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Biodegradation of beet molasses vinasse by a mixed culture of micro organisms: Effect of aeration conditions and pH control

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Abstract

The effect of aeration conditions and pH control on the progress and efficiency of beet molasses vinasse biodegradation was investigated during four batch processes at 38°C with the mixed microbial culture composed of *Bifidobacterium*, *Lactobacillus*, *Lactococcus*, *Streptococcus*, *Bacillus*, *Rhodopseudomonas*, and *Saccharomyces*. The four processes were carried out in a shake flask with no pH control, an aerobic bioreactor without mixing with no pH control, and a stirred-tank reactor (STR) with aeration with and without pH control, respectively. All experiments were started with an initial pH 8.0. The highest efficiency of biodegradation was achieved through the processes conducted in the STR, where betaine (an organic pollutant occurring in beet molasses in very large quantities) was completely degraded by the microorganisms. The process with no pH control carried out in the STR produced the highest reduction in the following pollution measures: organic matter expressed as chemical oxygen demand determined by the dichromatic method + theoretical COD of betaine (COD_{sum}, 85.5%), total organic carbon (TOC, 78.8%) and five-day biological oxygen demand (BOD_5, 98.6%). The process conditions applied in the shake flask experiments, as well as those used in the aerobic bioreactor without mixing, failed to provide complete betaine assimilation. As a consequence, reduction in COD_{sum}, TOC and BOD_5 was approximately half that obtained with STR.

Key words: aerobic biodegradation; batch process; beet molasses vinasse; mesophilic conditions; mixed culture of microorganisms

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Introduction

In the past decade, the need to reduce fossil fuel combustion has increased interest in ethanol as a renewable energy source (biofuel). In 2008 the proportion of ethanol fuel in the overall volume of ethanol produced approached 81% (HGCA, 2009; Licht, 2009). Ethanol is produced both from sugar-based (sugar cane, molasses, and sugar beets) and starch-based (mainly food grain) substrates (Coyle, 2007; Gopinathan and Sudhakaran, 2009; Krzywonos et al., 2009). The growing demand for such biofuel over the last decade has spurred a worldwide rise in its production, which has increased from 17.1×10^9 to 73.9×10^9 L (Licht, 2009; GRFA, 2010). However, bioethanol production still accounts for less than 8% of the world's gasoline consumption (Andersen, 2009). The predicted further rise in the production of bioethanol fuel will undoubtedly cause the volume of stillage (distillation residue) to increase dramatically, thus aggravating the global problem of its utilization since the quantity of stillage obtained is more than ten times the quantity of the bioethanol produced (Wilkie et al., 2000).

Economic considerations frequently suggest the simplest answers to the question of how to utilize stillage produced, specifically as either fodder or fertilizer. Such uses, however, do not resolve the problem because of the large scale of bioethanol production. Molasses-based stillage (vinasse) is characterized by high potassium content, and therefore limits its potential as farm animal fodder (Lewicki, 2001). Another use, particularly in the case of vinasse, consists in classifying stillage as wastewater and making it subject to anaerobic biodegradation (Wilkie et al., 2000; Satyawali and Balakrishnan, 2008).

Stillage contains many organic and mineral substances (Wilkie et al., 2000), including organic acids, reducing substances, glycerin and proteins (Krzywonos et al., 2010). Beet molasses vinasse additionally contains betaine (Parnaudeau et al., 2008). The organic pollution load of beet molasses vinasse expressed as chemical oxygen demand (COD) is high and often exceeds 100 g O_2/L (Ryznar-Luty et al., 2008), thus adding to the environmental issues of this

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high-strength wastewater. A recommended approach to the treatment of such effluents is to use aerobic biodegradation processes. However, the use of biodegradation for the treatment of distillery wastewater is still subject to laboratory tests, and so far no studies on their industrial applications have been reported. A previous laboratory study on biodegradation of beet molasses vinasse produced promising results (Ryznar-Luty, 2008). Over the temperature range of 27–54°C using a mixed culture of thermo- and mesophilic *Bacillus* bacteria, an 83.7%–88.7% reduction in COD_{sum} (COD of vinasse + theoretical COD of betaine) and 95.8%–99.5% reduction in BOD_5 was obtained. The positive results of beet molasses vinasse biodegradation under aerobic mesophilic conditions spurred our current research on the effect of aeration conditions and pH control on the progress and efficiency of biodegradation of the same stillage by a commercial mixed culture of microorganisms marketed under the brand name of SCD ProBio Original™ (formerly EM-Farming™).

1 Materials and methods

1.1 Microorganisms

In all experimental processes, a mixed SCD ProBio Original™ (formerly EM-Farming™) culture of microorganisms was used, occurring in a liquid medium obtained from ProBiotics Polska™ (formerly EM-WORLD® (Poland)). According to the supplier's specifications, the culture consisted of the following microorganisms: *Bifidobacterium animalis*, *Bifidobacterium bifidum*, *Bifidobacterium longum*, *Lactobacillus acidophilus*, *Lactobacillus bulgaricus*, *Lactobacillus casei*, *Lactobacillus delbrueckii*, *Lactobacillus plantarum*, *Lactococcus diacetylactis*, *Lactococcus lactis*, *Streptococcus thermophilus*, *Bacillus subtilis var natto*, *Rhodopseudomonas palustris* and *Saccharomyces cerevisiae*. In this study, the culture will be referred to as SCD ProBio Original™.

Upon 1:100 dilution, 1 mL of inoculum was obtained, added to the beet molasses vinasse medium (500 mL), and placed in the scrubber (non-stirred bioreactor) aerated at the rate of 1.0 vvm (volume of air/(volume of medium × min)). This aeration speed was based on the knowledge of the culture composition and our previous use of the same aeration speed and same scrubber to support (with success) the activity of a mixed culture of *Bacillus* (Ryznar-Luty, 2008), despite the oxygen deficiency in the medium. With the exception of *Bacillus subtilis*, all microorganisms colonized in the present study were anaerobic, but oxygen tolerant, and displayed low oxygen demand. It should be noted that the choice of aeration speed (1.0 vvm) was a compromised solution.

The medium in the scrubber consisted of beet molasses vinasse (Section 1.2). Every three days the microorganisms were inoculated onto a fresh medium, the volume of the inoculum amounting to 20 mL. The temperature in the scrubber was maintained at 38°C as specified by the supplier. After four weeks of adaptation, the inoculum (also a 72-hr culture), prepared via this route, was used in the experiments.

1.2 Preparation of the beet molasses vinasse medium

The beet molasses vinasse (referred to as stillage) was obtained from Zakłady Wytwórcze CHEKO, Włocławek, Poland. The chemical composition of this stillage is shown in Table 1.

Table 1 Characterization of beet molasses vinasse[a]

Parameter	Value
pH	4.97 ± 0.01
Density (°Bx)	9.40 ± 0.10
Suspended solids (SS) (g/L)	4.64 ± 0.44
$SCOD_{sum}$[b] (g O_2/L)	104.64 ± 2.48
SCOD (soluble chemical oxygen demand) (g O_2/L)	57.39 ± 0.35
BOD_5 (five-day biological oxygen demand) (g O_2/L)	36.40 ± 2.14
TOC (total organic carbon) (g/L)	30.75 ± 1.23
Betaine (g/L)	22.53 ± 1.17
Reducing substances without hydrolysis (g/L)	3.350 ± 0.071
Reducing substances with hydrolysis (g/L)	7.710 ± 0.221
Glycerol (g/L)	3.333 ± 0.117
Total nitrogen[c] (g/L)	4.004 ± 0.165
Ammonia nitrogen[c] (g/L)	0.187 ± 0.015
Total phosphorus[c] (g/L)	0.056 ± 0.007
Phosphate phosphorus[c] (g/L)	0.011 ± 0.003

[a] Besides pH, density and suspended solids, the parameters were determined after suspended solids separation; [b] SCOD determined by the dichromate method + the theoretical COD of betaine; [c] determined before enrichment with 0.9 g $NH_4H_2PO_4$/L.

The stillage was boiled for 15 min before use to avoid contamination. After cooling, samples were enriched with 0.9 g/L of $NH_4H_2PO_4$, and the pH was adjusted to 8.0 with 33% NaOH. The enrichment of the stillage with phosphorus was substantiated by previous biodegradation studies of beet molasses vinasse (Ryznar-Luty, 2008). However, there was a risk that if large quantities of the biomass were synthesized, NH_4^+-N might become a process limiting factor, as observed previously during biodegradation of potato stillage with a mixed culture of *Bacillus* bacteria (Cibis et al., 2004).

1.3 Biodegradation processes

Four biodegradation processes were conducted. One involved a shake flask culture (SFC), where a 750-mL volume flask was filled with 150 mL of stillage medium and shaken using an orbital platform shaker (GFL 3031, Germany; orbit diameter, 30 mm; platform size, 450 mm × 450 mm) at 150 r/min. The second process entailed a 500-mL working volume aerobic bioreactor without mixing (ABWM), with aeration set to 1.0 vvm. During both processes the pH was uncontrolled and its initial value was 8.0. The other two processes were performed in a 5-L working volume stirred-tank reactor (STR) of Biostat® B type (B. Braun Biotech International, Germany), at a stirrer speed of 900 r/min (stirrer shaft with two 6-bladed Rushton turbines) and aeration set to 1.6 vvm. One of these processes was conducted without pH control (stirred-tank reactor; non-controlled pH) (STRNC), at the initial pH 8.0; the other with pH control (stirred-tank reactor; controlled pH) (STRC), also at the initial pH 8.0, which was kept constant with 2 mol/L H_2SO_4 and 2 mol/L NaOH. The pH

8.0 was adopted according to previous work (Ryznar-Luty, 2008). Temperature, dissolved oxygen tension (DOT), and pH were measured in a continuous mode by the sensors built into the bioreactor.

Biodegradation experiments were conducted at 38°C for 168 hr. The volume of the inoculum (prepared as shown in Section 1.1) accounted for 4% of the stillage (starting material).

1.4 Analytical methods

The number of cells in the stillage medium was counted with a haemocytometer. After centrifugation at 18,500 ×*g* for 40 min (4K15, Sigma Laborzentrifugen GmbH, Germany), suspended solids (SS) were determined gravimetrically after the sample was dried at 50°C for 24 hr and then at 105°C until a constant weight was obtained. The supernatant was used in further analyses. Chemical oxygen demand, five-day biological oxygen demand (BOD_5), total organic carbon (TOC), total nitrogen (TN), total phosphorus (TP), and phosphate phosphorus (PO_4^{3-}-P) were established spectrophotometrically using Dr. Lange cuvette tests (Handbook of Photometrical Operation Analysis, 2000). Chemical oxygen demand was measured both in the supernatant (SCOD) and in the non-centrifuged medium (TCOD). Total nitrogen was determined by the Kjeldahl method (APHA, 1992) using C. Gerhardt GmbH & Co. KG apparatus (Germany). To measure ammonia nitrogen (NH_4^+-N) concentration, distillation (Williams, 1979) with water vapor in the Parnas apparatus was used. Glycerol concentration was prepared using spectrophotometry (Rebelein, 1957; Ogorodnik and Stupakova, 1981). The concentrations of reducing substances were measured by the Lane-Eynon method involving titration of the stillage by Cu(II) in an alkaline medium and at high temperature (about 100°C) (McDonald, 1954). Betaine was determined using the colorimetric method (Focht et al., 1956). Since dichromatic analysis of COD fails to detect betaine, the parameter COD_{sum}, defined as the sum of the stillage COD and the theoretical COD of betaine (2.097 g O_2/g betaine), was introduced as one of the pollution load parameters of the stillage examined.

2 Results

2.1 Biodegradation efficiency of beet molasses vinasse

As shown in Table 2, the SFC process and the ABWM process were characterized by very poor biomass production, with final cell numbers in the stillage medium of 1.9 × 10^9/mL and 1.3 × 10^9/mL, respectively. This indicated that the transfer from shake flasks to aerobic bioreactor without mixing failed to increase the quantity of biomass produced. A significant rise in biomass production was observed, however, when biodegradation was conducted in the stirred-tank reactor (STR). In the STR process with pH control (STRC) the final number of microbial cells totaled 10.7 × 10^9/mL, compared with the process with no pH control (STRNC) in which cells totaled 4.9 × 10^9/mL. In all processes, the initial number of cells in the vinasse medium approached 0.4 × 10^9/mL. In the STR processes,

Table 2 Effect of biodegradation method on the efficiency of beet molasses vinasse treatment (38°C, initial pH = 8.0)

Parameter	Biodegradation method			
	SFC	ABWM	STRNC	STRC
$SCOD_{sum}$ removal (%)	46.9 ± 3.1[a]	42.2 ± 3.1	85.5 ± 2.6	84.3 ± 2.7
Time after which 90% of the overall reduction in $SCOD_{sum}$ was attained (hr)	ND	ND	84.0	32.0
$SCOD_{sum}$ removal rate (g O_2/(L·hr))[b]	ND	ND	0.9	2.2
SCOD removal (%)	62.2 ± 1.4	35.8 ± 1.3	72.1 ± 0.8	67.1 ± 0.8
$TCOD_{sum}$ removal (%)	40.5 ± 3.0	40.2 ± 3.0	75.5 ± 2.4	70.1 ± 2.7
TCOD removal (%)	47.9 ± 1.1	32.6 ± 1.3	56.2 ± 0.9	41.3 ± 1.2
BOD_5 removal (%)	58.8 ± 0.9	40.4 ± 1.4	98.6 ± 0.1	98.4 ± 0.1
TOC removal (%)	49.9 ± 2.9	54.3 ± 2.2	78.8 ± 2.0	78.1 ± 2.9
Ammonia nitrogen removal (%)	–6.3 ± 8.2	–4.5 ± 5.2	48.5 ± 4.5	–314.7 ± 15.2
Total nitrogen removal (%)	34.6 ± 4.1	32.2 ± 4.1	59.3 ± 4.1	54.8 ± 3.8
Phosphate phosphorus removal (%)	54.0 ± 4.2	–39.7 ± 6.6	–28.0 ± 5.3	46.3 ± 4.3
Total phosphorus removal (%)	60.4 ± 5.2	7.6 ± 6.6	0.9 ± 6.5	49.0 ± 5.4
Betaine removal (%)	32.8 ± 6.2	48.1 ± 5.8	100.0 ± 0.0	100.0 ± 0.0
Glycerol removal (%)	94.0 ± 3.3	94.8 ± 3.3	90.3 ± 3.0	80.4 ± 3.3
Removal of reducing substances without hydrolysis (%)	77.2 ± 2.2	58.9 ± 2.3	78.6 ± 2.0	74.1 ± 2.2
Removal of reducing substances with hydrolysis (%)	88.1 ± 3.5	62.5 ± 3.7	87.7 ± 3.5	86.6 ± 3.5
Initial SS (g/L)	6.9 ± 0.6	6.9 ± 0.6	9.4 ± 0.7	6.9 ± 0.6
Final SS (g/L)	6.1 ± 0.6	3.3 ± 0.4	10.0 ± 0.8	12.4 ± 0.7
Final amount of SS formed (g/L)	–0.8 ± 0.8	–3.6 ± 0.7	0.6 ± 1.1	5.5 ± 0.9
SS removal (%)	11.4 ± 8.6	51.5 ± 7.1	–6.4 ± 6.8	–79.7 ± 13.2
Y_{SS} ((g final SS formed/g $SCOD_{sum}$ removed) × 100%)	–1.9 ± 2.0	–9.3 ± 2.0	0.7 ± 1.3	7.1 ± 1.2
Initial number of cells (× 10^9/mL)	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1	0.4 ± 0.1
Maximal number of cells formed (× 10^9/mL)	1.9 ± 0.2	1.3 ± 0.2	6.8 ± 0.8	10.7 ± 1.4
Final number of cells formed (× 10^9/mL)	1.9 ± 0.2	1.3 ± 0.2	4.9 ± 0.6	10.7 ± 1.8

SFC: shake flask culture; ABWM: aerobic bioreactor without mixing; STRNC: stirred-tank reactor, non-controlled pH; STRC: stirred-tank reactor, controlled pH. ND: not determined.

[a] Values are presented as mean ± SD (n = 3).

[b] The value of this parameter was calculated for the point in time after which 90% of the overall reduction in $SCOD_{sum}$ was attained.

[c] The sign minus indicates an increase in the content of the pollutant.

an increase was also observed in the content of suspended solids (79.7% increase in STRC and 6.4% in STRNC compared to initial values), while in SFC and ABWM the final SS content was lower than the initial one.

The variations in number of cells and DOT in the stillage medium during the two STR processes are shown in Fig. 1. Under STRNC conditions, the maximal number of cells (6.8×10^9/mL) was achieved after 20 hr at pH 8.95 (final pH 9.58). The increase in cell number was paralleled by a rapid decrease in DOT, whose value dropped to 0% after about 10 hr. In this case the phase of oxygen deficiency took about 5 hr. Thereafter DOT increased to almost 100% by the end of the process. The variations in DOT followed a different pattern in the process with pH control. At the initial stage of cell growth (which was not as rapid as in the biodegradation process with non-controlled pH) DOT fell to 0.3% after 10.25 hr but increased to 88% after 18 hr. After 33.75 hr, DOT fell to 2.9%, which was followed by an increase to 100%. The maximal number of bacterial cells was attained by the end of the process. As already mentioned, STRC produced the largest quantity of biomass. This, however, did not increase the efficiency of biodegradation expressed as $SCOD_{sum}$, $TCOD_{sum}$, BOD_5 and TOC, as the values of these parameters obtained in STRNC were slightly higher (without pH control: 85.5%, 75.5%, 98.6% and 78.8%, respectively; with controlled pH: 84.3%, 70.1%, 98.4% and 78.1%, respectively) (Table 2).

During the STR processes, betaine (which accounted for 37% of the organic pollutants expressed as TOC) was degraded completely by the SCD ProBio Original^{TM} culture. The extent of betaine biodegradation achieved with the microorganisms in the ABWM and SFC regimes was low, totaling 48.1% and 32.8%, respectively. As a consequence, reduction in $SCOD_{sum}$, $TCOD_{sum}$, BOD_5, and TOC, which included betaine, was noticeably lower than that obtained by STR processes (Table 2). As for the ABWM and SFC processes, higher efficiencies of reduction in $SCOD_{sum}$, $TCOD_{sum}$ and BOD_5, as well as in TCOD and SCOD, were reached with the SFC regime. Only TOC reduction was 4.4% higher in the ABWM than in the SFC process (Table 2).

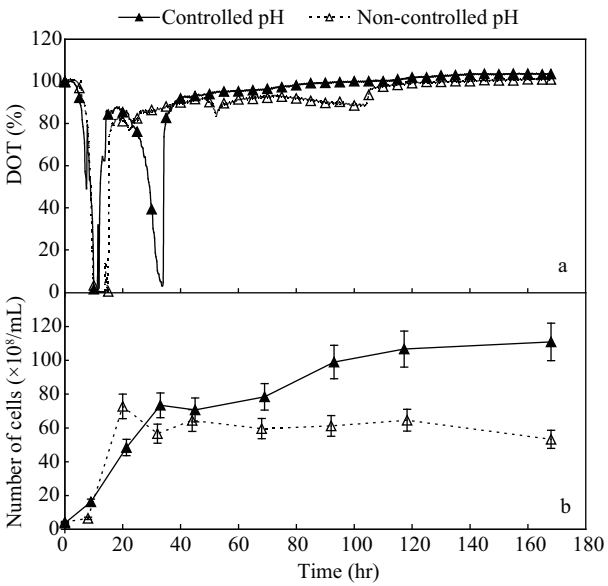

Fig. 1 Variations in DOT (a) and number of cells (b) in beet molasses vinasse during STR (stirred-tank reactor) processes with and without pH control. The length of each error bar is twice the standard deviation of the measured concentration (n = 3).

The plots of betaine and SCOD variations in the course of the STR processes (Fig. 2) imply that pH control contributed largely to the rate of betaine degradation by the microorganisms, but did not affect the rate of SCOD reduction, i.e., the overall rate of degrading organic pollutants other than betaine. In the STRC process, therefore, 100% degradation of betaine was observed after 33 hr compared to 92 hr in the STRNC process. As a consequence, after 32 hr in the STRC process, the extent of $SCOD_{sum}$ reduction reached 90% of the final value achieved after 168 hr. In the STRNC process, 90% reduction in $SCOD_{sum}$ was attained after 84 hr (Table 2). Thus, the $SCOD_{sum}$ removal rate for the STRC process (2.2 g O_2/(L·hr)) was more than twice as high as for the STRNC process (0.9 g O_2/(L·hr)), if the parameter was calculated at the point in time when 90% of the final reduction in $SCOD_{sum}$ was attained (Table 2). It is important to note that this method of determining the $SCOD_{sum}$ removal rate better reflects the dynamics of the process than the $\Delta SCOD_{sum}/t$ ratio, which relates the decrease in $SCOD_{sum}$ ($\Delta SCOD_{sum}$) to time elapsed. This finding holds particularly true for wastewater treatment processes conducted until the $SCOD_{sum}$ stabilization phase has been reached (or continued throughout that phase), because quotient $\Delta SCOD_{sum}/t$ is just another form of describing the efficiency of biodegradation, whose values decrease with the duration of the process.

2.2 Nitrogen and phosphorus removal

The extent of nitrogen and phosphorus removal was calculated taking into account the 0.9 g $NH_4H_2PO_4$/L added for stillage enrichment. After enrichment, the initial content of nitrogen and phosphorus were determined using the analytical methods described in Section 1.4.

The efficiency of total nitrogen removal varied according to the biodegradation method used. In ABWM and SFC processes the TN content of the stillage medium was reduced by 32.2% and 34.6%, respectively, while TN content decreased by 59.3% and 54.8% under STRNC and STRC conditions, respectively.

Figure 3 depicts the variations in NH_4^+-N content of the stillage medium with the STR processes. In STRNC mode, after reaching a maximal value (more than twice the initial one), ammonia nitrogen concentration decreased steadily until 48.5% removal efficiency was obtained with the termination of the process. In STRC mode, ammonia nitrogen followed a different pattern of removal. After 48 hr, its concentration in the stillage medium increased four-fold compared to the initial value and thereafter stabilized. The removal patterns were pH-dependent. In STRNC, after a defined time, the pH rose to 9.0 or higher, and ammonia nitrogen in the form of NH_3 volatilized at a rate faster than the rate of its production. In STRC, after 48 hr the rate of nitrogen volatilization in the form of NH_3 and the

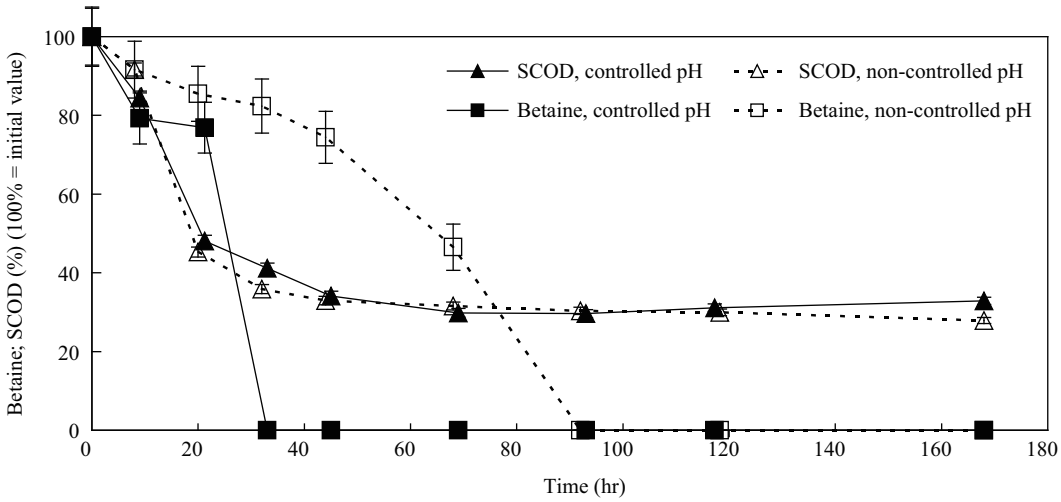

Fig. 2 Variations in SCOD and betaine concentration in beet molasses vinasse during STR processes with and without pH control. The length of each error bar is twice the standard deviation of the measured concentration (n = 3).

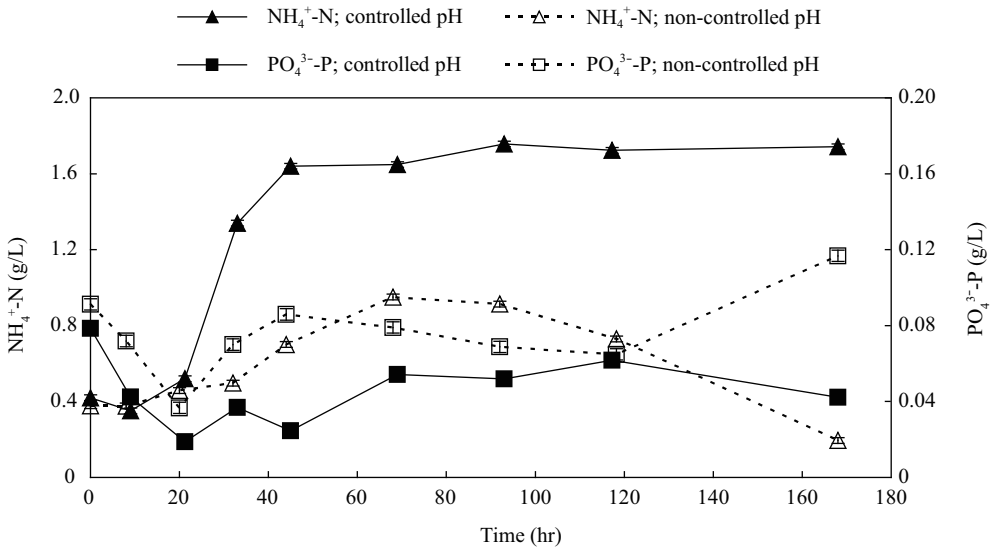

Fig. 3 Variations in ammonia and phosphate concentrations in beet molasses vinasse during STR processes with and without pH control. The length of each error bar is twice the standard deviation of the measured concentration (n = 3).

rate of NH_4^+-N production equalized. In the ABWM and SFC regimes, final ammonia nitrogen concentrations were higher than the initial ones (Table 2).

The highest efficiencies of total phosphorus (TP) removal from the stillage medium were in the SFC (60.4%) and STRC (49.0%) processes, and the lowest (0.9%) in the STRNC process (Table 2). Phosphate phosphorus concentrations in the stillage media for the two STR processes decreased steadily during biomass growth up to 20 hr (Fig. 3). Minimal phosphate phosphorus concentration amounted to 0.037 g/L in the STRNC mode and 0.019 g/L in the STRC mode, and was lower by 59.5% and 75.8%, respectively, than the initial values. In the STRNC regime, the decrease in number of microorganisms at the final stage of the process (Fig. 1b) was concomitant with a significant increase in PO_4^{3-}-P content (final value 0.12 g/L) (Fig. 3). This may be attributable to autolysis of the cells. Neither a rise in PO_4^{3-}-P concentration nor a simultaneous decrease in cell number was observed at the final stage of the STRC process.

2.3 Removal of reducing substances and glycerol

The lowest removal of reducing substances with and without hydrolysis totaled 62.5% and 58.9%, respectively, and was obtained in the ABWM process (Table 2), although it is important to add that the content of reducing substances with hydrolysis is an approximate measure of total sugar content, while without hydrolysis is a simplified measure of total monosugar content. The other biodegradation processes produced comparable reducing substances removal efficiencies, which ranged from 86.6% to 88.1% with hydrolysis, and from 74.1% to 78.6% without hydrolysis (Table 2). For the reducing substances and glycerol removal efficiencies, glycerol was removed to a higher extent (the STRC process being an exception). The highest extent of glycerol assimilation was obtained with the ABWM process (94.8%), followed by SFC (94.0%) (Table 2). In the STR processes, glycerol was assimilated at a faster rate (within the first 24 hr) compared to reducing substances with and without hydrolysis (Fig. 4). Those determined with hydrolysis were assimilated at a faster

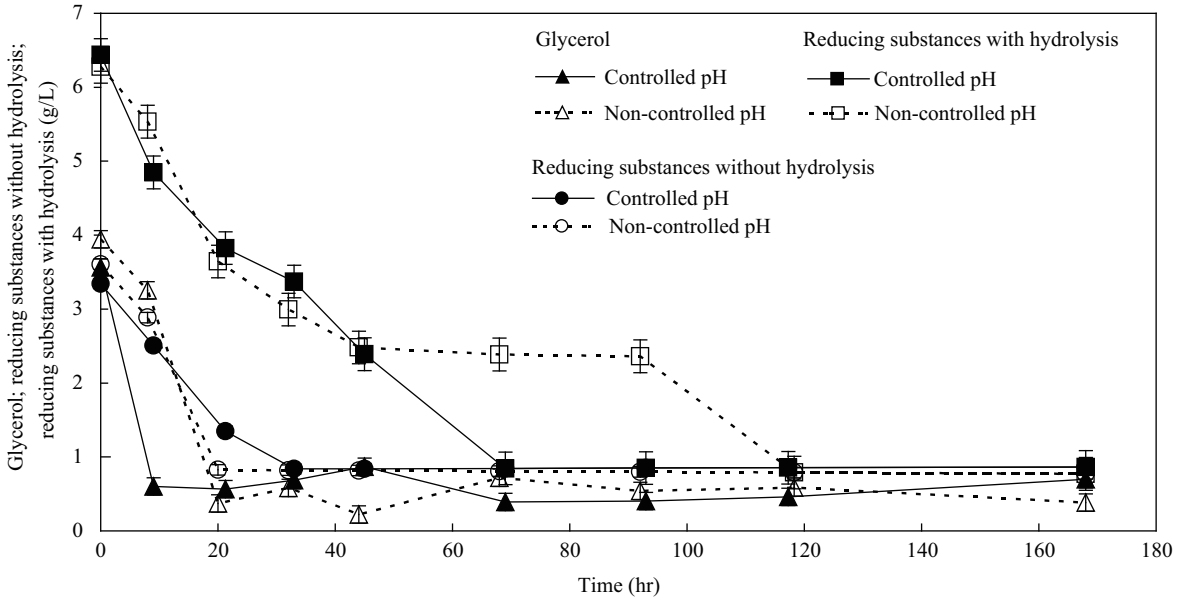

Fig. 4 Variations in the concentrations of glycerol and reducing substances in beet molasses vinasse during STR processes with and without pH control. The length of each error bar is twice the standard deviation of the measured concentration (n = 3).

rate under STRC than STRNC conditions, while those determined without hydrolysis were utilized at a faster rate in the STRNC regime. The lack of pH control in the STR processes did not decrease the assimilation of either reducing substances or glycerol. On the contrary, their removal from the stillage medium was visibly higher in the STRNC mode.

3 Discussion

Our results substantiate the potential use of the mixed microbial culture chosen for this study in the biodegradation of beet molasses vinasse under mesophilic conditions. The methods with which the stillage was biodegraded contributed largely to the progress and efficiency of the treatment process. The highest extent of removal was obtained with the aerated stirred-tank bioreactor (STR processes) (Table 2). It can be assumed that under such conditions the following aerobic and facultative aerobic strains of the mixed culture were active: *Bacillus subtilis* var *natto*, *Rhodopseudomonas palustris* and *Saccharomyces cerevisiae.* The removal efficiencies achieved with this mode of biodegradation were similar to those reported by Ryznar-Luty (2008), who used a mixed culture of thermo- and mesophilic *Bacillus* bacteria for the biodegradation of stillage under similar conditions. The $SCOD_{sum}$ reduction obtained with that culture at 36°C amounted to 86.44% for the process with pH control (pH 8.0) and 85.41% for the process with no pH control (initial pH 8.0). The high $SCOD_{sum}$ reduction achieved in the present study and by Ryznar-Luty (2008) were attributable to the 100% microbial degradation of betaine, the main organic stillage pollutant. It is important to note that under thermophilic conditions the assimilation of betaine proceeded at a slower rate, and at excessively high temperature (63°C) betaine was not assimilated (Ryznar-Luty, 2008).

The same study has also demonstrated that for processes conducted at pH 8.0, the bacteria removed betaine over a wider temperature range than in processes performed at pH 6.5 (27–45°C). Previous investigations reported in the literature have revealed that both aerobic and anaerobic microorganisms have the capacity for degrading betaine (Klotzbücher et al., 2007). However, the number of studies in this field is limited. Aerobic microorganisms such as *Rhizobium*, *Pseudomonas* and *Corynebacterium* bacteria (Kortstee, 1970) and anaerobic microorganisms such as *Clostridium, Eubacterium, Sporomusa* and *Desulfobacterium* bacteria (Oren, 1990) are classified as betaine degraders. All these microorganisms assimilate betaine under mesophilic conditions. As yet, however, no data have been reported on the degradation of betaine by any of the microorganisms present in the SCD ProBio Original^{TM} culture, although the Biocyc database (Caspi et al., 2010) contains computer-predicted pathways of betaine degradation for all except *Saccharomyces cerevisiae*.

In the majority of investigations into the treatment of betaine-containing wastewater, the efficiency of betaine assimilation was assessed only in terms of dichromate COD removal, where betaine is not included. Only a few reports on the extent of betaine removal have been found relating to removed betaine during anaerobic treatment (Gil-Peña et al., 1987; Thalasso et al., 1999) and under aerobic biodegradation conditions (Glanser-Soljan et al., 1985; Ryznar-Luty, 2008). In their studies Gil-Peña et al. (1987) used unidentified bacteria from rumen fluid, Thalasso et al. (1999) used anaerobic sludge from an industrial digester, Glanser-Soljan et al. (1985) applied the yeast *Trichosporon* and activated sludge, and Ryznar-Luty (2008) used a mixed culture of *Bacillus* bacteria.

The $SCOD_{sum}$ reduction obtained in this study was comparable with that obtained in previous studies on distillery effluents other than beet molasses vinasse, such as biodegradation of potato slops with a mixed culture

of *Bacillus* bacteria (Cibis et al., 2004, 2006), which achieved SCOD reduction between 80.4% and 89.8%, and biodegradation of rye stillage and maize stillage, which achieved SCOD reductions of 84.6% and 82.6%, respectively (Cibis, 2004). A comparable SCOD removal rate (81%) was also reported for treatment of anaerobically predigested cane molasses vinasse with *Bacillus cereus* (Jain et al., 2002). The literature also reports on aerobic biodegradation of other high-strength industrial effluents, although SCOD reduction efficiencies were lower. Kosseva et al. (2001) attained a 62.5% SCOD reduction during bioremediation of cheese whey. Lasik and Nowak (2007) obtained an extent of SCOD reduction from 66% to 68% during biodegradation of wastewater from a potato processing plant after protein recovery from potato juice.

The markedly lower biodegradation efficiency produced by SFC and ABWM processes than by STR was not surprising (Table 2), and probably should be attributed to the more advantageous aerobic conditions in the stirred-tank bioreactor. Similar observations were made by Krzywonos et al. (2002) during aerobic biodegradation of potato slops under moderate thermophilic conditions (45°C), as well as by Ryznar-Luty et al. (2008) during aerobic biodegradation of beet molasses vinasse under thermophilic conditions (58°C). In both studies the extent of SCOD reduction was noticeably higher (by 17% or more) in the STR than in the SFC processes.

The rate of $SCOD_{sum}$ reduction attained by STRC (2.2 g O_2/(L·hr)) (Table 2) was higher than in previous studies using other modes of stillage biodegradation. Ryznar-Luty (2008) reported that for beet molasses vinasse biodegradation by thermo- and mesophilic *Bacillus* bacteria, at 36°C and pH 8.0, $SCOD_{sum}$ was reduced by 1.69 g O_2/(L·hr). Krzywonos et al. (2008) achieved a SCOD removal rate of 2.02 g O_2/(L·hr) during potato slops biodegradation by the same mixed culture of *Bacillus* bacteria, also at 36°C but at pH 7.0. Kosseva et al. (2001) found that for thermophilic (65°C) bioremediation of whey by a different mixed culture of bacteria SCOD reduction was 1.57 g O_2/(L·hr).

In the present study, all biodegradation modes produced relatively high glycerol removal, with the ABWM process providing the highest removal efficiency (94.8%), followed by SFC (94.0%) (Table 2). This demonstrates that less advantageous aerobic conditions compared to those in the STR did not reduce the quantity of the glycerol removed from the stillage medium. Lower efficiencies of glycerol removal in the STR processes compared to those achieved in the SFC and ABWM process were also reported by Krzywonos et al. (2002) and Cibis et al. (2002), which totaled 90% and 86%, respectively. As for the efficiency of reducing substance removal (Table 2), the values attained did not differ much from those reported by Cibis et al. (2002), but they were lower than the value obtained by Krzywonos et al. (2002).

As for nitrogen and phosphorus removal achieved by the STR processes, we found that with the STRC mode the SCD ProBio Original™ culture reduced total nitrogen, total phosphorus, and phosphate phosphorus to a higher extent than did the mixed culture of thermo- and mesophilic *Bacillus* bacteria used by Ryznar-Luty (2008). Depending on the type of biogen, the extent of reduction produced by the SCD ProBio Original™ culture ranged from 4% to 20% higher (Table 2). This finding does not hold true for the STR processes with no pH control, where, with the exception of ammonia nitrogen removal, the SCD ProBio Original™ culture was far less efficient. The *Bacillus* culture produced a removal efficiency of more than 70% for both total phosphorus and phosphate phosphorus; however, the SCD ProBio Original™ culture only produced a 0.9% total phosphorus removal, while PO_4^{3-}-P content in the stillage medium increased. In the case of ammonia nitrogen, the extent of reduction obtained with the SCD ProBio Original™ culture was by 5% higher than the reduction achieved by the *Bacillus* culture (Table 2).

4 Conclusions

Upon aeration, the mixed microbial SCD ProBio Original™ culture can be used for the aerobic treatment of beet molasses vinasse under mesophilic conditions. The efficiency of organic matter removal achieved (more than 98% of BOD_5 and more than 84% of $SCOD_{sum}$) corroborates the suggestion that biodegradation with SCD ProBio Original™ culture may be used as the first stage in a two- or multi-stage system for the treatment of this high-strength distillery effluent.

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