Valorization of brewery wastewater by hydrogen and VFAs production through dark fermentation and bio-electrochemical systems.

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Introduction

Hydrogen has been recognized as an energy carrier of the future with a high energy content and non-polluting emissions (Keskin et al., 2012). It is usually produced from fossil fuels using various methods. However, it can also be produced through biological systems such as photofermentation and dark fermentation (Al-Mohammedawi et al., 2020), where by-products are used as substrates. Among them, we can mention different types of sludge from wastewater treatment plants, from sugar industry to beverage industry, chemical industry, distillery industry, and so on (Boodhun et al., 2017). However, there are just a few applications with brewery wastewater (BW) on hydrogen generation (Pachiega et al., 2019). One possible method for managing BW is treatment via dark fermentation (Sinbuathong et al., 2015). In this process, bio-H_2 is produced by the decomposition of organic matter through a series of metabolic interactions between different groups of microorganisms. Hydrogen-producing bacteria hydrolyze complex macromolecules (carbohydrates) into simpler organic compounds (e.g., volatile fatty acids, VFAs), producing H_2 and CO_2 (Garduno et al., 2023). VFAs will be used in the second phase of the project. They will be the substrate for the growth of microorganisms in the anode compartment of a bio-electrochemical reactor. Electrons derived from the oxidation of VFAs in anaerobic conditions are transferred to one electrode (the anode) and flow through a circuit to reach another electrode (the cathode) where they will be used by purple non-sulfur bacteria (PNSB) to produce H_2 in a bio-photocathode, also adsorbing initial emitted CO_2.

Materials and methods

Brewery wastewater (BW) was collected from two local brewery production plants in Radda in Chianti (Siena, Italy) and Certaldo (Florence, Italy). Table 1 shows the characteristics of the BWs used in this study.

	BW 1 (Radda in Chianti)	BW 2 (Certaldo)	BW 3 (Certaldo)
pH	4,50	4,70	4,97
Total solids (g/L)	1,05	41,40	36,12
Carbohydrates (g/L)	41,18	36,77	41,23
Lactic Acid (g/L)	0,352	0,212	0,359
Butyric Acid (g/L)	--	---	---
Propionic Acid (g/L)	0,621	1,043	0,995
Acetic Acid (g/L)	0,334	0,709	0,732
Total heterotrophs (CFU/ml)	$2{,}26*10^7$	$3{,}48*10^8$	$1{,}51*10^{10}$
Lactic acid bacteria (CFU/ml)	$3{,}5*10^4$	$8{,}88*10^7$	$7{,}70*10^8$

Table 1. Chemical and microbiological characterization of brewery by-products. BW 1 is the wash water of the brewers, at the end of fermentation. BW 2 and BW 3 are the yeast-containing wort from the end of fermentation.

The effect of BW concentration on gas and organic acid production was evaluated. The objective was to optimize H_2 and VFAs formation. Three BW concentrations were assessed: “tal quale” (tq, 100% BW), 1:2, and 1:4. Autoclaved mineral water was used for dilution. All media and bottles were autoclaved (121°C, 15 atm for 20 minutes) before inoculation. Triplicates were performed with 100 mL working volume in 120 mL Serum bottles tightly closed with a hollow cap and self-sealing rubber stopper. This allowed the insertion of syringes to measure the gas volume in the headspace (water displacement method) and to sample the medium without opening the bottle to measure carbohydrates and organic acids concentrations. Serum bottles were incubated at 35°C for at least 96h (or until no gas was detected) with orbital shaking (35 rpm).

The total solids content (TS) was determined by oven drying at 105 ◦C. Carbohydrate concentration was determined by calorimetry following Dubois' (1956) method. VFAs (Lactic acid, Butyric acid, Propionic acid and Acetic acid) were quantified by high-performance liquid chromatography (HPLC). The pH of the samples was measured using a pH meter. MRS and LB agarified media were used to determine the concentration of lactic acid bacteria and total heterotrophs, respectively. To measure the production of H_2 versus all other gases, 2M NaOH was added to the columns used for the water displacement method.

Results and discussion

Dark fermentation of BWs with approximately 40 g/L of carbohydrates was evaluated in the present study in terms of H_2 and VFAs production.

BW 3 yielded the best results. With this by-product, 2.14 g/L acetic acid, 7.54 g/L propionic acid, and 0.89 g/L lactic acid were obtained at the end of the fermentation (2 weeks) with tq (100% BW) (Fig. 1). The total gas produced was 1035.8 mL/L of solution for tq, 724.72 mL/L for the 1:2 dilution, and 363.92 mL/L for the 1:4 dilution (Fig. 2). The percentage of H_2 was 70 % for tq and 90 % for 1:2 and 1:4 dilutions. Carbohydrate content decreased by 32% in tq after two weeks of fermentation to a final concentration of 28.1 g/L.

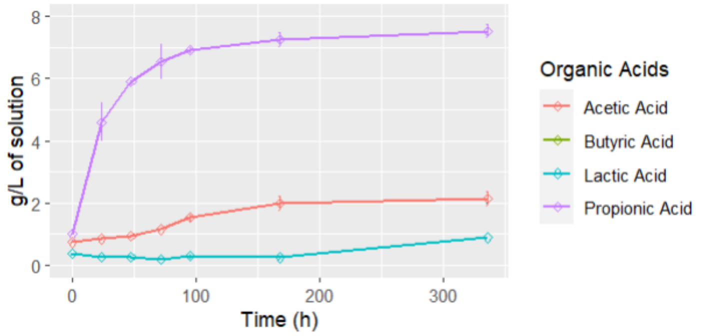

Figure 1. Production of organic acids (g/L of solution) during the dark fermentation phase (2 weeks)

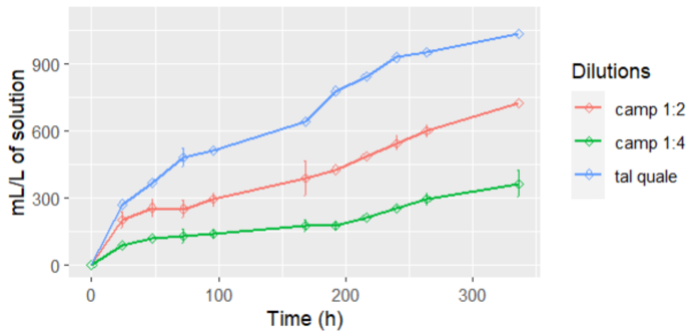

Figure 2. Gas production (mL/L of solution) during the dark fermentation phase (2 weeks).

Research on hydrogen production from BWs is scarce and the existing literature is difficult to compare. This is because BWs are usually highly diluted, supplemented with glucose or other molecules to achieve specific conditions, or fermented with an inoculum.

The results obtained in this study indicate the need to define the best experimental conditions to improve the production of H_2 and VFAs. Despite the high presence of lactic acid bacteria in the BWs, it is likely that they cannot compete with the large amount of yeast that is still present. Future experiments should consider centrifuging and freezing the BWs to remove all yeast and then fermenting it with a lactic acid bacterial inoculum.

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