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Aerobic biodegradation of wheat stillage (distillery wastewater) at an elevated temperature—Effect of solids separation

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ABSTRACT

The aim of the study was to determine how the separation of solids affects the course and efficiency of the batch process of wheat stillage (distillery wastewater) biodegradation using meso- and thermophilic bacteria of the genus *Bacillus*. The processes with and without solids separation were conducted for 144 h in a 5-L bioreactor, with aeration at 1.6 vvm, stirrer revolutions of 550/min, at a constant pH (pH = 6.5) and the temperature of 45 °C. The results have shown that the separation of solids is superfluous, because it had only a minor effect on the reduction in the chemical oxygen demand determined in the substrate upon solids separation (SCOD). The extent of SCOD reduction amounted to 88.25% for non-filtered and 92.85% for filtered stillage. Moreover, during biodegradation of the non-filtered stillage the bacterial consortium was able *per se* to remove more than 50% of the suspended solids present in the stillage in the amount of approx. 50 g/L (the biomass produced being neglected).

1. Introduction

The persistent rise in the prices of fossil fuels has triggered the trend towards substituting them by renewable natural energy sources. Among those substitutes is ethanol, a product obtainable from vegetable feedstock. Admittedly, this gave rise to a remarkable worldwide increase in ethanol production at the turn of the 21st century [1]. Take for example the overall ethanol volume of 29.2 billion liters produced in the year 2000, which rose to 79.01 billion liters in 2008, thus becoming more than 2.7-fold as high as in 2000 [2]. Changes were also observed in the structure of the feedstocks used for the production of ethyl alcohol. Until recently, preference had been given to sugar-based feedstocks. In 2006, 53% of the world's alcohol production was based on starch feedstocks, which means that the use of sugar substrates accounted solely for 47% [3]. In Europe the main feedstock used for the production of ethanol as fuel additive is crops, and this includes starch-based substrates, *e.g.* wheat. The point is that the rise in the production of ethanol is concomitant with the increase in the quantity of stillage, which is a troublesome by-product, the more so as the volume of the stillage produced can be twenty times as high as that of ethanol [4].

A conventional method of utilizing wheat stillage because of its nutritional value includes its direct use as fodder without any processing [5,6]. This is, however, technically feasible only in the case of small-sized rural distilleries that are combined with farms, since liquid stillage fails to have a long shelf life [7]. In order to overcome that shortcoming, the stillage is made subject to drying, which substantially extends its durability and at the same time reduces the transport charges. It should be noted, however, that in the case of wheat stillage the drying cost largely assets the value of the dried product [8].

One of the available methods for the management of the generated stillage is disposal by classifying it as an industrial waste and making it subject to aerobic or anaerobic biodegradation, depending on the microorganisms applied. A comprehensive account of researches into biological biodegradation of stillage has been presented by Pant and Adholeya [9]. From the review, it can be inferred that the use of thermophilic processes for the biodegradation of distillery effluents has not been sufficiently considered. Aerobic biodegradation at elevated temperature has been used with success by some of the authors of the present paper for the treatment of other types of starch stillage [10–15]. In those studies, biodegradation was conducted solely for the liquid phase of the stillage, using filtration paper. The aim of the present study was to assess the effect of solids separation from wheat stillage on the course and efficiency of its biodegradation performed at elevated temperature (45 °C), using a mixed culture of bacteria of the genus *Bacillus*.

2. Materials and methods

2.1. Microorganisms

The microorganisms used in the experiments (a mixed culture of bacteria of the genus *Bacillus*) were obtained from an industrial

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Table 1
Composition of a wheat stillage used in the present study.

Parameter	Unit	Value
pH	–	3.61
Density	°Blg	7.7
Suspended solids	g/L	54.66
TCOD	g O_2/L	154.2
SCOD	g O_2/L	76.8
Lactic acid	g/L	15.7
Propionic acid	g/L	0.022
Acetic acid	g/L	0.600
Succinic acids	g/L	3.010
Reducing substances	g/L	23.43
Maltotriose	g/L	4.352
Maltose	g/L	1.145
Glucose	g/L	0.461
Fructose	g/L	1.937
Glycerol	g/L	3.95
Total nitrogen	g/L	2.192
Ammonia nitrogen	g/L	0.238
Total phosphorus	g/L	0.896
Phosphate phosphorus	g/L	0.583

plant (in the UK), which processes food waste under thermo- and mesophilic conditions. The activity of the mixed culture was maintained at 45 ± 2 °C in an aerated 0.5 L volume non-stirred bioreactor (aeration rate = 1 vvm), packed with wheat stillage of an initial pH of 6.5. Every 72 h the biological material in the bioreactor was inoculated into the fresh medium prepared as described in Section 2.2 (the volume of the inoculum being 20 mL). The bacteria present in that mixed culture were identified making use of standard methods and, additionally, of the API 50CHB tests [12]. The culture of bacteria included seven strains belonging to the following species of the genus *Bacillus*: *Bacillus laterosporus*, *Bacillus circulans* (2 strains), *Bacillus filicolonicus*, *Bacillus stearothermophilus*, *Bacillus acidocaldarius* and *Bacillus licheniformis*.

2.2. Medium preparation

The medium for supporting the activity of the mixed culture in the 0.5 L volume bioreactor was prepared in the following mode: The wheat stillage (its chemical composition is shown in Table 1) was passed through a filtration paper, and the filtrate was boiled twice for 15 min. After each boiling procedure the pH of the stillage was adjusted to the value of 6.5 with a 33% NaOH solution. Finally, the filtrate was made up with distilled water to the volume it had prior to the first boiling procedure.

When the stillage was made subject to biodegradation after the solid phase had been separated, the preparation procedure was the same as the one used for the maintenance of bacterial activity. When the stillage was biodegraded without solids separation as a prior step, the preparation procedure consisted only in adjusting its pH to the level of 6.5 with the 33% NaOH solution.

2.3. Biodegradation conditions

The stillage was subjected to a 144-h batch biodegradation process conducted in a 5-L working volume Biostat® B (B. Braun Biotech International) stirred-tank bioreactor (total volume of the bioreactor 6.6 L), with aeration at 1.6 vvm, a stirrer speed of 550 rpm, a temperature of 45 °C and a constant pH (pH = 6.5). The process conditions were chosen based on our previous studies [11,14]. Temperature, pH and dissolved oxygen tension (DOT) were measured using the sensors incorporated in the bioreactor. The inoculum consisted of 200 mL of a 72-h-old biological material from the bioreactor, where the activity of the mixed bacterial culture was maintained (Section 2.1). The pH was kept automatically with 2 M H_2SO_4 and 2 M NaOH. Liquid loss in the bioreactor in response to evaporation was made up with distilled water automatically.

Table 2
Effect of solid phase separation on the parameters of wheat stillage biodegradation.

Parameter	Filtrated stillage	Non-filtrated stillage
TCOD removal (%)	84.63	71.38
SCOD removal (%)	92.85	88.25
COD removal rate (g O_2/(L h))[a]	0.8678[b]	1.4003[b]
Final SCOD (g O_2/L)	5.14	8.65
Final TCOD (g O_2/L)	11.05	43.30
Final SCOD/Final TCOD	0.4652	0.1998
Final BCOD (g O_2/L)	5.91	34.65
Final BCOD/Final TCOD	0.5348	0.8002
SS removal (%)	−233.08	44.29
Y_{SS} (g SS produced/g SCOD removed)	0.0444	–
Y_{B1} (g final BCOD/g SCOD removed)	0.0885	–
Y_{B2} (g BCOD produced/g SCOD removed)	0.0647	–

[a] The value of this parameter was calculated for the point in time at which 90% of the overall reduction in COD was attained.
[b] COD removal rate was calculated using TCOD for non-filtered and SCOD for filtered stillage.

2.4. Analytical methods

Bacterial cells (number of cells = NC) in the medium were counted with a haemocytometer. It is essential to note that the NC was determined only during biodegradation of the filtered stillage. 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. Chemical oxygen demand (COD), total phosphorus and phosphate phosphorus were established with the aid of Dr Lange spectrophotometric cuvette tests [16]. To measure ammonia nitrogen concentration, use was made of the distillation method with water vapor in the Parnas apparatus. Total nitrogen was determined by the Kjeldahl method. Organic acids (lactic, acetic, propionic and succinic), carbohydrates (maltotriose, maltose, glucose and fructose) and glycerol concentrations were measured by HPLC (Varian Pro Star, USA; column type, Aminex HPX-87H, column size 7.8 mm i.d. × 300 mm, eluent 0.004 M H_2SO_4, flow rate 1.2 mL/min). The content of reducing substances was determined upon hydrolysis, making use of the Lane–Eynon method [17]. COD was measured both for the supernatant (soluble COD = SCOD) and for the medium, *i.e.* before solids separation (total COD = TCOD).

3. Results

The extent of SCOD reduction was high in both biodegradation processes, with 88.25 and 92.85% for non-filtered and filtered wheat stillage, respectively. The reduction in TCOD amounted to 71.38 and 84.63%, respectively (Table 2).

The rate at which the pollutants, expressed as COD, were removed from the stillage was determined for the point in time at which 90% of the overall reduction in COD was attained. Such method seemed to be best suited for the determination of this parameter because after approximately one-half of the anticipated process duration had passed, the reduction in COD was found to be insignificant (Fig. 1a and b). Thus, if the whole length of process duration were taken into account, this would lead to an unjustified underestimation of the removal rate value. It is also essential to add that determining the value of the removal rate in this study, the SCOD was adopted as a measure of the pollution load for the filtered stillage, and the TCOD for the non-filtered one. If the rate of