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Production of biohydrogen from crude glycerol in an upflow column bioreactor

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HIGHLIGHTS

- Biohydrogen production from crude glycerol (CG) was investigated.
- Continuous packed bed reactor and mixed acidogenic consortia were used.
- pH was shown to be the crucial parameter affecting conversion efficiency.
- Hydrogen yield was affected by pH, HRT and organic loading rate.
- Hexanoate, a platform chemical, was produced to considerable amounts from CG.

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ABSTRACT

A continuous attached growth process for the production of biohydrogen from crude glycerol was developed. The process consisted of an anaerobic up-flow column bioreactor (UFCB), packed with cylindrical ceramic beads, which constituted the support matrix for the attachment of bacterial cells. The effect of crude glycerol concentration, pH and hydraulic retention time on glycerol conversion, hydrogen yield and metabolite distribution was investigated. It was shown that the most critical parameter for the efficient bioconversion was the pH of the influent, whereas the hydrogen yield increased with an increase in feed glycerol concentration and a decrease in the hydraulic retention time. The main soluble metabolite detected was 1,3-propanediol in all cases, followed by butyric and hexanoic acids. The latter is reported to be produced from glycerol for the first time. Acidification of the waste reached 38.5%, and the maximum H_2 productivity was 107.3 ± 0.7 L/kg waste glycerol at optimal conditions.

1. Introduction

Waste or crude glycerol (CG) is the main by-product of biodiesel production. Due the recent boost of the biodiesel industry, large quantities of CG are becoming dispensable annually. Indeed, the market demand for pure glycerol cannot cope with the astounding generation rate of waste glycerol, whereas the purification of the latter is not favorable economically. Given the high supply of CG, possible ways for its valorization are gaining interest. Among them the use of CG as feedstock for biofuels production has been proposed, with fermentative hydrogen production being a remarkable option (Murarka et al., 2008; Ntaikou et al., 2010a).

Biological H_2 production can be accomplished either through photo-fermentation, realized by photosynthetic microorganisms (Keskin et al., 2011) or through dark fermentation, realized by anaerobic microorganisms. During the dark fermentation of carbohydrate-based substrates, either via pure or mixed microbial cultures, hydrogen is liberated as a gaseous byproduct along with the production of short chain fatty acids, such as formate, acetate and butyrate (Antonopoulou et al., 2008; Ntaikou et al., 2009a,b; Ho et al., 2014). During the dark fermentation of glycerol, hydrogen is generated along with acetate, butyrate and ethanol, whereas 1,3-propanediol (PDO) is also produced, as a method of NADH regeneration for respiratory balance. Per 1 mol of PDO generated, 1 mol of H_2 is required and thus its production adversely affects H_2 gas yields (Selembo et al., 2009). The main challenge for efficient H_2 production from glycerol is thus the prevalence of efficient metabolic/microbial H_2 generating pathways, which can be secured through appropriate manipulation of operational

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parameters (such as the retention time, the temperature, the pH etc). On the other hand, bioreactor configuration is also quite important. Although the continuous stirred tank reactor (CSTR) is the most widely used bioreactor type for the production of biohydrogen from carbohydrate based substrates (Bakonyi et al., 2014), previous studies with glycerol as the main organic substrate have revealed that such a bioreactor has limited overall efficiency and unstable performance (Vlassis et al., 2012).

Hydrogen production using attached cultures (biofilm reactors) on the other hand can be advantageous as it allows for a small hydraulic retention time, while the biosolids retention time is large enough. When it comes to biohydrogen production, maintaining a high solids retention time has in principle, the potential obstacle of allowing hydrogen consuming methanogens to grow. This of course may be avoided if the pH in the reactor is maintained at a sufficiently low level (below 6) (Venkata Mohan et al., 2013).

In the present study, an anaerobic up-flow column bioreactor (UFCB), containing ceramic beads as the support matrix for the attachment of bacterial cells, was developed for the bioconversion of CG to hydrogen with a mixed acidogenic culture. The reactor was operated in continuous mode and exhibited a very stable performance for a wide range of operating conditions. The effect of the key parameters such as the feed glycerol concentration, the feed pH and the bioreactor hydraulic retention time (HRT) on glycerol conversion, hydrogen yield and metabolites distribution was investigated. It was shown that the most critical parameter for an efficient bioconversion was the pH of the influent, whereas the hydrogen yield increased as the substrate concentration was increased and as the HRT was decreased. In all cases, 1,3-propanediol (PDO) was the main soluble metabolite detected, followed by butyric and hexanoic acids. The latter is reported for the first time to be produced from glycerol fermentation.

2. Methods

2.1. Feedstock

The CG that was used as feedstock in the present study was the byproduct of biodiesel production from sunflower oil, and was kindly supplied by the biodiesel production company PETTAS SA. The main characteristics of the crude glycerol were as follows: purity 92.2 ± 0.3%, pH of 10% w/v aquatic solution 5.2, COD 1.28 ± 0.00 gO_2/g waste, ash content <6%. Feed solutions of crude glycerol were supplemented with 0.5–0.75 g/L yeast extract, 100 mL/L phosphate buffer solution (PBS), 10 mL/L $FeSO_4{\cdot}7H_2O$, (FES), and 10 mL/L trace elements solution (TES). The above solutions were prepared separately and were of the following composition (g/L): PBS, 37.4, 72.3 or 71.9 K_2HPO_4 and 86.8, 44.8 or 38.4 KH_2PO4 for obtaining a feed pH of 6, 6.5 and 7 respectively; TES, $CaCl_2{\cdot}2H_2O$, 22.5; NH_4Cl, 35.9; $MgCl_2{\cdot}6H_2O$, 16.2; KCl, 117; $MnCl_2{\cdot}4H_2O$, 1.8; $CoCl_2{\cdot}6H_2O$, 2.7; H_3BO_3, 0.51; $CuCl_2{\cdot}6H_2O$, 0.24; $Na_2MoO_4{\cdot}2H_2O$, 0.23; $ZnCl_2$, 0.19; $NiCl_2{\cdot}6H_2O$, 0.2; H_2WO_4, 0.01; FES, $FeSO_4{\cdot}7H_2O$, 0.07 g/L.

2.2. Microbial culture

An acidogenic mixed culture was obtained by thermally pretreating at 100 °C for 20 min a sludge taken from an anaerobic digester of the municipality of Patras. A thermal pretreatment secured inhibition of methanogens and domination of hydrogen producing bacteria, mainly clostridia (Antonopoulou et al., 2008). The bioreactor was filled with 20% (v/v) of sludge for start-up.

2.3. Experimental apparatus and setup

The experimental apparatus consisted of an up-flow, packed bed column bioreactor (internal diameter of 9 cm, height 35 cm), made of Plexiglas, a peristaltic pump and a heating system. The reactor was double-coated and temperature control (35 ± 0.5 °C) was achieved via recirculation of warm water in the outer jacket. Cylindrical porous ceramic beads with specific surface of 600 m^2/L, height of 1.5 cm, inner diameter of 1 cm and outer diameter of 1.5 cm, were used as support material for the attachment of bacterial cells. Fresh medium was fed at the bottom of the reactor continuously via a peristaltic pump, at a flow rate of 0.63 mL/min, 0.5 mL/min or 1 mL/min corresponding to an HRT of 36 h, 48 h or 24 h respectively. The effluent left the reactor through overflow at a height of 30 cm from the bottom. Volumetric gas production rates were measured using a liquid displacement method. The bioreactor was initially operated in batch mode for 48 h and was switched subsequently to continuous mode. Dissolved Chemical Oxygen Demand (d-COD), glycerol, total suspended solids (TSS), volatile suspended solids (TSS) and pH were measured daily both in the feed and in the fermentation broth. Moreover, in the latter, PDO, Short Chain Fatty Acids (SCFAs) and alcohols were also measured daily. Total biogas and hydrogen generated and mole % of hydrogen in the biogas were also measured on a daily basis.

2.4. Analytical methods

TSS, VSS and d-COD (via the closed reflux method) were determined according to Standard Methods (APHA, 1995), and SCFAs i.e. acetate, propionate, butyrate, isobutyrate, valerate, isovalerate, and hexanoate and alcohols, i.e. ethanol and butanol, acidified samples with 30 μL/mL 20% H_2SO_4 were analyzed in a Varian CP-3800 GC, equipped with a flame ionization detector. The hydrogen content in the gaseous phase of the reactor was quantified by an SRI 8610c GC, equipped with a thermal conductivity detector, while glycerol and PDO were measured by High-Performance Liquid Chromatography (DIONEX GP50), equipped with RI detector (Shodex RI-101) and an Aminex HPX-87H column, using an isocratic program at 60 °C with 6 mN H_2SO_4 as eluent at a flow rate of 0.7 mL min^{-1}.

2.5. Statistical data analysis

The statistical analysis of the obtained data was conducted with the use of the SPSS Inc.17 software package. After checking for homogeneity of the variance (Levene's test of equality of error variances), the significant differences among each treatment were assessed non-parametrically, using the Mann Whitney *u* test ($p < 0.05$, ANOVA).

3. Results and discussions

The UFCB was operated continuously for almost 9 months in total, during which three crucial operational parameters for biohydrogen production were studied; substrate concentration in the feed and HRT, corresponding to different organic loading rates (OLRs) and feed pH. The operational profile of the bioreactor regarding these parameters is summarized in Table 1. Eight distinct periods (A–G) with different feed characteristics were realized.

3.1. Effect of operational parameters on glycerol consumption

In Fig. 1a and b, glycerol concentrations (C_{gl}) and pH respectively, in the feed and the reactor are presented throughout the