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Thermo- and mesophilic aerobic batch biodegradation of high-strength distillery wastewater (potato stillage) – Utilisation of main carbon sources

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ABSTRACT

The aim of the study was to ascertain the extent to which temperature influences the utilisation of main carbon sources (reducing substances determined before and after hydrolysis, glycerol and organic acids) by a mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus* in the course of aerobic batch biodegradation of potato stillage, a high-strength distillery effluent (COD = 51.88 g O_2/l). The experiments were performed at 20, 30, 35, 40, 45, 50, 55, 60 and 63 °C, at pH 7, in a 5 l working volume stirred-tank bioreactor (Biostat®B, B. Braun Biotech International) with a stirrer speed of 550 rpm and aeration at 1.6 vvm. Particular consideration was given to the following issues: (1) the sequence in which the main carbon sources in the stillage were assimilated and (2) the extent of their assimilation achieved under these conditions.

1. Introduction

When high-strength wastewater is treated in the presence of microorganisms under aerobic thermophilic conditions, both organic and inorganic pollutants are transformed into environment-friendly by-products. The majority of the organic compounds are oxidised to carbon dioxide and water, and the heat produced during industrial-scale degradation in the bioreactors accounts for a rise in temperature to 50–70 °C (Kurisu et al., 2002). That is what enables the treatment processes to be conducted by the aerobic method with no temperature control (Kelly et al., 1993). The commonest treatment systems, however, work at ambient temperature (5–20 °C), and, not surprisingly, the effect of temperature on the kinetics of the process has only been examined over that temperature range (Droste and Sanchez, 1983). As yet, there is no satisfactory explanation regarding the effect of temperature on the metabolism of mesophilic and thermophilic bacteria that participate in wastewater treatment. Although there have been attempts to examine this phenomenon, they have concentrated primarily on the contribution of temperature to the efficiency of biodegradation achieved with high-strength effluents. In those studies use was made of both pure (Becker et al., 1999) and mixed bacterial cultures (LaPara and Alleman, 1999; Lim et al., 2001). The catabolic capacity of mixed bacterial cultures used for the biodegradation of wastewaters with a high chemical oxygen demand (COD) was examined by our research team (Cibis et al., 2002, 2006; Krzywonos et al., 2002, 2008), as well as by some of the researchers collaborating with our team under UE V Framework Programme, who are working on the bioremediation of cheese whey (Kosseva et al., 2001, 2003), and on the treatment of wastewater from potato processing (Lasik and Nowak, 2007). The literature contains only a few reports on the issue of how temperature contributes to the sequence in which the microorganisms assimilate the carbon sources that are found in the wastewater being biodegraded, or to the extent of assimilation achieved under this conditions (LaPara et al., 2000; Ugwuanyi et al., 2005a,b; Vogelaar et al., 2002). This is what prompted us to examine the influence of a wide range of temperature, from 20 to 63 °C, on the course of the biodegradation of potato stillage, a hot industrial effluent of a high COD, which in some instances exceeds 100 g/l (Cibis et al., 2002). Particular consideration was given to the sequence in which the main carbon sources in the stillage, i.e. reducing substances, glycerol and organic acids, were assimilated by the mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus* used in the present study. Such knowledge offers possibilities of enriching the mixed culture with other bacteria of *Bacillus* sp. that will utilize those substances at a faster rate. This will enhance not only the rate of biodegradation but also the efficiency of the process. The understanding of the mechanisms is a prerequisite not only for modelling the biodegradation process or computing the kinetic and dynamic process parameters, but also for ascertaining how this method of establishing the efficiency and the course of the biodegradation process compares with alternative methods.

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2. Methods

2.1. Microorganisms

The mixed bacterial culture used in the study was isolated from a food waste processing plant. After adaptation to the potato stillage (Cibis et al., 2002) the inoculum was stored at 48 ± 2 °C in a 0.5 l volume non-stirred bioreactor with aeration at 1vvm (volume of air/ (volume of medium × minute)), packed with potato stillage of an initial pH of 7.0. Every 72 h the biological material was inoculated (20 ml) onto fresh medium. The mixed culture of bacteria after adaptation to the potato slops was analysed and bacteria present in that population were then identified making use of standard methods and, additionally, on the API 50CHB tests (Cibis et al., 2006). We identified the population as consisting of the following seven strains of the genus *Bacillus: Bacillus laterosporus*, *Bacillus circulans* (two strains), *Bacillus filicolonicus*, *Bacillus stearothermophilus*, *Bacillus acidocaldarius* and *Bacillus licheniformis.*

2.2. Distillery wastewater

The potato stillage (Elipsa Ltd., Kąty Wrocławskie, Poland) was filtered through a filter paper. The liquid phase of the stillage was composed as follows (g/l): chemical oxygen demand (COD), 51.88; total organic carbon (TOC), 17.58; total nitrogen (TN), 0.525; ammonia nitrogen (N-NH_4), 0.154; total phosphorus (TP), 0.139; and phosphate phosphorus (P-PO_4), 0.083. The pH and density of the stillage was 3.88 and 3.95 °Blg, respectively. The composition of the main carbon sources was as follows (g/l): reducing substances determined before hydrolysis (RSBH), 12.85; reducing substances determined after hydrolysis (RSAH), 18.72; glycerol, 2.98; sum of all the organic acids determined, 12.24; lactic acid, 8.77; propionic acid, 1.318; acetic acid, 1.051; butyric acid, 0.410; malic acid, 0.215; formic acid, 0.135; valeric acid, 0.180; succinic acid, 0.077; isobutyric acid, 0.051; and citric acid, 0.035. After filtration stillage was boiled twice for 15 min. After each boiling procedure the pH was adjusted to 7.0 with 2 M NaOH and the precipitate was separated. After the filtrate was made up with distilled water to its initial volume, it was used as the medium for supporting the activity of the inoculum.

The medium to be used in the bioreactor was prepared in the same way and enriched with nitrogen and phosphorus in order to provide their excess in the substrate. The need to add N and P has been elucidated in a previous paper of ours (Cibis et al., 2004). The quantities of ammonium and phosphate salts added to the medium depended on the temperature applied. The sources for these biogens were 1 g/l portions of $(NH_4)_2HPO_4$ and 2 g/l portions of $(NH_4)_2SO_4$ added to the medium when the process was carried out at 30, 35, 40 and 45 °C. At 50 °C and higher temperatures, these portions were increased by 50%. At 20 °C (the lowest temperature applied) the addition of biogens was increased twofold.

2.3. Biodegradation

The processes were conducted in a 5 l working volume Biostat®B stirred-tank bioreactor (B. Braun Biotech International), with aeration at 1.6 vvm, a stirrer speed of 550 rpm, at the following temperatures: 20, 30, 35, 40, 45, 50, 55, 60, 63 °C. The bioreactor was inoculated with 200 ml of the medium prepared as described in Section 2.2. Each process had a duration of 125 h. Temperature, pH and dissolved oxygen tension (DOT) were measured using the sensors with which the bioreactor was equipped. The pH was kept at 7.0 automatically with 2 M H_2SO_4 and 2 M NaOH. The value of the pH adapted for the purpose of the present study comes from a previous paper of ours (Krzywonos et al., 2002)

Table 1
Major parameters characterising the biodegradation process.

Parameter	Removal (%)									
	20 °C	30 °C	35 °C	40 °C	45 °C	50 °C	55 °C	60 °C	63 °C
COD	84.10 ± 0.87	87.37 ± 0.68	89.14 ± 0.74	87.77 ± 0.85	85.40 ± 0.75	82.35 ± 0.96	77.57 ± 0.58	79.37 ± 0.42	80.37 ± 0.54
Reducing substances determined before hydrolysis	93.68 ± 0.92	90.01 ± 0.61	94.34 ± 0.93	93.92 ± 0.96	92.55 ± 0.81	92.58 ± 0.91	93.79 ± 0.61	93.54 ± 0.84	93.46 ± 0.71
Reducing substances determined after hydrolysis	94.61 ± 0.91	94.55 ± 0.73	95.98 ± 0.86	95.18 ± 0.92	95.37 ± 0.76	92.76 ± 0.94	84.32 ± 0.89	93.31 ± 0.76	89.86 ± 0.73
Glycerol	92.59 ± 0.84	94.82 ± 0.90	90.23 ± 0.93	95.94 ± 0.82	93.29 ± 0.70	93.36 ± 0.71	91.51 ± 0.84	95.56 ± 0.93	95.11 ± 0.88
Sum of organic acids	99.63 ± 0.96	97.79 ± 0.86	97.11 ± 0.88	98.89 ± 0.91	93.82 ± 0.81	96.55 ± 0.92	92.29 ± 0.87	91.70 ± 0.83	93.21 ± 0.85
Lactic acid	100 ± 0	100 ± 0	98.53 ± 0.95	100 ± 0	98.83 ± 0.94	100 ± 0	98.3 ± 0.93	100 ± 0	100 ± 0
Propionic acid	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	87.69 ± 0.87	100 ±0	100 ± 0
Acetic acid	100 ± 0	100 ± 0	99.18 ± 0.84	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Butyric acid	93.34 ± 0.88	98.09 ± 0.92	98.3 ± 0.95	100 ± 0	100 ± 0	100 ± 0	94.91 ± 0.91	34.98 ± 0.75	2.99 ±0.82
Malic acid	93.68 ± 0.86	100 ± 0	100 ± 0	100 ± 0	97.03 ± 0.75	79.07 ± 0.84	81.94 ± 0.83	57.07 ± 0.85	77.84 ± 0.78
Formic acid	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	100 ± 0	72.17 ± 0.74	100 ± 0	100 ± 0
Valeric acid	100 ± 0	100 ± 0	100 ± 0	100 ± 0	-151.74 ± 4.79	100 ± 0	100 ± 0	100 ± 0	100 ± 0
Succinic acid	100 ± 0	68.13 ± 0.77	64.41 ± 0.88	100 ± 0	100 ± 0	74.73 ± 0.94	62.51 ± 0.62	56.41 ± 0.69	64.73 ± 0.93
Isobutyric acid	100 ± 0	-2136.02 ± 53.35	-1130 ± 32.94	-645.93 ± 13.64	-125.93 ± 2.46	-303.48 ± 7.15	-1084.43 ± 31.06	-1642.61 ± 39.65	-277.63 ± 5.26
Citric acid	100 ± 0	71.32 ± 0.74	100 ± 0	100 ± 0	-203.57 ± 6.49	-357.73 ± 7.15	-488.7 ± 13.48	-1011.4 ± 27.2	-637.9 ± 16.25

Note: "-" before the number denotes that it was increment in value.