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Perspectives for the microbial production of methyl propionate integrated with product recovery

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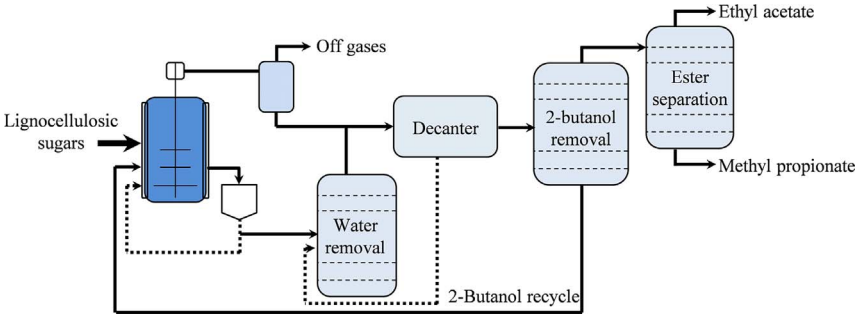
GRAPHICAL ABSTRACT



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ABSTRACT

A new approach was studied for bio-based production of methyl propionate, a precursor of methyl methacrylate. Recombinant *E. coli* cells were used to perform a cascade reaction in which 2-butanol is reduced to butanone using alcohol dehydrogenase, and butanone is oxidized to methyl propionate and ethyl acetate using a Baeyer-Villiger monooxygenase (BVMO). Product was removed by *in situ* stripping. The conversion was in line with a model comprising product formation and stripping kinetics. The maximum conversion rates were 1.14 g-butanone/(L h), 0.11 g-ethyl acetate/(L h), and 0.09 g-methyl propionate/(L h). The enzyme regioselectivity towards methyl propionate was 43% of total ester. Starting from biomass-based production of 2-butanol, full-scale ester production with conventional product purification was calculated to be competitive with petrochemical production if the monooxygenase activity and regioselectivity are enhanced, and the costs of bio-based 2-butanol are minimized.

1. Introduction

Polymethyl methacrylate (PMMA) is a valuable thermoplastic known for its excellent performance characteristics, and its vast application in several fields (Ali et al., 2015). Its market size is expected to exceed USD 11 billion by 2022 (Global Market Insights, 2016). Nonetheless, industrial production methods rely on fossil carbon sources and precious metal catalysts, and generate greenhouse gases (Clegg et al., 1999; Liu et al., 2006). To mitigate these issues, the producers seek opportunities for biomass-based approaches for methyl methacrylate production.

In the present work, we propose a pathway for biomass-based methyl methacrylate that comprises a series of reactions under distinct oxygen regimes, which is summarized in Fig. 1. First, fermentable sugars such as lignocellulosic hydrolysate are converted into butanone by anaerobic fermentation (Chen et al., 2015; Yoneda et al., 2014). This fermentation includes butanone conversion into 2-butanol by a suitable NADH-dependent alcohol dehydrogenase (ADH), to generate the

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Nomenclature

C	concentration (mol/L)
C^*	concentration at the liquid interface (mol/L)
D	diffusivity (cm^2/s)
F	mole flow rate (mol/h)
H	Henry coefficient bar/(mol L)
$k_L a$	volume-specific mass transfer coefficient (1/h)
K_i	distribution coefficient (bar/bar)
K_M	Michaelis constant (mol/L)
M_w	molecular mass (g/mol)
n	number of moles (mol)
P	pressure (bar)
P^{Sat}	saturation pressure (bar)
r	rate of formation mol/(L h)
t	time (h)
T	temperature (K)
V	volume (L)
x	mole fraction in the liquid phase (–)
y	mole fraction in the vapor phase (–)

Greek symbols

γ	activity coefficient (–)
μ	microbial growth rate (1/h)
θ	transfer rate from liquid to gas phase (mol/h)
ρ	density (g/L)

Subscripts

BuOH	2-butanol
But	butanone
C	carbon dioxide
ester	ester (methyl propionate or ethyl acetate)
EtAc	ethyl acetate
G	gas phase
i	component i
j	component j
L	liquid phase
max	maximum rate
MePr	methyl propionate
N	nitrogen
O	oxygen
S	substrate
sol	organic solvent
X	cell mass

essential NAD^+ required for closing the redox balance. The conditions for profitable bio-based 2-butanol production have been determined (Pereira et al., 2017). Next, in an aerobic biotransformation, 2-butanol is oxidized back to butanone by a $NADP^+$-dependent ADH, and butanone is further oxidized by O_2 using cyclohexanone monooxygenase (CHMO). CHMO inserts one oxygen atom in butanone, while the other atom is reduced with NADPH to water. Although oxygen insertion next to the most substituted carbon yields the so-called normal product, ethyl acetate, the regioselectivity of this reaction has been shifted to the abnormal product, methyl propionate (van Beek et al., 2017). Methyl propionate is finally condensed with formaldehyde to form methyl methacrylate, in a downstream chemistry step which is outside the scope of this study, as it resembles the last part of the existing Alpha process for methyl methacrylate production (Eastham et al., 2013).

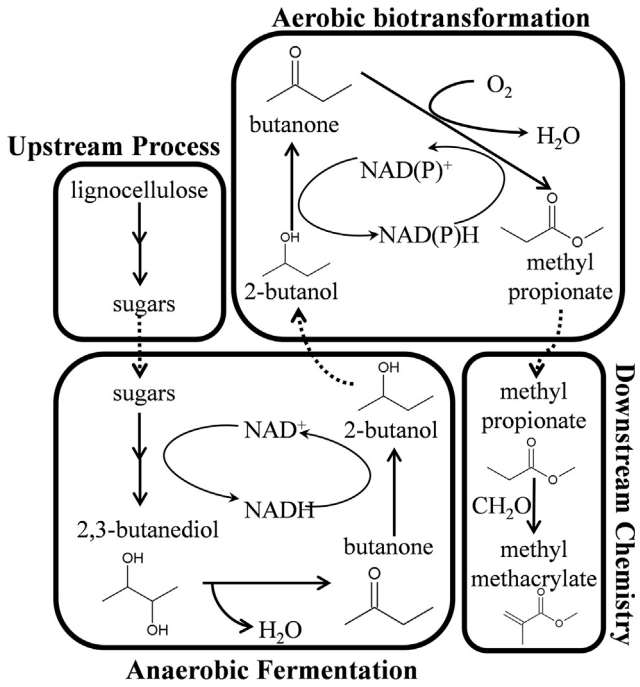

Fig. 1. Pathway for the production of methyl methacrylate from lignocellulose via 2-butanol and methyl propionate.

The current research evaluates an integrated biotransformation for methyl propionate production, aiming to identify the major process bottlenecks at an early stage of strain engineering and process design. Recombinant *Escherichia coli* cells expressing two distinct fused redox-complementary enzymes (Aalbers and Fraaije, 2017) have been used to determine the conversion rate of 2-butanol and identify the rate-limiting step in the cascade reaction.

As this is a strictly aerobic conversion, the continuous aeration promotes the stripping of the volatile biotransformation products along with the exhaust gas. Although stripping is useful to alleviate product toxicity and enhance product recovery costs (de Vrije et al., 2013; Xue et al., 2013), uncontrolled product loss makes the ester quantification a challenging task, demanding full understanding of the stripping process. Using stripping and product formation kinetics, the performance of a conceptual 120 kton/a process for bio-based methyl propionate production will be assessed.

The inevitable by-product, ethyl acetate, is an environmentally friendly solvent with broad application range, and its commercial value is close to that of methyl propionate itself (Straathof and Bampouli, 2017). Despite its current petrochemical-based production, numerous yeast species are natural ethyl acetate producers, particularly *Kluyveromyces marxianus* with an outstanding formation rate of 0.67 g/g/h, and 56% of the maximum theoretical yield (Urit et al., 2013). Recently, Kruis et al. (2017) reported ethyl acetate production using recombinant *E. coli*, reaching 33% of the maximum theoretical yield. In the two-step process proposed herein, the maximum achievable theoretical yield is 0.453 $g_{ester}/g_{glucose}$, similar to that of direct glucose conversion (Löser et al., 2014). This suggests the high potential of the proposed process for bio-based ester production.

2. Material and methods

2.1. Bacterial strains and culture media

Fusion constructs containing the cyclohexanone monooxygenase (TmCHMO) gene from *Thermocrispum municipale*, and either the alcohol