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Fermentative bio-hydrogen production from galactose

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ABSTRACT

Bio-hydrogen production through fermentation of waste biomass has considerable benefits both as a waste treatment process and a substitute for fossil fuels. Galactose, which can be the dominant component in various biomass wastes (such as marine red algae, cheese and dairy industry waste streams) was fermented by anaerobic fermentative bacteria to assess bio-hydrogen production. The impacts of pH, the YE/G (yeast extract/galactose) ratio and substrate concentration were investigated and optimised by response surface methodology. Hydrogen production was mainly via acetic and butyric acid pathways, while hydrogen consumption was via caproic acid and homoacetogenesis pathways. The hydrogen yield and production rate were improved to 278.1 mL/g galactose (2.23 mol/mol galactose) and 33.6 mL/g galactose/h, respectively, under the optimal conditions (pH value of 6.05, YE/G ratio of 0.56 and substrate concentration of 5 g volatile solid/L). The overall energy conversion efficiency from substrates to hydrogen and soluble metabolic products reached 68.6%.

1. Introduction

World primary energy consumption in 2013 was 12,730 Mtoe (535 EJ); an increase of 28% compared to 2003. Fossil fuels were still the dominant energy source, accounting for 87% of the total primary consumption [1]. This increasing energy demand is unsustainable due to finite fossil fuel supply and the environmental impact of fossil fuel combustion [2–4].

Hydrogen is considered as a potential alternative option due to its clean combustion and high energy density by mass [2,5,6]. Currently, steam reforming of natural gas is the dominant hydrogen production process; this process is still associated with extensive consumption of fossil fuels. For example, approximately 95% of hydrogen consumed in the United States is produced via steam reforming of natural gas [7,8]. In contrast, fermentative bio-hydrogen production from biomass wastes, which combines waste treatment and renewable clean fuel production, is attracting increased attention [8–12].

The main biodegradable organic compounds in biomass wastes include carbohydrates, proteins and lipids [13,14]. Carbohydrates are the main compounds responsible for fermentative bio-hydrogen production. Carbohydrate monomers can theoretically produce bio-hydrogen yields of 4 mol/mol hexose and 10/3 mol/mol pentose [15,16]. Proteins are not advantageous for direct bio-hydrogen production, but they can provide the essential nitrogen sources to improve anaerobic fermentative bacteria (AFB) growth and biological activities [5]. Protein monomers (amino acids) can hardly produce hydrogen under common fermentation conditions [10]. Triacylglycerol, which is the main component of lipids, is composed of ca. 10% glycerol and 90% long chain fatty acids (LCFAs) [17,18]. Glycerol is a readily available substrate for bio-hydrogen production (theoretical bio-hydrogen yield: 3 mol/mol glycerol) [19], while LCFAs degrade poorly during bio-hydrogen fermentation. Fermentation of LCFAs to shorter chain equivalents is thermodynamically unfavourable and non-spontaneous (positive standard free energy), unless coupled with methanogenesis [20]. However, methanogenic communities are usually inhibited by inoculum pre-treatments or operational parameters in bio-hydrogen fermentation systems [21–23]. Therefore, biomass wastes, which are rich in carbohydrates with a small amount of

Abbreviation: AFB, anaerobic fermentative bacteria; ECE, energy conversion efficiency; LCFAs, long chain fatty acids; SMPs, soluble metabolic products; VFAs, volatile fatty acids.

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proteins and little or no lipids, are recommended for bio-hydrogen production.

Galactose is one of the most abundant carbohydrate monomers, which can be the dominant component in marine red algae, and cheese and dairy industry waste streams [24,25]. Galactose and glucose have the same chemical formula ($C_6H_{12}O_6$) and are epimers. For bio-hydrogen production, galactose must first be converted into glucose-1-phosphate via the Leloir pathway (which requires chemical energy input) and subsequently be metabolised to produce various soluble metabolic products (SMPs) and hydrogen [25]. Bio-hydrogen production from glucose has been well studied [26–28], however, studies on galactose are quite limited. A previous study showed the bio-hydrogen production rate and yield by *Clostridium beijerinckii* from glucose were 321% and 47% higher than those from galactose [29]. This suggests galactose is less favourable for bio-hydrogen production than glucose. The impacts of the key fermentation parameters of bio-hydrogen from galactose (such as pH and substrate concentration) need to be assessed and optimised; this would be very beneficial for the understanding of galactose fermentation process, and for improving the bio-hydrogen performances from galactose-based substrates (such as red seaweeds and dairy wastes). The objectives of this paper are to:

- Optimise bio-hydrogen production from galactose using RSM (response surface methodology);
- Assess bio-hydrogen production and consumption associated with metabolic pathways;
- Analyse energy and carbon production efficiencies of the fermentation process.

2. Materials and methods

2.1. Inoculum and media

The mixed AFB were separated and enriched from anaerobic granular sludge sourced from an upflow anaerobic sludge blanket reactor treating creamery waste in Cork, Ireland. The AFB sludge was heated at 100 °C for 30 min in an autoclave to inactivate the methanogenic community. The sludge was subsequently acclimatised for 3 runs (3 days for each run) to activate spore-forming AFB such as *Clostridium butyricum*.

The composition of the acclimation medium was adapted from a previous study [30] as follows: galactose, 20.0 g/L; tryptone, 3.0 g/L; yeast extract, 1.0 g/L; NaCl, 3.0 g/L; K_2HPO_4, 2.5 g/L; $FeCl_2$, 0.1 g/L; L-Cysteine, 0.5 g/L; vitamin liquid, 10.0 mL/L; and trace element liquid, 10.0 mL/L. The compositions of vitamin and trace element liquids were described in a previous study [30].

The composition of the basal medium for batch bio-hydrogen production tests was as follows: NaCl, 3.0 g/L; K_2HPO_4, 2.5 g/L; $FeCl_2$, 0.1 g/L; vitamin liquid, 10.0 mL/L; and trace element liquid, 10.0 mL/L.

2.2. Bio-hydrogen production via fermentation

Batch tests for fermentative bio-hydrogen production were conducted in the Bioprocess Control AMPST II system. The glass bottles had a total volume of 600 mL. To this was added 30 mL of acclimated AFB. Concentrations of galactose and yeast extract (as indicated in Section 2.4 and Table 1) were mixed with basal medium until the total volume reached 300 mL. The initial pH was adjusted to 5.0, 6.5 or 8.0 (Table 1) using 6 M NaOH or HCl solution. The bottles were sealed with rubber stoppers, flushed with nitrogen gas and placed in a water bath maintained at 37 °C for bio-hydrogen production. A mixing system was attached to each bottle at a speed of 60 rpm, switching between on and off for 1 min periods. The produced biogas was passed through 3 M NaOH solution to remove carbon dioxide, and the volume of bio-hydrogen was subsequently recorded by a gas tipping device [31]. The gas results were automatically normalised at 1.0 standard atmospheric pressure, 0 °C and zero moisture content by the Bioprocess Control AMPST II system.

2.3. Instrumental analysis

To obtain the dry samples, the test samples were placed in an oven at 105 °C for 24 h. The volatile solid (VS) content was obtained by placing the dry samples in a furnace at 550 °C for 2 h. The contents of carbon (C), hydrogen (H) and nitrogen (N) were obtained by ultimate analysis using CE 440 element analyser [31]. The oxygen content was assumed to be the remaining VS content.

The concentrations of SMPs in the effluents of fermentation, including ethanol, acetic acid, propionic acid, iso-butyric acid, butyric acid, iso-valeric acid, valeric acid and caproic acid, were determined using an Agilent HP 6890 gas chromatograph equipped with a Nukol fused silica capillary column (30 m × 0.25 mm × 0.25 μm) and a flame ionisation detector [32]. The concentration of galactose in the effluents of fermentation was measured by the 3,5-dinitrosalicylic acid method using galactose as a standard [10].

2.4. Experimental design and data analysis

The design of the experiment was based on three values of three fermentation parameters (pH, yeast extract/galactose (YE/G) ratio and substrate concentration). The Box-Behnken method was employed to optimise these three fermentation parameters. Fifteen experimental runs, including 3 central runs (Code "0") were carried out as shown in Table 1. The ranges of pH, YE/G ratio and substrate concentration were 5–8 (central value: 6.5), 0–1 (central value: 0.5) and 5–35 g VS/L (central value: 20 g VS/L), respectively. The parameter levels were calculated via Eq. (1).

$$X_i = \frac{A_i - A_{i0}}{\Delta A_i} \tag{1}$$

where X_i is the code value of the ith variable; A_i is the real value of the ith variable; A_{i0} is the central value of ith variable; and ΔA_i is the step change of the ith variable. The experimental design for X_1 (pH), X_2 (YE/G ratio) and X_3 (substrate concentration) is shown in Table 1. To optimise fermentative bio-hydrogen production, a quadratic polynomial equation (Eq. (2)) was fitted to estimate the relationship between the variables and response (i.e., bio-hydrogen yield, mL/g galactose) using Design Expert software.

$$Y = \alpha_0 + \alpha_1 X_1 + \alpha_2 X_2 + \alpha_3 X_3 + \alpha_{12} X_1 X_2 + \alpha_{13} X_1 X_3 + \alpha_{23} X_2 X_3 + \alpha_{11} X_1^2 + \alpha_{22} X_2^2 + \alpha_{33} X_3^2 \tag{2}$$

where Y is the response value predicted by RSM; α_0 is the offset term; α_1, α_2, α_3 are the linear coefficients; α_{12}, α_{13}, α_{23} are the interaction coefficients; and α_{11}, α_{22}, α_{33} are the quadratic coefficients [33]. All the 15 experimental runs (as shown in Table 1) were carried out in duplicate. In order to validate the results for optimal settings predicted by the RSM, bio-hydrogen production was analysed under optimal conditions in a subsequent run in triplicate.