# Methanol-based chain elongation of acetate to i/n- butyrate at thermodynamic equilibrium of isomerization by an enriched microbiome

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## Authors

Mr. Kasper de Leeuw - Wageningen University & Research

Ms. Sanne de Smit - Wageningen University & Research

Ms. Sabine van Oossanen - Wageningen

Mr. Merijn Moerland - Wageningen University

Prof. Cees Buisman - Wageningen University

Dr. David Strik - Wageningen University

## Abstract

Kasper D. de Leeuw [*,a], Sanne M. de Smit [a], Sabine van Oossanen [a], Marinus J. Moerland [a], Cees J. N. Buisman [a], David P.B.T.B. Strik [a].

* Kasper de Leeuw, kasper.deleeuw@wur.nl

[a] Wageningen University & Research, The Netherlands

**HIGHLIGHTS:**

- An enriched methanol-based chain elongation microbiome was capable of producing isobutyrate and n-butyrate at a concentration ratio that approached thermodynamic equilibrium of isomerization.

The usage of isobutyrate as electron acceptor within an enriched ethanol-based chain elongation microbiome led to isocaproate formation. Similarly isovalerate addition led to isoheptanoate formation, however at a much lower selectivity.

- Carboxylates larger than acetate can be reduced to their corresponding alcohols with electrons derived from ethanol oxidation towards acetate. To drive this process the reactant to product ratios of the coupled reaction need to be sufficiently high.

**BACKGROUND**: Microbial chain elongation can be employed to convert organic residues into platform chemicals such as carboxylates and alcohols. Sometimes trace amounts of branched carboxylates are observed, but until now no branched chain elongation process was developed. We investigated how to enrich microbiomes to form branched carboxylates and thereby expand the product spectrum of microbial chain elongation and its application range. In earlier research it was shown that isobutyrate could be formed using a microbiome that performed methanol-based chain elongation[62]. Toxicity of high butyrate concentrations was suggested as one of the causes for isomerization. A hypothesis was formulated that by lowering the pH and thereby aggravating the butyrate toxicity, isobutyrate formation from acetate and methanol could be stimulated. A long term continuous reactor experiment was operated at various pH levels to enrich the microbiome towards production of n-butyrate and isobutyrate or mainly n-butyrate[63].

In the realm of ethanol-based chain elongation, it has extensively been shown that odd-chains (e.g. n-propionate and n-valerate) can be elongated by two carbon atoms at a time[64]. It was hypothesized that isobutyrate could be elongated to isocaproate in a similar fashion. We verified this hypothesis via a long-term continuous reactor experiment[65]. Additionally, we performed experiments to increase the selectivity of the discovered branched chain elongation. This led to more insights into alcohol formation during chain elongation.

**RESULTS & DISCUSSION**:

We showed with the methanol reactor experiment that the selectivity for i-$C_4$ and/or n-$C_4$ could be reversibly adjusted by operating at different reactor pH values. A reactor pH of 6.75 led to formation of (carbon per total carbon of products) 0.78 n-$C_4$ and 0.024 i-$C_4$, whereas a reactor pH of 5.2 led to a selectivity of 0.24 n-$C_4$ and 0.65 i-$C_4$. A microbial community analysis showed that a *Eubacterium* genus was responsible for the formation of n-$C_4$, whereas a *Clostridium luticellarii* strain was responsible for the formation of a mixture of i-$C_4$ and n-$C_4$. At low pH (5.2-5.5) the isobutyrate and n-butyrate concentration ratios approached thermodynamic equilibrium of isomerization. The highest achieved volumetric productivity for isobutyrate was 2.4 ±0.3 g $L^{-1}$.

The ethanol reactor experiment showed that isocaproate (4-methyl pentanoate, i-$C_6$) can be produced via ethanol based chain elongation of isobutyrate. The enriched microbiome was dominated by *Clostridium kluyveri* and formed isocaproate from

isobutyrate and ethanol at a rate of 1.4 ± 0.1 g $L^{-1}$ $day^{-1}$. This amounted to 20% of all formed compounds based on carbon atoms. The presence of other electron acceptors, besides isobutyrate, strongly reduced the selectivity of isocaproate formation; there was a strong preference for straight chain elongation. Because acetate elongation towards straight chains is competing with branched chain elongation for ethanol, reducing the available acetate was one of the strategies to increase selectivity for isocaproate formation. However, when acetate was low, while ethanol and larger carboxylates were abundantly available, this stimulated reduction of the larger carboxylates to their corresponding alcohols. Electrons for this reduction seemed to originate from ethanol oxidation. The effect was augmented when the $CO_2$ supply became limiting for hydrogenotrophic methanogenesis, allowing the reducing equivalents to be channelled towards formation of the alcohols instead.

**CONCLUSION**:

A microbiome was successfully enriched to perform methanol-based chain elongation of acetate to form both n-butyrate and isobutyrate (at pH 5.2 and 5.5). Further we show that ethanol-based chain elongation can used to elongate isobutyrate towards isocaproate and that alcohol formation can be stimulated during acetate and $CO_2$ limited operation. These results provide the pioneering basis to further develop new products from open culture chain elongation fermentation systems.