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Original Research

Aerobic Biodegradation of Vinasse by a Mixed Culture of Bacteria of the Genus *Bacillus*: Optimization of Temperature, pH and Oxygenation State

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Abstract

The aim of the study was to optimize the temperature, pH and oxygenation state for the aerobic biodegradation of vinasse using a mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus*. At the initial stage of the study a series of experiments was performed in shake flasks over the temperature range of 30 to 65°C and an initial pH of the medium ranging between 5.40 (the pH of vinasse) and 9.5 in order to determine the optimal values of the two parameters (T = 58°C, pH = 8.35) and thus maximize the extent of COD reduction (60.88%). At the subsequent stage, with the optimal values of temperature and initial pH, batch biodegradation processes were conducted in an STR with aeration at 1.0 vvm and two stirrer speeds, 550 rpm and 900 rpm, which provided a reduction in COD of 77.56% and 76.48%, respectively. With 900 rpm, a concomitant rise in the biodegradation rate was observed. The extent of COD reduction increased to 85.37% when biodegradation was conducted at 58°C (optimal temperature) and a stirrer speed of 900 rpm, the pH being maintained at 8.35 throughout the process.

Keywords: aerobic biodegradation; vinasse; batch process; bacteria of the genus *Bacillus*

Introduction

Vinasse, a by-product of ethanol production from molasses, is a high-strength effluent with a high content of organics, mainly organic acids, reducing substances, betaine (only in the case of beet-vinasse) [1], coloured matter and glycerol [2]. The wastewater is characterized by high concentrations of potassium, calcium, chloride and sulphate ions, a high content of suspended solids, a high COD (Chemical Oxygen Demand) level (15 to 176 g O_2/l) and a high temperature at the moment of generation [3].

The water content that ranges between 91 and 94% reduces the applicability of the stillage to direct grazing, as this is concomitant with the production of large amounts of liquid manure whose treatment is far more troublesome than the treatment of vinasse itself [4]. The high potassium content is responsible for the laxative properties of vinasse [5, 6]. The volume of the stillage produced (9 to 14 l per 1 l of ethanol) [7] is too high to allow its utilization as fodder in the proximity of the distillery. On the other hand, the transport of vinasse to the farms situated at a farther distance appeared not to be cost-effective [4]. Vinasse becomes viscous when concentrated, and hygroscopic when incinerated, and this makes its application as a fodder component very difficult. Furthermore, the incineration of this stillage requires its concentration as a prior step, and this is a costly procedure. Another mode of utilizing vinasse involves yeast cultivation, but this process fails to provide the desired removal of the organic substances included [2].

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Owing to the abundance of mineral matter, vinasse might be used as a soil fertilizer, if this were not associated with the requirement of applying corrosion-resistant equipment [5]. A major drawback inherent in direct land applications of vinasse is the concomitant release of a strong, objectionable odour and the proliferation of certain insects [6]. When vinasse is introduced into the soil, the proteins included decompose and release hydrogen sulphide and ammonia nitrogen, thus producing environmental contamination [4]. It is essential to note that direct land application of vinasse can be carried out only seasonally.

An alternative approach is the classification of vinasse as a waste and making it subject to biological treatment. So far, anaerobic utilization has been applied on an industrial scale mainly in India [8]. The process is moderate in energy demand, and the anaerobic bacteria involved convert organic compounds to biogas, producing only slight biomass amounts [7]. However, if the COD of the stillage exceeds 100 g O_2/l, dilution becomes a must, as the process is inhibited by the high concentrations of pollutants [8]. This inhibition seems to be in response to the presence of phenol compounds in the stillage that exert a toxic effect on methane bacteria. Another major factor affecting these organisms is the high salinity of vinasse [7]. When the stillage was treated by anaerobic methods, the extent of COD reduction ranged from 39% [9] to 95% [10]. The reduction in COD attained using aerobic-anaerobic treatment processes (aerobic degradation was performed with the fungus species *Penicillium decumbens*) amounted to 96.5% [7].

To treat high-strength high-salinity wastewater which contains toxic substances, use is made of thermophilic aerobic processes and the treatment effects are promising [11]. With a mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus,* the aerobic biodegradation of the stillage from distilleries using starch-based substrates for ethanol production brought about a COD reduction of 82.6%, 84.6%, 89.8% and 93.7% for maize slops, rye slops, potato slops and the slops coming from starch-based waste substrates, respectively [12]. It is essential to note, however, that no references to the aerobic biodegradation of vinasse with the use of bacteria have been found in literature. With these thoughts in mind, as well as considering the promising results obtained with starch-based substrates, we decided to examine the applicability of a mixed culture of bacteria of the genus *Bacillus* to the treatment of vinasse.

The aim of the study was to optimize the temperature, pH and oxygenation state for the process in question. The optimal values of temperature and initial pH were established in a series of shake flask experiments. Subsequently, with these optimal values in hand, experiments were conducted in an STR (Stirred-Tank Reactor) in order to determine such a stirrer speed that would provide an appropriate (non-limiting) quantity of dissolved oxygen in the medium. The STR was also used to investigate the influence of pH control on the efficiency and course of the biodegradation process.

Experimental Procedures

Microorganisms

The mixed culture of the bacteria made use of 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. The activity of the microorganisms was maintained at 45 ± 2°C in an aerated non-stirred 0.5 l (operating volume) reactor (scrubber) 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 not only in shake flasks but also in the STR.

Preparation of the Medium

The vinasse used in our study came from CHEKO, Włocławek, Poland. Its composition is characterized in Table 1. In order to eliminate the potentiality for contamination, the stillage was boiled for 15 mins not only before being used as the medium for bacterial activity maintenance in the scrubber but also prior to the biodegradation processes carried out in shake flasks and in the bioreactor. After cooling, the pH was adjusted (using 33% NaOH) either to 7.5, when the vinasse was to be used as the medium for the bacteria maintained in the scrubber, or to the values determined for the programme of the study when the vinasse was made subject to biodegradation in shake flasks or in the bioreactor.

Parameters of the Biodegradation Process

Optimization of Temperature and pH

Processes of 96 h duration, which aimed at determining the optimal temperature and initial pH, were carried out in 750 ml flasks containing 150 ml of the vinasse. The other process parameters were: rotation, 150 rpm, temperature, 30, 35, 40, 45, 50, 55, 60 and 65°C, initial pH of the culture medium, 5.40 (the pH of vinasse), 6.0, 6.5, 7.0, 7.5, 8.0, 8.5, 9.0 and 9.5. In sum, 72 experiments (8 temperature values x 9 values of initial pH) were performed. The inoculum consisted of 2 ml of the medium from the aerated non-stirred bioreactor which contained a three-day-old mixed culture of bacteria, as on the third day the number of bacterial cells in the scrubber was found to be the highest.

Biodegradation in the Bioreactor

168-hour batch processes were performed in a 5-litre (working volume) STR of Biostat®B type (B.Braun Biotech International) with an aeration rate of 1.0 vvm (volume per volume per minute) and two stirrer speeds, 550 rpm (rotations per minute) and 900 rpm. In the process with pH control, 2 M H_2SO_4 and 2 M NaOH were used to maintain the pH at a constant level. Liquid loss in the bioreactor in response to evaporation was made up automatically with distilled water. The inoculum was a 200 ml portion of the medium coming from the 0.5 l bioreactor where the three-day-old mixed culture of bacteria was maintained.

Methods of Analysis Used in the Course of Biodegradation

COD, total organic carbon (TOC), total nitrogen, total phosphorus and phosphate phosphorus (P-PO_4) were determined via Dr. Lange spectrophotometric cuvette tests [13]. Organic acid content was measured by HPLC (Varian Pro Star, USA; column type: Aminex HPX-87H; column size: 7.8 mm i.d. x 300 mm; eluent, 0.004 M H_2SO_4; flow velocity, 1.2 ml/min). Glycerol concentration was measured by spectrophotometry [14.15], the content of reducing substances being established by the Lane-Eynon method. The distillation technique was used to determine ammonia nitrogen concentration. Prior to the determination of betaine, the samples were decoloured via treatment with hydrochloric acid until pH equalled 1, followed by heating with activated carbon in a water bath, and by hot filtration involving a 3h filter paper (FILTRAK, Germany). Further colorimetric analysis using Reinecke salt was carried out according to the method developed by Sławiński et al. [16]. Potassium was determined by flame photometry. All the analyses mentioned above were performed after the separation of suspended solids, which involved centrifugation for 40 mins at 13,000 rpm (Sigma®4K15). Suspended solids 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 Thoma chamber.

Results

Biodegradation of Vinasse in Shake Flasks – Optimization of Temperature and Initial pH of the Medium

The process was carried out without controlling the pH of the medium. The number of bacterial cells was established eight times in the course of the process, according to the following pattern: at 30°C in hour 0, 12, 24, 36, 48, 72, 84 and 96 of the process; at 35 to 65°C every 12 hours during the first three days, and subsequently after 24 hours *i.e.* at the moment when the process terminated. Suspended solids and COD were measured at the beginning and at the end of each experiment.

When the biodegradation process was carried out at 30 or 35°C, cell growth occurred only at a high initial pH of the vinasse being treated (Fig. 1a). In that case, the duration of the lag phase varied from 35 to 50 h. At temperatures equal to, or higher than, 40°C, the period of microorganism adaptation became noticeably shorter, approaching 24 h. At 45 and 50°C, bacterial growth was observed over a wide range of the pH (Fig. 1b). This pattern changed at the temperature of 40 and 55–65°C, when bacterial growth was more intense in the medium of a neutral and an alkaline initial pH than in the medium of an acidic initial pH (Fig. 1c). Regardless of the process temperature applied, the highest increment in the cell number was always linked with high initial pH values of the medium.

For each of the biodegradation processes performed, the extent of COD reduction, the maximal number of bacterial cells and the final concentrations of suspended solids were expressed in terms of the experimental functions of two variables: temperature and initial pH. Making use of the software TableCurve® 3D ver. 3.12, the functions were approximated automatically by the theoretical relations. In all instances, the most promising results were obtained when the experimental data were approximated using cosine series bivariate order 7. The coefficient of determination for the extent of COD reduction, for the maximal number of cells and for the final concentration of suspended solids amounted to 0.9474, 0.8646 and 0.8282, respectively. The results of the approximation, as well as the experimental points, are depicted in Figs. 2, 3 and 4.

With the software mentioned, it was possible to determine the values of both temperature and initial pH at which the reduction in COD, the maximal number of bacterial cells in the course of the biodegradation process and the final amount of suspended solids were the highest. Thus, taking into account the maximization of the extent of COD reduction (which is 60.88%), the optimal values of temperature and pH were found to be 58°C and 8.35, respectively. Considering the maximal number of bacterial cells observed in the course of the process, as well as the final amount of suspended solids ($12.79 \cdot 10^8$/ml and 6.76 g/l, respectively), the optimal values of temperature and pH were 30°C and 9.5, respectively.

Biodegradation in the STR

Biodegradation processes in shake flasks seemingly suffer a deficiency of dissolved oxygen in the medium, and that is why the experiments were furthered in an aerated laboratory bioreactor. Three experiments were carried out at 58°C and a pH of 8.35, as these were the conditions that had provided the highest efficiency of COD reduction in the shake flask processes. During each process, samples for chemical analyses were collected nine

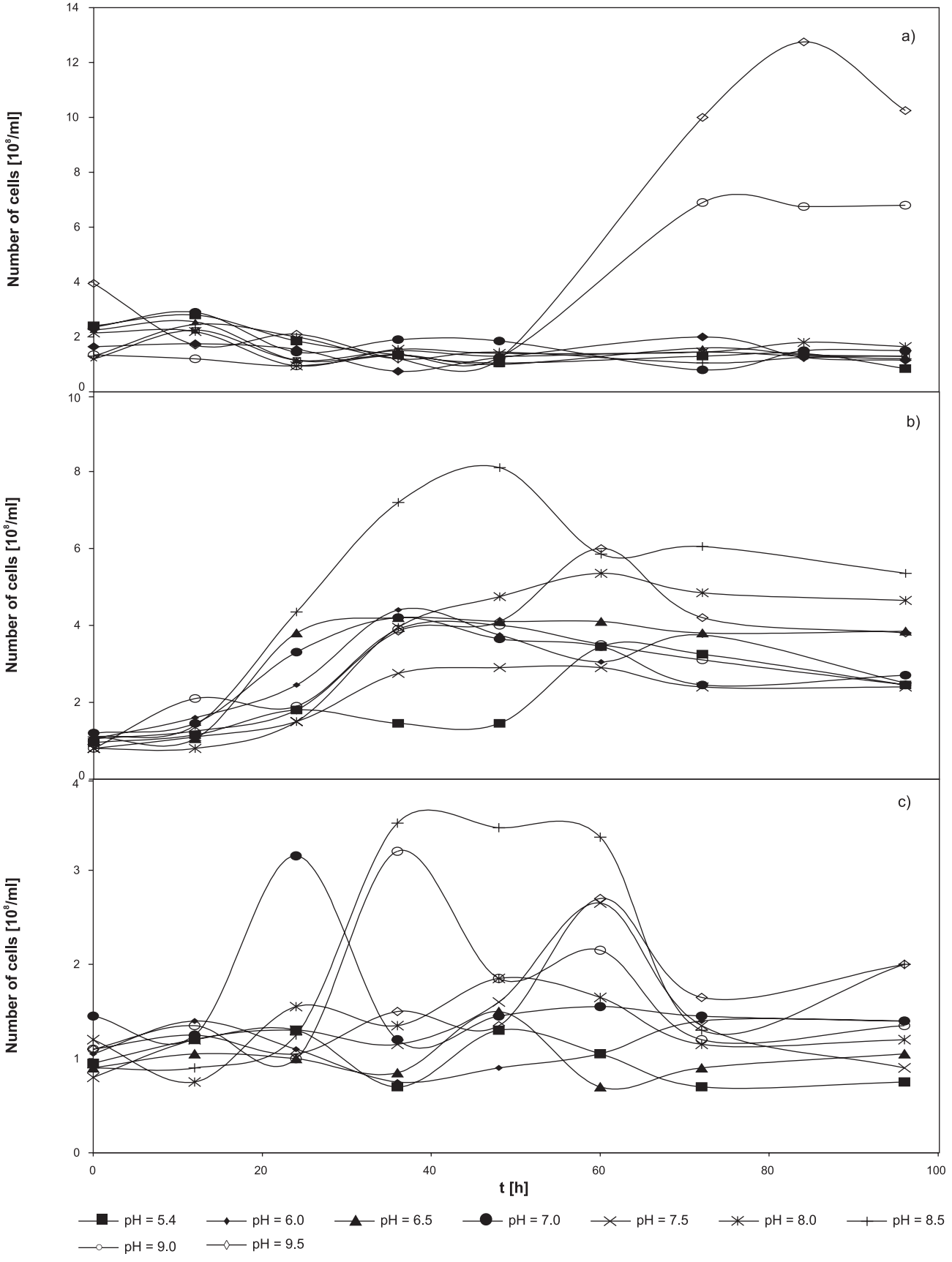

Fig.1. Number of bacterial cells in shake flask experiments.
a) 30°C, b) 50°C, c) 60°C.

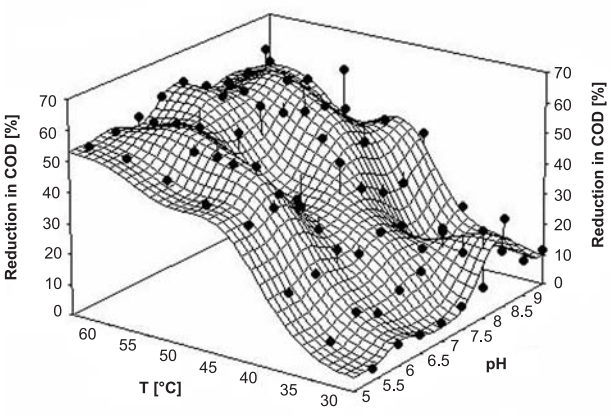

Fig. 2. Extent of COD reduction in shake flask processes related to temperature and initial pH.

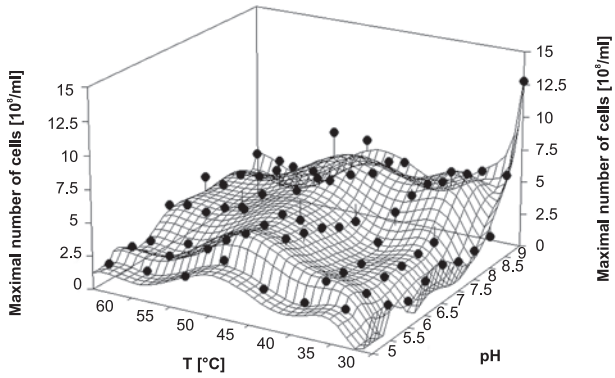

Fig. 3. Maximal number of bacterial cells in shake flask processes related to temperature and initial pH.

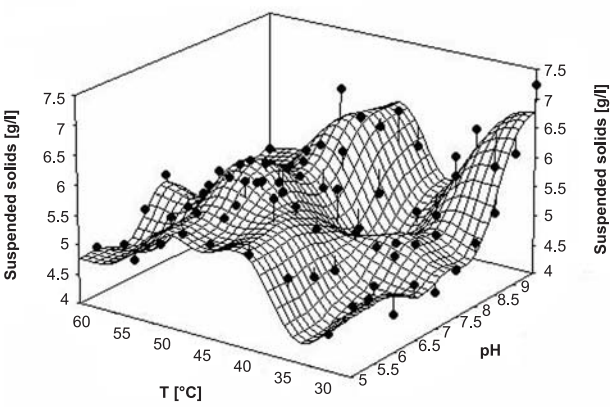

Fig. 4. Final concentration of suspended solids in shake flask processes related to temperature and initial pH.

times every 24 hours; an additional sample was taken on the second day of the process during the phase of enhanced microbial growth. Both at the commencement and the termination of the process COD was determined three times. In this way it was possible to obtain (for each of the processes performed) 9 different values (replicates) of the extent of COD reduction and, consequently, to assess statistically the significance of the differences between the mean values of the COD reduction obtained for the processes conducted.

The first experiment was performed with no pH control and with the stirrer speed of 550 rpm. There was an 18-hour period of dissolved oxygen deficiency (observed between the 17th and 35th hour of the process) (Fig. 5a), which is an indication that the stirrer speed applied failed to provide the oxygenation state desired. In the 20th hour of the process, the pH of the medium fell to its lowest level in this study, which was 6.72. Then a rapid rise was observed, and after the 38th hour of the process the pH exceeded 9.5 (Fig. 5a). Up to the 48th hour of the experiment the reduction in COD accounted for 93.4% of the total COD reduction attained in this process (77.56%).

In order to prevent the occurrence of oxygen-limited conditions, the second experiment involved a stirrer speed of 900 rpm. The minimal DOT (Dissolved Oxygen Tension) value then amounted to 8%, which was measured in the 25th hour of the process (Fig. 5b). The lowest pH of the medium (determined in the 16th hour of the process) equalled 7.48 and began to increase continually to exceed the value of 9.5 after the 30th hour of the process (sooner than in the first experiment) (Fig. 5b). Total reduction in COD was 76.48%. It is essential to note, however, that only after the 48th hour did the extent of COD reduction accounte for 97.24% of the organic substances removed throughout the experiment. Thus, the process ran at a faster rate than when the stirrer speed was 550 rpm. This can be inferred from Fig. 5, which, besides other parameters, includes the time profiles for COD in the three experiments with the STR.

In the third experiment the pH of the medium was maintained at 8.35 and the stirrer speed amounted to 900 rpm. As was the case with the second experiment, no phase of dissolved oxygen deficiency was observed in the medium. Between the 16th and 28th hour of the process, saturation with oxygen was lower than 60%, but never fell below 32.3% (Fig. 5c). The extent of COD reduction, which amounted to 85.37%, was higher as compared to the other two experiments.

The utilization of the main carbon sources by the bacteria, as well as the efficiency of total nitrogen, ammonia nitrogen, total phosphorus and phosphate phosphorus removal, is characterized in Table 1. A continual decrease in the concentration of ammonia nitrogen in the medium was observed in the processes with no pH control. When the pH was under control, the amount of ammonia nitrogen decreased to the 24th and after the 96th hour of the process. Between these hours the concentration of ammonia nitrogen increased to the value of 369 mg/l. Total nitrogen content in the medium decreased with time, the only exception being the experiment that involved the stirrer speed of 550 rpm, where the concentration of total nitrogen increased after the 72nd hour of the biodegradation process. In all the experiments the final concentrations of total phosphorus and phosphate phosphorus were found to be higher than the relevant initial values. Minimal con-

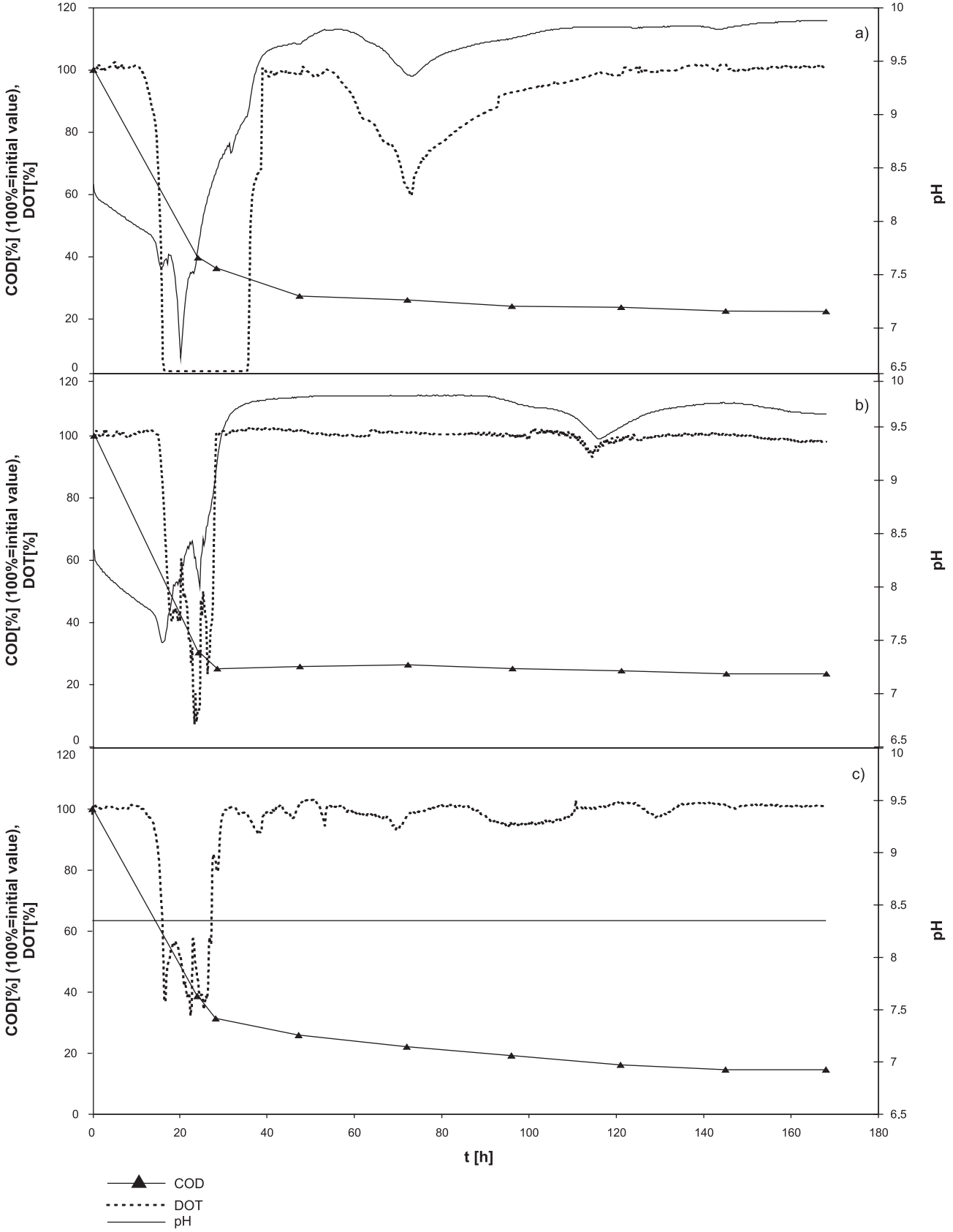

Fig. 5. Variations in the parameters chosen during the biodegradation of vinasse in the STR.
a) 550 rpm, no pH control, b) 900 rpm, no pH control, c) 900 rpm, pH = 8.35.

Table 1. Composition of vinasse and reduction in the content of the medium components during biodegradation in the STR.

Parameter	Unit	Value	Removal [%]		
			550 rpm, no pH control	900 rpm, no pH control	900 rpm, pH = 8.35 (controlled)
			(initial value = 8.35)		
pH	-	5.4	-	-	-
Density	°Blg	6.1	not determined	not determined	not determined
Suspended solids	g/l	5.3	ic	ic	21.22
COD	g O_2/l	126.7	*77.56 ± 1.08	*76.48 ±1.09	85.37 ± 0.79
TOC	g/l	54.25	not determined	not determined	not determined
Lactic acid	g/l	20.76	99.89	99.80	100
Acetic acid	g/l	12.11	79.81	81.01	100
Butyric acid	g/l	11.87	85.80	82.99	100
Isobutyric acid	g/l	1.65	60.18	55.77	100
Malic acid	g/l	0.243	53.73	ic	100
Reducing substances without hydrolysis	g/l	2.41	100	100	100
Reducing substances with hydrolysis	g/l	5.99	46.25	40.01	66.20
Glycerol	g/l	3.74	98.75	96.96	96.87
Betaine	g/l	20.71	0	0	0
Total nitrogen	g/l	3.63	17.78	20.97	16.51
Ammonia nitrogen	g/l	0.24	76.25	36.81	14.28
Total phosphorus	g/l	0.026	ic	ic	ic
Phosphate phosphorus	g/l	0.0057	ic	ic	ic
Potassium	g/l	9	0	0	0

ic = increased content

* Denotes the lack of a statistically significant difference for $\alpha \leq 0.05$.

centrations of total phosphorus and phosphate phosphorus were observed at enhanced bacterial growth. In every instance, these minimal concentrations were less than 30% those at the end of the process and slightly more than 50% those at the beginning of the process.

Of the organic acids that were present in the medium, lactic acid, acetic acid and butyric acid occurred in the largest quantities. When the experiments were carried out without pH control, all of the acids mentioned were utilized by the bacteria during the phase of increased oxygen demand (Fig. 6a, b) (lactic acid, over 90%; acetic acid and butyric acid, 83.24% and 74.13%, respectively, at the stirrer speed of 550 rpm, and 76.55% and 66.45%, respectively, at the stirrer speed of 900 rpm). After the 40th hour of the process, a rise was observed in the content of the three acids followed by another phase of their assimilation by the microorganisms. In both the processes citric acid was synthesized in the amount of 3.87 g/l and 5.01 g/l at the stirrer speed of 550 rpm and 900 rpm, respectively. Following the termination of the biodegradation process, 19.77% and 25.45% of the citric acid produced persisted in the medium in the first and the second experiment, respectively. In the experiment involving pH control an almost 100% assimilation of butyric acid was observed in the phase of intense microbial growth (Fig. 6c). This was followed by the synthesis of butyric acid in an amount exceeding half the initial content, which was then fully utilized by the microorganisms involved. As in that particular case no synthesis of lactic or acetic acid had occurred, the removal of both the species from the medium was complete.

The variations in the content of glycerol and reducing substances in the vinasse during the processes performed in the STR are plotted in Fig. 7. In each of the three experiments, glycerol was assimilated by the bacteria at a faster rate. Up to the 29th hour of the process its content in the medium decreased by 77.64% in the first experiment, by 89.73% in the second experiment, and by 93.18% in

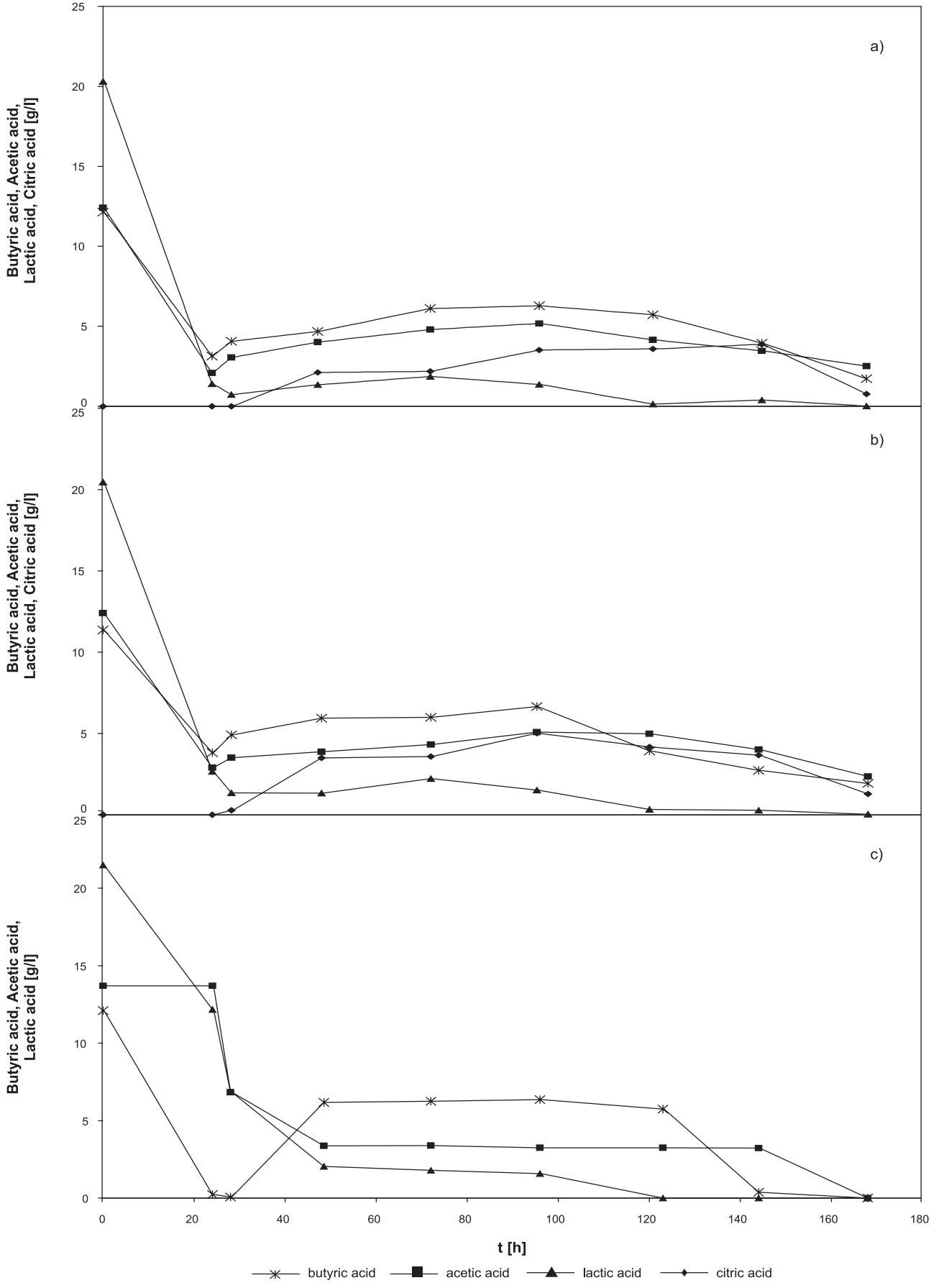

Fig. 6. Variations in the content of the organic acids chosen during biodegradation in the STR.
a) 550 rpm, no pH control, b) 900 rpm, no pH control, c) 900 rpm, pH = 8.35.

the third experiment (the one with pH control). Within the same time span, the reducing substances determined without hydrolysis were removed to a notably lower degree: by 37.77%, 49.07% and 33.12% in the first, second and third experiment, respectively. In sum, the extent of glycerol assimilation exceeded 96% in each experiment. A complete assimilation of the reducing substances determined without hydrolysis was in every instance achieved before the 72nd hour of the process. The removal of the reducing substances determined upon hydrolysis failed to exceed 47% in the first two experiments. In the third experiment (with controlled pH) the extent of reducing substances removal totalled 66.20%. It seems worth noting that during the processes with no pH control, when the reducing substances determined without hydrolysis had been exhausted, the removal of the reducing substances determined after hydrolysis practically did not undergo changes.

In each of the three experiments an increase in the amount of suspended solids was observed during the phase of enhanced oxygen demand. After that, the suspended solids content decreased continually, but only in the experiment with pH control was the final value lower than the initial one (by 21.22%). The decrease in the solids content that commenced at a certain point in time in the course of the process can be attributed to the autolysis of the cells and to the dissolution of a portion of the solids that were not part of the biomass.

Discussion

As can be inferred from the investigations reported in literature, the use of mixed cultures has become commonplace in biodegradation processes. Compared to monocultures, their nutritional requirements are lower and their activity is higher. Seemingly, this has to be attributed to the synergistic interaction between the microorganisms [17, 18, 19]. Another argument for the use of mixed cultures for biodegradation purposes is that these processes are conducted in open, non-sterile systems. And this means that it is practically impossible to avoid contamination and, consequently, to conduct the biodegradation process with a monoculture. The mixed cultures involved in biodegradation processes consist of both meso- and thermophilic (or thermotolerant) organisms [20, 21, 22]. Under industrial conditions, biodegradation processes are generally conducted with no control of temperature or pH in order to minimize the costs involved. As a consequence, the two parameters undergo variations over a wide range of values prior to their autostabilization. In the case of temperature, this range includes values that are optimal for both meso- and thermophilic organisms. That is why we decided to use a mixed culture of meso- and thermophilic bacteria in our study reported on in this paper. It has to be noted that the optimal temperature and initial pH values obtained in the shake flask processes are the resultants of the optimal values characteristic for particular strains.

The culture of bacteria of the genus *Bacillus* made use of in our study for the thermophilic biodegradation of vinasse produced a COD reduction (85.37%) that was comparable not only with the one attained in the treatment of starch-based stillage where the same culture was involved [12] but also with the COD reduction (ranging from 60 to 90%) obtained during the biodegradation of other industrial effluents under thermophilic conditions [23, 24]. It is essential to add, however, that such reduction in COD is still insufficient to allow discharge of the vinasse into watercourses or soil immediately after biodegradation. What can be done is mixing the effluent with wastewater of a lower strength and sending it to the sewer system. An alternative solution can be the inclusion of the aerobic biodegradation of vinasse, as a prior step, into a two-stage treatment process. The second stage in this process can involve anaerobic biodegradation. According to the COD reduction obtained, the vinasse can be passed either into the natural environment or into the sewer system.

In biological treatment methods, the COD:N_{tot}:P_{tot} ratio of 200:5:1 is believed to be optimal [25]. In our investigations into the biological treatment of vinasse this ratio amounted to 200:5.73:0.041. The minimal phosphorus content in the medium was measured in the phase of intense microbial growth during enhanced oxygen demand but no limitation of the process was found to occur in response to phosphorus. It can be assumed that the rise in the content of both total phosphorus and phosphate phosphorus after their minimal values have been achieved is attributable to the following two factors, the release of phosphorus from the bacterial cells undergoing autolysis and the transition of this substance from the suspended solids to the liquid phase. Seemingly, the latter phenomenon has to be blamed for the fact that phosphorus content in the liquid phase was higher at the termination than at the commencement of the process.

When the process in the STR was carried out with no pH control, the pH of the medium decreased and thereafter increased rapidly above the value of 9.5. The same pattern has been observed by Loll [20], Beaudet et al. [26] and Lasik et al. [27] in their research into the aerobic thermophilic treatment of high-strength industrial effluents. Malladi and Ingham [28] have noticed that the decrease in the pH is concomitant with an increase in microbial growth, and the subsequent rise in the pH value is attributable to the assimilation of the substrates and to the release of alkaline by-products. Tripathi and Allen [25] have ascribed the increase in the pH level to the formation of basic chemical compounds while McIntosh and Oleszkiewicz [29] have attributed this increase to the formation of a faintly amoniacal base resulting from the ammonia nitrogen generated in response to deamination. In our study the rise in pH cannot be explained in the way proposed by McIntosh and Oleszkiewicz [29] since it was parallelled by a decrease in the content of ammonia nitrogen. This decrease, however, does not exclude the occurrence of the deamination phenomenon. There is some evidence to sug-

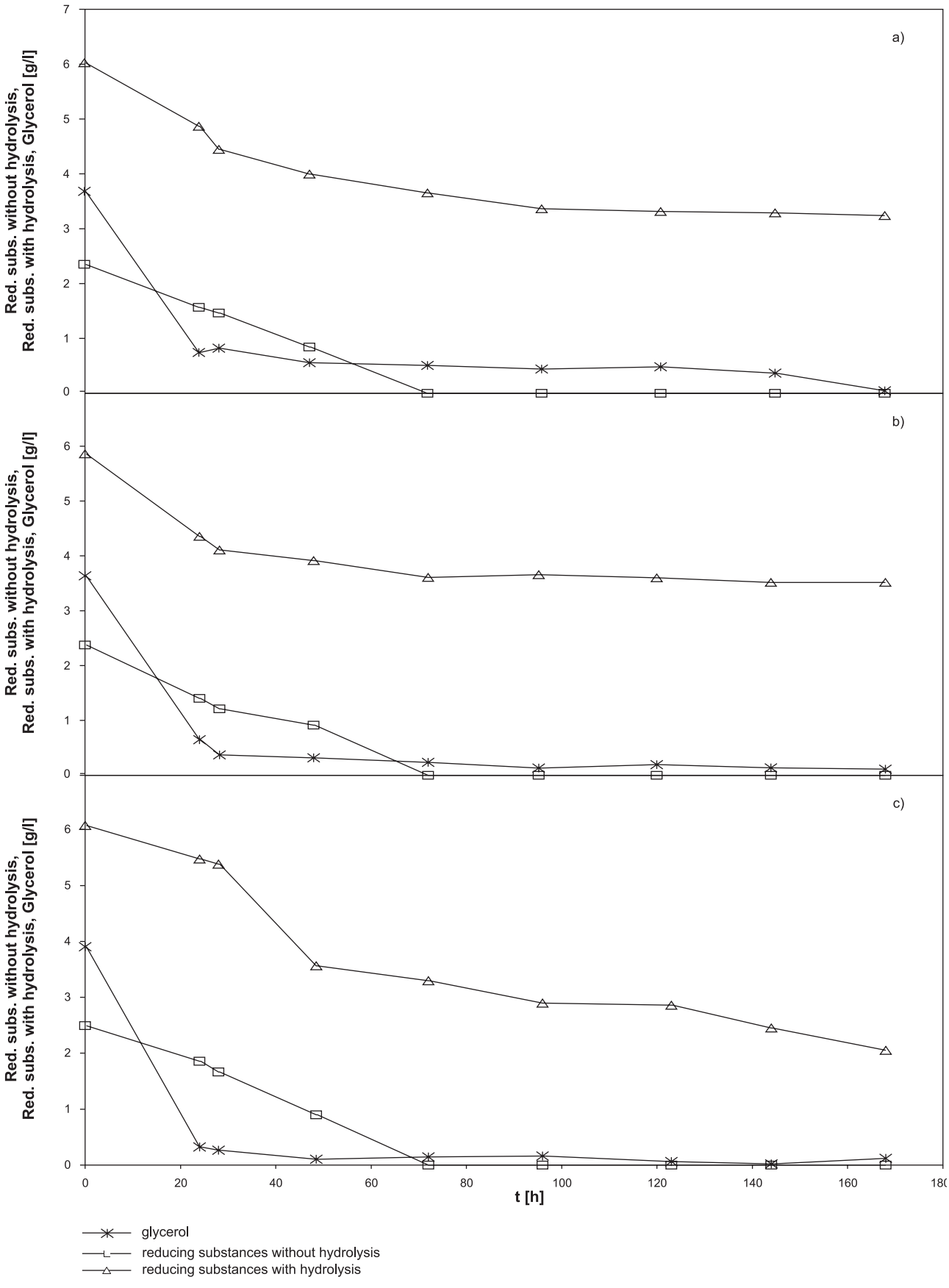

Fig. 7. Variations in the concentration of glycerol and the reducing substances during biodegradation of vinasse in the STR. a) 550 rpm, no pH control, b) 900 rpm, no pH control, c) 900 rpm, pH = 8.35.

gest that ammonia nitrogen was forming but, in response to the high pH and temperature, volatilized in the form of free ammonia, which has been detected using Nessler's reagent. The less distinct decrement in ammonia nitrogen in the course of the process with pH control (Table 1) was probably due to the following: the level of 8.35, at which the pH was maintained, was notably below the one observed after 48 h (over 9.5) in the other two processes. And this means that the experiments with no pH control provided more advantageous conditions for the volatilization of ammonia nitrogen. According to Henze et al. [30], biodegradation without pH control in the course of the process is justified when the wastewater under treatment is characterized by a high buffer capacity and the pH of the medium is maintained between 6.0 and 9.0, as within this pH range the activity of the bacteria is not inhibited. As reported by Beaudet et al. [26], there is no need to control the pH when COD does not exceed the value of 30 g O_2/l. Some investigators indicate that the biodegradation process involving pH control is normally carried out over the pH range of 6.0 to 8.0 [31, 32]. In our study the pH of the medium was maintained at 8.35 during biodegradation in the STR, which enabled the extent of COD reduction to be increased by 8.89%.

The phenomenon of volatile acid formation has been reported by many investigators dealing with aerobic thermophilic biodegradation of wastewater [29, 33, 34]. They have observed that acids are synthesized as a result of oxygen deficiency in the culture medium and that the largest quantities produced are those of acetic acid. According to Ugwuanyi et al. [33], this is the composition of the medium that decides which type of acid is synthesized in the course of the aerobic biodegradation of the wastewater. Chu et al. [35] have presented a biochemical model describing the synthesis of acetic acid under microaerobic conditions. As shown by this model, acetic acid is produced in response to the limited oxidation of NADH to NAD^+, which is attributable to the reduced efficiency of the respiratory chain. When the cells suffer a lack of NAD^+, they transform aceto-CoA (via acetyl phosphate) to acetate, and thus an ATP molecule is obtained without NAD^+ reduction. But if aceto-CoA is passed directly to the Krebs cycle, then the acquisition of energy is paralleled by the reduction of NAD^+ to NADH, which under aerobic conditions is oxidized to NAD^+. In our study, each of the three experiments in the bioreactor produced butyric acid in amounts larger than those of the other acids chosen. Butyric acid was synthesized primarily during the phase that followed the period of enhanced oxygen demand. The same phenomenon has been reported by Cibis [12] for aerobic thermophilic biodegradation of the stillage obtained from starch-based wastes, as well as for the biodegradation of rye-based or maize-based stillage.

A similar sequence of glycerol and reducing substances assimilation by the bacteria of the genus *Bacillus* has been reported by Cibis et al. [34], who made use of the same culture to biodegrade the stillage from another substrate. It should be noted, however, that their determinations included reducing substances only after hydrolysis. They have found that during the aerobic biodegradation of potato stillage glycerol was assimilated first and that its content in the medium decreased rapidly before the 20th hour of the process. Then the bacteria removed the reducing substances from the stillage. The removal of both glycerol and reducing substances from this medium exceeded 90%.

Conclusions

1. The maximal extent of COD reduction in the shake flask processes for the vinasse under study amounted to 60.88% and was achieved at a temperature of 58°C and initial pH of 8.35.
2. The highest maximal number of bacterial cells in the course of the process and the highest final content of suspended solids in the shake flask processes were obtained at 30°C and initial pH of 9.5.
3. The enhanced oxygenation state (achieved by increasing the stirrer speed from 550 to 900 rpm) was without any effect on the extent of COD reduction during biodegradation in the bioreactor but there was an increase in the rate of the biodegradation process.
4. When the pH of vinasse was constant throughout the biodegradation process in the bioreactor, the extent of COD reduction increased. With a controlled pH of 8.35, a temperature of 58°C and a stirrer speed of 900 rpm, the efficiency of reduction in COD amounted to 85.37%.

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