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Effect of temperature on the efficiency of the thermo- and mesophilic aerobic batch biodegradation of high-strength distillery wastewater (potato stillage)

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Abstract

The objective of the study was to assess the effect of temperature on the extent of aerobic batch biodegradation of potato stillage with a mixed culture of bacteria of the genus *Bacillus*. The experiments were performed in a 5-l stirred-tank reactor at 20, 30, 35, 40, 45, 50, 55, 60, 63 and 65 °C with the pH of 7. Only at 65 °C, no reduction in chemical oxygen demand (COD) was found to occur. Over the temperature range of 20–63 °C, the removal efficiency was very high (with an extent of COD reduction following solids separation that varied between 77.57% and 89.14% after 125 h). The process ran at the fastest rate when the temperature ranged from 30 to 45 °C; after 43 h at the latest, COD removal amounted to 90% of the final removal efficiency value obtained for the process. At 20, 55, 60 and 63 °C, a 90% removal was attained after 80 h. Two criteria were proposed for the identification of the point in time when the process is to terminate. One of these consists in maximising the product of the extent of COD reduction and the extent of N-NH_4 content reduction. The other criterion is a simplified one and involves the search for the minimal value of N-NH_4 concentration.

Keywords: Aerobic biodegradation; Temperature; Potato stillage; Batch process; *Bacillus*

1. Introduction

Recent years have witnessed a continuing worldwide increase in the production of ethanol (Cibis et al., 2006b). In 2005, global ethanol production approached 46 bn litres (Anon., 2006). Over 95% of that volume was produced by fermentation using starch-based (approximately 39%) and sugar-based (approximately 61%) substrates (Anon., 2007). The increase in the volume of the ethanol produced is paralleled by an increase in the quantity of the by-products released. Of these (when ethanol is obtained by fermentation), stillage represents the most severe hazard to the environment, owing to a very high COD (Cibis et al., 2002, 2006a; Krzywonos et al., 2002, 2008). It is essential to note that the volume of the stillage obtained exceeds more than 10-fold the volume of the ethanol produced (Wilkie et al., 2000). In 2005, for example, the total volume of the stillage generated on the global scale approached 500 bn litres. These quantities were too high to enable a complete utilisation of the stillage for conventional agricultural purposes (as a fodder or fertilising agent), which gave rise to the search for alternative modes. One of these includes biodegradation, a treatment process that seems to show great promise for the future (Krzywonos et al., 2008). Biological treatment involving anaerobic methods is already in use, particularly in India, where approximately 30% of the distilleries have been provided with the apparatus needed (Ranade et al., 1999). It is interesting to note, however, that in India cane molasses dominates as the substrate for alcohol production. But anaerobic methods are far less frequently applied to the biodegradation of starch-based stillage (so far, in 9 distilleries

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only) (Wilkie et al., 2000). In the recent past, encouraged by the positive results reported for the treatment of other high-strength wastewater (Chiang et al., 2001; Lim et al., 2001; Rozich and Bordacs, 2002; Lasik and Nowak, 2007), a number of investigators have directed their attention to laboratory-scale experiments involving the thermophilic aerobic method for the biodegradation of stillage (Cibis et al., 2002, 2006a; Krzywonos et al., 2002; Ferzik et al., 2004).

Thermophilic aerobic biodegradation of wastewater is an exothermic process with the inherent capability of temperature autostabilisation (Tripathi and Allen, 1999). According to literature data (Kelly et al., 1993), the temperature range stabilisable under industrial conditions varies from 20 to 70 °C. When the temperature stabilises at the value that guarantees the desired course of the biodegradation process, temperature control is not necessary, which considerably reduces the costs involved (Chiang et al., 2001; Rozich and Bordacs, 2002). The reports mentioned gave us encouragement to investigate the effect of temperature on the course and efficiency of the aerobic batch biodegradation of potato stillage using a mixed culture of thermo- and mesophilic bacteria of the genus *Bacillus*. The use of a mixed bacterial culture, which comprised strains differing in the optimal temperature of growth, corroborated our expectations that the results would be promising over a wide range of the temperature applied. In engineering applications, the biodegradation process would be immune to the influence of weather conditions, which eliminates the need for temperature control.

2. Methods

2.1. Microorganisms

The mixed bacterial population used in this study was isolated from a food waste processing plant. The microbial inoculum was stored at 48 ± 2 °C in a 0.5 l volume aerated, non-stirred bioreactor (Drechsler-type aerated bottle) 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 onto the fresh medium, the volume of the inoculum amounting to 20 ml. The used mixed culture of bacteria of the genus *Bacillus* consisted of the following strains: *B. laterosporus, B. circulans* (two strains), *B. filicolonicus, B. stearothermophilus, B. acidocaldarius* and *B. licheniformis*. The method for the identification of single strains has been described elsewhere (Cibis et al., 2006a).

2.2. Medium

The potato stillage (Elipsa Ltd., Kąty Wrocławskie, Poland) was filtered through a filter paper. The liquid phase of the medium 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. pH of the stillage was 3.88 and the density was 3.95 °Blg. After filtration, the potato 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 (Cibis et al., 2004). 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 2-fold.

2.3. Biodegradation

The processes were conducted in a 5 l working volume stirred-tank reactor (Biostat®B, B. Braun Biotech International), with aeration at 1.6 vvm, a stirrer speed of 550 rpm, and at the following temperatures: 20, 30, 35, 40, 45, 50, 55, 60, 63 and 65 °C. The bioreactor was inoculated with 200 ml of the medium stored at the above-mentioned Drechsler-type aerated bottle. Each process had a duration of 125 h. This means that such were the hydraulic retention time (HRT) and the sludge retention time (SRT) values. Temperature, pH and dissolved oxygen tension (DOT) were measured using the sensors incorporated in the bioreactor. The pH was kept at 7.0 automatically with 2 M H_2SO_4 and 2 M NaOH. The pH value adopted for the purpose of the present study comes from a previous paper of ours (Krzywonos et al., 2002). Liquid loss in the bioreactor in response to evaporation was made up with distilled water automatically.

2.4. Analytical methods

Bacterial cells (number of cells, NC) in the medium were counted with a haemocytometer. After the culture medium was centrifuged at 18,500*g* for 40 min, using a centrifuge of Sigma® 4K15 type, suspended solids (SS) were determined gravimetrically after having dried the sample at 50 °C for 24 h and then at 105 °C until a constant weight was obtained. The supernatant was used in the further analyses. COD, TOC, TN, TP and P-PO_4 were established spectrophotometrically using Dr. Lange cuvette tests (Anon., 2000). COD was measured both in the supernatant (SCOD) and in the non-centrifuged medium (TCOD). To measure N-NH_4 concentration, use was made of the distillation method with water vapour in the Parnas apparatus.