Butanol Fermentation from Low-Value Sugar-Based Feedstocks by *Clostridia*

Ye Ni, Zhihao Sun

School of Biotechnology, Jiangnan University
Wuxi, China

2012.9.10

Current Status of Bio-butanol in China

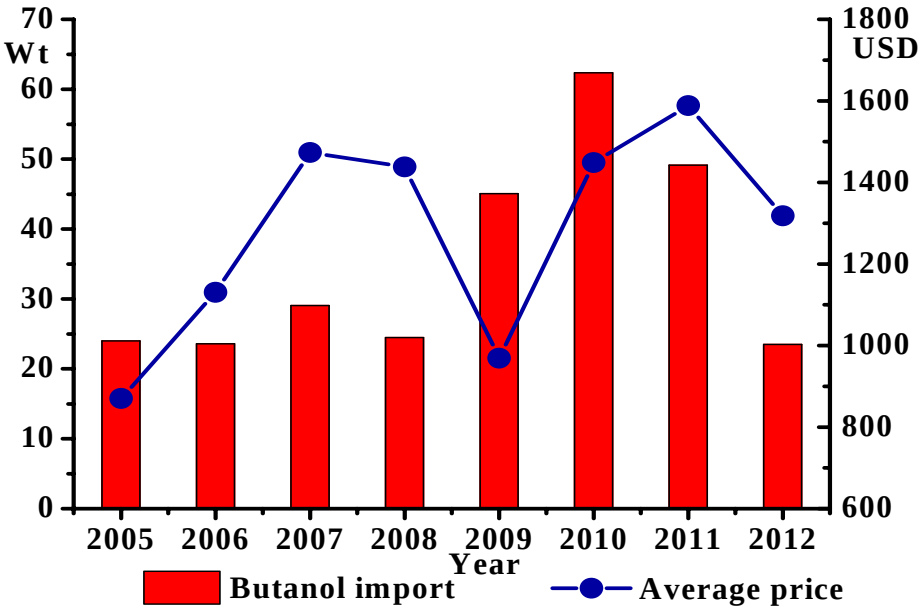
- **Market**
- **Factories**
- **Research**

Studies on ABE fermentation in Our Lab

- **Continuous Fermentation by *Clostridium saccharobutylicum***

Market

Butanol consumption and import in China



Year	Butanol import	Average price
	Ton	USD
2005	239926	870.5
2006	235834	1130.6
2007	290358	1473.6
2008	244661	1438.4
2009	450742	968.9
2010	623509	1448.9
2011	491246	1588.2
2012.1–6	235083	1318.5

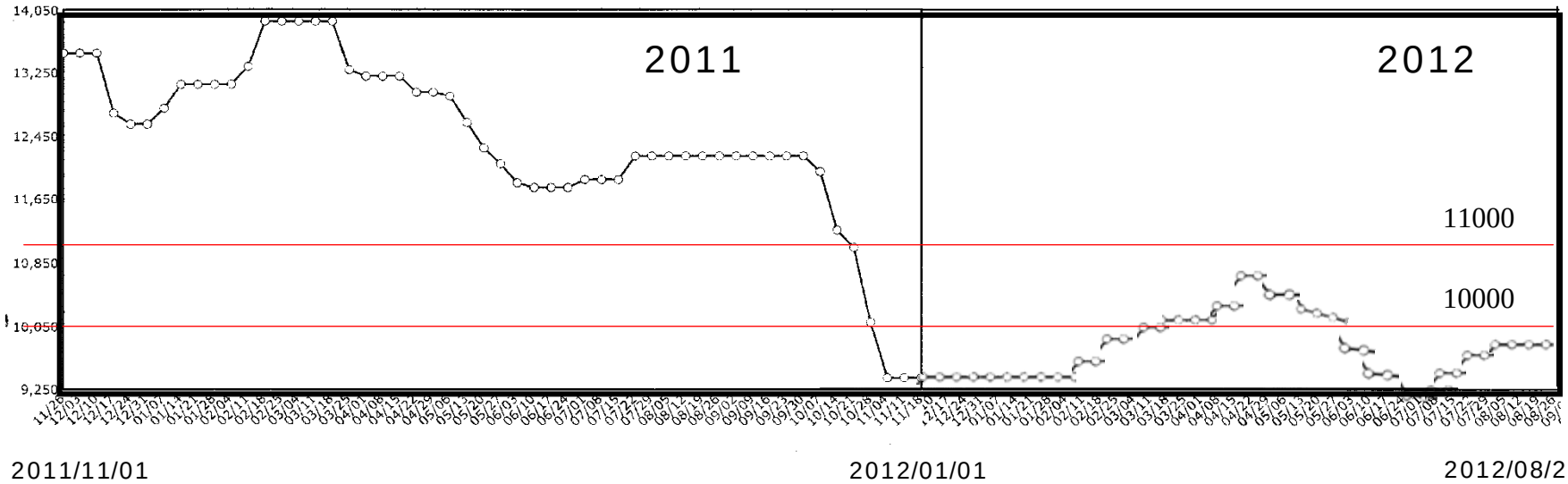
During the period of 2005–2011, around 50% of total annual butanol consumption was imported, and butanol consumption increased rapidly at an average rate of 6-8% in China.

(Data sources： http://chem.chem99.com/)

Market

Butanol Price Trend in China

Butanol Price (Jilin Petrochemical)



（ http://jiage.china.alibaba.com/price/trend/ ）

Market: butanol price downturn continuation …

Factories

江南大学 JIANGNAN UNIVERSITY

ABE Factories in China (data by 2012.8)

Factory	Current Capacity (t/y)	Starting date	Current Status	Address
Laihe Rockley Bio-Chemicals Ltd	150,000	2007.12.5	–	Songyuan, Jilin
Jilin Cathay Biotechnology Co. Ltd	100,000	2008.3.20	–	Jilin, Jilin
Guangxi Guiping JinYuan Alcohol Co. Ltd	30,000	2007.8	–	Guiping, Guangxi
Jimaoyuan Biochemical Co. Ltd	40,000	2008.3.2	producing ethanol	Lianyungang, Jiangsu
Lianhai Biological Co. Ltd	50,000	2008.10	–	Haimen, Jiangsu
Lianyungang Union of Chemicals Co. Ltd	40,000	2010.4	producing ethanol	Lianyungang, Jiangsu

All ABE factories stopped production or switch to ethanol ...

Choice of Feedstocks for ABE Fermentation

Starchy materials:
corn, **cassava**, wheat

Molasses:
cane and beet molasses

Cellulosic biomasses:
crop straw hydrolysate

Energy crops:
sweet sorghum, jerusalem artichoke

→ **Food materials**

Substitutes

→ **Low-value sugar-based feedstocks**

Cost issue

"Food versus fuel" debate

Corn material

Profit from corn utilization in ABE fermentation in China

Utilization of corn components (per ton corn)

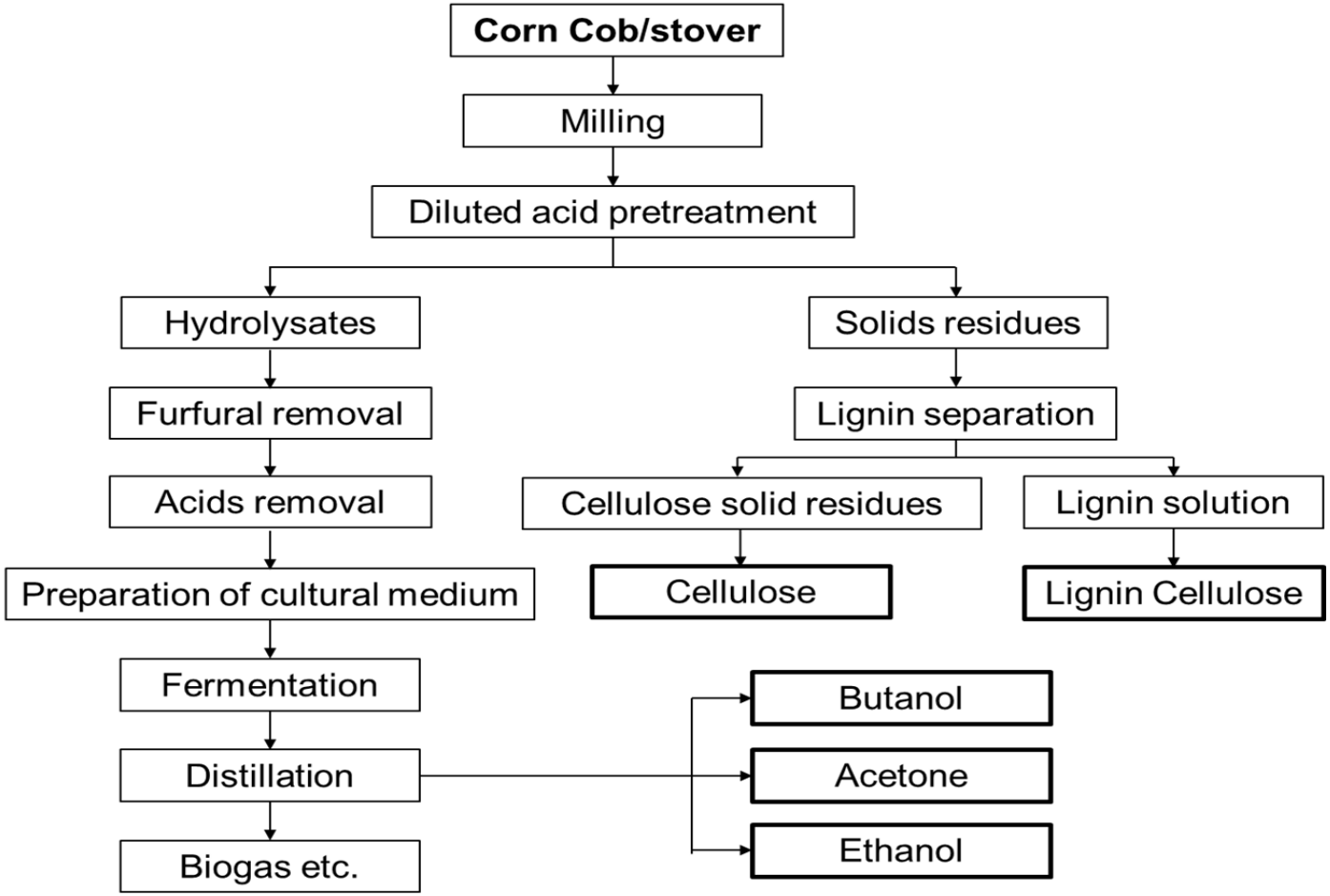
Corn components	Output (Kg)	Price (Yuan/t)	Profits (Yuan)	
Starch (10% water)	600	2860	1700	
Fiber	115~120	1350	160~200	720
Embryo	50~60	4500	270	
Protein	60	4300	250	
CSL (45% water)		350		
Total			**2420**	

*CSL: 10% solid content (3% protein), its profit was not considered.

- **In 2012.2, corn price in Jilin is 2,030 yuan/t (14% water), including shipping;**
- **4.5～4.7 t corn/t solvent, equal to a material cost of 10,080 yuan/t solvent;**
- **After utilization of corn component: 3,200 yuan/t solvent, the material cost: 7,000 yuan.**

Corn Stover material

Corn Stover Butanol Biorefinery



Corn Stover Butanol Biorefinery







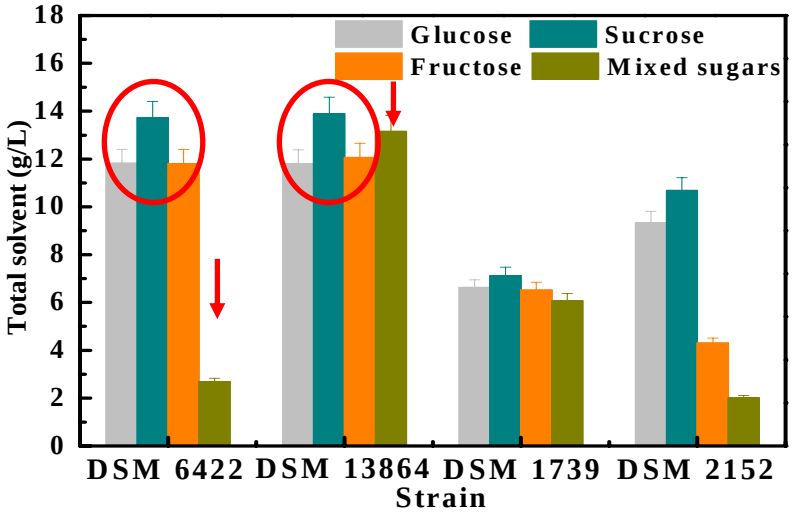

Corn stover cost when construction: 200 yuan/t; when production: 500–700 yuan/t

Studies on ABE in Our Lab

Continuous Butanol Fermentation from Low-Value Sugar-Based Feedstocks by *Clostridium saccharobutylicum* DSM13864

Sugar Utilization **of *Clostridial* Strains**

Sugars utilization of various *Clostridial* strains in ABE fermentation



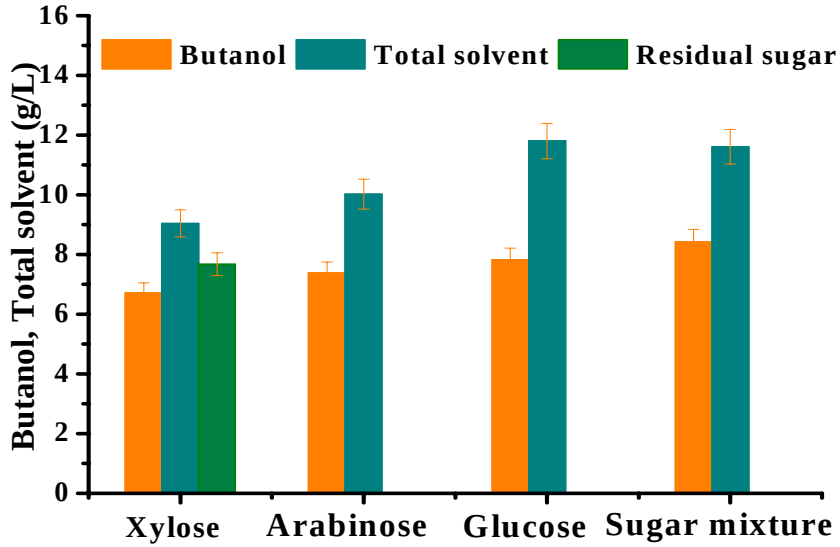
C. Beijerinckii DSM 6422、DSM 1739

C. Saccharobutylicum DSM 13864

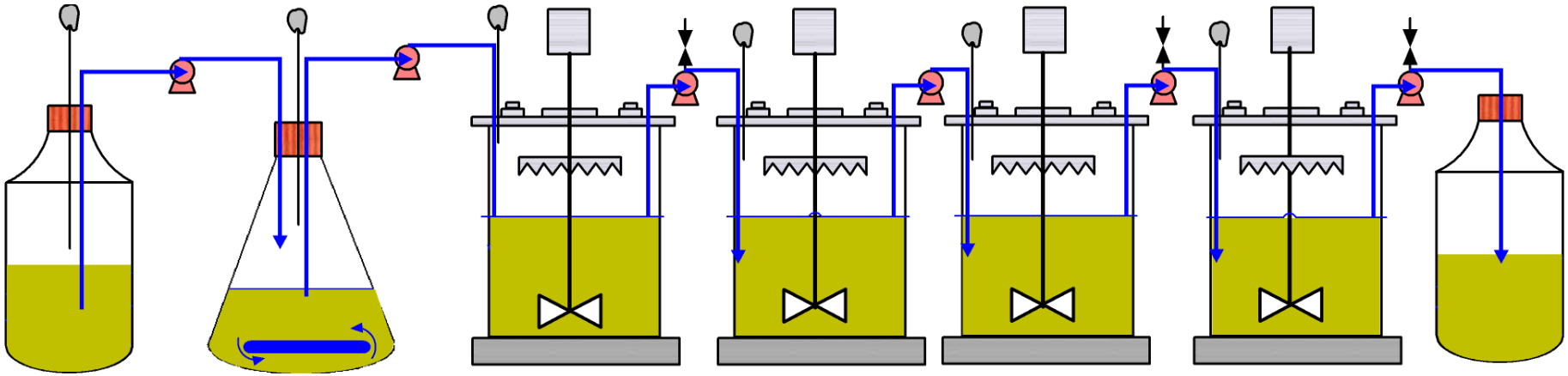
C. Saccharoperbutylacetonicum DSM 2152

C. saccharobutylicum DSM 13864 could be used for ABE fermentation from cane molasses.

C. saccharobutylicum DSM 13864 could be used for ABE fermentation from lignocellulosic hydrolysates.



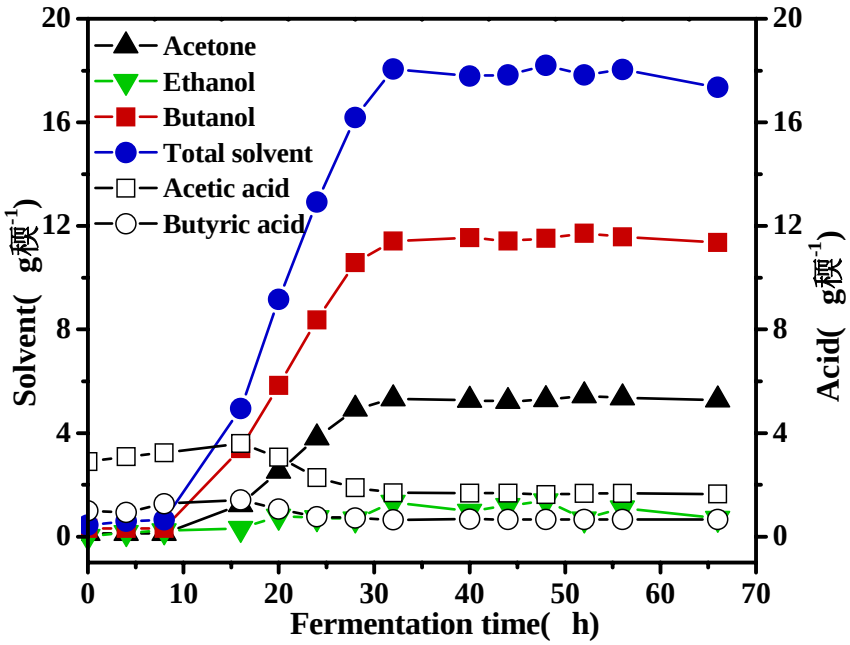
Scheme of 4-Stage Continuous ABE fermentation

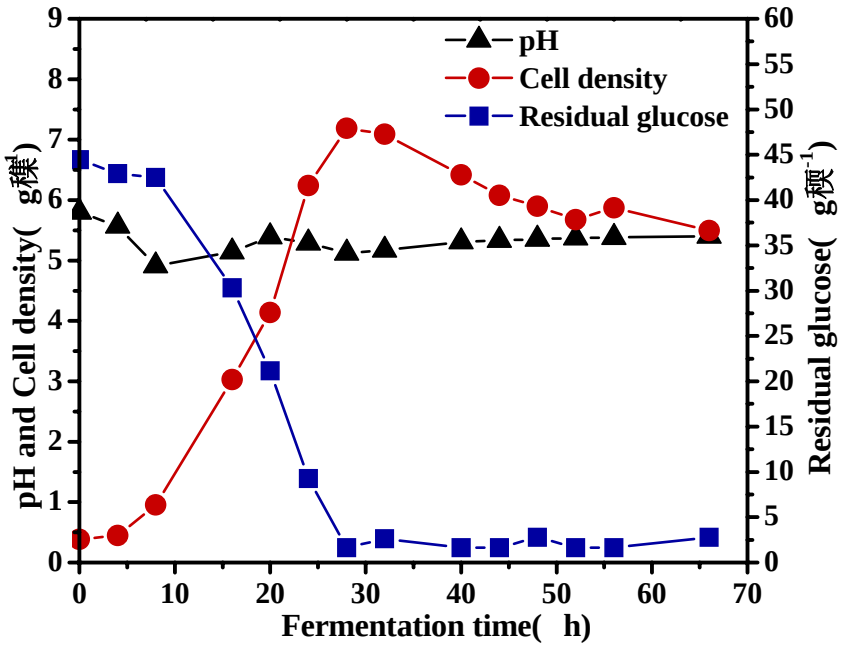


Feeding tank Blending vessel Bioreactor 1 Bioreactor 2 Bioreactor 3 Bioreactor 4 Collection tank

Substrate: glucose

Strain: DSM 13864
Batch fermentation in 3-L bioreactor

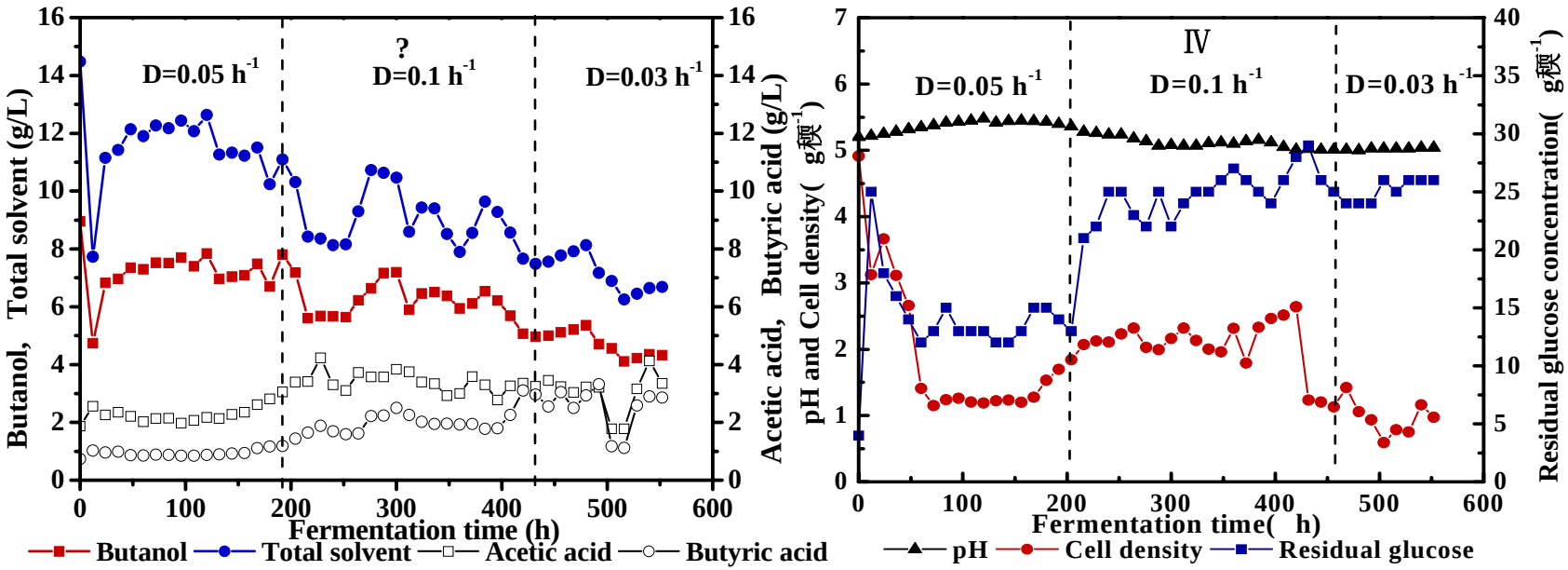




Total solvent: 18.20 g/L (butanol 11.53 g/L)
Productivity: 0.379 g/L/h
Yield: 0.314 g ABE/g sugar
Fermentation time: 48 h

Substrate: glucose

Strain: DSM 13864
4-Stage continuous fermentation (D=0.03, 0.05, 0.1 h^{-1})



At D = 0.05 h^{-1}:

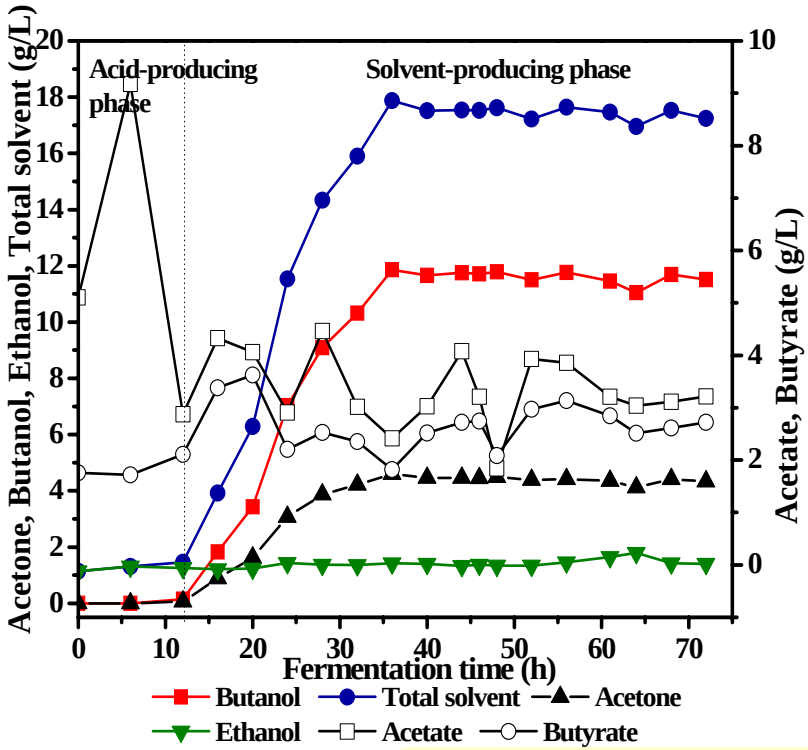
Total solvent: 11.57 g/L (butanol 7.29 g/L); **Productivity:** 0.145 g/L/h

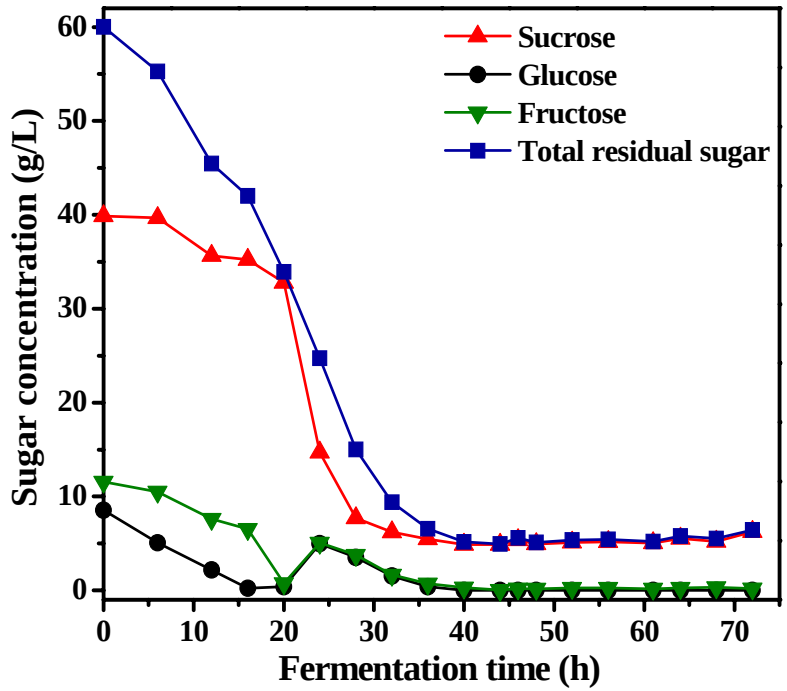
At D = 0.1 h^{-1}:

Total solvent: 8.99 g/L (butanol 6.14 g/L); **Productivity:** 0.225 g/L/h

Substrate: cane molasses

Strain: DSM 13864
Batch fermentation in 5-L bioreactor





Total solvent: 17.88 g/L (butanol 11.86 g/L)

Productivity: 0.50 g/L/h

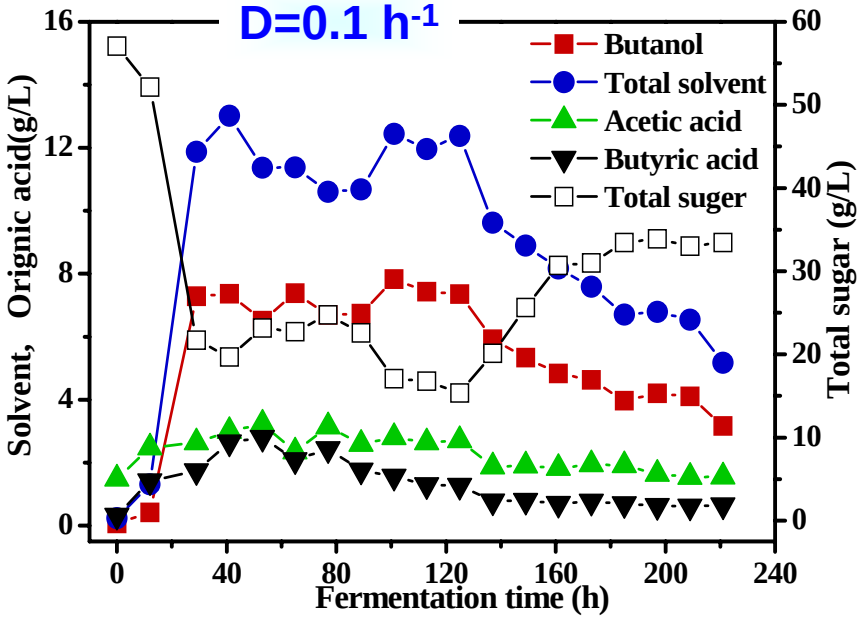
Yield: 0.33 g ABE/g sugar

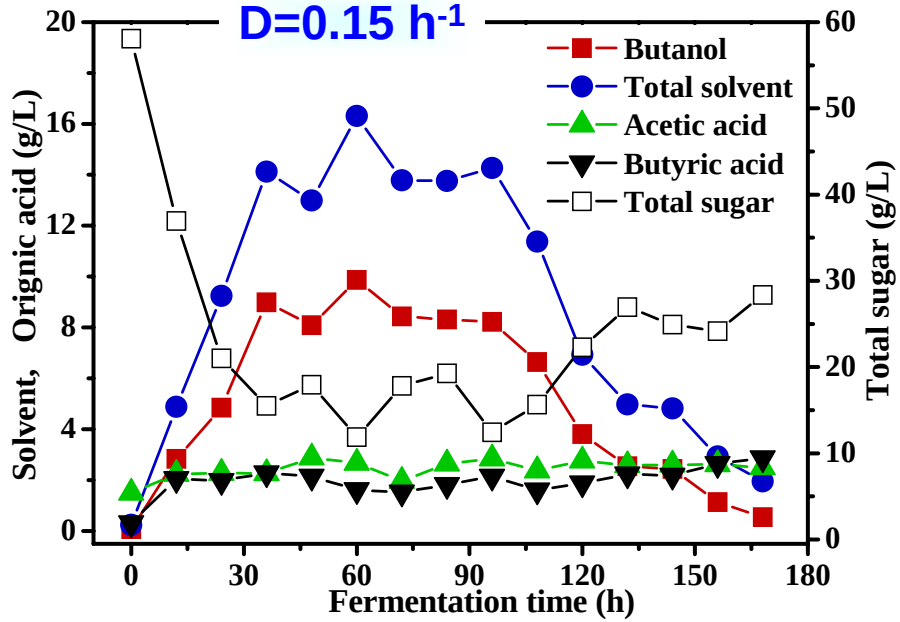
Fermentation time: 36 h

Substrate: cane molasses

Strain: DSM 13864

4-Stage continuous fermentation





At D = 0.1 h^{-1} (30–130 h)
Total solvent: 11.74 g/L (butanol 7.18 g/L); Productivity: 0.294 g/L/h
Fermentation time: 221 h

At D = 0.15 h^{-1} (30–100 h)
Total solvent: 13.75 g/L (butanol 8.37 g/L); Productivity: 0.439 g/L/h
Fermentation time: 170 h

Pretreatment conditions of various straw

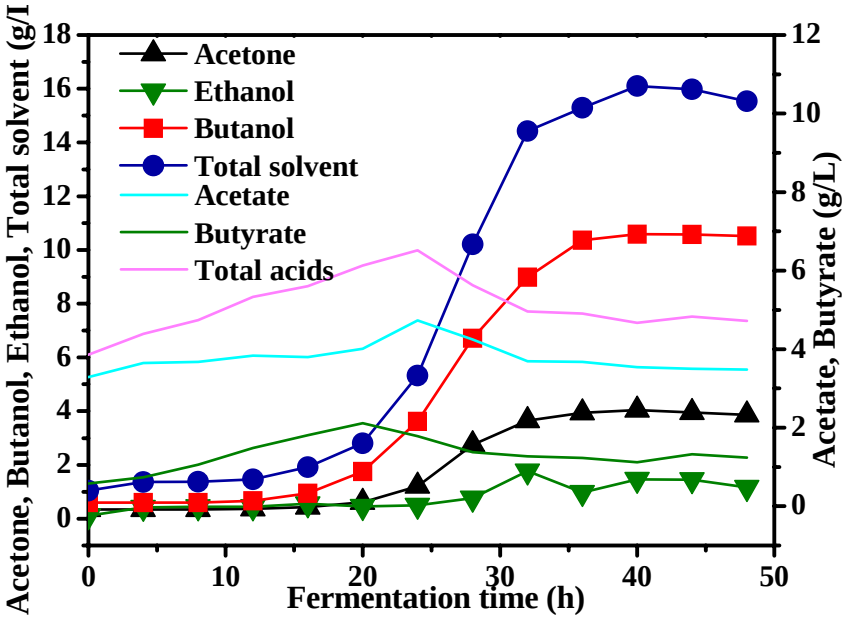
Straw types	g straw/L liquid	Glucose g/L	Xylose g/L	Arabinose g/L	Total sugar g/L	Enzymolysis ratio %
Corn straw	60	33.34	11.16	1.04	45.54	72.41
	70	42.88	14.32	1.33	58.52	80.51
	80	48.52	16.18	1.59	66.29	80.54
	90	57.19	18.84	2.03	78.06	85.08
Rice straw	60	37.09	11.70	0.65	49.43	78.59
	70	44.00	13.63	0.68	58.30	80.21
	80	49.53	14.71	0.59	64.84	78.78
	90	53.83	17.15	1.59	72.56	79.10
Wheat straw	60	38.34	12.50	0.44	51.27	81.52
	70	45.43	15.74	0.78	61.96	85.23
	80	42.02	14.74	0.83	57.58	69.96
	90	53.16	18.79	1.17	73.12	79.70

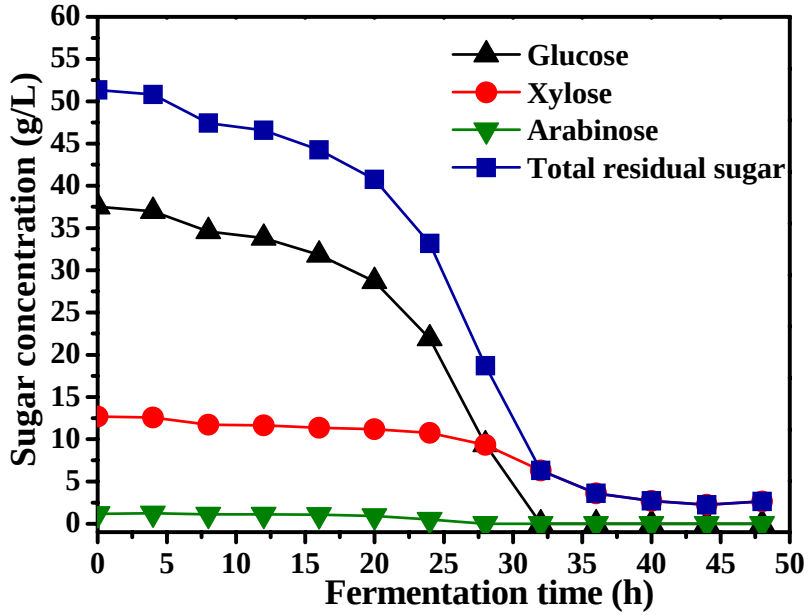
- **Alkaline Pretreatment:** 1% NaOH, 120 °C for 2 h
- **Cellulase hydrolysis:** 20–30 FPIU/g straw, pH 4.8 and 50 °C for 40 h

Substrate: corn stover hydrolyzate

Strain: DSM 13864

Batch fermentation in 3-L bioreactor





Total solvent: 16.1 g/L (butanol 10.59 g/L)

Productivity: 0.40 g/L/h

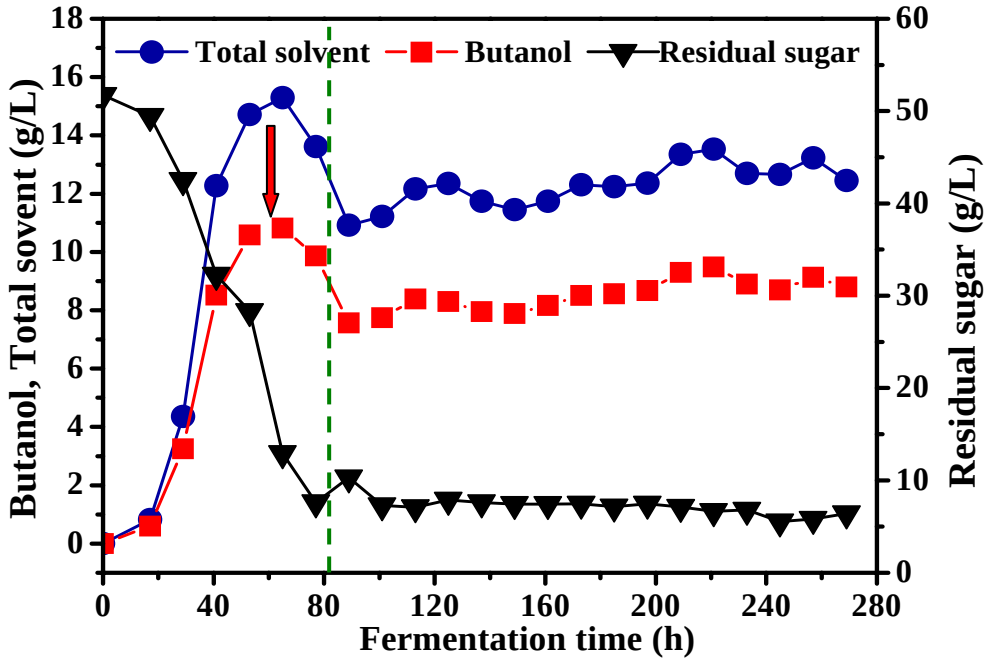
Yield: 0.33 g ABE/g sugar

Fermentation time: 40 h

Substrate: corn stover hydrolyzate

5-stage temperature-shifting continuous fermentation in 500-mL tanks

(Stage 1, 2: 37 ℃, Stage 3–5: 30 ℃)



Total solvent: 12.28 g/L (butanol 8.50 g/L) **after 80 h**

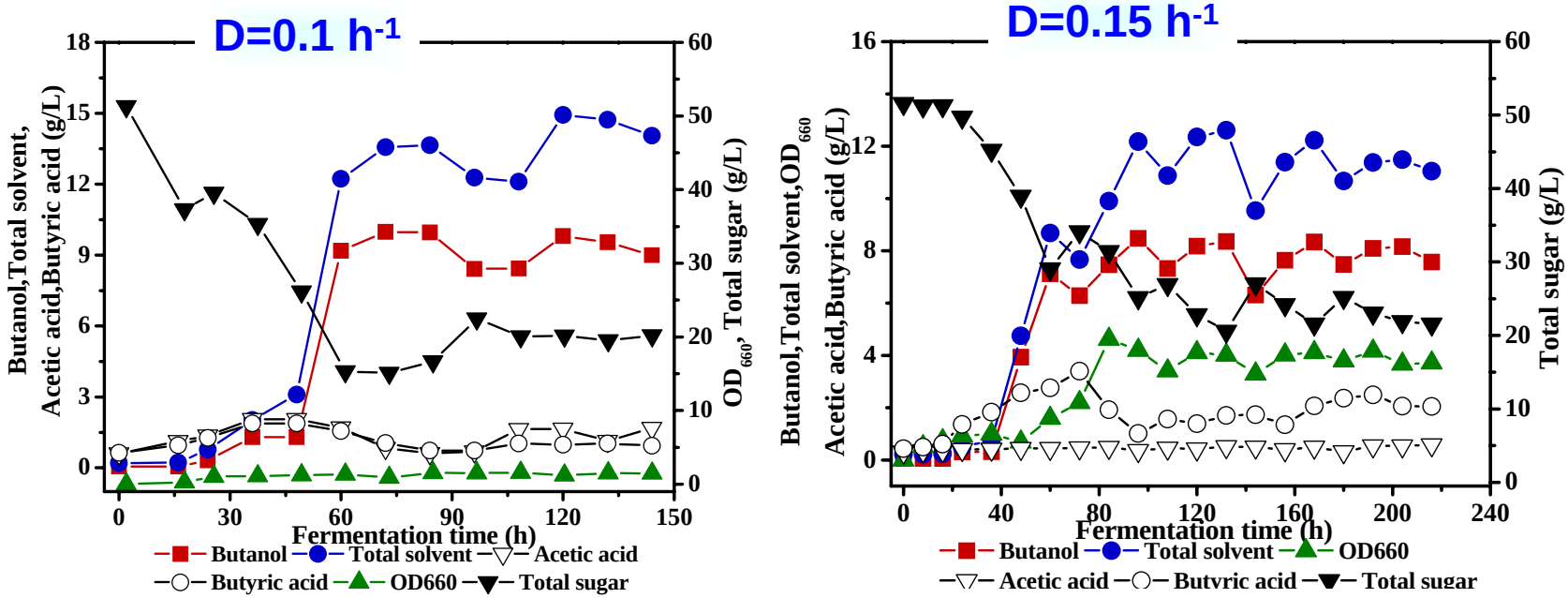
Productivity: 0.25 g/L/h

Yield: 0.33 g ABE/g sugar

Fermentation time: 270 h **(D=0.1 h^{-1})**

Substrate: corn stover hydrolyzate

Strain: DSM 13864
4-Stage Continuous fermentation



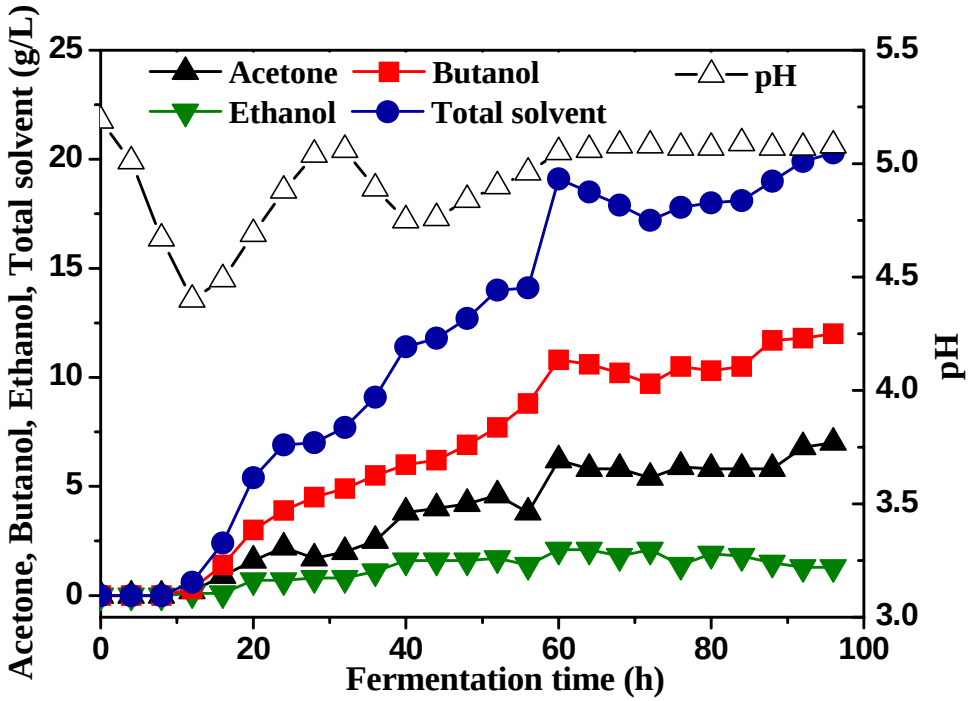
At D = 0.1 h^{-1}
Total solvent: 13.44 g/L (butanol 9.29 g/L); Productivity: 0.336 g/L/h
Fermentation time: 150 h

At D = 0.15 h^{-1}:
Total solvent: 11.43 g/L (butanol 7.81 g/L); Productivity: 0.429 g/L/h
Fermentation time: 220 h

Substrate: cassava

Strain: *C. acetobutylicum* A

Batch fermentation in 3-L bioreactor



Total solvent: 20.9 g/L (butanol 12.0 g/L)

Productivity: 0.211 g/L/h

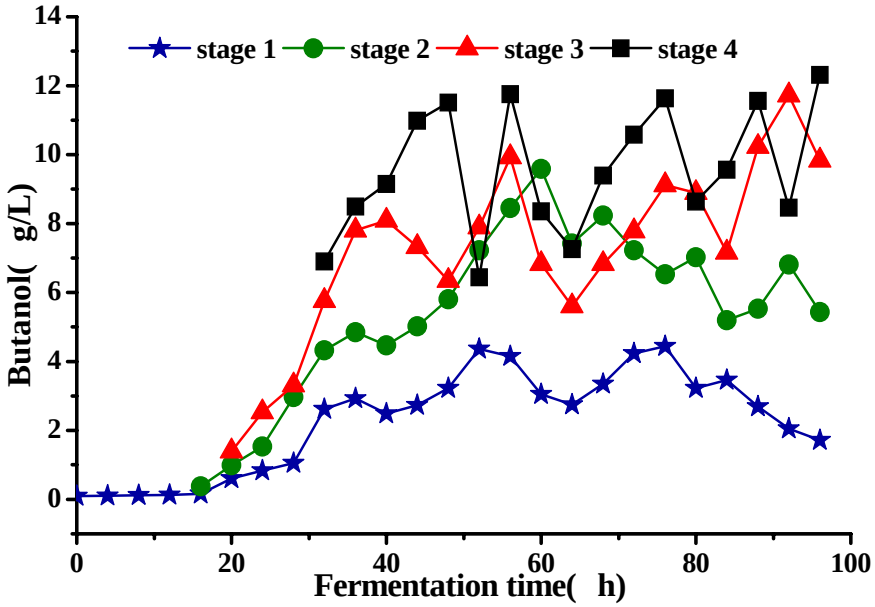
Yield: 0.33 g ABE/g sugar

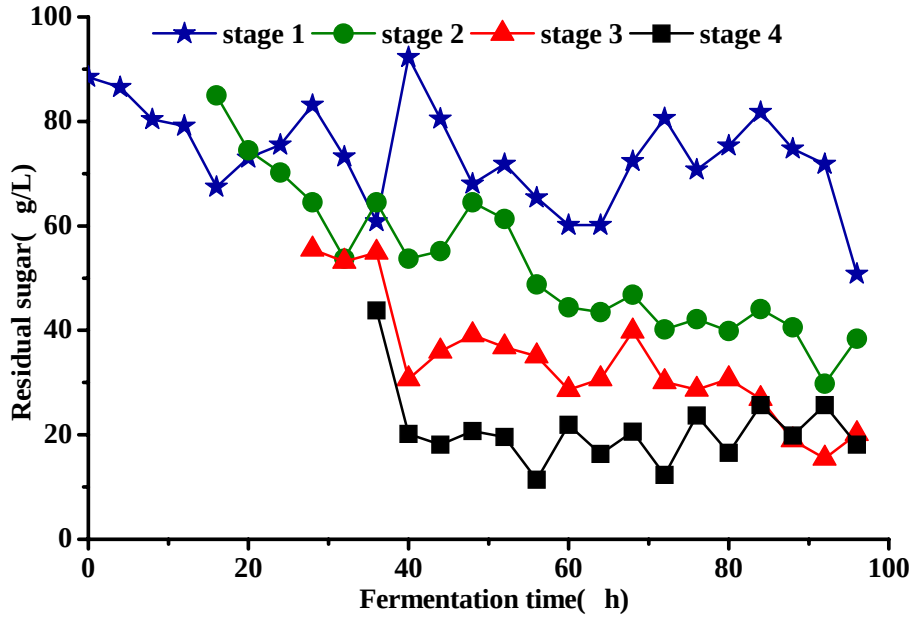
Fermentation time: 96 h

Substrate: cassava

Strain: *C. acetobutylicum* A

4-Stage continuous fermentation in 3-L bioreactor





Total solvent: 22.8 g/L (butanol 12.38 g/L)
Productivity: 0.57 g/L/h
Yield: 0.33 g ABE/g sugar
Fermentation time: 96 h (D=0.1 h^{-1})

Discuss

Batch and Continuous ABE fermentation

Operation mode	Strain	Substrate	Dilution rate (h^{-1})	Productivity (g/L/h)	Total solvent (g/L)	Reference
Batch Fermentation	*C. beijerinckii* P260	Corn stover	—	0.31	26.27	Qureshi et al. 2010
	C. acetobutylicum 2N	Beet molasses	—	0.34	16.32	Fan et al. 2010
	C. Saccharobutylicum DSM 13864	Cane molasses	—	0.50	17.88	Ni et al. 2012
	C. acetobutylicum XY16	Glucose	—	0.63	20.30	Guo et al. 2012
Continuous fermentation Immobilized cell	*C. beijerinckii* BA101	Glucose	2.0	15.80	7.9	Qureshi et al. 2000
	C. beijerinckii ATCC 55025	Glucose	0.2	1.76	8.99	Zhang et al. 2009
Continuous fermentation Membrane cell bioreactor	*C. pasteurianum* ATCC 6103	Glycerol	0.9	8.3	9.2	Malaviya et al. 2012
	C. Saccharoperbutylacetonicum N1-4	Glucose	0.11	7.55	8.58	Tashiro et al. 2005
Continuous fermentation Free cell	*C. saccharobutylicum* DSM 13864	Gelatinised sago starch	0.05	0.46	9.10	Liew et al. 2006
	C. acetobutylicum BCRC 10639	Glucose	0.054	0.37	6.85	Yen et al. 2011
	C. saccharobutylicum DSM 13864	Corn stover hydrolysate	0.1 0.15	0.336 0.429	13.44 11.43	Ni et al. Unpublished

Conclusions

- **Low-value sugar-based feedstock was utilized in batch and continuous ABE fermentation, including: cane molasses, corn stover hydrolyzate, etc.**

- **Using cane molasses, the ABE fermentation could be steadily operated for over 100 h at D=0.15 h^{-1}, the average total solvent of 13.75 g/L (butanol 8.37 g/L); and productivity of 0.439 g/L/h were obtained in a 4-stage continuous fermentation.**

- **Corn stover hydrolysate was prepared using alkaline pretreatment and enzyme hydrolysis. In a 4-stage continuous fermentation, the process was operated for 220 h at D=0.15 h^{-1}, the average solvent was 11.43 g/L (butanol 7.81 g/L), and the average solvent productivity was 0.429 g/L/h.**

Acknowledgement

江南大学 JIANGNAN UNIVERSITY

green biologics

Graduate students:

Yun Wang

Ziyi Xia

Gang Song

Shan Sun

Thank you!