Continuous process of aerobic biodegradation of beet molasses vinasse: focus on betaine removal

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Abstract

We analyzed the effect of hydraulic retention time (HRT) and pH control of beet molasses vinasse on the efficiency of its continuous aerobic biodegradation with a mixed culture of *Bacillus* bacteria. Two experimental series were conducted in a 2-L stirred-tank reactor, at an aeration rate of 1 vvm, stirrer speed of 900 rpm, and temperature of 36 °C. The first series was run without medium pH control, whereas the other with pH maintained at 8.0. In both series, HRTs were 26, 47, and 78 h, whereas vinasse pH was raised to 8.0. The extent of its treatment via aerobic biodegradation depended on betaine assimilation phenomenon. High removal of pollutants (over 84%) expressed by the SCOD_{sum} index (SCOD_{sum} = soluble COD determined with the dichromatic method + theoretical COD of betaine) was achieved at HRTs of 47 and 78 h, whereas medium pH control had no explicit effect on biodegradation efficiency.

Keywords Beet vinasse · *Bacillus* · Continuous biodegradation · Aerobic conditions

Introduction

The Directive 2003/30/EC (2003) of the European Parliament and of the Council has obliged the Member States to increase the share of biofuels used in transport to 20% by the year 2020. Bioethanol is deemed to be one of the significant sources of ecoenergy. Since 2015, its global production has been reported to exceed 100×10^9 L annually (World Fuel Ethanol Production 2018). Interestingly, on average, 13 L of stillage is generated per liter of produced bioethanol (Fuess and Garcia 2014); hence, over 1300×10^9 L of this environment-unfriendly by-product, characterized by acidic pH, high concentrations of macro- and microelements, and corrosive properties (Fuess and Garcia 2014), needs to be managed globally. In addition, the pollution concentration of the stillage may be over 100 times higher than that of municipal sewage.

Cereal stillages are used for the production of high-protein feed mixtures for livestock (Ethanol Industry Outlook 2017). However, this method of stillage management is

curbed by high energy consumption during the technological process. In turn, sugarcane vinasse finds application as an additive to feed mixtures and fertilizers; it may also be directly spray-irrigated on sugarcane plantations, which enables reducing volumes of fresh water used for soil hydration simultaneously with its fertilization. This practice is, however, left uncontrolled, which poses a serious threat of soil structure modification, contaminant migration to the local surface waters, and finally of the reduced loads of dissolved oxygen in water bodies. Many distilleries discharge the molasses vinasse directly into open waters, which has a negative impact on aquatic ecosystems due to the toxic compounds it contains (Fuess and Garcia 2014; Suhaili et al. 2018).

In most cases, an alternative to the agricultural management of distillery wastewater may be offered by physico-chemical (e.g., adsorption, coagulation–flocculation) and biological (e.g., aerobic and anaerobic digestion) treatment processes successfully conducted at the laboratory scale (Espana-Gamboa et al. 2011). In our earlier works, we have proposed a mixed culture of meso- and thermophilic bacteria of the genus *Bacillus*, isolated from a bioreactor in one of the fruit and vegetable processing plants in the UK, for the aerobic biodegradation of beet molasses vinasse (Ryznar-Luty et al. 2008; Cibis et al. 2011). Our experiments concerning the batch processes have demonstrated that the tested culture

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ensured the highest efficiency and—most of all—the highest rate of biodegradation at temp. 36–45 °C and pH 8.0 (Cibis et al. 2011; Ryznar-Luty et al. 2015). To determine the feasibility of employing the proposed method of biodegradation under industrial conditions, which require continuous management of distillery stillage, continuous biodegradation of beet molasses vinasse was performed at the subsequent stage of our research.

The study presented in this manuscript was aimed at determining the effect of hydraulic retention time and pH regulation of beet molasses vinasse on the efficiency and course of the continuous processes of its aerobic biodegradation with the use of a mixed culture *Bacillus* bacteria. Experiments were performed at 36 °C because at this temperature, the batch processes were characterized by a higher rate of pollutants removal than at 45 °C (Ryznar-Luty et al. 2008; Cibis et al. 2011). Biodegradation efficiency was evaluated based on pollution indicators such as COD (chemical oxygen demand), BOD₅ (5-day biological demand), and TOC (total organic carbon). We additionally analyzed the removal of betaine, i.e., an organic compound representing 37.6% of the pollution load of beet molasses vinasse expressed by the TOC value (Cibis et al. 2011), and of biogenic elements such as nitrogen and phosphorus.

Experimental

Microorganisms

The bacterial culture used in the study has been isolated from the material collected from some plant in the Great Britain which processes various types of waste from the food industry. The composition of the bacterial culture used in the experiment included two strains from the species *B. circulans* and one strain from each of the following species: *B. laterosporus*, *B. filicolicus*, *B. stearothermophilus*, *B. acidocaldarius*, and *B. licheniformis*. Storage conditions of the culture were presented in our earlier works (Cibis et al. 2011; Ryznar-Luty et al. 2015, 2018).

Vinasse characterization

The beet molasses vinasse to be biodegraded in the study originated from Zakłady Wytwórcze CHEKO, Włocławek, Poland. Its proximate composition is provided in Table 1. The vinasse was stored in 5-L closed plastic bottles at a temperature of − 18 °C.

Prior to the biodegradation process, beet molasses vinasse was cooked for 15 min to eliminate pollutants that could have appeared during its transport and preparation for storage. The cooking loss of liquid was completed with distilled water. After cooling, the vinasse was enriched with 0.9 g/L

Table 1 Vinasse characteristics

Parameter	Unit	Value
pH	—	4.97 ± 0.01 ^c
Density	°B _{lg}	9.40 ± 0.10
Suspended solids	g/L	4.641 ± 0.44
TCOD ^a _{sum}	g O ₂ /L	108.36 ± 2.65
TCOD	g O ₂ /L	61.16 ± 0.52
SCOD ^b _{sum}	g O ₂ /L	104.64 ± 2.48
SCOD	g O ₂ /L	57.39 ± 0.35
BOD ₅	g O ₂ /L	36.40 ± 2.14
TOC	g/L	30.75 ± 1.23
Betaine	g/L	22.53 ± 1.17
Total nitrogen	g/L	4.004 ± 0.165
Ammonia nitrogen	g/L	0.187 ± 0.005
Total phosphorus	g/L	0.056 ± 0.0021
Phosphate phosphorus	g/L	0.011 ± 0.0002

^aTCOD determined by the dichromate method+the theoretical COD of betaine

^bSCOD determined by the dichromate method+the theoretical COD of betaine

^cValues following the sign “±” denote standard deviation, *n* = 3

of NH₄H₂PO₄. The necessity of vinasse supplementation with an additional source of phosphorus was demonstrated in our earlier investigations. Afterwards, vinasse pH was adjusted to the value resulting from study design using 33% NaOH.

Biodegradation processes

Two series of three biodegradation processes were carried out. The experiments were conducted in a stirred-tank reactor (STR) having a working volume of 2.0 L under the following conditions: an aeration rate of 1.0 vvm [volume of air/(volume of medium × minute)], temperature of 36 °C, and stirrer speed of 900 rpm (rotations per minute). In both series, pH of vinasse fed into the bioreactor was raised to 8.0 using 33% NaOH and the processes were carried out at a hydraulic retention time (HRT) of 26, 47, and 78 h. In the first series, medium pH in the bioreactor was kept constant at 8.0 using 2.0 M NaOH, whereas the second series of processes was performed without pH control in the bioreactor. Temperature, pH, and dissolved oxygen tension were measured continuously using sensors mounted in the bioreactor. Each of the two experimental series was preceded with a batch process (run at temp. 36 °C, and at pH regulated to 8.0), with 80 mL of the medium (containing a 3-day mixed culture of bacteria) from the Drechsler-type aerated bottle used as the inoculum (Ryznar-Luty et al. 2015). After 28 h of the batch process, a feeding pump was switched on to feed fresh

vinasse to the bioreactor, and another pump was switched on to discharge the treated vinasse from the bioreactor, to launch a series of continuous processes. Each of these processes was continued at least until the steady state has been reached, i.e., for the period equal to 4.3–5.0 HRT, depending on the process.

Analytical methods

The content of suspended solids (SS) was determined after their separation from the biodegraded medium (by centrifugation at 13,000 rpm for 40 min in a Sigma® 4K15 type centrifuge). The supernatant was used for further analyses. The analytical methods used to determine SS, COD, BOD₅, TOC, total phosphorus and orthophosphate as phosphorus, total ammonia, ammonia nitrogen, number of bacterial cells, and betaine were described in detail in our earlier works addressing the batch biodegradation processes of beet molasses vinasse (Cibis et al. 2011; Ryznar-Luty et al. 2015, 2018). COD was measured both in the supernatant (SCOD) and in the non-centrifuged medium (TCOD).

Considering the fact that betaine content is not determined using a dichromate method for COD determination (Thalasso et al. 1999), the efficiency of processes conducted in our study was evaluated based on SCOD_{sum} and TCOD_{sum} indices. Their indices were calculated as sums of SCOD and TCOD, respectively, determined with the dichromate method and of the theoretical COD of betaine accounting for 2.097 g O₂/g, which was computed based on the assumption that betaine nitrogen is converted into

ammonia nitrogen, usually upon betaine biodegradation (Caspi et al. 2008).

Results and discussion

The removal of the pollution load of the beet molasses vinasse by a mixed culture of *Bacillus* genus bacteria, expressed by the extent of TCOD_{sum} and SCOD_{sum} reduction, was strongly affected by betaine assimilation from the medium. In the vinasse subjected to biodegradation, this compound represented 45.15% of pollution load expressed by the SCOD_{sum} index. In the processes during which the microorganisms removed over 99% of betaine (Fig. 1), TCOD_{sum} reduction accounted for over 67% and SCOD_{sum} reduction for over 84%. These reductions were reported at HRT of 47 and 78 h (Table 2). The same dependency was observed in the batch processes of beet molasses vinasse biodegradation performed with and without medium pH regulation (Cibis et al. 2011; Ryznar-Luty et al. 2015). In most of the works of other authors concerning treatment processes of betaine-containing wastewater, the efficiency of these processes was evaluated only based on COD reduction determined with the dichromate method, i.e., with no consideration given to betaine (Satyawali and Balakrishnan 2008).

A comparative analysis of the reductions noted in values of SCOD, TOC, and BOD₅ indices at all hydraulic retention times tested reveals significant differences between HRT = 26 h and HRT = 47 h, with more beneficial results achieved at the longer HRT. Extending HRT from 47 to 78 h had only a negligible effect upon the outcome of the biodegradation process. For both HRT values, the reductions

Fig. 1 Effect of hydraulic retention time (HRT) and pH regulation on betaine removal during aerobic biodegradation of beet molasses vinasse

Table 2 Effect of hydraulic retention time (HRT) and pH regulation on beet molasses vinasse biodegradation

Parameter	HRT = 26 h		HRT = 47 h		HRT = 78 h	
	NR ^a	R ^b	NR	R	NR	R
TCOD _{sum} removal (%)	38.05 ± 1.22	57.11 ± 0.81	68.25 ± 0.60	67.35 ± 0.64	72.04 ± 0.54	70.06 ± 0.97
SCOD removal (%)	70.71 ± 0.17	66.80 ± 0.68	73.79 ± 0.40	75.40 ± 0.85	74.99 ± 1.12	78.75 ± 0.43
SCOD _{sum} removal (%)	47.35 ± 1.04	72.42 ± 0.61	84.54 ± 0.41	86.09 ± 0.49	85.65 ± 1.05	87.98 ± 0.75
TOC removal (%)	59.14 ± 1.58	72.56 ± 0.60	87.89 ± 0.23	85.93 ± 0.32	87.83 ± 0.59	87.43 ± 0.93
BOD ₅ removal (%)	62.83 ± 1.75	70.74 ± 2.57	97.03 ± 0.36	99.41 ± 0.22	97.56 ± 0.24	99.61 ± 0.12
Y _{SS} (g final SS formed/g SCOD _{sum} removed)	0.1646 ± 0.008	0.1440 ± 0.013	0.1166 ± 0.008	0.1214 ± 0.020	0.0935 ± 0.006	0.1183 ± 0.017
SS formed (g/L)	7.73 ± 0.51	10.27 ± 1.07	9.58 ± 0.64	11.90 ± 0.82	7.43 ± 0.47	10.88 ± 1.32
Final pH	8.81 ± 0.007	8.06 ± 0.006	8.98 ± 0.016	8.05 ± 0.005	9.12 ± 0.003	8.05 ± 0.005
Total nitrogen removal (%)	22.40 ± 3.57	26.87 ± 2.58	49.46 ± 3.40	42.07 ± 2.03	48.57 ± 2.67	45.75 ± 2.44
Ammonia nitrogen removal (%)	− 64.04 ± 4.43	− 304.24 ± 9.95	− 285.07 ± 13.44	− 310.89 ± 12.08	− 262.36 ± 9.19	− 318.39 ± 9.02
Total phosphorus removal (%)	57.29 ± 2.91	67.46 ± 1.84	80.27 ± 0.84	69.28 ± 2.16	71.27 ± 1.51	82.58 ± 0.92
Orthophosphate as phosphorus removal (%)	55.01 ± 2.53	69.93 ± 1.56	65.32 ± 1.37	69.32 ± 2.71	74.84 ± 1.92	86.22 ± 0.85

^aProcesses with non-regulated pH^bProcesses with regulated pH

in SCOD and BOD₅ values were by a few percent higher in processes with controlled medium pH than in these with non-controlled pH. In the case of TOC, more beneficial results were obtained in the experiment with non-controlled pH (Table 2). Similar results were achieved by Ghosh Ray and Ghangrekar (2019) in the process of aerobic biodegradation of the effluent from anaerobic degradation of distillery wastewater. They reported reductions in COD and BOD₅ values by 74 and 96%, respectively, at HRT = 24 h in the case of pollution load of 3.6 kg COD/(m³ day), and by 43 and 84% in the case of pollution load of 9.0 kg COD/(m³ day). Worthy of notice is that in our study the pollution load of the bioreactor, expressed by SCOD_{sum}, was significantly higher and reached 96.72 kg SCOD_{sum}/(m³ day), 53.52 kg SCOD_{sum}/(m³ day), and 32.16 kg SCOD_{sum}/(m³ day) for HRTs of 26, 47, and 78 h, respectively.

The batch processes of beet molasses vinasse biodegradation conducted with the same bacterial culture as that used in the reported study were characterized by the sequential removal of organic pollutants from the medium. If the bacteria assimilated betaine, its intensive removal occurred usually when easily available carbon sources were depleting in the medium (Cibis et al. 2011; Ryznar-Luty et al. 2018).

In the reported experiments, the extent of betaine removal from the biodegradable medium was high [it reached 100% at HRT = 78 h in both processes and at HRT = 47 h in the process with controlled pH, and over 99% at HRT = 47 h in the process with non-controlled pH (Fig. 1)] in the processes in which SCOD reduction was higher than 73% and

reductions of BOD₅ and TOC values exceeded 97% and 85% (Table 2). These reductions were indicative of the depletion of easier assimilable sources of carbon by the tested microorganisms. Apart from our earlier works, the scientific literature lacks reports on the aerobic processes of betaine biodegradation. The only available publications present results from investigations on the anaerobic methods used for the biodegradation of this compound (Thalasso et al. 1999; Gil-Pena et al. 1987; Nakai et al. 2013). The first-mentioned research group investigated degradation of protein compounds (including betaine) during continuous acidogenesis of sugar beet vinasse. In turn, the second group monitored betaine content in the inflow and in the effluent during biodegradation of eluates generated during the production of yeast biomass and citric acid from molasses, whereas the third research group studied betaine degradation under in vitro conditions by microorganisms autochthonous to the rumen of dairy cows. Nakai et al. (2013) reported a stable concentration of betaine sustaining for a few hours during its assimilation by the microflora of cow rumen in the medium free of other sources of carbon. It was not until 6 h, when betaine concentration began to decrease. In turn, Thalasso et al. (1999) observed a dependency between betaine removal time from the medium and betaine concentration in the mineral medium in processes of its anaerobic biodegradation. With the initial betaine concentration of 18 g/L, its complete removal was noted after ca. 22 days, whereas when the initial betaine concentration ranged from 2 to 6 g/L, its 100% reduction was observed after 5 days. In our study, the

Fig. 2 Effect of hydraulic retention time (HRT) on betaine removal process during aerobic biodegradation of beet molasses vinasse without pH regulation

Fig. 3 Effect of hydraulic retention time (HRT) on betaine removal process during aerobic biodegradation of beet molasses vinasse with pH regulation

process of betaine removal from the beet molasses vinasse began as early as in the first hours of the biodegradation process (Figs 2, 3).

Betaine biodegradation was accompanied by a decreasing content of total nitrogen in the medium. In the processes in which betaine removal reached 99 and 100%, it exceeded 42%. Simultaneously, an increase was observed in the concentration of ammonia nitrogen in the medium, but still its lower concentration in the medium was determined in the processes without the control of biodegraded medium pH (Table 2). According to Liu et al. (2012), the reduction in nitrogen concentration observed in the processes of aerobic biodegradation of wastewater is due to the release of

ammonia from the medium having a high pH value. This phenomenon was likely to occur in the presented study, wherein medium pH ranged from 8.05 to 9.12. The increase in ammonia nitrogen concentration along with betaine concentration decrease was also observed by Nakai et al. (2013). Betaine concentration decrease by 6% after 12 h of the process and by 15% after 24 h of the process, determined in their study, resulted in 1.4- and 2.7-fold, respectively, increase in the concentration of ammonia nitrogen (Nakai et al. 2013). In the case of the smaller assimilation of betaine by the bacteria (HRT = 26 h), the decrease in total nitrogen concentration was remarkably lesser (Table 2), which is obvious because a lower amount of ammonia nitrogen was

produced under these conditions, part of which was next volatilized in the form of ammonia.

Concentrations of total phosphorus and orthophosphate as phosphorus in the biodegraded medium were observed to decrease in all processes. In most cases, higher by over 10% removal of both forms of phosphorus was determined in the processes with controlled pH compared to those with uncontrolled pH of the medium (Table 2). An exception was observed in the experiments with HRT of 47 h, in which 80.27% of total phosphorus was removed in the process conducted without pH control and 69.28% in the process with medium pH control. An opposite observation was made during batch processes of aerobic biodegradation of molasses vinasse with the same bacterial culture (Cibis et al. 2011; Ryznar-Luty et al. 2015), i.e., pH regulation contributed to a lower extent of total phosphorus removal. Changes in phosphorus concentration during wastewater biotreatment may be due to various causes. For example, microorganisms consume phosphorus contained in the medium for biomass growth. In addition, bacteria from the genus *Bacillus* are capable of accumulating this element in the form of polyphosphates. It is released from cells in the processes of intracellular respiration and also as a result of cell breakdown. Changes in concentrations of both mentioned forms of nitrogen are also due to the hydrolysis and precipitation of suspensions during the biodegradation process (Ryznar-Luty et al. 2015).

Because of the necessity of managing the bacterial biomass produced during biodegradation process, its as low as possible production is highly advised. In our study, the production of lower volumes of suspended solids was observed in all processes with uncontrolled pH compared to those with controlled pH of the medium, at each HRT. The lowest suspended solid load produced per 1 g of removed SCOD_{sum} (Y_{SS}) was determined in the process with uncontrolled pH at HRT of 78 h. Interestingly, the Y_{SS} value decreased along with extending hydraulic retention time (Table 2). In the steady state, the lowest bacterial cell count, reaching $6 \times 10^9/\text{mL}$, was determined in the process with HRT of 26 h, whereas the highest one, reaching $12.3 \times 10^9/\text{mL}$, in the process with HRT of 78 h. Both processes were conducted with the non-regulated pH value of the medium being biodegraded.

Periods of oxygen concentration drop to 0% in the medium during biodegradation were observed only in the processes with HRT of 26 h. In both cases, this decrease was noted before the steady state had been reached, i.e., during intense proliferation of the bacterial culture. In the steady state, the level of dissolved oxygen reached 60% in the process without pH regulation, whereas in the process with regulated medium pH, it ranged from 6 to 26%. In the case of the other process, the concentration of dissolved oxygen in the steady state exceeded 50%.

The mixed bacterial culture used in our experiment was efficient in degrading the beet molasses vinasse in processes with both controlled and uncontrolled pH of the medium, at HRTs of 47 and 78 h (Table 2). Although at a significance level of $p=0.05$, the differences in the reduction of pollution load (expressed by the SCOD_{sum} value) between the corresponding processes were statistically significant, they accounted only for ca. 2%. No need for pH regulation is an undoubted advantage of the proposed method because it allows reducing the costs of the treatment process. According to Henze et al. (2002), wastewater biodegradation without pH regulation in the course of the process is feasible when the buffer capacity of wastewater is high and when medium pH falls between 6.0 and 9.0. Such conditions do not impair bacterial activity. In processes of aerobic biodegradation of high-strength wastewater from the food industry conducted without pH control, its values are observed to increase and exceed 8.0. This increase in the pH value may be due to the release of ammonia and carbon dioxide (Jang et al. 2013). In our study, the pH value also increased during biodegradation conducted without its regulation. In the steady state, degraded medium pH attained values from 8.81 to 9.12. In addition, the pH values measured in the steady state increased along with HRT elongation (Table 2).

Conclusions

Study results indicate that the high efficiency of the continuous aerobic biodegradation of beet molasses vinasse by a mixed culture of *Bacillus* genus bacteria may be achieved at the hydraulic retention time of ca. 2 or more days. Within this time period, bacteria are capable of assimilating the main organic pollutant of the beet molasses vinasse—betaine. The regulation of the pH value of the biodegraded medium has no significant effect on the outcome of the treatment process.

Results presented in this manuscript prompt us to consider employing the developed method as the first stage of beet molasses vinasse treatment on the industrial scale.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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