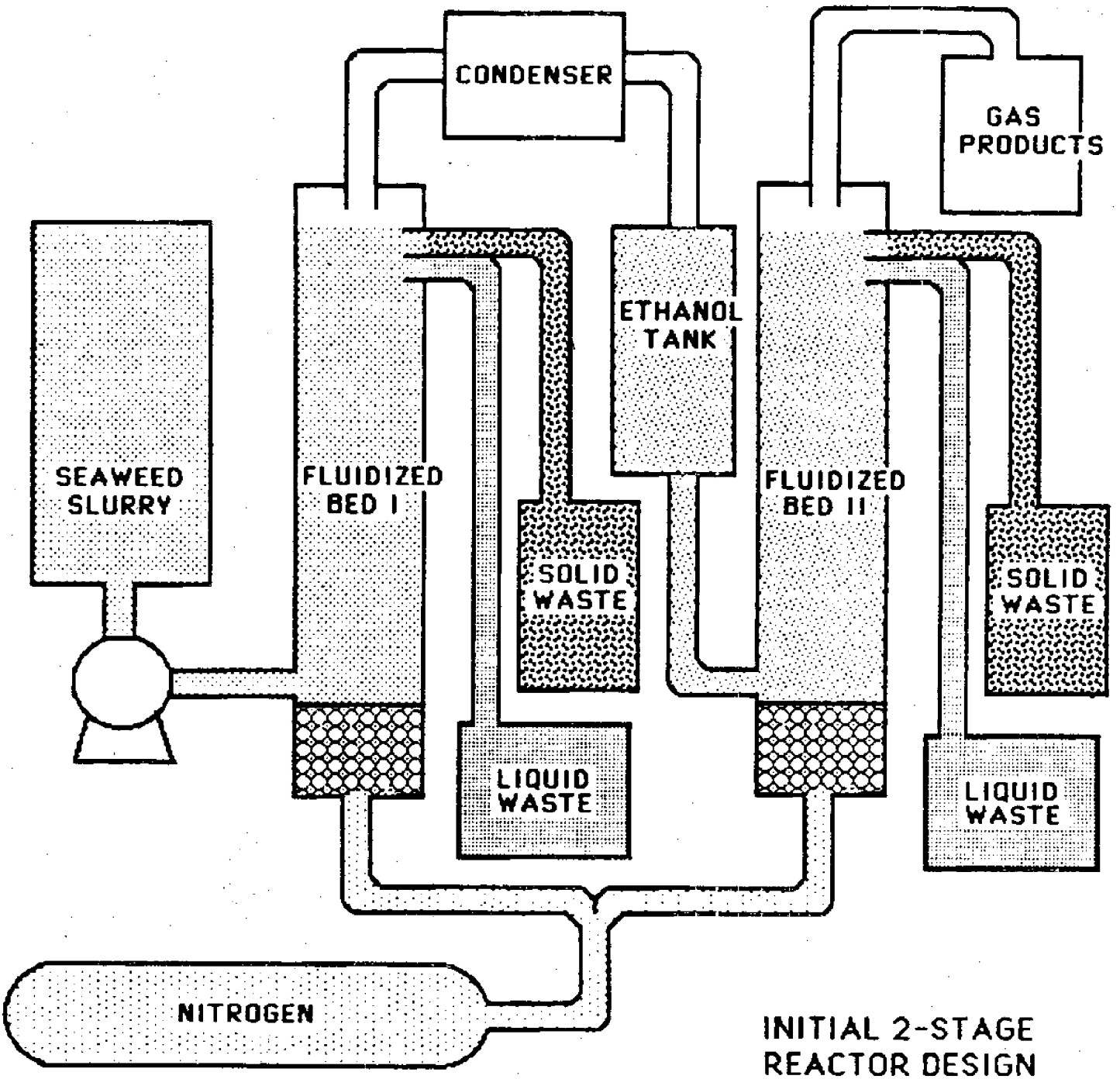

CONDENSER
GAS PRODUCTS
ETHANOL TANK
SEAWEED SLURRY
FLUIDIZED BED I
FLUIDIZED BED II
SOLID WASTE
SOLID WASTE
LIQUID WASTE
LIQUID WASTE
NITROGEN
INITIAL 2-STAGE REACTOR DESIGN

figure 1

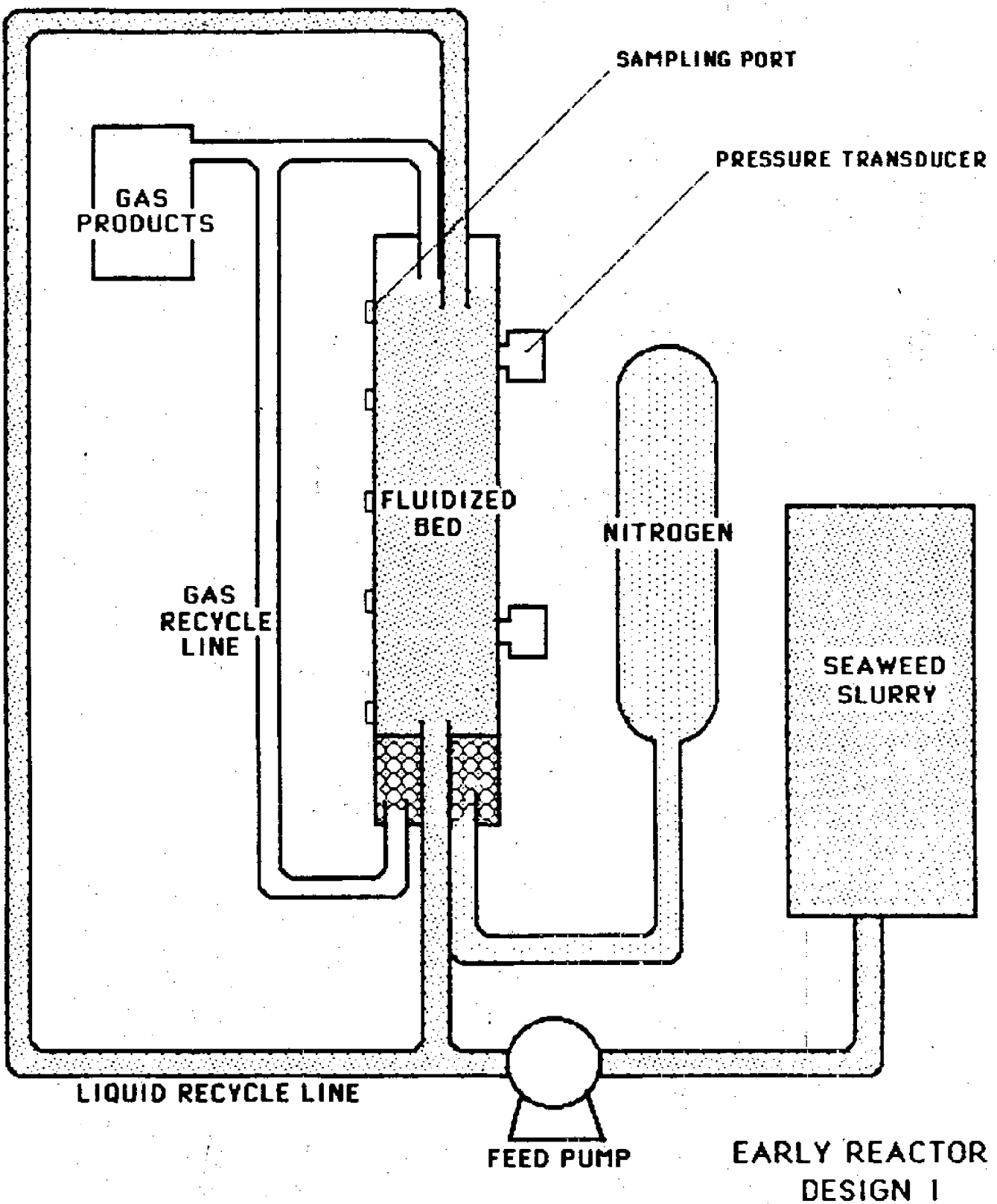

SAMPLING PORT
PRESSURE TRANSDUCER
GAS PRODUCTS
FLUIDIZED BED
NITROGEN
GAS RECYCLE LINE
SEAWEED SLURRY
LIQUID RECYCLE LINE
FEED PUMP
EARLY REACTOR DESIGN I

figure 2

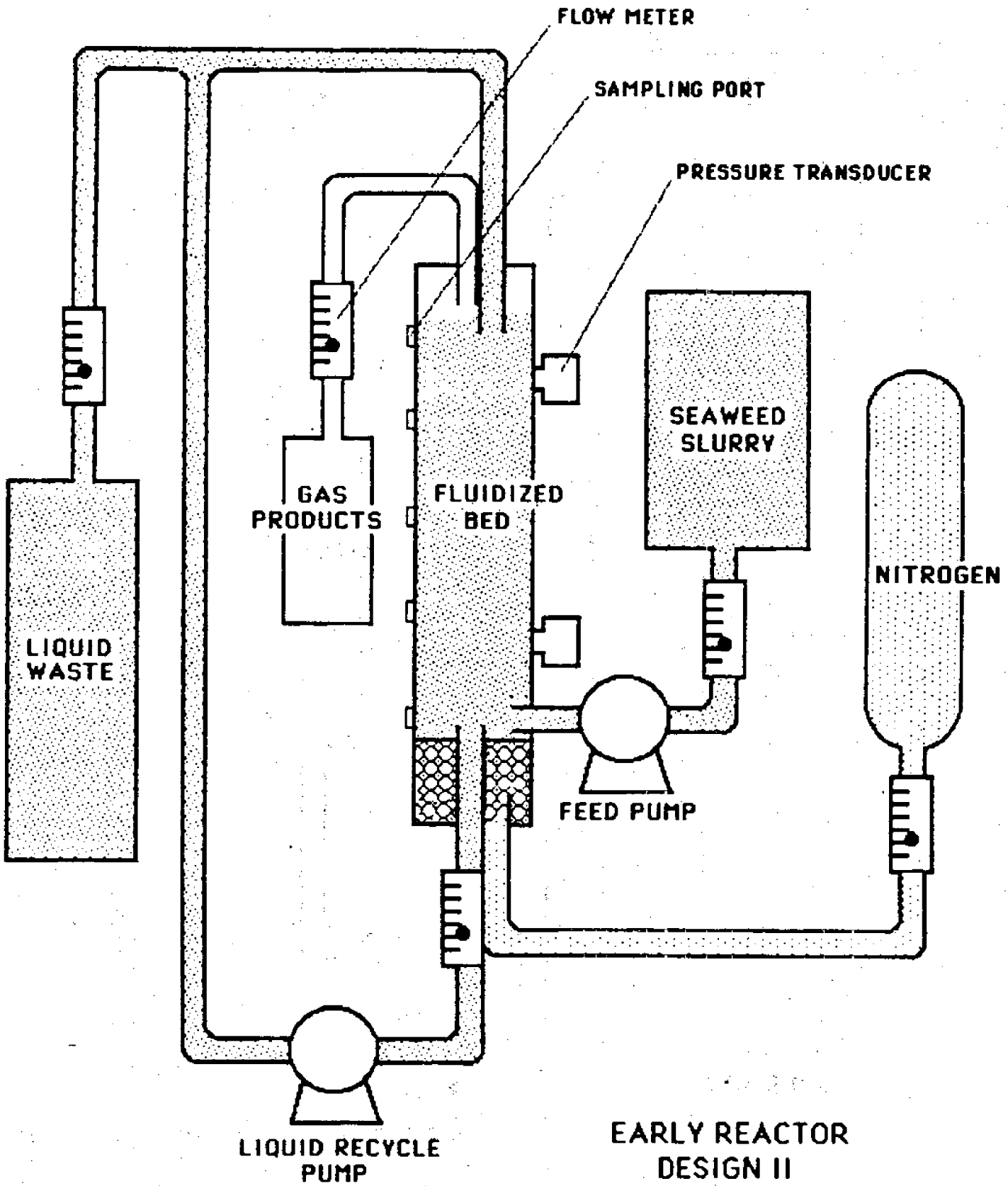

FLOW METER
SAMPLING PORT
PRESSURE TRANSDUCER
SEAWEED SLURRY
NITROGEN
GAS PRODUCTS
FLUIDIZED BED
LIQUID WASTE
FEED PUMP
LIQUID RECYCLE PUMP
EARLY REACTOR DESIGN II

figure 3

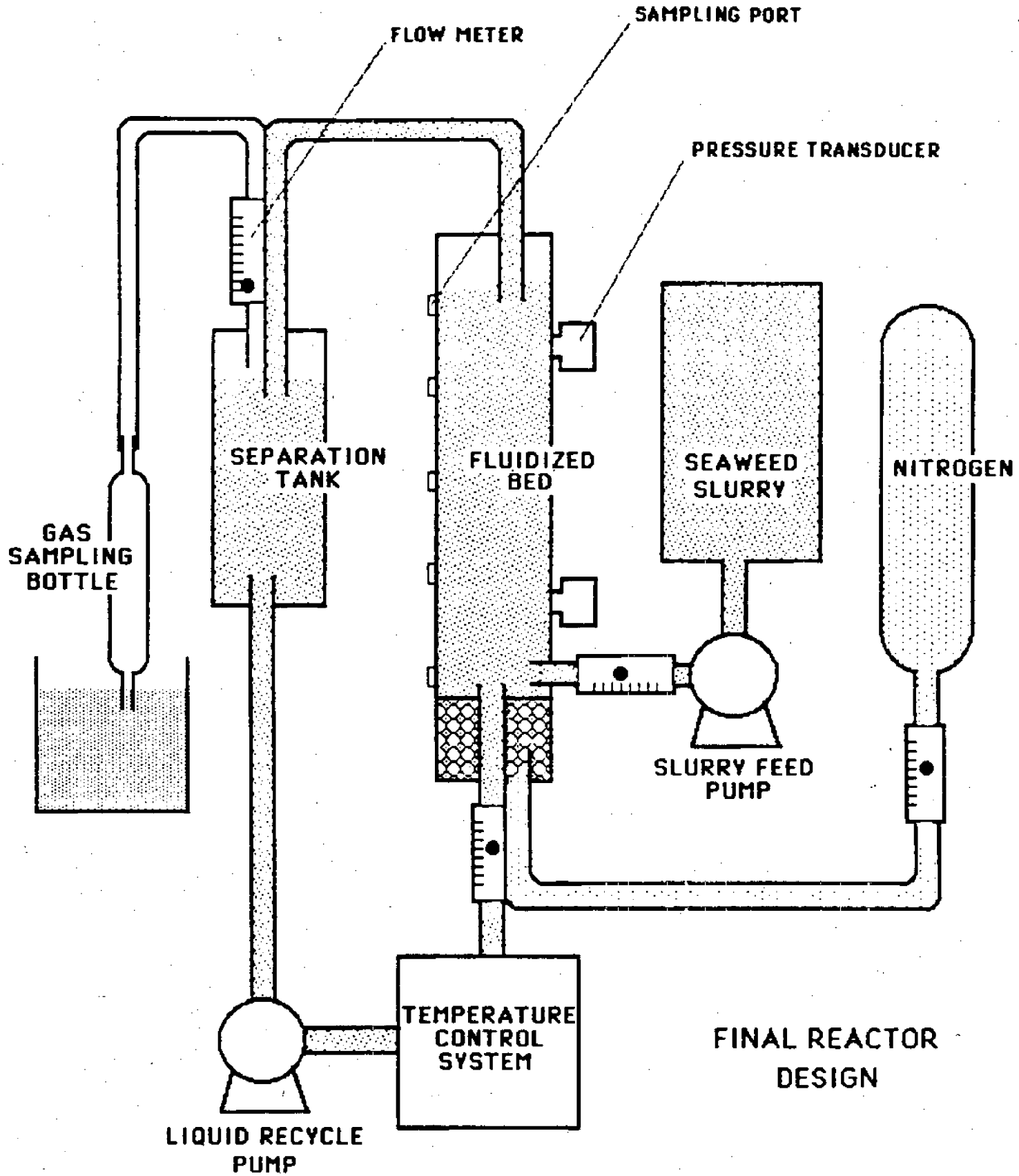

FLOW METER
SAMPLING PORT
PRESSURE TRANSDUCER
SEPARATION TANK
FLUIDIZED BED
SEAWEED SLURRY
NITROGEN
GAS SAMPLING BOTTLE
SLURRY FEED PUMP
TEMPERATURE CONTROL SYSTEM
LIQUID RECYCLE PUMP
FINAL REACTOR DESIGN

figure 4

APPENDIX II

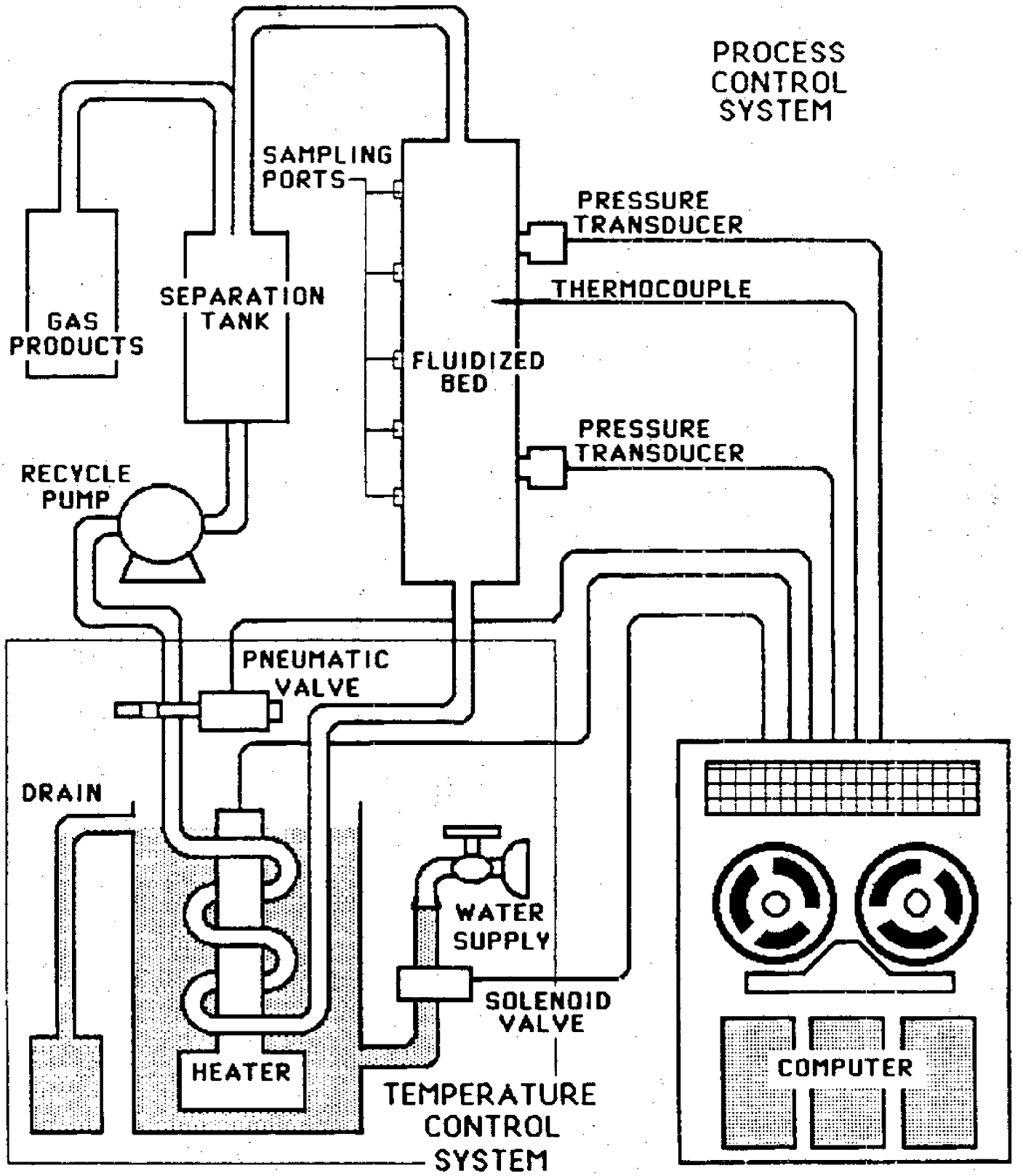
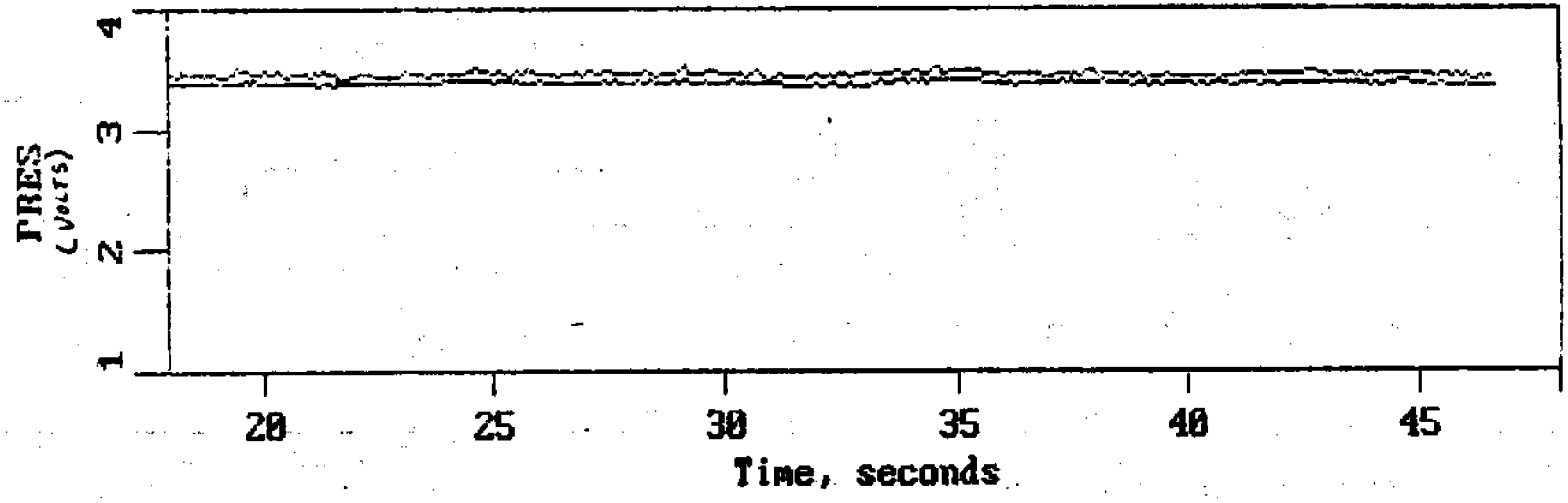
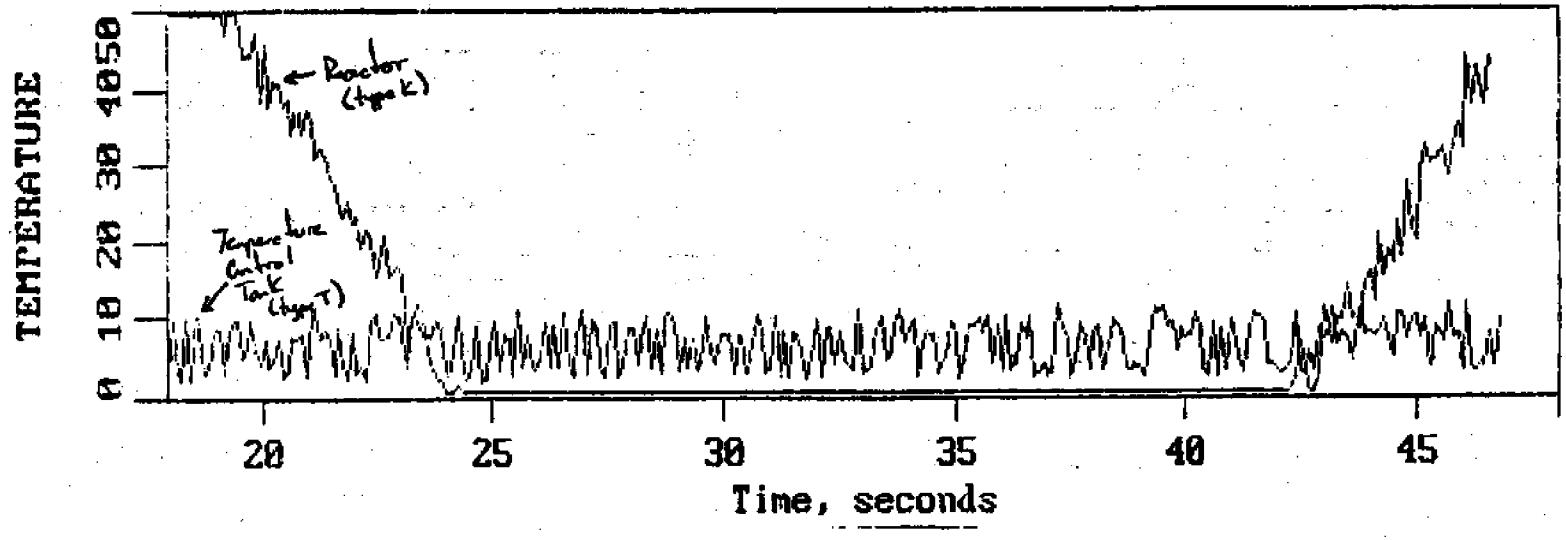

PROCESS
CONTROL
SYSTEM
SAMPLING
PORTS
PRESSURE
TRANSDUCER
THERMOCOUPLE
SEPARATION
TANK
GAS
PRODUCTS
FLUIDIZED
BED
PRESSURE
TRANSDUCER
RECYCLE
PUMP
PNEUMATIC
VALVE
DRAIN
WATER
SUPPLY
SOLENOID
VALVE
HEATER
COMPUTER
TEMPERATURE
CONTROL
SYSTEM

figure 5

REACTOR TEMPERATURE AND PRESSURE AS READ BY THE COMPUTER FOR PROCESS CONTROL





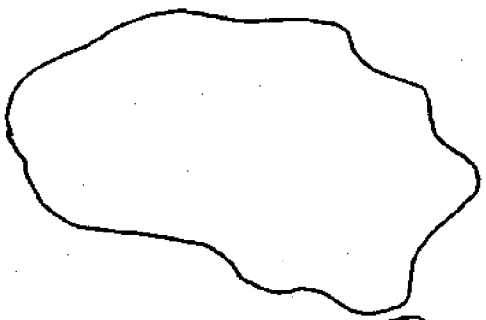
APPENDIX III

METHANOGENIC BACTERIA

AS OBSERVED UNDER A MICROSCOPE (using a polyclonal antibody [redacted] probe)

(100 X times 1.25 X)

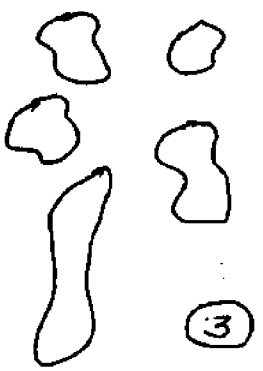
GREEN FLUORESCENCE



Methanosarcina Barkeri (1)

(2)

Methanobrix-like bacterium (unclassified)

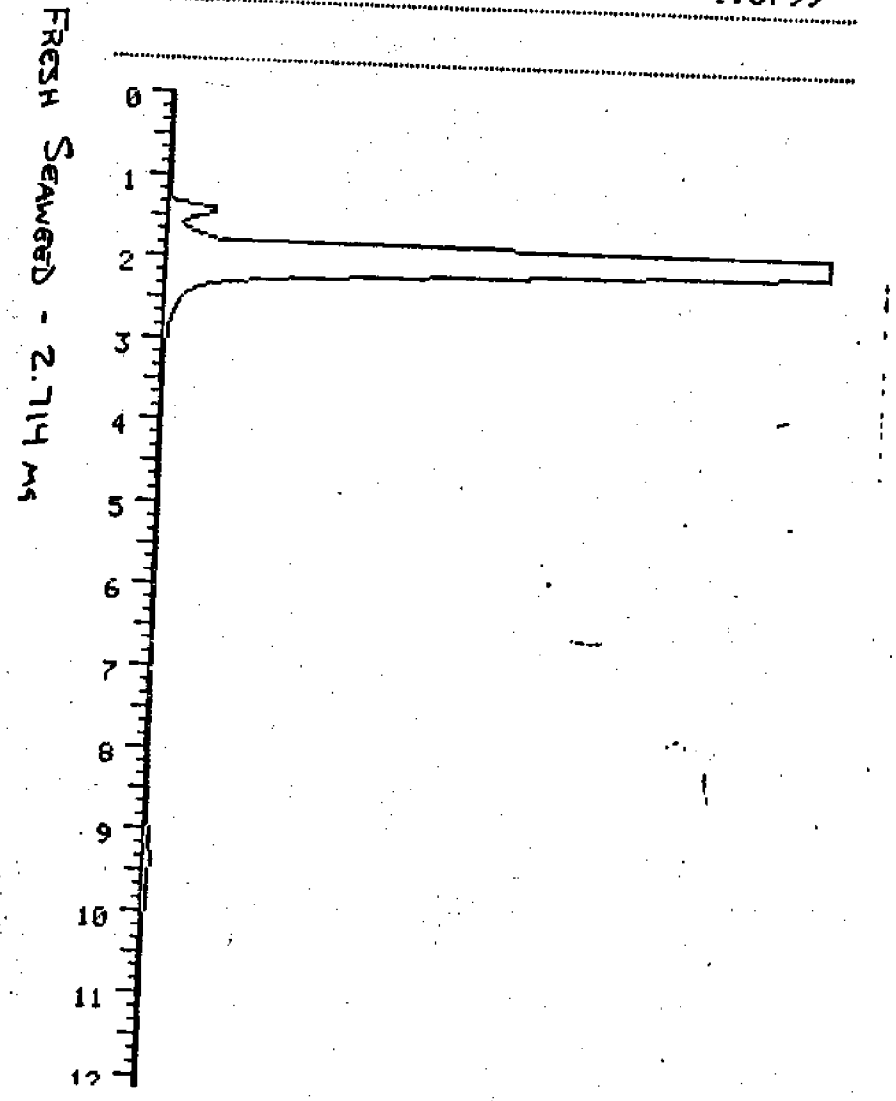


(3)

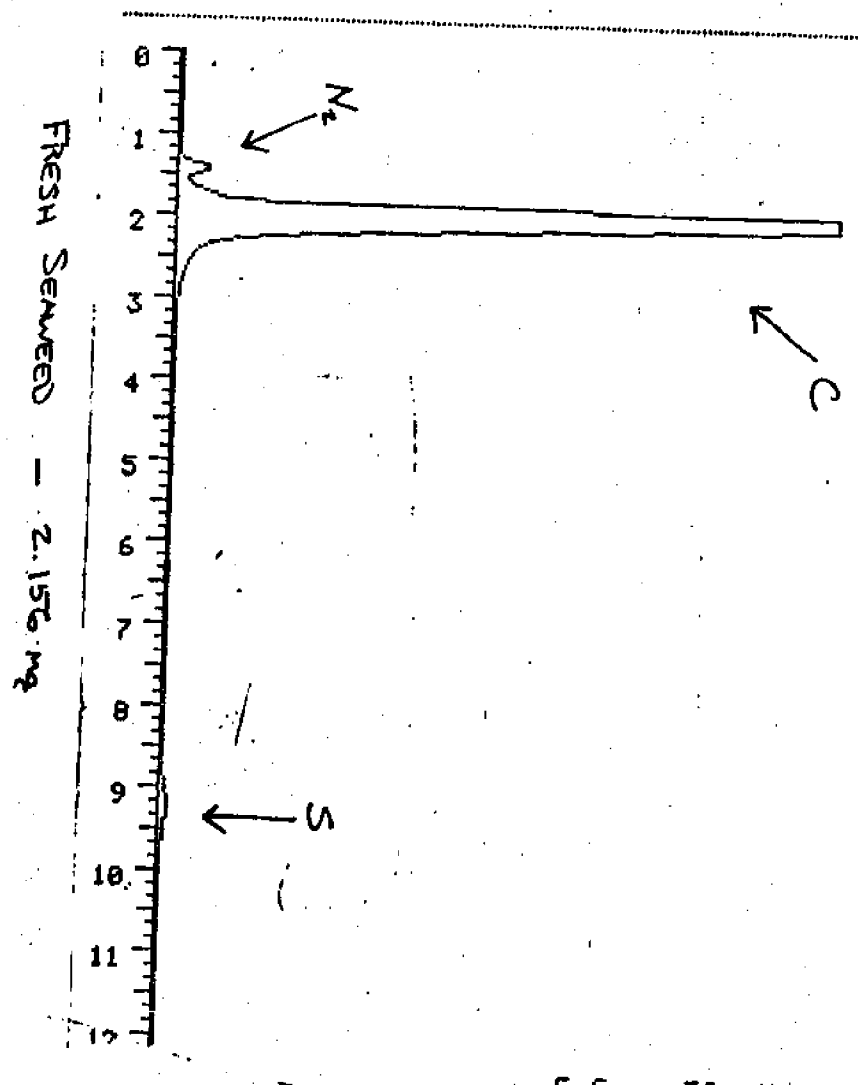
Methanobacterium smithii

SAMPLE 20 # RUNTIME 730 SEC.
INHTIM 70 SEC. BLDRIFT 1 µV

PEAK	RT SEC.	AREA µV*SEC.	AREA%
1	88	25908	2.4236
2	116	1025105	95.8964
3	565	17958	1.6799



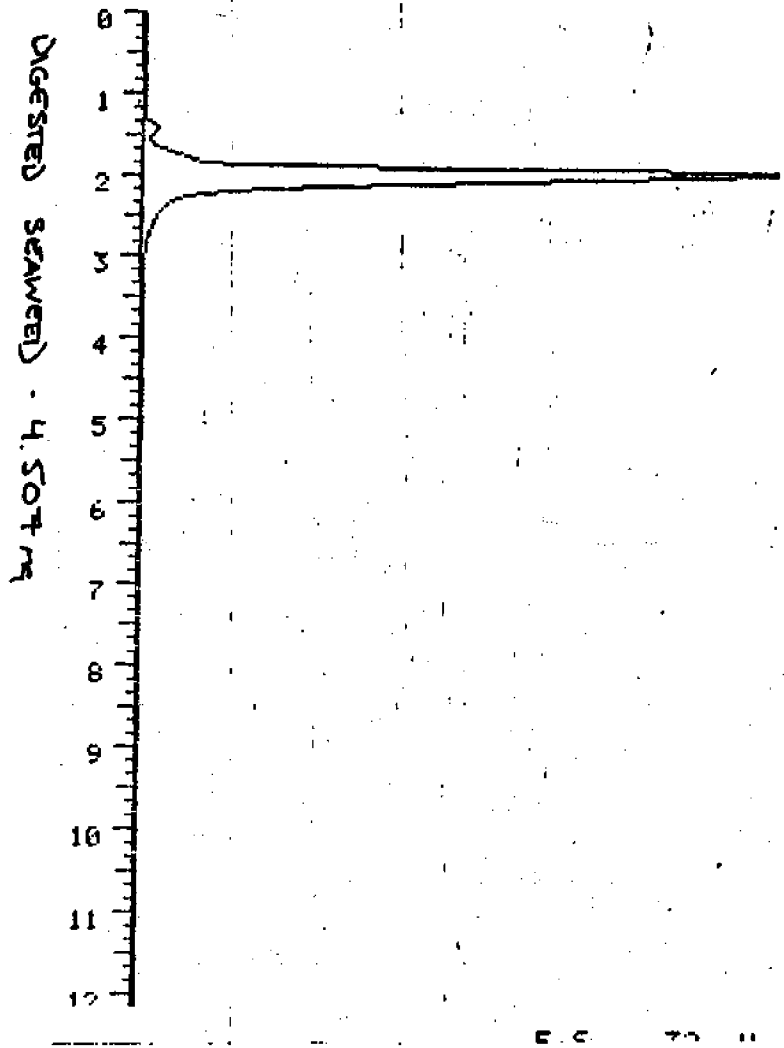
F.S. 32 mV



F.S. 32 mV

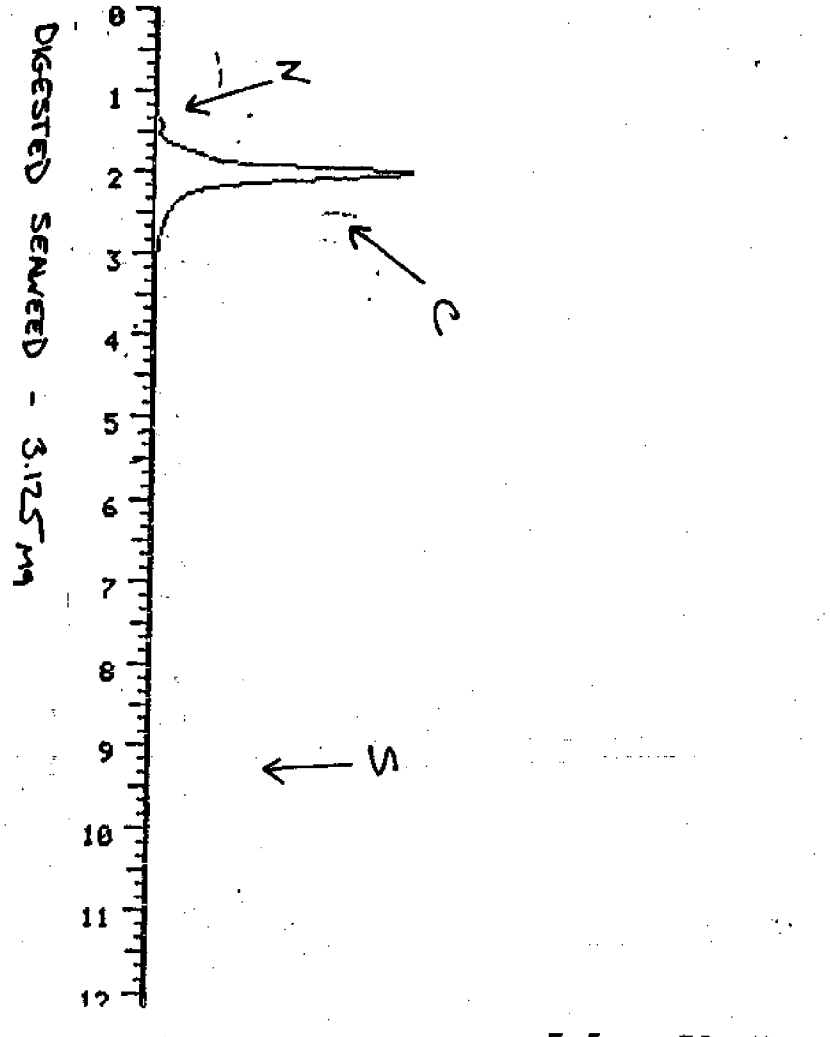
SAMPLE	22 #	RUNTIME	730 SEC.
INHTIM	70 SEC.	BLDRIFT	1 μV

PEAK	RT SEC.	AREA μV*SEC.	AREA%
1	119	433540	99.2666
2	556	3203	.7333



SAMPLE	21 #	RUNTIME	730 SEC.
INHTIM	70 SEC.	BLDRIFT -	7 μV

PEAK	RT SEC.	AREA μV*SEC.	AREA%
1	121	214800	99.3533
2	554	1398	.6466



F.S. 32 mV

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