Butyric acid production from lignocellulosic biomass hydrolysates by engineered *Clostridium tyrobutyricum* overexpressing xylose catabolism genes for glucose and xylose co-utilization

Hongxin Fu^{1,2} Shang-Tian Yang^{2,*} Mingqi Wang³ Jufang Wang¹ I-Ching Tang⁴

¹School of Bioscience and Bioengineering, South China University of Technology, Guangzhou 510006, China

²Department of Chemical and Biomolecular Engineering, The Ohio State University, 151 West Woodruff Avenue, Columbus, Ohio 43210, USA

³School of Animal Science and Technology, Zhejiang University, Hangzhou, Zhejiang, China

⁴Bioprocessing Innovative Company, 4734 Bridle Path Ct., Dublin, Ohio 43017, USA

*Corresponding author: Phone: 614-2926611; fax: 614-2923769; Email: yang.15@osu.edu

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Abstract

Clostridium tyrobutyricum can utilize glucose and xylose as carbon source for butyric acid production. However, xylose catabolism is inhibited by glucose, hampering butyric acid production from lignocellulosic biomass hydrolysates containing both glucose and xylose. In this study, an engineered strain of *C. tyrobutyricum* Ct-pTBA overexpressing heterologous xylose catabolism genes (*xylT*, *xylA*, and *xylB*) was investigated for co-utilizing glucose and xylose present in hydrolysates of plant biomass, including soybean hull, corn fiber, wheat straw, rice straw, and sugarcane bagasse. Compared to the wild-type strain, Ct-pTBA showed higher xylose utilization without significant glucose catabolite repression, achieving near 100% utilization of glucose and xylose present in lignocellulosic biomass hydrolysates in bioreactor at pH 6. About 42.6 g/L butyrate at a productivity of 0.56 g/L·h and yield of 0.35±0.02 g/g was obtained in batch fermentation, demonstrating the potential of *C. tyrobutyricum* Ct-pTBA for butyric acid production from lignocellulosic biomass hydrolysates.

Keywords: Butyric acid; *Clostridium tyrobutyricum*; glucose carbon catabolite repression; lignocellulosic biomass hydrolysates; xylose

1. Introduction

Butyric acid, a specialty chemical widely used in food and pharmaceutical industries, is produced mainly from petroleum-based feedstocks via chemical synthesis (Dwidar et al., 2012; Zhang et al., 2009). However, the demand for bio-based products from renewable resources and concern over environmental pollution by chemical synthesis have revived the interest for butyric acid fermentation (Baumann and Westermann, 2016; Wang et al., 2016; Yang et al., 2013). To date, *Clostridium tyrobutyricum* has been studied extensively for butyric acid production from a variety of fermentable sugars and starch- and sucrose-based substrates including cane molasses (Jiang et al., 2009), wheat flour (Fayolle et al., 1990), Jerusalem artichoke (Huang et al., 2011), sweet sorghum, and beet molasses (Sjoblom et al., 2015), achieving a high final titer (55.2 to 62.8 g/L), yield (0.38 to 0.52 g/g), and productivity (1.1 to 3.2 g/L·h) in fed-batch fermentation. However, the relatively high cost of these substrates, which account for ~50% of the overall cost is limiting the industrial production of bio-based butyric acid.

More recently, attention has turned to using non-food feedstocks such as lignocellulosic biomass including agricultural residues for economical and sustainable production of bio-based butyric acid (Baroi et al., 2015a; 2015b; Huang et al., 2016a; Lee et al., 2015a; Liu et al., 2013; Wei et al., 2013; Zhu et al., 2002). However, there are several problems in using lignocellulosic biomass for fermentation, including difficulty in breaking down the recalcitrant plant biomass for cellulose hydrolysis, growth inhibition by the toxic compounds present in the hydrolysates, and low xylose utilization efficiency (Jönsson et al., 2013; Menon and Rao, 2012). Although many microorganisms, including *C. tyrobutyricum*, is able to utilize xylose as carbon source (Jiang et

al., 2010; 2011; Liu and Yang, 2006; Zhu and Yang, 2004), xylose catabolism is usually inhibited by glucose (Baroi et al., 2015a), which is commonly referred as carbon catabolite repression (CCR) (Görke and Stülke, 2008; Yao and Shimizu, 2013). Simultaneous utilization of glucose and xylose, two major sugars in lignocellulosic biomass hydrolysates, is the prerequisite for exploiting economically competitive and sustainable biological process for fuels and chemicals production (Gu et al., 2014; Ho et al., 1998; Liu et al., 2013; Wang et al., 2014; Wei et al., 2016).

Engineering cells to overexpress xylose catabolism genes (*xylT*, *xylA* and *xylB*) could eliminate or alleviate the glucose-mediated CCR and thus enhance xylose utilization and butanol production by *Clostridium* (Gu et al., 2010; Xiao et al., 2011; Yu et al., 2015). Recently, we have also overexpressed *xylT*, *xylA* and *xylB* in *C. tyrobutyricum* for butyric acid production from both glucose and xylose (Fu et al., 2017). The objective of the present study was to further evaluate the feasibility of using the engineered *C. tyrobutyricum* Ct-pTBA capable of co-utilizing glucose and xylose for butyric acid production from lignocellulosic biomass. Batch fermentations with free cells in serum bottles containing hydrolysates of soybean hull, sugarcane bagasse, corn fiber, wheat straw, and rice straw were studied first, and the best two hydrolysates from soybean hull and sugarcane bagasse were further studied in bioreactor with pH controlled at 6.0. The results showed that Ct-pTBA was able to simultaneously use glucose and xylose present in the undetoxified hydrolysates, achieving the highest butyric acid titer of 42.6 g/L ever reported in a free-cell fermentation using lignocellulosic biomass hydrolysate. This study demonstrated the potential of *C. tyrobutyricum* Ct-pTBA for bio-based butyric acid production.

2. Materials and methods

2.1. Cultures and media

C. tyrobutyricum Ct-pTBA used in this study was obtained by transforming the strain ATCC 25755 with the plasmid pTBA for overexpressing three xylose catabolism genes (*xylT*, *xylA*, and *xylB*) from *C. acetobutylicum*. The construction of Ct-pTBA followed similar procedures described elsewhere (Fu et al., 2017). Unless otherwise noted, the strain was cultured anaerobically at 37 °C in Clostridial Growth Medium (CGM) (Liu et al., 2006a; Zhu et al., 2005) supplemented with 30 µg/mL thiamphenicol. The CGM contained (g/L): 50 glucose, 2 yeast extract, 4 tryptone, 2 (NH₄)₂SO₄, 1 K₂HPO₄·3H₂O, 0.5 KH₂PO₄, 0.1 MgSO₄·7H₂O, and trace minerals.

2.2. Preparation of lignocellulosic biomass hydrolysates

Lignocellulosic biomass, including soybean hull (yellow pellets), corn fiber (yellow pellets), wheat straw (brown particles), rice straw (yellow particles) and sugarcane bagasse (brown powder), was pretreated with dilute acid and then hydrolyzed to obtain hydrolysates containing fermentable sugars (glucose, xylose and arabinose). Briefly, biomass was mixed with dilute acid (0.04 M HCl or 0.01–0.02 M H₂SO₄) at a solid/liquid ratio of 10% (w/v) and autoclaved at 121 °C for 30 min. The acid and its concentration used in the pretreatment of a biomass were chosen for maximum sugar yield based on data from previous studies (Dong 2014; Du 2013; Lee et al., 2015a; Liu et al., 2013). After cooling and adjusting the pH to 5.0 with NaOH, the pretreated biomass was treated with cellulases (Cellic® CTec2 5.4% and HTec2 0.6% w/w

biomass, Novozymes North America, Inc., Franklinton, NC) for 72 h at 50 °C and 150 rpm. After removing insoluble solids by centrifugation at 8000 rpm for 20 min, the supernatant was concentrated by evaporation to a desirable total sugar concentration of ~40 g/L and then stored at 4 °C.

2.3. Butyric acid fermentation

Butyric acid production in batch fermentation was first studied in serum bottles (50 mL), with glucose, xylose, the mixture of glucose and xylose (~1:1), and hydrolysates of various biomass, including soybean hull (SHH), corn fiber (CFH), wheat straw (WSH), rice straw (RSH), and sugarcane bagasse (SBH), respectively, as the carbon source and 40 g/L calcium carbonate as buffer. The medium, which had an initial pH value of ~6.5, was autoclaved at 121 °C for 30 min, sparged with nitrogen for ~30 min, and then inoculated with an active seed culture (~24 h) at a volume ratio of 5%. Batch fermentation kinetics was then studied in 1-L stirred-tank bioreactors (600 mL) with selected hydrolysates and pH controlled at 6.0 with 30% (v/v) ammonium hydroxide. Broth samples were taken periodically throughout the fermentation. All fermentation conditions were studied in duplicate.

2.4. Analytical methods

Cell density was monitored by measuring the optical density (OD) at 600 nm using a spectrophotometer (UV-16-1, Shimadzu, Columbia, MD). Organic acids and sugars were determined by HPLC (LC-20AD, Shimadzu, Columbia, MD) with an organic acid analysis column (HPX-87H, Bio-Rad, Hercules, CA) at 65 °C and 0.005 M sulfuric acid at 0.6 mL/min as

the mobile phase following the previously described method (Liu et al., 2006b).

3. Results and discussion

3.1. Effects of overexpressing xylose catabolism genes

To evaluate the effects of overexpressing the xylose catabolism genes *xylT*, *xylA*, and *xylB* on *C. tyrobutyricum* growth and sugar metabolism, fermentation in CGM containing glucose, xylose, and glucose/xylose mixture, respectively, as carbon source was studied with Ct-pTBA and its parental strain (wild type Ct without pTBA). **Figure 1** shows the fermentation kinetics with Ct and Ct-pTBA cultured in serum bottles. It is noted that the fermentation stopped before all substrate was consumed because the culture pH had dropped from the initial ~6.5 to below 5.0 due to acids production. Compared to glucose, xylose utilization was more sensitive to the pH and was inhibited at acidic pH. Ct-pTBA was able to use xylose at a faster rate either with or without the presence of glucose. In fact, the wild type strain Ct was not able to use xylose in the presence of glucose due to glucose-induced CCR. Clearly, overexpressing *xylT*, *xylA*, and *xylB* alleviated glucose repression and afforded Ct-pTBA the ability to use glucose and xylose simultaneously, although glucose as the preferred substrate was consumed at a faster rate than xylose. Compared to the wild-type, Ct-pTBA grew slower on glucose, probably because of the additional metabolic burden imposed by the constitutive expression of heterologous genes not required for growth with glucose as the sole carbon source.

3.2. Butyric acid fermentation with hydrolysates in serum bottles

Figure 2 shows the fermentation kinetics of Ct-pTBA utilizing sugars (glucose, xylose, and

arabinose) present in various biomass hydrolysates (SHH, CFH, WSH, RSH and SBH) in serum bottles. As expected, glucose and xylose were consumed simultaneously in these fermentations. However, xylose consumption was much slower compared to glucose, especially when the pH was allowed to decrease to lower than 6.0 (**Fig. 2A-E**). Except for RSH, almost all glucose in the hydrolysates was consumed in 72 h, whereas only 27%–52% of xylose was consumed in 120 h when the fermentation stopped due to low pH. About 10.6–14.4 g/L butyrate was produced with a yield of 0.37–0.40 g/g sugar consumed in these fermentations. With daily pH adjustment to 6.0 (**Fig. 2F-J**), xylose utilization increased to 65%–94%, resulting in a significantly higher final butyrate titer (12.3–15.3 g/L) and productivity (0.15 ± 0.01 g/L·h vs. 0.12 ± 0.01 g/L·h). For CFH, WSH, SHH and SBH, the increased xylose utilization rate was at the expense of decreased glucose consumption rate. Clearly, both glucose and xylose catabolisms were inhibited by acidic pHs, but the inhibition was more pronounced on xylose. The higher pH sensitivity for xylose catabolism could be attributed to that xylose transport is mainly through the xylose-proton symporter while glucose is via the phosphoenolpyruvate (PEP)-dependent glucose phosphotransferase system (PTS). It should be mentioned that cell growth and fermentation were inhibited by the undissociated acids (Tang et al., 1989), especially butyric acid (Wu and Yang, 2003), which with a pK_a value of 4.82 dominated at pH <5.0. The cytotoxicity of the undissociated butyric acid can be attributed to its impacts on the transmembrane pH gradient, ATP generation, and activities of acid-forming enzymes (Zhu and Yang, 2003). Our result was consistent with a previous study showing 50% reduction in the xylose consumption rate at pH 5.3 as compared to at pH 6.3 in batch fermentations by *C.*

tyrobutyricum (Zhu and Yang, 2004).

Table 2 summarizes the fermentation results with the five hydrolysates studied. Among them, RSH gave the lowest butyrate productivity, probably because of the high ash content (10–17%; of which ~75% was SiO₂) in rice straw, which is known to inhibit fermentation (Binnod et al., 2010; Liu et al., 2013). The fermentation could also be inhibited by the phenolic compounds present in RSH (Lee et al., 2015a; 2015b). SHH gave the highest butyrate yield of 0.43 g/g sugar consumed. The higher butyrate yield could be attributed to the protein present in SHH, which can also be used for butyrate production by *C. tyrobutyricum* (Sjoblom et al., 2015). The higher butyrate yield could also be attributed to the re-utilization of acetate (~2 g/L) in the fermentation. *C. tyrobutyricum* produces butyrate from butyryl-CoA through a CoA-transferase, which transfers CoA to acetate, allowing its reassimilation in the metabolic pathway (Lee et al., 2016). Adding acetate in the medium thus could induce acetate reassimilation and increase butyrate yield (Jaros et al., 2012; Fu et al., 2017). The utilization of acetate present in the biomass hydrolysates during butyrate fermentation has also been reported for wheat straw (Baroi et al., 2015a), cane molasses (Jiang et al., 2009), sweet sorghum stalk (Sjoblom et al., 2015), and Japanese larch (Kim et al., 2016).

3.3. Butyric acid fermentation in bioreactor at pH 6

The serum bottle fermentation results suggested that pH control at ~6.0 would be crucial for efficient co-utilization of glucose and xylose by *C. tyrobutyricum* Ct-pTBA. Butyric acid fermentation of glucose and xylose with Ct-pTBA was thus further studied in a stirred-tank

bioreactor with its pH controlled at 6.0 (**Fig. 3A**). At pH 6, glucose and xylose were consumed simultaneously at a similar consumption rate of 1.05 ± 0.05 g/L·h in the fermentation and 37.5 g/L butyrate was produced at a productivity of 0.72 g/L·h and yield of 0.34 g/g. Clearly, both sugar consumption and butyrate productivity improved significantly with the pH controlled at 6.0 as compared to the fermentations in serum bottles without pH control. The feasibility of using concentrated hydrolysates of soybean hull (~45 g/L glucose) and sugarcane bagasse (~60 g/L glucose) for butyric acid production was then studied in the bioreactor at pH 6. For easier comparison with the results from glucose/xylose mixture, we supplemented SHH and SBH with additional xylose to make the initial glucose/xylose ratio close to 1. After a long lag phase of ~80 h, both glucose and xylose in the concentrated hydrolysates were used simultaneously by Ct-pTBA (**Fig. 3B, 3C**). The long lag phase was to allow the cells to adapt to the harsh environment containing the hydrolysate inhibitors ([Baroi et al., 2015a](#); [Jönsson et al., 2013](#)). In the end, 29.7 and 42.6 g/L of butyric acid was produced from SHH and SBH, respectively, with a high xylose utilization efficiency (90% and 97%) comparable to that obtained with glucose/xylose mixture (95%). Compared to the glucose/xylose medium, the butyrate yield from the hydrolysates was slightly higher (0.35–0.36 g/g vs. 0.34 g/g), while acetate production decreased (4.0–5.4 g/L vs. 8.2 g/L). Butyrate accounted for 48.2–49% (mol/mol) of the substrate carbon and 3.3–6.4% substrate carbon was captured in the produced acetate in the fermentations with SBH and SHH, whereas 46% of the substrate carbon was present in butyrate and 7.2% in acetate in the fermentation with glucose/xylose (**Table 3**). Clearly, the carbon flux shifted to favor butyrate biosynthesis from acetate biosynthesis in the fermentations with SHH and SBH

1 containing a relatively high amount of acetate, which inhibited acetate production and facilitated
2 the butyrate biosynthesis reaction catalyzed by CoA transferase (Fu et al., 2017; Lee et al., 2016).
3 However, the total carbon accounted by butyrate and acetate was similar (~53% of the total
4 substrate carbon) for all three fermentations.

5 It is noted that a higher cell density, as indicated by the OD₆₀₀ value, was also reached in the
6 fermentations with SHH and SBH, probably because these hydrolysates contained additional
7 nitrogen sources for cell growth. However, both the specific growth rate (0.055–0.086 h⁻¹ vs.
8 0.142 h⁻¹) and butyrate productivity (0.56–0.67 g/L·h vs. 0.72 g/L·h) were significantly lower
9 with the concentrated hydrolysates, which might contain weak acids, furan derivatives and
10 phenolic compounds toxic to cells (Cheng et al., 2008; Ezeji et al., 2007; Liu et al., 2013; Zhang
11 et al., 2016). The type and amount of inhibitors present in the hydrolysate determine its toxicity
12 and are dependent on the feedstock and pretreatment method used (Jönsson et al., 2013). In
13 general, a mild pretreatment method applied to a less recalcitrant feedstock would generate fewer
14 inhibitors in the hydrolysate. It has been reported that the hydrolysates obtained from dilute acid
15 pretreated lignocellulosic biomass, including corn stover (Liu et al., 2013), rice straw (Lee et al.,
16 2015a), and wheat straw (Baroi et al., 2015a) were highly toxic to *C. tyrobutyricum*, resulting in
17 poor cell growth and little butyrate production. Nevertheless, butyric acid production could be
18 improved significantly after detoxification of the hydrolysates (Jönsson et al., 2013; Lee et al.,
19 2015a; 2015b) and adaptation of cells to better tolerate the inhibitors (Baroi et al., 2015a).

20 **3.4. Comparison to other studies**

Butyric acid fermentation with glucose or fermentable sugars as carbon source can achieve high final product titer (>60 g/L), yield (>0.46 g/g) and productivity (>2 g/L·h) (Yang et al., 2013). On the other hand, similar fermentations using hydrolysates of lignocellulosic biomass such as wheat straw, corn stover, corn fiber, rice hull, and switchgrass usually gave poor performance, including inefficient xylose utilization and low productivity (see Table 4). In our study, we showed that Ct-pTBA overexpressing xylose catabolism genes was able to co-utilize glucose and xylose in un-detoxified hydrolysates, such as SHH and SBH, for butyric acid production, achieving a high final butyric acid concentration of 29.7–42.6 g/L, which was much higher than those previously reported with similar biomass hydrolysates in a free-cell fermentation (9–20 g/L) or even some immobilized-cell fermentations (Wei et al., 2013; Zhu et al., 2002) (see Table 4).

However, it should be mentioned that butyric acid fermentation can be improved by immobilizing cells in a bioreactor, such as the fibrous bed bioreactor (FBB), which can increase cell density and tolerance to butyric acid and hydrolysate inhibitors, and thus greatly increase final butyrate titer and productivity (Jiang et al., 2011; Huang et al., 2016a; 2016b; Zhu and Yang, 2003). Interestingly, cell immobilization in the FBB also facilitated the co-utilization of glucose and xylose by *C. tyrobutyricum* ATCC 25755 (Wei et al., 2013; Zhu et al., 2002). In addition, butyric acid production can also be improved by *in situ* removal of the inhibiting butyric acid from the fermentation broth (Baroi et al., 2015b; Wu and Yang, 2003). These approaches should also be implemented in future industrial production of butyric acid from lignocellulosic biomass such as sugarcane bagasse, the most abundant industrial waste (280 kg bagasse/ton sugarcane)

from alcohol and sugar industries (Cardona et al., 2010) and soybean hull, the major byproduct of the soybean processing (accounting for ~8% of the whole seed) with an annual output of 17.3 million tons (Schirmer-Michel et al., 2008).

4. Conclusions

C. tyrobutyricum Ct-pTBA overexpressing heterologous xylose catabolism genes (*xylT*, *xylA*, and *xylB*) showed excellent ability to co-ferment glucose and xylose present in undetoxified lignocellulosic biomass hydrolysates for butyric acid production, achieving a high product titer, yield, and productivity. The fermentation can be further improved with cell adaptation and process optimization, providing a sustainable process for cost-effective production of butyric acid from abundant lignocellulosic biomass.

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Table 1. Compositions of various lignocellulosic biomass hydrolysates obtained after acid pretreatment, enzymatic hydrolysis, and concentration.

Biomass	Pretreatment* acid	Glucose (g/L)	Xylose (g/L)	Arabinose (g/L)	Total sugar (g/L)	Acetate (g/L)
Soybean hull	0.04 M HCl	31.50	12.69	0.01	44.20	1.99
Corn fiber	0.01 M H ₂ SO ₄	37.45	6.62	5.67	49.74	0.54
Wheat straw	0.02 M H ₂ SO ₄	33.60	11.03	2.19	46.82	0.90
Rice straw	0.02 M H ₂ SO ₄	31.03	5.89	0.63	37.55	0.21
Sugarcane bagasse	0.04 M HCl	24.75	17.10	1.28	43.13	1.60

*The type of inorganic acid and concentration were chosen for maximum sugar yield based on data from previous studies (Dong 2014; Du 2013; Lee et al., 2015a; Liu et al., 2013).

Table 2. Butyrate production from various biomass hydrolysates by *C. tyrobutyricum* Ct-pTBA in serum bottles with and without daily adjustment of pH to 6.0.

Substrate	Xylose consumed (%)		Butyrate titer (g/L)		Butyrate yield (g/g)		Butyrate productivity (g/L·h)	
	Without	With	Without	With	Without	With	Without	With
Soybean hull	30.6±0.6	73.6±0.9	12.33±0.23	15.34±0.03	0.38±0.01	0.43±0.01	0.13±0.01	0.15±0.01
Corn fiber	36.9±0.4	70.5±0.7	14.38±0.11	13.95±0.37	0.37±0.01	0.37±0.01	0.12±0.01	0.14±0.00
Wheat straw	35.3±0.9	84.4±0.6	14.10±0.35	15.18±0.18	0.38±0.01	0.40±0.01	0.11±0.01	0.15±0.01
Rice straw	52.0±0.9	64.8±0.6	6.40±0.12	12.30±0.01	0.40±0.01	0.41±0.01	0.05±0.01	0.09±0.01
Sugarcane bagasse	27.0±0.8	93.5±0.7	10.55±0.28	15.21±0.07	0.38±0.01	0.40±0.01	0.11±0.01	0.16±0.01

Table 3. Carbon balance in batch fermentations by Ct-pTBA with glucose/xylose, SHH, and SBH as carbon sources, respectively.

	Glucose/xylose	SHH	SBH
Substrates			
Glucose	1.89	1.46	2.00
Xylose	1.83	1.34	1.96
Total	3.72	2.80	3.96
Products			
Acetic acid	0.27 (7.2%)	0.18 (6.4%)	0.13 (3.3%)
Butyric acid	1.71 (46.0%)	1.35 (48.2%)	1.94 (49.0%)
Total	1.98 (53.2%)	1.53 (54.6%)	2.07 (52.3%)

Values are given in C-mol. Values in parentheses are percentages of substrate carbon recovered in the products. SHH: soybean hull hydrolysate; SBH: sugarcane bagasse hydrolysate.

Table 4. Butyrate production from various biomass hydrolysates by *C. tyrobutyricum* in fermentation

Strain	Substrate	Fermentation Condition	Xylose utilization	Titer (g/L)	Yield (g/g)	Productivity (g/L·h)	Reference
Ct-pTBA	Glucose/Xylose	Batch, pH 6	Yes, 95%	37.5	0.34	0.72	This study
	Soybean hull		Yes, 90%	29.7	0.35	0.67*	
	Sugarcane bagasse		Yes, 97%	42.6	0.36	0.56*	
RPT-4213	Wheat straw	Batch, pH 6	NR	9.87	0.44	0.41	Liu et al., 2013
	Switchgrass		NR	7.05	0.42	0.29	
C. sp. S1	Soft wood	Batch, pH 6	Repressed	21.2	0.47	0.66	Kim et al., 2016
DSMZ 2637	Wheat straw	Batch, pH 7	Low, 20%	20	0.33	0.21	Baroi et al., 2015a
ATCC 25755	Rice straw	Batch	Low, 17%	8.7	0.40	0.18	Lee et al., 2015a
ATCC 25755	Sugarcane bagasse	FBB, fed-batch	Yes, ~80%	20.9	0.48	0.51	Wei et al., 2013
ATCC 25755	Corn fibre + CSL	FBB, batch	Yes, 82%	29.0	0.47	2.91 ⁺	Zhu et al., 2002
ZJU 8235	Oilseed rape straw	FBB, fed-batch	NR	50.2	0.38	1.08 ⁺	Huang et al., 2016a
ZJU 8235	Waste paper	FBB, fed-batch	Yes, ~80%	53.4	0.40	1.33 ⁺	Huang et al., 2016b

CSL: Corn steep liquor; FBB: Fibrous bed bioreactor; NR: Not reported

*Based on the exponential phase data; ⁺Based on the FBB volume

1 **List of Figures**

2 **Figure 1.** Batch fermentations of *C. tyrobutyricum* wild-type (Ct) and mutant (Ct-pTBA)
3 overexpressing xylose-catabolism genes *xylT*, *xylB*, *xylA* in CGM with glucose, xylose
4 and glucose/xylose mixture (1:1) as substrates in serum bottles. Each fermentation
5 condition was repeated at least twice and typical fermentation kinetics is shown here.

6 **Figure 2.** Batch fermentations of Ct-pTBA grown in CGM with hydrolysates of soybean
7 hull (SHH), corn fiber (CFH), wheat straw (WSH), rice straw (RSH) and sugarcane
8 bagasse (SBH), respectively, as substrates in serum bottles. (A-E) with 40 g/L calcium
9 carbonate as buffer to maintain the pH above 5.0. (F-J) with the pH adjusted to 6.0 with
10 NaOH daily. The initial arabinose concentration was low (<1 g/L) in SHH, RSH and SBH,
11 and is thus not shown in the figure. Each fermentation condition was repeated at least
12 twice and typical fermentation kinetics is shown here.

13 **Figure 3.** Batch fermentations of Ct-pTBA grown in CGM with glucose/xylose mixture
14 (A), SHH (B), and SBH (C), respectively, as substrates in bioreactor at pH 6.0.

15

Figure 1

Figure 2

Figure 3

Graphical Abstract

Soybean hull, Corn fiber, Wheat straw,
Rice straw, Sugarcane bagasse