0273-2289/99/76/0193/$12.25

Anaerobic Upflow Fixed-Film Bioreactor for Biomethanation of Salty Cheese Whey

Priti Patel, Chirag Patel, and Datta Madamwar*

Post Graduate Department of Biosciences, Sardar Patel University, Vallabh Vidyanagar 388 120, Gujarat, India

Received December 10, 1996; Accepted July 28, 1997

Abstract

In order to develop a suitable reactor for the biomethanation of high-strength salty cheese whey, the performance of anaerobic upflow fixed-film reactors packed with different support materials, such as charcoal, gravel, brick pieces, pumice stones, and PVC pieces, has been studied. The charcoal-bedded reactor gave the best performance, with the maximum gas production (3.3 L/L digester/d) and an enriched methane content (69% CH_4). Temperature and hydraulic retention time were optimized, with the ultimate aim of improving biomethanation. Maximum gas production (3.3 L/L digester/d) was achieved at a hydraulic retention time of 2 d at 40°C.

Index Entries: Salty cheese whey; biomethanation; anaerobic digestion; methane; energy; fixed-film bioreactor; charcoal.

Introduction

The dairy industry, like most other agroindustries, generates strong wastewaters characterized by high chemical oxygen demands (COD), reflecting their organic matter content. Dairies in India are no exception, and a large number of dairies dispose of their waste, especially cheese whey, into the environment in enormous quantities. Cheese whey has high organic strength, with a COD of about 70–80 g/L, and its disposal remains a major problem. Anaerobic digestion of cheese whey offers an excellent approach toward considerations related to both energy conservation and pollution control *(1–3)*.

Cheese whey, a byproduct from the manufacture of cheese and casein, represents a potential energy source, and possesses several advantages if it is subjected to anaerobic digestion. Cheese whey produces a renewable effluent slurry that has a high manure capacity and converts organic

*Author to whom all correspondence and reprint requests should be addressed.

residues, thus reducing the environmental pollution. The major advantages of this process are low cost, high energy efficiency, and process simplicity, compared to other wastetreatment methods. However, despite the waste reduction and energy potential, anaerobic digestion is not a highly regarded process in the dairy industry, chiefly because of the problems of the anaerobic microbes' slow growth rate, which requires longer hydraulic retention times (HRT), and because of poor process stability using conventional mixed reactors. In order to solve this problem, several high-rate configurations have been developed for treating soluble wastewater at relatively short HRT of less than 4 d *(4–6)*. Among the advanced technologies of anaerobic digestion, the fixed-film reactor allows efficient digestion of high and low-strength (in terms of suspended solids and organic materials) soluble waste at shorter HRT *(7–11)*.

Previous study has shown gas production of 6.7 L/L digester/d, with enriched methane content (72%), when sweet cheese whey (COD = 70 g/L) was subjected to fixed-film digestion at HRT of 2 d, using charcoal as bedding material *(4)*. Charcoal was found to be long-lasting with good strength *(4)*.

There is further growing interest in maximizing the extraction of methane for energy recovery from acidic cheese whey, especially whey that has high Na^+ concentrations (concentration of NaCl is about 4–6% [w/v]). The presence of high concentrations of sodium ions have been found to be detrimental to the performance of anaerobic bioreactors *(12)*. This problem could be overcome by diluting cheese whey with total dairy wastewater having a low total-solid (TS) content. Admixing total dairy wastewater with salty cheese whey reduces the salt content of the whey and supports the growth of methanogens. This dilution would also provide an operational advantage because of the reduction in the TS content. As such, no detailed information is available for processing salty cheese whey for biomethanation. This has prompted the authors to seek the appropriate support material for anaerobic fixed-film reactor that could handle salty cheese whey after dilution with total dairy waste.

Materials and Methods

Resources

All chemicals used were of AR grade. Salty cheese whey and total dairy wastewater were collected from AMUL Dairy of Anand in India (cheese-manufacturing unit). Salty cheese whey characteristics are given in Table 1. The salty cheese whey was diluted with total dairy wastewater in order to get final TS of 3% (w/v) (salty cheese whey and total dairy waste in the proportion of 1:2 was normally found to be suitable for getting TS of 3% [w/v]). The total dairy wastewater was alkaline, having low COD and TS content. The pH of the influent was adjusted to 7 by lime before loading into the reactor.

Table 1
Characteristics of Salty Cheese Whey

Components	g%
Lactose	4.5–5.0
Protein	0.6–0.7
Salt (NaCl)	4.0–6.0
Total solids	10.0–12.0[a]
Volatile solids	4.0–5.0
COD	6.0–8.0

[a]This was because of high salt content; otherwise, without salt, TS was 4.5–6.0% (w/v). pH 4.5–5.5.

In addition to the above, salty cheese whey also contains varying amount of minerals and water-soluble vitamins.

Experimental Setup

Laboratory-scale anaerobic-upflow, fixed-film reactors were used. Each reactor was made of a glass column with a void volume of 1 L, id of 50 mm, od of 55 mm and having a packing height of 900 mm and packing volume of 1.5 L. Reactors were packed with one of the bedding materials (charcoal, brick pieces, pumice stones, gravel, and PVC pieces having an average size of $5 \times 5 \times 5$ mm) as biological support. Biofilms were allowed to develop on bedding materials for 60 d, using effluent from another operating whey reactor, after mixing it with samples obtained from loose sand saturated with salty water from near-coastal areas (obtained from about 60 cm below the surface) as initial inoculum. The initial inoculum was slowly replaced by salty cheese whey diluted with total dairy wastewater in the proportion of 1:2, which was filtered through muslin cloth to remove the flocs formed after adjusting the pH to 7 with lime. Salty cheese whey and total dairy wastewater in the proportion of 1:2 was found to be suitable for getting TS of 3% (w/v). This dilution has also provided an operational advantage because of reduction in the TS. The reactors were operated at 40°C unless indicated, and all other conditions were kept constant for all reactors. Steady-state condition was attained in 45 d. Judgment was based on constant gas production and constant COD of effluent. All reactors were run for 60 d after reaching steady-state condition. The feed was pumped upward continuously and flow rate was adjusted with the aid of a peristaltic pump (Gilson Miniplus 3 Model, Villiess-le-Bel, France). The reactors were operated at desired HRT.

Experiments were also performed to study the effect of temperature on process performance. The temperature ranged from 20 to 50°C, in 5°C-increments between the reactors. Desired temperature ± 1°C was maintained using a thermostat.

Experiments were repeated using a larger bioreactor having a void volume of 10 L for scale-up study after finding out the optimum HRT and best bedding material for maximum energy recovery and process performance.

Analytical Methods

Gas production was measured from the displacement of acidified saturated salt solution, making due correction for atmospheric pressure and temperature. Gas composition was analyzed with a Sigma-make (Baroda, India) gas–liquid chromatograph equipped with 2-m long stainless-steel Porapak R (80–100 mesh) column at 40°C and thermal conductivity detector. Nitrogen was used as a carrier gas at a flow rate of 40 mL/min. The temperatures of injector and detector were kept at 125°C. Feed and effluent samples were routinely analyzed for pH, fatty acids (volatile fatty acid [VFA]), COD, TS, and volatile solids (VS), according to standard procedure *(13)*.

Various types of fatty acids were analyzed using the same chromatogram as above, with a 10% FFAP column and flame ionization detector. The column temperature was maintained at 170°C and injector and detector temperatures at 250°C. Nitrogen served as a carrier gas. Identification and percentage of different fatty acids were based on comparison of retention time and peak area of unknowns with standard amounts of each acids *(14)*. Experiments were repeated four times and average data were recorded with standard deviation.

Results and Discussion

Table 2 exhibits the data of steady-state performance of fixed-film reactors operated at 40°C and hydraulic retention time (HRT) of 2 d using different support materials, such as charcoal, pumice stones, brick pieces, gravel, and PVC pieces, under the same set of conditions (in terms of height, width, total volume, void volume, amount of bedding material, and their average size).

Among the reactors examined, the highest total gas production was obtained in the reactor packed with charcoal (3.3 L/L of digester/d) and the lowest was in the PVC-packed reactor (2.1 L/L of digester/d). In addition to high gas production, the charcoal-bedded reactor showed improved methane production, presented as methane percentage (69%) and methane yield. As shown in Table 2, a maximum methane yield of about 0.20 L/g of COD utilized in charcoal-bedded reactor was obtained, compared with methane yield of 0.14 L/g of COD utilized in the PVC-bedded reactor. Methane content was the highest in the charcoal fixed-film reactor, followed by pumice stone, brick pieces, gravel, and PVC pieces.

This may be because charcoal provides a better surface for the attachment of methanogens and other anaerobic bacteria, in comparison to other supporting materials, resulting in good biofilm development on the sup-

Table 2
Steady-State Profile of Anaerobic Upflow Fixed-Film Reactors Operated at 40°C with Hydraulic Retention Time of 2 d, Using Different Bedding Materials

Bedding materials	Charcoal	Pumice stone	Brick pieces	Gravel	PVC pieces
Total gas production (L/L of digester/d)	3.300	2.800	2.700	2.300	2.100
Gas production (L/g of TS Load/d)	0.220	0.180	0.180	0.150	0.140
Methane (%)	69.000	65.000	65.000	63.000	62.000
Methane yield (L/g of COD utilized)	0.200	0.170	0.180	0.150	0.140
Total volatile fatty acids (g/L)	1.100	1.220	1.250	1.570	1.610
Acetic acid (g/L)	0.560	0.709	0.800	0.910	1.010
Propionic acid (g/L)	0.162	0.210	0.232	0.323	0.346
Isobutyric acid (g/L)	0.120	0.088	0.095	0.105	0.096
Butyric acid (g/L)	0.227	0.213	0.123	0.232	0.158
Effluent COD (g/L)	7.500	9.600	10.800	10.800	12.000
COD removal (%)	75.000	68.000	64.000	64.000	60.000
pH	6.900	6.800	6.700	6.700	6.400

COD in influent was 30 g/L.

porting media. Charcoal works in two ways: by providing surfaces for the growth of anaerobes, and also by providing adsorption sites where substrates can accumulate, thereby providing high localized substrate concentrations and producing a more favorable growth environment for bacteria substrate system *(15)*. Similar observations were previously recorded with activated charcoal as adsorbent, with improved gas production from the anaerobic digestion of water hyacinth *(16)*. It is also known that all anaerobic digesters have a pH optimum around 7.0, and that a pH below 6 adversely affects waste degradation and methane formation *(17)*. In this study, pHs were almost unaffected, even in the face of the variation in bedding materials, except with PVC pieces. Process performance can also be judged by residual COD. As seen in Table 2, residual COD values were lowest in the charcoal-packed reactor, indicating greater biodegradation in this reactor. Table 2 also shows the data on percentage of COD removal in the steady-state anaerobic-upflow fixed-film reactors. This parameter is important because values suggest the bacterial efficiency of converting COD into methane gas *(18)*. COD removal was highest (75%) in the charcoal-bedded reactor, followed by those packed with pumice stones, brick pieces, gravel, and PVC pieces. Concentration of organic acids is a sensitive parameter used to determine fermentor stability *(19)*. Average volatile fatty acid concentrations ranged from 1.61 g/L in PVC pieces packed-bed reactor to 1.10 g/L in charcoal-bedded reactor, indicating proper balancing between the formation of acids and their consumption. At 2 d HRT, it also

Table 3
Steady-State Profile of Anaerobic Upflow Fixed-Film Reactor Operated at 40°C and Bedded with Charcoal at Different Hydraulic Retention Times

HRT (d)	1	2	3	4	5
Total gas production (L/L of digester/d)	4.480	3.300	2.300	1.710	1.440
Gas production (L/g of TS Load/d)	0.149	0.220	0.230	0.228	0.240
Methane (%)	65.000	69.000	68.000	66.000	63.000
Methane yield (L/g of COD utilized)	0.140	0.200	0.190	0.180	0.180
Total volatile fatty acids (g/L)	1.620	1.100	1.210	1.280	1.370
Acetic acid (g/L)	0.970	0.560	0.643	0.805	0.823
Propionic acid (g/L)	0.400	0.162	0.210	0.253	0.260
Isobutyric acid (g/L)	0.099	0.120	0.117	0.076	0.130
Butyric acid (g/L)	0.151	0.227	0.240	0.146	0.157
Effluent COD (g/L)	10.200	7.500	6.300	6.000	5.700
COD removal (%)	66.000	75.000	79.000	80.000	81.000
pH	6.500	6.900	6.900	6.800	6.300

COD in influent was 30 g/L.

shows low levels of propionate and butyrate, and in turn favors a methane-forming step of the digestion process, which is the slowest and the most rate-limiting stage.

Thus, overall best performance was exhibited by the reactor packed with charcoal. Experiments were then carried out to determine the optimum HRT, by increasing it from 1 to 5 d.

Table 3 illustrates the data of total gas and methane production at various HRTs. When HRT was increased from 1 to 5 d, there was a gradual decrease in gas production (expressed as L of gas/L of digester/d). This was mostly because organic load decreased with the increase in HRT. However, when the same data were recorded as L of gas/g of TS loaded, a significant increase in gas production, with higher methane percentage, was observed when HRT was increased from 1 to 2 d. However, further increase in HRT did not proportionately increase gas production with TS intake. Methane content also increased from 65 to 69% when HRT was increased from 1 to 2 d, and methane content declined thereafter. Similarly, methane yield improved with increase in HRT, reaching maximum value of 0.20 L/g of COD utilized, indicating highest methane production at 2 d HRT. In general, process stability was evident by lower volatile fatty acids *(19)*. Table 3 shows that the concentration of volatile fatty acids decreased with an increase in HRT from 1 to 2 d. Maximum process stability, identified by the lowest volatile fatty acid concentrations, was achieved at 2 d HRT (volatile fatty acids = 1.10 g/L). Process performance can be judged from residual COD values, which were reduced when HRT was increased from

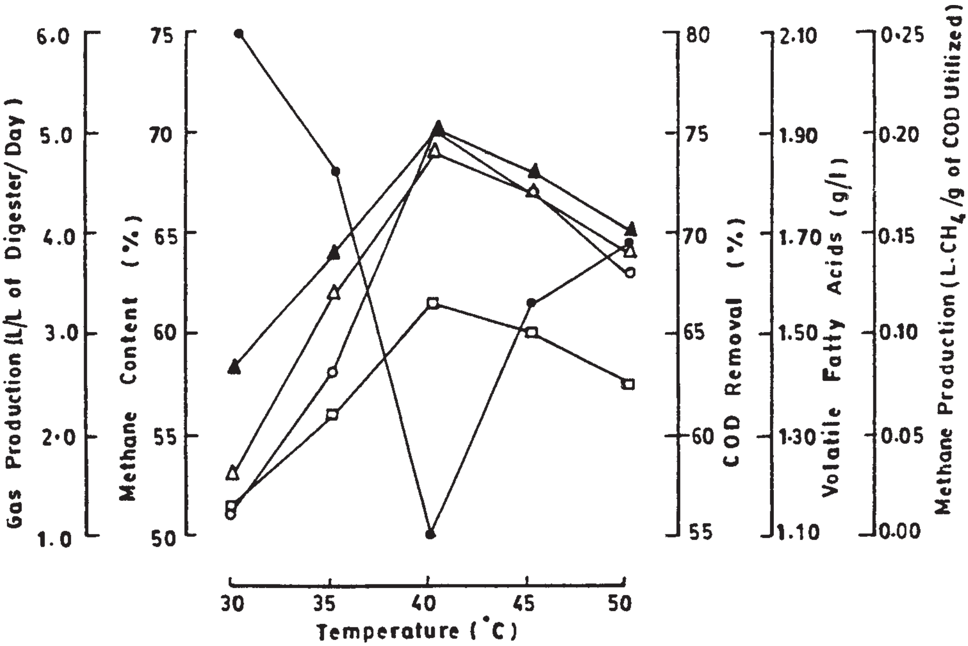

Fig. 1. Steady-state profile of anaerobic upflow fixed-film reactor packed with charcoal at 2 d hydraulic retention time under various temperatures. □, gas production (L/L digester/d); △, methane content (%); ○, COD removal (%); ●, volatile fatty acids (g/L); ▲, methane yield (L/g of COD utilized). COD of influent was 30 g/L.

1 to 2 d. Percentage of COD removal also improved when HRT increased from 1 to 2 d; however, on further increase in HRT, the increase in percentage of COD removal was low. Similar observations were also recorded during the study of biomethanation of sweet cheese whey under the same set of conditions *(4)*. Wildenauer and Winter *(20)* achieved 95% COD reduction of acidic whey at 5 d HRT, using porous clay beads. However, the authors were successful in achieving rapid anaerobic digestion of salty cheese whey by using a charcoal fixed-film reactor at a comparatively shorter HRT (2 d), with a COD loading of 15 g/L/day.

All other conditions were optimized using a charcoal-bedded reactor operated with 2 d HRT. Temperature data are presented in Fig. 1. When the temperature was increased from 30 to 40°C, there was a gradual increase in gas production and higher methane content, followed by a decrease between 45 and 50°C. A similar trend was also observed by Pfeffer *(21)* (in anaerobic digestion of domestic refuse). Thus, gas production showed a temperature optimum at 40°C. This temperature not only resulted in maximum gas production, but also higher methane content and improved digestion, as evident from COD and volatile fatty acid values, indicating better process performance, high process stability, and high rate of biodegradation. This study proves that the anaerobic fixed-film reactor bedded with charcoal can be utilized for high-strength dairy waste management and energy recovery by operating at 40°C with a HRT of 2 d.

Table 4
Steady-State Profile of Large-Scale (10 L Void Volume) Anaerobic Upflow Fixed-Film Reactor Operated at 40°C at 2 d HRT, Using Charcoal as Bedding Material as Scale-Up Study

Total gas production (L/L digester/d)	Gas production (L/g of TS Load/d)	Methane (%)	Methane yield (L CH_4/g of COD utilized)	Volatile fatty acids (g/L)					Effluent COD (g/L)	COD removal (%)	pH
				Total VFA	Acetic acid	Propionic acid	Isobutyric acid	Butyric acid			
3.5	0.233	68	0.21	1.16	0.672	0.155	0.121	0.212	7.65	74.5	6.8

COD in influent was 30 g/L.

In order to scale up and to examine the efficiency of fixed-film reactors, the above studies were linked with the demonstration of a 10-L, anaerobic-upflow fixed-film reactor bedded with charcoal. Table 4 describes the performance of the steady-state anaerobic reactor operated at 40°C with HRT of 2 d. The data from the larger reactor exhibited a similar pattern of performance, with high process stability, high rate of biodegradation, and improved gas production with enriched methane content.

References

1. Guiot, S. R., Safi, B., Frigon, P. M., Mulligan, C., Tremblay, R., and Samson, R. (1995), *Biotechnol. Bioeng.* **45,** 398–405.
2. Yan, J. Q., Lo, K. V., and Liao, P. H. (1989), *Biol. Wastes* **27,** 289–305.
3. Desai, M., Patel, V., and Madamwar, D. (1994), *Environ. Pollution* **83,** 311–315.
4. Patel, P., Desai, M., and Madamwar, D. (1995), *J. Ferment. Bioeng.* **79,** 398–399.
5. Balaguer, M. D., Vicent, M. T., and Paris, J. M. (1991), *Environ. Technol.* **12,** 1167–1173.
6. Callender, I. J. and Barford, J. P. (1983), *Proc. Biochem.* **18,** 24–30.
7. Denac, M. and Dunn, I. J. (1988), *Biotechnol. Bioeng.* **32,** 159–173.
8. Liao, P. H. and Lo, K. V. (1985), *Biotechnol. Bioeng.* **27,** 266–272.
9. Murray, W. D. and van den Berg, L. (1981), *Appl. Environ. Microbiol.* **42,** 502–505.
10. Sarner, E. (1995), *Vatten* **51,** 304–313.
11. Keith, B. and Tom, S. (1996), *Biotechnol. Bioeng.* **49,** 601–610.
12. Backus, B. D., Clanton, C. J., Goodrich, P. R., and Morris, H. A. (1988), *Trans. ASAE* **31,** 1274–1283.
13. American Public Health Association (1989), *Standard Methods for the Examination of Water and Wastewater*, 17th ed. American Public Health Association, Washington, D.C.
14. Ranade, D. R. and Gadre, R. V. (1988), in *Microbiological Aspects of Anaerobic Digestion, Laboratory Manual*, Ranade, D. R. and Gadre, R. V., eds. Maharashtra Association for the Cultivation of Science, Pune, India, pp. 79–93.
15. Andrews, G. F. and Tien, C. (1981), *Al. Chem. J.* **27,** 396–402.
16. Madamwar, D., Patel, V., and Patel, A. (1992), *Bioresource Technol.* **40,** 179–181.
17. De Beer, D., Huisman, J. W., Heuuel, V., and Ottengraf, S. P. P. (1992), *Water Res.* **26,** 1329–1336.
18. Britz, T. J. and Van der Merwe, W. (1993), *Biotech. Lett.* **15,** 755–760.
19. Cobb, S. A. and Hill, D. T. (1991), *Trans. ASAE* **34,** 2564–2571.
20. Wildenauer, F. X. and Winter, J. (1985), *Appl. Microbiol.* **22,** 367–372.
21. Pfeffer, J. T. (1974), *Biotechnol. Bioeng.* **16,** 771–787.