Conversion of fecal waste to biofuels by engineered microbes

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Motivation

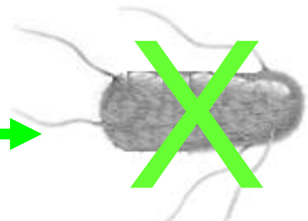
Fecal waste

Pathogens

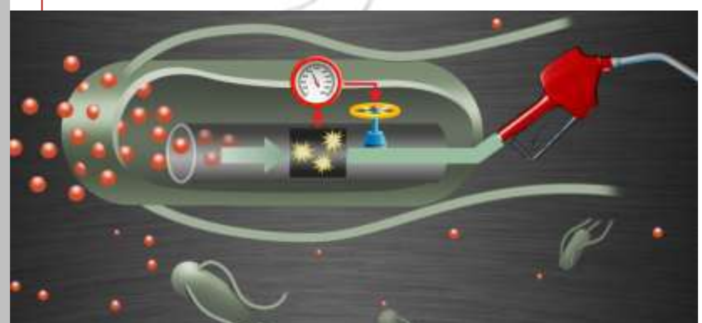
Fats and oils
Protein
Sugar polymers
Cellulose

N and P sources
Salts

Use fecal waste economically and environmental friendly



1. Kill pathogens



3. Generate advanced biofuel (beyond biogas)

2. Recycle N&P as fertilizer

Research

Fecal waste

Fats and oils
Protein
Sugar polymers
Cellulose

1. Direct utilization of fecal waste via "robust" microbes (fungi)

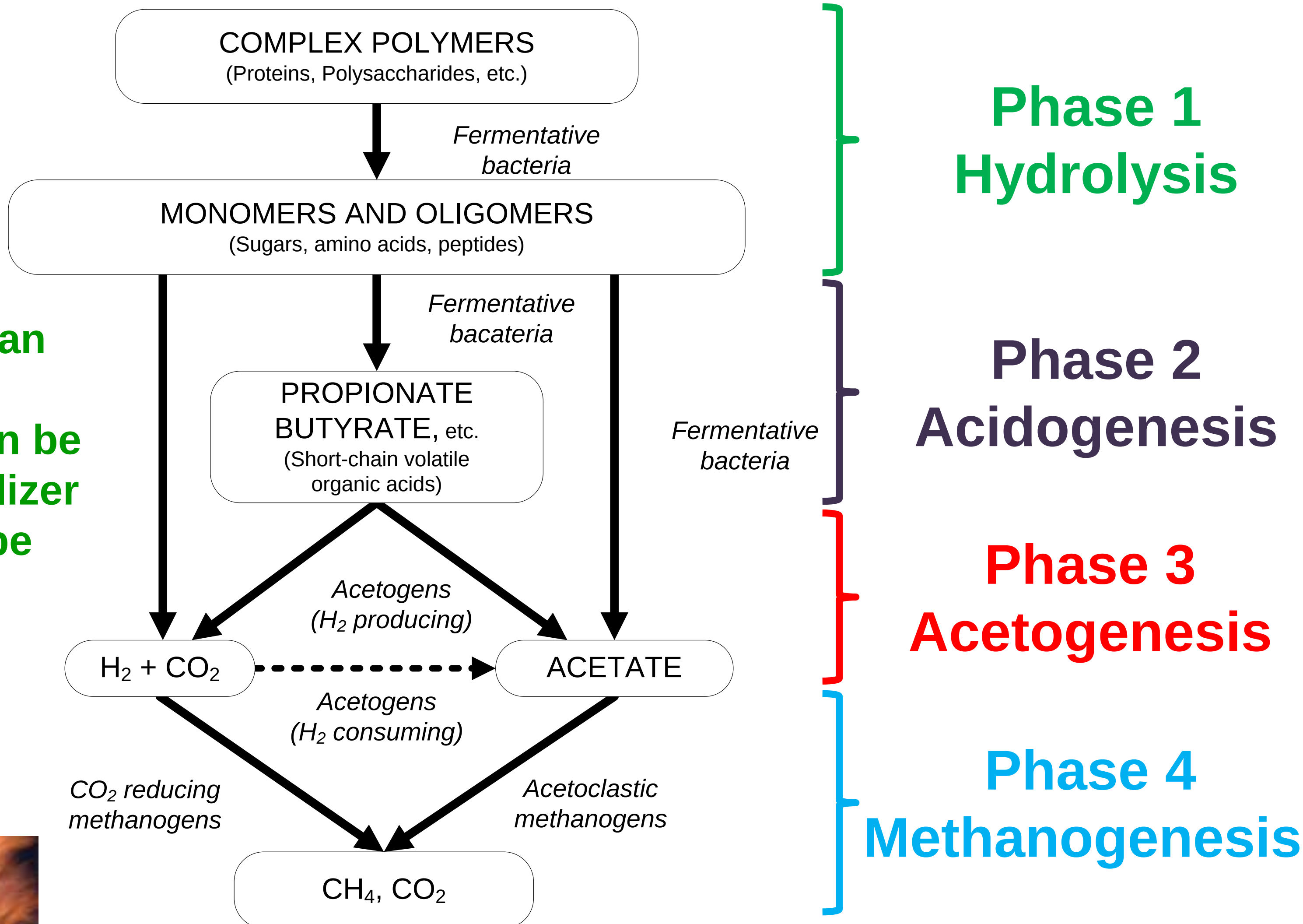
We tried different fungal species, *Aspergillus nidulans*, *Aspergillus oryzae*, *Aspergillus niger* and *Phanerochaete chrysosporium*

2. Anaerobic digestion + Microbial conversion

Some fungal species may grow on AD fecal waste, but very slow. Exogenous sugar is necessary to promote fungal growth

3. A new anaerobic digestion + microbial conversion approach

Anaerobic Digestion



Benefits

1. Pathogens can be removed.
2. Digestate can be used as fertilizer
3. Biogas can be used as fuel

Source: Syed Hashsham, PhD, lecture notes, Michigan State University

Anaerobic Digestion Biochemistry

Acetogenesis

$CH_3CH_2COO^- + 3H_2O \rightarrow CH_3COO^- + H^+ + HCO_3^- + 3H_2$

$C_6H_{12}O_6 + 2H_2O \rightarrow 2\ CH_3COOH + 2CO_2 + 4H_2$

$CH_3CH_2OH + 2H_2O \rightarrow CH_3COO^- + 2H_2 + H^+$

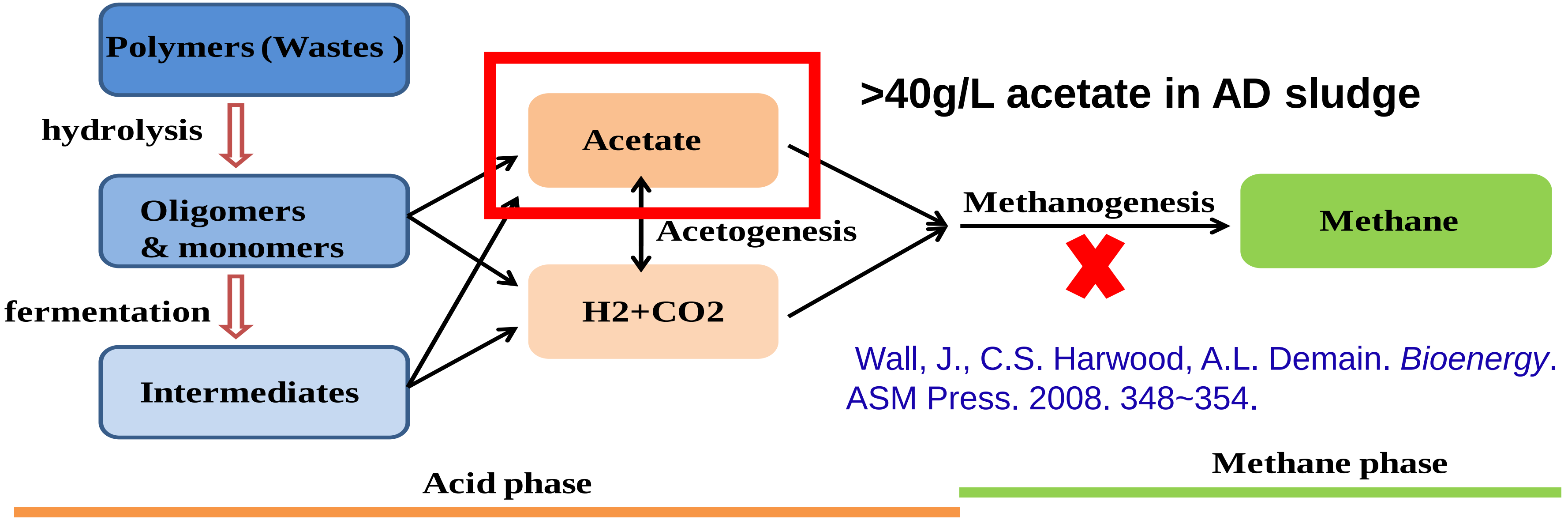
$2HCO_3^- + 4H_2 + H^+ \rightarrow CH_3COO^- + 4H_2O$

Methanogenesis

$2CH_3CH_2OH + CO_2 \rightarrow 2\ CH_3COOH + CH_4$

$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O$ $CH_3COOH \rightarrow CH_4 + CO_2$

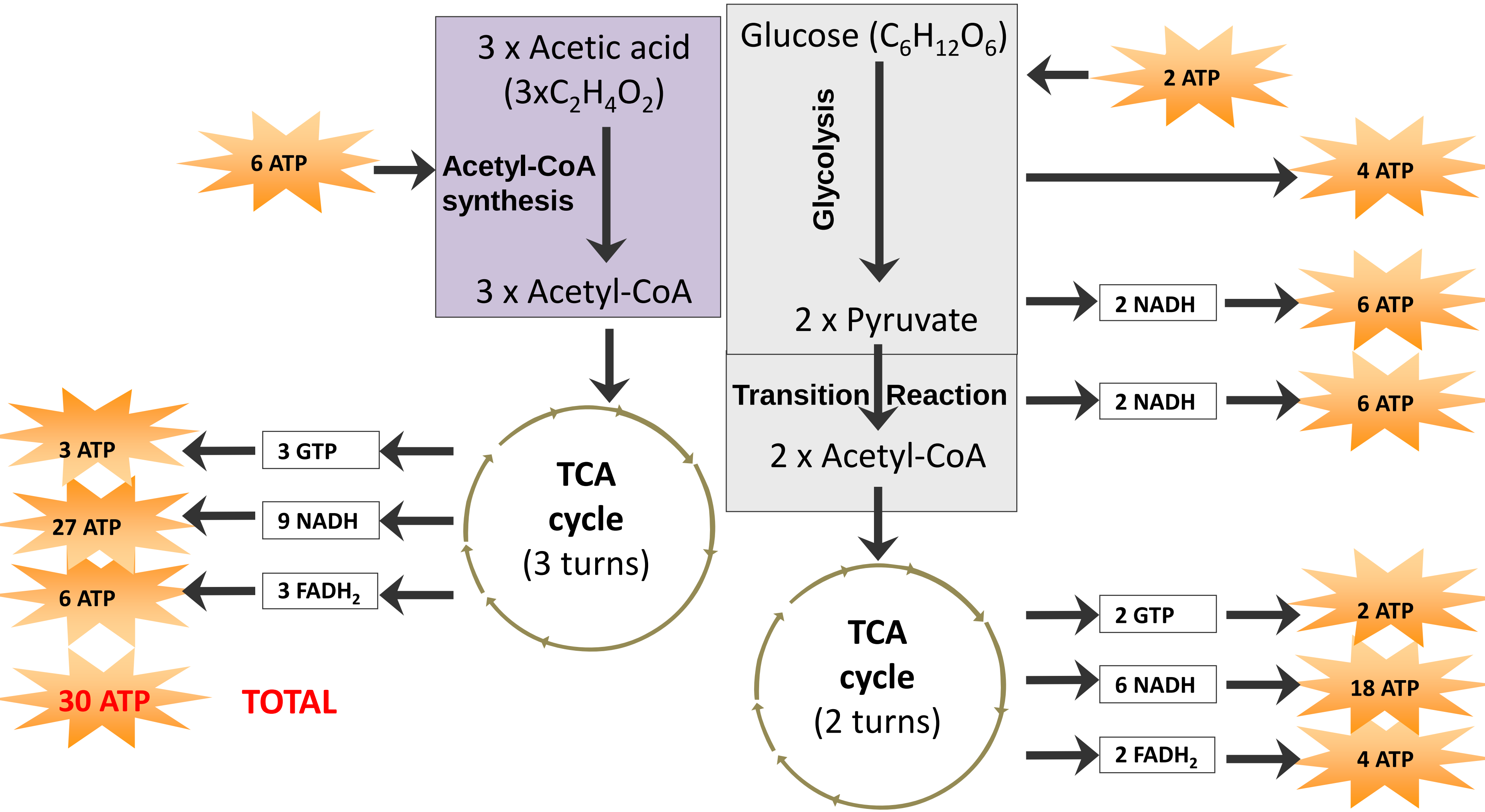
$CH_3OH + H_2 \rightarrow CH_4 + H_2O$



>40g/L acetate in AD sludge

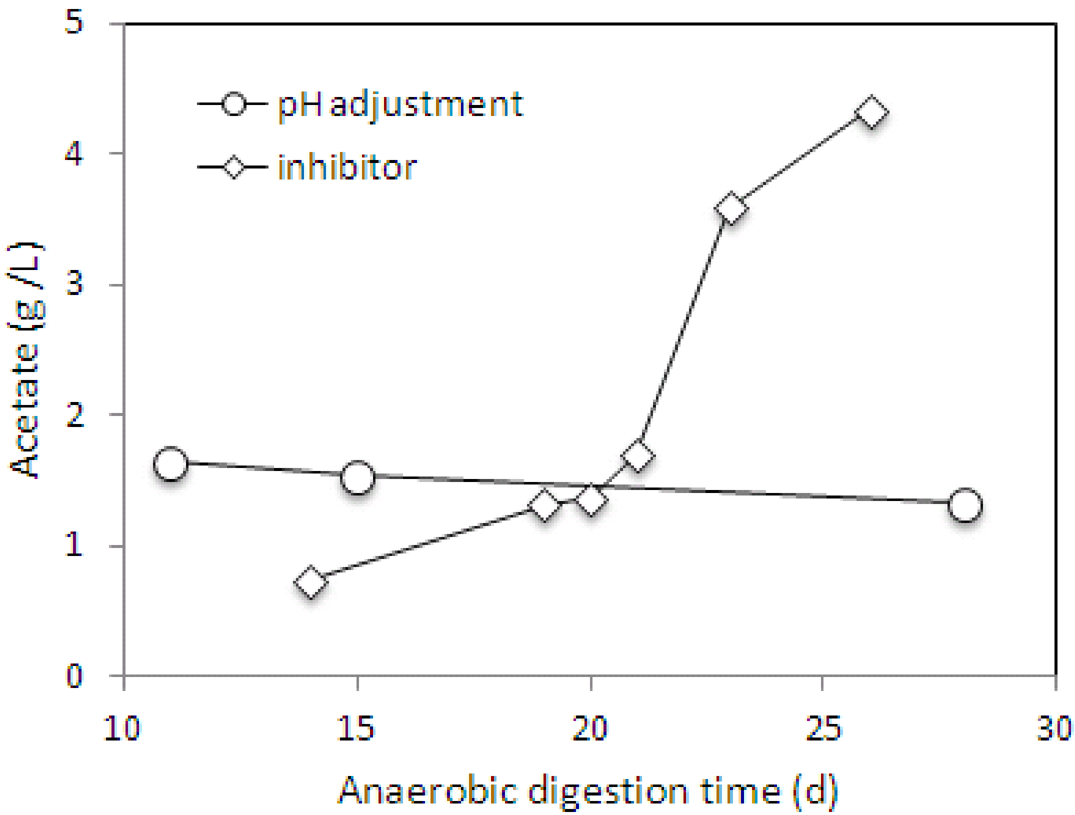
Wall, J., C.S. Harwood, A.L. Demain. *Bioenergy*. ASM Press. 2008. 348~354.

Acetate can serve as a good carbon source



8 acetyl-CoA+7ATP+14NADPH→ fatty acid (FAs) [biodiesel fuel] ***Theoretical yield :*** 0.29 g FAs/g acetic acid

Anaerobic digestion for acetate production



Acetate accumulation in AD fecal sludge

(iodoform 8 mg/L, Room Temperature)

Strategies for limiting methanogen activities: ***sudden changes in pH, temperature and air exposure, and the addition of chemical inhibitors (iodoform)***

Note: AD (inhibition of methanogens) may accumulate acetate 40g/L (55 ^{o}C)

Wall, J., C.S. Harwood, A.L. Demain. *Bioenergy*. ASM Press. 2008. 348~354.

B) Oil producing fungi growing with acetate

Substrates	Initial acetate (g/L)	Final acetate (g/L)	Lipid(g/L)	Lipid in biomass (w/w%)	Conversion (% of theoretical value)
Pure acetate	2.34	0	0.05	3.75	7.5 %
	4.8	0	0.15	9.93	10.9 %
	7.11	0.25	0.23	13.44	11.4 %
Acetate from AD	4.34	2.5	0.03	3.44	**5.8 %**

Mortierella isabellina

Collaborative work with Michigan State University

MICHIGAN STATE UNIVERSITY

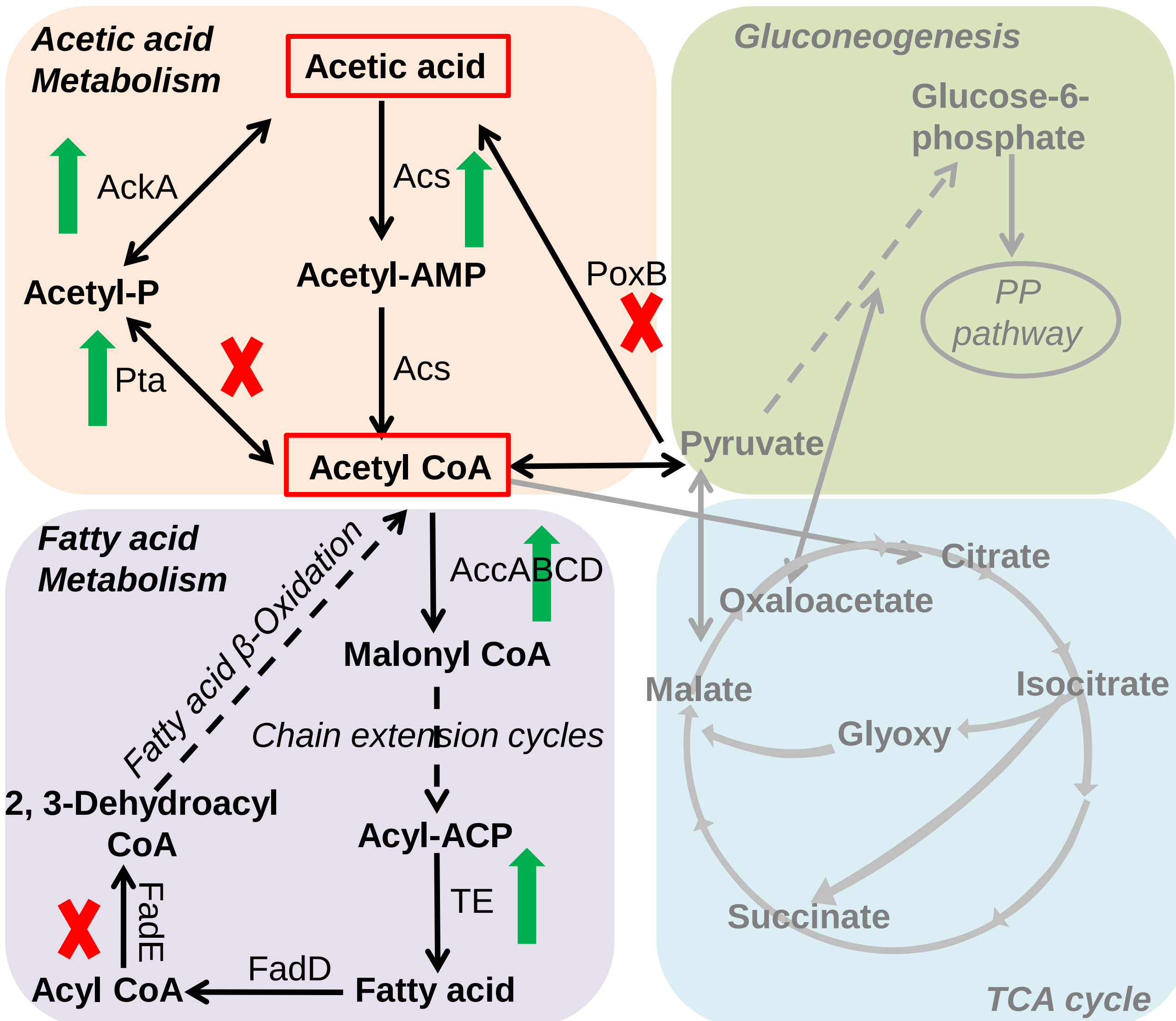
Department of Biosystems and Agricultural Engineering

Wei Liao

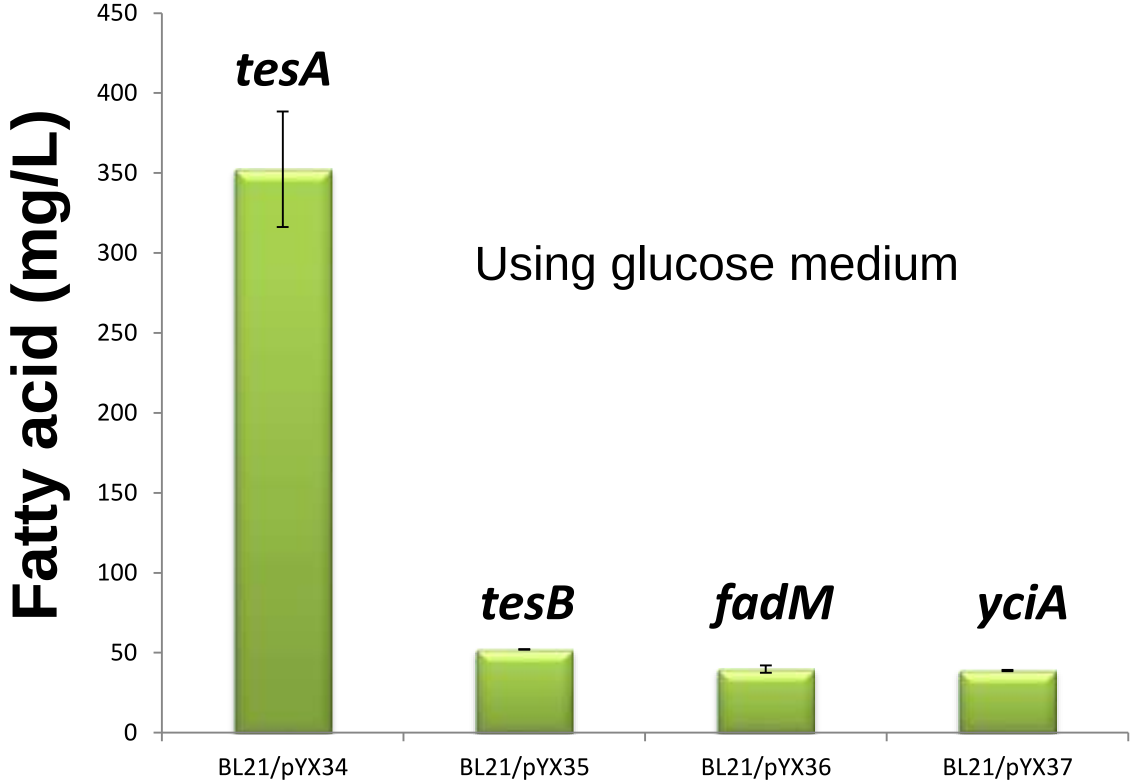
Liu et al, Bioresource Technology, under review

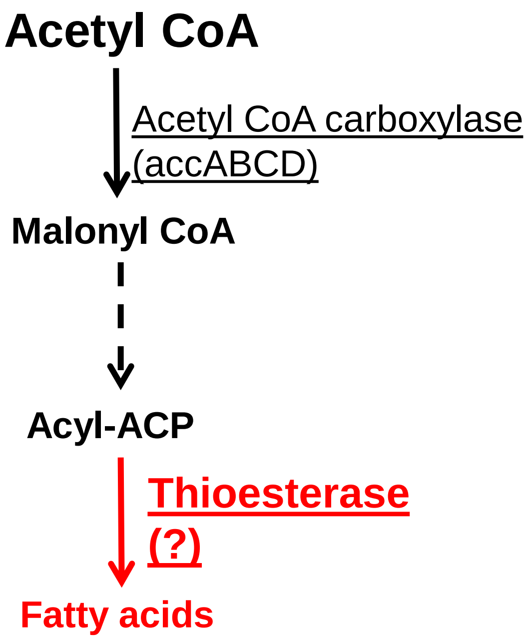
Engineer biosynthetic pathway for fatty acid production from acetic acid

E. coli BL21



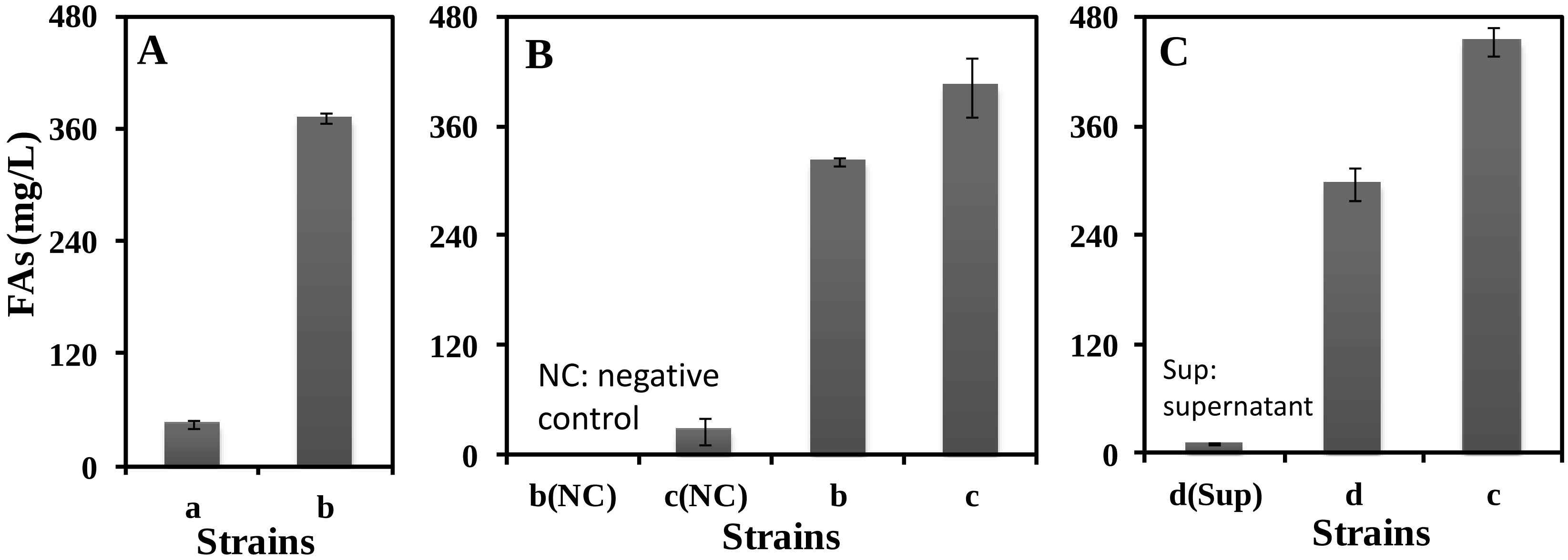
Comparison of four reported acyl-ACP thioesterases from E. coli.





***tesA* is the key enzyme for optimal fatty acid production.**

Create E. coli mutants for fatty acids (FAs) production using acetic acid

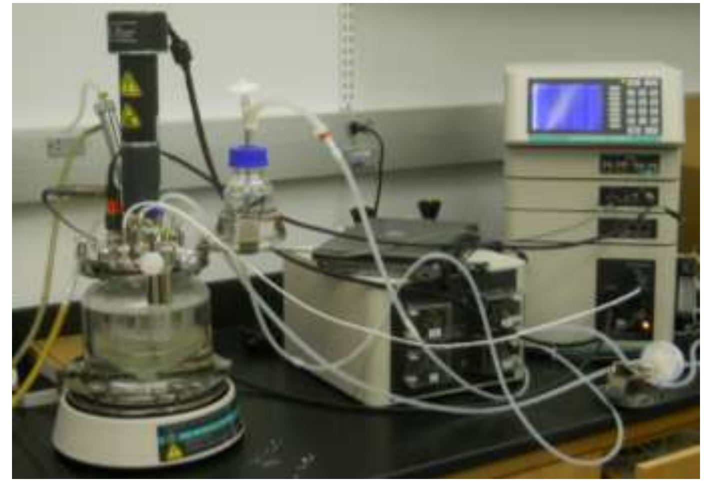


	A: a	A: b	B: b(NC)	B: c(NC)	B: b	B: c	C: d(Sup)	C: d	C: c
acs	+	+	+	+	+	+	+	+	+
tesA	-	+	+	+	+	+	+	+	+
accABCD	-	-	-	-	-	-	+	+	-
ΔfadE	-	-	-	+	-	+	+	+	+

The mutant with acs and tesA produced 360mg/L fatty acids using acetate.

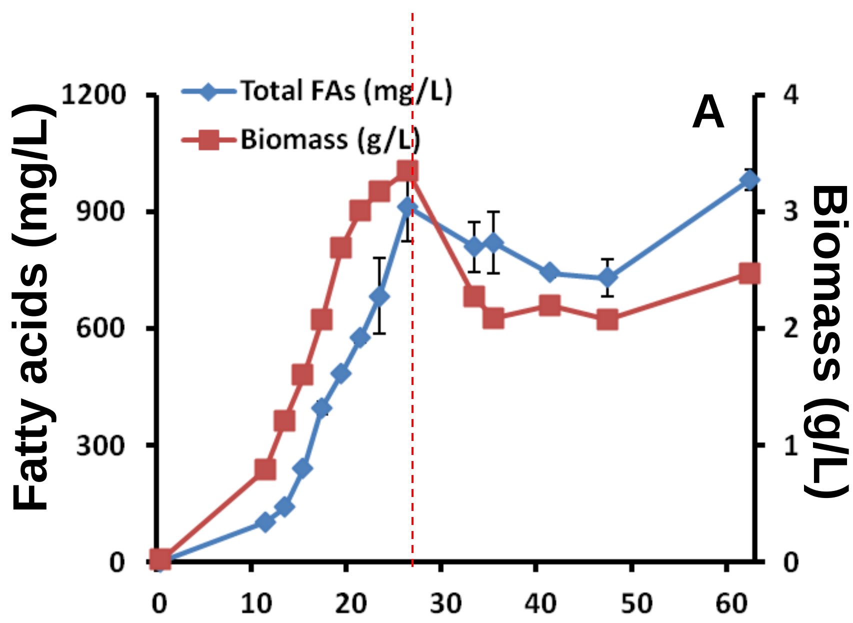
The mutant with ΔfadE, acs and tesA produced ~450 mg/L fatty acids using acetate.

Acetic acid feeding fermentation

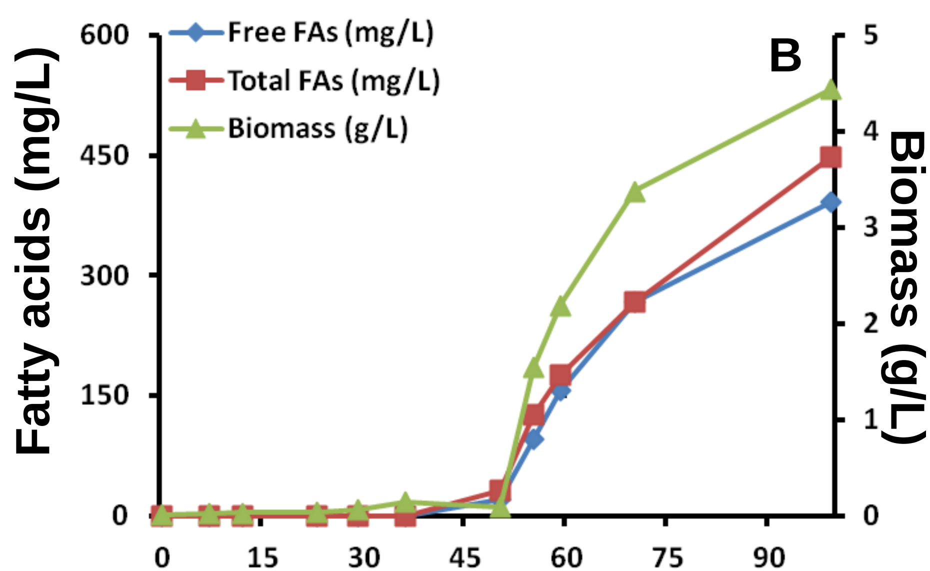


$$CH_3COO^- + 2O_2 \rightarrow 2CO_2 + H_2O + OH^-$$

1. **Fatty acid production is growth associated .**
2. **Yeast extract promote growth and fatty acid production**

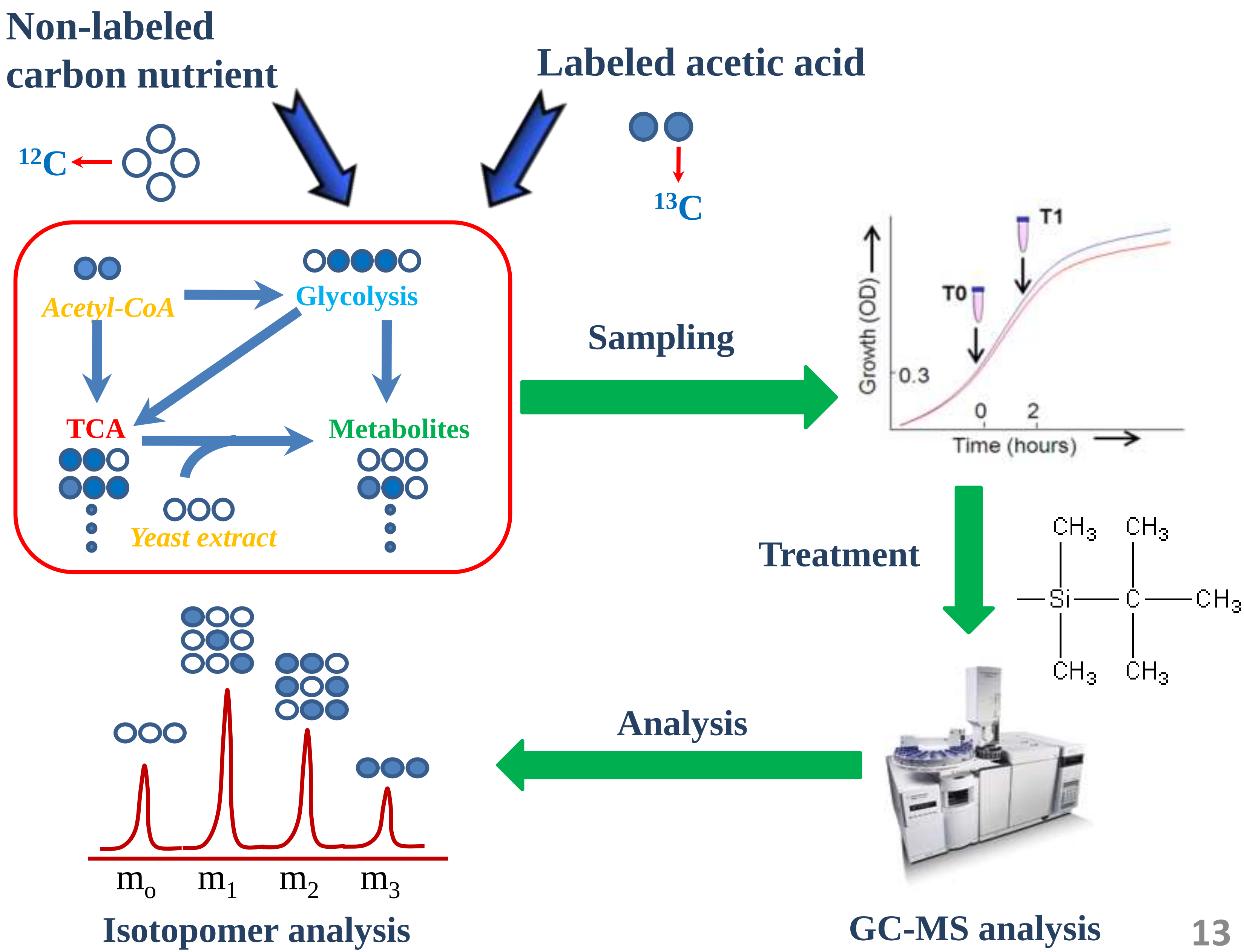


Rich Medium with 1% yeast extract

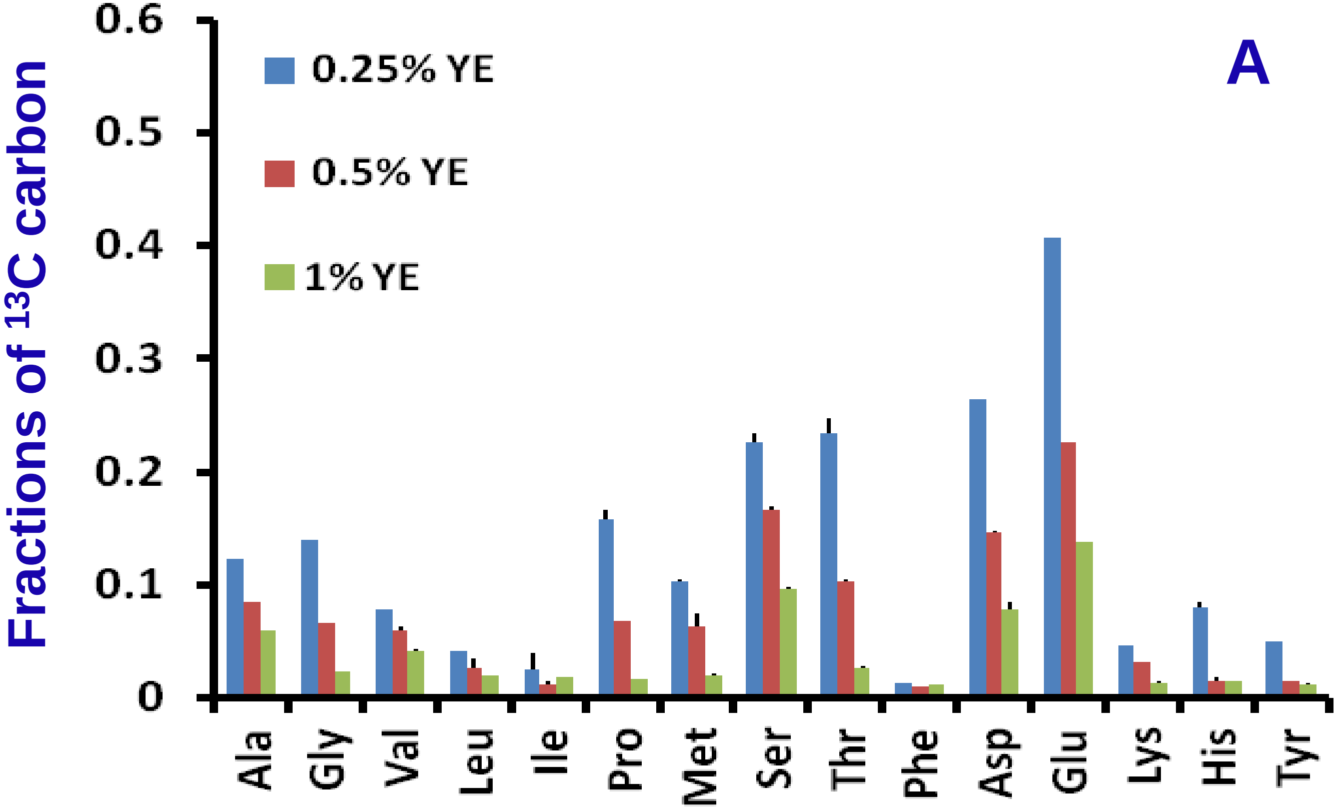


Minimal Medium

^{13}C-isotopic experiments to offer metabolic insights

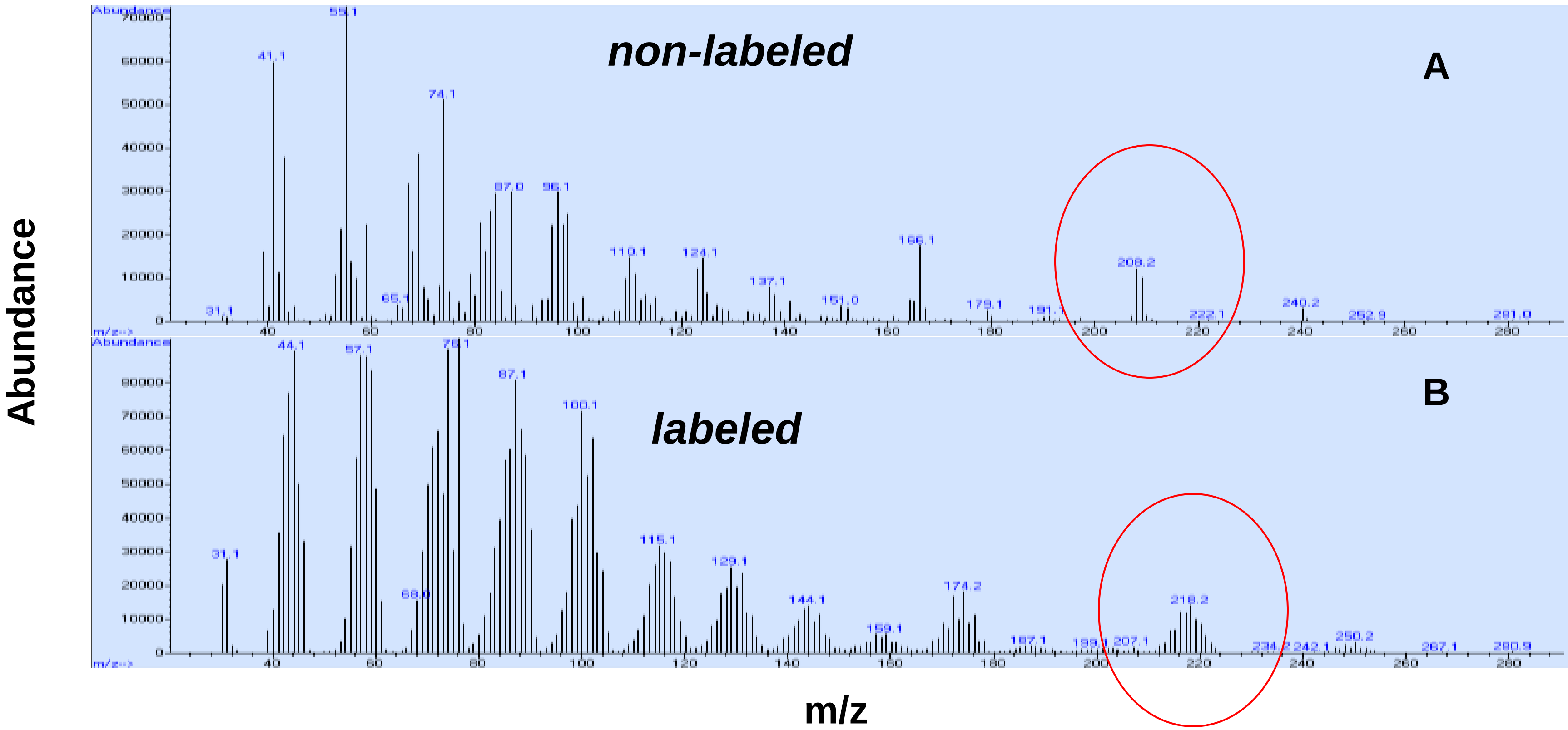


^{13}C-abundance in the proteinogenic amino acids



E.coil biomass growth is mainly dependent on yeast extract

Fatty acids biosynthesis yields



% ^{13}C contributed by HAc	
FAs-C14:0 (20h)	**59.8 %**
FAs-C14:1 (20h)	**65.3 %**
FAs-C12:0 (20h)	**64.2 %**
Average (20h)	**63 %**

$$R_F = \frac{n+1}{n}\left(\frac{100}{98.9}\frac{1}{n+1}\sum_{x=0}^{n+1}\left(x\frac{A_x}{\sum_{x=0}^{n+1} A_x}\right) - \frac{1.1}{98.9}\right)$$

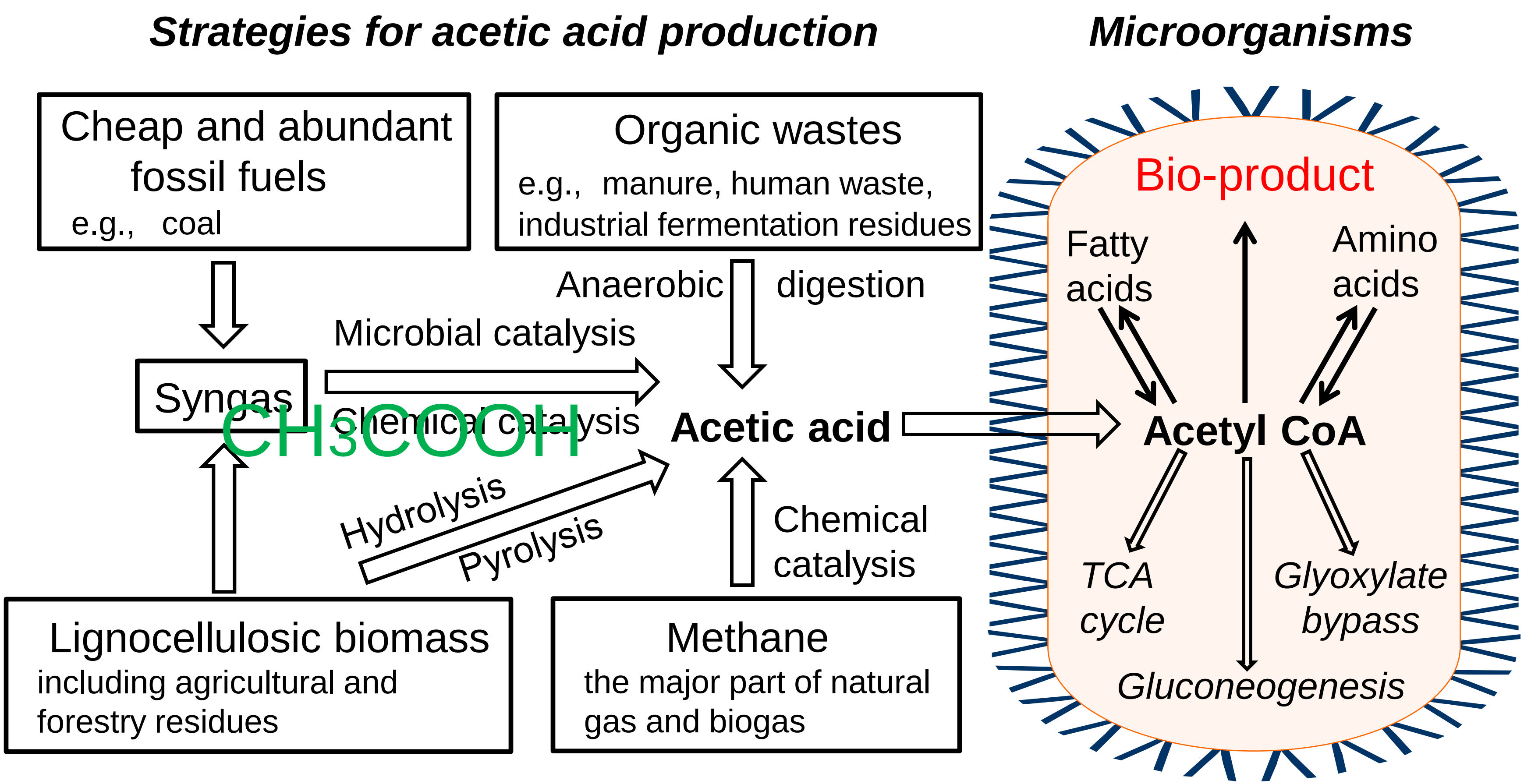
The actual yield :
~ 20% of theoretical yield

Fatty acids produced from pure acetic acid and AD effluent

	Pure acetic acid		AD effluent	
	M.Isabellina	*E.coli*	*M.isabellina*	*E.coli*
Initial acetate (g/L)	7.2	10.0	5.0	4.0
Acetate Consumption (g/L)	7.1	10.0	3.1	4.0
Composition of fatty acids				
C12:0 (mg/L)	-	142.3	-	41.0
C12:1 (mg/L)	-	40.4	-	13.8
C14:0 (mg/L)	-	176.1	-	54.8
C14:1 (mg/L)	-	47.4	-	16.5
C16:0 (mg/L)	55.1	39.0	21.9	21.3
C16:1 (mg/L)	4.7	85.0	2.0	32.2
C18:0 (mg/L)	11.3	8.0	4.9	0.0
C18:1 (mg/L)	69.3	14.8	27.3	5.1
C18:2 (mg/L)	21.4	-	4.7	-
C18:3 (mg/L)	10.9	-	3.4	-
Total fatty acid (mg/L)	172.7	553.0	64.2	184.7
Conversion (g fatty acid/g acetate consumed)	0.02	0.06	0.02	0.09
Yield (% of theoretical yield)	6.9	20.7	6.9	31.0

Jet fuel

Acetic acid feedstock



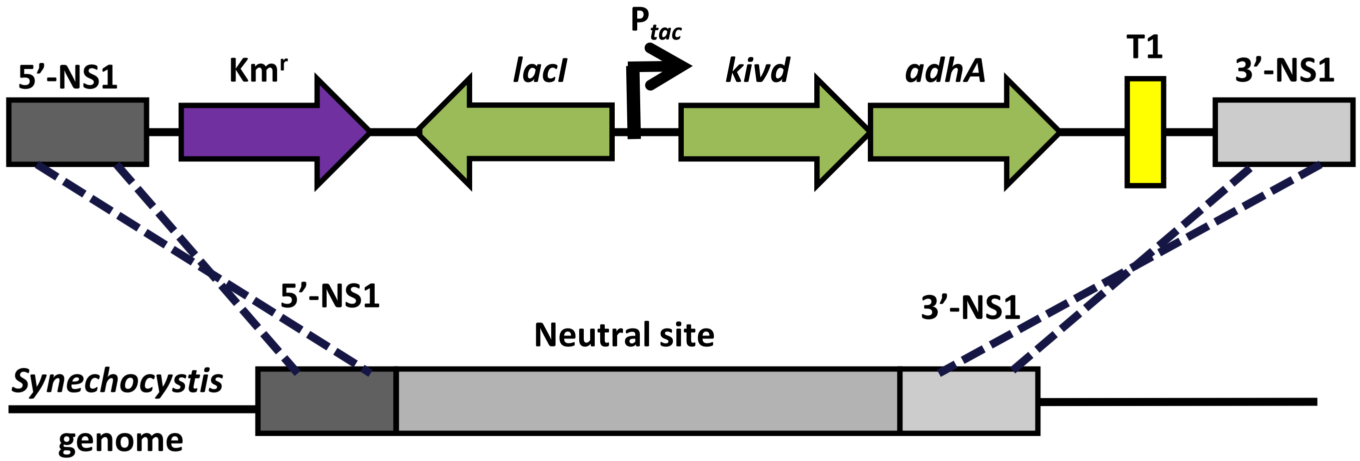
Advantages

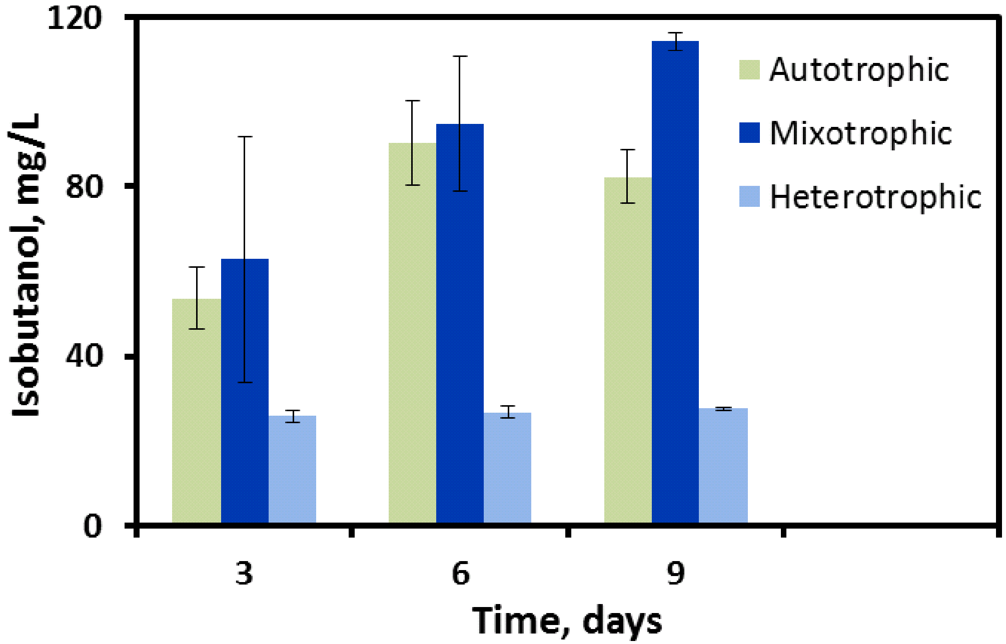
- **Sources: various, cheap, and plentiful**
- **Energy rich**
- **High water solubility (easy mass transfer)**

Disadvantages

- **Toxicity**
- **Slow metabolic utilization**

CO_2 capture *by Synechocystis* 6803 and isobutanol production

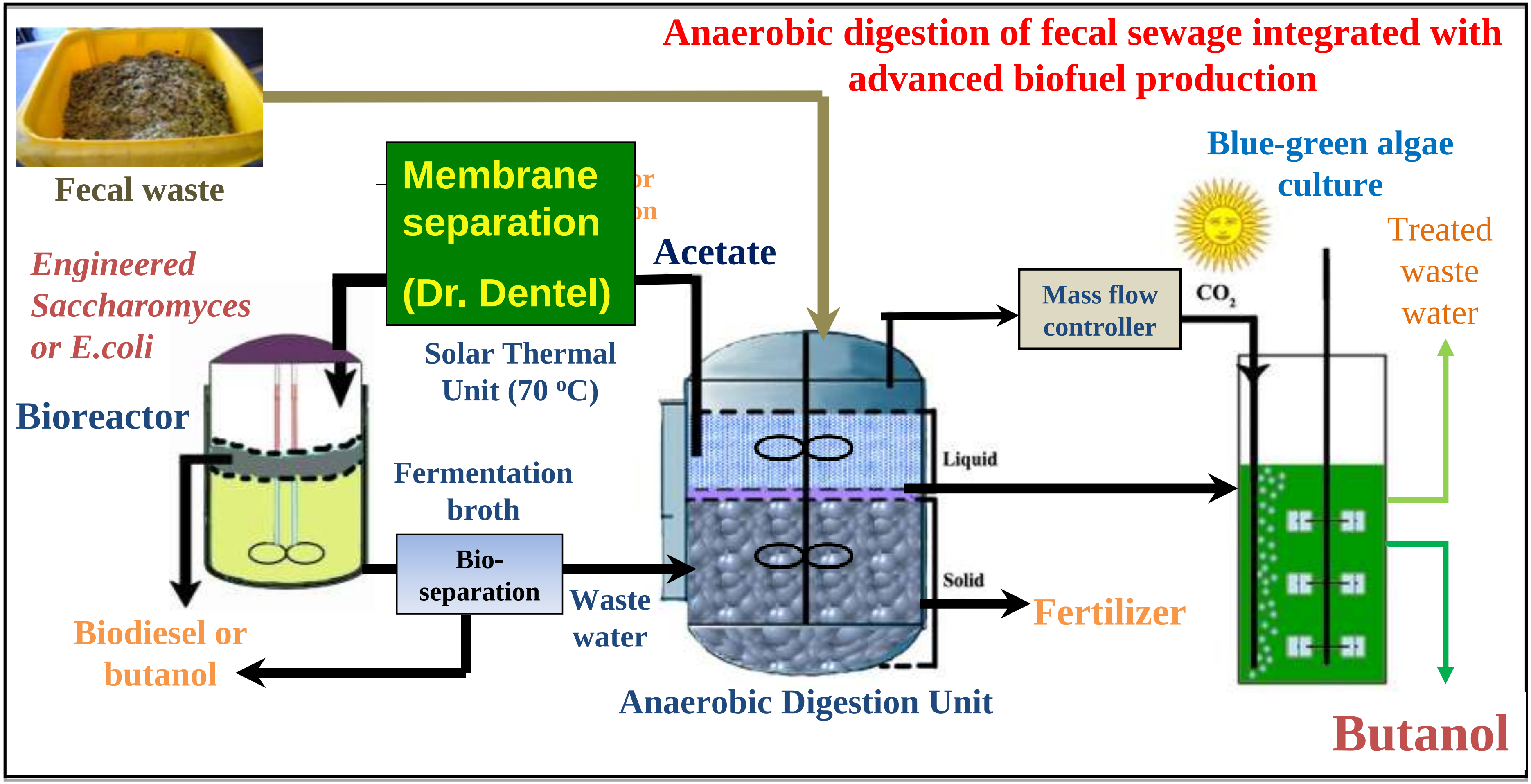




This strain does not require any inducer or antibiotics to maintain its isobutanol production.

Varman, AEM, under review

Integration of anaerobic digestion with microbial conversion



Future Work: 1. Enhance AD acetate accumulation
2. Increase cell metabolic conversion of AD waste
3. Improve product recovery and water reuse

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