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Caproate formation in mixed-culture fermentative hydrogen production

Hong-Bo Ding, Giin-Yu Amy Tan, Jing-Yuan Wang *

School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

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ABSTRACT

Caproate always appears during fermentative H_2 production but its formation was not well explained. It possibly results from the secondary fermentation of ethanol and acetate or butyrate by some special species like *Clostridium kluyveri*. This study attempts to elucidate caproate formation during the fermentation H_2 production by using *C. kluyveri* as an example and evaluating several possible pathways of caproate formation. A detailed energetic analysis of the empirical data of an H_2-producing reactor demonstrated that caproate can be formed from two substrates, either ethanol and acetate or ethanol and butyrate. The analysis showed that at least 5 mol ethanol per mole reaction was essential to support caproate formation under the experimental condition. The analysis also indicated that the secondary fermentation by *C. kluyveri* might be another pathway to spontaneously produce H_2, butyrate, and acetate in addition to the butyrate-acetate pathway. Co-production of caproate and H_2 from ethanol was thermodynamically feasible and contributed to at least 10–20% of total H_2 production in the reactor studied. It is also clarified that caproate formation is hydrogenogenic rather than hydrogenotrophic.

1. Introduction

Fermentative H_2 production is a special case of acidogenesis in anaerobic digestion (AD). In this process, hydrogenotrophs such as methanogens are severely inhibited while hydrogenogenic acidogens are enriched to convert carbohydrates to H_2, volatile fatty acids (VFAs), and alcohols. Due to the accumulation of VFAs and alcohols and the inhibition of methanogens, some interesting phenomena emerge. One of these phenomena is formation of high-carbon VFAs such as caproate, valerate and heptanoate. The concentrations of caproate and valerate reported are summarized in Table 1. Significant amounts of caproate and valerate were always found in several granule-based upflow anaerobic sludge blanket reactors (Yu and Mu, 2006; Zhao and Yu, 2008; Zhao et al., 2008) and also in other granule-based reactors (Lee et al., 2004; Zhang et al., 2007).

Caproate is a unique product of only a few species including *Eubacterium alactolyticus*, *E. biforme*, *E. limosum* and *E. pyruvativorans*, as well as *Clostridium kluyveri*, *Peptococcus niger* and *Megasphaera elsdenii* (Dworkin et al., 2006). Among all the caproate-producing species mentioned thus far, the spore-forming *C. kluyveri* appears to be the most possible species resistant to heat treatment or chemical treatment used for the enrichment of H_2-producing bacteria. Additionally, ethanol produced under acidic environment in fermentative H_2 production only favors the growth of *C. kluyveri*, while inhibiting the growth of other caproate-producing species, especially *M. elsdenii* (Dworkin et al., 2006).

Caproate formation by *C. kluyveri* can be simply described by three coupled reactions in Eqs. (1)–(3) (Thauer et al., 1968; Schoberth and Gottschalk, 1969; Seedorf et al., 2008):

$$CH_3CH_2OH + H_2O = CH_3COO^- + H^+ + 2H_2 \quad (1)$$
$$\Delta G_r^{0\prime} = +9.7 \text{ kJ mol}^{-1}$$

$$CH_3CH_2OH + CH_3COO^- = CH_3(CH_2)_2COO^- + H_2O \quad (2)$$
$$\Delta G_r^{0\prime} = -38.7 \text{ kJ mol}^{-1}$$

$$CH_3CH_2OH + CH_3(CH_2)_2COO^- = CH_3(CH_2)_4COO^- + H_2O \quad (3)$$
$$\Delta G_r^{0\prime} = +38.7 \text{ kJ mol}^{-1}$$

Eq. (1) describes the dehydrogenation of one ethanol leading to ATP synthesis via substrate-level phosphorylation (SLP) and H_2 formation. Eqs. (2) and (3) describe the dehydrogenation of another ethanol leading to formation of butyrate and caproate from ethanol and acetate and ethanol and butyrate, respectively. The combination of Eqs. (2) and (3) gives Eq. (4) which describes caproate formation via butyrate formation:

$$2CH_3CH_2OH + CH_3COO^- = CH_3(CH_2)_4COO^- + 2H_2O \quad (4)$$
$$\Delta G_r^{0\prime} = -77.5 \text{ kJ mol}^{-1}$$

Valerate is formed in fermentation of ethanol and propionate according to the reaction in Eq. (5) which also has to be coupled with the reaction in Eq. (1):

* Corresponding author. Tel.: +65 6790 4100; fax: +65 6792 7319.
E-mail addresses: ding0012@ntu.edu.sg (H.-B. Ding), amytangy@ntu.edu.sg (G.-Y.A. Tan), jywang@ntu.edu.sg (J.-Y. Wang).

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Table 1
Formation of caproate and valerate in the fermentative hydrogen production.

Reactors	Growth mode	Substrate	HRT, h	pH	EtOH	HA	HB	HC	HP	HV	Units	References
UASB	Granule	Sucrose	13	6.1–6.5	11–12	27–33	30–38	15–18	6–10	0.4–2.1	% of mass of VFAs and ethanol	Zhao and Yu (2008)
				8.0–8.5	11–13	15	48–51	7–8	4–5	11–12		
		Sucrose	3–6	4.4	8	8–11	10–11	8–9	0.6–1.0	0.8	mM	Yu and Mu (2006)
			10–14		7–8	7–8	5–10	11–15	0.4–0.7	0.6–1.3		
		Sucrose	12	3.9	770	380	1110	1500	100	160	mg L^{-1}	Zhao et al. (2008)
		Lactose		4.5	500	1500	1060	250	750	450		
CIGSB	Granule	Sucrose	1–4	6–7	3–5	5–10	15–25	N.A.	5–20	2–3	% of COD sucrose consumed	Lee et al. (2004)
UFBR	Biofilm	Glucose	2–3	5–6	28–49	18–33	18–32	11–23	1–2	Low	mM	This study
AFBR	Granule	Glucose	1–4	4	20–25	6–17	10–15	5–6	2–3	N.A.	mM	Zhang et al. (2007)

Note: UASB, upflow anaerobic sludge blanket; CIGSB, carrier-induced granular sludge beds; UFBR, upflow fixed-bed reactor; AFBR, anaerobic fluidized bed reactor; EtOH, ethanol; HC, caproate; HA, acetate; HB, butyrate; HP, propionate; HV, valerate.

$$CH_3CH_2OH + CH_3CH_2COO^- = CH_3(CH_2)_3COO^- + H_2O \qquad (5)$$
$$\Delta G_r^{0\prime} = -38.7 \text{ kJ mol}^{-1}$$

A comparison between the $\Delta G_r^{0\prime}$ of Eqs. (2) and (4) shows that the increment in carbon numbers (from butyrate to caproate) gives additional free energy. It implies that caproate formation can be spontaneous since it yields more energy than butyrate formation.

Several other pathways (Reactions 1–5 in Table 2) were suggested for caproate formation in the fermentative H_2 production (Yu and Mu, 2006; Zhao and Yu, 2008). The argument is that butyrate is the primary source of caproate formation pathways in Reactions 1–3, and H_2 is the electron donor of Reactions 2–4 in Table 2. However, Reaction 1 in Table 2 is unlikely to happen due to its positive Gibbs free energy change of the reaction ($\Delta G_r^{0\prime}$) under the physiological condition (1 atm, pH 7, 25 °C, concentrations: 1 atm if the reactants are gases or 1 M if the reactants are solutes). The major difference between Reactions 2–4 in Table 2 and caproate formation pathways by *C. kluyveri* is the source of reducing equivalents. In *C. kluyveri*, $NADH_2$ ($NADH + H^+$), the major carrier of reducing equivalents, is derived from ethanol dehydrogenation. As for Reactions 2–4 in Table 2, molecular H_2 is directly be utilized. Ethanol would be a much better source of reducing equivalents than H_2 to support high VFA synthesis and biomass growth (Kenealy and Waselefsky, 1985). Reaction 5 in Table 2 has ethanol and acetate as reactants and is superficially the same as the reaction in Eq. (4). However, Reaction 5 in Table 2 gives a simplified picture and does not accurately describe the observed quantitative relations among the substrates and products because H_2 formation is also observed along with caproate production (Schoberth and Gottschalk, 1969). Its occurrence for exergonic caproate formation has to be energetically coupled with endergonic ethanol dehydrogenation in Eq. (1) (Seedorf et al., 2008).

In this study, the secondary fermentation pathways by *C. kluyveri* were used to explain caproate formation during the fermentative H_2 production. Energetic analysis and thermodynamic efficiency were used to determine caproate formation pathways in an upflow fixed-bed reactor (UFBR). The mutual influence between caproate formation and H_2 production was discussed.

2. Methods

The UFBR had a working volume of 0.8 L with a diameter of 10 cm in diameter and a height of 25 cm. The cylindrical activated carbon pellets (3 mm in diameter and 7 mm in length) were used as biofilm carriers with a void ratio of 60–70%. The reactor was operated at 30 ± 1 °C. Seed sludge (45 g – VSS L^{-1} and pH 7.8) was obtained from the methanogenic reactor of a pilot-scale two-phase AD system established on the campus of the university.

Table 2
Possible pathways for butyrate and caproate formation.

No.	Pathways	Combinations	References	Remark
Reaction 1	$2CH_3(CH_2)_2COO^- = CH_3(CH_2)_4COO^- + CH_3COO^-$		Yu and Mu (2006)	Unlike to occur
Reaction 2	$CH_3(CH_2)_2COO^- + CH_3COO^- + 2H_2 + H^+ = CH_3(CH_2)_4COO^- + 2H_2O$			H_2 is Used as electron and proton donor
Reaction 3	$CH_3(CH_2)_2COO^- + 2CO_2 + 6H_2 = CH_3(CH_2)_4COO^- + 4H_2O$			
Reaction 4	$3CH_3CH_2OH + 4H_2 + 2H^+ = CH_3(CH_2)_4COO^- + 4H_2O$			
Reaction 5	$2CH_3CH_2OH + CH_3COO^- = CH_3(CH_2)_4COO^- + 2H_2O$			Incomplete expression
Eq. (6)	$(k+1)CH_3CH_2OH + (k-1)CH_3COO^- = kCH_3(CH_2)_2COO^- + H^+ + 2H_2 + (k-1)H_2O$	Eq. (1)+k×Eq. (2)	Schoberth and Gottschalk (1969)	*C. kluyveri produces butyrate only from ethanol and acetate*
Eq. (7)	$(k+1)CH_3CH_2OH + (\frac{k}{2}-1)CH_3COO^- = \frac{k}{2}CH_3(CH_2)_2COO^- + H^+ + 2H_2 + (k-1)H_2O$	Eq. (1)+k/2×Eq. (4)		*C. kluyveri produces caproate from ethanol and acetate. Butyrate is considered as an intermediate product.*
Eq. (8)	$(k+1)CH_3CH_2OH + kCH_3(CH_2)_2COO^- = kCH_3(CH_2)_4COO^- + CH_3COO^- + H^+ + 2H_2 + (k-1)H_2O$	Eq. (1)+k× Eq. (3)	This study	*C. kluyveri produces caproate only from ethanol and butyrate.*