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Performance and Microbial Community Analysis of a full-scale Hybrid Anaerobic–Aerobic Membrane System for Treating Molasses-Based Bioethanol Wastewater

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ABSTRACT: We evaluated the efficacy of a full-scale combined biophysicochemical system for treating molasses-based bioethanol wastewater in terms of organic substances, nutrient, and dark brown color removal. The main organic removal unit, i.e., the upflow anaerobic sludge blanket (UASB) reactor, achieved 80.7% removal and 4.3 Nm^3 methane production per cubic meter of wastewater with a hydraulic retention time of 16.7 h. Downflow hanging sponge (DHS) reactors were important in reducing the biochemical oxygen demand (BOD), and the lowest possible organic waste intake prevented excessive biomass formation. The BOD removal efficiency was 71.2–97.9%. The denitrification upflow anaerobic fixed bed (UFB) reactor achieved 99.2% total nitrogen removal. Post-physicochemical membrane treatment reduced the total phosphate, color, and remaining organic matter by 90.4%, 99.1%, and 99.8%, respectively. We analyzed the microbial diversity of the sludge from the UASB reactors. *Methanosaeta* was the dominant archaeal genus in the system, followed by *Methanolinea*, *Methanomicrospillum*, *Caldiserica*, *Bacteroidetes*, and *Deltaproteobacteria*.

Key words: Molasses, Wastewater, DHS, Membrane filtration, Decolorization, Microbial diversity

INTRODUCTION

Recently, the demands and prices of fuel have increased tremendously and molasses-based bioethanol has become an alternative renewable bioenergy source for reducing the use of gasoline. However, this has generated vast amounts of wastewater, which has caused severe environmental problems such as its odor, contamination of groundwater, and depletion of the dissolved oxygen when released to the fresh water (Mohana *et al*., 2009). In Miyakojima, a small island in Okinawa prefecture where brown sugar from sugarcane is the major agricultural product and its by-product, blackstrap molasses, which contains high carbon source, is used as raw material for bioethanol manufacturers and local Awamori (Okinawan alcoholic beverage) distillers. During production, the volume of wastewater discharged after washing the fermentation tanks is about 15 times that of every unit of bioethanol produced (Satyawali *et al*., 2008). Groundwater is the only water source on the island so contamination with pesticides or harmful substances is a concern to the inhabitants and government. Molasses-based wastewater contains a high concentration of organic compounds, nutrients, and dark color pigments. Conventional treatments such as biological processes, including aerated lagoons or ferti-irrigation of cropland, are the most common methods used in most

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Asian and South American sugar- and ethanol-producing countries. However, these methods lead to the emission of greenhouse gases and have a high area requirement, while they also produce groundwater contamination and odor problems (Nandy *et al*., 2006). To overcome the aforementioned problems, cost-effective and eco-friendly remedies have been widely developed (Wilkie *et al*., 2000). In particular, a high-rate closed biological system has been developed for the treatment of molasses-based bioethanol waste, which consists of an upflow anaerobic sludge blanket (UASB), downflow hanging sponge (DHS), and an upflow anaerobic fixed bed (UFB) followed by a series of microfiltration (MF), ultrafiltration (UF), and nanofiltration (NF) stages.

The objective of this study was to evaluate the performance of a full-scale combined biological (UASB+DHS+UFB) system for treating molasses-based bioethanol wastewater in terms of removing the major organic substances, suspended solids (SS), nitrification (in DHS), and denitrification (in UFB), and a physicochemical (MF+UF+NF) treatment system for removing the particles that remained after the UFB (by MF), as well as phosphate (UF and NF) and color (NF). We also observed the recovery of methane for bioenergy and analyzed the microbial diversity in different operating conditions.

MATERIALS & METHODS

The wastewater used in this investigation was discharged directly from a local bioethanol plant and stored in a 200-m^3 substrate tank. The raw wastewater was mainly the washing waste from the fermentation tanks and blackstrap molasses containers, which had the following characteristics: chemical oxygen demand (COD) = 4,800–36,000 mg/L, biological oxygen demand (BOD) = 2,600–16,300 mg/L, SS = 200–2,450 mg/L, total nitrogen (TN) = 70–450 mg/L, total phosphorus (TP) = 10–40 mg/L, sulfate of 120–520 mg-S/L and acidic pH = 3.7–4.9. The UASB reactors were inoculated with sludge from a local beer brewery wastewater treatment plant. The sludge concentrations of the UASBs were 35.5 gMLVSS/L and 35.3 gMLVSS/L. The MLVSS/MLSS ratios were 0.87 in UASB1 sludge and 0.84 in UASB2 sludge.

COD was determined using a colorimetric method (HACH DR5000 spectrophotometer). Nutrients, i.e., PO_4^{3+}-P, NH_4^+-N, NO_2^--N, and NO_3^--N, were also analyzed using a colorimetric method (HACH DR5000). Biogas production was monitored automatically by measuring the volume of biogas entering storage while the composition was determined using gas chromatography (Shimadzu GC1700 and GC8A). The samples were filtered through a φ0.4-μm pore size filter paper to eliminate the turbidity, which would enable the measurement of the true color (Nippon Denshoku, NDR2000). Other analytical parameters were monitored in accordance with the Standard Methods for the Examination of Water and Wastewater (APHA, 2005). The system was operated at full-scale in ambient temperature (18–32°C) conditions. The biophysicochemical system was located on-site at Miyakojima Bioethanol Plant, Okinawa, Japan. The biological process involved a pre-adjustment tank, UASB reactors, DHS reactors, and a UFB reactor. The pre-adjustment tank was used to balance the acidic pH and increase the temperature during winter by pre-heating the raw wastewater with a gas boiler, which used the biogas produced by the UASB reactors. The UASB reactors were constructed of plate steel in a cylindrical shape with a working volume of 17.3 m^3 (UASB1) and 2.8 m^3 (UASB2), based on a maximum influent flow of 15 m^3/d. The design flow per day was calculated using the remaining volume of the generated raw wastewater (Table 1), which was fed intermittently from 2 m^3/d to 8 m^3/d. In-series UASBs were designed mainly to remove organic substances from the processing manufacturer followed by two DHS reactors, which were mainly applied for BOD, SS, and nitrogen species removal. At the end of the biological process, a UFB was installed to ensure that the denitrification process would occur and nitrate (NO_3) will be reduced before entering the membrane modules.

Table 1. Operational condition of the biological process

Flow [m^3 d^{-1}]	HRT [day]				Substrate conc. (STD) [mgCOD L^{-1}]	Substrate TN (STD) [mgN L^{-1}]	Influent TP (STD) [mgP L^{-1}]	UASB1+2 Loading [kgCOD m^{-3} d^{-1}]	DHS Loading (STD) [kgCOD m^{-3} d^{-1}]	Duration [day]
	Total	UASB1+2	DHS	DN						
2	20.8	5.0	7.1	2.0	15,900 (9,009)	15,900 (2,296)	15,900 (2,296)	1.3 (0.8)	0.3 (0.13)	58 - 117
4	10.5	2.5	3.6	1.0	8,986 (3,475)	9,000 (1,203)	9,000 (1,203)	1.3 (0.4)	0.4 (0.07)	155 - 192
6	7.0	1.7	2.4	0.7	8,463 (981)	8,500 (996)	8,500 (996)	2.2 (0.3)	0.6 (0.05)	133 - 142
8	5.2	1.2	1.8	0.5	14,522 (9,101)	14,800 (5,965)	14,800 (5,965)	4.4 (2.4)	1.0 (0.15)	118 - 132

Following the biological process, the effluent was treated with a series of membranes, i.e., the MF, UF, and NF units. MF was a hollow-type 60 m^2 surface area membrane with a 0.4 μm pore size polyvinylidene difluoride (PVDF) membrane, which was equipped with a low pressure suction pump, a 1.6-Nm^3/min air blower, and activated carbon filters. The MF module flow was set at 0.8 m^3/h. Two eight-inch spiral-wound polyamide UF membranes modules with a molecular weight cutoff (MWCO) range of 2500 Da, each with a working surface area of 34.4 m^2, were packed inside separate fiber reinforced plastic (FRP) tubes. The UF permeate flow rate was 0.72 m^3/h and the concentrate flow rate was 0.04 m^3/h, which were maintained throughout the operation. The spiral wound membrane was also applied to the NF module but it had a smaller MWCO range (200 Da), a membrane surface area of 28.0 m^2, and a permeate flow rate of 0.36 m^3/h. To improve the performance of the membrane and prevent fouling problems, we used two types of cleaning once a week, i.e., water flushing and chemical cleaning (100 L of sodium hydroxide solution followed by 100 L of hydrochloric acid solution). The pH was pre-adjusted to 6.5–7.0 by adding concentrated hydrochloric acid before the wastewater feed entered the UF units. Sludge samples were collected from UASB1 and UASB2 on the 200th day of operation. DNA extract from the sludge samples were prepared using FastDNA Spin Kit for soil (MP Biomedicals, Irvine, CA). The 16S rRNA gene sequences were amplified using a One-Shot LA PCR Mix (Takara Bio, Otsu, Japan) with 0.3 μM of each PCR primer. The PCR primer pairs EUB338F (Hatamoto *et al.*, 2007a; Amann *et al.*, 1990; Daims *et al.*, 1999)/Uni1490R (Hatamoto *et al.*, 2007b) and Ar109f (Imachi *et al.*, 2006)/Uni1490R were used for the bacterial and archaeal 16S rRNA genes, respectively. The PCR products were purified with a QIAquick PCR Purification Kit (QIAGEN Inc. Valencia, CA) and subsequently cloned using a TOPO TA cloning kit (Invitrogen, Carlsbad, USA). The partial 16S rRNA gene sequences were sequenced using Uni907R primer (Hatamoto *et al.*, 2007). The sequencing was performed at Dragon Genomics Center (Takara Bio Inc., Otsu, Japan). Partial 16S rRNA gene sequences with more than 97% identity were grouped in the same Operational Taxonomic Units (OTUs). The phylogenetic affiliations were determined using the NCBI BLAST search tool (BLASTN; http://www.ncbi.nlm.nih.gov/BLAST/), the Ribosomal Database Project (http://rdp.cme.msu.edu), and the ARB program package (http://www.arb-home.de/).

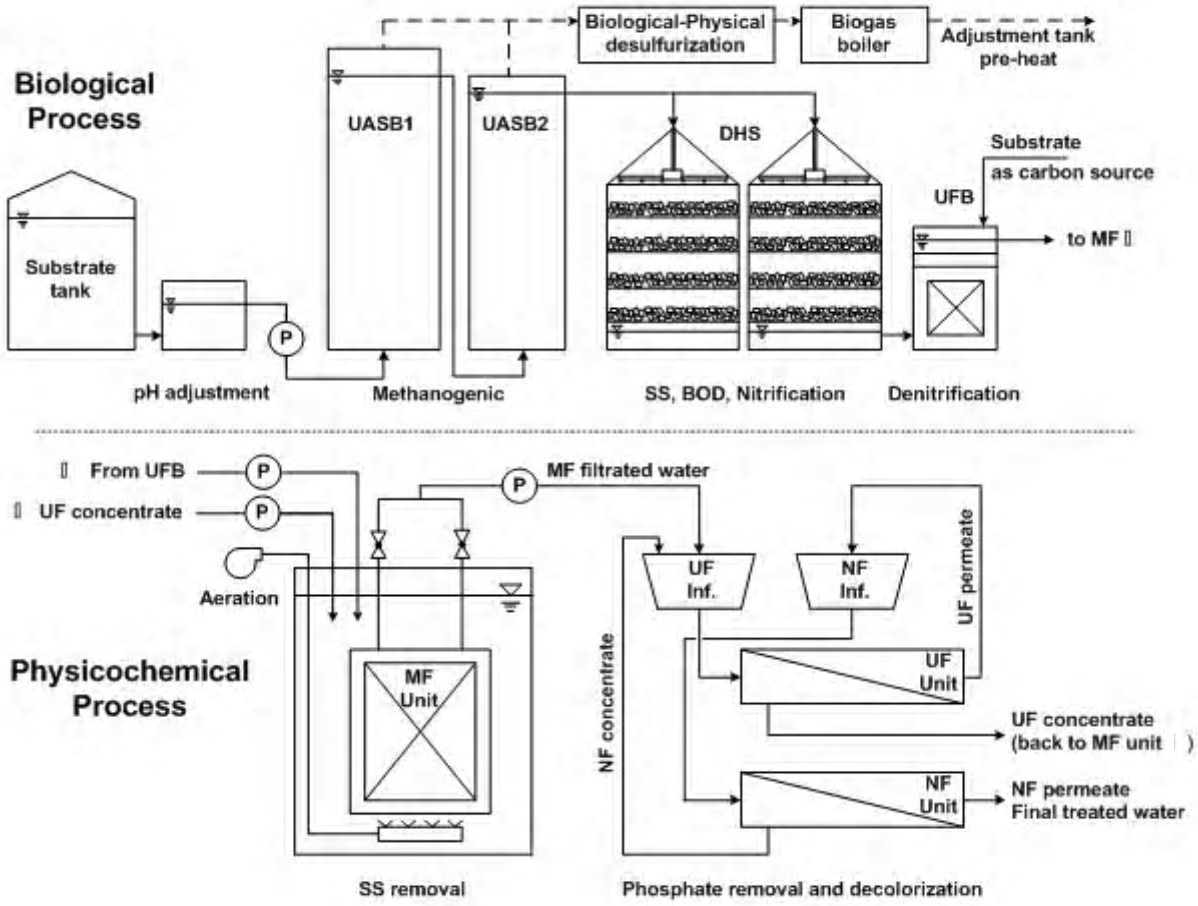


Fig. 1. Systematic of the biophysicochemical processes

RESULTS & DISCUSSION

The full-scale UASB-DHS-UFB system achieved continuous biogas generation and high strength molasses wastewater treatment at the same time. The average COD removal efficiency of each unit during the 200-day operation period is shown in Fig. 2. The data indicated that each unit had a significant effect on COD removal during molasses wastewater treatment, although the COD removal effect of each unit was distinct. When the system was operated in the first concentration gradient, the COD removal efficiency of each UASB reactor unit was approximately 60–80%. As the influent into the system increased (because of the periodic production and fermentation tank cleaning conditions), the efficiency of the UASB reactors was enhanced because an adequate carbon source was available for the anaerobic microbes (the COD removal efficiency reached up to 90% with an influent concentration of 35,000 mgCOD/L). Indeed, the COD removal efficiency of the DHS unit did not appear to decrease when the concentration of the treated water was even higher than 2000 mgCOD/L. The performance of the DHS unit was maintained throughout the entire operation. However, the UFB effluent concentration was moderately increased after the addition of the carbon source (raw wastewater) before denitrification occurred (Fig. 3). The substrate BOD:COD ratio of approximately 0.6 was because of the molasses-based bioethanol wastewater containing readily soluble biodegradable material, which is reported to have a higher denitrification rate compared with particulate substrates (Griffiths *et al.*, 1994). The use of molasses as a carbon source and denitrification have been studied by several researchers and it was shown that long-chain polysaccharides cannot be readily utilized by denitrifying bacteria so they need to be reduced to monosaccharides such as glucose and fructose (Najafpour and Shan, 2003). The molasses used in this process was fermented into ethanol; it could be presumed that certain amount of the spentwash from the fermentation tank contained the hydrolyzed form of this carbon source, which explained the biodegradability of the substrate. The wastewater SS was reduced by 17–44% with a flow of 2, 6, and 8 m^3/d (except 4 m^3/d) using UASB. Most of the SS was absorbed by DHS sponges, which reduced it to approximately 100–250 mg/L, although it increased again to approximately 400–800 mg/L with all flows after treated by UFB because of the anaerobic sludge and additional raw wastewater. During the 200 days of operation, 86% of the nitrate was significantly removed by the UFB reactor, i.e., from 55.5 to 7.7 mg-N/L. The total nitrogen was reduced by 73% (flow = 2 m^3/d), 25% (flow = 4 m^3/d), 28% (flow = 6 m^3/d) and 24% (flow = 8 m^3/d) reduced by DHS then 80% (flow = 2 m^3/d), 50% (flow = 4 m^3/d), 30% (flow = 6 m^3/d) and 10% (flow = 8 m^3/d^1) removed in UFB (Fig. 5). The process was maintained in anaerobic conditions so that the denitrifying organisms could use nitrate instead of dissolved oxygen as an oxygen source during their metabolism and organic substance oxidation. Ammonium was accumulated during anaerobic degradation, which was 87–99% oxidized by the nitrification process in the DHS reactor with all feed conditions (Fig. 6). Almost 50% of the substrate and UASB effluent comprised of organic and ammonium forms of nitrogen in oxygen-depleting conditions. During the aerobic stage (DHS), most of the ammonium was oxidized to nitrite-N and nitrate-N, whereas the organic form of nitrogen still persisted. After denitrification in the UFB tank, the anaerobic bacteria used nitrate-N for respiration and converted it to the gaseous form of nitrogen, which was used as a carbon source (Fig. 6). Phosphate was retained by the DHS sponge (DHS- treated water = 6.8 mg-P/L; the phosphate level of the UFB-treated water was higher than that of the DHS-treated water because of the addition of raw wastewater) to yield an allowable dischargeable level of <8 mg-P/L (Fig. 7). The substrate contained dark brown color pigments, which were mostly phenols and high molecular weight (MW) amino–carbonyl melanoidins with an MW value in the range 20–35 kDa. Unfortunately, they remained untreated during the biological process, whereas the UASB reactor appeared to remove color from the molasses-based wastewater (5% to 7%), although the effluent was 26.9% darker after DHS treatment, possibly because of the polymerization of colored substances under aerobic conditions (Fig. 8). Biogas with methane contents > 70% was produced by methanogens in UASBs at a production rate of 0.38 Nm^3/m^3-reactor/d which was relatively exceptional for high-rate anaerobic treatment (data not shown).

The performance of the three membrane filtrations stages was measured in terms of the percent rejection ($\%R_j$) of COD, SS, nitrogen, phosphate, and color. MF showed a potential of rejecting 33.5% of total COD mostly in the form of particulate COD when UF, which has typical operating range between 0.005 to 0.2 μm, removed 61% of the remaining COD to 275 and 244 mg/Lin total COD form. NF showed the advantage of polishing the organic substances up to 32, 25, and 0 mg/Lin terms of total COD of the final residue with functional micropores of <2 nm at the pressure range of 0–0.2 MPa (Fig. 3). MF (filrer pore size = 0.1–5μm) was carried out mainly for the purpose of suspended solid removal. Most of the colloidal particles were filtered by the φ0.4-μm PVDF membrane (similar to the φ0.4-μm pore size of the glass fiber filter used for SS

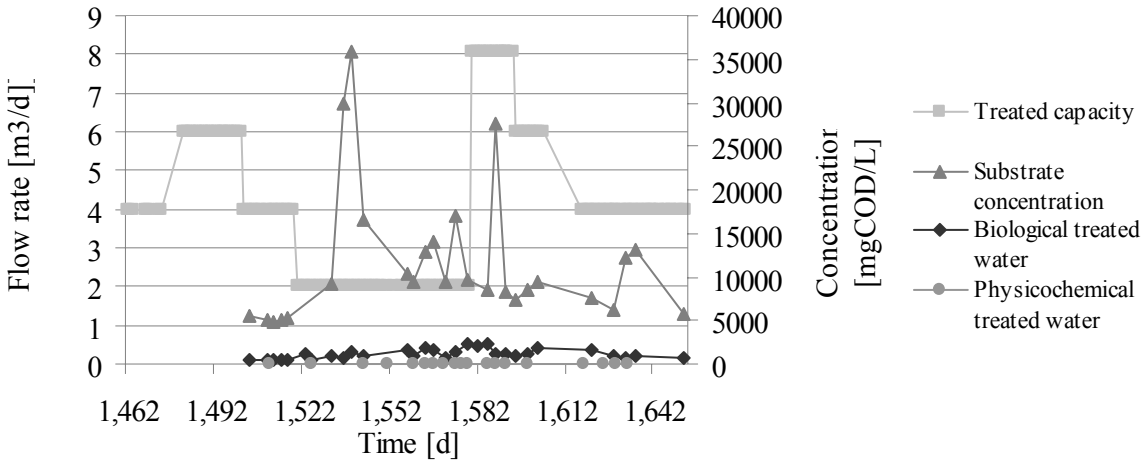


Fig. 2. Treated water concentration of the biological and physicochemical processes

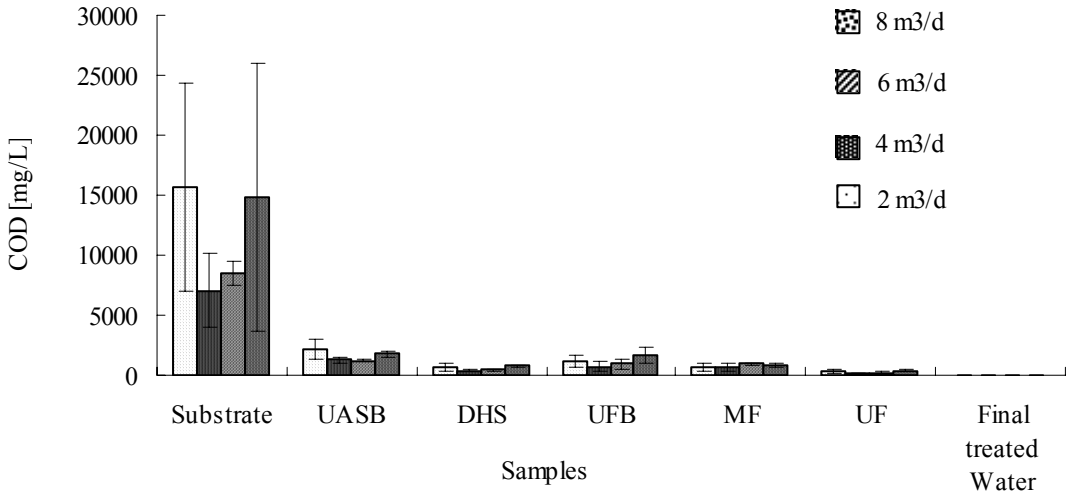


Fig. 3. Average COD removal by each process

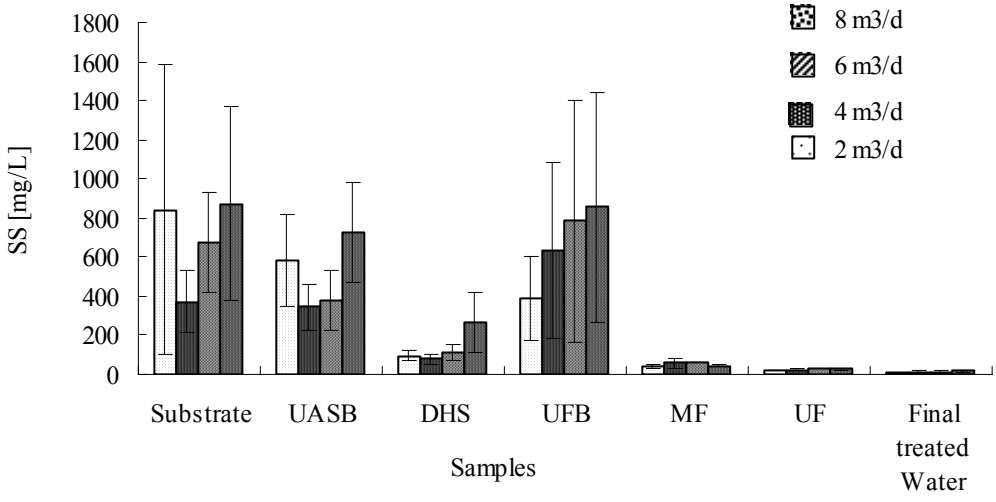


Fig. 4. Average suspended solids removal by each process

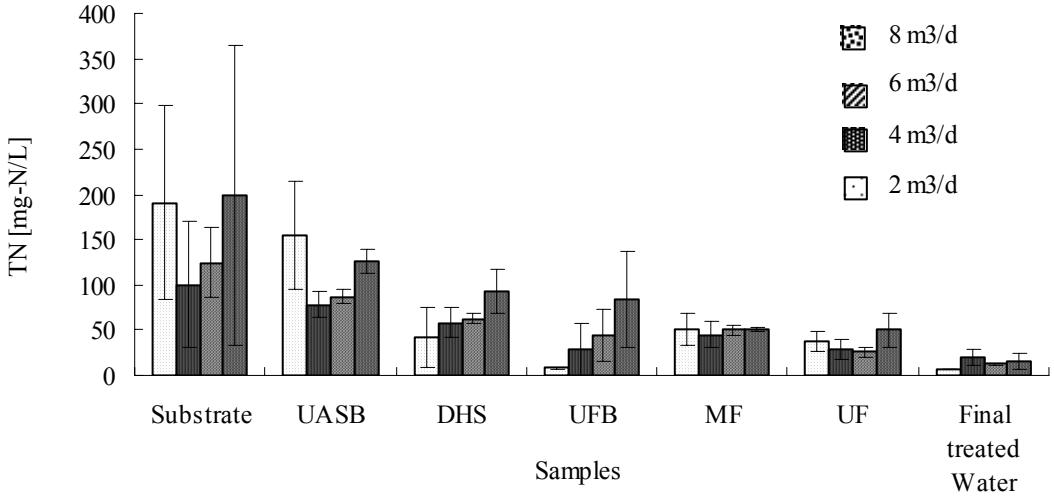


Fig. 5. Average total nitrogen removal by each process

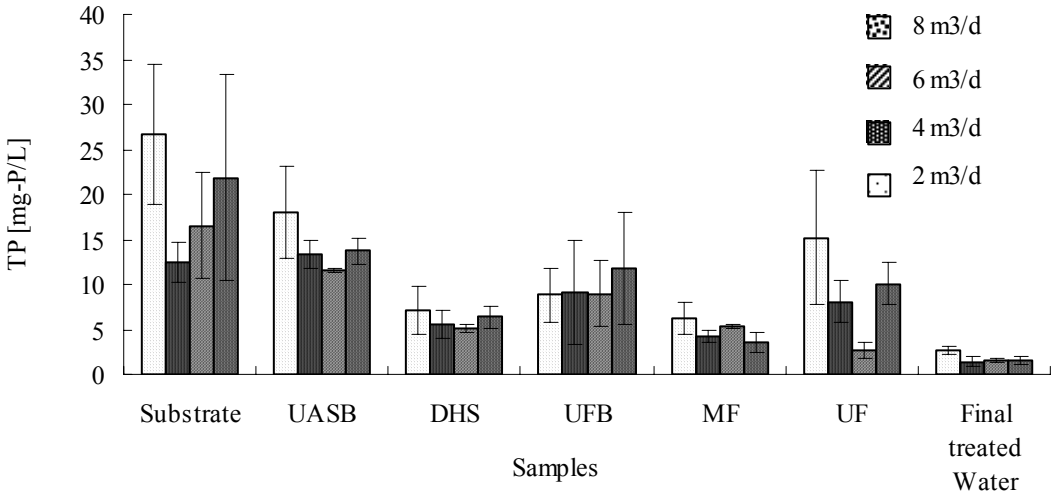


Fig. 6. Average total nitrogen removal by each process

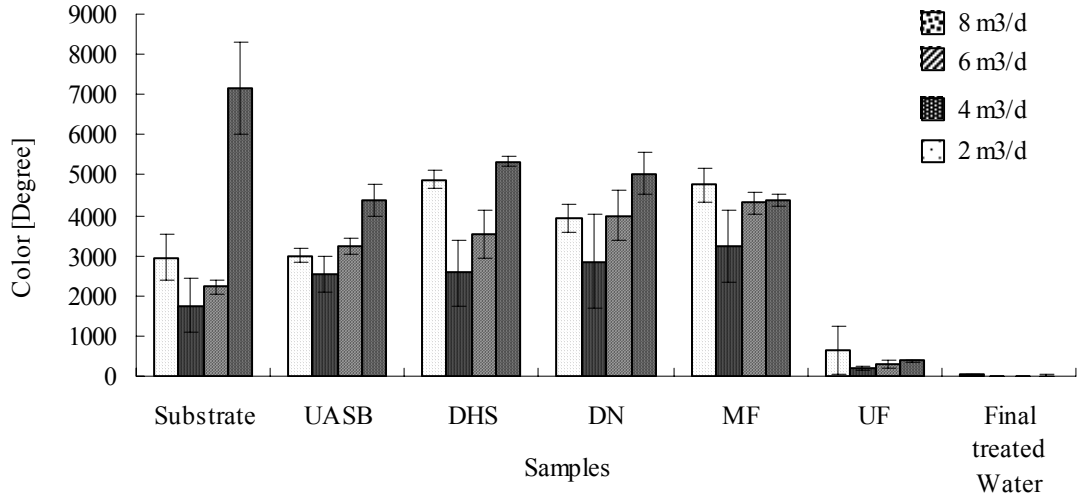


Fig. 7. Average color removal by each process

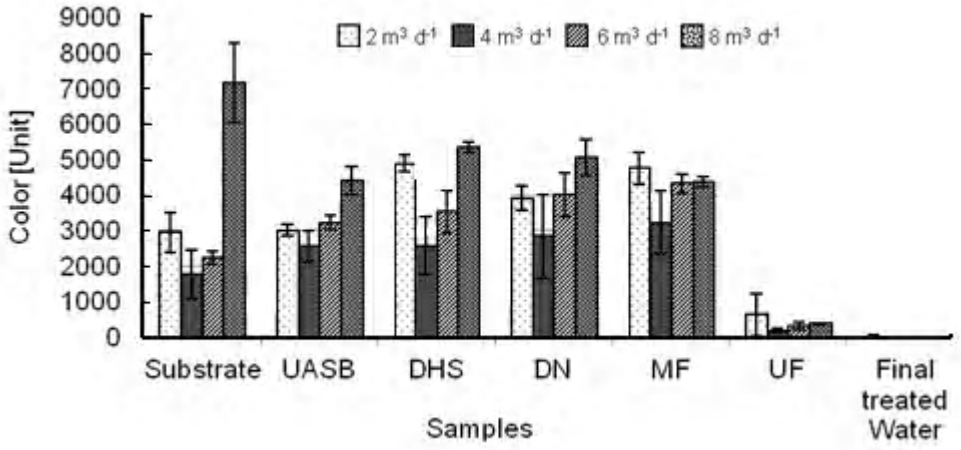
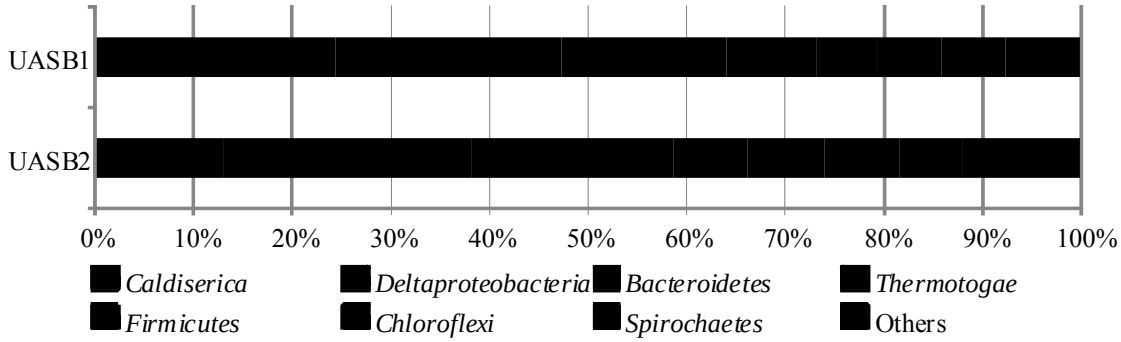


Fig. 8. Average color removal by each process



Others; Actinobacteria, Synergistetes, Verrucomicrobia, Epsilonproteobacteria, Planctomycetes, Candidate division OP8, Candidate division OP9, Candidate division WS3 and unclassified.

Fig. 9. 16S rRNA analysis of the microbial diversity in the UASB1 and UASB2 reactors

Table 2. Summary of the biological and physicochemical processes parameters used to treat the wastewater

Biological treated water								Physicochemical treated water					
Flow [$m^3 d^{-1}$]	SS [$mg L^{-1}$]	COD_{cr} [$mgCOD L^{-1}$]	BOD [$mg L^{-1}$]	TN [$mgN L^{-1}$]	TP [$mgP L^{-1}$]	Color [Unit]	pH	SS [$mg L^{-1}$]	COD_{cr} [$mgCOD L^{-1}$]	TN [$mgN L^{-1}$]	TP [$mgP L^{-1}$]	Color [Unit]	pH
2	404 (220)	1,194 (544)	269 (183)	44.4 (34.8)	8.9 (3.0)	3,914 (345)	8.1 (0.6)	11 (3.4)	36.7 (22.9)	13.9 (7.1)	2.7 (0.4)	26 (20)	7.2 (0.2)
4	633 (447)	703 (418)	115 (92)	25.4 (12.9)	9.2 (5.7)	2,839 (1,172)	8.4 (0.4)	13 (3.2)	36.6 (5.7)	17.2 (10.5)	1.4 (0.6)	9 (4)	7.4 (0.2)
6	785 (621)	1,276 (445)	233 (28)	44.0 (27.9)	9.0 (3.7)	3,994 (614)	8.4 (0.2)	14 (3.8)	19.0 (7.0)	12.5 (0.7)	1.6 (0.2)	14 (7)	7.3 (0.5)
8	855 (587)	1,621 (579)	483 (277)	84.8 (53.0)	11.8 (6.1)	5,045 (502)	8.6 (0.1)	17 (3.0)	19.0 (12.7)	15.5 (9.2)	1.5 (0.4)	25 (5)	7.1 (0.3)

and VSS measurements). The MF module reduced the suspended particles from 496 to 42 mg/L(%R_j = 89.9) and the UFB-treated water was used to feed the UF (Fig. 4). However, the influent (UFB-treated water) with an average total nitrogen concentration of 43 mg-N/L was not removed during MF, but slightly decreased by UF and NF to the level of 34 and 17 mg-N/L, respectively (Fig. 5). The ammonia-N, nitrite-N, and nitrate-N were not measured during the physicochemical process, but it was found that the membrane configuration reduced the levels of some ions (NH_4^+-N and NO_3^--N) by NF. Thus, the permeate phosphate (PO_4^{3+}-P) concentration was reduced from 15.2 mg-P/L (UF effluent, 2 m^3/d flow) to 2.7 mg-P/L (NF effluent, 2 m^3/d flow) (Fig. 7). This showed that the NF membrane was highly efficient in removing trivalent cations including phosphate and certain metals. The MF membrane (MF filtered water: 4750 units with 2 m^3/d flow; 3219 units with 4 m^3/d flow; 4316 units with 6 m^3/d flow; and 4360 units with 8 m^3/d flow) had a micropore size of >50 nm so it could only filter suspended particles and not the colored pigments remaining in the molasses-based effluent from the UFB. However, the color (true color) of the permeates from the UF (634 units with 2 m^3/d flow; 185 units with 4 m^3/d flow; 303 units with 6 m^3/d flow; and 376 unit with 8 m^3/d flow) appeared to be transparent light yellow and crystal clear in the NF permeate (26 units with 2 m^3/d flow; 9 units with 4 m^3/d flow; 14 units with 6 m^3/d flow; and 25 units with 8 m^3/d flow) (Fig. 8). We analyzed 78 (UASB1) and 92 (UASB2) bacterial 16S rRNA gene clones. Fig. 9 shows the phylogenetic affiliation of the bacterial clones. Over 50% of the bacterial clones belonged to common dominant bacteria such as *Caldiserica*, *Bacteroidetes*, and *Deltaproteobacteria*, although others such as *Thermotogae*, *Firmicutes*, *Chloroflexi*, and *Spirochaetes* were also found. Most bacteria known from the phylum *Caldiserica* were isolated from a hot spring mat (Skirnisdottir *et al.*, 2000), hydrothermal vent (Inagaki *et al.*, 2006), and anaerobic wastewater treatment reactors (Kaksonen *et al.*, 2004; Chen *et al.*, 2004). The dominant *Caldiserica*-related OTUs (UASB1B_D11 = 16 clones and UASB2B_E12 = 11 clones) were retrieved from the UASBs and they shared 99% sequence similarity with a clone isolated from a muddy hot spring in southwestern Taiwan (FJ638586). *Caldisericum exile* (Mori *et al.*, 2009) shared 81% sequence similarity with a known isolate (NR041680). However, there was no significant proof of an important role for the identified microorganisms in both UASBs. Nevertheless, the *Caldiserica*-related clones were the most abundant in UASB1 (Fig. 9), which suggested that *Caldiserica* had a role in breaking down complex organic compounds (e.g., carbohydrate, protein, and lipids) in this system. Bacteria of the phylum *Bacteroidetes* were also found and they are known to be complex organic compound-degrading anaerobic microorganisms (Kragelund *et al.*, 2008). The dominant *Bacteroidetes*-related OTUs (UASB1B_A03 = three clones, UASB1B_E01 = three clones, UASB2B_C06 = four clones, and UASB2B_B10 = three clones) in this system shared high sequence similarity with others collected from anaerobic bioreactors (U81712, FJ228431, DQ661703, and GQ182907). *Firmicutes*-related clones and other complex organic macromolecules-degrading bacteria were less abundant than the *Bacteroidetes*-related clones in both UASBs (Fig. 9). Thus, it can be assumed that bacteria from the phylum *Bacteroidetes* played a more important role in degrading complex organic compounds in both UASBs compared with *Firmicutes*. The dominant syntrophic bacterial OTUs from the *Deltaproteobacteria* were affiliated to *Syntrophobacter* (UASB1B_G09 = one clone and UASB1B_C10 = three clones), *Syntrophus* (UASB2B_C11 = four clones), and *Syntrophaceae* (UASB2B_A03 = three clones). *Syntrophobacter* is known to be a propionate-degrading bacteria (Boone *et al.*, 1980; Chen *et al.*, 2005; Harmsen *et al.*, 1998; Wallrabenstein *et al.*, 1995) while *Syntrophaceae* is a long chain fatty acid-degrading bacteria (Jackson *et al.*, 1999; Grabowski *et al.*, 2005, Hatamoto *et al.*, 2007). Moreover, *Syntrophaceae* and *Syntrophus* were reported to have an ability of degrading benzoic acid and butyric acid (Mountfort *et al.*, 1984; Jackson *et al.*, 1999; Wallrabenstein *et al.*, 1995). Anaerobic chemoorganotroph-related clones from the phyla *Thermotoga*, *Chloroflexi* and *Spirochaetes* were also retrieved, which can biodegrade various organic compounds by fermentation (Balk *et al.*, 2002; Yamada *et al.*, 2006; Breznak and Warnecke, 2008). The dominant *Thermotogae*-related OTUs (UASB1B_D07 = seven clones and UASB2B_F08 = seven clones) had 100% sequence similarity to an uncultured bacterium retrieved from crude oil-contaminated soil (HQ689254). Despite all cultured members of *Thermotogae* are thermophilic anaerobic bacteria (Nunoura *et al.*, 2010). *Thermotogae* 16S rRNA genes have been retrieved from mesophilic anaerobic digesters. Therefore, these OTUs also appeared to be low-temperature-adapted *Thermotogales* (Nesbo *et al.*, 2006). In UASB2, the dominant OTU within *Chloroflexi* was *Anaerolineaceae* (UASB2B_F11 = three clones). An *Anaerolineaceae*-related OTU shared 99% sequence similarity with an uncultured bacterial clone retrieved from an anaerobic digester used to treat feedstock (GU389465). *Anaerolineaceae* consisted of only a few isolates and those were filamentous bacteria. In addition, these bacteria may play an important role in granulation in this system (Yamada *et al.*, 2005). Other bacterial clones within *Actinobacteria*, *Synergistetes*,

Verrucomicrobia, *Epsilonproteobacteria*, *Planctomycetes*, Candidate division OP8, Candidate division OP9, and Candidate division WS3 were retrieved from one or both UASBs at low frequencies (Fig. 9).

In total, 91 archaeal 16S rRNA gene clones were analyzed from UASB2. The acetoclastic methanoarchaeal genus *Methanosaeta* was dominant in granular sludge samples (54 clones), which showed that *Methanosaeta* could overcome *Methanosarcina* (undetected), another major acetotrophic archaean encountered in most anaerobic digesters, in a low acetate environment (Koster *et al*., 1987). We also found 16 clones of *Methanolinea*, which (Imachi *et al.*, 2008) has a line-shaped morphotype and utilizes H_2 and formate to produce methane. Other H_2-utilizing *Methanobacterium* clones were also found (ten clones). The remainder were classified as *Thermoplasmata* (three clones), *Methanomethlovorans* (one clone), *Methanospillirum* (one clone), *Thermoprotei* (one clone), and others (six clones).

CONCLUSION

This study demonstrated that a series of combined biological and physicochemical treatments effective for treating medium to high concentration molasses-based wastewater. The biological UASB-DHS-UFB had an important role as the main organic treatment, especially for reducing COD, SS, BOD, and nitrogen species, while the membranes (MF, UF, and NF) were a functional alternative treatment that enhanced the removal of untreated nutrients and color. In addition, the biogas recovered from UASBs could be used to increase the treatment performance and reduce the operational energy costs. The application of hybrid biophysicochemical systems could be a pragmatic environment-friendly solution for removing >95% of the overall organic substances, nutrients, and color. Finally, the molecular microbiological analysis of sludge samples from UASB1 and UASB2 produced similar results because the same 16S rRNA gene sequences of meso-thermophilic bacteria were the dominant microbial species. In particular, an unusual *Caldiserica* isolate was detected in both reactors, which is generally found in thermophilic hot spring environments. It is possible that *Caldiserica* existed along in the seed sludge, which existed prior to the brewery wastewater, and could adapt themselves to the new environment of the molasses-based bioethanol wastewater.

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