Development of Combined Anaerobic-Aerobic System for Treating Industrial Molasses Wastewater

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ABSTRACT

High-concentration industrial molasses wastewater treatment was examined using biological reactors coupled with physicochemical filtration membranes. The biological processes combined two mesophilic upflow anaerobic sludge blanket (UASB) reactors, a multi-stage upflow anaerobic sludge blanket (MS)-UASB, and a regular UASB for primary anaerobic pre-organic removal, and a downflow hanging sponge (DHS) reactor, equipped with polyurethane sponge media for post-aerobic treatment. Concentrated blackstrap molasses was diluted [12,000 – 1,500 mg of chemical oxygen demand (COD)/L] with organic loading rate (OLR) of 4.5 – 57.7 kg-COD/m³/d (MS-UASB), 2.3 – 34.7 kg-COD/m³/d (UASB), and 0.2 – 6.0 kg-COD/m³/d (DHS). A 1:1.3 recirculation ratio within the MS-UASB was evaluated at different influent concentrations for COD, biogas (CH₄) production, and nitrogen, phosphate, and color removal. The average total organic COD removal was over 92% with and without recirculation. A total of 150 NL/d of biogas with 64 – 75% methane content was collected at the maximum loading rate and influent concentration. Ammonia was reduced from 30 mg-N/L to 5 mg-N/L in the DHS reactor. The dark influent could not be reduced biologically; however, ultrafiltration and nanofiltration removed 98% of the color.

Keywords: biomethanation, decolorization, downflow hanging sponge reactor, molasses wastewater, multi-stage upflow anaerobic sludge blanket reactor, nutrient removal

INTRODUCTION

Molasses, a dark colored by-product of the sugar refining process, is considered to be one of the most recalcitrant agricultural products with a high concentration of organic matter (Satyawali and Balakrishnan, 2008). Due to the world's high consumption of molasses, a large amount of wastewater is generated during its manufacturing process (Saha *et al.*, 2005). Conventional molasses wastewater treatments, including anaerobic lagoons and continuous stirred-tank reactor, have several major disadvantages such as large space requirements, longer hydraulic retention times (HRTs), and emission of methane and carbon dioxide as greenhouse gases into the atmosphere (Nandy *et al.*, 2002). To avoid these pitfalls, biological anaerobic processes such as upflow anaerobic sludge blanket (UASB) reactors accompanied by an aerobic system, have been widely applied to industrial molasses-based distillery wastewater treatment (Mohana *et al.*, 2009). The UASB system has been developed to successfully treat alcohol distillery wastewater with an organic loading rate of 20 – 30 kg-COD/m³/d under thermophilic conditions and produces a considerable amount of biogas (Harada *et al.*, 1996). Other

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mesophilic anaerobic treatments for molasses using reactors are reported to have an average organic loading rate (OLR) of 12 g-COD/L/d with an average treatment efficiency of removed biochemical oxygen demand (BOD) and chemical oxygen demand (COD) and methane productivity of 79%, 71%, and 3.8 L/L-reactor/d, respectively (Kucivilize, 2004). However, this system is incapable of treating highly concentrated wastewater to industrial discharge standards, and an aerobic post-treatment system is necessary to further treat the remaining substances. Thus, an aerobic downflow hanging sponge (DHS) system, designed for sewage treatment in developing countries, has been considered for combined operation with a UASB reactor (Tandukar *et al.*, 2005). Wilkie *et al.* (2000) also summarized that thermophilic anaerobic digestion of alcohol stillage achieved similar treatment efficiencies and methane yields compared to mesophilic treatment. Furthermore, the physicochemical technology of ultrafiltration (UF) and nanofiltration (NF) spiral-wound membranes has been used to investigate the feasibility of degrading the dark brown pigments (Pant and Adholeya, 2007) and recovering remaining nutrients, since these membranes have recently been reported to have advantages over traditional approaches for large-scale removal of organics, nutrients, and dark-colored substances (Nataraj *et al.*, 2006).

In this study, we evaluated the performance of combined biological processes and the feasibility of optional physicochemical systems using UASB/DHS and UF/NF membrane filtration, with particular focus on COD, biogas production, nutrients (N and P), and color removal. We also observed the sludge morphology to improve our understanding of the formation of granular sludge bulking (Li *et al.*, 2008) in carbohydrate-rich molasses-based wastewater treatment reactors.

MATERIALS AND METHODS

Experimental apparatus

Figure 1 shows a schematic diagram of the biological process unit, which was a

Fig. 1 - Flow diagram of the biological and physicochemical treatment processes.

combination of a multi-stage (MS) UASB reactor (10 L) followed by regular-type (Reg) UASB (11 L) and DHS (18-L sponge capacity) reactors. The DHS reactor was filled with $\varnothing 3.5 \times H 3.5$ cm polyurethane sponge media attached to the plastic sieve supporter. In the physicochemical process, 4 inches of spiral-wound UF and NF modules were employed to treat the effluent from the DHS reactor. Ultrafiltration modules outer vessel was made of fiber reinforced plastics (FRP) and installed with GE thin-film spiral-wound membrane GH8040F1001 which had molecular weight cut off (MWCO) of 2,500 Da (GE, USA), while NF module consisted of 4 inch-Bekaert side port PRO-4-300-SP outer vessel and TORAY spiral-wound SU220 polyamide composite membrane (TORAY, Japan). The microorganism biomass used as seeding for the reactors was digested sludge collected from a local municipal sewage treatment plant mixed with sludge from a non-molasses wastewater treatment reactor.

Substrate

The substrate used in the study was a dark brown, caramel-like form of blackstrap molasses with an original COD of 600,000 – 800,000 mg-COD/L and was diluted to the setup concentration. Sodium bicarbonate (NaHCO_3 ; 2,500 mg/L) was also added as a buffer. The substrate was stored at the control temperature of 4°C.

Operational conditions

The operational conditions of the biological processes were divided into nine phases P1 – P9 (Table 1), with P1 as the startup and steady period, and the influent concentration was gradually raised from 1,000 to 8,000 mg-COD/L. The influent concentration was increased to 10,000 mg-COD/L in P2 without recirculation. In P3, the recirculation ratio of 1:1.3 was started with the same influent concentration as P2. The substrate concentration was reduced to 5,000 mg-COD/L in P4 with 1:1.3 recirculation ratio for the recuperation. The substrate concentration was again constantly increased to 8,000 mg-COD/L as steady state. In P6, the same conditions as presented in P2 were conducted for the maximum organic loading without recirculation. The same substrate concentration of 10,000 mg-COD/L was continuously applied without circulation in P7, however HRT was increased two-fold from P6. In P8, substrate concentration was reduced to 5,000 mg-COD/L and maintained at recovery state before the final shock loading in P9.

Table 1 - Operational conditions for the biological processes.

Phase no.	Opt.period [day]	COD conc. (Before rec.) [mg/L]	Rec. ratio [Rec.Q/Q]	HRT				MS-UASB COD Loading [kg-COD/m ³ /d]
				Total [h]	MS-UASB [h]	UASB [h]	DHS [h]	
1	1 – 196	1,000 – 8,000	–	~ 24.1	~ 5.7	~ 6.7	~ 11.9	5 – 36
2	197 – 231	10,000	–	21.7	5.3	6.1	10.3	51
3	232 – 245	10,000	1.3	18.7	2.3	6.1	10.3	87
4	246 – 309	5,000	1.3	18.7	2.3	6.1	10.3	29
5	310 – 339	6,000 – 8,000	1.3	18.7	2.3	6.1	10.3	35 – 60
6	340 – 363	10,000	–	21.7	5.3	6.1	10.3	50
7	364 – 391	10,000	–	43.4	10.6	12.2	20.6	23
8	392 – 498	5,000	–	21.7	5.3	6.1	10.3	23
9	499 – 577	10,000	1.3	18.7	2.3	6.1	10.3	76

rec.: recirculation, opt.: operation, Q: flow rate, conc.: concentration

Biodegradability test

The biodegradability test was conducted in order to investigate the substrate degradability of the seed sludge taken on day 0 and incubated in 122-mL vials at 35°C. All test sludge were withdrawn from reactor and purged with N₂ gas to maintain anaerobic condition. Test sludge was rinsed with 25-mM phosphate buffer at the test temperature and homogenized (Physoctron, Nichion, Japan). Hydrogen/carbon dioxide (80:20, v/v), acetate, propionate and diluted molasses were used as substrate.

Analytical methods

Chemical oxygen demand concentration was determined using colorimetric method (DR5000 spectrophotometer, HACH, USA). Volatile fatty acids (VFA) were analyzed by gas chromatography (GC-1700, Shimadzu, Japan). Nutrients including PO₄³⁻-P, NH₄⁺-N, NO₂⁻-N, NO₃⁻-N were analyzed by high performance liquid chromatography (HPLC) (Prominence, Shimadzu). Biogas production was measured by a wet gas meter, and the composition was determined by gas chromatography (GC-8A, Shimadzu). The samples were filtered through a ø0.45-µm pore size filter paper to avoid fluctuating turbidity when measuring the color with a drainage coloration and turbidity meter (NDR2000; Nippon Denshoku, Japan). Morphological analysis of granular sludge was performed by scanning electron microscopy (SEM). Other analytical factors were monitored in accordance with Standard Methods for the Examination of Water and Wastewater (APHA, 2005).

RESULTS AND DISCUSSION

Performance

Figure 2 shows the time course of COD removal efficiency in the biological process, which achieved an average organic removal of more than 90% of COD and 95% of

Fig. 2 - Time course of COD removal efficiency in the biological process.

BOD. The maximum OLR of 57 kg-COD/m³/d (MS-UASB), 19 kg-COD/m³/d (UASB) and 2.1 kg-COD/m³/d (DHS) corresponded to a maximum influent concentration of 12,000 mg COD/L with an HRT of 5 h in the MS-UASB, 6 h in the UASB, and 10 h in the DHS, respectively. In the membrane process, with 910 mg-COD/L of effluent from the DHS as a feed, the UF and NF membranes achieved final effluent concentrations of 48 mg-COD/L and 18 mg-COD/L, respectively.

On the 140th day, the buffer was reduced (shock loading), and all three reactors showed the same effects from the pH drop, but recuperated in 14 days after the pH was adjusted to normal. During the pH shocking period, acetate accumulated from 15.1 mg/L to 1043.9 mg/L and propionate from 0 mg/L to 720.2 mg/L, which was assumed to be due to the temporary inhibition of methane-producing bacteria. Chemical oxygen demand removal by the UASB and DHS reactors appeared to fluctuate. This resulted from the MS-UASB performance when most of the organic compounds were treated in the MS-UASB and the remaining compounds had relatively low concentrations (less than 1,000 mg-COD/L) that could lower the F/M ratio of the system. In P2, the influent concentration was raised to 12,000 mg-COD/L, and the MS-UASB performance deteriorated due to the high OLR after the 200th day, which resulted in the decrease of COD removal efficiency to 60%. However, the UASB and DHS could recover the excessive loading and support a total removal of 90%. When the recirculation ratio was 1:1.3 within the MS-UASB in P3 and the same 10,000 mg-COD/L of influent concentration was applied, COD removal was almost the same, but accumulated propionate decreased from its highest value of 1,120 mg/L in P2 (pH shocks) to 670 mg/L in P3 when recirculated (data not shown). In P4, the influent concentration was reduced to 5,000 mg-COD/L with the same overall HRT to lessen the loading stress and stabilize the system. Consequently, the recovery rate of the MS-UASB increased again to nearly 80%, which led to a decrease of 50 – 55% in UASB and 30% in DHS removal efficiencies from the 246th day. After system re-stabilization, the loading of each reactor was raised with the recirculation mode, and the performances of all reactors in the second half-period were different. As a result, the MS-UASB appeared to be enfeebled several times by the direct high loadings with concentrations of about 10,000 mg-COD/L and the amount of the granular seed sludge was enlarged and turned into bulking sludge floating out of the reactor. In contrast, the UASB and DHS efficiently recovered untreated organic matter, as revealed in P6. The removal percentages of the UASB and DHS reactors were higher, in contrast to the MS-UASB performance, which gradually decreased and remained in the range of 45 – 67%. At the OLR of 30 kg-COD/m³/d, a substrate concentration of 6,000 mg-COD/L at the HRT of 5 h appeared to be the optimum condition for the MS-UASB to remove 80% of the COD removal percentage in this operation (Fig. 3).

Consequently, the UASB functioned as the pre-adjustment reactor before preceding the high COD concentration effluent entering the DHS reactor. The most suitable influent concentration for the UASB was between 2,000 mg-COD/L and 6,000 mg-COD/L, with a removal rate of 70% when the OLR could be raised to 20 kg-COD/m³/d (Fig. 4).

To further study the reactors' performance, the COD balances of both the UASB and DHS reactors at 5,000 and 10,000 mg-COD/L influent concentrations with and without recirculation were assessed. At a 5,000 mg-COD/L influent concentration (Fig. 5),

wastewater was treated and converted by the main MS-UASB unit to methane gas more effectively with recirculation (67%) than the non-recirculation mode (36%) and the percentage removal was 75 – 80% compared with 65% removal efficiency in the non-recirculation mode. At a higher concentration of 10,000 mg-COD/L (Fig. 6), recirculation within the MS-UASB slightly increased the methane conversion from 51% to 61%. This could have resulted from an insufficient recirculation ratio against the high concentration influent.

Fig. 3 - MS-UASB COD % removal vs. influent (inf.) concentration (conc.) (a), OLR (b), and HRT (c).

Fig. 4 - UASB COD % removal vs. influent (inf.) concentration (conc.) (a), OLR (b), and HRT (c).

Fig. 5 - COD balance at 5,000 mg/L concentration.

Biodegradability test

This test was performed to evaluate the biodegradability of the wastewater and the adaptability of sludge cultivated on different wastes for molasses wastewater treatment. Figure 7 indicates that molasses wastewater could be degraded and converted to methane gas with an initial 0.3 g-COD-CH₄/g-VSS/d compared with other substrates.

Color and nutrient removal

Anaerobic bacteria are reported to have the ability to decompose the colored substance melanoidin in molasses spent wash (MSW). In fact, in Fig. 8, MS-UASB and UASB system decolorized approximately 10 – 15% of the substrate, it was reported that anaerobic bacteria produced some enzymes that could degrade melanoidin substances (Sirianuntapiboon *et al.*, 2004). The color turned almost 50% darker after treatment with the DHS reactor, which could have been due to repolymerization of the pigments. The UF/NF system showed significant removal of the molasses wastewater color, from

Fig. 6 - COD balance at 10,000 mg/L concentration.

Fig. 7 - Biodegradability test.

3,600 color units in the DHS effluent to 1 color unit (colorless; 99% removal). Moreover, the experiments revealed the reduction of $\text{PO}_4^{3-}\text{-P}$ from 48 mg-P/L to 6 mg-P/L and of total nitrogen from 64 mg-N/L to 37 mg-N/L, which were not removed by biological processes (Table 2).

Microbial community

Figure 9 shows SEMs of granular sludge taken from the 75th day seed sludge. Clusters of round microorganisms morphologically similar to *Methanosarcina* sp. could be distinguished (Fig. 9a). These microorganisms were probably the predominant organisms from the previous treatment environment. In the 175th day sludge sample, when sodium bicarbonate was added to the substrate to adjust the pH (7.5 – 8.0), *Methanosaeta*-like microorganisms were observed (Fig. 9b). Figure 9c shows filamentous microorganisms covering the outer surface of wash-out sludge taken at the 335th day of operation. These microorganisms were possibly related to acid-forming bacteria that caused the biogas to be trapped inside the granular sludge and to flow up along with the stream.

Fig. 8 - Color profile of biological processes.

Table 2 - pH, COD, color, and nutrient removal of the total system.

Subject		COD [mg/L]	BOD [mg/L]	pH	Color [Degree]	$\text{PO}_4\text{-P}$	$\text{NO}_2\text{-N}$	$\text{NO}_3\text{-N}$ [mg/L]	$\text{NH}_4\text{-N}$	TKN
Substrate		12,700	5,520	7.1	3,310	119	2	0	1	132
Biological phase	MS-UASB	4,930	1,890	7.8	2,620	258	0	0	27	107
	Reg-UASB	1,160	280	7.9	2,340	111	0	0	30	45
	DHS	911	110	8.8	3,600	48	0	31	5	28
Physicochemical phase	UF	48	N.D.	8.5	33	12	18	22	9	9
	NF	18	N.D.	8.5	1	6	8	27	1	1
Industrial wastewater Standard		120	160	5.8–8.6	100*	16		120 as TN		

* Standard: not objectionable ; 100 unit is an author's objective value, N.D. : Not Determined

Fig. 9 - Morphological SEM observations of granular sludge from the MS-UASB reactor: (a) *Methanosarcina*-like microorganisms, scale bar = 7.50 μm ; (b) *Methanosaeta*-like microorganisms, scale bar = 10.0 μm ; (c) filamentous microorganisms at the surface of wash-out sludge, scale bar = 30.0 μm .

CONCLUSIONS

Over the 500 days of operation, the performance of the UASB/DHS process for the treatment of molasses-based spent wash wastewater was satisfactory, with 90% COD removal efficiency at a shortest total HRT of 18 h and the ability to cover from various critical conditions. The MS-UASB, which performed the main biological treatment, was able to treat MSW wastewater with a concentration of 6,000 mg-COD/L and OLR of 20 kg-COD/m³/d at an HRT of 7 h at optimum stability. The UASB and DHS reactors also played significant roles by effectively backing up the MS-UASB in case of excessive loading at an OLR of 58 kg-COD/m³/d. The application of the physicochemical membrane (UF/NF) proved to be a pragmatic approach, with 98% COD removal and 99% color removal from the DHS effluent. The typical anaerobic microorganisms reported in the literature could be observed by SEM. *Methanosarcina*-like microorganisms, *Methanosaeta*-like microorganisms and filamentous microorganisms were distinguished, but these will require further analysis by microbiological methods, such as 16S rRNA, to elucidate the phylogenetic details. The biological process appeared to be capable of treating the MSW wastewater for organic matter and nitrogen removal with nitrification within the DHS at a total OLR of 15 kg-COD/m³/d and an HRT of 24 h; whereas the membrane process demonstrated remarkable color and phosphate removal efficiency. Therefore, this combined biological and physicochemical treatment system could achieve optimum performance in meeting the industrial wastewater standard.

REFERENCES

- APHA (2005) *Standard Methods for the Examination of Water and Wastewater*, 21st ed. American Public Health Association, Washington DC, USA.
- Harada H., Uemura S., Chen A. C. and Jayadevan J. (1996) Anaerobic treatment of a recalcitrant distillery wastewater by a thermophilic UASB reactor. *Biores. Technol.*, **55**(3), 215-221.
- Kucivilize P. (2004) Super High Rate Anaerobic Wastewater Treatment by Thermophilic Multi-Stage UASB Reactor. PhD thesis, Department of Environmental System Engineering, Nagaoka University of Technology, Japan.
- Li J., Hu B., Zheng P., Qaisar M. and Mei L. (2008) Filamentous granular sludge

- bulking in a laboratory scale UASB reactor. *Biores. Technol.*, **99**(9), 3431-3438.
- Mohana S., Acharya B. K. and Madamwar D. (2009) Distillery spent wash: Treatment technologies and potential applications. *J. Hazardous Materials*, **163**(1), 12-25.
- Nandy T., Shastry S. and Kaul S. N. (2002) Wastewater management in a cane molasses distillery involving bioresource recovery. *J. Environ. Manage.*, **65**(1), 25-38.
- Nataraj S. K., Hosamani K. M. and Aminabhavi T. M. (2006) Distillery wastewater treatment by the membrane-based nanofiltration and reverse osmosis processes. *Water Res.*, **40**(12), 2349-2356.
- Pant D. and Adholeya A. (2007) Biological approaches for treatment of distillery wastewater: A review. *Biores. Technol.*, **98**(12), 2321-2334.
- Saha N. K., Balakrishnan M. and Batra V. S. (2005) Improving industrial water use: case study for an Indian distillery. *Resources Conservation Recycling*, **43**(2), 163-174.
- Satyawali Y. and Balakrishnan M. (2008) Wastewater treatment in molasses-based alcohol distilleries for COD and color removal: A review. *J. Environ. Manage.*, **86**(3), 481-497.
- Sirianuntapiboon S., Phothilangka P. and Ohmomo S. (2004) Decolorization of molasses wastewater by a strain No.BP103 of acetogenic bacteria. *Biores. Technol.*, **92**(1), 31-39.
- Tandukar M., Uemura S., Machdar I., Ohashi A. and Harada H. (2005) A low-cost municipal sewage treatment system with a combination of UASB and the "fourth-generation" downflow hanging sponge reactors. *Water Sci. Technol.*, **52**(1-2), 323-329.
- Wilkie A. C., Riedesel K. J. and Owens J. M. (2000) Stillage characterization and anaerobic treatment of ethanol stillage from conventional and cellulosic feedstocks. *Biomass Bioenergy*, **19**(2), 63-102.