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# Selective carboxylate production by controlling hydrogen, carbon dioxide and substrate concentrations in mixed culture fermentation

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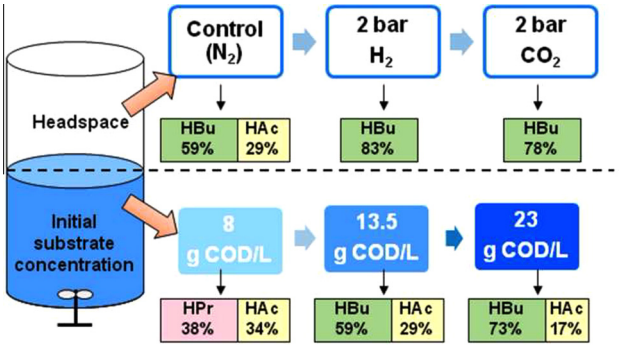
## HIGHLIGHTS

- Study $H_2$ and $CO_2$ addition on carboxylate spectrum at increasing substrate concentrations.
- 2 bar $CO_2$ in the headspace directs the fermentation process towards *n*-butyrate.
- At elevated initial substrate concentrations, the effect of 2 bar headspace becomes limited.
- 2 bar of $CO_2$ and elevated substrate concentration steered fermentation to *n*-butyrate.

## GRAPHICAL ABSTRACT

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## ABSTRACT

This research demonstrated the selective production of *n*-butyrate from mixed culture by applying 2 bar carbon dioxide into the headspace of batch fermenters or by increasing the initial substrate concentration. The effect of increasing initial substrate concentration was investigated at 8, 13.5 and 23 g COD/L with potato processing waste stream. Within 1 week of incubation, *n*-butyrate fraction selectively increased up to 83% by applying 2 bar hydrogen or 78% by applying carbon dioxide into the headspace whereas it was only 59% in the control reactor. Although the fraction of *n*-butyrate was elevated, the concentration remained lower than in the control. Both the highest concentration and fraction of *n*-butyrate were observed under the highest initial substrate concentration without headspace addition. The concentration was 10 g COD/L with 73% fraction. The operational conditions obtained from batch experiments for selective *n*-butyrate production were validated in a continuous process.



## 1. Introduction

The 'carboxylate platform' concept has been introduced by Holtzapple et al. (1999) to generate energy intense chemicals, specifically carboxylic acids, in single stage bioconversion processes by using undefined mixed cultures. Since then, a variety of chemical and biological conversions in which carboxylates can be used as building block has been shown by different researchers. The principle of the platform is a partial anaerobic digestion of organic matters using mixed cultures in which mainly acetate (C2), propionate (C3) and *n*-butyrate (C4) in the aqueous phase and hydrogen in the gas phase are produced. It is, however, important to selectively produce one end compound or a favorable ratio of a carboxylates mixture in the first stage, since this will be the precursor for the generation of the target compounds in the second stage (Li and Yu, 2011). For example, in the polyhydroxyalkanoate (PHA) production, the type of carboxylates and mixture composition produced in the carboxylate platform determines the type of PHA produced (Serafim et al., 2008).

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Organic waste streams are attractive candidates to use as a substrate for generation of biobased chemicals. The main advantage of using waste streams is that they are cheap materials (sometimes with no cost or with a gate fee). Anyhow, large amounts of organic waste are being generated and need to be treated. Fortunately, fermentation of organic waste materials provides both treatment of waste matters and simultaneous generation of carboxylates like acetate, propionate and *n*-butyrate (Gajdos, 1998).

Organic waste materials are mainly composed of carbohydrate, protein and lipid type polymers. Given this complex composition of the starting material, a mixed consortium is required to reduce each polymer to valuable end products (Angenent and Wrenn, 2008). Further, using mixed cultures makes the fermentation process economically more interesting because of elimination of a costly sterilization process. The overall process becomes more stable and flexible without the risk of contamination (Ueno et al., 1995). However, using mixed consortia degrading a complex substrate leads to an effluent containing a mixture of various acids and alcohols in different concentrations. As a result downstream processing is more challenging which increases the production costs of the target products. It is thus crucial to maximize both the concentration and the fraction of a single compound in total products. When more than one product is required the fermentation process needs to be steered towards a certain ratio of desired compounds.

Increasing concentrations and tuning the product distribution among fermentation products can be achieved in several ways. In literature, substrate type, concentration of substrate in the fermenter, retention time, reactor design, temperature, and pH were found to alter the concentrations and also the type of products. Table 1 presents reported product concentrations and distributions from different studies in literature. In our previous work, it was shown that headspace manipulation with externally added hydrogen and/or carbon dioxide in variable ratios can increase product concentrations and result in the selective production of a single compound depending on the polymer type in mixed culture fermentation (Arslan et al., 2012).

Previously, the effect of feed concentration in batch and continuous mode operation on fermentation products was investigated by Lim et al. (2008), Gomez et al. (2009), Coats et al. (2011) and Badiei et al. (2011) (Table 1). These studies aimed to maximize the total concentration of carboxylates rather than targeting selective generation of a single compound. Yet, in their results it was shown that carboxylate concentrations and distributions were affected by changing the substrate concentration in the fermenter.

The aim of this work was to investigate the combined effect on the carboxylate product spectrum by applying only hydrogen or only carbon dioxide at 2 bar to the headspace at increasing substrate concentrations.

## 2. Methods

### 2.1. Inocula and waste streams

As inoculum, granular sludge from a potato wastewater anaerobic digester (Opure, Ede, The Netherlands) was used. To avoid methanogenic growth during fermentation, the granules were first washed with 20 mM potassium phosphate buffer at pH 5 and sieved through 500 μm for three times. They were left in potassium phosphate buffer solution overnight at room temperature. The next day, the granules were heat-treated by boiling in water for 15 min.

Carbohydrate rich waste streams were collected from a potato processing company.

### 2.2. Batch experiments

Batch reactors (5 L) with 750 ml working volume were run as described in Arslan et al. (2012). The organic waste stream was diluted to three different initial substrate concentrations: 8, 13.5 and 23 g Chemical Oxygen Demand (COD substrate/L) through the addition of basal medium solution. The basal medium was prepared according to Phillips et al. (1993). To avoid sulfate reduction during fermentation, sulfate salts were omitted. The reactors were inoculated with 8 g of wet weight (0.6 g Volatile Solids (VS) content) of pre-treated granular sludge. Pressures in the reactors were increased to 2 bar by applying only hydrogen or only carbon dioxide. With a manometer, the headspace pressure of each reactor was monitored on-line. If the headspace pressure dropped during the experiment, it was adjusted back to 2 bar with the same original headspace gases. A control reactor was prepared in the same way. However, it was flushed with $N_2$ only at the beginning of the experiment and connected to a volumetric gas counter (Milligascounter, BnC-Ritter) working under atmospheric pressure. Reactors were stirred at 100 rpm by a magnetic drive stirrer (Büchi-cyclone 075) and kept at 30 °C with hot water circulation. The experiments lasted 30 days with weekly gas and liquid sampling. The sampling amount was calculated in a way that the liquid to headspace ratio was not disturbed or no underpressure was occurring.

### 2.3. Continuous experiment

A continuous reactor (5 L) with 3 L working volume was prepared in the same way as the batch reactors. The initial wet weight of inocula in the continuous reactor was kept at the same concentration as in the batch experiments. The headspace was flushed with $N_2$ at the beginning of the experiment and then connected to a volumetric gas counter under 1 bar of atmospheric pressure. The reactor was kept at 30 °C with hot water circulation. The reactor pH was initially not controlled. When the pH spontaneously dropped to 4, it was kept above 3.9 by dosing 2 M NaOH. The hydraulic retention time (HRT) of the reactor was 2 days. The substrate load to the reactor was 23 g COD/L d. Waste was diluted with the same basal medium solution as in the batch experiments. The feed tank was kept at 4 °C to avoid that fermentation already started inside the tank and was continuously stirred to prevent settling of particles. A grinder pump (Cat DK 40, drive unit X 1740) was connected in a closed loop to the influent tank and continuously circulated the influent solution to reduce the size of the particles coming from the waste material. A peristaltic pump (Watson

**Table 1**
Product concentrations and distributions reported in the studies on maximizing total VFA or hydrogen production with mixed culture.

| Substrate | TVFA (g/L) | Ac (%) | Pr (%) | Bu (%) | Va (%) | Ca (%) | Reactor type | Ref. |
|---|---|---|---|---|---|---|---|---|
| Municipal solid waste | 5.7 | 38 | 0 | 62 | – | – | Batch | Okamoto et al. (2000) |
| Manure | 3.8 | 70 | 17 | 11 | 1 | – | Batch | Coats et al. (2011) |
| Cafeteria food waste | 13.5 | 28 | 28 | 22 | 21 | – | Fed-Batch | Lim et al. (2008) |
| Palm oil mill | 1 | 34 | 0 | 65 | – | 0 | Fed-Batch | Badiei et al. (2011) |
| Glucose | 1.6 | 32 | 2 | 64 | – | 2 | Continuous | Fang and Liu (2002) |
| Sucrose | 8.4 | 24 | – | 76 | – | – | Continuous | Kyazze et al. (2005) |
| Food waste | 3.5 | 49 | 9 | 21 | – | 21 | Continuous | Gomez et al. (2009) |