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Efficiency of aerobic biodegradation of beet molasses vinasse under non-controlled pH: conditions for betaine removal

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Abstract: The aim of the study was to establish such conditions that would provide high-efficiency aerobic biodegradation of beet molasses vinasse with a mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus* in batch processes without controlling the pH of the medium. Particular consideration was given to the betaine removal (the main pollutant of vinasse), which accounted for as much as 37.6% of total organic carbon. Biodegradation was performed in a stirred tank reactor at 27–63°C with initial pH (pH_0) of 6.5 and 8.0. Efficiency of biodegradation was expressed in terms of reduction in $SCOD_{sum}$, which is a sum of SCOD (soluble chemical oxygen demand, *i.e.* COD determined after suspended solids separation) and theoretical COD of betaine. The values achieved at 27 and 36°C with pH_0 = 8.0 exceeded 77.7%, whereas those obtained at 36 and 45°C with pH_0 = 6.5 were higher than 83.6%. The high biodegradation efficiency obtained in the four processes is attributable to the betaine removal by the bacterial strains used in the study. Maximal extent of reduction in $SCOD_{sum}$ (85.41%), BOD_5 (97.91%) and TOC (86.32%), and also the fastest rate of biodegradation (1.17 g O_2/l·h) was achieved at 36°C and pH_0 = 8.0.

Introduction

The rapid growth of global ethanol production, which in the time span of 2006 to 2010 has more than doubled (from 39 192 to 85 763 million litres (GRFA 2011)), has triggered serious problems with the utilization of stillage by methods that have been used so far. It is essential to note that the production of one litre of ethanol generates from 9 to 14 litres of stillage (Jimenez et al. 2003). This means that efficient agricultural use of this by-product in its natural form becomes infeasible, especially in the proximity of the distillery. Stillage transport to farmsteads situated at a farther distance is uneconomic (Murphy and Power 2008). Owing to the high content of mineral substances, particularly potassium (Rutkowska et al. 2008), vinasse (sugar-based stillage) exerts a laxative effect and, also, increases sodium and magnesium retention. This is what limits its use as a fodder additive to less than 10% of the diet for ruminants, and to less than 2% of the diet for pigs and poultry (Wilkie et al. 2000). Agricultural use of plants grown on soil fertilized with vinasse is also limited. They cannot be used as fodder in any amount due to the accumulation of potassium from the vinasse. Furthermore, upon penetration into the soil, the protein compounds that are present in the vinasse decompose, releasing hydrogen sulphide and ammonia, and thus pollute the environment. Direct fertilization of the fields with vinasse can be carried out only seasonally, because of the vegetation pattern of the plants to be grown, and the meteorological conditions.

Taking into account the content of mineral compounds, as well as that of organic substances easily available to the microorganisms, vinasse can be used as a component in compost production together with solid wastes where the content of the substances mentioned is poor. Agricultural application of composts prepared using molasses vinasse as an additive has the advantage of increasing the concentrations of organic matter, humus-forming substances and Kjeldahl nitrogen in the soil (Madejon et al. 2001). Nevertheless, also this method has its limitations (related to, *e.g.*, transport, storage or seasonality), which are a major hindrance to solving the problem of vinasse utilization on a larger scale.

In the distilleries of India, where ethanol is produced mainly from cane molasses, vinasse is treated primarily by anaerobic biodegradation (Nandy et al. 2002, Wilkie et al. 2000). However, the removal efficiencies achieved so far have not been very high. According to Nandy et al. (2002), the reduction in BOD_5 obtained in those processes approaches 75%, and that in COD only 25%. Other researchers have reported higher extents of COD reduction (Satyawali and Balakrishnan 2008), some of them exceeding 60% under industrial conditions (Wilkie et al. 2000). But these results can nevertheless be regarded as satisfactory. That is why in those distilleries of India where vinasse is treated anaerobically, the

anaerobic treatment process is regarded only as a prior step in the utilization of this troublesome effluent, and is generally followed by the activated sludge process, composting or drying in the open air (Satyawali and Balakrishnan 2008).

Anaerobic biodegradation is moderate in energy demand and yields a value added product, namely biogas. Another major benefit is the production of a very low biomass volume requiring further utilization (Henze et al. 2002). Nevertheless, it is essential to emphasize that the high organic pollution load which is typical of vinasse exerts an inhibiting effect on the anaerobic treatment process. And this necessitates the dilution of the vinasse to the COD level of approximately 50 g O_2/l with wastewater of other origin, which, however, is not available in distilleries in the amounts desired. Moreover, the high salinity of vinasse (attributable to the high content of potassium, metal ions and sulphates) as well as to the presence of phenol compounds that are toxic to methane bacteria reduces the effectiveness of the process (Jimenez et al. 2003, Wilkie et al. 2000). For these reasons, extensive laboratory investigations were carried out to raise the extent of organic matter removal during anaerobic treatment of vinasse (Jain et al. 2001, Jimenez et al. 2003, Jimenez et al. 2006, Martin et al. 2002).

In this context, the problem of finding other methods for distillery wastewater utilization, especially for the utilization of vinasse (which, compared to starch stillage, is less valuable when applied as fodder) has taken on a sense of significance.

Thermo- or mesophilic aerobic bacteria have been used with success for the biodegradation of high-strength effluents from food processing or chemicals producing plants, as well as for the stabilization of wastewater sludge (LaPara and Alleman 1999, Suvilampi and Rintala 2003). In most instances they are mixed cultures characterized by a higher activity as compared to monocultures, which is probably due to the synergistic interactions between the microorganisms (LaPara et al. 2002, Lasik and Nowak 2006). In technological terms, aerobic thermo- and mesophilic biodegradation processes are far less complex than the anaerobic ones. Moreover, when the effluent to be treated has been hot since the time of origin (and such has been vinasse), it is recommended, in economic terms, that biodegradation should be carried out at high temperature. This will eliminate the need for cooling down such wastewater.

Previous studies performed by the authors of the work reported on here have revealed that aerobic biodegradation involving a mixed thermo- and mesophilic culture of *Bacillus* bacteria can be successfully applied as a prior step in the treatment of distillery wastewater (Cibis 2004, Cibis et al. 2006, Krzywonos et al. 2008). In the case of starch-based stillage, the extent of COD removal varied from 82.6% for maize stillage to 93.7% for wheat-potato stillage (Cibis 2004). In our preliminary studies into biodegradation of vinasse carried out with the same bacterial culture, the reduction in COD was 85.37% and 76.48% at controlled pH and uncontrolled pH, respectively. In both the instances the microorganisms failed to remove betaine (the main pollutant of beet molasses vinasse not detected by the dichromate COD method (Thalasso et al. 1999, Ryznar-Luty et al. 2008). In the biodegradation processes conducted in the stirred tank reactor over the temperature range of 27 to 63°C (step 9°C) at controlled pH of 6.5 and 8.0, the highest extent of COD reduction was 88.73% (at 36°C and the pH of 6.5), while betaine was removed within the range of 27 to 54°C at the pH of 8.0, as well as over 27 to 45°C at the pH of 6.5 (Cibis et al. 2011).

Apart from the investigations conducted by the authors of this work here, only two references in the literature pertaining to the biodegradation of betaine-containing effluents include information on the removal efficiency obtained for this compound. The both papers deal with anaerobic biodegradation processes. The paper by Gil-Pena et al. (1987) reports on 65% removal of betaine in a laboratory-scale process of vinasse treatment by bacteria isolated from the digestive system of ruminants; the paper by Thalasso et al. (1999) informs about 100% betaine removal achieved in a large-scale anaerobic biodegradation of effluents originating during yeast and citric acid production from molasses. As can be inferred from the analysis of literature data, only a few microorganisms display betaine metabolizing activity (Leblanc et al. 2001). No references have been found to the removal of betaine by the bacteria of the genus *Bacillus* but the database Biocyc (Caspi et al. 2008) contains computer-predicted pathways of betaine degradation for some *Bacillus* strains.

The aim of the present study was to establish such conditions that would provide high-efficiency aerobic batch biodegradation of beet molasses vinasse in processes without pH control, and thus abate the treatment costs. Particular consideration was given to the removal of betaine, which accounted for as much as 37.6% of total organic carbon. The culture used was the same as in our previous studies, *i.e.* a mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus*.

Materials and methods

Microorganisms

The mixed culture of bacteria used in this study was isolated from the material supplied by a plant processing waste products from food industry. The culture included seven strains of the genus *Bacillus*. Two belonged to the species *B. circulans*, the other five belonged to the species *B. laterosporus*, *B. filicolonicus, B. stearothermophilus*, *B. acidocaldarius* and *B. licheniformis,* respectively. Bacteria were identified by standard methods and, additionally, by API 50CHB tests (Cibis et al. 2006, Cibis et al. 2004). The activity of the microorganisms was maintained at 45 ± 2°C in a 0.5 l volume, aerated, non-stirred bioreactor (Drechsler-type aerated bottle), in a medium prepared as presented in the subsection "Preparation of the medium". Every three days the bacteria were inoculated onto a fresh medium, the volume of the inoculum amounting to 20 ml. Using the mode described above, it was possible to maintain the activity of the mixed culture throughout the experiments.

Preparation of the medium

The vinasse used in our study was obtained from CHEKO, Włocławek, Poland. Its composition is characterized in Table 1. Since betaine is not detected by the dichromatic analysis of COD (Thalasso et al. 1999), the parameter COD_{sum}, defined as the sum of the vinasse COD and the theoretical COD of betaine (2.097 g O_2/g betaine), was introduced as one of the parameters describing the pollution load of the stillage examined.

To avoid contamination, the vinasse was boiled for 15 mins not only before use as the medium for bacterial activity maintenance in the Drechsler-type aerated bottle, but

also prior to the biodegradation processes in the bioreactor. After cooling, as the content of phosphorus in the vinasse was insufficient (which was checked experimentally), the medium was enriched with $NH_4H_2PO_4$ (0.9 g/l) and then the pH was adjusted (33% NaOH) either to 7.5, when the vinasse was used as the medium for maintaining bacteria activity, or to 6.5 and 8.0, when the vinasse was biodegraded.

Biodegradation processes

Two series of five biodegradation processes were performed. Each process lasted 168 h. The processes were conducted in a 5.0 l working volume stirred tank reactor (STR) (Biostat type B. Braun Biotech International) with an aeration rate of 1.0 vvm (volume per volume per minute) and stirrer speed of 900 rpm (rotations per minute). Both experimental series were performed at the following temperatures (T): 27, 36, 45, 54 and 63ºC. The pH was non-controlled. The initial pH (pH_0) of the culture medium was 6.5 in the first series and 8.0 in the second series. Initial pH values and process temperatures were chosen based on the results of previous investigations into aerobic biodegradation of distillery effluents with the same mixed culture of *Bacillus* bacteria as the one used in this work here (Cibis 2004, Cibis et al. 2011, Krzywonos et al. 2008, Ryznar-Luty et al. 2008).

Each process was conducted once. The frequency of the analysis varied according to the following pattern: three samples on the first day, two samples on the second day, and one sample on each of the other days of the process. All chemical analyses were performed in triplicate. Temperature, pH and dissolved oxygen tension (DOT) were measured continuously using the sensors incorporated in the bioreactor. Liquid loss in the bioreactor in response to evaporation was made up automatically with distilled water. The inoculum was a 0.2 l portion of the medium taken from the Drechsler-type aerated bottle, where the three-day-old mixed culture of bacteria was maintained.

Analytical methods

Chemical oxygen demand (COD), biological oxygen demand (BOD_5), total organic carbon (TOC), total phosphorus and phosphate phosphorus were determined using Hach--Lange spectrophotometric cuvette tests (Handbook 2000). Total nitrogen was determined by the Kjeldahl method. The distillation technique was used to determine ammonia nitrogen concentration. Betaine was determined using the colourimetric method developed by Focht et al. (1956). Coloured substances (alkaline products of invert sugar degradation, products of saccharose carmelization, and melanoidins) were determined by a spectrophotometric method presented by Sapronov (1963). Potassium and magnesium concentrations were measured by flame photometry. All analyses were performed after suspended solids separation, which involved centrifugation for 40 mins at 18 500g (Sigma®4K15). Suspended solids (SS) were determined by weight, drying each sample at 50°C for one day and thereafter at 105°C until a constant weight was attained. The number of bacterial cells was established in the haemocytometer.

Results and discussion

Oxygenation

Many literature reports on the use of aerobic methods in wastewater treatment have demonstrated that the extent of biodegradation obtained in this way is strongly influenced by the quantity of dissolved oxygen in the medium. They have also revealed that in the phase of enhanced pollutant removal oxygen demand increases dramatically. Consequently, when the aeration conditions remain constant throughout the process, the level of dissolved oxygen is frequently close or equal to zero (Cibis et al. 2002, Cibis et al. 2011, Kosseva et al. 2001, Krzywonos et al. 2002, LaPara and Alleman 1999, Lasik et al. 2010). In three out of ten experiments conducted within the scope of our study the DOT value declined to zero (T = 63°C,

Table 1. Characteristics of vinasse[a]

Parameter	Unit	Value
pH	-	4.97 ± 0.01[b]
Density	°Bx	9.40 ± 0.10
Suspended solids	g/l	4.641 ± 0.44
$SCOD_{sum}$	g O_2/l	104.64 ± 2.48
BOD_5	g O_2/l	36.40 ± 2.14
TOC	g/l	30.75 ± 1.23
Betaine	g/l	22.53 ± 1.17
Alkaline products of invert sugar degradation	g/l	16.45 ± 1.41
Products of saccharose carmelization	g/l	2.401 ± 0.208
Melanoidins	g/l	0.879 ± 0.115
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
Potassium	g/l	7.160 ± 0.201
Magnesium	g/l	0.011 ± 0.0004

[a]Besides pH, density and suspended solids the parameters were determined after suspended solids separation.
[b]Values following the sign "±" denote standard deviation, n = 3.

pH_0 = 6.5; T = 63°C, pH_0 = 8.0; T = 36°C, pH_0 = 8.0). In every instance, however, the phases of oxygen deficit were of a short duration (1.25 h) (data not shown).

Variations in the pH

In all of the biodegradation processes examined, pH showed the following pattern of change: upon initial decline, it increased continuingly to reach a value higher than 9 (Fig. 1). Similar behaviour was also observed when aerobic methods were used in the treatment of effluents from mechanical peeling of potatoes (Malladi and Ingham 1993), from the biodegradation of whey (Kosseva et al. 2001), from the utilization of paper mill wastewater (Tiirola et al. 2003), dairy wastewaters (Carta-Escobar et al. 2004) and potato slops (Cibis et al. 2004), as well as from the biodegradation of vinasse, where the concentrations of pollutants were noticeably higher than those dealt with in the present study (Ryznar-Luty et al. 2008). The acidification of the medium is attributable to the production of organic acids during enhanced microorganism growth (Cibis et al. 2004, Fang et al. 2001, Krzywonos et al. 2009), while the increase in the pH value that followed is due to the assimilation of the acidic products of bacterial metabolism, to the synthesis of alkaline by-products by the bacteria (as suggested by Malladi and Ingham (1993), and Tripathi and Allen (1999)), or to the hydrolytic release, followed by the acidic-basic conversion of reduced nitrogen (as reported by Staton et al. (2001)).

Major parameters of biodegradation efficiency

In this study, the biodegradation processes were divided into two groups according to their efficiency. Group one included experiments at 54 and 63°C with pH_0 = 6.5 and pH_0 = 8.0, as well as those at 27°C with pH_0 = 6.5, and at 45°C with pH_0 = 8.0. In this group, reduction in $SCOD_{sum}$ (soluble COD_{sum}, *i.e.* COD_{sum} determined after suspended solids separation) did not exceed 41%. Group two encompassed two processes at 36°C and experiments at 45°C with pH_0 = 6.5, and at 27°C with pH_0 = 8.0. Compared with Group one, each of these processes produced $SCOD_{sum}$ reduction higher than 77%. Substantially higher was also the reduction in BOD_5 and TOC (Tables 2 and 3). The cause underlying the significant differences in biodegradation efficiency was the betaine removal in the processes of Group two (Table 4), where the proportion of this compound in the $SCOD_{sum}$ of the vinasse being treated was as high as 45.15%. As for the experiments of Group two, the removal of betaine was complete (100%) in three of them, namely those with the highest extent of $SCOD_{sum}$ reduction, which exceeded 83%. In one experiment of Group two, with 77.74% removal of $SCOD_{sum}$, the betaine removal was 86.37% (Tables 2, 3 and 4). The results obtained in the present work were slightly lower compared with those obtained in a previous study where vinasse was biodegraded with the same bacterial culture under the same conditions but at a controlled pH of the medium (pH = 6.5 and 8.0) (Cibis et al. 2011). This finding indicates that maintaining the pH at a constant level only slightly increases the extent of vinasse biodegradation. However, the reduction in organic pollutants ($SCOD_{sum}$ > 83%, BOD_5 > 95 %, TOC > 76%) directly linked with betaine removal was obtained over a wider temperature range (from 27 to 54°C at pH = 8.0 and from 27 to 45°C at pH = 6.5, as mentioned in the "Introduction" section) (Cibis et al. 2011) than under conditions without pH control (Tables 2 and 3).

The highest extent of reduction in $SCOD_{sum}$ (85.41%), BOD_5 (97.91%) and TOC (86.32%) was achieved with biodegradation at 36ºC and pH_0 = 8.0. A similar optimal temperature value, 35ºC, has been reported by Tripathi and Allen (1999) (for the biodegradation of bleached kraft pulp mill effluents), as well as Krzywonos et al. (2008) (for the biodegradation of potato slops). Our present study, and also some previous investigations on meso- and thermophilic biodegradation (Krzywonos et al. 2008, Suvilampi and Rintala 2003, Tripathi and Allen 1999) have revealed that higher removal of pollutants from the wastewater is attained when biodegradation is conducted at lower temperatures. Biodegradation involving higher temperatures provides higher removal of some specific organic compounds (Becker et al. 1999, Tripathi and Allen 1999). This finding conflicts with the results obtained in our present study, where betaine, an organic compound typical of the effluent from sugar beet

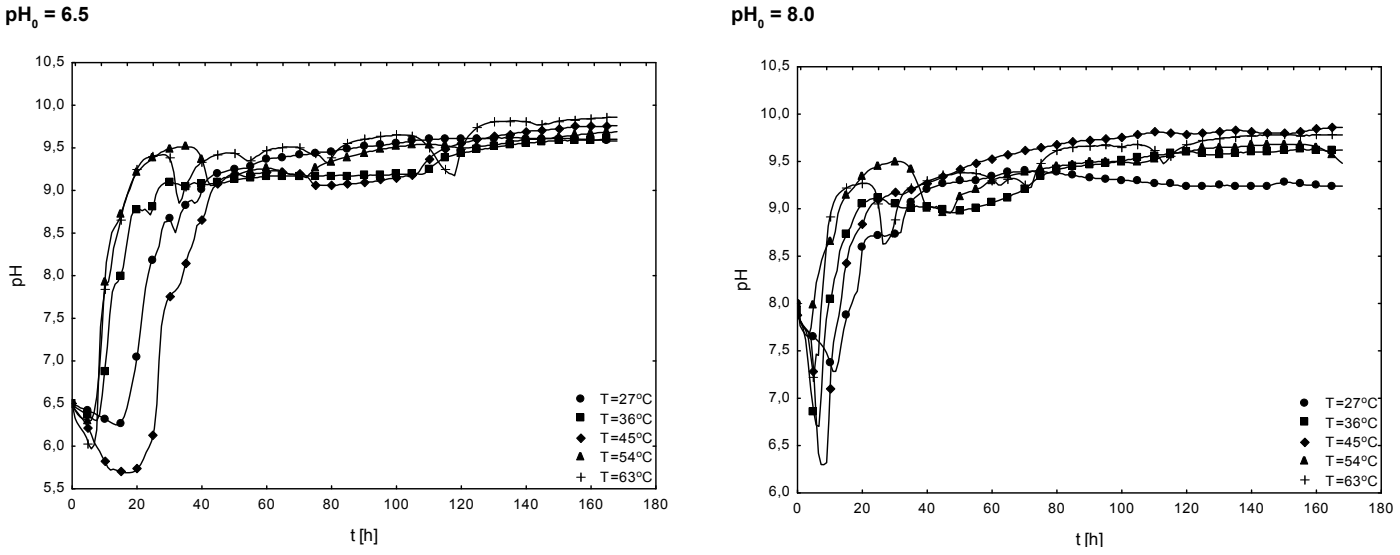

Fig. 1. Effect of temperature and initial pH on the pattern of change in the pH of the medium during biodegradation of vinasse

Table 2. Effect of temperature on the results of vinasse biodegradation carried out at pH_0 = 6.5

Parameter	Temperature (°C)				
	27	36	45	54	63
$SCOD_{sum}$ removal (%)[c]	36.42 ± 2.78[b]	83.86 ± 0.53	83.61 ± 0.45	40.72 ± 2.47	33.72 ± 3.87
$SCOD_{sum}$ removal rate (g O_2/(l·h))[a]	0.4754	0.7920	0.8199	0.3165	0.2813
Time after which 90% of the overall reduction in $SCOD_{sum}$ was attained (h)	69.49	87.78	92.13	115.48	110.23
BOD_5 removal (%)	73.86 ± 1.54	97.54 ± 0.54	97.50 ± 0.50	43.91 ± 5.95	53.38 ± 5.30
TOC removal (%)	55.06 ± 3.86	82.43 ± 0.43	86.04 ± 0.33	48.10 ± 1.40	43.28 ± 1.49
Final SS (g/l)[d]	12.76 ± 0.59	10.63 ± 0.97	5.59 ± 0.54	8.36 ± 0.64	8.24 ± 0.67
Final amount of SS formed (g/l)[e]	9.59 ± 0.65	7.73 ± 0.78	1.08 ± 0.91	4.80 ± 0.63	4.60 ± 0.40
Maximal amount of SS formed (g/l)	11.56 ± 0.86	10.48 ± 0.67	5.88 ± 0.54	5.76 ± 0.60	5.15 ± 0.63
Y_{SS} ((g final SS formed/g $SCOD_{sum}$ removed)·100%)	26.11 ± 1.22	10.01 ± 1.19	1.28 ± 0.93	11.83 ± 1.29	13.34 ± 1.03
Maximal number of cells formed (10^8/ml)	41.70 ± 4.26	33.41 ± 3.83	24.75 ± 2.79	25.03 ± 4.01	15.75 ± 4.82
Final number of cells formed (10^8/ml)	24.54 ± 2.84	20.18 ± 2.45	17.50 ± 3.57	21.12 ± 2.65	13.30 ± 2.23

[a]The value of this parameter was calculated for the point in time after which 90% of the overall reduction in $SCOD_{sum}$ was attained.
[b]Values following the sign "±" denote standard deviation, n = 3.
[c]The value of this parameter was calculated: ((removed SCOD determined by the dichromate method + the theoretical COD of the removed betaine amount) / (the initial SCOD determined by the dichromate method + the theoretical COD of the initial betaine amount))*100%.
[d]Suspended solids determined in the medium after the biodegradation process.
[e]The difference between suspended solids determined in the medium after the biodegradation process and suspended solids determined before the biodegradation process.

Table 3. Effect of temperature on the results of vinasse biodegradation carried out at pH_0 = 8.0

Parameter	Temperature (°C)				
	27	36	45	54	63
$SCOD_{sum}$ removal (%)[c]	77.74 ± 0.82[b]	85.41 ± 0.59	39.47 ± 2.70	35.44 ± 2.67	35.02 ± 3.78
$SCOD_{sum}$ removal rate (g O_2/(l·h))[a]	0.4623	1.1684	1.0197	0.2781	0.3610
Time after which 90% of the overall reduction in $SCOD_{sum}$ was attained (h)	147.40	65.50	34.26	118.81	87.78
BOD_5 removal (%)	85.09 ± 0.51	97.91 ± 0.39	63.96 ± 4.68	50.05 ± 1.34	40.45 ± 4.88
TOC removal (%)	70.76 ± 1.23	86.32 ± 0.38	57.16 ± 2.08	49.86 ± 1.27	49.52 ± 0.79
Final SS (g/l)[d]	14.69 ±0.96	9.49 ± 0.69	4.04 ± 0.57	8.43 ± 0.61	7.34 ± 0.53
Final amount of SS formed (g/l)[e]	10.86 ± 0.74	6.05 ± 0.91	0.19 ± 0.73	3.37 ± 0.55	1.79 ± 0.34
Maximal amount of SS formed (g/l)	11.71 ± 0.78	9.41 ± 0.55	4.59 ± 0.32	4.81 ± 0.48	2.98 ± 0.51
Y_{SS} ((g final SS formed/g $SCOD_{sum}$ removed)·100%)	14.35 ±1.14	7.10 ± 0.63	0.49 ± 0.91	9.17 ± 0.85	5.07 ± 0.46
Maximal number of cells formed (10^8/ml)	41.85 ± 4.06	43.25 ± 4.62	16.10 ± 3.14	19.70 ± 2.28	19.90 ± 2.17
Final number of cells formed (10^8/ml)	26.25 ± 2.65	22.82 ± 2.84	11.33 ± 2.19	15.90 ± 1.99	15.18 ± 1.86

[a]The value of this parameter was calculated for the point in time after which 90% of the overall reduction in $SCOD_{sum}$ was attained.
[b]Values following the sign "±" denote standard deviation, n = 3.
[c]The value of this parameter was calculated: ((removed SCOD determined by the dichromate method + the theoretical COD of the removed betaine amount) / (the initial SCOD determined by the dichromate method + the theoretical COD of the initial betaine amount))*100%.
[d]Suspended solids determined in the medium after the biodegradation process.
[e]The difference between suspended solids determined in the medium after the biodegradation process and suspended solids determined before the biodegradation process.

Table 4. Removal of main vinasse components under various biodegradation conditions

Component	pH_0	Temperature (°C)				
		27	36	45	54	63
Betaine	6.5	0 ± 6.66[a]	100 ± 0	100 ± 0	0 ± 6.90	0 ± 9.88
	8.0	86.37 ± 0.95	100 ± 0	0 ± 6.79	0 ± 7.23	0 ± 9.77
Alkaline products of invert sugar degradation	6.5	31.71 ±2.17	46.76 ±3.26	33.21 ± 2.20	16.55 ± 1.28	ic
	8.0	48.39 ± 3.16	51.33 ± 3.45	27.92 ± 1.89	0.12 ± 0.37	7.93 ± 2.55
Products of saccharose carmelization	6.5	39.04 ± 7.92	54.00 ± 4.91	40.79 ± 8.15	38.26 ± 8.04	20.51 ± 4.63
	8.0	46.70 ± 4.25	51.42 ± 4.37	36.05 ± 7.14	25.52 ± 5.23	ic
Melanoidins	6.5	ic	ic	ic	ic	ic
	8.0	ic	ic	ic	ic	ic
Total nitrogen	6.5	6.01 ± 4.47	64.85 ± 3.46	56.99 ± 4.77	40.08 ± 3.58	28.66 ± 2.87
	8.0	47.53 ± 3.98	61.49 ± 3.38	39.98 ± 3.53	8.33 ± 4.15	24.70 ± 2.92
Ammonia nitrogen	6.5	ic	21.85 ± 2.96	52.17 ± 3.15	65.49 ± 1.69	100 ± 0
	8.0	ic	42.86 ± 3.34	66.95 ± 2.76	64.71 ± 3.51	100 ± 0
Total phosphorus	6.5	60.45 ± 2.96	47.82 ± 3.19	62.90 ± 2.17	56.19 ± 1.98	58.06 ± 2.89
	8.0	80.13 ± 1.51	70.64 ± 1.74	48.09 ±3.18	44.47 ± 2.39	39.35 ± 3.18
Phosphate phosphorus	6.5	64.69 ± 0.87	66.73 ± 1.19	79.86 ± 0.79	75.89 ± 1.55	70.87 ± 0.86
	8.0	78.90 ± 2.15	72.47 ± 0.96	79.65 ± 1.58	42.70 ± 1.23	20.14 ± 1.59

ic = increased content

[a]Values following the sign "±" denote standard deviation, n = 3.

processing, was removed, as already mentioned, primarily at lower temperatures.

In this study here, not only the temperature at which the biodegradation processes were conducted, but also the initial pH of the medium had an appreciable effect on the rate of $SCOD_{sum}$ reduction (Tables 2 and 3) (the rate were determined for the time when the parameter reached 90% of the value obtained in the entire process). With either of the two pH_0 values, 6.5 or 8.0, the rate of $SCOD_{sum}$ reduction in the experiments at 27, 54 and 63ºC was markedly lower than at 36 and 45ºC. In the majority of these biodegradation processes the reduction rate for $SCOD_{sum}$ was higher at pH_0 = 8.0. The highest rate of $SCOD_{sum}$ reduction was achieved at 36 and 45ºC with pH_0 = 8.0. Under such conditions, the $SCOD_{sum}$ reduction rate amounted to 1.17 g O_2/(l·h) and 1.02 g O_2/(l·h), respectively (in the process at T = 45ºC and pH_0 = 8.0 betaine was not removed). These values are lower than the maximal SCOD reduction rate values obtained by Cibis et al. (2011) during biodegradation of vinasse with pH control ($SCOD_{sum}$ removal rate = 1.69 g O_2/(l·h)), Kosseva et al. (2001) during biodegradation of whey (1.57 g O_2/(l·h)), as well as by Krzywonos et al. (2008) (2.02 g O_2/(l·h)) and Cibis et al. (2006) (1.215 g O_2/(l·h)) during biodegradation of potato stillage. In the experiment at 35ºC performed by Krzywonos et al. (2008) with the same culture of bacteria as the one used in our present study, the rate of SCOD reduction obtained was twice as high.

In the mesophilic processes, at 27 and 36°C, both with pH_0 = 6.5 and pH_0 = 8.0, not only the highest values of the final and the maximal quantity of suspended solids formed were achieved, but also an appreciably higher maximal number of bacterial cells (as compared to the other experiments) was observed with the progress of the process (Tables 2 and 3). It is essential to note that in contrast to the processes at 45, 54 and 63°C, where the number of bacterial cells decreased slightly after the maximal value had been reached, in the mesophilic biodegradation processes this decrease was more pronounced (Fig. 2). A downward trend in the quantity of suspended solids (mainly biomass) with the rise in process temperature was also observed by Tripathi and Allen (1999) during treatment of a bleached kraft pulp mill effluent at non-controlled pH. Thermophilic processes are characterized by a lower biomass production as compared to mesophilic processes, since thermophilic microorganisms require a larger quantity of energy for life support than do mesophilic microorganisms. They utilize a major portion of the energy acquired from the oxidation of organic compounds for metabolism than for growth (Lasik and Nowak 2006, Tripathi and Allen 1999).

With both pH_0 values, the lowest quantity of suspended solids formed in relation to organic matter removed (Y_{ss}), which amounted to 1.28% and 0.49% at pH_0 = 6.5 and pH_0 = 8.0, respectively, was obtained at 45°C. This is an indication that the removal of organic pollutants practically occurred without

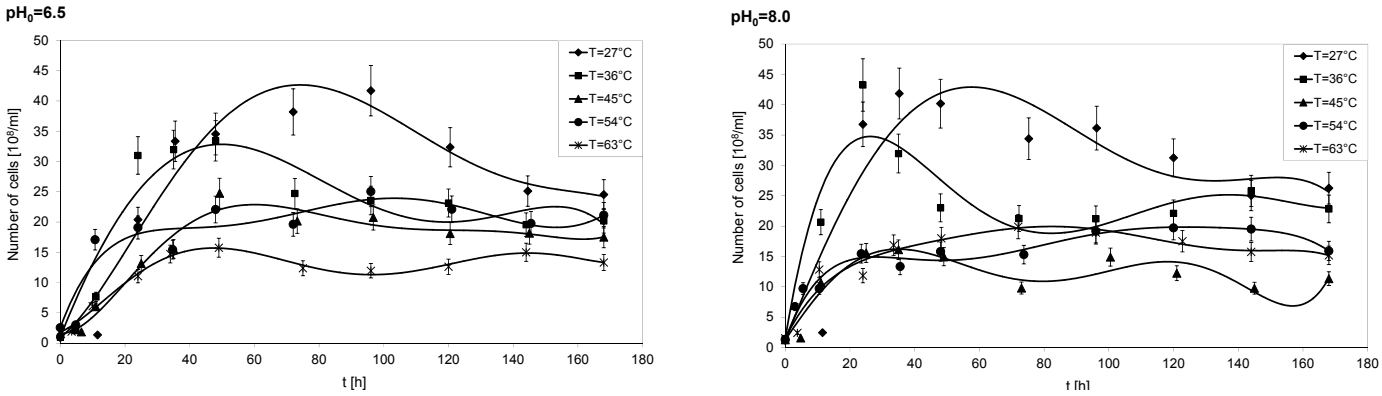

Fig. 2. Effect of temperature and initial pH on the pattern of change in the number of bacterial cells during biodegradation of vinasse
(The length of each error bar is twice the standard deviation of the measured concentration, n = 3)

suspended solids formation, which is a nuisance in wastewater biodegradation. Of the two results, the value of 1.28% deserves particular consideration as it was obtained in the process where betaine was removed. For each of the temperatures applied, the Y_{ss} value was lower at $pH_0 = 8.0$ than at $pH_0 = 6.5$ (Tables 2 and 3). No such relation was noted during vinasse biodegradation with pH control. However, similarly to what was observed in the present study, the processes had low Y_{ss} values when the bacteria removed betaine. At 45°C, the value of Y_{ss} was 2.53% (for pH = 6.5) and 2.35% (for pH = 8.0), whereas at 54°C and pH = 8.0 the Y_{ss} value amounted to 0.22% (Cibis et al. 2011).

Removal of coloured substances

Apart from the high concentrations of organic pollutants, it is also the dark colour of the vinasse that raises environmental problems. When discharged into surface waters, the dark brown effluent inhibits the photosynthesis process by blocking the access of solar radiation. According to Cibis et al. (2011) and Mohana et al. (2009), the biodegradation process (both aerobic and anaerobic) generally fails to provide decolourization of the vinasse effluent, and in some instances there is even an increase in colour intensity.

In all of the experiments conducted within the scope of our study, a rise in the content of melanoidins was observed in the course of the biodegradation process. In the majority of experiments there was a decrease in the quantity of alkaline products of invert sugar degradation (maximal reduction amounted to 51.33%), as well as in the quantity of saccharose carmelization products (maximal reduction amounted to 54%). The highest extent of removal for alkaline products of invert sugar degradation and for the products of saccharose carmelization was achieved at 36ºC. Experiments at 54 and 63ºC were characterized by a lower reduction in colorant content as compared to the other experiments, and even by an increase in the content of some coloured substances (Table 4).

Nitrogen removal – effect of betaine removal

The efficiency of the wastewater treatment process is also assessed in terms of biogens removal (primarily that of nitrogen and phosphorus) as an indicator. If the biogens content of the wastewater is excessively high, this will lead to the eutrophication of the recipient stream. As was the case with the study reported by Cibis et al. (2011), in our experiments the variations in total nitrogen and ammonia nitrogen concentrations were linked with betaine removal. Variations in the content of betaine, total nitrogen and ammonia nitrogen are compiled in Table 4. Fig. 3 presents the amount of ammonia nitrogen formed versus a betaine concentration decrease in the medium during the biodegradation of vinasse. The correlation coefficients for these two variables amounted to 0.9036 for T = 36°C and $pH_0 = 6.5$, 0.8974 for T = 45°C and $pH_0 = 6.5$, 0.9478 for T = 27°C and pH_0 =8.0, 0.9526 for T = 36°C and pH_0 = 8.0. The values of these correlation coefficients and also the course of the graphs in Fig. 3 confirmed that there was a strong correlation between the amount of ammonia nitrogen released to the environment and the amount of betaine removed.

The highest reduction in total nitrogen concentration was observed in the processes where betaine underwent removal. In the vinasse under study betaine nitrogen accounted for as much as 67.5% of total nitrogen. However, the assimilation by the bacteria of the nitrogen originating from betaine decomposition does not seem to be the underlying cause of the appreciable decrease in total nitrogen concentration noted in the processes with betaine removal. In the two processes at 36ºC, as well as in the experiment at T = 45ºC and $pH_0 = 6.5$, the increase of ammonia nitrogen concentration in the medium proceeded faster from the point in time when the quantity of betaine began to decrease. Ammonia nitrogen concentration reached a maximal value after betaine had already been exhausted. Once the maximal value was achieved, ammonia nitrogen concentration began to decrease (data not shown). During biodegradation at 27ºC and $pH_0 = 8.0$, the ammonia nitrogen concentration followed an upward pattern throughout the process, which was concomitant with a continuing betaine removal by the bacteria (data not shown). From the findings described above it can be inferred that betaine decomposition by the bacterial strains chosen was the contributory factor in the formation of $N\text{-}NH_4$ from the betaine's nitrogen component. This fact is also confirmed by the suggestions of Caspi et al. (2008) according to which ammonia nitrogen is released in the last stage (L-serine → pyruvate) of the metabolic pathway of betaine decomposition. Owing to the increased temperature and the alkaline pH, ammonia nitrogen volatilized in the form of NH_3 (NH_3 volatilization was confirmed using Nessler's reagent). And this is what seems to offer a sensible explanation

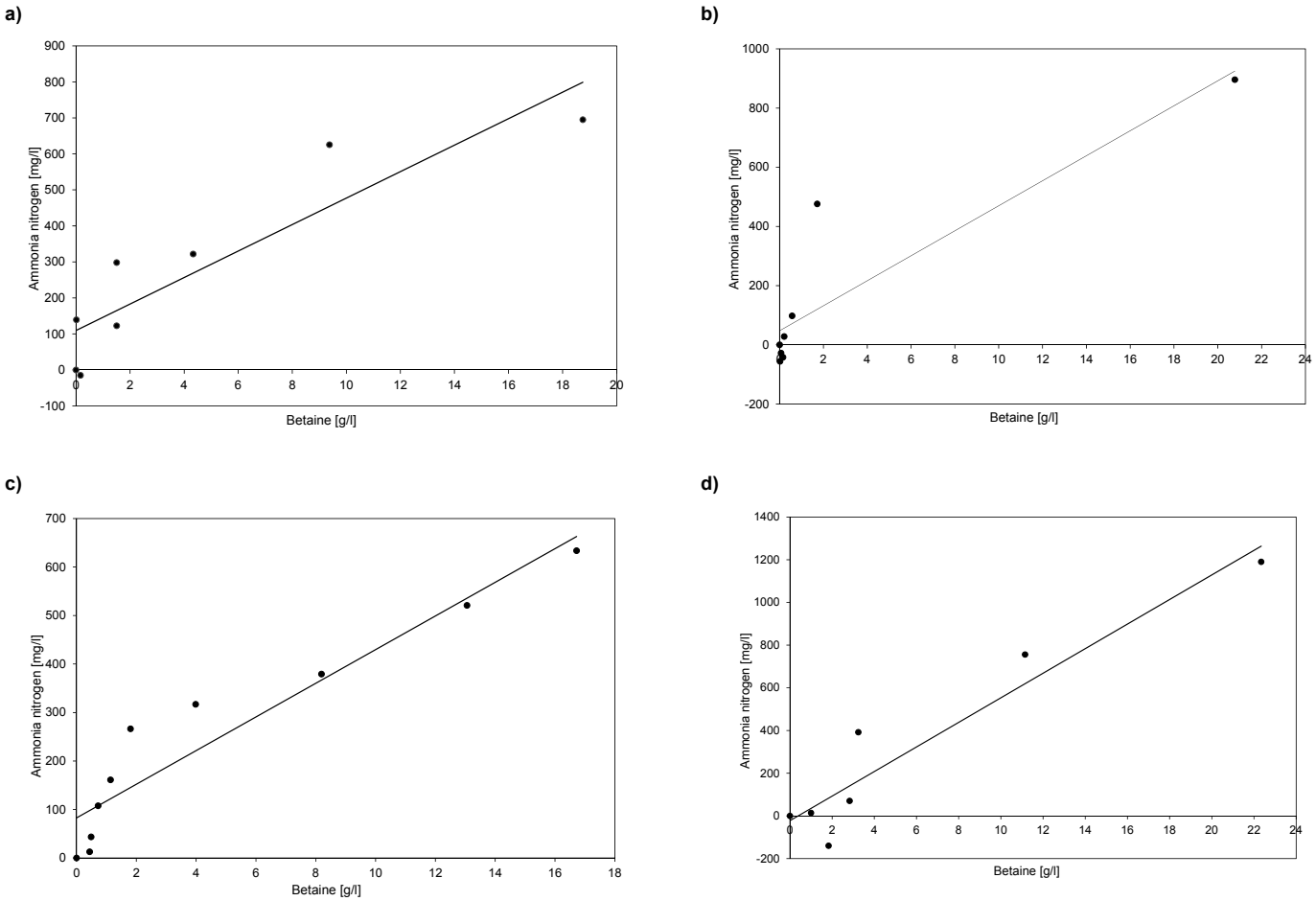

Fig. 3. The amount of ammonia nitrogen formed versus betaine concentration decrease in the medium during the biodegradation of vinasse

a) pH_0=6.5, T=36°C
b) pH_0=6.5, T=45°C
c) pH_0=8.0, T=27°C
d) pH_0=8.0, T=36°C

for the higher extent of total nitrogen removal as compared with the processes where betaine removal failed to occur.

Regardless of whether or not betaine was removed, the extent of ammonia nitrogen removal from the medium increased with the rise in process temperature. Only with pH_0 = 8.0 was the extent of N-NH_4 removal by approximately 2% higher at 45ºC than 54ºC (Table 4). At 63ºC ammonia nitrogen was removed completely. It can safely be assumed that the main contributory factor in the 100% removal of N-NH_4 at this temperature was the volatilization of NH_3, not the synthesis of the biomass, because the highest number of bacterial cells was measured when a considerable quantity of N-NH_4 still persisted in the medium (data not shown). A high loss of ammonia nitrogen (more than 64%) was also observed in the other processes, which were performed at elevated temperature (≥ 45ºC) and did not provide betaine removal. For the same reason it should be assumed (as was the case with the process at 63ºC) that the high extent of N-NH_4 removal in these processes is attributable to the volatilization of NH_3.

In the experiments the NH_3 that volatilized into the atmosphere had originated not solely from the mineral ammonia nitrogen added to, or being a component of, the vinasse but also from the decomposed betaine. It is essential to take into account the fact that an inherent part of any aerobic biodegradation conducted with a sufficiently long retention time is the deamination of proteins – those present in the medium and those owing their origin to the autolysis of bacterial cells (Cibis 2004). Hence, deamination of proteins was an additional, though less important, contributing factor in the increase of N-NH_4 concentration with the progress of the processes where betaine was removed.

In their study of vinasse biodegradation with the same bacterial culture and over the same temperature range as those applied in this work here but with pH control at 6.5 and 8.0, Cibis et al. (2011) noted a rise in the N-NH_4 content of the medium in all of the experiments. Reduction in total nitrogen content observed in the majority of experiments with pH control was lower than in the corresponding processes carried out in our present study without pH control (Table 4).

The literature contains references to aerobic biodegradation of wastewater under thermophilic conditions with no pH control (high temperature, high pH), where ammonia nitrogen removal from the medium ranged from 52% (Couillard and Zhu 1993, Juteau et al. 2004) to about 100% (Beaudet et al. 1990). These values compare with those obtained in the present study.

Phosphorus removal

The extent of total phosphorus removal decreased with the rise in temperature at pH_0 = 8.0. Such trend of change failed to occur in the experiments at pH_0 = 6.5 (Table 4). The highest extent of reduction in total phosphorus concentration and the highest final number of bacterial cells were observed at 27ºC and pH_0 = 8.0 (Tables 3 and 4). The lowest concentrations of both total and phosphate phosphorus in the vinasse being treated were generally measured before the point in time at which the number of bacterial cells reached the maximal value (Fig. 2 and Fig. 4, variations in phosphate phosphorus concentration). In most instances the extent of reduction in total phosphorus and phosphate phosphorus was higher than the one obtained in the corresponding processes performed by Cibis et al. (2011) with controlled pH of the medium.

Phosphorus concentration in the medium decreases when the demand for this biogen increases during biomass synthesis by bacteria (Couillard and Zhu 1993). Bacteria of the genus *Bacillus* have the activity for accumulating phosphorus in the form of polyphosphates (Merzouki et al. 1999), and this phosphorus is then released during intracellular respiration (Couillard and Zhu 1993). In the study reported on in this paper, however, the variations in total and phosphate phosphorus concentrations were attributable not only to the activity of the microorganisms but also to the hydrolysis and reprecipitation of suspended solids. It is a well-established fact that in the suspended solids found in distillery wastewater, as well as in other industrial effluents, phosphorus generally occurs in large amounts.

Conclusions

During biodegradation of beet molasses vinasse with a mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus* in batch processes without pH control betaine was removed at T = 27 and 36°C with pH_0 = 8.0 and at T = 36 and 45°C with pH_0 = 6.5. The removal efficiencies achieved in these processes were high.

Maximal reduction in $SCOD_{sum}$ (85.41%), BOD_5 (97.91%) and TOC (86.32%) was achieved during biodegradation at 36°C and pH_0 = 8.0. Under the same temperature and pH regime, betaine was removed completely, while biodegradation proceeded at the fastest rate (1.1684 g O_2/(l·h) for $SCOD_{sum}$ removal), providing efficiencies of total nitrogen and total phosphorus removal from the medium of 61.49% and 70.64%, respectively.

The high extent of organic matter degradation parallelled by the high efficiency of biogen removal from the medium encourages potential use of the biodegradation process described here as a prior step in the treatment of beet molasses vinasse on an industrial scale. An unquestionable advantage of such approach is that the pH of the medium does not need to be controlled, which reduces the process costs involved.

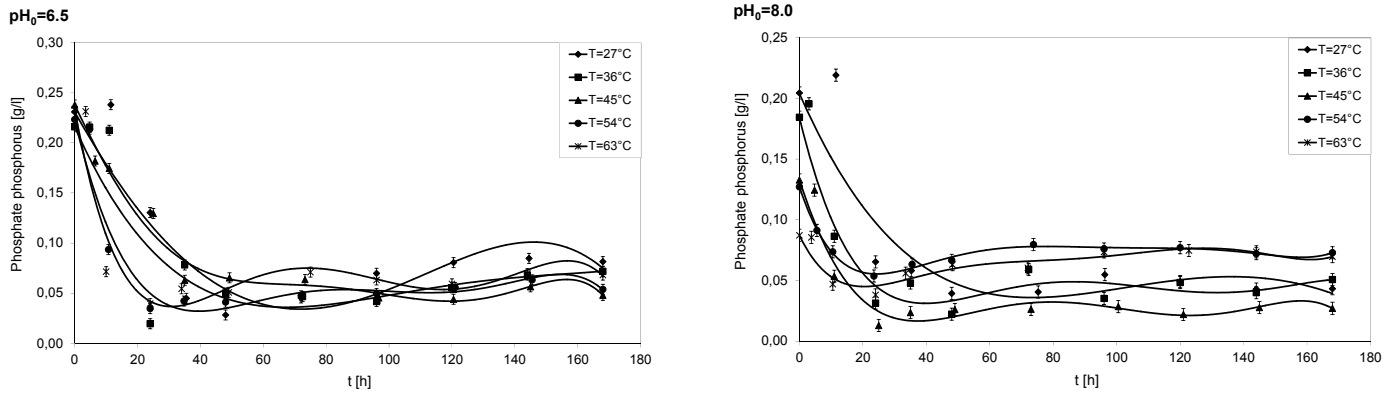

Fig. 4. Pattern of change in phosphate phosphorus concentration in the medium during biodegradation of vinasse. (The length of each error bar is twice the standard deviation of the measured concentration, n = 3.)

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Efektywność tlenowej biodegradacji buraczanego wywaru melasowego przy nieregulowanym pH podłoża: określenie warunków usunięcia betainy

Celem pracy było określenie warunków zapewniających wysoką efektywność tlenowej biodegradacji buraczanego wywaru melasowego za pomocą mieszanej kultury termo- i mezofilnych bakterii z rodzaju *Bacillus* w procesach okresowych prowadzonych bez regulacji pH podłoża. Główną uwagę poświęcono usunięciu betainy (głównego zanieczyszczenia buraczanego wywaru melasowego). Stanowiła ona aż 37,6% zawartości ogólnego węgla organicznego w biodegradowanym wywarze. Procesy biodegra-

dacji prowadzono w 5-litrowym bioreaktorze z układem mieszania w temperaturze 27–63°C, co 9°C, dla pH początkowego (pH_0) równego 6,5 i 8,0. Wysoką efektywność biodegradacji wyrażoną poprzez redukcję $SChZT_{całk}$ (suma SChZT (ChZT oznaczane po oddzieleniu części stałych) i teoretycznego ChZT betainy) uzyskano w procesach prowadzonych w temperaturze 27 i 36°C dla pH_0 = 8,0 (redukcja ponad 77,7%) oraz 36 i 45°C dla pH_0 = 6,5 (redukcja ponad 83,6%). Przyczyną wysokiej efektywności biodegradacji w wymienionych czterech procesach było usunięcie przez bakterie betainy. Maksymalny stopień redukcji $SChZT_{całk}$ (85,41%), BZT_5 (97,91%) i OWO (86,32%), jak również największą szybkość biodegradacji (1,17 g O_2/l·h) uzyskano w eksperymencie prowadzonym w temperaturze 36°C dla pH_0 = 8,0.