Report Number XB-9-8263-1-3 July, 1981

Final Report
Preliminary Feasibility Overview

MICROALGAE SEPARATION, CONCENTRATION, AND CONVERSION TO FUEL WITH AN ANAEROBIC EXPANDED BED REACTOR

by

William J. Jewell and Gosse Schraa
Department of Agricultural Engineering*
Riley-Robb Hall
Cornell University
Ithaca, New York 14853

Prepared for the U. S. Department of Energy
under Contract No. XB-9-8263-1
with the Solar Energy Research Institute
Dr. Larry Raymond
1536 Cole Blvd.
Golden, Colorado 80401

*in the College of Agriculture and Life Sciences,
a statutory division of the State University of New York.

NOTICE

MICROALGAE SEPARATION, CONCENTRATION, AND CONVERSION TO FUEL WITH AN ANAEROBIC EXPANDED BED REACTOR

ABSTRACT

The use of microscopic algae to store solar energy has long been an attractive possibility, both for wastewater purification, food production and fertilizer synthesis. The total potential energy available from these sources would appear to exceed 2 quads of energy annually on a renewable basis. Presently there are thousands of algal stabilization ponds that discharge many tons of organic matter to receiving waters that cause subsequent downstream pollution. The major reason why microscopic algae are not being used is because they are difficult and expensive to harvest and convert to useful products. This preliminary study was conducted to investigate the possibility of using a new process referred to as the "Anaerobic Attached Film Expanded Bed" (AAFEB) to separate, concentrate and digest microscopic algae.

Early results with the expanded bed process indicate that it is an efficient fine solids filter that encourages sewage solids to accumulate within the bed and flocculate on top of the expanded bed and to be rapidly converted to methane. Retention times for sewage purification as short as 30 minutes have resulted in complete conversion of sewage and retention of suspended solids down to less than 10 mg/ℓ. Since algal cells are of an approximate size similar to many of the fine solids in primary sewage that has been tested extensively, it was felt that the harvesting and processing of algal cells could be possible with the expanded bed.

Since the algal concentrations produced in waters are extremely low, it is necessary that the conversion process to fuel be accomplished in a two-stage process. The first stage must concentrate algae to a level that can be handled and processed for efficient methane recovery. Experiments using a variety of natural algal sources with concentrations varying from 20 to 130 mg/ℓ influent resulted in efficient separation of the algae down to effluent concentrations as low as 6 mg/ℓ of effluent solids in a retention time of several hours. The best results have been obtained using a two-stage AAFEB with the facultative anaerobic unit, the first unit, followed by an aerobic secondary unit. Strong flocculated aggregates of the algae formed a blanket on top of the aerobic expanded bed at concentrations exceeding 1% solids. This separation and concentration appears to represent a successful application of the expanded bed in order to achieve a three-fold increase of algal cells in a short retention time.

Continuously fed expanded bed digesters were operated at a decreasing hydraulic retention time for a short period. A 2% solids feed stream of algae resulted in a 28% volatile solids destruction at a 2.3-day hydraulic retention time at 35°C. These results demonstrate that the expanded bed can process algae at a retention time significantly shorter than the reproduction period of the methanogenic bacteria. Existing kinetic analysis would indicate that a conventional completely mixed digester would require 10 to 30 days at 35° to achieve similar results. It is concluded that the results are sufficiently positive to warrant comprehensive testing of the expanded bed for the separation and concentration of microscopic algae.

TABLE OF CONTENTS

TABLE OF CONTENTS (CONTINUED)

LIST OF FIGURES

LIST OF TABLES

ACKNOWLEDGEMENTS

This study was partially supported by a U. S. Department of Energy contract administered by the Solar Energy Research Institute, Contract No. XB-9-8263-1. Mr. Daniel Jantzen was SERI's project manager at the beginning of this study. His early interest and support were appreciated.

This was a small "seed funded" project that was intended to test the feasibility of algae conversion with the expanded bed. Follow-up support was being sought during the preparation of this report.

Many individuals in the Department of Agricultural Engineering assisted in the conduct of this study. Three key individuals are Mr. Randolph M. Kabrick, who assisted in project management and administration, Mr. J. A. Spada, who helped prepare this report, and Mrs. Diane M. Harbert, who assisted throughout this effort.

INTRODUCTION

Of the various waste treatment systems in use today, oxidation ponds and aerobic and facultative lagoons are perhaps the simplest. Stabilization of organic matter in these systems is accomplished generally by bacteria while nutrient removal (nitrogen and phosphorous) is achieved by algae. Figure 1 illustrates the possible biological interactions in an oxidation pond. In these systems, solar energy is converted to algal biomass at a relatively high rate of efficiency, ~ 2%. Because algae grow rapidly, a number of schemes have been proposed to use algae as solar energy collectors to produce food and/or fertilizer and energy. Three possibilities for using algal cells as solar collectors are shown in Figure 2. An important technical limitation to the use of these interesting alternatives is the difficulty of harvesting the small microalgae and subsequently converting it to energy. A new process referred to as the anaerobic attached microbial film expanded bed (AAFEB) appears to be capable of harvesting fine particles from wastewater while converting them to methane and carbon dioxide. This preliminary feasibility study was conducted to test this process with algae.

Energy from existing sewage lagoons could generate several hundred million dollars' worth of energy per year. It is interesting to note that the total U. S. nitrogen fertilizer production (8×10^6 tons/yr) could be produced using 4 million acres of algal ponds (Benemann et al., 1977). Energy production from these ponds would equal one quad. The energy saved from the manufacture of the nitrogen fertilizer would equal about one quad also (Shelef, 1976). Thus, there are significant potentials for contribution to the U. S. energy picture. However, major limitations of this concept appear to include costs and difficulties of harvesting/concentrating the microalgae and providing a nutrient environment capable of supporting maximum growth rates. Existing data indicate that a new methane generation process may be able to drastically reduce these restrictions.

The new system, a high rate methane production system, was developed for application to soluble organics and then applied to a complex substrate in an earlier DOE sponsored study (Jewell et al., 1978). This study indicated that an AAFEB such as that shown in Figure 3 could process high solids streams (2 percent TS) at hydraulic retention times as short as three hours, as compared to a minimum of about five days with the highest rate conventional process.

Data on sewage treated with the AAFEB (Jewell et al., 1981) is shown in Figure 4. These data were obtained at ambient air temperatures at 20°C with primary raw domestic sewage. The very high treatment efficiencies were achieved because of the large attached microbial biomass. Obviously, since bacterial concentrations were removed to below 10 mg/ℓ at hydraulic retention times as short as 30 minutes, similar removal efficiencies would probably be achieved with algae substrate. Such a process would make all of the alternatives shown in Figure 2 potentially feasible.

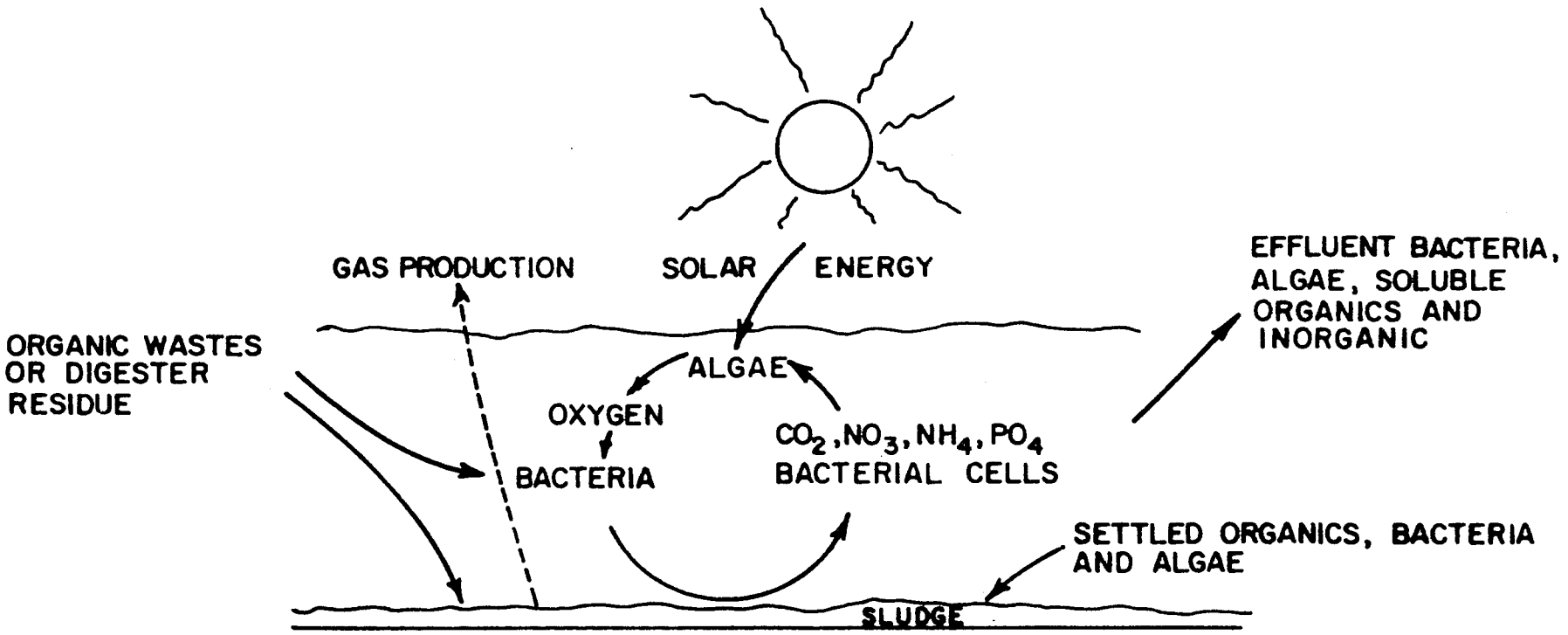

Figure 1. Biological interactions in an algal-bacterial system.

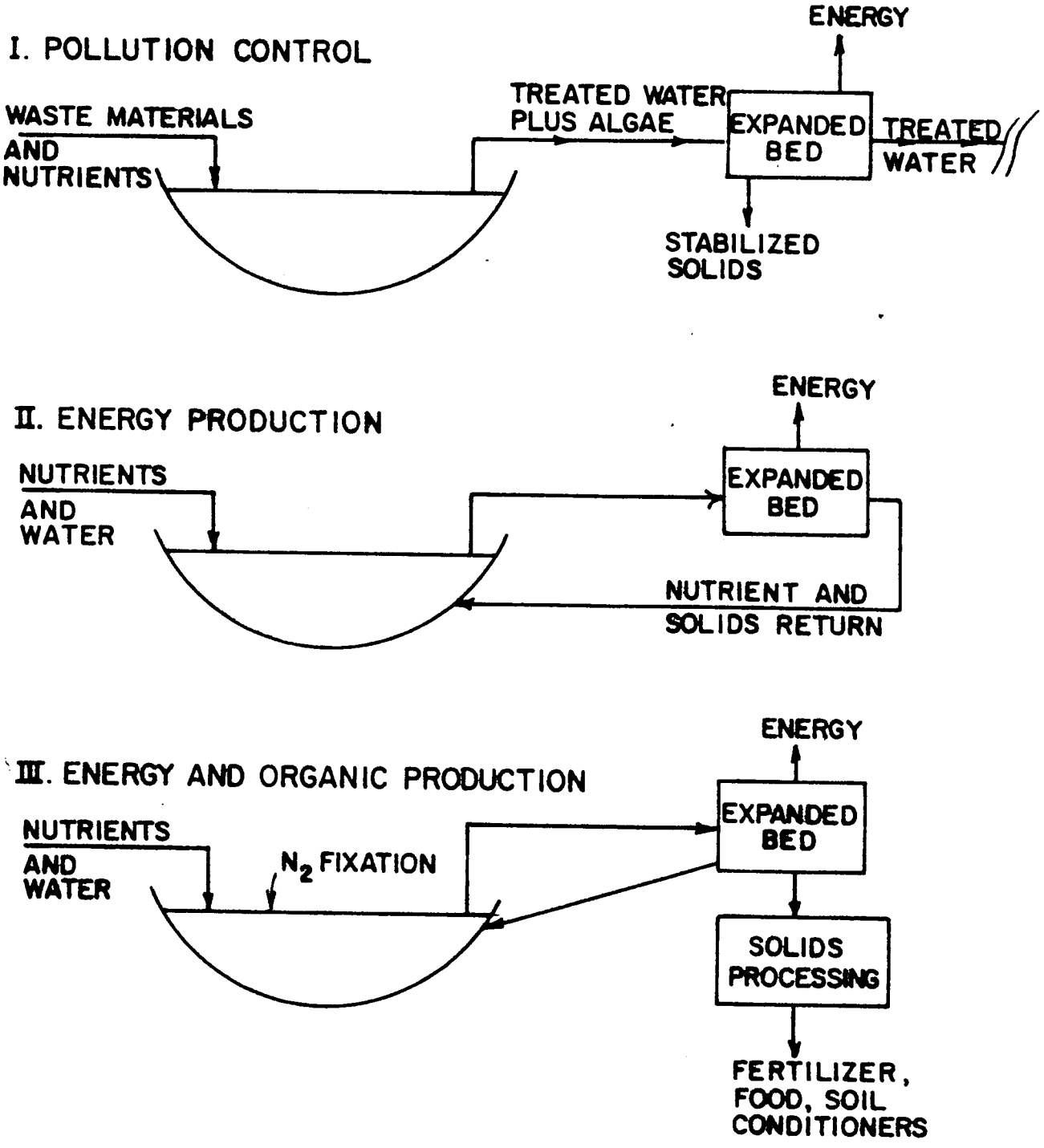

Figure 2. Algal pond alternatives for wastewater purification, energy production, and fertilizer synthesis.

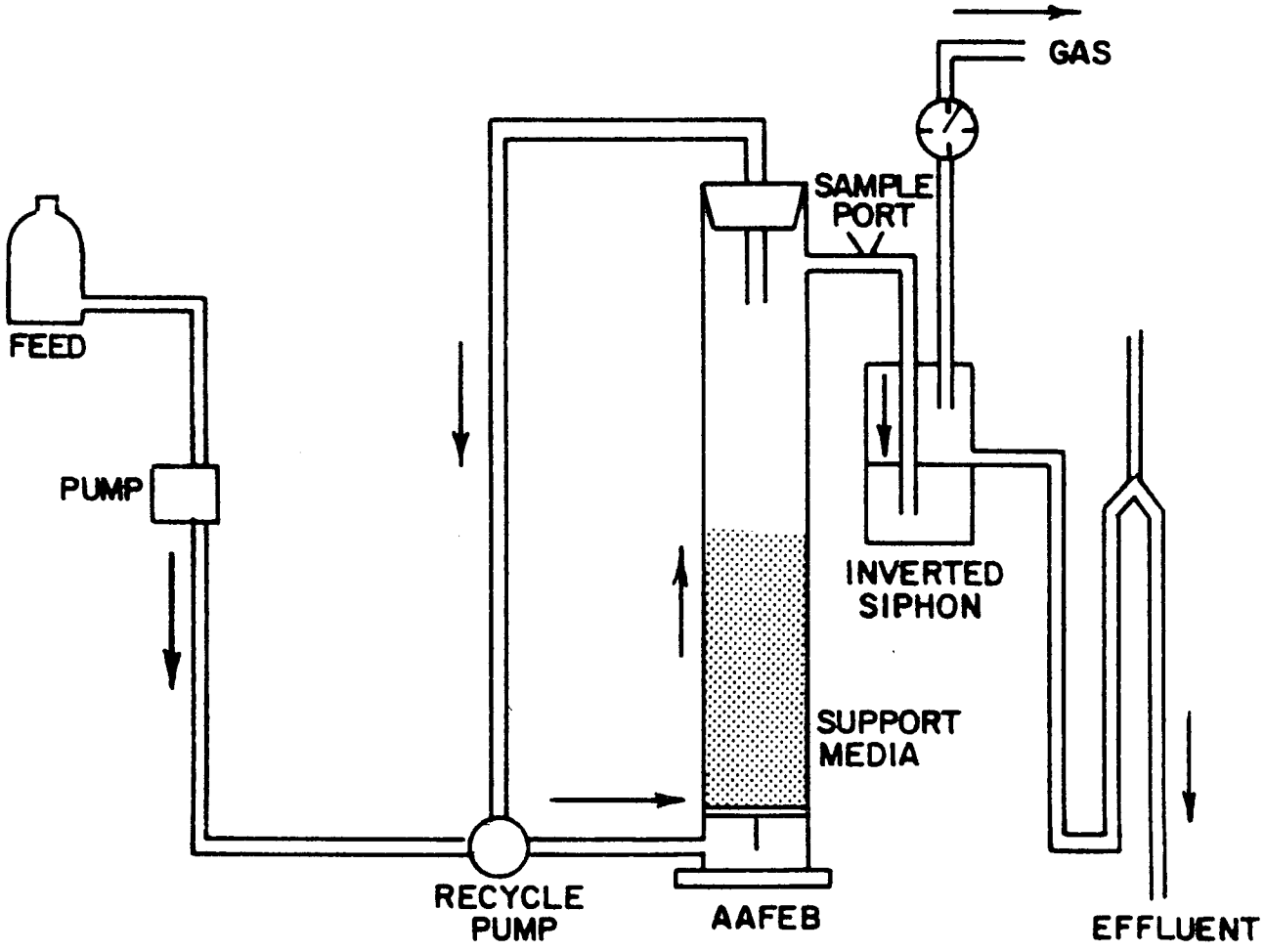

Figure 3. Schematic diagram of AAFEB system.

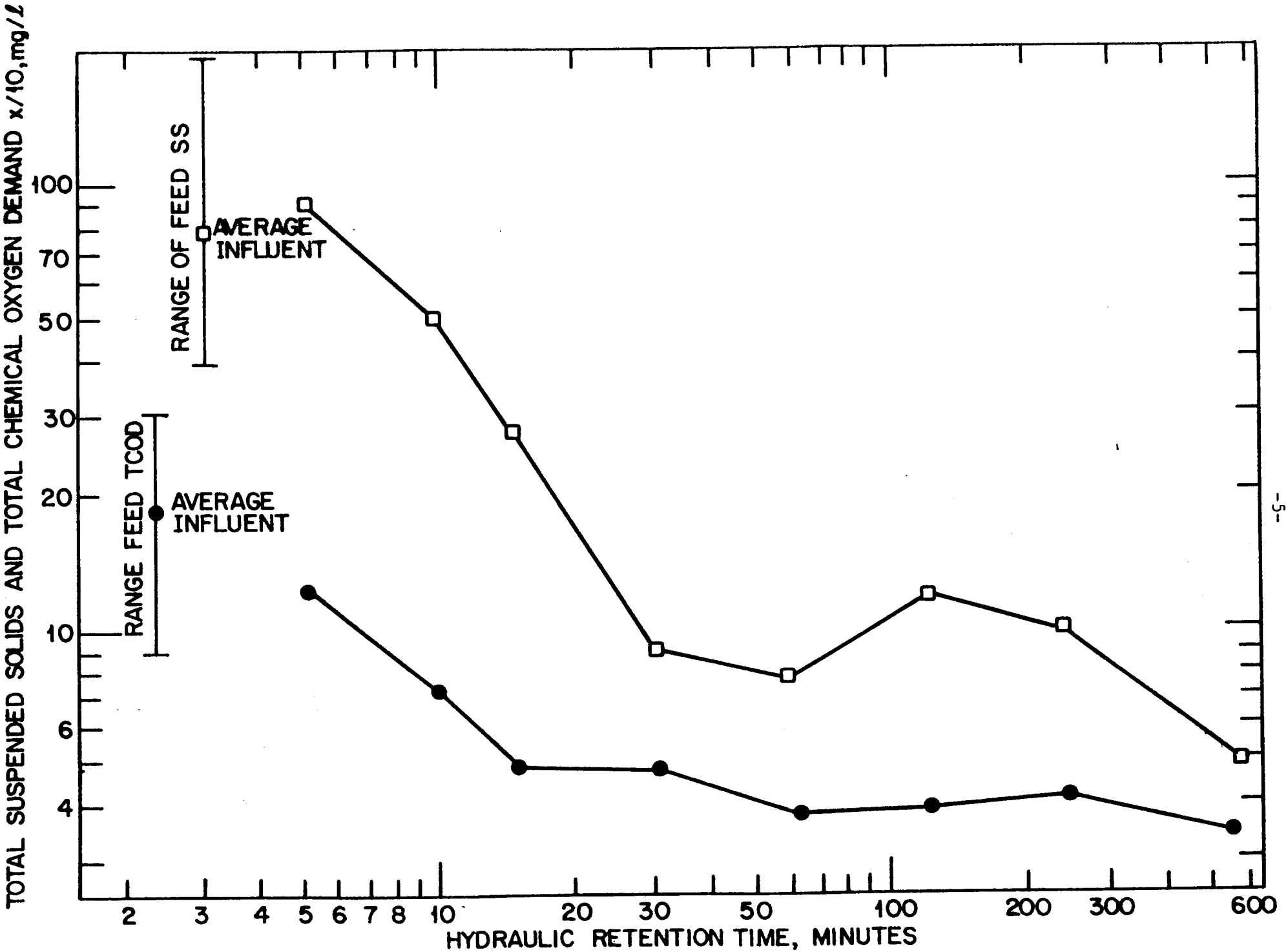

Figure 4. Results of a nine-month study of the treatment of primary settled domestic sewage with an anaerobic attached film expanded bed reactor at 19°C showing the impact of decreasing hydraulic retention times (empty bed volume) on the effluent suspended solids and chemical oxygen demand.

OBJECTIVES

The general goal of this study is to determine the feasibility of microscopic algae harvesting and conversion to methane, utilizing laboratory anaerobic attached film expanded bed reactors. Specific objectives of this study will be to:

1. design, construct, and operate several anaerobic attached film expanded bed reactors under various conditions, thereby developing information on the conversion efficiency of algal cells to methane and on the kinetics of the process;

2. determine the feasibility of separating and digesting heterogeneous microscopic algal cells produced under rapid growing conditions similar to those that exist in aerobic stabilization ponds, using the anaerobic attached film expanded bed process;

3. determine the feasibility of scaling up the optimized system, found in this study to harvest and convert microscopic algae to methane.

BACKGROUND

Microalgae grow symbiotically with bacteria in most kinds of nutrient rich outdoor waters. Algal nutrients comprised of inorganic substrates are provided by the bacteria which decompose organic materials to carbon dioxide, ammonium, phosphate and other products in forms available to algae. Algal photosynthesis provides the oxygen for aerobic bacterial decomposition and algae utilize solar energy to convert the inorganic substances into algal cell material. In such systems sunlight energy can be converted to algal biomass at a relatively high rate of efficiency, about 2% (Benemann et al,. 1978b). One of the first systems used by mankind was and still is the oxidation pond. These ponds are relatively shallow, diked structures with a large surface area to maintain aerobic conditions. Oxidation ponds as a waste treatment system have not only found widespread acceptance in the southern part of the United States, but also in Asia, South America and Africa. The popularity of these ponds has been due to the favorable climate in those areas, the relatively low cost and easy operation of the pond system and the land availability.

Algae stabilization ponds have a significant disadvantage in that the algae contained in the effluent can cause pollution problems themselves. Public Law 92-500, passed in 1972, set minimum effluent standards of 30 mg/ℓ 5-day biochemical oxygen demand (BOD_5) and 30 mg/ℓ total suspended solids (TSS) as a monthly average for municipal waste treatment plants, effective July 1977. Suspended solids concentrations of 100 mg/ℓ or more have been reported frequently in effluent from algal ponds. This meant that solutions had to be found for decreasing suspended solids algal pond effluents.

In the fifties, Golueke et al.,(1957) considered anaerobic digestion as an applicable method of disposal of the large amounts of algae generated in high-rate shallow lagoons or oxidation ponds. Green unicellular algae, grown on sewage, were found to be roughly comparable to sewage sludge digestion. Methane production of the algae grown in oxidation ponds could result in large quantities of sludge, especially if most of the nutrients were converted to plant biomass. This led to the idea of creating a methane producing bioconversion process (Oswald and Golueke, 1965) in which the bioconversion part-- the microalgal pond --is controlled by nutrients, solar light and temperature.

Microalgae have certain inherent advantages in bioconversion. They can be cultivated in a completely hydraulic system over a wide range of temperatures and salinities. The latter is of prime importance since brackish or saline water resources, unsuitable for crop plant production, are often available at marginal sites. Microalgal cultivation is a low energy-demanding process suitable for large-scale systems. It allows process control and maintenance of optimized conditions for high productivity. The currently available information indicates that an ideal design for high-productivity algal-bacterial systems in warmer climates includes a depth of 20-50 cm and a retention time of 2 to 4 days. Slow mixing at a linear velocity of 10 cm sec^{-1} for 18 to 22 hrs. day^{-1}, and, possibly, fast mixing at a linear velocity of 20 cm sec^{-1} for 2 to 6 hrs. day^{-1} appear adequate. Under these conditions, the annual productivity should average 55 metric tons ha^{-1} $year^{-1}$ (15 gm m^{-2} day^{-1}) of volatile suspended solids in temperate climates, and slightly higher in the tropics (Oswald and Benemann, 1980). Research has been mainly focused on two algal biomass generation systems: 1) unicellular green algae generation by recycling nutrients and adding of a carbon source, and 2) algal biomass + nutrient generation nitrogen by a system which is favorable for N_2 fixing algae, as shown in Figure 2.

Several critical problems exist in the low-cost cultivation of microalgae for fuels. The dilute (50-600 mg/$liter^{-1}$ SS) nature of the algae cultures requires constant harvesting (concentrating) of large volumes of the culture while the size of the unicellular green algae puts a severe limitation on the harvesting method. Another major problem is the lack of processes for maintaining algal culture stability. Finally, microalgal biomass production systems will be limited by the capital costs of the pond system and the digester. In fact, Eisenberg et al. (1979) reported that conventional digestion techniques and concentration processes would be economically unattractive for the generation of energy from algal biomass. They proposed a low cost earthen basin design for digestion of algal sludges at 2% volatile solids. However, at the retention times studied (16 to 38 days), with a maximum loading rate of 1.0 kg VS m^{-3} day^{-1}, land requirements for such a covered lagoon would be significant, and volumetric gas production rates would be small and economically unattractive.

Research at Cornell University in the last decade has generated data from a new methane generation process which indicate that this process may be able to drastically reduce these restrictions. The new system, a high rate methane production system, was developed for

application to soluble organics and then applied to a complex substrate in an earlier study (Jewell et al., 1978). This study showed that an anaerobic microbial attached film expanded bed (AAFEB) such as that shown in Figure 3 could process high solids streams (2% T.S.) at hydraulic retention times as short as three hours, as compared to a minimum of about five days with the highest rate of conventional process.

ALGAL HARVESTING

The scientific basis for mass cultures of algae was comprehensively reviewed 25 years ago, with the engineering design and practice put forward by 1960. (Burlew, 1953 and Oswald, 1960). Yet only few commercial large-scale pond systems now exist for the purpose of producing algae. The principal reason is the lack of a cost-effective algal harvesting method for the microscopic pond algae. The methods in use at the moment are briefly discussed.

Chemical Coagulation-Sedimentation

Algae harvesting and removal were studied extensively by Golueke, et al. (1964) and Oswald (1968). A large number of inorganic and organic coagulants were tested, with alum and lime being the most effective. The resultant algal product is greatly contaminated with the coagulating chemical. Besides the contamination, the method is also expensive in capital and energy utilization. Partial alum recovery by acidification was tested in Thailand (McGarry, et al., 1972) and is being used in Israel (Shelef, et al., 1976).

Nigam, et al. (1980) used chitosan as a potential cationic flocculant to concentrate cultures of the alga Scenedesmus acutus. With a dose of 50 mg ℓ^{-1} chitosan, the algal concentration dropped from 560 mg ℓ^{-1} to 40 mg ℓ^{-1} in 30 minutes. Since chitosan is a natural carbohydrate polymer it appears to have greater potential in concentration of algae in mass cultures without affecting the toxicological safety.

Centrifugation

Centrifugation was found to be effective at laboratory scale but very expensive in capital and energy utilization (Golueke, 1965).

Filtration

Intermittent sand filters with 0.17 mm effective size filter sand were used to upgrade stabilization lagoon effluent. The overall average of suspended solids applied was 31 mg/ℓ with a low single value of 6 mg/ℓ and a high single value of 130 mg/ℓ. The effluent consistently contained an effluent with a suspended solids concentration of less than 10 mg/ℓ (Harris, et al., 1978). Three full scale prototype intermittent sand filters were tested for three consecutive 30 day periods during different seasons throughout a sixteen-month period by Russell, et al. (1980). The range of mean suspended solids applied was 31 to 89 mg/ℓ and the overall removal efficiency 69%.

Rock filtration, in which sedimentation is probably the primary mechanism of algal removal, was shown to be an effective, low cost unit process for removing algae from lagoon effluents and correspondingly upgrading lagoon treatment (Swanson, 1980). Weekly average total suspended solids removals were larger than 70%, while the weekly average effluent TSS did not exceed 15 mg/ℓ. Experiments for the removal of suspended solids from the effluent of a facultative wastewater lagoon system with rock filters by O'Brien and McKinney (1979) showed a mean 44% SS removal. The average lagoon effluent was 60 mg/ℓ suspended solids.

Microstraining

Conventional microstrainers with pore openings of minimal 23 μm are not suitable to filter effectively most of the unicellular green species found in high rate ponds. A solution to this problem would be the development of algal species control techniques which allow cultivation of the desirable algal types. Two methods were examined by Benemann, et al. (1977)--the theory of size selective biomass recycle and the selection and cultivation of nitrogen-fixing blue-green algae. Their conclusions were that: (1) selective biomass is not a sufficient tool in itself for sustained species control, and (2) although the harvestability of the blue-green algae Spirulina by microstraining is excellent (90-95%), it can only be grown on settled sewage at very long, and thus impractical, retention times.

The use of new, fine filter fabrics will filter the small unicellular green species. One day experiments by Kormanik and Cravens (1979) showed that microscreening with 1 mm polyester fabric was able to reduce average lagoon effluent from 58 and 126 mg/ℓ total suspended solids to 15 and 19 mg/ℓ total suspended solids respectively (74 and 85% removal). However, the fine filter fabrics blind quickly and are difficult to clean.

Flotation

Research in Thailand (McGarry, et al., 1974), Israel (Shelef, 1976) and recently in the U.S.A. (Lincoln, 1980) has demonstrated the reliability of dissolved air flotation as a viable harvesting technique. After extensive dewatering in a float thickener, the solids concentration of float can be 7 to 9%. However, the cost of proven chemicals required for flocculation (which has to take place before flotation) is a major component of the overall cost. Furthermore, the harvested algae contain a substantial amount of the flocculating agent, commonly alum, which may have toxicological significance.

Betzer, et al. (1980) tested separation of algae from oxidation pond effluents in a bench scale flotation column using ozone-enriched oxygen. Experimental results indicated that the process could produce a clear, colorless liquid, and over 98% removal of suspended solids and final values of less than 15 mg ℓ^{-1} SS. Ozone dosage ranged from 15 to 50 mgℓ^{-1}. Through settling and thickening by gravity, the froth formed a sludge with a solids level of 2 to 7%, depending on the ozone concentration of the gas (0.05 to 8%).

Continuous-Belt Filtration

The paper precoated belt filter was tested at pilot scale in Australia (Bureau of Environmental Studies, 1975, Dodd, et al., 1977). The belt filter incorporates a coarse fabric belt on which a precoat of paper fibers is deposited, forming a continuously renewed filter medium. After filtration, the algae are separated under vacuum from the precoat and the paper fibers are washed and recycled. This eliminates the problem of progressive blinding and loss of throughput found with conventional fine fabric media. The algae-paper mat is sandwiched between the main and secondary belts during separation of algae from the precoat (Dodd, 1979).

While the precoat filtration approach was technically successful, the use of a precoat caused undesirable operational complexity and increased costs. As a result of the experience gained during the previous work and the availability of new fine-weave synthetic fabrics (5 mm range), fine-weave cloth rather than the precoated filter belt is being investigated in harvesting trials in Singapore (Dodd, 1980). This latter system is very efficient when harvesting the larger species of algae common to high-rate ponds, such as Micractimum, but has problems of blinding with the smaller species such as Chlorella and is incapable of removing the tiny blue-green Synechocystis. (International Development Research Centre, 1980).

Autoflocculation/Bioflocculation

The use of flocculation and settling of microalgae without chemical additions has been repeatedly suggested as a method of algal harvesting from oxidation pond effluent. That algae spontaneously flocculate and settle is well known from both the ecological and sanitary engineering literature. The factors and processes by which algae are induced to agglutinate or flocculate have not been satisfactorily elucidated. Nutrient limitation seems to be one important parameter. Nitrogen limitation has been established to be a cause for algal flocculation in the laboratory and to be of possible ecological significance (Fogg, 1965). Carbon limitation (resulting in high pH in the afternoons) resultsin autoflocuulation of alfal cultures in high rate ponds (Golueke, 1965). Autoflocculation appears to involve a co-precipitation of magnesium, calcium, phosphate and carbonate salts with microalgae (Moellmer, 1970). Bioflocculation, in contrast, is reserved as a term to denote algal flocculation not caused by co-precipitation with mineral salts. This mechanism probably involves the excretion of polysaccharides by the algae and bacteria.

One apparent bioflucculation process, termed "dark sedimentation," has been described in which a 90% removal of TSS was achieved by the operation of facultative high-rate and covered settling ponds in series; the mechanism of this process is uncertain (Oswald, 1977). An "activated algae" process involving cell recycle and algal flocculation-sedimentation has been the subject of extensive laboratory investigations (Regan, 1977 and Humenik, 1970).

Recently, in the operation of a high-rate pond system in the Philippines using screened, low-strength sewage, a minimum 75% TSS removal was observed in a settling pond receiving the high-rate pond effluent (Oswald, 1976). In more recent experiments good results have been achieved with the auto/bio-flocculation process. With certain limits of hydraulic retention time and mixing, a self-flocculating culture was obtained which settles within 2 to 20 hours to yield an algal sludge of over 1% solids and a 75 to 90% clarified supernatant. The key concern, however, is whether the flocculation-settling process can be sufficiently controlled to perform reliably throughout a full year of operation. Present experience suggests that it may be difficult to reliably remove more than 75% of the TSS in oxidation ponds through a rapid settling process (Benemann, et al, 1978a).

Other Methods

High gradient magnetic separation (HGMS) is a technology which enables the separation of only weak magnetic particles from water; these particles include biological colloids (e.g. viruses, bacteria), dissolved nutrients, and algae. However, pretreatment of the water to be processed with magnetic seeding material (1000 ppm Fe_3O_4) and a flocculating agent (aluminum sulphate) is required. Because of the chemical additions the quality of the algal product will be severely decreased (DeLatour, 1973).

Non-fouling membranes in the membrane ultrafiltration method promise to increase operational life and flux rates, but their use involves highly mechanized ultrafiltration systems requiring extra pumping (minimum filtration pressure is 20 psi) (Cruver, 1972).

ALGAL DIGESTION

The feasibility of anaerobically fermenting the algae produced in algal ponds was first demonstrated by Golueke, et al. (1957). Experiments with 11-liter digesters provided data on temperature effects, loading rates, retention times and the effect of using alum-flocculated algae (Table 1). Mixing was done once daily, prior to effluent removal and feeding. At loading rates of 1.44 kg volatile solids (VS) m^{-3} day^{-1} and with a retention time of 30 days, CH_4 production was 0.25 and 0.32 ℓ/gm VS introduced/day at 35° and 50°C, respectively. By varying the concentration of solids in the feed they studied retention times from 3.3-30 days, as shown in series 1B experiments. Golueke, et al. (1957) found that gas production from algae decreased steadily at retention times less than 11 days at 50°C, although little increase was observed at retention times in excess of 15 days. In series 1C experiments, loading rates were doubled from 1.44 to 2.88 kg VS m^{-3} day^{-1}. The change in gas production was minimal. They also found that the aluminum, used for flocculation of the algae, had neither a detrimental nor a beneficial effect on the microorganisms functioning in the digestion process. The use of aluminum flocculated feed also had no effect on the physical quality of the digester sludge.

TABLE 1. SUMMARY OF ALGAL DIGESTION DATA.

Experiment[1]	Temp °C	Loading $kg/m^3/day$	Det. Time Days	Methane Production ℓ/gm VS Introduced/day	% CH_4	VSS Destruction %
1A	35	1.44	30	.25	63	45
	50	1.44	30	.32	63	55
1B	50	1.44	22	.31	63	
	50	1.44	11	.32	63	
	50	1.44	7	.21	63	
	50	1.44	3.3	.17	63	
1C	50	1.44	22	.33	63	
	50	2.88	22	.31	63	
2A	37	0.80	10, 15, 20 days[2]	.38	71	
2B	37	1.60	"	.36	71	
2C	37	3.20	"	.36	71	
2D	37	4.00	"	.33	71	
2E	37	6.40	"	.29	71	
3A	36	0.58	28	.34	67	55

[1]Experiments 1A - 1C from Golueke, et al.(1957). Primarily Chlorella and Scenedesmus
Experiments 2A - 2E from Uziel, et al.(1975). Primarily Scenedesmus
Experiments 3A from 1400 liter digestion experiments from Benemann, et al.(1978). Primarily Scenedesmus.

[2]Retention time had no systematic effect on gas production, so the average of the 5, 10, and 15-day runs are given.

Experiments 2A through 2E in Table 1 are from the work of Uziel, et al. (1975), with 1.5 liter, well mixed cultures at 37°C. He attributed the higher gas production, compared to experiment 1A, to the vigorous mixing of the digesters. His work also established the fermentability of other harvestable genera, including Micractinium, Spirulina and Oscillatoria. All experiments indicated that algal digestion cultures were quite stable. In experiment 3A, a 1400-liter mixed and heated digester which was fed sun-dried algae at the rate of 0.58 kg of volatile solids per m^3 per day, produced 344 mℓ CH_4/gram of volatile solids introduced (Benemann, et al., 1978a). All of the above data were obtained from the digestion of dried algae. In addition, most previous studies, used dry or frozen algae as a substrate for convenience. Early work by Golueke and Oswald (1957) raised serious questions about the digestibility of live algae at mesophilic temperatures. Benemann, et al. (1980) did some live algal digestion experiments, and the data showed that this live algal digestion was equivalent to or better than that obtained when sun-dried algae was the substrate.

Eisenberg, et al. (1979) showed that methane production of fresh algae, which was harvested and concentrated by two-stage sedimentation, refrigerated, and fed to the digesters within two weeks after harvesting, was lower than that of sun-dried algae (.33 m^3CH_4 per kg VS introduced and .28 m^3CH_4 per kg VS introduced, respectively). The fresh algae (Scenedesmus) is apparently less readily degraded than the dried algae. They also reported that a minimum retention time of sixteen days was necessary. Gas production decreased to zero when the retention time became smaller than sixteen days at 35°C.

MICROALGAE DECOMPOSITION CHARACTERISTICS--AEROBIC AND ANAEROBIC

The most comprehensive studies of algal conversions in biological conversions were conducted in the late 1960's by Jewell and Foree. The general objective of these efforts was to be able to describe the conversion of algae in natural waters under aerobic conditions (Jewell and McCarty, 1971) and anaerobic conditions (Foree and McCarty, 1971). These laboratory studies defined for the first time the biodegradability of microalgae and the rate at which the biodegradable matter was made available by the decomposing community, i.e., the total decay kinetics were defined.

The surprising outcome of these decay studies showed that algal cells often contained a large fraction of all biomass that resisted microbial conversion; i.e., was nonbiodegradable. The nonbiodegradable portion of algal cells (i.e., the refractory fraction, f) was reported to average 0.4 for both aerobic and anaerobic decomposition. In other words, 40 percent of the organic matter (expressed as chemical oxygen demand) resisted conversion to either carbon dioxide and water or to methane. A summary of the algae conversion characteristics is given in Table 2. These two studies provide a sound basis for predicting the fate of carbon and nutrients in the energy systems under consideration in this study. The following discussion indicates how the data shown in Table 2 can be used to predict the results in anaerobic decomposition of microalgae.

Table 2. Summary of Microalgae Decomposition Characteristics at 20°C (from Jewell and McCarty, 1971 and Foree and McCarty, 1971).

		Aerobic Decomposition	Anaerobic Decomposition
I.	Decay Rate, k, first order decay of biodegradable volatile solids, day^{-1}		
	Range	0.01 to 0.15	
	Typical Value	0.02	0.02
II.	Refractory fraction, fraction of volatile solids remaining after one year for aerobic studies, 200 days for anaerobic studies		
	Average	0.40	0.40
	Range	0.12 to 0.87	0.18 to 0.64

KINETIC ANALYSIS OF MICROALGAE BIOLOGICAL CONVERSIONS

The decomposition kinetics as defined by Jewell and McCarty (1971) and used by Foree and McCarty was as follows:

$$M = (M_o - fM_o)\, e^{-kt} + fM_o$$

where f is defined as the refractory organic fraction and was expressed as the decimal fraction of the initial particulate COD, M_o, remaining after a decay period of about a year (250 days for Foree's study). The average value of the ratio of TCOD to VSS was 1.48, with a standard deviation of 0.27 and a range of 1.1 to 2.0 for the anaerobic study.

Most of the comprehensive testing with the decomposition of algae was conducted at 20°C, since the researchers were mainly interested in interpretation of reactions in natural waters. Several short-term tests on the influence of temperature on the anaerobic decay kinetics showed that the rate of decay was affected by the temperature, but this did not affect the refractory fraction (Foree and McCarty, 1971). The relationship between k and temperature was expressed as

$$k_T/k_o = e^{C_k\,(T-To)}$$

where k_T is the first order decay coefficient of the biodegradable fraction of COD at temperature T in °C, k_o is the decay coefficient at temperature T_o, and C_k is the influence of temperature and was found to be 0.055 per °C.

If one were to operate a mesophilic anaerobic fermentor, the predicted conversion rate from the above relationship would be:

$$k_{35}/0.02 = e^{0.055\,(35-20)}$$

$$k_{35} = 0.046\ day^{-1}$$

Example Anaerobic Conversion Estimates

Using the above analysis, the potential conversion of microalgae at varying temperatures can be estimated. If the algae are concentrated to a 2% total dry solids mixture, the following estimates can be made.

TSS = 20 gms/ℓ, assume TVSS = 0.9 TSS

TVSS = 0.9 x 20 = 18 gm/ℓ

Assume ratio TCOD/TVSS = 1.48
M_o = 1.48 (TVSS) = 1.48 (18) = 26.6 gm COD/ℓ

If it is assumed that the batch kinetics determined by Jewell and Foree applied to continuously operated digesters, the predicted effluent quality from reactors operated at 20° and 35°C and at 30-day HRT would be as follows:

At 20°C digester operation, 30-day HRT:

$$M = (26.6 - 0.4\ (26.6))e^{-0.02(30)} + 0.4(26.6)$$
$$M = 19.4 \text{ gm COD/}\ell$$

Effluent TSS would be 14.5 gm/ℓ, resulting in a 27.5% conversion efficiency of the TSS. About 45% of the biodegradable solids were converted under these conditions.

At 35°C digester operation, 35-day HRT:

$$M = (26.6 - 0.4\ (26.6))e^{-0.046(30)} + 0.4(26.6)$$
$$M = 14.6 \text{ gm COD/}\ell$$

Effluent TSS would be 11 gm/ℓ resulting in a 45% conversion efficiency of the TSS. About 75% of the biodegradable solids were converted under these conditions.

At 35°C digester operation, 10-day HRT:
Effluent TSS would be 15.5 gm/ℓ and the solids conversion efficiency would be about 22.5 percent. The above values will be useful to compare to the efficiencies achieved with the expanded bed studies.

MATERIALS AND METHODS

INTRODUCTION

Since the AAFEB has been shown to be both an efficient fine solids separator and fermentor, one might attempt to collect, to separate, concentrate and convert the algae in one unit process. There are several limitations that indicate that this is not the most efficient method of operation. The first consideration relates to the amount of organic matter that will result in production of gaseous methane from a high rate of flow expanded bed system. Before the methane is given off from the liquid as a gas bubble, it is necessary to first saturate the water with methane. A comparison of the saturation values of water at ambient temperature indicates that complete conversion of greater than 200 mg/ℓ of the biodegradable fraction of algae would be required just to saturate the water with the methane gas. Therefore, in most applications with the expanded bed a one-step process would not yield a significant amount of methane. Rather, all of the methane produced in the efficient anaerobic digestion process would leave in the effluent as a dissolved gas. Therefore, it is necessary to consider the process of microalgal separation and digestion from stabilization pond effluents as a two-stage process. A secondary consideration is that processing of the algae in a one-step process results in a low rate of conversion, as noted earlier in the literature survey, thus making the one-step conversion also unattractive from a kinetics viewpoint.

GENERAL EXPERIMENTAL APPROACH

Due to the preliminary nature of these experiments, it was felt that it was necessary to test a wide range of variables without comprehensive testing of any of the parameters considered. The study was divided into two general areas--first, the separation, and second, the fermentation of concentrated algae. In order to obtain a measure of effectiveness of separation and concentration of algae in the expanded bed, a number of sources of algae were tested on a continuous flow basis for over a year. Since it is unclear as to what mechanisms are responsible for the fine solids separation, both expanded beds with and without attached films were tested. A number of sources of natural algae were obtained from operating algal ponds and other small bodies of water contaminated with algae.

After the feasibility of the expanded bed for separation and concentration of algae was demonstrated, the process then was tested for conversion of the algae at elevated temperatures. Increased temperatures were considered viable alternatives because it was possible to concentrate the algae to a significant level, and in full scale applications it would be possible to reclaim heat from the effluent to achieve efficient digestion. It should be noted that the conversion studies used microbial films that had not achieved mature attached films. For this reason, the data should be considered to be preliminary in nature and only a conservative estimate of the feasibility of the system.

REACTORS FOR SEPARATION AND CONCENTRATION

Three laboratory scale attached film expanded bed reactors were constructed for the separation and concentration experiments. Styrene-acrylonitrile Imhoff settling cones were used for these units (Figure 5). The cones were 40 cm tall and had an inside diameter which increased from 2 cm at the bottom to 9 cm at the top, where the effluent line was connected. The total volume (liquid plus media) was one liter. This tapered bed design reduced the "short-circuiting" in the reactors to a minimum. Each column was filled with 500 mℓ of diatomaceous earth. During operation the bed was expanded to 10 to 20% of its volume by means of a recycle pump. The media in the columns used for the attachment of bacteria were diatomaceous earth particles (AIROX, Diatomite product produced by AIROX EARTH RESOURCES, Santa Maria, California) in the size range of 0.197 to 0.595 mm. The bulk density of the dry particles was 0.54 g/cm^3, and one gram of the particles displaced 0.6 cm^3 of water. The particles were rather light and provided a large surface area for attachment. Diatomaceous earth is inexpensive and may be useful in full scale applications.

Feed solutions were pumped (Dial-a-Pump, Model 12 AP, 12-channel liquid pump) into the recycle lines. The pumps used for recycling were Masterflex Variable Speed Drives with a solid state controller (Model 7545, Cole Parmer Instrument Company). Tygon tubing was used to connect the influent reservoirs, pumps and reactors. The experiments took place at room temperature, 20°C.

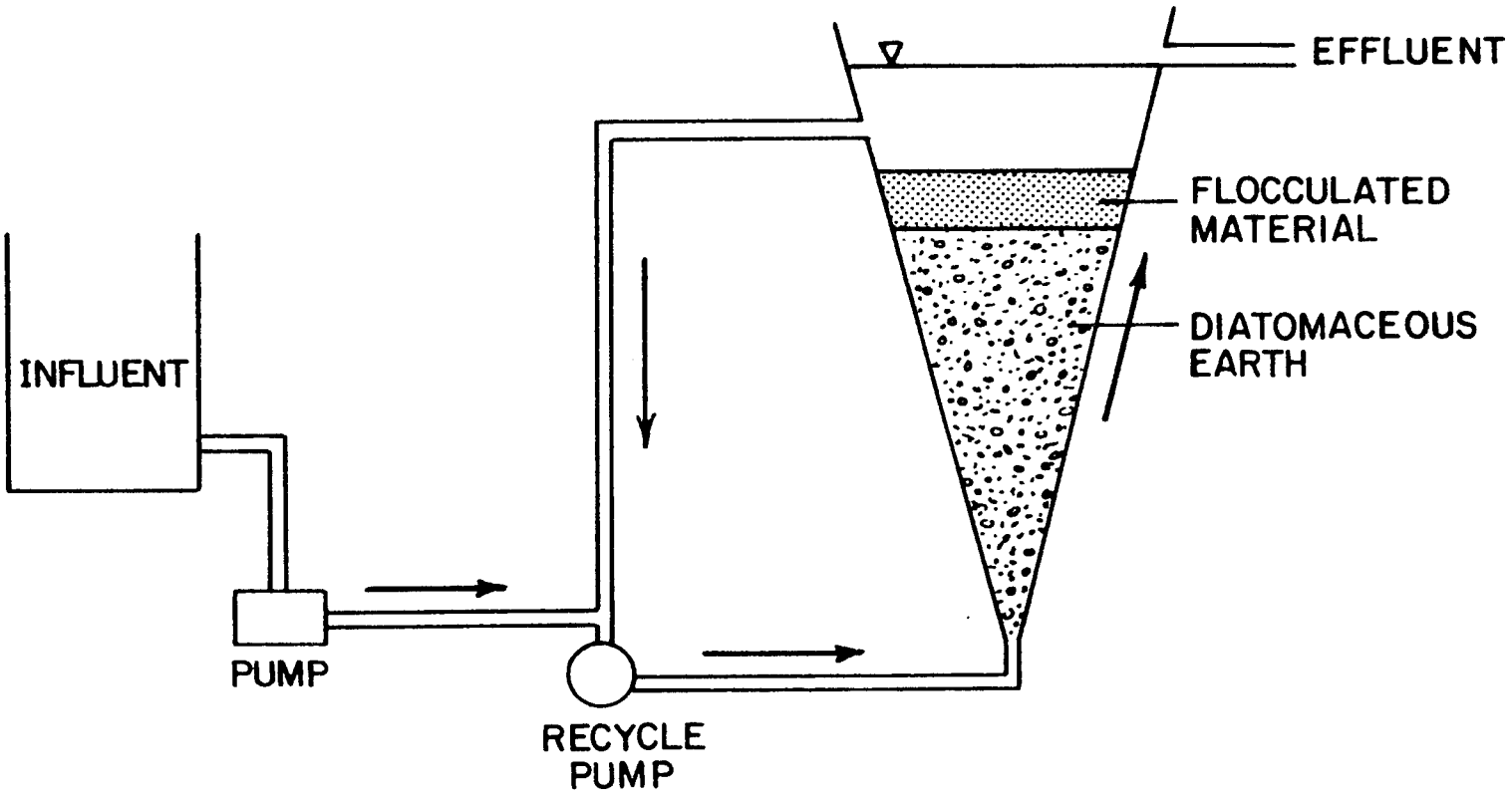

Figure 5. Schematic diagram of a reactor to separate and concentrate microscopic algae.

SOURCES OF ALGAE FOR SEPARATION AND CONCENTRATION

During the separation and concentration experiments, water containing algae from three different ponds was used. The first one was a small outdoor garden pond (2.5 m^2 surface area). The second one was a pond located at the Cornell facilities (400 m^2 surface area); while the third one was located 45 kilometers north of Ithaca. This last source was a facultative treatment pond for wastewater from a dairy milking center (0.085 ha of surface area). In all three ponds most of the algae present were unicellular green algae; e.g., Chlorella, Scenedesmus, Chlamydomonas and Closteriopsis.

REACTORS FOR DIGESTION

Two laboratory scale attached film expanded bed reactors were constructed for the digestion experiment. The reactors were constructed similar to the solid separation units. On top of the settling cone a plexiglass pipe was placed (9 cm in inside diameter and 30 cm tall). This part acted as the clarifier zone. Each column was filled with one liter of diatomaceous earth. The top of each reactor was sealed by means of a rubber stopper. During operation the bed was continuously expanded 10 to 20% by means of a recycle pump. A schematic diagram of the anaerobic attached film expanded bed reactor is shown in Figure 6. The pumps used for feeding and recycling were Masterflex Variable Speed Drives with solid state controllers (Model 7545, Cole Parmer Instrument Company).

Gas was collected in an inverted, calibrated cylinder in a column filled with water. At the top of each cylinder was a septum from which a gas sample could be obtained to determine the composition of the gas. The total volume of each reactor was 2750 mℓ: 1000 mℓ of media in the bed and 1750 mℓ liquid in the clarifier zone.

One laboratory scale, completely mixed, semi-continuous reactor was constructed to act as a control unit. The reactor was constructed of a 4-liter glass bottle. A constant operating volume of three liters was maintained in the reactor. Mixing of the reactor's contents was accomplished twice daily by manual agitation. The mouth of the reactor bottle was sealed with a rubber stopper. A schematic diagram of the control reactor is shown in Figure 7. The pump used for feeding and for effluent removal was a Masterflex Variable Speed Drive with a solid state controller (Model 7545, Cole Parmer Instrument Company). Gas was collected in the same way as described for the attached film expanded bed reactors.

For all three reactors Tygon tubing was used while the units were kept at 35°C.

SOURCE OF ALGAE FOR DIGESTION

Algae for the digestion experiments were obtained from Mr. D. Eisenberg, Department of Civil Engineering at the University of California, Berkeley. This algae was from a high-rate oxidation pond and was obtained by centrifuging until a paste of ~15% total solids

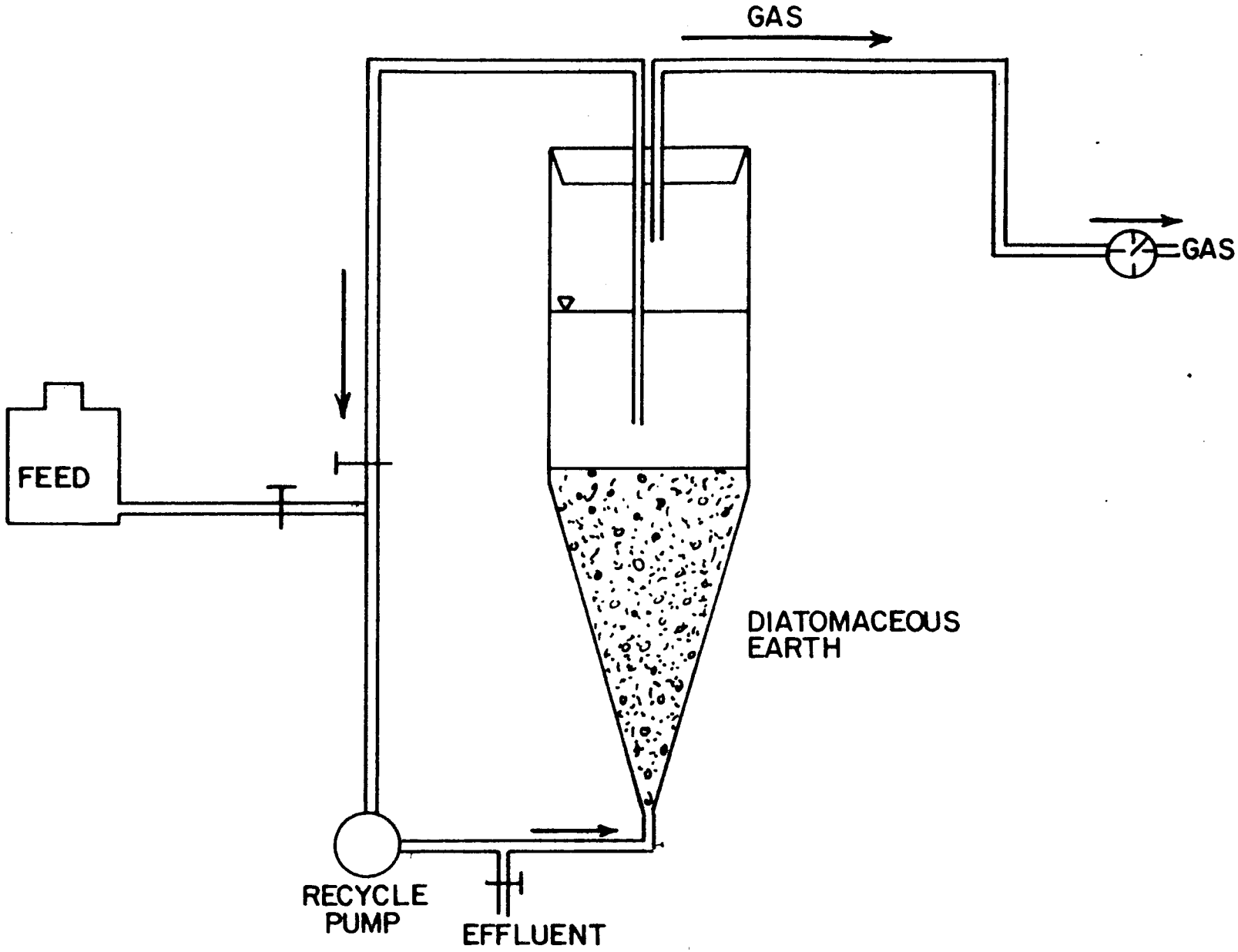

Figure 6. Schematic diagram of AAFEB reactor.

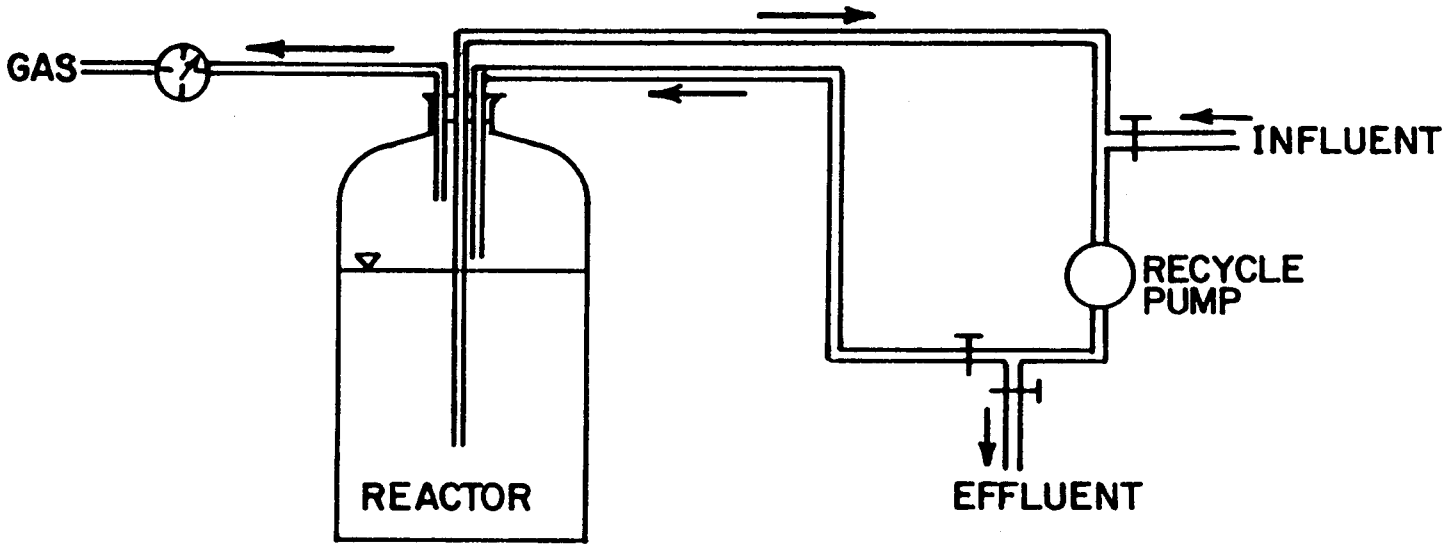

Figure 7. Diagram of the completely-mixed, semi-continuous reactor.

was obtained. Five gallons of this paste was sent by air freight to Ithaca,where it was stored at 4°C and diluted to the appropriate volatile solids concentration when needed as reactor feed. The majority of the algae were unicellular, green algae.

SEPARATION AND CONCENTRATION OF ALGAE

In the first experiment the previously described reactor was filled with 500 mℓ of diatomaceous earth which was devoid of attached microorganisms. Facultative/anaerobic conditions existed since no oxygen was provided. The reactor was fed continuously and at varying hydraulic retention times with water from the small outdoor garden pond for a period of 12 days.

In the second experiment the two reactors connected in series were used (see Figure 8). One was filled with 500 mℓ of diatomaceous earth, taken from an acclimated anaerobic attached film column and was operated under anaerobic conditions. The acclimated column was a plexiglass column, 90 cm tall and 8.5 cm in inside diameter, and filled with 1250 mℓ diatomaceous earth. This column had been operated as an anaerobic attached film expanded bed reactor for six months at room temperature. The feed for this column had been a mixture of sucrose and cellulose solutions, to which nitrogen and phosphorus were added. Regular additions of supernatant from an anaerobic digester, rumen fluid of a cow and anaerobically digested cow manure were also conducted. These last three substances served as seed and as a source of micronutrients. When particles of this column were transferred to the separation and concentration reactor, they had an initial film of attached anaerobic microorganisms as observed under a light microscope.

The second aerobic reactor was filled with 500 mℓ of diatomaceous earth, taken from an aerobic acclimation column. Aeration was achieved by air injection into the recycled water. These particles had a well established film of aerobic and facultative microorganisms, which was grown during two months in an aerobic acclimation column. The reactor feed was comprised of a mixture of sucrose and cellulose, to which nitrogen and phosphorus were added, while primary effluent sewage had been used as seed.

Prior to the experiment, two weeks for acclimation of the reactors was conducted by feeding them water from the pond located at the Cornell facilities at a hydraulic retention time (HRT) of 24 hours. The hydraulic retention time was taken to be the volume of substrate added per unit of time divided by the total volume of the reactor (empty volume). The experiment lasted 40 days, during which time the reactors were operated in parallel or in series.

The following parameters were measured regularly: influent total suspended solids and volatile suspended solids, effluent total suspended solids and volatile suspended solids, pH and turbidity.

DIGESTION OF ALGAE

Two AAFEB reactors were operated semi-continuously during 55 days at three different hydraulic retention times (HRT's). Prior to feeding

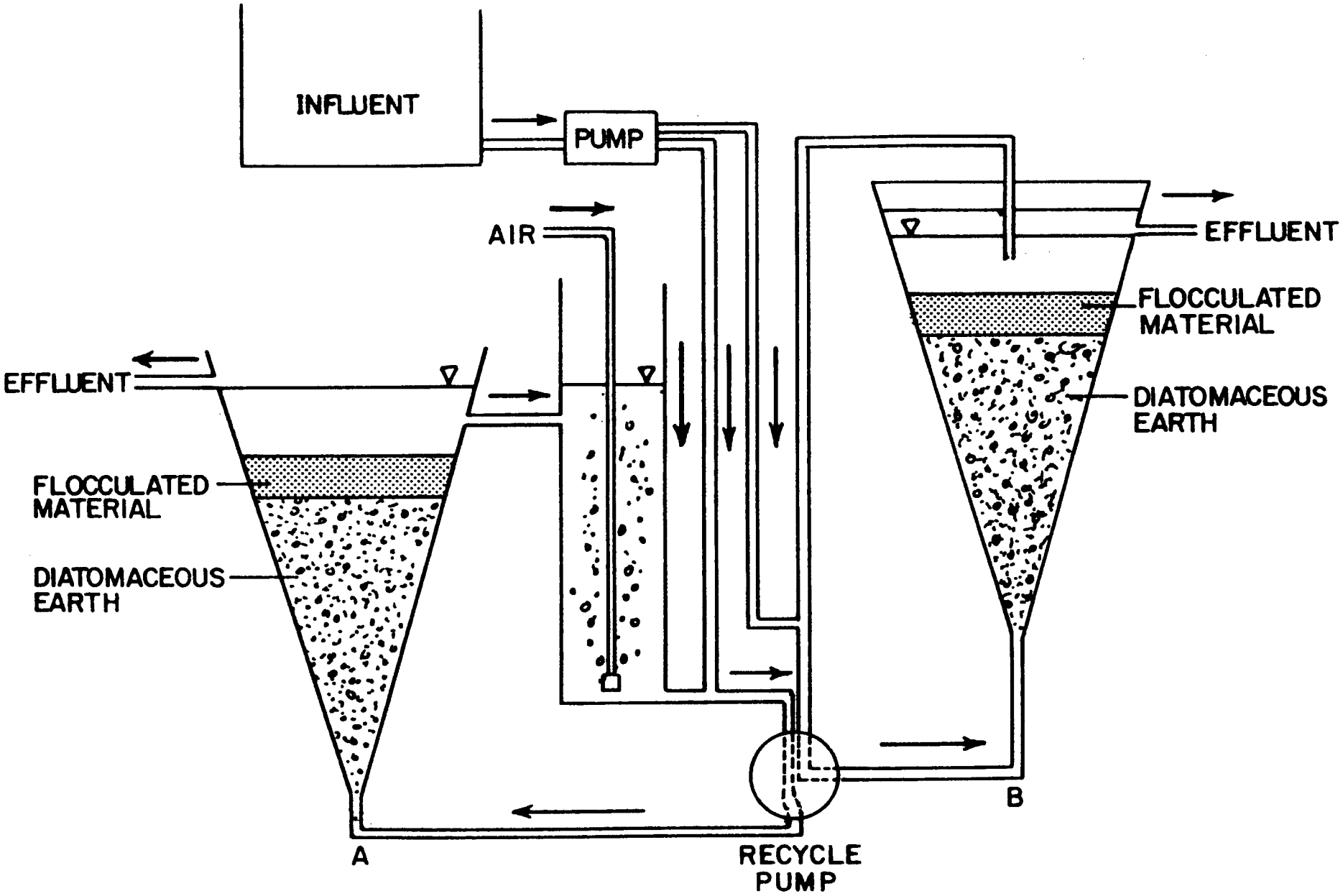

Figure 8. Schematic diagram of aerobic (A) and anaerobic (B) reactor to separate and concentrate microscopic algae.

the reactor, the feed was allowed to reach a temperature of 35°C, while a volume of liquid equal to the feed volume was withdrawn from the reactor with the help of the recycle pump. The first 14 days of operation were used for acclimation, then followed 10 days of operation with an HRT of 9.2 days, 15 days with an HRT of 4.6 days, and 16 days with an HRT of 2.3 days (reactor volume was 1750 mℓ). It was assumed that the conditions under which the volatile solids and other performance parameters which were obtained during the last days of each period approximated a steady state condition. Periods four to five times as long would be required to actually obtain an estimate of steady state.

A 2 % solids feed solution was prepared, of which 63% was volatile.

The control reactor was operated at an HRT of 10 days for the full 55 days of this experimental run. A volume of 300 mℓ was removed from the reactor with the recycle pump before 300 mℓ of feed was added. The control reactor was kept operating in a batch mode after the 55 days of semi-continuous operation to determine the ultimate biodegradability of the algae. The following parameters were measured on a regular basis: influent total solids and volatile solids, effluent total solids and volatile solids, pH, gas production, gas composition and alkalinity. At the beginning of the experiment the COD of the 15% total solids algae paste was determined.

DETERMINATION OF ALGAE REFRACTORY FRACTION

For the determination of the refractory fraction (R) of the algae, two different methods were employed. The first one was used by Anthonisen et al., (1968) and Wood et al., (1974) and modified by Morris (1976). For this method samples were withdrawn from the control reactor at various intervals (after 55, 152 and 229 days of digestion) and analyzed for total volatile solids. The assumption was that as the solids retention time (SRT) approached infinity, the biodegradable fraction of the algae would become zero, leaving only the refractory fraction. A plot of Effluent VS/Influent VS versus 1/Influent VS•SRT was made (as shown in Figure 9). This would produce a linear relationship with the ordinate intercept being the refractory fraction of the organic matter.

The second method was the chemical-physical procedure, developed by Chandler (Chandler et al., 1979), which predicts the biodegradability based on substrate lignin content. A fiber analysis was done to determine the lignin_s content. The formula used to calculate the refractory fraction is presented in Equation 1.

$$1-B = -0.028 \times +0.830 \qquad (1)$$

where B = biodegradable fraction
X = VS lignin_s content (percent)

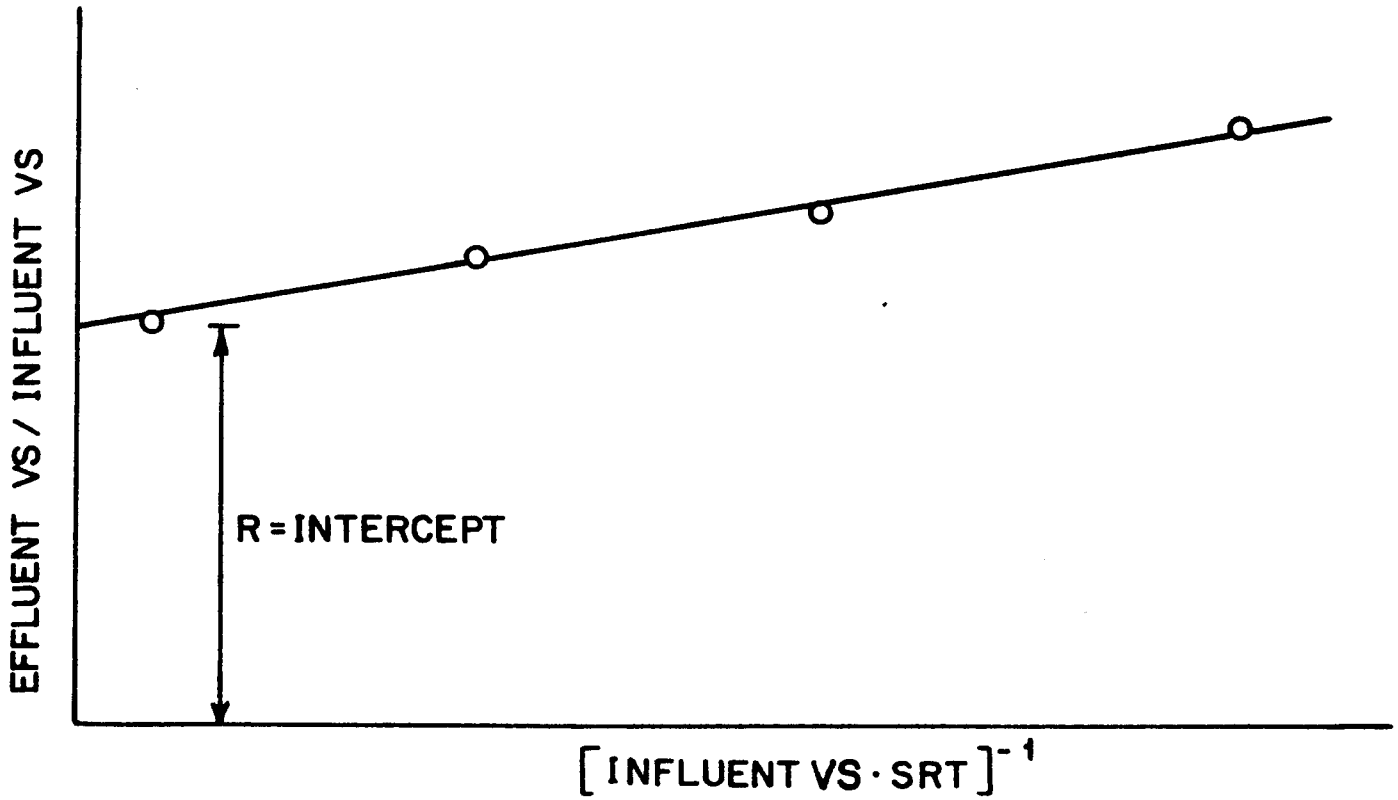

Figure 9. Graphical determination of the refractory fraction, R.

ANALYTICAL METHODS

pH

pH was determined by the glass electrode method using an Orion Model 701 pH meter. The sensitivity of the meter was 0.01 pH units.

Total Suspended and Volatile Suspended Solids

The glass fiber filter method was used to measure the suspended solids in this study. An appropriate sample volume was filtered through a fiber filter supported on a Millipore filtration apparatus (Millipore Corporation, Bedford, Massachusetts). The filter was then washed with an equal amount of distilled water. After filtration, the filter pad and retained solids were dried at 103°C for one hour, cooled in a desiccator, and weighed. To determine the volatile portion of these solids, the dried pad and solids were placed in a muffle furnace at 600 °C, ashed overnight, cooled in a desiccator, and weighed. A blank pad was carried through all steps in order to correct for moisture changes or volatilization of the filter. The glass fiber filters used were 5.25 cm Whatman Glass papers, grade GFC.

Turbidity

The turbidity was measured with a Hellige turbidimeter (Hellige Inc., Garden City, New York) in AHPA turbidity units. It works on the principle that light which passes upwards through a sample of 10 mm high is scattered. The higher the intensity of the scattered light, the higher the turbidity.

Total and Volatile Solids

A well mixed, 100 mℓ sample, was evaporated in a weighed dish and dried to constant weight in an oven at 103°C. The increase in weight over that of the empty dish represented the total solids. To determine the volatile portion of these solids, the dried dishes with sample were placed in a muffle furnace at 600°C, ashed overnight, cooled in a desiccator, and weighed. A dish with 100 mℓ tap water was carried through all steps to account for solids in the dilution water.

Gas Analysis

Total gas production was measured continuously by buoyancy displacement described previously. Determinations for methane and carbon dioxide content were made using a Gow Mac-550 thermal conductivity gas chromatograph.

Alkalinity

Alkalinity was determined by the potentiometric titration to endpoint pH = 4.3 method as described in Standard Methods for the Examination of Water and Wastewater, 14th edition (1976).

Chemical Oxygen Demand

A colormetric method described by Knechtel (1978) was used. Sample and reagent volumes proportional to, but smaller than, those used in Standard Methods were added to Kimax 25 x 150 mm culture tubes with teflon-lined caps. Tubes were then inverted several times for mixing, placed in a 150°C oven for two hours and cooled in a waterbath. The appearance of Cr III in standards, blanks, and samples was determined by absorbance readings at 600 mm on a Bausch and Lomb Spectronic 70 spectrophotometer. Culture tubes were placed directly in the spectrophotometer after cooling to room temperature.

Fiber Analysis

The lignin value found for the algae samples was determined by the 72% sulfuric acid method and was designated as $lignin_g$. The analysis was performed using a Labconco Crude Fiber Reflex Unit, Thermolyne hot plate and a Tecator Fiber Tec #1010 heat extractor.

EXPERIMENTAL RESULTS

SEPARATION AND CONCENTRATION OF ALGAE

The data gathered in the first experiment are presented in Table 3. Total suspended solids and volatile suspended solids were determined for the reactor influent and effluent on the 5th, the 8th and the last day (day 12) of the experiment. The hydraulic retention time was based on a reactor volume of 1000 mℓ (media and liquid).

TABLE 3. TSS AND VSS DATA FOR INFLUENT AND EFFLUENT OF THE REACTOR USED IN THE FIRST ALGAE SEPARATION EXPERIMENT.

Day	Influent TSS (mg/ℓ)	Influent VSS (mg/ℓ)	Effluent TSS (mg/ℓ)	Effluent VSS (mg/ℓ)	HRT (hr)
5	12	12	6	6	37
8	27	22	26	19	1
12	41	33	10	18	10

Prior to day 5 the hydraulic retention time was kept at 37 hours. Between day 5 and day 10 the HRT was brought down to 5 hours, then to 1 hour for short periods of time. This was done only for short periods of time because the VS removal efficiency dropped significantly at these short retention times. At day 10 the HRT was adjusted to 10 hours, and on day 12 final measurements were conducted. On day 12, a representative sample was taken from the layer of flocculated material on top of the bed. The volatile solids concentration in this layer was found to be 10.6 g/ℓ.

In the second experiment the two reactors were either run parallel or in series during 40 days of experimentation at different HRT's with algae from the facultative treatment pond. Results are summarized in Tables 4 and 5.

TABLE 4. TURBIDITY DATA IN INFLUENT AEROBIC EFFLUENT AND ANAEROBIC EFFLUENT OF HARVESTING UNIT. UNITS OPERATING IN SERIES-- AEROBIC/ANAEROBIC.

Day	Influent (ppm SiO_2)	Aerobic Effluent (ppm SiO_2)	Anaerobic Effluent (ppm SiO_2)	Hydraulic Retention Time (hr)
1	15	5	6	2
2	15	11	11	1
3	15	9	8	1
4	72	31	27	2
5	62	23	27	2
6	50	17	17	2

TABLE 5. TSS AND VSS DATA IN INFLUENT, AEROBIC EFFLUENT AND ANAEROBIC EFFLUENT OF HARVESTING UNIT. UNITS OPERATING IN SERIES AEROBIC/ANEROBIC

Day	Influent TSS (mg/ℓ)	Influent VSS (mg/ℓ)	Aerobic Effluent TSS (mg/ℓ)	Aerobic Effluent VSS (mg/ℓ)	Anaerobic Effluent TSS (mg/ℓ)	Anaerobic Effluent VSS (mg/ℓ)	Hydraulic Retention Time (hr)
1	20	15	3	2	7	8	2
5	77	46	30	23	33	28	2

During the first 6 days the reactors were operated in series: the effluent of the aerobic reactor was pumped into the anaerobic reactor. From day 6 through day 16 the effluent of the anaerobic reactor was pumped into the aerobic one. Data from these experiments are shown in Tables 6 and 7.

TABLE 6. TURBIDITY DATA IN INFLUENT, ANAEROBIC EFFLUENT AND AEROBIC EFFLUENT OF HARVESTING UNIT. UNITS OPERATED IN SERIES-ANAEROBIC/AEROBIC.

Day	Influent (ppm SiO_2)	Anaerobic Effluent (ppm SiO_2)	Aerobic Effluent (ppm SiO_2)	Hydraulic Retention Time (hr)
7	54	48	21	2
8	44	50	25	2
9	39	44	19	2
12	79	102	11	12
13	53	86	14	12
14	40	54	12	12
15	56	56	14	12
16	34	46	11	12

TABLE 7. TSS AND VSS DATA IN INFLUENT, ANAEROBIC EFFLUENT AND AEROBIC EFFLUENT OF HARVESTING UNIT. UNITS OPERATED IN SERIES-ANAEROBIC-AEROBIC.

Day	Influent		Anaerobic Effluent		Aerobic Effluent		Hydraulic Retention Time (hr)
	TSS (mg/ℓ)	VSS	TSS (mg/ℓ)	VSS	TSS (mg/ℓ)	VSS	
8	52	49	57	49	25	20	2
12	83	69	139	107	16	11	12
16	55	34	49	24	40	29	12

From day 17 through 23 the reactors were run in parallel at an HRT of 8 hours. Starting at day 24 the reactors were set in series again with the effluent of the anaerobic reactor pumped into the aerobic one. Because of unexplained poor removal of the solids the feed pump was turned off for 4 days beginning at the 25th day, while recycling was maintained. During these 23 days turbidity data were taken (Table 8).

TABLE 8. TURBIDITY DATA IN INFLUENT AND EFFLUENT OF HARVESTING UNITS FOR DAYS 17 TO 40.

Day	Influent (ppm SiO_2)	Anaerobic Effluent (ppm SiO_2)	Aerobic Effluent (ppm SiO_2)	Hydraulic Retention Time (hr)
17	66	58	29	8
18	66	72	29	8
19	60	56	34	8
20	129	91	51	8
21	129	124	93	8
22	142	122	117	8
23	139	124	114	8
24	154	154	100	8
25	Feed pump off			
27	106	11	38	-
28	86	6	17	-
29	New HRT: 56 hours			
31	81	14	9	56
32	58	11	36	56
35	New HRT: 24 hours			
39	97	28	12	24
40	105	34	6	24

On day 23 a representative sample was taken from the layer of flocculated material on top of the bed in the aerobic reactor. The volatile solids concentration was found to be 6 g/ℓ (0.6%). No sample was taken from the anaerobic one. Figure 10 shows the pH variation during this study for the influent, aerobic reactor and anaerobic reactor.

DIGESTION OF ALGAE

Results from the control reactor show that it took nearly 40 days before reactor start-up occurred (Figure 11). The total gas production data were reduced to methane production data at standard temperature and pressure (STP) with the following equations:

$$V_{stp} = V_{35°C} \cdot \frac{T_{stp}}{T_{35°C}}$$

where, V_{stp} = volume at standard temperature and pressure (0°C, 1 atmosphere)

$V_{35°C}$ = volume at 35°C

T_{stp} = temperature at STP in °R

$T_{35°C}$ = temperature of 35°C in °R

and methane production = V_{stp} • % methane

The average CH_4 content during day 14 through 55 was 61% with a minimum of 57% and a maximum of 68%.

Figures 12 and 13 show the methane production, pH, volatile solids for influent and volatile solids for effluent during day 14 through 55 for the two AAFEB reactors. The above equations were used to convert total gas production to methane production at STP. Because of the difference in operating reactor I and II, only reactor II was able to give useful data for daily gas production and volatile solids destruction.

Prior to effluent withdrawal, the liquid above the bed in reactor II was mixed thoroughly using an increased recycle rate. The solids in the liquid above the bed in reactor I were allowed to settle for 5 minutes before liquid was withdrawn. This was intended to increase the SRT in the unit.

The average CH_4 content for reactor I during day 14 through 55 was 63% with a minimum of 50% and a maximum of 73%. For reactor II, the average CH_4 content during day 14 through 55 was 60%, with a minimum of 48% and a maximum of 69%.

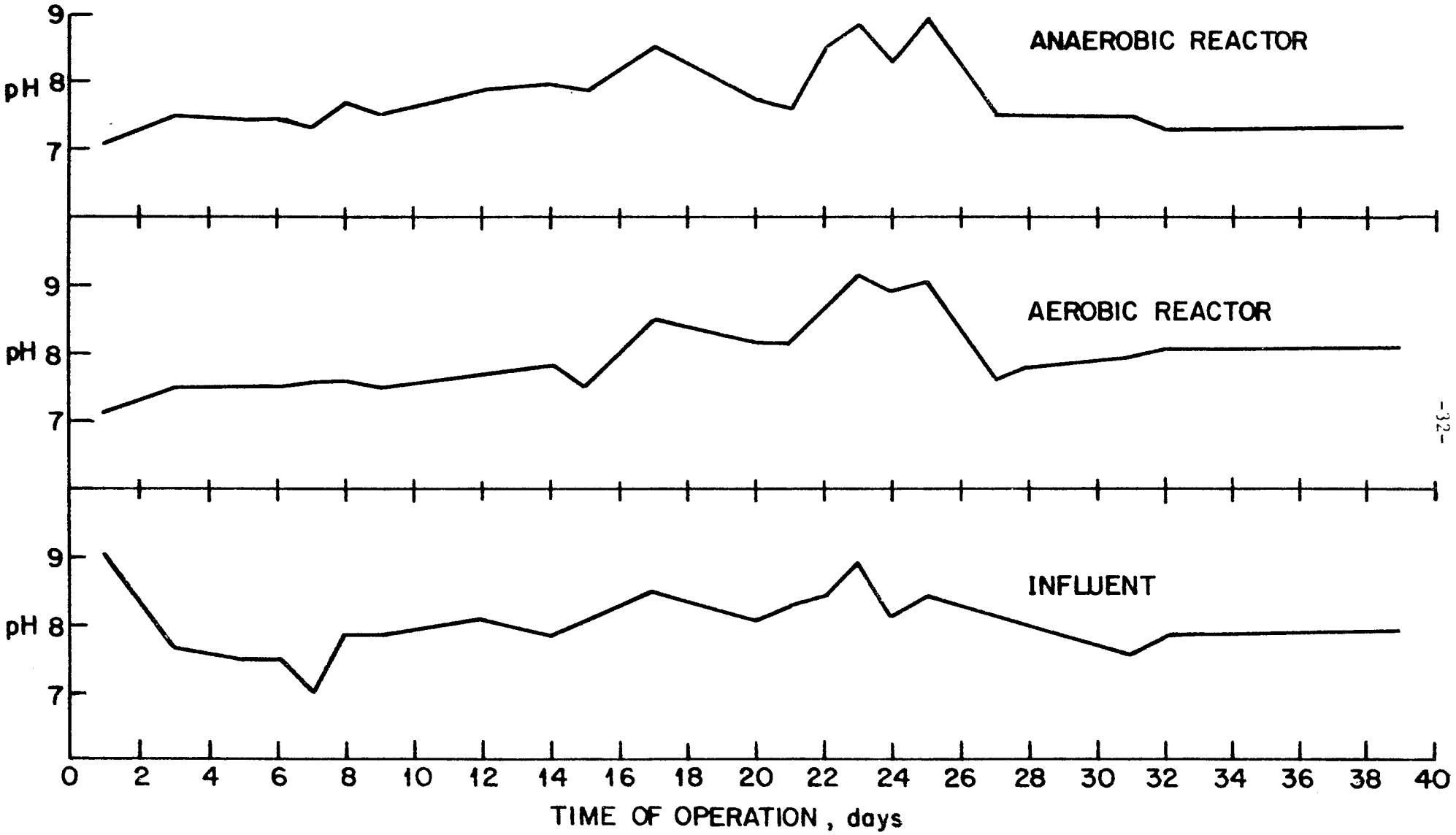

Figure 10. pH variation during 40-day experiment in influent, aerobic reactor and anaerobic reactor.

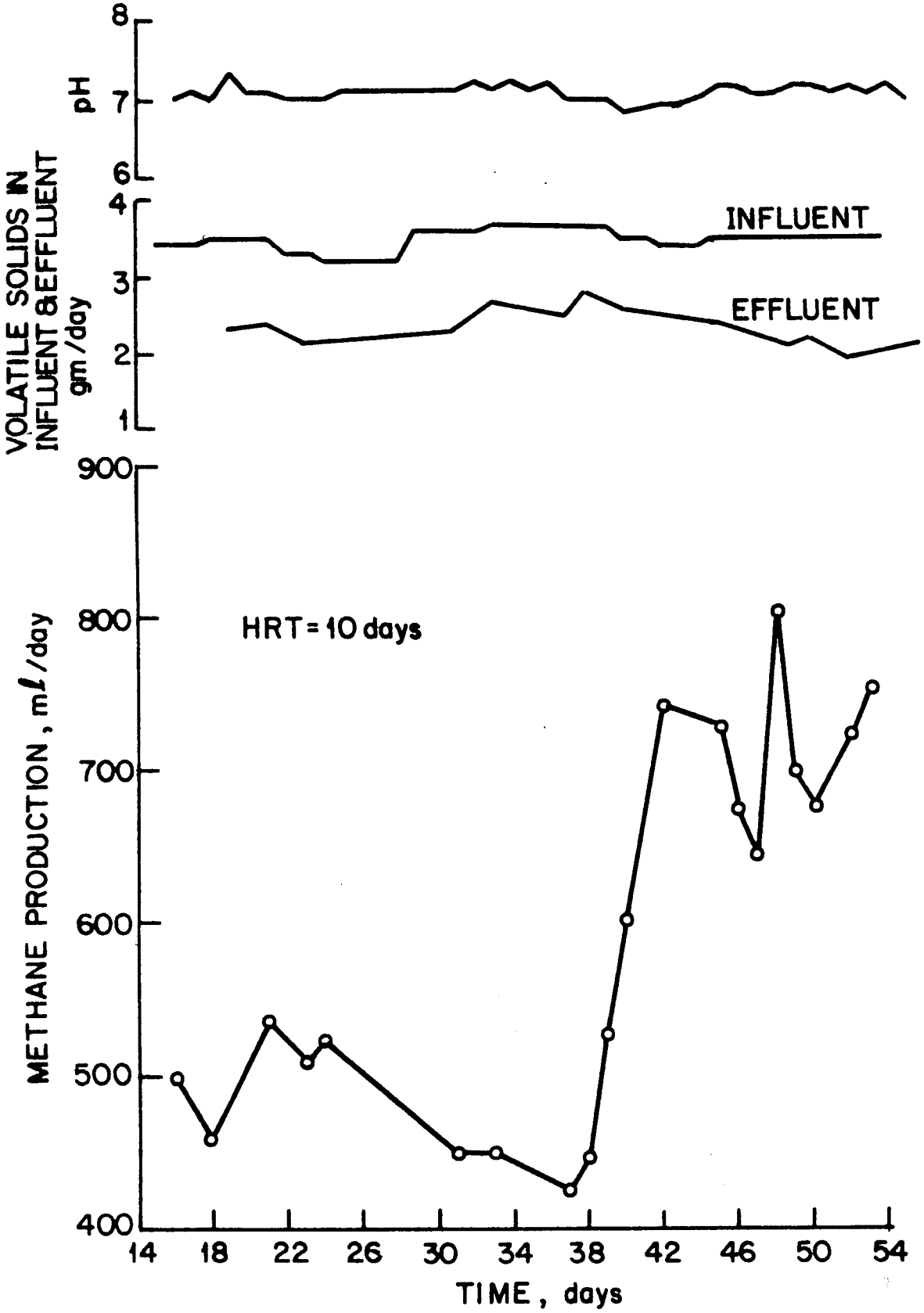

Figure 11. Methane production, volatile solids removal and pH variation for the control reactor.

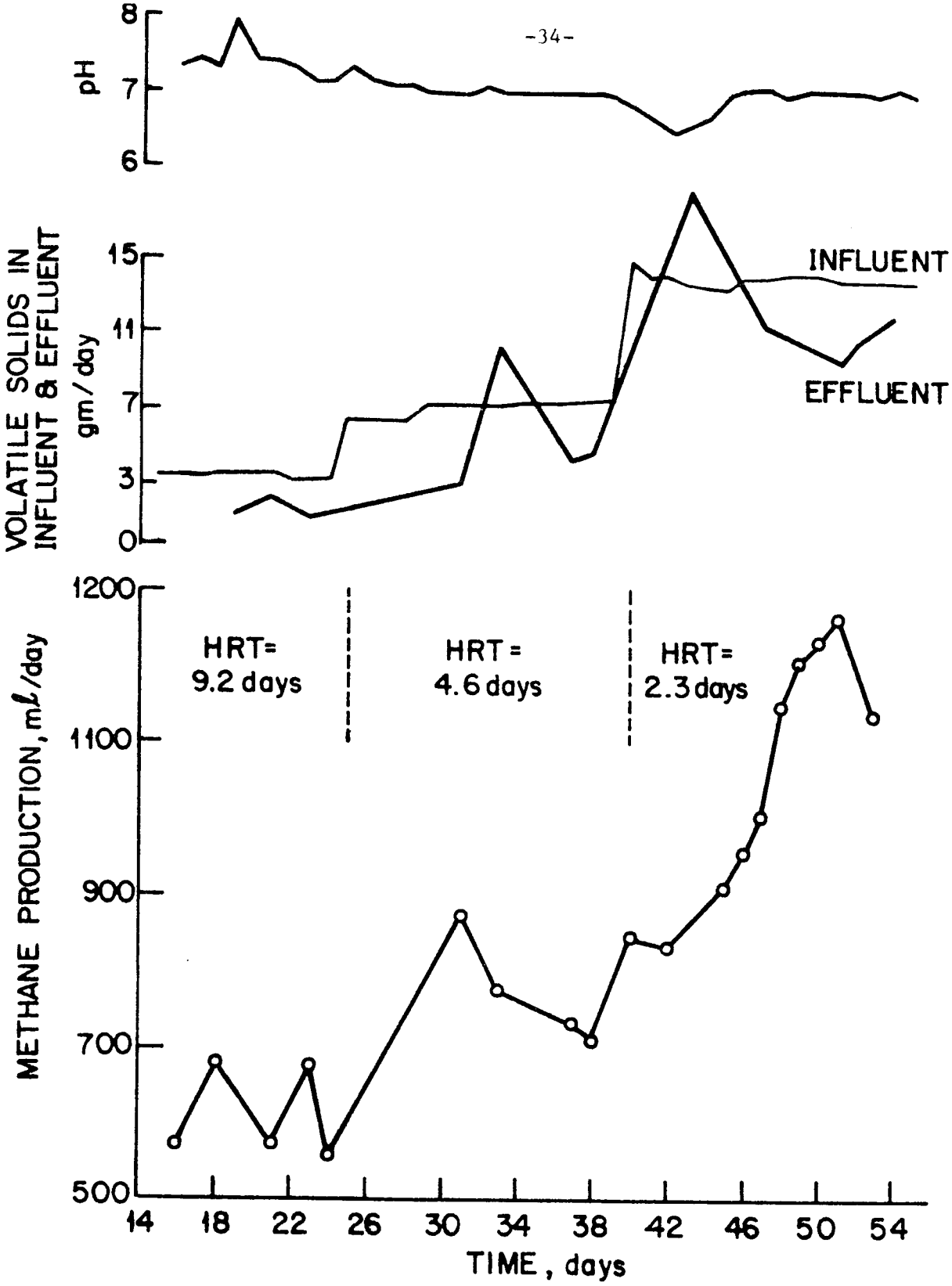

Figure 12. Methane production, volatile solids removal and pH variation for AAFEB Reactor I.

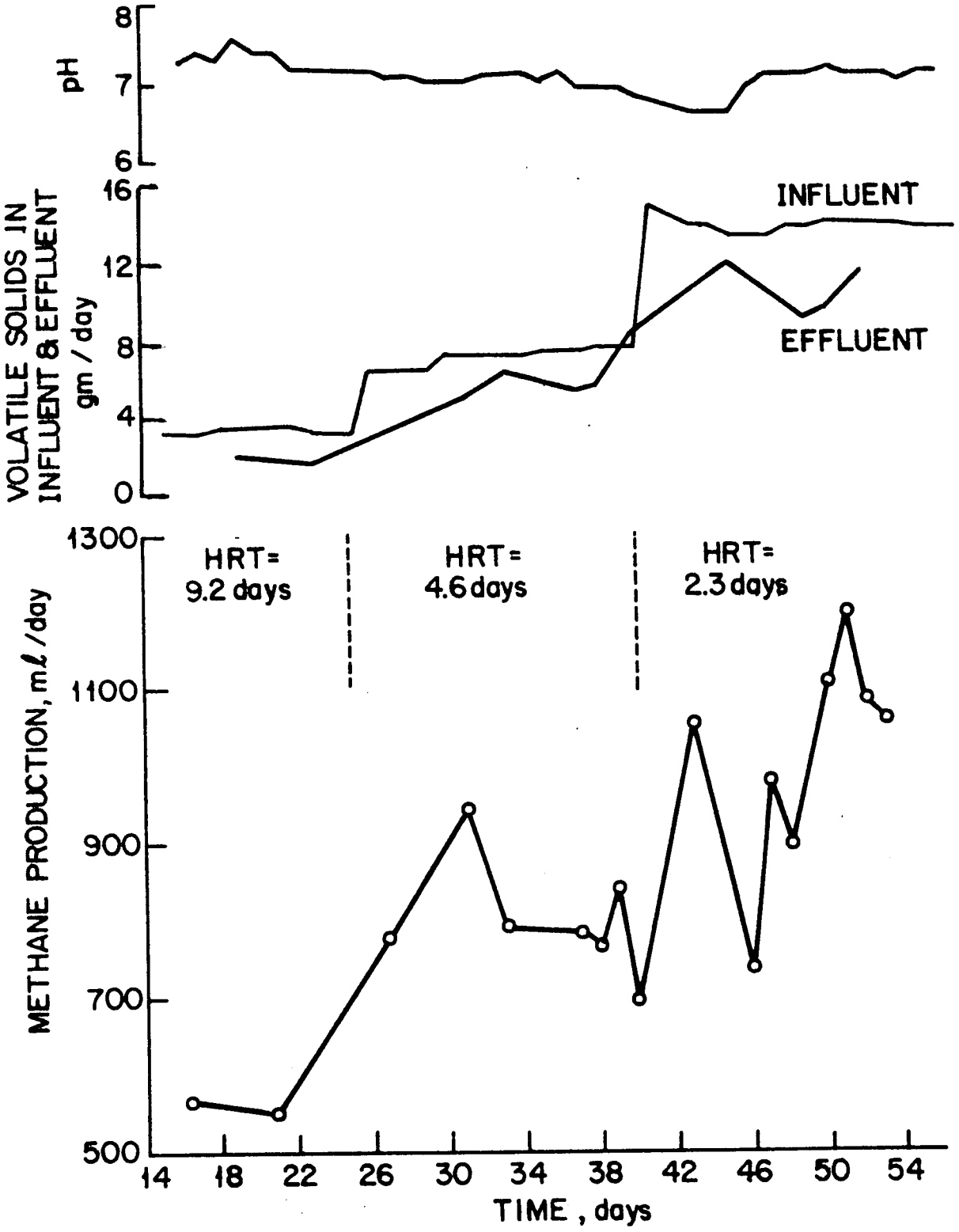

Figure 13. Methane production, volatile solids removal and pH variation for AAFEB Reactor II.

TABLE 9. PERFORMANCE OF AAFEB REACTOR II AND COMPLETE-MIX REACTOR ON AN ALGAL SUBSTRATE WITH A 2% T.S. COMPOSITION AT 35°C..

Reactor Type (and volume)	HRT, days	Average Volatile Solids, Loading Rate Kg VS/m^3-day	Average Volumetric Gas Production Rate,			Average Volatile Solids Destruction %
			ℓCH_4/ gm VS introd.-day	ℓ CH_4/ℓ reactor day	ℓCH_4/ gm VS destr.-day	
Complete Mix (days 15-40)	10.0	1.16	0.14	0.16	0.45	26
Control (3 ℓ) (days 41-55)	10.0	1.16	0.21	0.24	0.55	38
AAFEB (2.75ℓ	9.2	1.13	0.16	0.20	0.35	44
including	4.6	2.35	0.11	0.28	0.54	23
clarifier zone)	2.3	4.62	0.08	0.40	0.31	28

A comparison between the control reactor and reactor II was made for volatile solids loading rate, for volatile solids destruction, and for volumetric gas production rate per gm volatile solids introduced, per gm volatile solids destroyed and per volume of reactor (Table 9). Two sets of data for the control reactor are shown--data gathered prior to day 40 and after day 40. The data presented for reactor II were taken during the last 5 days of each HRT. Reactor I was not included in these calculations because of its nonfavorable mixing conditions.

It should be noted here that when the HRT of the two AAFEB reactors was decreased to 2.3 days, the pH dropped to 6.5 to 6.7. Therefore, some alkalinity was added in the form of $NaHCO_3$ to the feed solution before feeding to the reactor. As a result the alkalinity of the effluent was increased to ~2500 mg/ℓ (as $CaCO_3$).

REFRACTORY FRACTION OF THE ALGAE USED FOR DIGESTION

For the first method three samples were taken to determine the refractory fraction of the algae, after 55, 152 and 229 days of operation (Figure 14). Graphically, it was found that, based on three data points the refractory fraction was 0.375.

The second method gave the following results: of the volatile part of the algae, 69.5% was cell soluble material and 30.5% was cell wall. $Lignin_s$ = 10.0%, cellulose = 13.5%, hemicellulose = 8.6% and $Lignin_k$ = 8.4%. Total protein content was 55.8%. Using equation 1, a refractory fraction of 0.450 was determined.

DISCUSSION AND CONCLUSIONS

Typical results obtained from efforts to separate dilute microalgal cultures from wastewater to subsequently digest them are shown in Table 10. In several cases, exceptionally clear effluent was obtained with one pass system through a facultative anaerobic expanded bed. However, it appeared that short-term aerobic treatment was necessary to encourage efficient algal separation. In all cases, the algae separated within the expanded bed gradually and then gradually aggregated into large flocculated particles. These particles gradually migrated to the top of the expanded bed and became a flocculant layer on top of the reactor.

Measurements of the solids concentration from the flocculated layer indicated that the concentration varied from .6% to greater than 1% solids, or greater than 10 g/ℓ. It is interesting to note that for much of the testing period with several of the reactors the influent concentrations varied from a low of around 20 to 30 mg/ℓ suspended solids up to a maximum concentration of around 130 mg/ℓ. Thus the increase in concentration to greather than 10,000 mg/ℓ reflects a three- to four fold order of magnitude in concentration in the system. The short retention time required to achieve this concentration is encouraging. Furthermore, the fact that the algae aggregates in a dense, flocculated mass on top of the expanded bed leads to the possibility of easy transfer and storage prior to further digestion. These results tend to indicate that this is the first process capable of

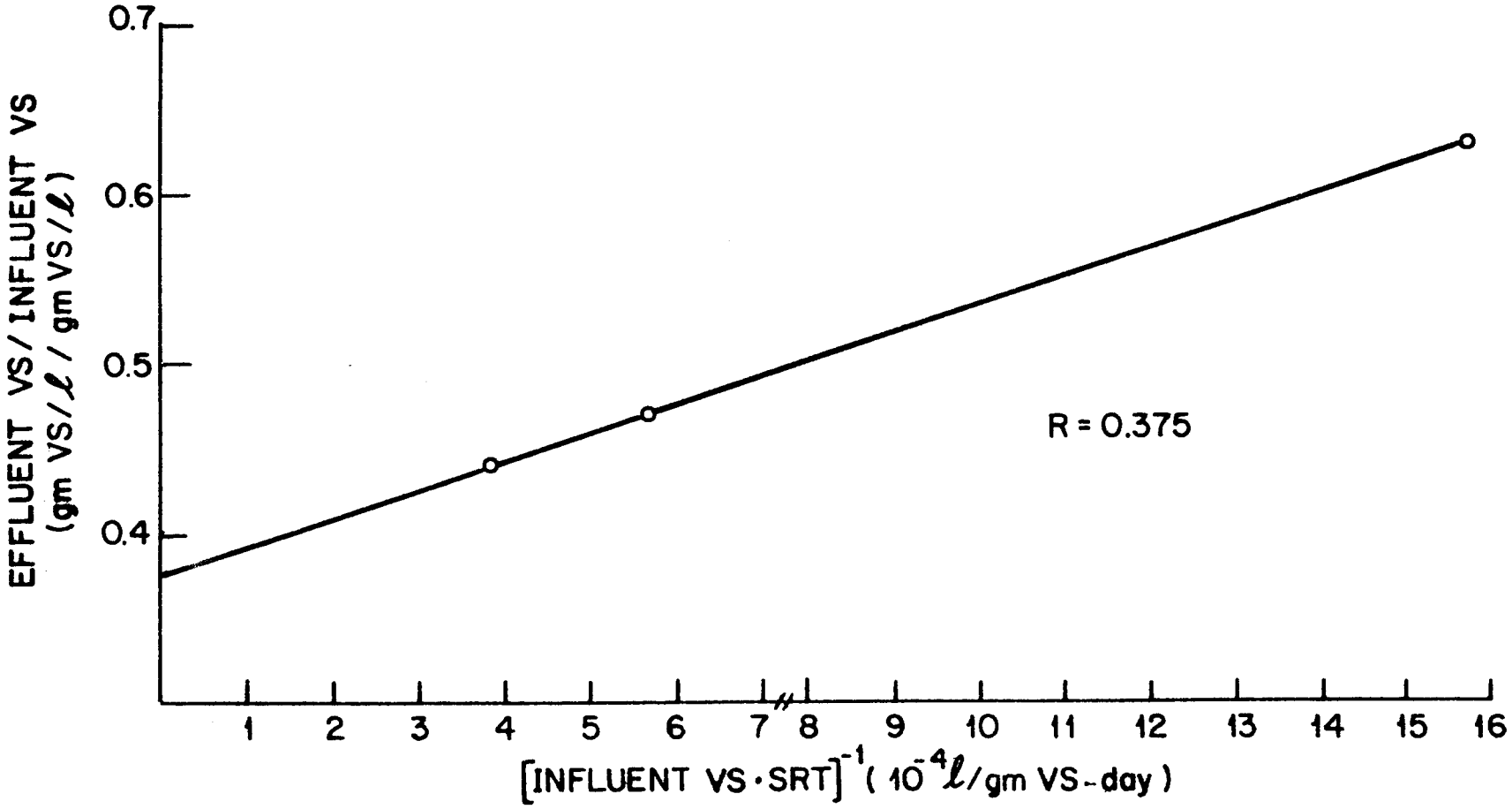

Figure 14. Graphical analysis of VS data to determine R, algae refractory fraction.

TABLE 10. TYPICAL RESULTS OBTAINED WITH ALGAE HARVESTING AND CONVERSION WITH THE EXPANDED BED PROCESS.

I. Separation and Concentration

A. Type System

	HRT (hr)	Algal Suspended Solids Removal Eff. (%)	Effluent Solids (mg/ℓ)
Single Anaerobic Stage	2	50	6 to 91
Two Stage--Anaerobic-Aerobic	12	75	7 to 51

II. Digestion

HRT = 2.3 days, 35°C, volatile solids conversion = 28%

efficiently harvesting microalgae without the addition of significant quantities of chemicals or without long hydraulic retention times.

Although the digestion experiments were not conducted for long periods of time, the possibility of using the expanded bed to concentrate methane-forming bacteria is demonstrated by the efficient conversion of solids at a retention time of 2.3 days--a retention time significantly lower than the minimum reproduction time for the methanogenic bacteria. The volatile solids conversion of 28% observed at this short retention time compares with that predicted using literature values discussed in the earlier review of completely mixed digesters having retention times of 10 to 30 days at the mesophilic condition. Thus, the digestion studies clearly indicate interesting possibilities for the application of the expanded bed.

The total system suggested by the experiments conducted in this preliminary overview indicates that an algal harvesting system composed of three different expanded bed units could achieve good algal separation. A facultative anaerobic high-rate unit with a retention time of one to two hours followed by a facultative aerobic unit would result in algal accumulation and a flocculated mass at the top of the aerobic unit. This material would then be transferred from the top of the aerobic unit into a temporary storage system that would feed into a mesophilic expanded bed with a retention time on the order of several days. It should be emphasized that this design is far from optimum since the tests completed here are only approximations of the data that could be achieved once a mature biological system is achieved.

In previous testing with the expanded bed it is clear that long acclimation periods for the films are required before equilibrium conditions are approached. It would be estimated that the time of testing for all systems would be 50% of what would normally be thought to be required.

In conclusion, it can be seen that the harvesting, separation and conversion of microscopic algae has received considerable attention in the past. The main obstacle that prevents microscopic algae from serving as a major energy/food/fertilizer source is the efficient and cost-effective collection of these small cells. The results of this study indicate that a simple process can be used to harvest algae from dilute wastewaters and can achieve effluent concentrations lower than secondary standards. These microscopic algae then can be converted efficiently and rapidly by the expanded bed system. The results appear to provide a new collection and digestion process that promises to lower costs to the point where microscopic algae can be used for a wide range of purposes. This study appears to warrant comprehensive investigation of the possibilities for algal separation and conversion to fuel.

REFERENCES

Anthonisen, A. and E. A. Cassell. 1968. Kinetic Model for High-Rate Anaerobic Digestion, In Studies on Chicken Manure Disposal: Part I Laboratory Studies. E. A. Cassell, Ed. Research Report No. 12, N. Y. State Dept. of Health.

Benemann, J. R., et. al. 1977. Cultivation on Sewage of Microalgae Harvestable by Microstrainers. Final Report. Sanitary Eng. Research Lab., Univ. of California, Berkeley.

Benemann, J. R., et. al. 1977. Species Control in Large-Scale Algal Biomass Production. Final Report. Sanitary Eng. Res. Lab., Univ. of California, Berkeley. Prepared for Energy Research and Development Administration Division of Solar Energy Fuels from Biomass Program. SAN/7405-77/1.

Benemann, J. R., et. al. 1978a. Large-Scale Fresh Water Microalgal Biomass Production for Fuel and Fertilizer. Final Report. Sanitary Eng. Res. Lab., Univ. of California, Berkeley. Prepared for the U. S. Dept. of Energy Fuels from Biomass Systems. SAN/0034-1.

Benemann, J. R., et. al. 1978b. Fuels from Microalgae Biomass. Sanitary Eng. Res. Lab., Univ. of California, Berkeley. Presented at 2nd Annual Symposium on Fuels from Biomass, June 20-22, Troy, NY.

Betzer, N., Y. Argaman, and Y. Kott. 1980. Effluent Treatment and Algae Recovery by Ozone-Induced Flotation. Water Research 14, 1003-1009.

Bureau of Environmental Studies. 1975. Department of Environment, Housing and Community Development. Algae Harvesting from Sewage. Environmental Study report No. 1, Australian Government Publishing Service, Canberra.

Burlew, J. S. (ed.). 1953. Algal Culture from Laboratory to Pilot Plant, Carnegie Institute of Washington, Pub. 600, Washington DC.

Chandler, J. A., W. J. Jewell, J. M. Gosset, P. J. Van Soest, J. B. Robertson. 1979. Predicting Methane Fermentation Biodegradability. In: Biotechnology and Bioengineering Symp. No. 10, 93-107.

Cruver, J. E. 1972. Membrane Processes, Chapter 7. In Physiochemical Processes for Water Quality Control. W. J. Weber, (ed.), NY Wiley-Interscience.

DeLatour, C. 1973. Magnetic Separation in Water Pollution Control. Paper presented at the 1973 Intermag Conference. Washington DC, April 24-27.

Dodd, J. C. and J. L. Anderson. 1977. An Integrated High-rate Pond-algae Harvesting System. Progress in Water Technology, 9, 713-726.

Dodd, J. C. 1979. Algae Production and Harvesting from Animal Waste waters. Agricultural Wastes, 1, 23-27.

Dodd, J. C. 1980. Harvesting Algae Grown on Pig Wastes in Singapore. Paper presented at a workshop on High-rate Algae Ponds, Singapore, Feb. 27-29.

Eisenberg, D. M. et. al. 1979. Methane Fermentation of Microalgae. Presented at the First International Symposium on Anaerobic Digestion, Univ. College, Cardiff, U.K., Sept. 18.

Fogg, G. E. 1965. Algal Cultures and Phytoplankton Ecology. The Univ. of Wisconsin Press.

Foree, E. G., W. J. Jewell and P. L. McCarty. 1971. The Extent of Nitrogen and Phosphorus Regeneration from Decomposing Algae. Proceedings, 5th International Water Pollution Research Conf., Pergamon Press.

Foree, E. G. and P. L. McCarty. 1971. The Rate and Extent of Algal Decomposition in Anaerobic Waters. Proceedings, 24th Industrial Waste Conf., Purdue Univ. Engineering Extension Service No. 135. Part I, pp. 13-36.

Goldman, Joel C. 1978. Fuels from Solar Energy: Photosynthetic Systems - State of the Art and Potential for Energy Production. Final Report. Woods Hole Oceanographic Institution, Woods Hole, Mass., Prepared for the Dept. of Energy. COO-4151-2.

Golueke, C. G., W. J. Oswald and H. B. Gotaas. 1957. Anaerobic Digestion of Algae. Applied Microbiology 5, 1.

Golueke, C. G. and W. J. Oswald. 1965. Harvesting and Processing Sewage-grown Planktonic Algae. Y.W.P.C.F. 37(4), 471-498.

Golueke, C. G. and W. J. Oswald. 1965. Y.W.P.C.F. 37, 471 and 478.

Golueke, C. G., et. al. 1964. Harvesting and Processing Sewage Grown Planktonic Algae. Sanitary Eng. Research Lab Report No. 64-8, Univ. of California, Berkeley.

Harris, S. E., D. S. Filip, J. H. Reynolds and E. J. Middlebrooks. 1978. Separation of Algal Cells from Wastewater Lagoon Effluents. Vol. I: Intermittent Sand Filtration to Upgrade Waste Stabilization Lagoon Effluent. Report EPA 600/2-78-033.

Humenik, F. J. 1970. Respiratory Relationships of a Symbiotic Algal-bacterial Culture for Wastewater Nutrient Removal. Biotech. and Bioeng. XII(4), 541.

Imhoff, K. and G. M. Fair. 1940. Sewage Treatment, New York. John Wiley, 220-227.

International Development Research Centre. 1980. Wastewater Treatment and Resource Recovery. Report of a workshop on high-rate algae ponds. Singapore, 27-29 February. Report No. 154e.

Jewell, W. J. and P. L. McCarty. 1971. "Anaerobic Decomposition of Algae". J. Environmental Science and Technology. 5 (No. 10) Oct. pp. 1023-1031.

Jewell, W. J., et. al. 1978. Anaerobic Fermentation of Agricultural Residue: Potential for Improvement and Implementation. U. S. Department of Energy Report No. HCP/T2981-07. Available from the National Technical Information Service.

Jewell, W. J., M. S. Switzenbaum and J. W. Morris. 1981. Municipal Wastewater Treatment with the Anaerobic Attached Microbial Film Expanded Bed Process. J. Water Pollution Control Federation, 53 (4): 482-490.

Kormanik, R. A. and J. B. Cravens. 1979. Cost Effective Algae Removal Possible with Microscreening. Water and Sewage Works 9, 31-35.

Lincoln, E. P. and T. W. Hall. 1980. Critical Factors in the Large-scale Production of Microalgae. Paper presented at a workshop on high-rate algae ponds. Singapore, 27-29 February.

McGarry, M. G., et. al. 1972. Photosynthetic Yields and By-product Recovery from Sewage Oxidation Ponds. Paper presented at the 6th International Water Pollution Research Conference, Jerusalem.

McGarry, M. G., K. S. Ng, N. H. Leung and T. L. Lee. 1974. Wastewater Reclamation under Tropical Conditions. Process Biochem. 9(7), 14-24.

Moellmer, W. O. 1970. Factors Controlling Autoflocculation of Algae and Algal Nutrient Removal, Ph.D. Dissertation, University of California, Berkeley.

Morris, G. R. 1976. "Anaerobic Fermentation of Animal Wastes: A Kinetic and Empirical Design Evaluation", Cornell University, Master of Science Thesis.

Nigam et al. 1980. Application of Chitosan as a Flocculant for the Cultures of the Green Alga: Scendeomus Acutus. Arch. Hydrobid. 88 (3), 378-387.

O'Brien, W. J. and R. E. McKinney. 1979. Removal of Lagoon Effluent Suspended Solids by a Slow-Rock Filter. Report EPA-600/2-79-011.

Oswald, W. J. 1960. Light Conversion Efficiency of Algae Grown in Sewage. Proc. ASCE San. Eng. Div. SA 4:71-95.

Oswald, W. J. 1968. Harvesting and Processing of Waste Grown Microalgae. In: Algae, Man and the Environment. Jackson, D. F. (ed.), Syracuse University Press, New York.

Oswald, W. J. 1976. Pilot Plant High-rate Pond for Study of Waste Treatment and Algae Production. Report for W.H.O., Regional Office for the Western Pacific, United Nations Ave., PO Box 2932, 12115 Manila, Philippines, March 16.

Oswald, W. J. 1977. Method of Waste Treatment and Algae Recovery, U. S. Patent No. 4005546, February 1.

Oswald, W. J. and J. R. Benemann. 1980. Algal-bacterial Systems. In: Biochemical and Photosynthetic Aspects of Energy Production. A. San Pietro (ed.). Academic Press, New York.

Regan, R. W. and R. E. McKinney. 1977. Proc. Wat. Tech. 8(4/5)451-466.

Russell, J. S., E. Y. Middlebrooks and J. H. Reynolds. 1980. Wastewater Stabilization Lagoon-Intermittent Sand Filter Systems. Report EPA-600/2-80-032.

Shelef, G., R. Moraine, A. Meydan and E. Sandbank. 1976. Combined Algae Production Wastewater Treatment and Reclamation Systems. In Schlegel, H. G. and Barnes, J., ed., Microbial Energy Production. Gottingen, Erich Goltze, K. G. 427-442.

Swanson, G. R. and K. J. Williamson. 1980. Rock Filters for Removal of Algae from Lagoon Effluents. Report EPA-600/2-80-038.

Uziel, M., W. J. Oswald and C. G. Golueke. 1975. Solar Energy Fixation and Conversion with Algal Bacterial Systems. Final Report. Sanitary Eng. Research Lab., Univ. Of California, Berkeley.

Wood, J. L. and J. R. O'Callaghan. 1974. "Mathematical Modeling of Animal Waste Treatment". Jour. Agric. Eng. Res., 19:245.