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Evaluation of anaerobic co-digestion of spent brewery grains and an azo dye

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ABSTRACT

Anaerobic batch biodegradation of spent brewery grains (SBG) was investigated in the presence of co-substrates and a monoazo dye (Acid Orange 7 – AO7) under mesophilic and thermophilic regimes. The highest values for the yield coefficient of biogas (STP) on substrate (Y_{bs}) were obtained under mesophilic conditions (0.381–0.516 L_{biogas}/g $COD_{removed}$ and 0.147 to 0.475 L_{biogas}/g $COD_{removed}$ for mesophilic and thermophilic regimes, respectively). A stimulation of the degradation of SBG associated with microbial growth was observed in the presence of co-substrates (glucose and acetate). Supplemented co-substrates also lowered the residual COD leading to an increase in the COD removal efficiency, particularly under thermophilic regime (from 41% to 70%). Although biogas yield (Y_{bs}) indicates a decrease in the presence of the dye, suggesting that it has inhibitory effects, the overall COD removal was not significantly altered. An increase of colour removal was observed when the temperature of the operation was increased (87 ± 2% and 93 ± 1% for mesophilic and thermophilic reactors, respectively), which could be explained by both faster adsorption and biotic reductive cleavage of azo dye bond mechanisms. These results indicate that raw SBG is more prone to biodegradation under an anaerobic mesophilic regime; hence its bio-energetic valorisation is possible.

1. Introduction

Agro-food industries produce large amounts of solid residues, such as spent brewer's grain (SBG), one of the main by-products of brewing industries (approximately 85%), which may have potential as a source of renewable energy. In addition the interest in SBG as a raw material has been increasing due to its low cost and high availability [1–3].

As a lignocellulosic material, SBG is composed primarily (on dry weight basis) of cellulose (16.8–25.4%), hemicellulose, particularly arabinoxylans (21.8–28.4%), lignin (11.9–27.8%) and protein (15.2–24.0%). Biological conversion of lignocellulosic compounds by enzymes and fungi has been exhaustively studied particularly for the production of bioethanol. Both cellulose and hemicellulose can undergo bioconversion by hydrolysis of polysaccharides into soluble sugars. Lignin has a heterogeneous chemical structure that is recalcitrant for most bacteria. However, a few lignin-degrading bacteria have been reported in the literature, making it suitable to be used as a substrate in biofuel production [3–8]. In this context, anaerobic digestion could play an important role converting lignocellulosic hydrolysates and fermentable sugars straight into biogas.

Mesophilic anaerobic bacteria are capable of producing extracellular enzymes named cellulosomes that degrade cellulose and hemicellulose (52%). Until now, although CH_4 and CO_2 generation resulting from anaerobic lignin depolymerisation has been observed, the process is still not well understood [9]. In general, it is assumed that anaerobic lignin degradation is slow and mainly related to low molecular weight compounds. However, in the presence of other co-substrates, the degradation of lignin by bacteria may be improved. Several aromatic compounds including aromatic amines and phenol are amenable to degradation by strict anaerobes. Because lignin is a polyphenolic macromolecule, delignification may also occur under anaerobic regime, even via anaerobic respiratory reactions using electron acceptors such as nitrate or sulphate.

SBG hydrolysis produces mainly sugars, phenolic compounds, proteins, fatty alcohols and acids. Although several SBG pretreatments have been tried to enhance the hydrolysis of complex substrates, results show that there are some additional problems associated with inhibition of anaerobic trophic groups by the resulting intermediates (benzene, phenol, cresol, ferulic acid,

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vanillin, gallic acid, etc.) [10]. Particularly, the accumulation of phenolic compounds due to their low rate of degradation could be, aside delignification, another drawback of lignocellulosic breakdown, because it could cause the inhibition of several anaerobic populations when present at high levels.

In terms of feeding used in anaerobic digestion, batch systems are easier to be operated than the continuous mode and have been widely used at full scale in biofuels production. Batch digestion systems at lab scale performed prior to the scale-up offer a cheap and preliminary via to evaluate the biodegradability of wastes simultaneously to their energetic valorisation. The identification of the critical process parameters could be properly done during this stage avoiding lengthy and costly tests. The results obtained provide useful information to be used at a full scale for both batch and continuous bioreactors in order to improve their efficiency (for instance, possible causes of process inhibition and how to overcome them, organic load to be used, yields, kinetics, reaction time and retention time, among others) [11].

Taking into account the above-mentioned considerations, the influence of the temperature and the presence of soluble co-substrates in anaerobic degradation of SBG were studied in batch mode at lab-scale. Innovative ways for value-added end-uses of SBG were also explored; therefore, in addition to its potential as a resource for biogas production, the co-remediation of xenobiotics (azo dyes) was assessed in the present work. It is well-known from the literature that azo dyes and their biotransformation products could be toxic to aquatic life and mutagenic to humans; therefore, investigating different strategies for degrading these pollutants, often considered recalcitrant, is still a matter of concern [12,13]. To date, neither the effect of direct carbon sources of acidogens and methanogens on the anaerobic degradation of SBG nor the co-remediation of azo dyes and SBG have been reported.

2. Materials and methods

2.1. Raw material

The raw spent brewery grains (SBG) used in the experiments are 2–4 mm in size. The SBG composition is approximately as follows (% dry weight, w/w): cellulose – 17, hemicellulose – 50.2, lignin – 16.9, extractives (in ethanol/toluene) – 9.5 and ashes – 4.6 [14]. Prior to be stored and used, SBG were dried at 60 °C for 12 h to reach a moisture content under 10%. These were kindly supplied by a brewery in the Estremadura (Portugal) region.

2.2. Anaerobic biomass

Anaerobic biomass was collected from mesophilic (37 ± 2 °C) and thermophilic (55 ± 2 °C) batch lab-scale reactors, fed with synthetic wastewater containing glucose (1.5 g/L) as the sole carbon source. Mesophilic anaerobic seed sludge had a VSS content ranging from 7.2 to 12.0 g/L and thermophilic inoculum of 15.5 to 28.4 g/L. As both cultures were grown with glucose three main types of anaerobic microorganisms should be present, namely hydrolytic fermentative (acidogenic) bacteria, syntrophic acetogenic bacteria and methanogenic *archaea*. Mixed cultures have the advantage over pure cultures of producing diverse enzymes; therefore an increase of the degradation rate of complex substrates is expected to occur.

2.3. Feed solutions

The feed solutions for inocula development were all based on a mineral medium containing macro and micronutrients [15]. For biodegradation tests, the mineral medium was prepared in a phosphate buffer solution (8.0 g/L Na_2HPO_4 and 2 g/L NaH_2PO_4). Phosphate was selected instead of bicarbonate to minimise interferences in the biogas production measurements.

The addition of co-substrates to the basal medium aimed to increase the biodegradation of raw SBG and the yield of biogas on the substrate. Glucose, a monomer of cellulose, was used as a direct substrate of fermentative acidogens to enhance their concentration. Sodium acetate was supplemented to increase the activity of syntrophic acetate-oxidising bacteria and methanogenic *archaea* so an improvement of acidogenesis and methanogenesis steps in SBG biodegradation tests was expected. Both carbon sources are present in a broad range of industrial wastewaters.

2.4. Dye

Azo dye Acid Orange 7 (*AO7*) was purchased from Sigma–Aldrich (Germany) and was supplemented in the feed medium at a concentration of 50 mg AO7/L (Fig. 1). The xenobiotic Acid Orange 7 was selected for this study, as azo dyes are common in several types of industrial wastewaters, produced namely by the textile, food and cosmetics industries, by the manufacturing of pharmaceutical drugs and personal care products, and its biodegradation was followed.

2.5. Analyses

Colour was measured spectrophotometrically with a Helios Alpha spectrophotometer (Unicam, UK) at the maximum visible absorbance wavelength of the dye (482 nm for AO7). Absorbance at this wavelength correlated with dye concentration was used to quantify decolourisation.

Acid precipitable polymeric lignin (APPL) was quantified in the supernatant liquor after acidification with 72% H_2SO_4 (w/w) and correction for the acid insoluble ash and protein [16,17]. Protein content was estimated by the Kjeldahl method [17], using the N × 6.25 and N × 4.2 conversion factors for SBG and anaerobic microorganisms, respectively.

Chemical oxygen demand (COD), suspended solids (SS), volatile suspended solids (VSS), ash and pH of the samples were determined according to standard procedures [18]. All analyses were carried out in duplicate.

2.6. Anaerobic activity and anaerobic biodegradation assays

Anaerobic biodegradability was assessed for raw SBG (used as a control) and SBG with medium augmentation (glucose or sodium acetate as co-substrates) under mesophilic and thermophilic regimes. Experiments were also run in the presence of an azo dye.

The specific biomass activity (SBA) and anaerobic biodegradation tests were performed in duplicate or triplicate in 250 mL bottles sealed with rubber septa. After washing with phosphate buffer solution (1.28 g/L Na_2HPO_4 and 0.42 g/L NaH_2PO_4) to

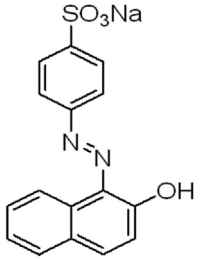

Fig. 1. Chemical structure of dye Acid Orange 7.