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# Acetone-butanol-ethanol production in a novel continuous flow system

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#### HIGHLIGHTS

• A novel continuous fermentation system with clarifier was used for ABE production.

• Mixed culture was used for ABE fermentation.

• Retention of ABE fermenters in the system improved ABE yield and production rate.

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#### 1. Introduction

## ABSTRACT

This study investigates the potential of using a novel integrated biohydrogen reactor clarifier system (IBRCS) for acetone–butanol–ethanol (ABE) production using a mixed culture at different organic loading rates (OLRs). The results of this study showed that using a setting tank after the fermenter and recycle the settled biomass to the fermenter is a practical option to achieve high biomass concentration in the fermenter and thus sustainable ABE fermentation in continuous mode. The average ABE concentrations of 2.3, 7.0, and 14.6 g ABE/L which were corresponding to ABE production rates of 0.4, 1.4, and 2.8 g ABE/L<sub>reactor</sub> h were achieved at OLRs of 21, 64, and 128 g COD/L<sub>reactor</sub> d, respectively. The main volatile fatty acids components in the effluent were acetic, propionic, and butyric acids. Acetic acid was the predominant component in the OLR-1, while butyric acid was the predominant acid in OLRs 2 and 3.

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Worldwide energy demand is increasing exponentially as population and human activities grow substantially. This increase in the energy demand has been a major challenge worldwide, since the fossil fuel sources are decreasing day by day. Therefore, biofuels production using various waste utilized biotechnologies became very attractive due to their dual benefits in terms of renewability and sustainability. Acetone–butanol–ethanol (ABE) fermentation using renewable carbohydrates is very promising biotechnology to meet the demand of conventional gasoline type fuels. Butanol and ethanol produced via ABE fermentation can be used as fuel or fuel additives, and can easily fit our existing fuel infrastructure. The global bio-butanol market is estimated to be \$250 billion by 2020 (Green, 2011).

ABE fermentation uses the metabolism of solventogenic Clostridia, strictly anaerobic spore forming bacteria. Many species of Clostridia have been widely used in anaerobic biotechnologies due to their capability to use a wide range of carbon sources to nol, ethanol, acetate, and butyrate. Various species of Clostridia such as Clostridium beijerinckii, Clostridium acetobutylicum, Clostridium saccharobutylicum, and Clostridium butyricum have been studied for ABE fermentation (Gao and Rehmann, 2014; Chang et al., 2014; Qureshi et al., 2012). Table 1 presents a list summarizing ABE productivity reported in different studies. Most of the studies conducted in batch with pure culture using a wide variety of feedstock including glucose, corn stover hydrolysate, cane molasses, wheat bran, sago pith residues, degermed corn, corncobs, and cassava chip hydrolysate. The yield of ABE production was ranging from 0.10 to 0.45 g ABE/g sugar. Gao and Rehmann (2014) recently reported almost similar ABE yield for pretreated corncobs and mixture of glucose and xylose which suggest that a wide variety of carbohydrate rich waste streams can be successfully adopted for this process. However, the ABE production rate seems to be one of the major bottlenecks for commercialization. The ABE production rate was ranging from 0.08 to 0.54 g ABE/L h, see Table 1. Batch operation typically involves longer lag phase and final product inhibition (Gheshlaghi et al., 2009; Jin et al., 2011). Interestingly, most of the studies conducted in batch possibly due to avoid the complexities (e.g., biomass washout)

produce a variety of value-added products including acetone, buta-







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## Table 1

Literature review of ABE fermentation using batch and continuous flow systems.

Inoculum	g Sugar/L (Substrate)	Operating condition (pH/Temp./ Mode)	A (g/L)	B (g/L)	E (g/L)	Total ABE (g/L)	Residual Glucose (g/L)	Yield (g total ABE/g sugar added)	Rate (g/L-h)	VSS (g/L)	Ref.
Clostridium saccharobutylicum DSM 13864	55 (NaOH-pretreated corncobs)	–/37 °C/batch	6.86	12.27	0.32	19.44	22	0.35	0.54	-	Gao and Rehmann (2014)
Clostridium saccharobutylicum DSM 13864	55 (glucose and xylose)	–/37 °C/batch	6.09	10.26	0.46	16.8	-	0.30	0.47	-	Gao and Rehmann (2014)
Clostridium acetobutylicum ABE 1201	60 (glucose)	–/37 °C/batch	6.97	14.02	2.27	23.26	-	0.41	0.37	-	Chang et al. (2014)
C. beijerinckii p260	60 (glucose)	6.5/35 °C/batch	1.38	15	5	21.38	2	0.36	0.30	2.45	Qureshi et al. (2012)
C. beijerinckii p260	60 (Glucose with 0.10 g/L furfural)	6.5/35 °C/batch	1.05	15	6	22.05	3	0.36	0.36	2.45	Qureshi et al. (2012)
C. saccharoperbutylacetonicum N1-4	65.1 (Cassava chip hydrolysate)	6.2/30 °C/batch	6	16.4	0.7	23.1	1	0.36	-	2.5	Thang et al. (2010)
C. saccharoperbutylacetonicum N1-4	65.9 (Glucose)	6.2/30 °C/batch	7	16.2	1	24.2	_	0.37	-	-	Thang et al. (2010)
C. beijerinckii IB 4	60 (Glucose)	4.9–6/ 35 °C/batch	3.01	10.96	0.16	14.13	-	0.24	-	-	Jiang et al. (2014)
C. beijerinckii IB 4	60 (Glucose)	5.5/35 °C/batch	8.63	15.68	0.32	24.63	_	0.41	-	-	Jiang et al. (2014)
C. acetobutylicum ATCC 824	23 (Sago pith residues)	-/37 °C/batch	1.73	2.23	0.26	4.22	1.6	0.18	-	-	Linggang et al. (2013)
C. acetobutylicum ATCC 824	40 (Sago pith residues)	-/37 °C/batch	3.05	4.04	0.26	7.35	8.7	0.18	0.06	-	Linggang et al. (2013)
C. acetobutylicum ATCC 824	57 (Sago pith residues)	-/37 °C/batch	2.09	3.48	0.41	5.98	12	0.10	0.1	-	Linggang et al. (2013)
C. acetobutylicum ATCC 824	23 (Glucose)	-/37 °C/batch	1.43	2.1	0.27	3.8	2.7	0.17	0.08	-	Linggang et al. (2013)
C. beijerinckii BA101	60 (Glucose)	5/35 °C/batch	5.2	11.9	0.5	17.6	14.5	0.29	0.28	-	Ezeji et al. (2013)
C. acetobutylicum strain ATCC 824	60 (Glucose)	4.5/ 35 °C/continuous	6.3	10.1	1.7	18	3.4	0.3	0.13	-	Hecke et al. (2012)
C. saccharobutylicum DSM 13864	57 (cane molasses)	4.8/ 37 °C/continuous	3.32	7.18	1.25	11.75	20.3	0.21	0.29	-	Ni et al. (2013)
C. saccharobutylicum DSM 13864	58 (corn stover hydrolysate)	4.8/ 37 °C/continuous	3.99	8.26	1.49	13.74	15.8	0.24	0.43	-	Ni et al. (2013)
Clostridium beijerinckii P260	58.3 (corn stover hydrolysate)	6.5/-/Batch	8	14.5	3.8	26.3	-	0.45	0.31	0.77	Qureshi et al. (2010)
C. acetobutylicum DSM 792	35 (SO <sub>2</sub> -ethanol-water spent liquor)	6.5/37 °C /Batch	3	5	0.79	8.79	2.64	0.20	-	-	Survase et al., 2011
C. acetobutylicum DSM 792	45 (SO <sub>2</sub> -ethanol-water spent liquor)	6.5/ 37 °C/continuous	3.2	7.1	1.7	12	7.9	0.27	-	-	Survase et al. (2011)
Clostridium beijerinckii BA101	25 (Xylose)	6.8/35 °C/batch	-	-	-	9.6	-	0.39	0.16	1.44	Qureshi et al. (2008)
C. beijerinckii ATCC 55025 c	53.1 (wheat bran)	6-6.5/ 37 °C/batch	2.2	8.8	0.8	11.8	-	0.22	0.16	-	Liu et al. (2010)
C. saccharoperbutylacetonicum N1-4 (ATCC 13564)	40 (diluted eucalyptus hydrolysate)	6.5/30 °C/batch	4.07	7.72	0.47	12.3	10	0.40	0.10	-	Zheng et al. (2015)
Clostridium beijerinckii DSM 6422	30 (glucose and xylose)	–/35 °C/batch	4.23	7.21	_	11.44	_	0.40	-	-	Bellido et al. (2014)

A, Acetone; B, Butanol; E, Ethanol.

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