Bioresource Technology 151 (2014) 378–382

Contents lists available at ScienceDirect

Bioresource Technology

journal homepage: www.elsevier.com/locate/biortech

Short Communication

Upgrading dilute ethanol from syngas fermentation to *n*-caproate with reactor microbiomes

Divya Vasudevan, Hanno Richter, Largus T. Angenent *

Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY 14853, USA

HIGHLIGHTS

- *n*-Caproic acid was produced from actual syngas fermentation effluent.
- The carboxylate platform was integrated with the syngas platform.
- Detrimental effect of methanogenesis on *n*-caproic acid concentration established.

ARTICLE INFO

Article history:
Received 14 August 2013
Received in revised form 21 September 2013
Accepted 25 September 2013
Available online 3 October 2013

Keywords:
n-Caproic acid
Chain elongation
Reversed beta-oxidation
Carboxylate platform
Syngas platform

ABSTRACT

Fermentation of syngas from renewable biomass, which is part of the syngas platform, is gaining momentum. Here, the objective was to evaluate a proof-of-concept bioprocessing system with diluted ethanol and acetic acid in actual syngas fermentation effluent as the substrate for chain elongation into the product *n*-caproic acid, which can be separated with less energy input than ethanol. Chain elongation is performed with open cultures of microbial populations (reactor microbiomes) as part of the carboxylate platform. The highest concentration of *n*-caproic acid of ~1 g L^{-1} was produced at a pH of 5.44 and a production rate of 1.7 g L^{-1} day^{-1}. A higher *n*-butyrate production rate of 20 g L^{-1} day^{-1} indicated that product toxicity was limiting the chain elongation step from *n*-butyric acid to *n*-caproic acid. This result shows that the syngas and carboxylate platforms can be integrated within a biorefinery, but that product separation is necessary.

1. Introduction

Bio-based fuels research and development has flourished during the last decade with the goal to develop renewable and sustainable alternatives to fossil fuels. Although solar and wind energy have proven to be a sustainable alternative to fossil fuels, there is still a need for sustainable liquid fuels. Worldwide, ethanol has remained the premier biofuel. However, ethanol distillation is an energy-intensive process that considerably impacts the ratio of energy returned vs. energy invested (Schrier, 2012). It is, therefore, desirable to circumvent ethanol distillation as a method for product recovery (Agler et al., 2012).

An anaerobic fermentation process with open cultures (reactor microbiomes) and fed with dilute, undistilled ethanol can catalyze a chain-elongating reaction via a well-understood reversed β-oxidation pathway, leading to the production of *n*-caproic acid (Agler et al., 2011; Kenealy et al., 1995). This process is part of the carboxylate platform and includes bacteria in the reactor microbiome, such as *Clostridium kluyveri*, which are known to elongate carboxylic acid carbon chains (Agler et al., 2012). *n*-Caproic acid is a 6 carbon chain carboxylic acid that is more hydrophobic in comparison to ethanol, and therefore easier to extract (Steinbusch et al., 2011). A recent study has demonstrated *n*-caproic acid separation by in-line, liquid–liquid extraction (i.e., pertraction) from recycled liquor from a bioreactor (Agler et al., 2012), providing a potential method to avoid or reduce the use of ethanol distillation. Production of *n*-caproic acid has at least two other benefits compared to ethanol. First, *n*-caproic acid is more energy dense than ethanol (Steinbusch et al., 2011) and can be converted to liquid biofuels (Agler et al., 2011). Second, *n*-caproic acid is a more valuable commodity that is already used: (i) in animal feed rations; (ii) as an antimicrobial (Skřivanová and Marounek, 2007); and (iii) as a feedstock for esterification into food products (Minich, 1960).

An anaerobic fermentor, which is optimized for chain elongation, has a different reactor microbiome compared to an anaerobic digester (both in the carboxylate platform). For efficient chain

* Corresponding author. Tel.: +1 607 255 2480; fax: +1 607 255 4449.
E-mail address: la249@cornell.edu (L.T. Angenent).

0960-8524/$ - see front matter © 2013 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.biortech.2013.09.105

elongation, the acetoclastic methanogens must be mostly inhibited to prevent the intermediate acetic acid from being converted into methane. In addition, it is important to maintain high enough hydrogen partial pressures to prevent oxidation of short- and medium-chain carboxylic acid, and thus hydrogenotrophic methanogens must be curtailed, possibly by limiting the availability of carbon dioxide. In conventional anaerobic digestion, the pH is maintained near neutral to increase methane production. In chain-elongating fermentors, methanogenesis can be suppressed by the addition of an inhibitor such as 2-bromoethanesulfonate (Zinder et al., 1984; Steinbusch et al., 2011). However, this is an expensive chemical and would not be economically feasible to be used for large-scale production of *n*-caproic acid. As an alternative, methanogens can be inhibited by maintaining the pH at 5.5 (Agler et al., 2012; VanKessel and Russell, 1996); or by completely excluding the presence of methanogens through the use of an inoculum devoid of methanogens and the use of synthetic substrates (Grootscholten et al., 2013a).

Several reactor configurations have been used to study chain elongation with reactor microbiomes, including a batch reactor (Kenealy et al., 1995), an upflow fixed bed reactor (Ding et al., 2010), an anaerobic sequencing batch reactor (Agler et al., 2012), and the anaerobic filter (AF) reactor (Grootscholten et al., 2013a). The highest production rates, thus far, have been achieved with continuously-fed AFs. Grootscholten et al. (2013a) achieved an *n*-caproic acid production rate of 16 g L^{-1} day^{-1} with a product selectivity of 85%, and in a later study even an *n*-caproic acid production rate of ~55 g L^{-1} day^{-1} with a product selectivity of ~80% (Grootscholten et al., 2013b). It is important to realize that the substrate for these two studies consisted of a synthetic substrate that is a mixture of procured ethanol and acetic acid with added growth factors and nutrients.

On the other hand, the study by Agler et al. (2012) fed a ~1/6 diluted stream of real, complex substrate (fermentation beer with 15% [w/w] ethanol) to a 5-L anaerobic fermentor. This substrate was withdrawn from the beer well at a corn kernel-to-ethanol plant and contained leftover corn kernel solids and yeast cells (Agler et al., 2012). Besides base (NaOH), no other growth factors or nutrients were added to the influent. In the quest to find other actual substrates, dilute ethanol and acetic acid obtained in effluent from a synthesis gas fermentation setup was identified as a potential substrate for the chain-elongating process.

Synthesis gas, which is referred to as syngas and comprises of a mixture of carbon monoxide, hydrogen, and carbon dioxide, can be produced sustainably with the syngas platform through a thermochemical step with biomass feedstock such as wood waste or willow (Munasinghe and Khanal, 2010). The produced syngas is converted to ethanol and acetic acid using carboxydotrophic bacteria, including *Clostridium ljungdahlii*, *Clostridium autoethanogenum*, *Acetobacterium woodii*, *Clostridium carboxidivorans*, or *Peptostreptococcus productus*. Syngas fermentation setups with a pure culture of one of these carboxydotrophic bacteria can achieve ethanol concentrations of 2–4% (w/w) (Richter et al., 2013). The two-stage, 5-L syngas fermentation system that was operated in the Angenent Lab has achieved ethanol concentrations of up to 3% and a volumetric production rate of 7.3 g L^{-1} day^{-1} (~0.3 g L^{-1} h^{-1}) (Richter et al., 2013). It is anticipated that effluent from syngas fermentation systems could consist of many of the growth factors and nutrients for chain elongation, even though further studies are necessary to ascertain this.

Here, a proof-of-concept study was evaluated to answer the question whether real, filtered syngas fermentation effluent, including dilute ethanol and acetic acid, can be used as a substrate to a continuously fed, chain-elongating bioreactor to circumvent ethanol distillation. In this project, it has been shown that the integration of the syngas platform with the carboxylate platform may be technically feasible after system improvements are made, and a discussion of these improvements is provided.

2. Methods

2.1. Inoculum and substrate for the bioreactor

The inoculum for the chain-elongating bioreactor was obtained from the effluent of a 5-L chain-elongating fermentor, which was fed corn beer for ~2 years and included a liquid–liquid extraction system (Agler et al., 2012). The substrate for the chain-elongating bioreactor was the effluent from a two-stage, 5-L syngas fermentor with a pure culture of *C. ljungdahlii* strain ERI-2 (Richter et al., 2013). The synthetic syngas to the syngas fermentor consisted of a procured gas mixture of 65% carbon monoxide, 5% carbon-dioxide, and 30% hydrogen (Airgas East, Ithaca, NY). The substrate that was fed to the chain-elongation bioreactor during days 0–23 of the operating period (Batch I) included ethanol and acetic acid concentrations of 11.4 and 2.3 g L^{-1}, respectively. The substrate that was fed during Days 24–29 (Batch II) consisted of considerably lower ethanol and acetic acid concentrations that were artificially maintained at 1.8 and 1.2 g L^{-1}, respectively, with procured chemicals, because of an unanticipated crash in the clostridia production rates during that period in the syngas fermentation system. Both Batch I and II substrate solutions were supplemented with 10 mL L^{-1} trace metal solution (Rajagopalan et al., 2002), 5 mL L^{-1} 2× vitamin solution (Rajagopalan et al., 2002), 1 mL L^{-1} yeast extract, and 2.9 g L^{-1} of potassium bicarbonate (it was not investigated whether these growth factors and nutrients were necessary). Bicarbonate was added because *C. kluyveri* (one of the microbial species that performs reversed β-oxidation pathway) requires carbon dioxide for its metabolism (Kenealy et al., 1995). 5 mM resazurin was added as an oxygen indicator.

2.2. Operating conditions for the anaerobic filter

The AF, with a total volume of 700 mL, was made from plastic with a cone at the bottom, and contained porcelain berl saddles with an outside diameter of 8 mm (CG-1284, Chemglass Life Sciences, Vinelan, NJ), resulting in an active volume of 425 mL. The bioreactor was operated in upflow mode and the feed substrate was pumped with a peristaltic pump (model 7528-10, Cole-Parmer, Vernon Hills IL) from a refrigerator (4 °C) to the bottom of the AF at a flow rate of 0.5 mL min^{-1}, resulting in a hydraulic retention time (HRT) of 14 h (Fig. 1). The mixed liquor of the AF was continuously recirculated using a peristaltic pump (model 7553-10, Cole-Parmer) at a flow rate of 22.7 mL min^{-1}. The temperature was maintained at 30 °C with a custom-built recirculating water heater and a hose wrapped around the bioreactor. The pH of the system was maintained at 5.5–5.7 for the first 6 days of the operating period, was increased to close to 6.5 between days 6 and 10, and was decreased again to below 5.5 after day 10. 0.5 M KOH or 0.5 M HCl was pumped for maintaining the pH at the set point by using a controller (alpha-pH 8000, Eutech Instruments, Singapore).

The offgas was passed through a bubbler and into a gas meter (MilliGascounter type MGC-1 PVDF, Ritter, Bochum, Germany) to determine the gas production rate (Fig. 1). At the start of the operating period, the bioreactor was completely filled with inoculum and the recirculation pump was operated for 24 h without the addition of substrate. After 24 h, the bioreactor was fed with substrate and sample volumes of 2 ml were collected daily during the operating period and centrifuged at 9.3*g* for 10 min. The supernatant was frozen at −20 °C for further analysis.