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Short Communication

High rate heptanoate production from propionate and ethanol using chain elongation

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HIGHLIGHTS

- Heptanoate production rate from propionate was improved by more than 25 times.
- Obtained heptanoate concentrations higher than heptanoic acid solubility in water.
- Limited heptanoate selectivity mainly due to caproate and valerate production.
- A higher ethanol load increased heptanoate, caproate and propanol production.
- First time that traces of pelargonate were measured during chain elongation.

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ABSTRACT

Heptanoate (or enanthate), a saturated mono-carboxylate with seven carbon atoms, is a commercially produced biochemical building block with versatile applications. Currently, heptanoate is mainly derived from the oxidation of heptaldehyde, which can be obtained after pyrolysis of castor oil. The objective of this investigation was to achieve efficient high rate heptanoate production using a mixed culture chain elongation process based on propionate and ethanol. An efficient high rate heptanoate production using chain elongation could offer an alternative for heptanoate production from castor oil. The investigation was performed in an upflow anaerobic filter with a hydraulic retention time of 17 h. A heptanoate production rate of 4.5 g l^{-1} d^{-1} was achieved with a heptanoate concentration of 3.2 g l^{-1}. These results show sufficient potential to consider this approach as an alternative for heptanoate production from castor oil. Future research should make heptanoate production from propionate and ethanol more cost-effective.

1. Introduction

Heptanoate (or enanthate), a saturated mono-carboxylate with seven carbon atoms, is a commercially produced biochemical building block with versatile applications, including biodiesel production (Renz, 2005), antimicrobials (e.g. Woolford, 1975) and bioplastic production (e.g. Liebergesell et al., 1991). Currently, heptanoate is mainly derived from the oxidation of heptaldehyde, which can be obtained after pyrolysis of castor oil (Das et al., 1989). In this thermochemical step, the castor oil is cleaved into undecylenic acid as major product and heptaldehyde as by-product.

However, heptanoate production from castor oil does have some disadvantages. First, the castor oil production itself is inefficient, only the seeds of the plants are used for castor oil production instead of the complete plant[1]. Second, most of the castor oil production is currently located in India, China, Brazil and Thailand (Ogunniyi, 2006). For castor oil importing countries, these limited production areas could lead to high prices due to lack of competition. Finally, the overall heptanoate selectivity from castor oil does not exceed 25% (castor oil as 100% Ricinoleic acid assumed). An alternative heptanoate production process could avoid these disadvantages.

Chain elongation could be such an alternative process. It is a fermentation that produces medium chain fatty acids (MCFAs) from volatile fatty acids (VFAs) and ethanol, in which undistilled ethanol can be used (Agler et al., 2012). Both VFAs and ethanol can be produced from lignocellulosic (waste) materials (Agler et al., 2011; Sarkar et al., 2012), which can contain a larger part of the plant than the seeds. Moreover, chain elongation is an efficient fermentation with a MCFA selectivity of more than 80% (Agler et al., 2012; Grootscholten et al., 2013a). In the studies of Steinbusch et al. (2011) and Agler et al. (2012), *Clostridium kluyveri* was found as dominant chain elongating micro-organism. However, it is possible that other (unknown) micro-organisms could perform chain elongation as well.

So far, research on the chain elongation process has mainly focussed on caproate (Steinbusch et al., 2011; Agler et al., 2012;

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[1] The plant (*Ricinus communis*) has other applications that are not discussed here.

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Grootscholten et al., 2013a), a straight mono-carboxylate with six carbon atoms, resulting in caproate production rates of 15.7 g caproate per litre per day (4.3 mol e eq l^{-1} d^{-1}). However, it is known that heptanoate can be produced by chain elongation as well, if propionate and ethanol are used (Bornstein and Barker, 1948).

Heptanoate is often found in low concentrations (up to 0.4 g l^{-1}(3 mM)) during acidification of the organic fraction of municipal solid waste (OFMSW) without ethanol additions (e.g. Grootscholten et al., 2013b). Bornstein and Barker (1948) were the first to report heptanoate production from propionate and ethanol with a pure culture of *C. kluyveri*. They achieved a heptanoate concentration of 0.3 g l^{-1}(2 mM) in their batch experiments. Smith and McCarty (1989) found temporarily elevated heptanoate concentrations (up to 1.5 g l^{-1}(12 mM)) after perturbations with ethanol in mixed culture propionate fed continuous reactors. Grootscholten et al. (2013b) produced heptanoate from OFMSW after ethanol additions during acidification (up to 1.5 g l^{-1}(12 mM)) in dry anaerobic batch reactors, but the maximum heptanoate production rate was very low (0.3 g C7 l^{-1} d^{-1} (0.1 mol e eq l^{-1} d^{-1})). Toxic effects of undissociated MCFAs and/or ethanol on hydrolysis could be reasons for the low productivity. Consequently, the VFA production rate is lowered and less substrate for chain elongation is available.

The objective of this investigation was to achieve efficient high rate heptanoate production using a mixed culture chain elongation process based on propionate and ethanol. Propionate can be produced from several precursors, including glucose, lactate (Agler et al., 2011) and glycerol (Schauder and Schink, 1989). For this investigation, a synthetic medium with a high propionate concentration (7.4 g l^{-1}(100 mM)) was used to be able to compare heptanoate production with caproate production (Grootscholten et al., 2013a) in terms of conversion rates, concentrations and selectivity. An efficient high rate heptanoate production using chain elongation could offer an alternative for heptanoate production from castor oil.

2. Methods

2.1. Reactor set-up and experiment

The reactor set-up and methods were the same as in Grootscholten et al. (2013a). In addition, pelargonate, a straight mono-carboxylate with nine carbon atoms, and propanol were measured as well. We applied a mixed culture upflow anaerobic filter with a hydraulic retention time (HRT) of 17 h. The temperature was controlled at 30.0 ± 0.1 °C and the pH was maintained between 6.5 and 7.0. No chemical agent for methane inhibition, such as 2-bromoethanosulfonate (2-BES), was used. The substrate consisted of acetate, propionate and ethanol instead of acetate and ethanol. Table 1 shows the substrate load during the experiment. Acetate was supplemented to sustain biomass concentrations as it is a required compound for protein synthesis in *C. kluyveri* (Tomlinson and Barker, 1954). After one week, the acetate load was lowered as sufficient acetate was internally produced (Fig. 1b). Two weeks after the start of the experiment, the ethanol load was increased to eliminate the ethanol limitation, because the ethanol concentration was below 1.0 g l^{-1}.

Table 1
Experimental overview of the investigation in different phases.

Phase	Days after start experiment	Acetate load (g l^{-1} d^{-1})	Propionate load (g l^{-1} d^{-1})	Ethanol load (g l^{-1} d^{-1})
I	Day 0 to day 7	1.7	10.4	13.0
II	Day 8 to day 14	0.9	10.4	13.0
III	Day 15 to day 21	0.9	10.4	19.5

2.2. Calculations

2.2.1. Selectivity

The selectivity is defined as the concentration of electrons in the formed product divided by the net consumed electrons from the fed acetate, propionate and ethanol (1). Acetate contains 8 mol electrons per mole, propionate 14 mol electrons per mole, ethanol 12 mol electrons per mole, butyrate 20 mol electrons per mole, valerate 26 mol electrons per mole, caproate 32 mol electrons per mole, heptanoate 38 mol electrons per mole and caprylate 44 mol electrons per mole.

$$\text{Selectivity} = [\text{Product}]\,(\text{mol e eq } l^{-1}) / \text{consumed acetate, propionate and ethanol}\,(\text{mol e eq } l^{-1}) \quad (1)$$

2.2.2. Volumetric production rate

The volumetric production rate of the products (butyrate, valerate, caproate, heptanoate and caprylate) is defined by its concentration divided by the HRT (2). The unit of the volumetric production rate is g l^{-1} d^{-1} and moles electron equivalents l^{-1} d^{-1} (mol e eq l^{-1} $d^{-1)}$.

$$\text{Volumetric production rate} = [\text{Product}]\,(\text{g } l^{-1} \text{ or mol e eq } l^{-1}) / \text{HRT}\,(\text{d}) \quad (2)$$

3. Results and discussion

3.1. High concentrations of heptanoate and traces of pelargonate were produced

High rate heptanoate production was demonstrated in a steady state operating continuous flow bioreactor. A heptanoate concentration of 3.2 g l^{-1} (25 mM) was obtained at a production rate of 4.5 g l^{-1} d^{-1} (1.3 mol e eq l^{-1} d^{-1}) with a selectivity of 23%. The obtained concentration is higher than the solubility of heptanoic acid (undissociated form) in water under standard conditions, which is 2.4 g l^{-1} (18 mM). In other words, this concentration shows sufficient potential to develop separation methods for heptanoate removal from fermentation broths. Compared to the work of Smith and McCarty (1989), we doubled the maximum heptanoate concentration and improved the heptanoate production rate by more than 25 times. Moreover, the formed heptanoate was not degraded probably due to absence of hydrogenotrophic methanogens (methane percentages were below 0.1% in the headspace of the reactor). The absence of methanogens could be related to the inoculum which may not have contained active methanogens (Grootscholten et al., 2013a). If present, hydrogenotrophic methanogens could lower the partial hydrogen pressure and can make heptanoate oxidation thermodynamically feasible (e.g. Stams, 1994).

During the two last days of the experiment, low concentrations of pelargonate (<25 mg l^{-1}) were found as well. In analogy with caproate and caprylate (Steinbusch et al., 2011), pelargonate could be formed from heptanoate and ethanol. Pelargonate formation from caproate and propanol might also be a possibility, as propanol can also be used by *C. kluyveri* (Table 3, reaction 3; Kenealy and Waselefsky, 1985).

Before the start of the experiment, the reactor produced mainly caproate from acetate and ethanol (Grootscholten et al., 2013a). Directly after adding propionate, valerate and heptanoate were produced (Fig. 1). The absence of a lag phase indicates the biomass in the reactor did not need to adapt from acetate elongation to propionate elongation.