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BIODEGRADATION OF POTATO SLOPS WITH A MIXED POPULATION OF BACTERIA OF THE GENUS *BACILLUS* – DETERMINATION OF THE PROCESS CONDITIONS

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ABSTRACT

The study has been carried out in order to optimise the conditions for the biodegradation of potato slops with a mixed population of thermophilic and mesophilic bacteria.

Biogen-enriched ($N\text{-}NH_4$ and $P\text{-}PO_4$) and non-enriched potato slops samples were subjected to biodegradation in shake-flasks and in a stirred-tank bioreactor at 45°C, controlled pH (at the level of 7.0) and uncontrolled pH (initial pH = 7.0). The high efficiency of the biodegradation process was found to be accounted for by the application of biogen-enrichment, pH control and the stirred-tank reactor, which provides more advantageous aeration than do shake-flasks. Under such conditions, the process yielded a COD reduction of 84.2%. When biodegradation was carried out in a shake-flask with non-enriched slops and with no pH control, the reduction in COD amounted to 21.3%.

Key words: potato slops, thermo- and mesophilic bacteria, aerobic biodegradation, batch process

INTRODUCTION

Until recently, potato slops from rural distilleries in Poland have been used as fodder for farm animals. However, Polish farmers show no interest in that sort of feed, as these days the fodder market is offering more attractive items. In this context, the search for other slops utilisation methods has taken on a sense of urgency, notably because the volume of this distillation residue (a nuisant high-strength waste product displaying COD levels of up to 100 g $O_2 \cdot l^{-1}$ at times) continues to increase [6, 24]. So far, the following alternative uses for potato slops have been proposed: as fertilisers, as fodder components obtained after thickening and drying [19], or as fillers in the production of rigid polyurethane foams [8]. There are, however, a number of drawbacks which make such applications less promising – the thickening and drying of the slops is paralleled by very high energy demand; fertilisation is carried out only over a short period of the year, and application as filling material for polyurethane foams provides the utilisation of only a certain part of the overall slops volume produced.

Another approach to the problem consists in viewing the potato slops as wastewater and attempting to bioremediate it. Aerobic biodegradation with a mixed population of thermo- and mesophilic bacteria seems to be well suited for this purpose. Many investigations reported in the literature have demonstrated that high-strength effluents can be successfully treated by thermo- or mesophilic aerobic bioremediation processes [1, 2, 14, 21]. What is more, the potato slop is a high-temperature effluent, which eliminates the need of its cooling down when thermo- and mesophilic bacterial populations are involved. Besides, we may expect that the exothermic character of the biodegradation process will enable self-stable temperature maintenance under industrial conditions owing to the large volume of the bioreactor, as demonstrated in the literature referring to other industrial effluents [4, 10].

The objective of our study reported on in the present paper was to establish the conditions for the aerobic biodegradation of potato slops with a mixed population of thermo- and mesophilic bacteria of the genus *Bacillus*.

MATERIALS AND METHODS

Microorganisms

We used a mixed bacterial population, isolated from an industrial plant processing a variety of wastes from the food industry. Making use of standard methods, based on Bergey's key [7] and, additionally, on the API 50CHB tests [16], we identified the population as consisting of seven strains of the genus *Bacillus*. Two of these belonged to the species *B. circulans* and the other five to the species *B. laterosporus*, *B. filicolonicus, B. stearothermophilus, B. acidocaldarius* and *B. licheniformis*.

Biological activity was maintained throughout at 48±2°C in an aerated 0.5 l volume bioreactor at initial pH=7.0, with a medium as described in the Section Preparation of the medium. Every three days, a portion of the culture was inoculated on fresh medium. The volume of the inoculum amounted to 20 ml.

Preparation of the medium

The potato slops came from a potato-processing plant (Elipsa Ltd., Kąty Wrocławskie). Following filtration via a fluted filter paper (the composition of the liquid phase obtained is shown in Table 1), the slops were diluted 1:1 with distilled water. Dilution was performed in order to increase the volume of the medium coming from the same slop batch. In a previous study [12] we found that 1:1 dilution had basically no influence on the extent of COD reduction.

Table 1. Liquid phase of potato slops

Parameter	Unit	Value
pH	-	3.88
Density	^{o}Blg	7.9
COD	$g\ O_2 \cdot l^{-1}$	103.76
TOC	$g \cdot l^{-1}$	35.15
Lactic acid	$g \cdot l^{-1}$	17.531
Propionic acid	$g \cdot l^{-1}$	2.635
Acetic acid	$g \cdot l^{-1}$	2.101
Formic acid	$g \cdot l^{-1}$	0.270
Butyric acid	$g \cdot l^{-1}$	0.811
Isobutyric acid	$g \cdot l^{-1}$	0.101
Valeric acid	$g \cdot l^{-1}$	0.359
Succunic acid	$g \cdot l^{-1}$	0.430
Malic acid	$g\ l^{-1}$	0.154
Citric acid	$g \cdot l^{-1}$	0.070
Reducing substances	$g \cdot l^{-1}$	37.44
Glycerol	$g \cdot l^{-1}$	5.96
Total nitrogen	$g \cdot l^{-1}$	1.05
Ammonia nitrogen	$g \cdot l^{-1}$	0.308
Total phosphorus	$g \cdot l^{-1}$	0.277
Phosphate phosphorus	$g \cdot l^{-1}$	0.165

The diluted filtrate was boiled twice for 15 min. After each boiling procedure, the pH was adjusted to the level of 7.0 with 2 $mol \cdot l^{-1}$ NaOH and the precipitate was separated by filtration via a filter paper. Finally, the filtrate was made up with distilled water to the volume it had prior to the boiling procedure.

The potato slops prepared in this way were used as the medium for the bacteria stored in the aerated washer and for all the experiments performed in the course of the study, except the last one. In the last experiment, the slop used as the medium was not filtered after pH adjustment to the volume of 7.0. Whenever the programme of the experiments required the enrichment of the medium with ammonia nitrogen and phosphate phosphorus, use was made of $(NH_4)_2SO_4$ (2 $g \cdot l^{-1}$) and $(NH_4)_2HPO_4$ (1 $g \cdot l^{-1}$).

Parameters of the biodegradation process

A previous experimental study with shake-flask cultures and uncontrolled pH has shown that the optimal temperature and the optimal initial pH for the biodegradation process amounts to 45°C and 7.0, respectively [13]. And that is why our present experiments involved the same values. During biodegradation with controlled pH, the pH value was also kept at the constant level of 7.0, using 2 $mol \cdot l^{-1}$ H_2SO_4 and 2 $mol \cdot l^{-1}$ NaOH as neutralising agents. Shake-flask experiments (flask volume = 750 ml; volume of the medium=300 ml) were carried out at 150 rpm, whereas the processes conducted in the stirred-tank reactor (Biostat®B, B.Braun Biotech International, working volume = 5 l) involved an aeration rate of 1.6 vvm and a stirrer speed of 550 rpm. All experiments had a duration of 96 h.

Analytical methods

COD, BOD_5, TOC, total nitrogen, total phosphorus and phosphate phosphorus were determined spectrophotometrically, using Dr. Lange cuvette tests. In some of the samples, total nitrogen was also determined by the Kjeldahl method. Organic acids concentrations were measured using the HPLC method (Varian Pro Star, USA; column type: Aminex HPX-87H; column size: 7.8 mm i.d. x 300 mm; temperature: 65°C; UV detection). Glycerol content was determined by spectrophotometry [20, 22]. The content of reducing substances (mainly products of enzymatic decomposition of starch) was analysed by the Lane-Eynon method. The distillation method was used to determine ammonia nitrogen.

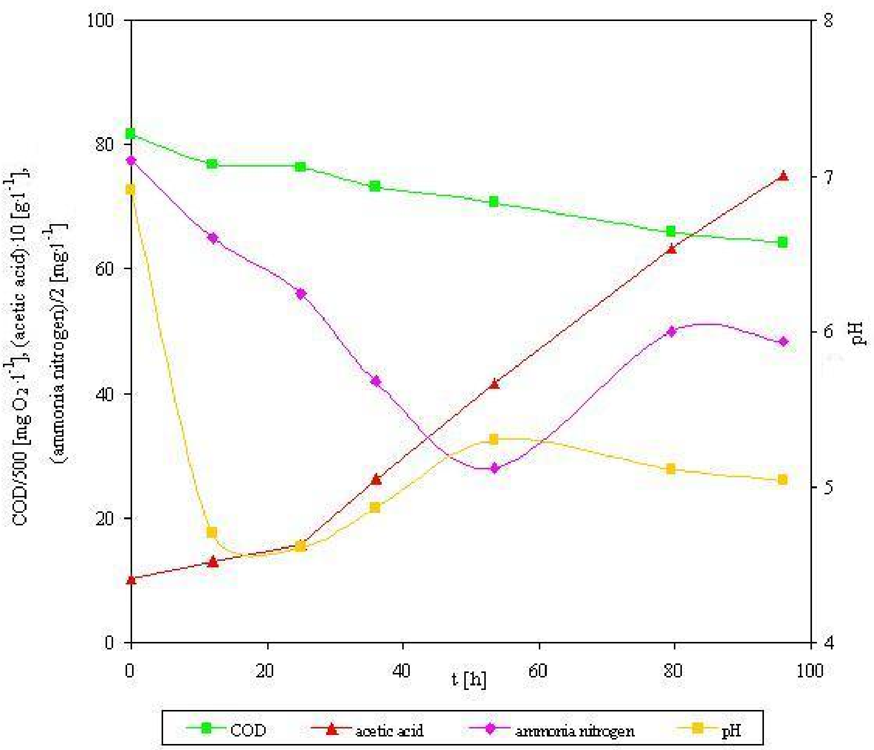
RESULTS

Biodegradation processes in shake-flasks

The efficiency of biodegradation in the shake-flasks was assessed in terms of the extent of COD and BOD_5 reduction, as well as in terms of the removal of reducing substances, organic acids and glycerol. The process itself was carried out with three variations of the pH: uncontrolled pH; pH kept constant throughout the experiment, and controlled pH paralleled by the enrichment of the slops with ammonia nitrogen and phosphate phosphorus (Section Preparation of the medium). The slops had to be enriched because in the shake-flask experiment with the constant pH the medium was found to be deficient in ammonia nitrogen and displayed a poor phosphate phosphorus content (5.5 $mg \cdot l^{-1}$). Phosphate phosphorus was added because of the risk that the increase in the biomass promoted by the lack of ammonia nitrogen limitation might account for the occurrence of phosphate phosphorus limitation. The extent of COD reduction measured after biomass separation in the shake-flask experiment with the uncontrolled pH amounted to 21.3%. The other two shake-flask experiments yielded COD reduction of 29.3% and 26.2%, respectively.

Figure 1 depicts the variations in of four major parameters of the biodegradation process conducted in a shake-flask at uncontrolled pH, with no addition of nitrogen and phosphorus. As shown by these plots, the low extent of COD reduction results primarily from the synthesis of acetic acid, which was also found to occur in the other two shake-flask experiments. According to Chu et al. [5], microaerobic conditions stimulate thermophilic bacteria to synthesise acetic acid from lactic, propionic, butyric, isobutyric and valeric acids, as well as from glucose, i.e. from the majority of carbon sources contained in the slops. The synthesis of acetic acid and the lack of increase in the extent of COD reduction (in spite of the slop enrichment with ammonia nitrogen and phosphate phosphorus) indicate that the biodegradation process in the shake-flasks may have been limited primarily by oxygen deficit. For this reason, further investigations were carried out in a stirred-tank bioreactor.

Fig. 1. Major parameters of biodegradation in shake flask at uncontrolled pH (initial pH=7.0), with no $N\text{-}NH_4$ and $P\text{-}PO_4$ enrichment



Biodegradation processes in the stirred-tank bioreactor

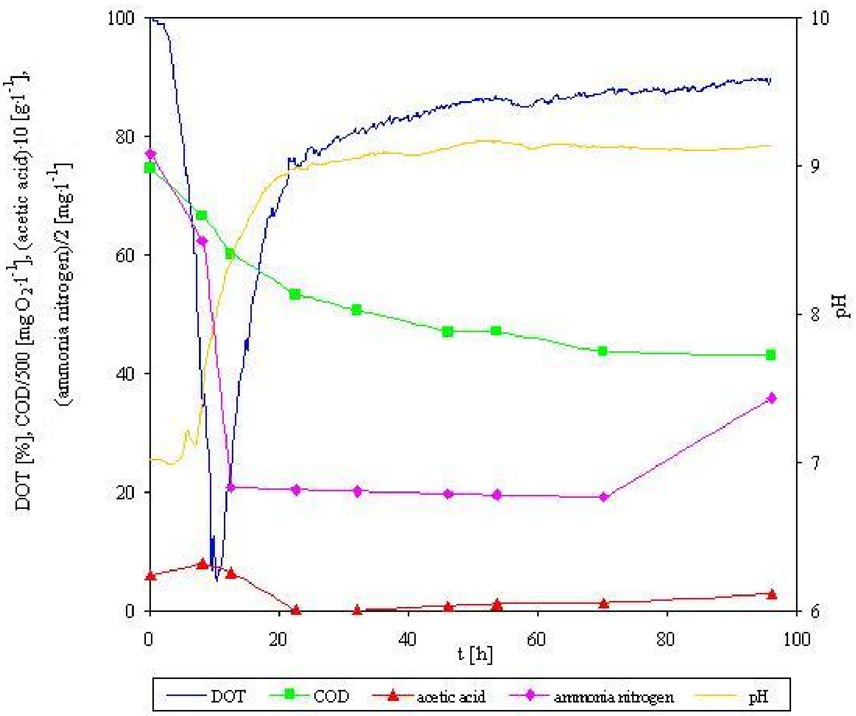
In each of the five experiments, biodegradation was assessed in terms of the extent of COD and BOD_5 reduction, as well as in terms of the removal of reducing substances, organic acids and glycerol, which are the major carbon sources for the microorganisms involved.

The first experiment in the stirred-tank bioreactor was carried out for the biodegradation of potato slops with no addition of ammonia nitrogen and phosphate phosphorus, and with no pH control. The extent of COD reduction obtained under such conditions amounted to 42.8% and was significantly higher than that achieved in the shake-flask experiments. As it can be inferred from the data in Table 2, this should be attributed primarily to the greater reduction in the content of organic acids in the medium, since the extent of glycerol reduction increased only slightly, from 84.9% to 93.7%, and the extent in the reduction of the reducing substances showed even a slight decrease, as compared to the biodegradation process conducted in the shake-flasks under the same conditions. From the analysis of the plots for acetic acid in Figs. 1 and 2 we can conclude that the increased reduction in the content of organic acids during biodegradation in the stirred-tank bioreactor is due to the lack of acetic acid synthesis. The plots in Fig. 2 show that biodegradation in the bioreactor has not been limited either by oxygen or ammonia nitrogen. Analysis of the behaviour of phosphate phosphorus (not presented in Fig. 2) has revealed that this compound has occurred in the slops also in excess amounts. A characteristic feature of this experiment is a rapid rise in the pH to values higher than 9, which suggests a notable contribution of this parameter to the inhibition of the biodegradation process. For this reason, the subsequent experiment was performed with pH 7, which was kept constant throughout.

Table 2. Efficiency of potato slops degradation related to process conditions

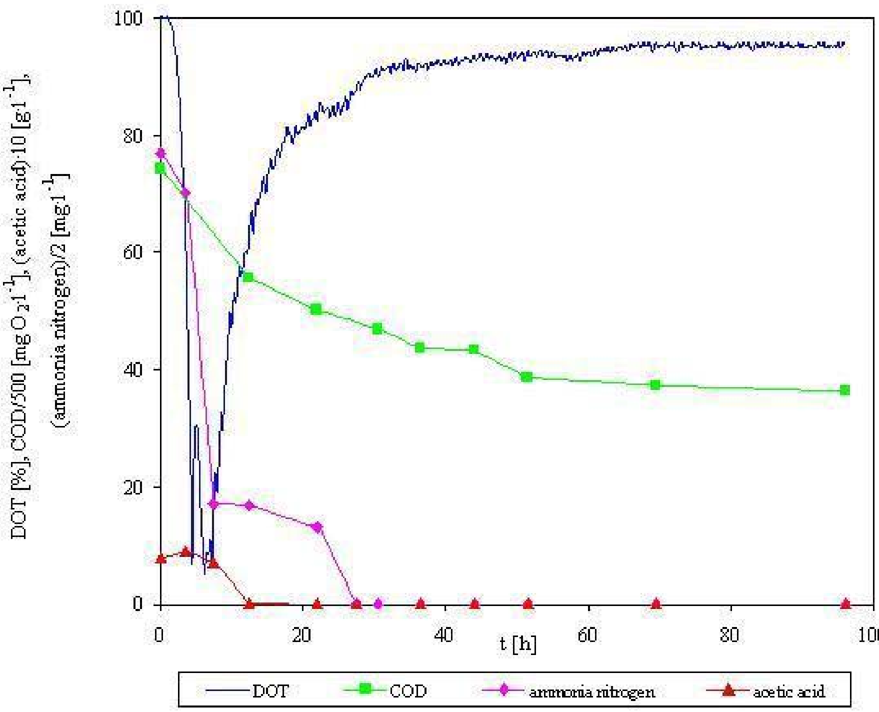
No. of experiment	Process conditions	Reduction in COD [%]	Reduction in BOD_5 [%]	Reduction in organic acids content [%]	Reduction in reducing substances content [%]	Reduction in glycerol content [%]
1	Shake-flask, no pH control, no $N\text{-}NH_4$ and $P\text{-}PO_4$ enrichment	21.3	16.4	increase in content	54.2	84.9
2	Bioreactor, no pH control, no $N\text{-}NH_4$ and $P\text{-}PO_4$ enrichment	42.8	49.7	70.4	50.8	93.7
3	Bioreactor, controlled pH, no $N\text{-}NH_4$ and $P\text{-}PO_4$ enrichment	51.0	52.0	79.0	51.0	100
4	Bioreactor, controlled pH, enrichment with $N\text{-}NH_4$ and $P\text{-}PO_4$	84.2	88.6	93.8	95.4	91.5
5	Bioreactor, no pH control, enrichment with $N\text{-}NH_4$ and $P\text{-}PO_4$	24.3	32.2	24.2	53.6	28.5
6	Bioreactor, controlled pH, enrichment with $N\text{-}NH_4$ and $P\text{-}PO_4$; slops not filtered after pH adjustment	83.1	87.9	98.4	92.6	89.5

Fig. 2. Major parameters of biodegradation in bioreactor at uncontrolled pH (initial pH=7.0), with no N-NH_4 and P-PO_4 enrichment



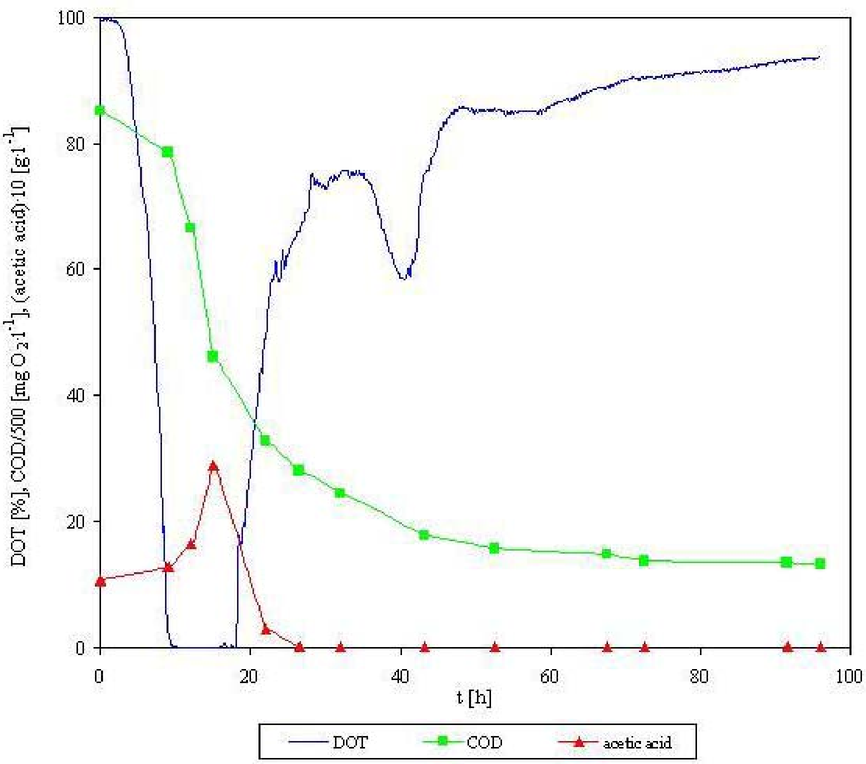
The application of pH control raised the extent of COD reduction to 51%. From the plots in Fig. 3 we understand that dissolved oxygen was present in excess throughout the process. This finding implies that dissolved oxygen cannot be viewed as a factor limiting biodegradation, but ammonia nitrogen can, because of its complete exhaustion only after 24 h.

Fig. 3. Major parameters of biodegradation in bioreactor at controlled pH (pH=7.0), with no N-NH_4 and P-PO_4 enrichment



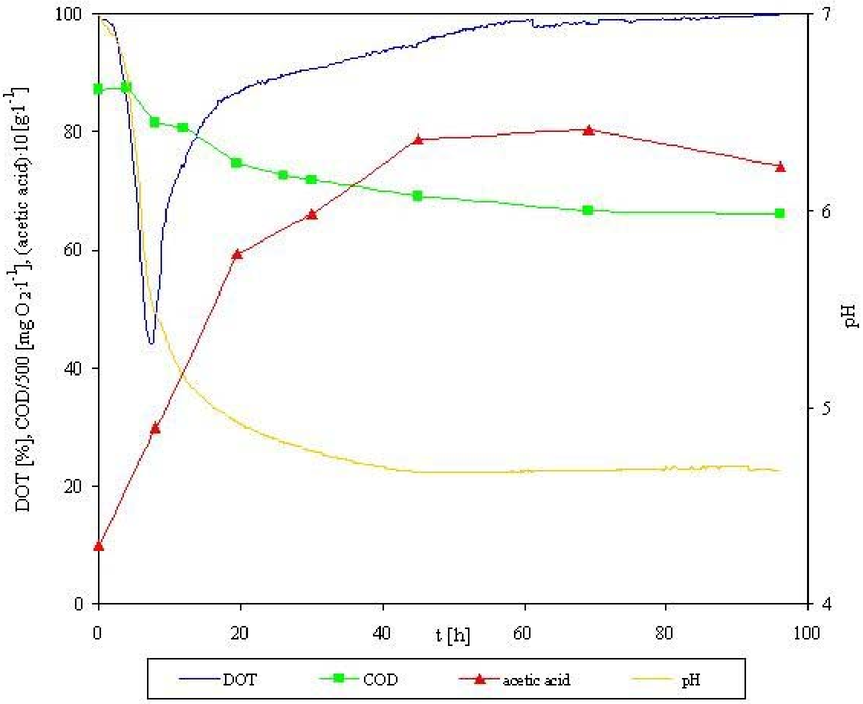
For these reasons, the third experiment in the bioreactor involved potato slops enriched with ammonia nitrogen and phosphate phosphorus. The latter was added, since it could be expected that this compound would become a limiting factor. As in the second experiment, the pH was kept constant at the level of 7.0. Under these conditions, the reduction in COD increased to 84.2%, while the extent of removal for organic acids, reducing substances and glycerol exceeded 90%. Over a period of several hours, the process suffered an oxygen deficiency (dissolved oxygen tension (DOT) = 0%), which manifested in the synthesis of acetic acid at the same time (Fig. 4). Before the termination of the process, or – to be more precise – after 24 h, the acetic acid was completely assimilated by the bacteria involved.

Fig. 4. Major parameters of biodegradation in bioreactor at controlled pH (pH=7.0); slops enriched with N-NH_4 and P-PO_4



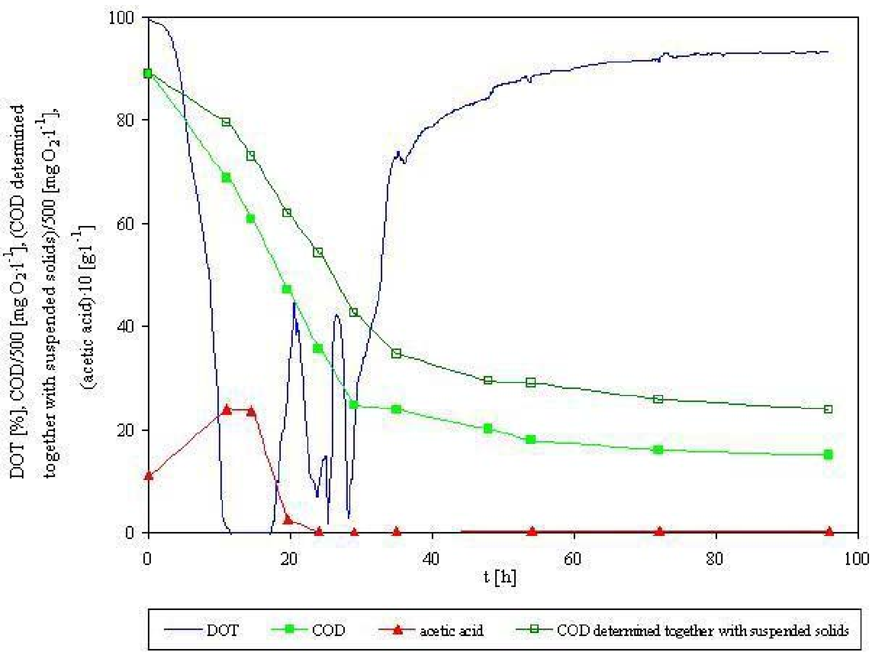
The fourth experiment was carried out for the biodegradation of the slops enriched with ammonia nitrogen and phosphate phosphorus under conditions of uncontrolled pH. The removal efficiencies were very low, amounting to 24.3%, 24.2%, 28.5% and 53.6% for COD, organic acids, glycerol and reducing substances, respectively. Such poor effects are likely to be due to the considerable drop in the pH, which fell below 5.5 after only 7 hours of the process (Fig. 5). Although no limitation of the dissolved oxygen content in the medium was found to occur, the process was characterised by a large amount of acetic acid produced, which rose from about 1.0 $g{\cdot}l^{-1}$ to about 8.0 $g{\cdot}l^{-1}$ and persisted till the end of the experiment with practically no variations (Fig. 5).

Fig. 5. Major parameters of biodegradation in bioreactor at uncontrolled pH (initial pH=7.0); slops enriched with $N\text{-}NH_4$ and $P\text{-}PO_4$



In the fifth experiment, biodegradation was performed with potato slops which had not been filtered following pH adjustment to the value of 7.0. Like the third experiment, this one was carried out with nitrogen and phosphorus enrichment, and with a controlled pH (at the level of 7.0). The plots for the two processes (Figs. 4 and 6) are similar in shape. The fifth experiment also had a period of several hours of oxygen deficiency paralleled by acetic acid production in amounts similar to those observed in the third experiment and completely utilised by the microorganisms after 24 h of the process. The extent of COD reduction totalled 83.1%, being only slightly lower than that obtained with the filtered slops. In the fifth experiment the extent of COD reduction was also determined for the slops with no separation of suspended solids (mainly biomass) prior to analysis. This value was 73.2%.

Fig. 6. Major parameters of biodegradation in bioreactor at controlled pH (pH=7.0); slops enriched with $N\text{-}NH_4$ and $P\text{-}PO_4$, not filtered after pH adjustment



Analysis of the behaviour of phosphate phosphorus indicates that if the slops had not been enriched with $(NH_4)_2HPO_4$ in experiments 3 and 5, there would have been a deficiency of this compound; in both experiments the maximal drop in the phosphate phosphorus content was much greater (about 100 to 110 $mg \cdot l^{-1}$) than was the initial content in the non-enriched medium (about 60 $mg \cdot l^{-1}$).

In spite of the over 80% COD reduction obtained in the biodegradation of the potato slops with the addition of mineral salts and with controlled pH, the final COD value approached 7 g $O_2 \cdot l^{-1}$. Considering the difference between the total nitrogen content determined by the Kjeldahl method and the ammonia nitrogen content, we calculated that approximately half the value had been contributed by proteins and free amino acids. Thus, if the investigated culture was enriched with bacteria of a greater capability to assimilate these compounds, it would be possible to attain an over 90% reduction in COD.

DISCUSSION

When the biodegradation of the slops was carried out with uncontrolled pH, this was concomitant with variations in this parameter. In the shake-flask experiment with the non-enriched slops, as well as in the bioreactor experiment with N-NH_4 and P-PO_4 enriched slops, the pH fell from 7.0 to about 5.0 and 4.7, respectively (Figs. 1 and 5). A decrease in the pH from 6.0 to about 3.5 during whey degradation in the shake-flasks at 45°C in the presence of a mixed bacterial population of the genus *Bacillus* has been reported by Kosseva et al. [11]. Fang *et al.* [9] have observed a drop in the pH after 24 h of sewage sludge composting, where use was made of thermophilic bacteria of the genus *Bacillus* (*B. brevis, B. coagulans, B. licheniformis*). In their opinion, such decrease should be attributed to the production of organic acids. In our experiments (both in the shake-flasks and in the bioreactor) we observed an enhanced production of acetic acid; its final content was found to be, respectively, 7 times and 7.5 times as high as its initial content.

When the potato slops were treated in the bioreactor with no addition of ammonium salts, the pH rose to higher values than 9 (Fig. 2). A rise in pH from 7.0 to about 9.6 during biodegradation of deproteined potato juice in the shake-flask experiments involving a mixed population of bacteria of the genus *Bacillus* has been reported by Lasik *et al.* [15]. Beaudet *et al.* [1], Cheubarn and Pagilla [3], Malladi and Ingham [17] have observed a similar pattern. In their study on aerobic treatment of potato-processing wastewater (where use was made of thermophilic bacteria of the genus *Bacillus* besides other thermophilic organisms), Malladi and Ingham suggested that the low pH at the initial stage of the process should be attributed to the growth of the culture, which coincided with the maximal increment in the biomass. The rise in the pH level at a further stage can be explained as being due to the assimilation of the substrates by the microorganisms, which was paralleled by the release of alkaline by-products. Tripathi and Allen [23] believe that the increase in the pH may be induced by the formation of alkaline compounds, or by the assimilation of organic acids (e.g. acetic acid) by the bacteria involved. A rise in the pH during aerobic thermophilic processes have also been observed by McIntosh and Oleszkiewicz [18]. In their opinion, the pH value increases owning to the formation of a weak ammonium base from the ammonia produced in the course of the deamination process.

CONCLUSIONS

1. There are two major requisites for an efficient aerobic biodegradation of potato slops with a mixed population of thermo- and mesophilic bacteria of the genus *Bacillus* – an appropriate aeration of the medium and the enrichment of the slops with a source of ammonia nitrogen and phosphate phosphorus.
2. Because of an insufficient buffer capacity, the pH of the slops during aerobic biodegradation should be kept at the optimal level, which is 7.0.
3. The precipitate produced during pH adjustment to the level of 7.0 does not affect the course of the biodegradation process.

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