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Application of microbial electrolysis cells to treat spent yeast from an alcoholic fermentation

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HIGHLIGHTS

- Electron recovery from spent yeast was improved by ethanol addition in a MEC.
- Current density and hydrogen production increased with higher organic loads.
- Spent yeast treatment was mostly fermentative explaining the drift of electrons.
- Ethanol and experimental conditions could have induced spent yeast autolysis.
- This is the first study, as far as we know, of spent yeast treated in a MEC.

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ABSTRACT

Spent yeast (SY), a major challenge for the brewing industry, was treated using a microbial electrolysis cell to recover energy. Concentrations of SY from bench alcoholic fermentation and ethanol were tested, ranging from 750 to 1500 mg COD/L and 0 to 2400 mg COD/L respectively. COD removal efficiency (RE), coulombic efficiency (CE), coulombic recovery (CR), hydrogen production and current density were evaluated. The best treatment condition was 750 mg COD/L SY + 1200 mg COD/L ethanol giving higher COD RE, CE, CR (90 ± 1%, 90 ± 2% and 81 ± 1% respectively), as compared with 1500 mg COD/L SY (76 ± 2%, 63 ± 7% and 48 ± 4% respectively); ethanol addition was significantly favorable (p value = 0.011), possibly due to electron availability and SY autolysis. 1500 mg COD/L SY + 1200 mg COD/L ethanol achieved higher current density (222.0 ± 31.3 A/m^3) and hydrogen production (2.18 ± 0.66 L_{H_2}/day/$L_{Reactor}$) but with lower efficiencies (87 ± 2% COD RE, 71.0±.4% CE). Future work should focus on electron sinks, acclimation and optimizing SY breakdown.

1. Introduction

The brewing industry generates significant volumes of by-products and solid waste, the disposal and management of which represent important operational costs and environmental challenges. Approximately 1.5–3% of the total volume of beer produced corresponds to spent yeast (SY) (Fillaudeau et al., 2006) which is considered the second major by-product from breweries (Huige, 2006). Before disposal, brewer's SY requires an inactivation treatment, which can be energy intensive as it requires the addition of toxic substances and heating at high temperatures. It is difficult to treat SY as a liquid waste since it increases the biological oxygen demand (BOD) of any body of water where it is released, and due to the yeast cell bodies and to the residual ethanol from fermentation, it is considered a pollutant (Doubla et al., 2007).

SY is recovered almost at the end of the brewing process and only a fraction of it can be reused (Olajire, 2012). This by-product includes yeast solids, beer solids, soluble ethanol, and sediment of hops and particles of grains (Rocha et al., 2014) and it has a high content of protein, vitamins and amino acids (Mussato, 2009). After a drying treatment, a small fraction of SY is commercially used for animal feed or nutritional supplement (Fillaudeau et al., 2006; Mussato, 2009), while the fraction that is not used is currently disposed of in landfills or, less frequently, directed to anaerobic digestion treatment plants (Neira and Jeison, 2010).

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There are only a few publications reporting how SY has been treated to obtain methane through anaerobic digestion; the best results having been obtained when it was co-digested (Bocher et al., 2008; Neira and Jeison, 2010; Zupančič et al., 2012). Using a pretreatment neither improved methane production, nor the speed of SY biodegradation, that requires long retention times (Neira and Jeison, 2010).

When high volumes of SY are produced, alternative uses *in situ* have to be explored, because the costs of transport and storage could become significant. Before inactivation, SY can contain both viable and dead cells (Bokulich et al., 2013), leading to high variability in its composition. In addition, a fraction of the SY cells undergo autolysis after the brewing process, leading to the release of internal cellular contents (Steckley et al., 1979), while the viable yeast cells can still perform their metabolic processes, consuming some of the residual carbohydrates in the media and releasing products like ethanol. These SY characteristics could be advantageous for the degradation process that needs to occur in a microbial electrolysis cell (MEC).

MEC is a developing technology that can allow hydrogen production from organic matter degradation (Logan et al., 2008). In these systems, electrochemically active bacteria can oxidize organic matter and generate CO_2, electrons and protons. These electrons are transferred to an electrode by a specific group of bacteria called anode respiring bacteria (ARB) which can only oxidize specific simple products such as acetate or hydrogen. The electrons travel through a circuit to the cathode where, with a minimum added voltage, hydrogen gas is produced. Fermentation of complex substrates is also possible in an MEC using a chain of microbial anaerobic reactions (Parameswaran et al., 2009) to break those compounds down and generate usable products, obtaining an added value from complex organic wastes (Rittmann, 2008). One of the products thus generated is hydrogen, which is considered a profitable, clean, sustainable and renewable fuel (Kadier et al., 2014). Thus, there is a growing interest in using this technology.

So far, there are no reports about bio-electrochemical systems that include MECs being used to treat brewer's SY. Some authors have reported the use of brewery waste water as a substrate for microbial fuel cells (MFC) (Pant et al., 2010; Wen et al., 2010), finding that the buffering capacity, temperature, and organic load have an important effect on the performance of the process (Feng et al., 2008). Ethanol is one of the main components of the brewery wastes and it has been used as a sole electron donor in MECs to establish a mass and electron balance for the process and to confirm methanogenesis as an important sink of electrons (Parameswaran et al., 2009); this was also confirmed in a pilot-scale MEC using winery waste water, the main component of which was ethanol (Cusick et al., 2011). Since fermentation of complex substrates can occur simultaneously in MEC (Parameswaran et al., 2009), when using mixed cultures (Kadier et al., 2014), an effective acclimation of the inoculum to the new substrate is needed for the best performance of the process. Other factors affecting MEC anode performance fed with complex wastes include organic loading rate and the availability of simple electron donors, such as ethanol or acetate for the ARB rapid utilization (Feng et al., 2008).

The objective of this work was to use MECs to treat SY from a bench scale alcoholic fermentation, and to evaluate the effect of SY and ethanol concentration on MEC performance. The performance of this treatment was evaluated in terms of the organic matter removed (COD) as removal efficiency (COD RE), coulombic efficiency (CE), coulombic recovery (CR), maximum current density (A/m^2 or A/m^3, current normalized to the anode active surface and the current normalized to the anode effective volume respectively) and the rate of hydrogen production (H_2 in $L_{H_2}/day/L_{Reactor}$ or mol/day). This is the first study, as far as we know, that considers the application of MEC for SY treatment.

2. Methodology

2.1. Bench scale alcoholic fermentation

In order to produce a consistent SY sample, a bench scale alcoholic fermentation was performed. Malt extract at 75 g/L (CBW® Pilsen, Briess Malt & Ingredients Co. Chilton, WI, USA) was added to previously boiled water. Once the solution was cold, brewer's yeast (*Saccharomyces cerevisiae*) (0.57 g/L of Safale S-04, Fermentis, France) was added and mixed in a clean container that was closed with an air-lock. The fermentation was carried out at room temperature (23 ± 2 °C) for 2 weeks. At the end of the process, solids were recovered by centrifuging at 3600 rpm for 10 min. The recovered fraction was then diluted in 200 mL of distilled water and stored at 4 °C.

2.2. Chemical characterization of SY

The chemical characterization of the SY was done immediately after the sample was collected and two weeks after that, immediately before the MEC experimental setup, to avoid uncertainty from composition changes during storage. This characterization allowed to monitor the sample stability during storage and to obtain evidence of yeast activity. The characterization included the quantification of total chemical oxygen demand (TCOD), that was measured initially and at the end of each experiment, using HACH procedure and spectrophotometer DR 2010 (HACH, Ames, IA, USA). Total suspended solids (TSS) and volatile suspended solids (VSS) were measured according to Standard Methods (APHA, 1998). A colorimetric method was used to determine carbohydrate concentration (DuBois et al., 1956) that involved placing 2 mL of sample in a 15 mL culture tube, followed by the addition of 50 μL of 80% phenol solution (w/w) and consecutively 5 mL of 95.5% sulfuric acid. The mixture was incubated for 30 min at room temperature. The specific absorbance at a wavelength of 485 nm was measured with a spectrophotometer, using a previously developed standard calibration curve with glucose. The Lowry method (Lowry et al., 1951) was used to determine proteins concentration. A standard curve with bovine serum albumin was used, with a specific absorbance recorded at 562 nm wavelength. Ammonia ($N\text{-}NH_3$ in mg/L) concentration was determined using the Nessler-HACH method and spectrophotometer DR 2010 (HACH, Ames, IA, USA). The pH was measured using a potentiometer (Orion Star A111, Thermo Scientific, Waltham, MA, USA) and the volatile fatty acids (VFA) and ethanol composition was determined using a gas chromatograph (Model Varian 3300) equipped with a FID detector according to (Buitrón and Carvajal, 2010) and pretreating the samples by filtration (glass filter 0.45 μm, PVDF GD/X, Whatman, GE Healthcare, Ann Arbor, MI, USA) and by acidification with 1 μL of HCl (2 M). The VFAs determined were acetate, butyrate, isobutyrate, iso-valerate and propionate.

2.3. Configuration and MEC operation

H-type MEC reactors (fabricated by Adams & Chittenden Scientific Glass, Berkeley, CA, USA) were used for all the experiments (Fig. 1), each chamber had an effective volume of 310 mL. The anodes were made of brushes of graphite fiber (fabricated by Millrose, Mentor, OH, USA) 9 cm long and 6.5 cm diameter, with approximately 160,000 fibers (Panex 35, Zoltec) and mounted to a titanium wire. To increase the oxidation sites the brushes were