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Pre-treating anaerobic mixed microflora with waste frying oil: A novel method to inhibit hydrogen consumption

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ABSTRACT

An innovative method was introduced to inhibit methanogenic H_2 consumption during dark fermentative hydrogen production by anaerobic mixed cultures. Waste frying oil was used as an inhibitor for hydrogenotrophic methanogens. Simultaneous effect of waste frying oil concentrations (0–20 g/L) and initial pH (5.5, 6.5 and 7.5) on inhibition of methanogenic H_2 consumption and enhancement of H_2 accumulation were investigated using glucose as substrate. Enhanced hydrogen yields with decreased methane productions were observed with increasing the waste frying oil concentrations. On average, CH_4 productions from glucose in the cultures received 10 g/L WFO were reduced by 88%. Increased WFO concentration up to 20 g/L led to negligible CH_4 productions and in turn enhanced H_2 yields. Hydrogen yields of 209.26, 195.35 and 185.60 mL/g $glucose_{added}$ were obtained for the cultures pre-treated with 20 g/L waste frying oil with initial pH of 5.5, 6.5 and 7.5 respectively. H_2 production by pre-treated cultures was also studied using a synthetic food waste. Anaerobic mixed cultures were pre-treated with 10 g/L WFO and varying durations (0, 24 and 48 h). A H_2 yield of 71.46 mL/g VS was obtained for cultures pre-treated with 10 g/L WFO for 48 h that was 475% higher than untreated control. This study suggests a novel and inexpensive approach for suppressing hydrogenotrophic methanogens during dark fermentative H_2 production.

1. Introduction

Dark fermentation is considered as one of the most favoured technologies for H_2 production with a high potential for commercialization in the near future (Bundhoo et al., 2015). Dark fermentative H_2 production may be conducted either by using pure or mixed cultures; though, the latter is more demanded for full scale applications (Luo et al., 2010). Using mixed microflora is less expensive and more practical compared to pure cultures due to the elimination of sterilization costs, improved substrate degradation, and easier process control. Therefore, using mixed microbial communities is economically and technically feasible for simultaneous waste reduction and clean energy production. A major problem for H_2 production by anaerobic mixed communities would be the presence of H_2 consuming microorganisms such as hydrogenotrophic methanogens, homoacetogens and propionate producers in the raw inoculum, which convert H_2 to CH_4, acetic acid, and propionic acid respectively. Among the H_2 consumers, hydrogenotrophic methanogens are recognized with the major contribution for H_2 consumption (Bundhoo et al., 2015; Saady, 2013).

Many investigations have been performed to suppress methanogenic H_2 consumption and enrich H_2 producing bacteria. Various pre-treatment methods have been employed to fulfil this aim include acid or alkaline pre-treatment (Zhang et al., 2011), chemical inhibition (Pendyala et al., 2012), irradiation (Dong et al., 2010) and heat shock (O-Thong et al., 2009; Pendyala et al., 2012). However, intermittent treatment would be an indispensable issue in full scale due to the subsequent proliferation of anaerobic and facultative H_2 consumers which are present in non-strile feedstocks. Regarding the pre-treatments, using acid or alkali needs periodical pH adjustment, heating and irradiationn are energy intensive and chemical inhibitors are discouraged due to their toxicity. Therefore, none of the mentioned methods are regarded as a perfect solution for full-scale application when the pre-treatment is repeated.

The inhibitory effect of long chain fatty acids (LCFAs) on anaerobic digestion has been recognized since many years ago (Hanaki et al., 1981; Lalman and Bagley, 2002). LCFAs could be adsorbed on the cell wall of some microbial species including acetoclastic and hydrogenotrophic methanogens, interfere metabolites transportation and subsequently hinder their growth (Dasa et al., 2016). LCFAs can be obtained from the hydrolysis of vegetable oils and animal fats. Using LCFAs as inhibitors for methanogenesis can be considered as a promising pre-treatment since they are

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inexpensive and non-toxic and moreover may be easily found in many wastes and waste streams (Rafieenia et al., 2017b). Several studies have used pure LCFAs such as palmitic and stearic acid (Saady et al., 2012), oleic acid (Chaganti et al., 2012), lauric acid (Shanmugam et al., 2014), and linoleic acid (Chowdhury et al., 2007; Shanmugam et al., 2016) to limit growth of hydrogenotrophic methanogens. According to these studies, lipid rich wastes which contain mixtures of LCFAs may be utilized as inhibitors for H_2 consumption. A potential option could be waste frying oil (WFO) which is generated by restaurants, households, canteens, and food processing industries worldwide. This study is aimed at evaluating the simultaneous effect of initial pH and WFO concentration on inhibition of H_2 consumption and subsequently enhancement of H_2 yield. In order to better analyse the experimental results, a quadratic model also was developed to predict the simultaneous effect of initial pH and WFO concentration on H_2 production. In the next step, H_2 production from a synthetic food waste was also investigated to confirm the impact of WFO on H_2 production performances using complex substrates. The significance of this study would be introducing an inexpensive and practical method to inhibit methanogenic H_2 consumption during dark fermentation of mixed microbial cultures.

2. Materials and methods

2.1. Inoculum pre-treatment using waste frying oil

Granular sludge was collected from a full-scale Up-flow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy. WFO (sunflower oil) was chosen in this study as it is widely used as cooking oil in Italy and collected from a local restaurant in Padova, Northern Italy. In order to solubilise WFO, 100 g of the oil was added to 14 g NaOH (98%) and mixed rigorously at 55 °C. Fatty acid composition of WFO was analyzed using gas chromatography and the main fatty acids detected were as follows: linoleic acid (52%), oleic acid (30.24%), palmitic acid (7.04%) and stearic acid (3.22%). For the fermentation tests performed using glucose, different concentrations of saponified WFO solution (0–20 g WFO/l) were added to the reactors contained 10 gVS/l of granular sludge. After 24 h, 5 g/L glucose was added to granular sludge plus WFO and control cultures. Untreated cultures were selected as controls. For the tests performed using synthetic food waste, granular sludge cultures were pre-treated with 10 g/l WFO with different durations (0, 24 and 48 h).

2.2. Hydrogen production studies

The first series of experiments were designed to assess the effect of varying concentrations of WFO on H_2 production from glucose. Since this study is the first report on inoculum pre-treatment with WFO, glucose was chosen as substrate in order to confirm reproducibility of the results which would be impossible using complex substrates due to the composition variability of organic wastes. Laboratory scale tests were performed using 1-liter glass reactors with a working volume of 500 mL. The reactors were nitrogen injected after substrate addition for 3 min to ensure anaerobic conditions and then incubated at 37 ± 1°C. Different initial pH conditions were applied (5.5, 6.5 and 7.5) before incubation using NaOH (3M) and HCl (3M). The start of the process was considered as the time of glucose addition. The tests were done in triplicate. H_2 and CH_4 volumes produced during the dark fermentation were calculated according to VanGinkel et al. (2005). In order to better demonstrate if inhibitory effect of WFO on hydrogenotrophic methanogens can be remained even after removing the WFO, an additional test was also performed. Granular sludge cultures were pre-treated with 10 g/L WFO for 48 h. Then, the cultures were washed twice in order to remove the WFO before adding 5 g/L glucose as substrate and incubation with pH 5.5.

The second series of the experiments were performed to study H_2 production from food waste in order to investigate the impact of WFO on H_2 production performances using complex substrates. A synthetic food waste was prepared with the aim of reproducibility of the results. The synthetic food waste was mainly composed of vegetables (14.7%), meat (13%), fruits (54%), cheese (5.5%), bread and pasta (10.8%) to simulate the food waste composition in Italy. After preparation, the samples were shredded in a kitchen mill to make a homogeneous mixture and analyzed. The characteristics of the synthetic food waste based on wet weight were as follows: Total solids (30.10%), Volatile solids (28.59%), Total Organic Carbon (14.11%) and Total Kjeldahl Nitrogen (0.99%). H_2 and CH_4 productions were studied for four conditions: untreated cultures (U), cultures received WFO and substrate at the same time (A), cultures pre-treated with WFO for 24 h before substrate addition (B) cultures pre-treated with WFO for 48 h before substrate addition (C). The initial pH for food waste fed cultures was adjusted at 5.5.

2.3. Analytical methods

The composition of the gas in the headspace of the bottles in terms of H_2, CH_4 and CO_2, was analyzed using a micro-GC (Varian 490-GC) equipped with a MS5A column to measure H_2 and CH_4 and a PPU column for CO_2 and two Thermal Conductivity Detectors. Argon was used as the carrier gas with a pressure of 60 kPa. Temperatures of column and injector were set to 80 °C.

VFA concentrations in the liquid phase were measured at the end of the process after filtration of the samples. A gas chromatograph (Varian 3900) equipped with a CP-WAX 58 WCOT fused silica column and a Flame Ionization Detector was used for this goal. The carrier gas was nitrogen with a flow of 4 mL/min in the column. The oven temperature was set at 80 °C for the first minute and then increased at a rate of 10 °C/min to 180 °C for two minutes and temperatures of column and injector were set to 250 °C.

H_2 and CH_4 volumes produced during dark fermentation were calculated according to Eq. (2) (VanGinkel et al., 2005):

$$V_{c,t} = C_{c,t} * V_{b,t} + V_H * (C_{c,t} - C_{c,t-1}) \quad (2)$$

where

$V_{c,t}$ = H_2 or CH_4 volume produced in the interval between t and t − 1,
$V_{b,t}$ = Volume of total biogas produced in the interval between t and t − 1,
V_H = Volume of the headspace of bottles,
$C_{c,t}$ = H_2 or CH_4 concentrations measured at times t,
$C_{c,t-1}$ = H_2 or CH_4 concentrations measured at times t − 1.

2.4. Data analysis

A quadratic model (Eq. (2)) was fitted in this study to analyse the effect of concentration of WFO and pH on cumulative H_2 production. Curve fitting was performed using Matlab (The Mathworks Inc., version 2016a).

$$Y = a_0 + a_1X_1 + a_2X_2 + a_{11}(X_1)2 + a_{22}(X_2)^2 + a_{12}X_1X_2 \quad (1)$$

where X_1 and X_2 are input variables (WFO concentration and initial pH respectively) which influence Y (H_2 production), a_0 is the offset term, a_1 and a_2 linear coefficients and a_{11} and a_{22} quadratic coefficients and a_{12} interaction coefficient. Minitab 17 statistical software (Minitab Inc., State College, PA, 2010) was used to obtain main effect plots for experimental factors.

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