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Dark fermentation metabolic models to study strategies for hydrogen consumers inhibition

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ABSTRACT

A Flux Balance Analysis (FBA) metabolic model of dark fermentation was developed for anaerobic mixed cultures. In particular, the model was applied to evaluate the effect of a specific inoculum pre-treatment strategy, addition of waste frying oil (WFO) on H_2-producing and H_2-consuming metabolic pathways. Productions of volatile fatty acid (VFAs), CO_2, H_2 and CH_4 measured through triplicate batch experiments, were used as constraints for the FBA model, to compute fluxes trough different metabolic pathways. FBA model could estimate the effect of pre-treatment with WFO on major microbial populations present in the mixed community (H_2 producing bacteria, homoacetogen and methanogens). Results revealed that low concentrations of WFO did not completely inhibited hydrogenotrophic methanogens. FBA showed that acetoclastic methanogens were more sensitive to WFO, in comparison to hydrogenotrophic methanogens. The proposed model can be used to study H_2 production by any other mixed microbial culture with similar substrates.

1. Introduction

In next generation bio-based refineries, hydrolysis and primary (or extractive) fermentations by mixed microbial cultures (MMC) are precursors of secondary bio-transformations, in which H_2, CO_2 and mixed carboxylates are used as substrate for achieving added-value target products (e.g. bio-based chemicals, bio-plastics and pigments) (Agler et al., 2012). Dark fermentation (DF) is the simplest MMC-driven process that include hydrolysis and primary fermentations to extract gaseous and soluble mixtures of compounds from raw biomass (Manzini et al., 2015). Dark fermentation of organic wastes is considered as a promising process in terms of sustainable waste management and simultaneous biofuel production. Carbohydrates are one of the main fractions of organic wastes with the highest contribution to hydrogen production. According to the previous studies, H_2 yield is strongly related to the carbohydrate content of organic wastes (Alibardi and Cossu, 2016; Ghimire et al., 2015). Using mixed inocula instead of pure cultures seems to be more practical and economically viable for full-scale applications. This is mainly due to the capability of mixed cultures for converting organic wastes and elimination of sterilization costs. Mixed microbial communities are very complex biological systems that are affected significantly by changes in environmental conditions or substrates. Anaerobic fermentation is mediated by complex microbial populations including H_2 producers, homoacetogens, methanogens, propionate producers and lactic acid bacteria. If the growth of H_2 consumers is not controlled, the H_2 produced by H_2 producing bacteria cannot be accumulated due the presence of H_2 consumers (Chaganti et al., 2011). Understanding the syntrophic association between different microbial populations enables the design strategies to inhibit unfavoured populations and subsequently optimize the favoured product. Mixed cultures should be pre-treated by different means to suppress hydrogen consuming species and enrich H_2 producing bacteria. The most extensively used inoculum pre-treatment methods include heat shock (Ding et al., 2017; Zhang et al., 2011), acid/alkaline treatment (Pendyala et al., 2012; Wang et al., 2015), Irradiation (Elbeshbishy et al., 2010; Yin and Wang, 2016a) and chemical inhibition (Shanmugam et al., 2016). A recently published study suggested that waste frying oil (WFO) can be used successfully as an inhibitor for H_2 consumption and therefore inoculum pre-treatment with WFO could be a promising technology to improve H_2 production (Rafieenia et al., 2018). In order to better investigate the effect of inoculum pre-treatment on the H_2 yield, contribution of H_2 producing and H_2 consuming pathways before and after pre-treatment should be quantified. Hydrogenotrophic methanogens that convert H_2 and CO_2 to CH_4 are considered as the main group of H_2 consumers in anaerobic mixed cultures (Eq. (1)).

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CH_4 production in anaerobic digestion is mediated by hydrogenotrophic and acetoclastic methanogens (Eqs. (1) and (2) respectively). Cumulative CH_4 production could be measured by gas chromatography method; however, contribution of hydrogenotrophic and acetoclastic methanogens in total CH_4 produced by a mixed microbial community remains unknown. Quantification of hydrogenotrophic methane production in a bioreactor could help to optimize the H_2 yield.

$$4H_2 + CO_2 \rightarrow CH_4 + 2H_2O \quad (1)$$

$$CH_3COOH \rightarrow CH_4 + CO_2 \quad (2)$$

During dark fermentation of carbohydrate-rich substrates, acetic acid and butyric acid constitute the highest proportion of the produced soluble metabolites (Eqs. (3) and (4)).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (3)$$

$$C_6H_{12}O_6 \rightarrow 2CH_3(CH2)_2COOH + 2CO_2 + 2H_2 \quad (4)$$

Although the theoretical H_2 yield of glucose for acetic acid production is twice of that for butyrate, several studies reported that there was no correlation between higher acetic acid production and increased H_2 yield (Cappai et al., 2014; Kim et al., 2006; Rafieenia et al., 2017). This could be because of possible acetic acid production from H_2 by homoacetogenic bacteria (Eq. (5))

$$4H_2 + 2CO_2 \rightarrow CH_3COOH + 2H_2O \quad (5)$$

Therefore, quantification of the acetate produced by H_2 producing bacteria and that produced by H_2 consumers is of great importance to analyze the activities of the two mentioned microbial populations and optimizing the inoculum pre-treatment conditions. The net acetate productions by a mixed culture could be quantified by measuring the acetate concentrations in the liquid phase of the reactors at the end of fermentation. Acetate production by H_2 producing bacteria is in favour of high H_2 yield (Eq. (3)) while acetate production by homoacetogens results in lower H_2 accumulation (Eq. (5). Therefore, contribution of acetate produced by H_2 producers and that is produced by homoacetogens in total acetate production could not be defined with measuring the net acetate production. Several studies have used metabolic network models for H_2 production using pure microbial cultures (Cai et al., 2010; Cheng et al., 2013; Rafieenia and Chaganti, 2015; Sarma et al., 2017). Metabolic network model construction for a mixed culture is more challenging compared to a pure culture since the syntrophic relationships between different microbial populations should be considered. To the best of our knowledge, there are only two studies on metabolic network modelling of hydrogen production for mixed cultures (Chaganti et al., 2011; Gonzalez-Garcia et al., 2017). Among these two studies, the latter did not include methanogenic and homoacetogenic H_2 consumption since they used a heat pre-treated mixed culture. Chaganti et al. (2011) used a simplified model without considering pentose-phosphate (PP) and Tricarboxylic Acid (TCA) pathways. Moreover, CO_2 flux was not included in the models presented by Chaganti et al. (2011) and Gonzalez-Garcia et al. (2017) while it is well understood that a major part of the initial carbon is emitted as CO_2 and therefore should be considered for carbon mass balance. Considering the lack of information about the mentioned issues, developing a comprehensive model, which addresses the missing information, seems to be an important issue.

Metabolic network models can be beneficial in dark fermentation studies of mixed cultures to give a more comprehensive understanding of metabolic pathways involved in H_2 production and consumption. Flux Balance Analysis (FBA) is an interesting approach that can be utilized to investigate how any changes in substrate or operational parameters can change the metabolic flux distribution towards different metabolic pathways and different products. FBA method is generally used for pure cultures to study the flux distribution through different metabolic pathways. The concept of universal bacterium was firstly introduced by Rodriguez et al. (2006) to apply FBA for mixed microbial communities. According to this concept, the mixed microbial community is regarded as a single bacterium with all the possible metabolites of single bacteria present in the community. It also considers the syntrophic effects of different bacteria where products of some bacteria are used as the substrate by the other populations. FBA approach can increase our understanding of the complex metabolic reactions occurring in a mixed culture and define the contribution of a substrate to products by quantification of intracellular fluxes that is quite difficult with experimental methods (Orth et al., 2010). In our work, significant changes were introduced compared to the work of Chaganti et al. (2011) since they did not consider a complete three carboxylic acid (TCA) cycle and pentose phosphate (PP) pathway for the universal bacterium. Moreover, CO_2 flux was also considered in the model while it was not presented by Chaganti et al. (2011) and Gonzalez-Garcia et al. (2017). All the known possible products from the dark fermentation of glucose and their metabolic routes were considered in the model (Rafieenia and Chaganti, 2015; Chaganti et al., 2011; Dias et al., 2008; Cai et al., 2010; Sarma et al., 2017; Gonzalez-Garcia et al., 2017; Kaushal et al., 2018). In this regard, the main products included in the metabolic network model are acetate, butyrate, lactate, propionate, valerate, caproate, ethanol, hydrogen, carbon dioxide and methane.

The objectives of the present study are 1) to develop a metabolic network model for dark fermentation of anaerobic mixed cultures and provide a comprehensive insight into the H_2 producing and H_2 consuming pathways and 2) to investigate the effect of inoculum pre-treatment with WFO on flux distribution towards different metabolic pathways compared to the untreated culture. To achieve these goals, triplicate batch experiments were designed to measure production of VFAs, CO_2, H_2 and CH_4 and these results were used as constraints for the FBA model to compute fluxes trough different metabolic pathways. The main focus will be on the estimation of H_2 consumption by hydrogenotrophic methanogens and homoacetogens, two major H_2 consuming populations.

2. Materials and methods

2.1. Inoculum and culture conditions

Granular sludge was used as the inoculum in this study and it was collected from a full-scale Up-flow Anaerobic Sludge Blanket (UASB) digester of a brewery factory located in Padova, Italy. Granular sludge was characterised by a Total Solids (TS) concentration of 15.13%, Volatile Solids (VS) concentration of 52.12% TS, Total Organic Carbon (TOC) content of 29.2% TS and Total Kjeldahl Nitrogen (TKN) content of 43.19 %TS.

WFO was used as a stressing agent to enrich H_2 producing bacteria, collected from a local restaurant in Padova, Italy. In order to solubilise WFO, a saponified WFO solution was prepared according to the method previously described by Rafieenia et al. (2018).

Batch fermentation tests were performed using 1-liter glass bottles received 5 g/L glucose as substrates. No other substrate, micro and macro nutrients were added to the bottles. In order to pre-treat the sludge with WFO, 10 gVS/L of granular sludge was mixed with varying concentrations of WFO (0, 5, 10 and 20 g/L) for 24 h before glucose addition. After glucose addition, tap water without filtration was added to the bottles to reach the working volume of 500 mL. All the bottles were sealed with silicone rubber and incubated in a water bath at a temperature of 35 °C for 72 h. Mesophilic conditions (35 °C) were chosen as it is less energy intensive compared to thermophilic conditions. The duration of fermentation (72 h) was chosen according to our previous studies to ensure the conclusion of H_2 production (Rafieenia et al., 2017; Rafieenia et al., 2018). The initial pH for untreated and pre-treated cultures was set at 5.5 using NaOH (3M) or HCl (3M) after glucose addition. All the tests were done in triplicate.