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Chain elongation of acetate and ethanol in an upflow anaerobic filter for high rate MCFA production

T.I.M. Grootscholten *, K.J.J. Steinbusch, H.V.M. Hamelers, C.J.N. Buisman

Sub-Department of Environmental Technology, Wageningen University, P.O. Box 17, 6700 AA Wageningen, The Netherlands

HIGHLIGHTS

- A new reactor system for chain elongation in non-sterile conditions was investigated.
- The volumetric MCFA production rate is in the range of other fermentation processes.
- The volumetric caprylate production rate was improved more than 16 times.
- Highest reported caprylate concentration by chain elongation was achieved.
- By increasing the ethanol load, the selectivity of MCFA production improved.

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ABSTRACT

Recently, interest has regained for medium chain fatty acids (MCFAs) as a low cost feedstock for bio-based chemical and fuel production processes. To become cost-effective, the volumetric MCFA production rate by chain elongation should increase to comparable rates of other fermentation processes. We investigate the MCFA production process at a hydraulic retention time of 17 h in an upflow anaerobic filter to improve the volumetric MCFA production rate. This approach resulted in a MCFA production with a volumetric production rate of 16.6 g l^{-1} d^{-1}, which is more than seven times higher than the current production rate. Moreover the rate is now in the range of other fermentation processes like methane, butanol and ethanol production. Increasing the ethanol load lead to higher volumetric production rates and a high MCFA selectivity of 91%. During operation, methane percentages lower than 0.1% were detected in the headspace of reactor.

1. Introduction

In the search for bio-based chemical and fuel production processes, often costs are limiting the transition from fossil to bio-based chemicals and fuels. Fermentations are one the key processes for the production of bio-based chemicals and fuel. However, fermentations have a high water content and their products are often soluble in water. The extraction of miscible fermentation products in water (like ethanol) is a large cost factor, because it is energy intensive. Ethanol requires significant amount of the energy for its distillation from the fermentation broth. As alternative feedstock for fuel production, medium chain fatty acids (MCFA) could be produced. The MCFA fermentation got recently more interest, because its products can be produced in high concentrations close to the solubility of their undissociated form in water (Steinbusch et al., 2011). Furthermore, the MCFA fermentation can use diluted ethanol directly (without its ineffective distillation) as a substrate (Agler et al., 2012). Moreover, MCFAs, straight carboxylic acids with six to eight carbon atoms, can be produced in a non-sterile environment and have more versatile applications than ethanol, including antimicrobials (directly), biofuels or bioplastics (both indirectly) (Levy et al., 1981; Witholt and Kessler, 1999). By producing these biochemical in a non-sterile environment, no costly sterilisation step is required.

MCFAs can be produced from intermediate biochemical produced in the anaerobic digestion process, like acetate, propionate and other volatile fatty acids (VFAs). These intermediate products can be produced from low grade biomass (Agler et al., 2011; D'Addario et al., 1993). However, they are mostly completely miscible in water and are difficult to extract. By adding a second fermentation step with addition of diluted ethanol, the carbon chain length of the VFAs is elongated to six, seven or even eight carbon atoms. This elongated carbon chain makes the MCFAs more hydrophobic than VFAs and drastically decreases their solubility in water. This MCFA production process, which elongates VFAs with ethanol, is called the chain elongation process and is considered

* Corresponding author. Tel.: +31 317 483227; fax: +31 317 482108.
E-mail address: Tim.Grootscholten@wur.nl (T.I.M. Grootscholten).

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to be a breakthrough, because of better separation properties from water than volatile fatty acids (VFAs) and ethanol and promising selectivity (Agler et al., 2011; Angenent and Kleerebezem, 2011).

To become a cost-effective platform technology for the production of biochemicals, several limitations of the non-sterile chain elongation process have to be improved. First, the MCFA volumetric production rate should be improved with high MCFA concentrations for easier separation. Second, the ethanol consumption should lead to selective MCFA production otherwise valuable ethanol is wasted. Because we work in non-sterile conditions, competitive processes like methanogenesis from acetate should be limited to obtain this selective MCFA production. According to Agler et al. (2012), chain elongation and methanogenesis from carbon dioxide and hydrogen are not competitive processes. Hence, the need to limit hydrogenotrophic methanogens does not seem to be as essential as the required limitation of acetotrophic methanogens. Finally, the operation of the chain elongation process should be robust and stable without additional chemical agents, like 2-Bromoethanosulfophate (2-BES), for methane inhibition.

Steinbusch et al. (2011) achieved a volumetric caproate rate of 0.5 g l^{-1} d^{-1} (0.1 mol e eq l^{-1} d^{-1}) at a concentration of 8.2 g l^{-1} (71 mM) from acetate and ethanol. Additionally, their maximum volumetric caprylate production rate reached 54 mg l^{-1} d^{-1} (16 mol e eq l^{-1} d^{-1}) with a concentration of 0.3 g l^{-1} (2 mM). Recently, Agler et al. (2012) achieved a volumetric caproate production rate of 2.1 g l^{-1} d^{-1} (0.6 mol e eq l^{-1} d^{-1}) from dilute ethanol in yeast-fermentation beer. In contrast to Steinbusch et al. (2011), Agler et al. (2012) mentioned no significant amount of caprylate production. A possible explanation for the lack of significant caprylate production in their study could be the removal of caproate from the fermentation broth by use of in-line extraction, which was not used in Steinbusch et al. (2011). However, Agler et al. (2012) produced caproate without an additional chemical agent for methane inhibition, while Steinbusch et al. (2011) applied 2-BES.

To increase the volumetric productivity of a MCFA production process, better retention of MCFA producing biomass is desired. Although, reactor systems with (internal) settlers retain more MCFA producing biomass, they retain unwanted microbial populations, such as acetotrophic methanogens, as well. Consequently, an ideal MCFA producing system should retain MCFA producing biomass and avoid accumulation of methanogenic biomass.

A possible reactor system to increase biomass concentrations selectively is the upflow anaerobic filter (or anaerobic fixed film bioreactor), which has a carrier material for biomass support. These reactors are well-known in mixed culture fermentations and have been used for various applications in environmental biotechnology, including caproate production from glucose (Ding et al., 2010) and methane production systems (Raynal et al., 1998; Hamoda et al., 1996). For methane production, these were not applied as much as reactor systems having internal settlers, like upflow anaerobic sludge blankets (UASBs) and expanded granular sludge beds (EGSBs), because less biomass was retained in the anaerobic filters (*e.g.* Ruiz et al., 1997). Moreover, the additional cost of packing materials in anaerobic filters makes them less favourable for methane production than internal settler systems. An explanation for lower biomass retention in methanogenic anaerobic filters can be found in the type of biomass formation, which cannot resist large shear stress in case the upflow velocity larger than 1 m s^{-1} is applied (Smith et al., 1996; Suraruksa et al., 2003). Once the methanogenic biomass is detached from its carrier, it could be washed-out of the reactor if the hydraulic retention time (HRT) is low enough.

In this research, we investigate an anaerobic filter to improve the volumetric MCFA production rate of the chain elongation process based on acetate. The filter was filled with polyurethane cubes as carrier material. For selective MCFA production, the reactor had a HRT of 17 h to retain detached MCFA producers and to reduce the impact of free methanogens. Furthermore, an upflow velocity of 1.2 m s^{-1} was used to stress methanogens that could be attached to the carrier material. Additionally, we want to investigate the effect of the ethanol concentration in the reactor, because Steinbusch et al. (2011) noticed that the volumetric MCFA production rate was low in case of low ethanol concentrations in the reactor.

2. Methods

2.1. Reactor set-up and inoculum

The experiments were executed in one litre glass upflow reactor filled with polyurethane cubes (Recticel, Belgium) to promote biomass retention. A liquid recycle with a recirculation rate of 2.5 l/h was applied for mixing the reactor. For both influent and liquid recycle peristaltic pumps were used (Marlon Watson 403 U/R1, U.K.). The temperature of the reactor was controlled at 30.0 ± 0.1 °C with a water bath (Julabo, Germany). The influent was prepared in an anaerobic hood and stored anaerobically in a refrigerator. Fig. 1 shows the schematic overview of the reactor set-up. The inoculum was derived from a mixed culture continuous stirred tank reactor

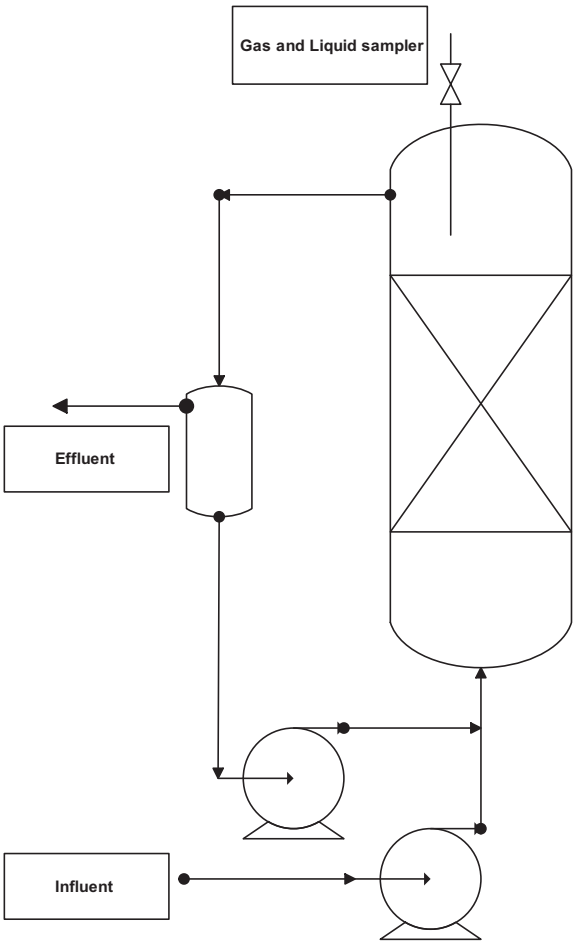

Fig. 1. Schematic overview of reactor set-up. Influent is pumped up from the refrigerator (not shown) into the bottom of the upflow anaerobic filter. On the top, the liquid and the gas in the reactor overflow into a small vessel, in which the excess of gas and liquid is removed. The other liquid (and gas) is recycled to the bottom of the reactor. Liquid samples are taken from inside the anaerobic filter, while gas samples are taken in the headspace of reactor (by pulling up the needle to the headspace).