0273-2289/94/4701—0089$04.40

Continuous Propagation of *Kluyveromyces fragilis* in Cheese Whey for Pollution Potential Reduction

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Received April 8, 1993; Accepted November 2, 1993

ABSTRACT

A 25-L working volume, upright cylindrical fermenter made of stainless steel was used to investigate cheese whey yeast fermentation for pollution potential reduction. The effluent total and soluble chemical oxygen demand, total and volatile solids, and total Kjeldahl and ammonium nitrogen concentrations were significantly affected by the hydraulic retention time, air flow rate, and mixing speed. The system removal efficiencies were 15.90–58.61%, 25.20–69.33%, 12.43–49.90%, 9.22–51.77%, 1.66–10.06%, and 54.82–72.22% for total chemical oxygen demand, soluble chemical oxygen demand, total solids, volatile solids, total Kjeldahl nitrogen, and ammonium nitrogen, respectively, depending on the hydraulic retention time, air flow rate, and mixing speed used.

Index Entries: Continuous fermentation; cheese whey; pollution potential; COD; solids; nitrogen.

INTRODUCTION

The largest pollutants in waste waters from diary food plants are whey from cheese production operations followed by wash water and pasteurization water. Manufacturing of cheese from either whole or skim milk produces a greenish yellow fluid known as whey. Disposal of cheese whey is a major problem for the dairy industry. Of the 1.7 billion kg of liquid whey produced annually in Canada, 17% was dumped in sewers and 26%

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was disposed of on land (*1*). Jones observed that for each kilogram of cottage cheese manufactured, a total of 0.2 kg of biochemical oxygen demand is either pumped into the river, waste water treatment plants, or other water bodies, or loaded on the land (*2*).

Because of its high biochemical oxygen demand value (40,000–60,000 ppm), whey may disrupt the biological process of sewage disposal plants (*3–5*). Hacking estimated that 3870 L/d of raw whey (equivalent to the output of a small creamery) discharged into the municipal sewage system can impose a load equivalent to 1800 people (*6*). Also, long-term land disposal of whey can cause environmental pollution problems as reported by several authors (*1,7*). The nitrogen in cheese whey is water soluble and may be subject to leaching into ground water, thus becoming a threat to human and animal health. Several authors reported that continuous land disposal of cheese whey can endanger the physical and chemical structure of the soil, decrease the crop yield, and lead to serious water pollution problems (*8–10*).

However, aerobic yeast fermentation of cheese whey can be used to reduce its pollution potential as well as to produce single cell protein (*11,12*). Mickle reported 82–92% of the reduction in the chemical oxygen demand (COD) of cottage cheese whey was removed by *K. fragilis* under aerobic conditions (*13*). Ghaly and Singh reported that aerobic fermentation of cheese whey using the yeast *K. fragilis* was successful in reducing the total chemical oxygen demand by 42%, the soluble chemical oxygen demand by 65%, the total solids by 53%, and the ammonium nitrogen by 90% (*14*). A reduction of up to 81% in chemical oxygen demand was observed by Burgess when using tower fermentation for the production of yeast protein from lactose permeate (*15*).

OBJECTIVES

The aim of the study was to investigate the effectiveness of yeast fermentation for the reduction of the pollution potential of cheese whey as measured by the reductions in COD, solids, and nitrogen concentrations under various hydraulic retention times, air flow rates, and mixing speeds.

EXPERIMENTAL APPARATUS

The experimental apparatus used in the study (Fig. 1) consists of a bioreactor, an air supply system, and a whey feeding and effluent removal system.

A 25-L working volume, upright cylindrical fermenter was constructed of 6.35-mm thick stainless steel material. The fermenter was designed with a water jacket for temperature control. The ratio of the diameter to

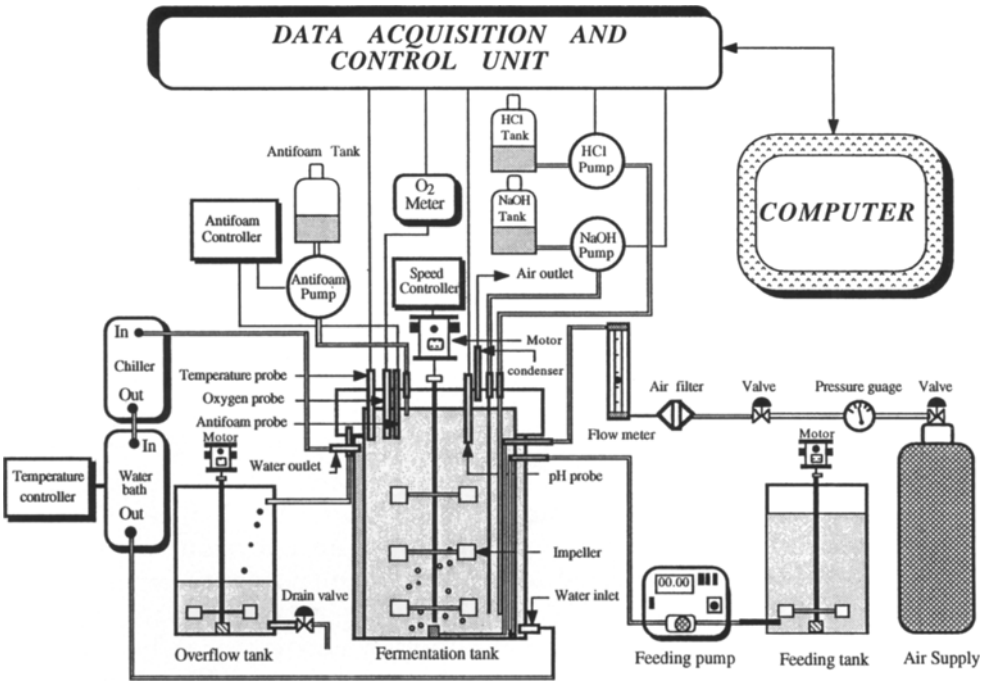

Fig. 1. Experimental apparatus.

height chosen for the final design of the fermenter was about 1:2. The fermenter was designed to be completely mixed and, hence, a stainless steel mixing shaft of 10-mm diameter and 700-mm length was installed through the center of the lid. Three, six-vaned flow disk impellers were used to ensure adequate mixing in the vertical direction. A heavy duty electric motor (G. K. Heller, model no. 99P46-18) with a gear head reducer was mounted on the lid of the fermenter to drive the mixing shaft and impellers. The electric motor was connected to a speed controller (Cole-Parmer, cat. no. J-004407-00). Four baffles (positioned every 90°) were used in the fermenter to reduce vortexing and to improve the top-to-bottom turnover.

Compressed air (Medigas Atlantic Limited cat. no. T100172) was supplied to the fermenter through a flowmeter with high-resolution valve using tygon tubing of 10-mm diameter. The moisture and impurities in the air were less than 10 and 2 ppm, respectively. A microfilter (Cole-Parmer, cat. no. L-29701-00) was used to reduce the risk of cross-contamination. The air was introduced from the bottom of the fermenter through a gas diffusion stone (Fisher Scientific, cat. no. 11-139B).

The whey feeding and effluent removal system included a cheese whey feeding tank (6-mm thickness, 298-mm diameter and 560-mm height) made from PVC material, a feeding pump and an effluent collection tank. An electric motor (Franklin Electric, model no. 6105121401) with a speed-reducing gear arrangement was mounted on the lid of the feeding tank to

drive the mixing shaft and a flat-bladed turbine impeller of 150-mm diameter. A pump with a variable speed motor (1–100 rpm) and a precision optical tachometer (Cole Parmer, DIGI-STALTIC Digital Flow Controller cat. no. N-07525-30) was used to feed the cheese whey into the reactor. The effluent collection tank was constructed from PVC material (the thickness, diameter, and height of tank were 6, 298, and 463 mm, respectively). A plastic tube of about 20-mm diameter connected the fermenter outlet to the lid of the overflow collection tank.

EXPERIMENTAL PROCEDURE

Whey Collection, Storage, and Preparation

The cheese whey was obtained from Farmer's Cooperative Dairy Plant in Truro, Nova Scotia. No dilution or concentration was made to the cheese whey used in this study. It was pumped from the plant storage tank into 60-L plastic containers, which were sealed and transported to the Cold Storage Facility of the Biotechnology Laboratory at the Technical University of Nova Scotia in Halifax where they were stored at −25°C until required. Some characteristics of the cheese whey used in this study are presented in Table 1. These analyses were performed according to the procedures described in the Standard Methods for the Examination of Water and Wastewater (*16*). Prior to placing the cheese whey into the fermenter, it was allowed to completely thaw at room temperature for 24 h.

Inoculum Preparation

Fifteen liters of raw cheese whey were first pasteurized in several 4-L reagent bottles by heating the whey to 70°C for 45 min and suddenly cooling it to 1°C for 60 min. The processes of heating and cooling were repeated three times. The pasteurized cheese whey was transferred to several 250-mL sterilized Erlenmeyer flasks (150-mL per flask). The yeast culture was then transferred from a previously prepared stock culture (*17*) to the pasteurized cheese whey in the sterilized Erlenmeyer flasks (two Petri dishes of pure culture of *K. fragilis* were added to each flask containing 150-mL pasteurized cheese whey). The Erlenmeyer flasks were capped with nonabsorbent cotton plugs and mounted on a controlled environment reciprocating shaker. The shaker was operated at a speed of 250 rpm for 48 h. Following the 48-h growth period, 15,000 mL of the yeast cultures were collected from the flasks, transferred to a large container, and then mixed thoroughly. The yeast culture was then divided into three equal parts of 500 mL each and was then stored at 4°C until needed.

Table 1
Some Characteristics of the Raw Cheese Whey Used in the Study

Characteristics	Measured value
Total solids	63835 mg/L
Fixed	9100 mg/L
Volatile	54738 mg/L
Percent volatile	85.74 %
Percent fixed	14.26 %
Suspended solids	22150 mg/L
Fixed	185 mg/L
Volatile	21965 mg/L
Percent volatile	99.16 %
Percent fixed	0.84 %
Total Kjeldahl nitrogen	1690 mg/L
Ammonium	270 mg/L
Organic	1420 mg/L
Percent organic	84.02 %
Percent ammonium	15.98 %
Total chemical oxygen demand	74220 mg/L
Soluble	59640 mg/L
Insoluble	14580 mg/L
Percent soluble	80.36 %
Percent insoluble	19.64 %
Lactose	5.0 %
pH	4.9

Experimental Protocol

The fermenter and all accessories (mixing system, tubing, and feeding tank) were chemically sterilized using 2% potassium meta-bisulfite solution, and then washed with hot water several times before starting the experiment in order to remove any chemical traces. The fermenter was filled to two-thirds of the working volume (16.0 L) with cheese whey and then immediately inoculated using 5.0 L of inoculum.

The air flow (1 vvm), turbine drive (200 rpm), computer, and data acquisition-and-control unit were started immediately. The temperature of the fermentation medium was maintained at 33 ± 2°C by circulating cooling water through the fermenter jacket using a temperature-controlled water bath. The pH was maintained at 4.5 ± 0.2 with the aid of a computer-based pH measurement-and-control system (*18*). The dissolved

Table 2
The Experimental Factors and Their Levels

Retention time, h	Air flow rate, vvm	Mixing speed, rpm
12.0	1.0	200.0
		400.0
		600.0
	3.0	200.0
		400.0
		600.0
18.0	1.0	200.0
		400.0
		600.0
	3.0	200.0
		400.0
		600.0
24.0	1.0	200.0
		400.0
		600.0
	3.0	200.0
		400.0
		600.0

oxygen concentration was continuously monitored. The remaining 9 L (to a full capacity) were made up with a continuous addition of cheese whey at a hydraulic retention time of 24 h (a flow rate of 1.04 L/h) for 2 d. When the fermenter reached the steady state condition (constant COD, solids, nitrogen, and lactose concentrations), 12 samples were collected and analyzed every 12 h for 6 d. The hydraulic retention time, mixing speed, and air flow rate were changed according to Table 2 and the same procedure was used for samples collection and analysis during the steady state condition of all the hydraulic retention time, air flow rate, and mixing speed combinations.

RESULTS AND DISCUSSION

Chemical Oxygen Demand

For each experimental run, the mean, standard deviation, and coefficient of variation of both the total and soluble chemical oxygen demand of the effluent were calculated (Table 3). The COD data indicated that the fermenter was operating at the steady state condition for all hydraulic

Table 3
The Effluent Chemical Oxygen Demand Concentrations at Various Hydraulic Retention Times, Air Flow Rates, and Mixing Speeds[a]

Retention time, h	Air flow rate, vvm	Mixing speed, rpm	TCOD			SCOD		
			Mean, mg/L	SD, mg/L	CV, %	Mean, mg/L	SD, mg/L	CV, %
12	1	200	62413	617	1.0	44608	426	1.0
		400	58300	797	1.4	40802	731	1.8
		600	54849	555	1.0	37573	636	1.7
	3	200	59712	568	0.9	43427	454	1.1
		400	52524	389	0.7	37500	328	0.9
		600	49967	571	1.1	34655	474	1.4
18	1	200	55992	567	1.0	36378	622	1.7
		400	50346	664	1.3	32369	320	1.0
		600	48110	409	0.9	28610	397	1.4
	3	200	51088	602	1.2	33298	444	1.3
		400	47817	378	0.8	28952	423	1.5
		600	45941	518	1.1	25244	481	1.9
24	1	200	45517	638	1.4	25240	259	1.1
		400	39731	937	2.4	21556	661	3.1
		600	35825	321	0.9	18850	405	2.1
	3	200	41069	448	1.1	22188	378	1.7
		400	34033	347	1.0	18290	285	1.6
		600	30720	452	1.5	15290	345	2.3

[a]The values are the average of 12 determinations.
Raw whey total chemical oxygen demand = 74,220 mg/L.
Raw whey soluble chemical oxygen demand = 59,640 mg/L.
SD = Standard deviation.
CV = Coefficient of variation.

retention times, air flow rates, and mixing speeds as there was no fluctuation in the performance of the fermenter (CV in the range of 0.8–3.1%). Figure 2 shows the total and soluble chemical oxygen demand of the effluent at various hydraulic retention times, air flow rates, and mixing speeds. Generally, increasing the hydraulic retention time and/or the air flow rate and/or the mixing speed decreased the effluent total and soluble chemical oxygen demand.

The total and soluble chemical oxygen demand reductions were calculated at all hydraulic retention times, air flow rates, and mixing speeds. The results shown in Fig. 3 indicated that the total and soluble chemical oxygen demand reductions increased as the hydraulic retention time and/or air flow rate and/or mixing speed increased. For all hydraulic retention times, air flow rates, and mixing speeds, the reductions in the SCOD were higher than the reductions in TCOD. This indicated that some of the soluble material were synthesized into insoluble microbial cells. The reductions

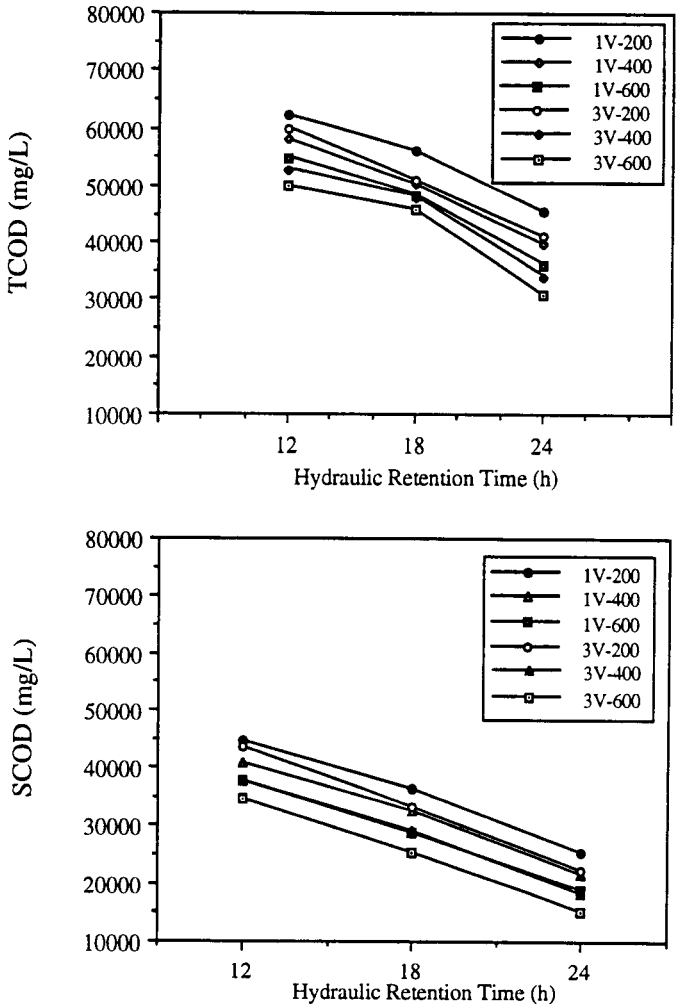

Fig. 2. Effect of hydraulic retention time on the effluent total and soluble chemical oxygen demand at various air flow rates and mixing speeds.

in the total and soluble chemical oxygen demand varied from 15.90–53.61% and from 25.20–74.36% for the total and soluble chemcial oxygen demand, respectively. Burgess reported that increasing the air flow rate from 0.5 L/min to 3.0 L/min increased the reduction in the TCOD from 71 to 81% (*15*). Vananuvat and Kinsella reported a COD reduction of 60% under a continuous culture condition (*19*). Ghaly and Singh reported that increasing the hydraulic retention time from 6 to 24 h increased the reduction in the SCOD and TCOD from 6.03 to 64.41% and from 3.83 to 42.08%, respectively (*14*).

Solids

For each experimental run, the mean, standard deviation, and coefficient of variation of the total and fixed solids were calculated. The volatile

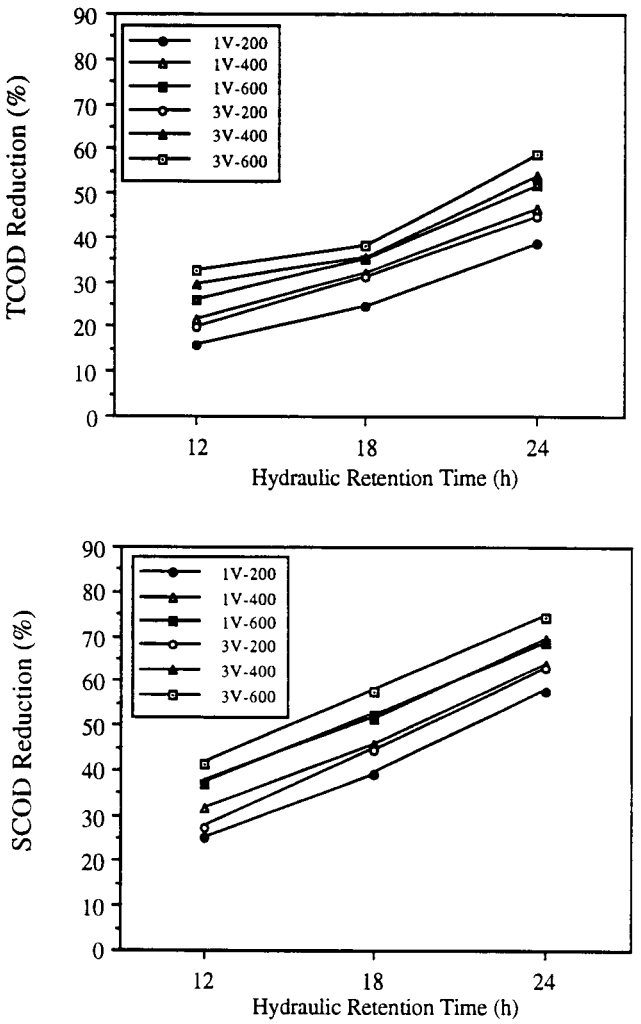

Fig. 3. Effect of hydraulic retention time on the total and soluble chemical oxygen demand reductions at various air flow rates and mixing speeds.

solids were calculated by subtracting the values of the fixed solids from those of the total solids. The results are shown in Table 4. The relatively low values of the coefficient of variation indicated that the fermenter was operating at the steady state condition and there was no fluctuation in the performance of the fermenter (CV in the range of 0.8–2.8%). Figure 4 shows the effluent total, fixed, and volatile solids concentrations at various hydraulic retention times, air flow rates, and mixing speeds. Generally, increasing the hydraulic retention time and/or the air flow rate and/or the mixing speed decreased the effluent solids.

The total, fixed, and volatile solids reductions were calculated at all hydraulic retention times, air flow rates, and mixing speeds. The results shown in Fig. 5 indicated that the total and volatile solids reductions increased as the hydraulic retention time and/or air flow rate and/or mixing

Table 4
The Effluent Solids Concentrations at Various Hydraulic Retention Times, Air Flow Rates, and Mixing Speeds[a]

Retention time, h	Air flow rate, vvm	Mixing speed, rpm	Total solids			Fixed solids			Volatile solids	
			Mean, mg/L	SD, mg/L	CV, %	Mean, mg/L	SD, mg/L	CV, %	mg/L	%
12	1	200	55,903	271	0.5	6217	169	2.7	49,686	88.88
		400	49,788	413	0.8	6330	109	1.7	43,458	87.29
		600	45,622	603	1.3	6112	47	0.8	39,510	86.60
	3	200	50,136	499	1.0	6052	62	1.1	44,084	87.83
		400	46,851	459	1.0	6205	84	1.4	40,645	86.75
		600	43,053	344	0.8	6100	62	1.0	36,952	85.49
18	1	200	51,011	837	1.6	6114	98	1.6	44,897	88.01
		400	46,603	242	0.5	6072	69	2.8	40,532	86.97
		600	43,098	509	1.2	5863	132	2.3	37,235	86.40
	3	200	49,543	603	1.2	6029	55	0.9	43,513	87.83
		400	44,140	477	1.1	6107	60	1.0	38,033	86.16
		600	40,088	233	0.6	6130	86	1.4	33,957	84.71
24	1	200	33,757	708	2.1	5716	228	3.9	28,041	83.07
		400	32,888	780	2.4	5882	79	1.4	27,006	82.12
		600	32,273	358	1.1	5867	163	2.8	26,406	81.82
	3	200	33,099	291	0.9	5899	108	1.8	27,200	82.18
		400	32,587	700	2.1	5710	81	1.4	26,877	82.48
		600	32,003	636	2.0	5876	71	1.2	26,128	81.64

[a]The values are the average of 12 determinations.
Raw whey total solids = 63835 mg/L.
Raw whey fixed solids = 9100 mg/L.
Raw whey volatile solids = 54735 mg/L.
SD = Standard deviation.
CV = Coefficient of variation.

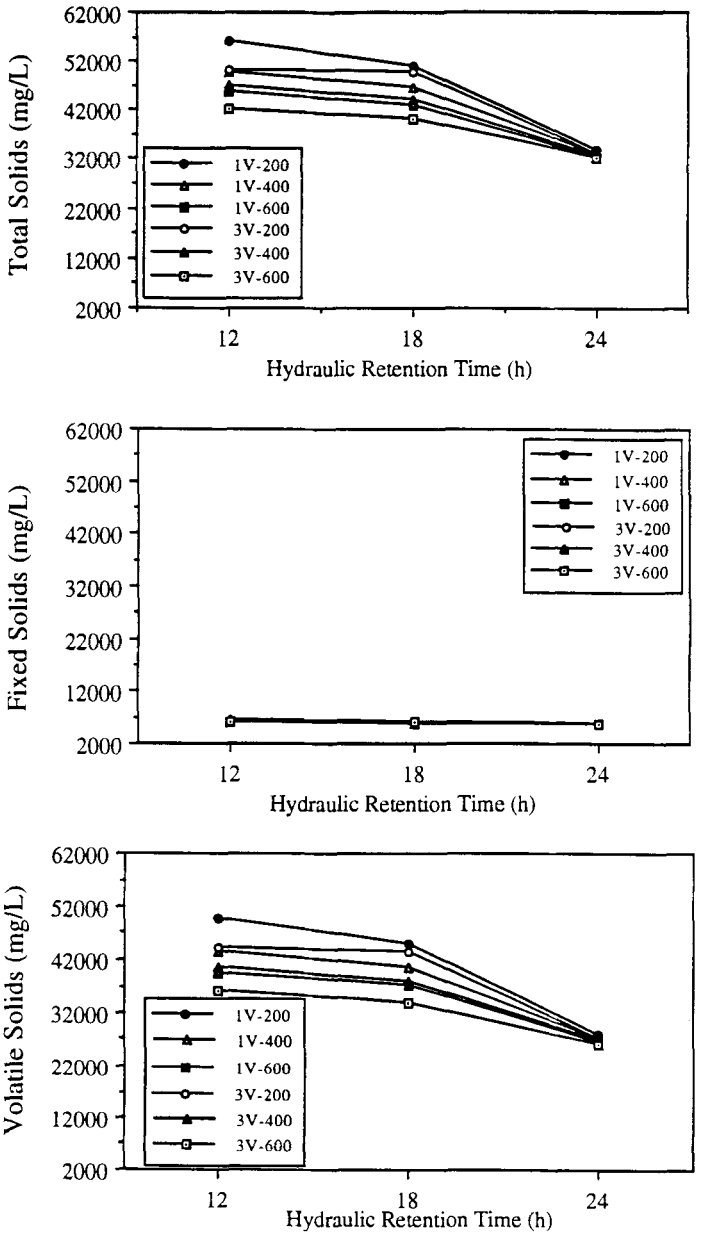

Fig. 4. Effect of hydraulic retention time on the effluent total, fixed, and volatile solids concentrations at various air flow rates and mixing speeds.

speed increased. The reductions varied from 12.43–49.90% and from 9.22–52.27% for the total and volatile solids, respectively. Ghaly and Singh reported solids reductions of 36.97–52.82 and 43.84–62.42 for the total and volatile solids, respectively (*14*). The average value of the fixed solids in the effluent was 6015 mg/L, which is (33%) lower than that of the influent

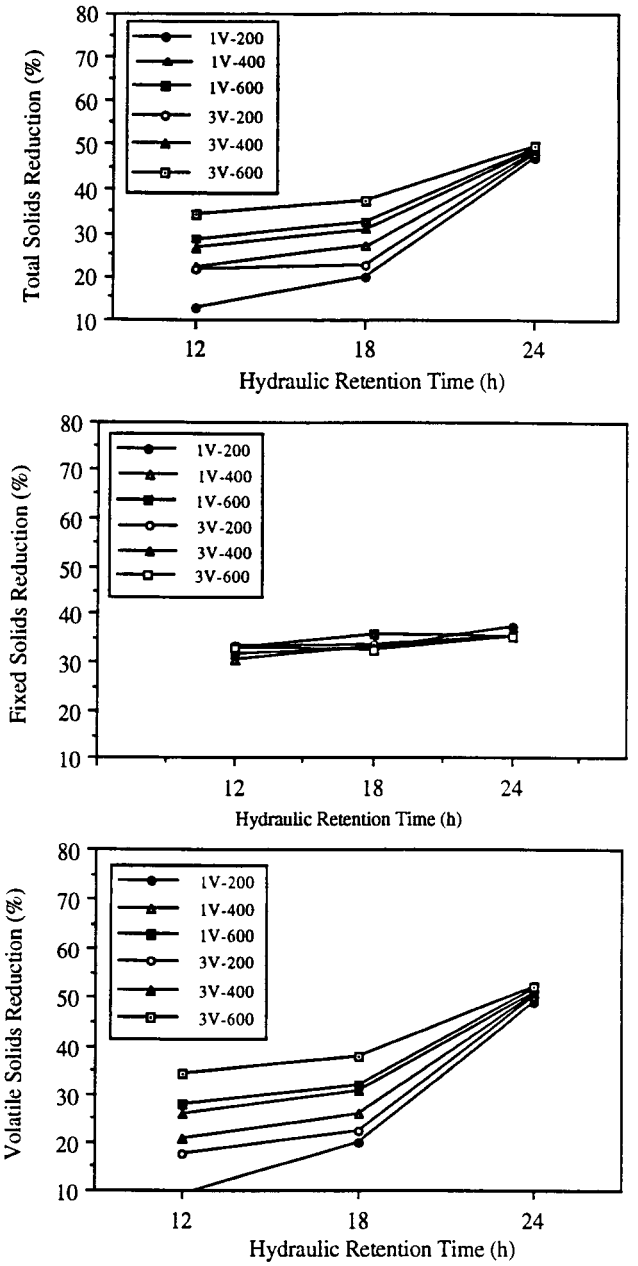

Fig. 5. Effect of hydraulic retention time on the total, fixed, and volatile solids reductions at various air flow rates and mixing speeds.

(9100 mg/L). Although, the reactor was continuously mixed, the lower value of the fixed solids in the effluent could be owing to the precipitation of some minerals into the bottom of the fermenter. Calcium and phosphorous may have been precipitated as $CaCO_3$ and $Ca_3(PO_4)$ so that they were not presented in the samples taken from the effluent. The hydraulic retention time and mixing speed seemed to affect the amount of precipitants. Similar results were reported by Ghaly and Singh (*14*).

Table 5
The Effluent Nitrogen Concentrations
at Various Hydraulic Retention Times, Air Flow Rates, and Mixing Speeds[a]

Retention time, h	Air flow rate, vvm	Mixing speed, rpm	Total Kjeldahl nitrogen			Ammonium nitrogen		
			Mean, mg/L	SD, mg/L	CV, %	Mean, mg/L	SD, mg/L	CV, %
12	1	200	1620	15	0.9	110	3	2.5
		400	1630	12	0.8	113	2	2.0
		600	1645	13	0.8	118	2	2.9
	3	200	1638	17	1.1	115	2	1.6
		400	1649	11	0.7	119	4	3.5
		600	1662	14	0.9	122	4	2.9
18	1	200	1578	7	0.5	94	2	1.7
		400	1585	8	0.5	98	2	2.4
		600	1597	13	0.8	103	1	1.3
	3	200	1588	7	0.4	100	3	3.2
		400	1600	14	0.9	104	1	1.4
		600	1615	17	1.1	108	2	1.9
24	1	200	1520	13	0.9	75	1	1.7
		400	1528	8	0.6	80	2	1.9
		600	1545	10	0.7	85	2	2.8
	3	200	1535	10	0.7	82	2	2.7
		400	1555	8	0.5	86	2	2.3
		600	1572	9	0.6	90	3	3.5

[a]The values are the average of 12 determinations.
Raw whey total Kjeldahl nitrogen = 1690 mg/L.
Raw whey ammonium nitrogen = 270 mg/L.
SD = Standard deviation.
CV = Coefficient of variation.

Nitrogen Compounds

The total Kjeldahl nitrogen as measured in this study included both the ammonium and organic nitrogen but did not include nitrite and nitrate. Nitrification (conversion of NH_4 to NO_2 and NO_3) did not take place since nitrifying bacteria were absent from the system. For each experimental run, the mean, standard deviation, and coefficient of variation of both the total Kjeldahl nitrogen and ammonium nitrogen were calculated (Table 5). The lower coefficient of variation (0.5–3.5) of the nitrogen results indicated that the system was operating at the steady state condition. Figure 6 shows the effluent total Kjeldahl and ammonium at nitrogen various hydraulic retention times, air flow rates, and mixing speeds. Generally, increasing the hydraulic retention time and/or decreasing the air flow rate and/or the mixing speed, increased both the effluent total Kjeldahl nitrogen and the ammonium nitrogen.

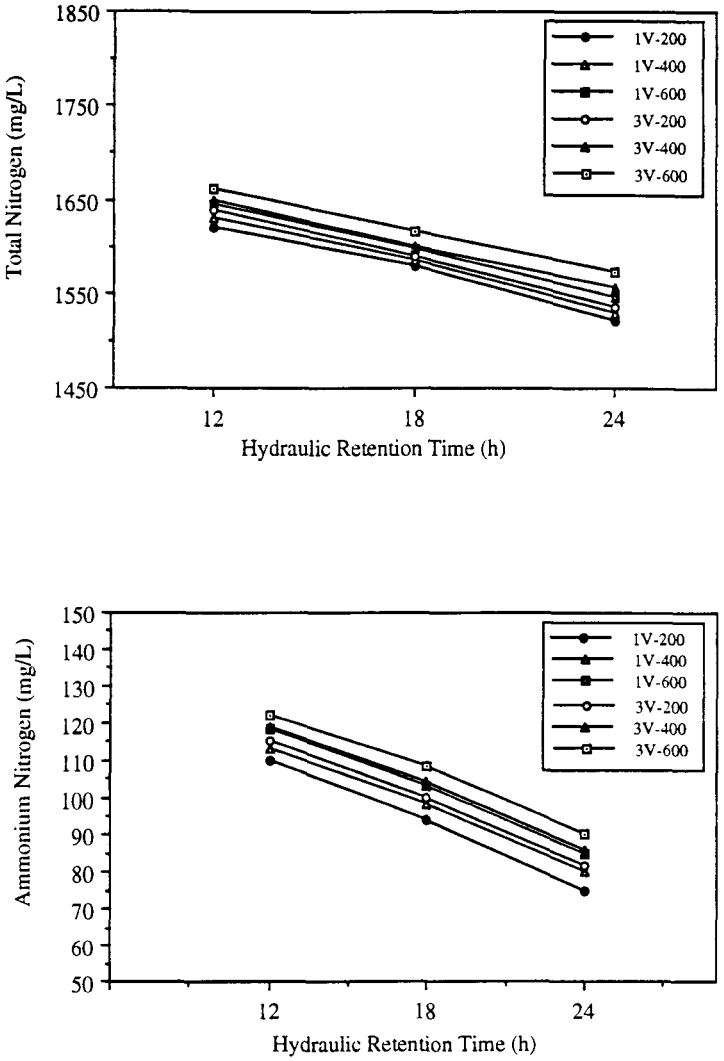

Fig. 6. Effect of hydraulic retention time on the effluent total and ammonium nitrogen concentrations at various air flow rates and mixing speeds.

The reductions in the total Kjeldahl and ammonium nitrogen are shown in Fig. 7. The results indicated that the reductions in the total Kjeldahl and ammonium nitrogen increased as the hydraulic retention time and/or the air flow rate and/or the mixing speed were increased. Reductions of 1.66–10.66% and 54.82–72.22% for the total Kjeldahl nitrogen and ammonium nitrogen were achieved, respectively. For all hydraulic retention times, air flow rates, and mixing speeds, the reductions in the ammonium nitrogen were higher than the reductions in the total Kjeldahl nitrogen. This was owing to the conversion of ammonium nitrogen to organic nitrogen in a form of microbial cells. In an aerobic treatment unit, ammonium

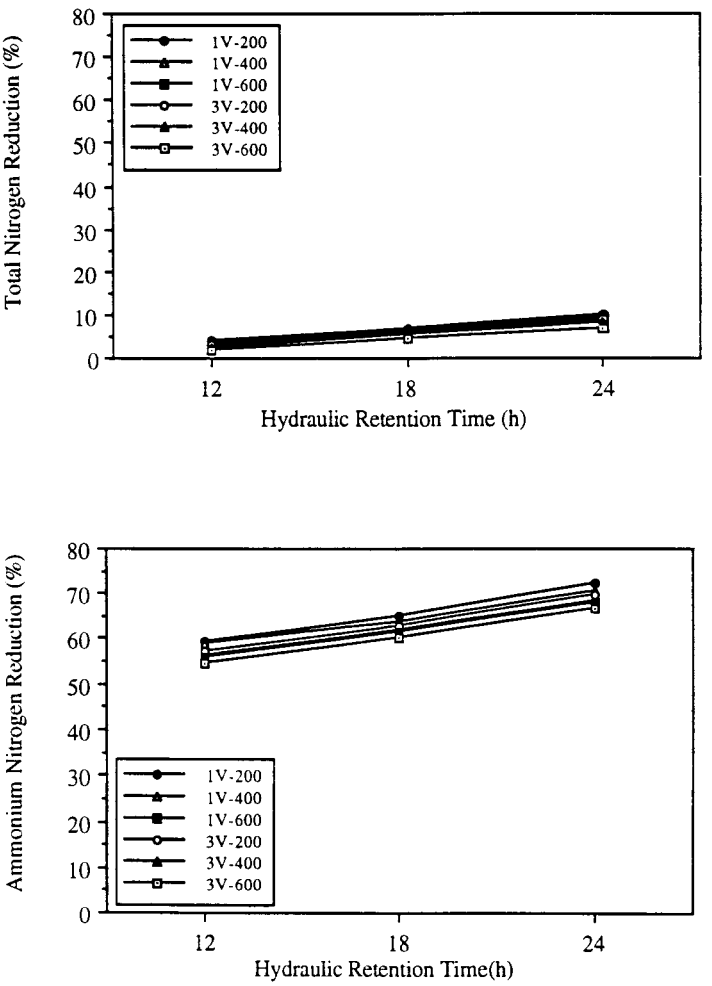

Fig. 7. Effect of hydraulic retention time on the total Kjeldahl and ammonium nitrogen reductions at various air flow rates and mixing speeds.

is converted to microbial cells through the assimilation process. Ammonium reduction can also be achieved through a nonbiological process that is referred to as *ammonia volatilization*. In this process, ammonium ion is decomposed to ammonia gas and is released to the atmosphere at a rate dependent on the concentration of the ammonia in the medium, the pH of the medium, and the degree of agitation. About 12.82–81.08% of the ammonium reduction was utilized by microbial cells through the assimilation process whereas about 18.92–87.18% of the ammonium reduction was lost through the ammonia volatilization process, depending on the hydraulic retention time, air flow rate, and mixing speed used. Higher values of nitrogen assimilation were obtained at shorter hydraulic retention times, higher air flow rates, and higher mixing speeds. The longer

retention time resulted in a reduction of yeast population owing to the death of some yeast cells, and as a result more nitrogen was lost from the system through ammonia volatilization.

CONCLUSIONS

The COD, solids, and nitrogen data indicated that the system was operating at the steady conditions for all hydraulic retention times, air flow rates, and mixing speeds. The effluent TCOD, SCOD, total solids, total Kjeldahl nitrogen, and ammonium nitrogen were significantly affected by the hydraulic retention times, air flow rate, and mixing speed. Increasing the hydraulic retention time and/or the air flow rate and/or the mixing speed decreased the effluent TCOD, SCOD, total solids, total Kjeldahl nitrogen, and ammonium nitrogen. The reductions in TCOD, SCOD, total solids, volatile solids, total Kjeldahl nitrogen, and ammonium nitrogen were dependent on the hydraulic retention time, air flow rate, and mixing speed. Reductions of 15.90–58.91%, 25.70–74.36%, 12.43–49.90%, 9.22–52.27%, 1.66–10.06%, and 54.82–72.22% were achieved for the TCOD, SCOD, total solids, volatile solids, total Kjeldahl nitrogen, and ammonium nitrogen, respectively. The reduction in the SCOD was higher than the reduction in the TCOD for all hydraulic retention times, air flow rates, and mixing speeds owing to the conversion of soluble materials into insoluble microbial cells.

ACKNOWLEDGMENT

The authors wish to express their sincere gratitude to John B. Pyke, Research Scientist, Agricultural Engineering Department, Technical University of Nova Scotia and Estelene Hasan, Visiting Technologist, Institute of Applied Science and Technology, Guyana, for their assistance with the chemical and microbiological analyses.

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