



Production of Acetone-Butanol-Ethanol (ABE) using distillers dried grains with solubles (DDGS): Pentose sugar utilization and impact of degradation products

University of Illinois
Dr. Hans Blaschek
Dr. Thaddeus Ezeji



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Acknowledgements

- Flavia Ramirez, Pratibha Ramanu and John Jerrell - University of Illinois
- Other Consortium Members
- Award #DE-FG36-04GO14220



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Why DDGS Utilization?

- Number of dry-grind ethanol plants is growing rapidly in the U.S.
- Currently, dry-grind ethanol plants produce the majority of fuel ethanol (ca 60%) in the U.S. By-products from dry-grind ethanol include wet and dry distiller's grains with solubles.
- More than 7 and 1.1 million metric tons of DDGS is expected to be produced in the U.S. and EU, respectively by the end of this year.
- Some industrial experts are predicting that DDGS production in the U.S. will reach up to 15 million metric tons in few years
- By finding alternative uses for DDGS, ethanol plants can improve their profitability and position themselves to withstand stronger competition (from petrochemical industry)



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Why Bio-Butanol?

Butanol offers significant advantages as a second generation liquid fuel or industrial feedstock chemical:

- Higher energy content than ethanol
- Better solubility characteristics - can be stored under humid conditions
- Can be used in internal combustion and diesel engines
- Niche market in the food and flavor industry as flavor extractant
- Higher overall value as a commodity chemical
- Very large market demand (4.5×10^9 lb/year) which is petrochemical based (except China)



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Liquid Fuel Characteristics

Characteristic	Gasoline	Diesel	Methanol	Ethanol	Butanol
Formula	C4-C12 hydrocarbons	C14-C20 hydrocarbons	CH ₃ OH	CH ₃ CH ₂ OH	CH ₃ (CH ₂) ₃ OH
Boiling Point					
°C	32-210	204-343	65	78	118
°F	90-410	400-650	149	173	244
Lower heating value*					
MJ/kg	44.5	43.0	19.6	26.9	33.1
Btu/gal	114,800	140,000	55,610	76,100	96,100



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Objectives:

- Screen various solventogenic clostridia for their ability to ferment mixed hexose and pentose sugar streams for ABE production
- Carry out studies on the utilization pattern of mixed sugar streams i.e. determine whether sugar utilization occurs sequentially, simultaneously and if the microbes have a preference for one sugar over another
- Test for the effect of potential inhibitory compounds [(A). products of hemicellulose hydrolysis, and (B). degradation products] on growth and ABE production by solventogenic clostridia e.g. *Clostridium beijerinckii* BA101



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Objectives (cont.)

- Test for the ability of DDGS which has been subjected to different pre-treatment regimes to be fermented into ABE



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Composition of DDGS

Dry matter content	88.8
Total glucan	23.4
Cellulose	18.3
Starch	5.1
Xylan	6.9
Arabinan	5.3
Ash & others	29.8



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Composition of DDGS hydrolysates

- Hexose sugars: glucose, mannose, galactose, fructose
- Pentose sugars: xylose and arabinose
- Proteins
- Fat and oils
- Lignins
- Acetate
- Uronic acids (e.g glucuronic acid)
- Other acids: ferulic, p-coumaric acids, etc



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Pretreatment and Hydrolysis of DDGS

- Hot water
- Ammonia fiber explosion (AFEX)
- Other Treatments
- Enzymatic



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Composition of DDGS hydrolysates: potential degradation products

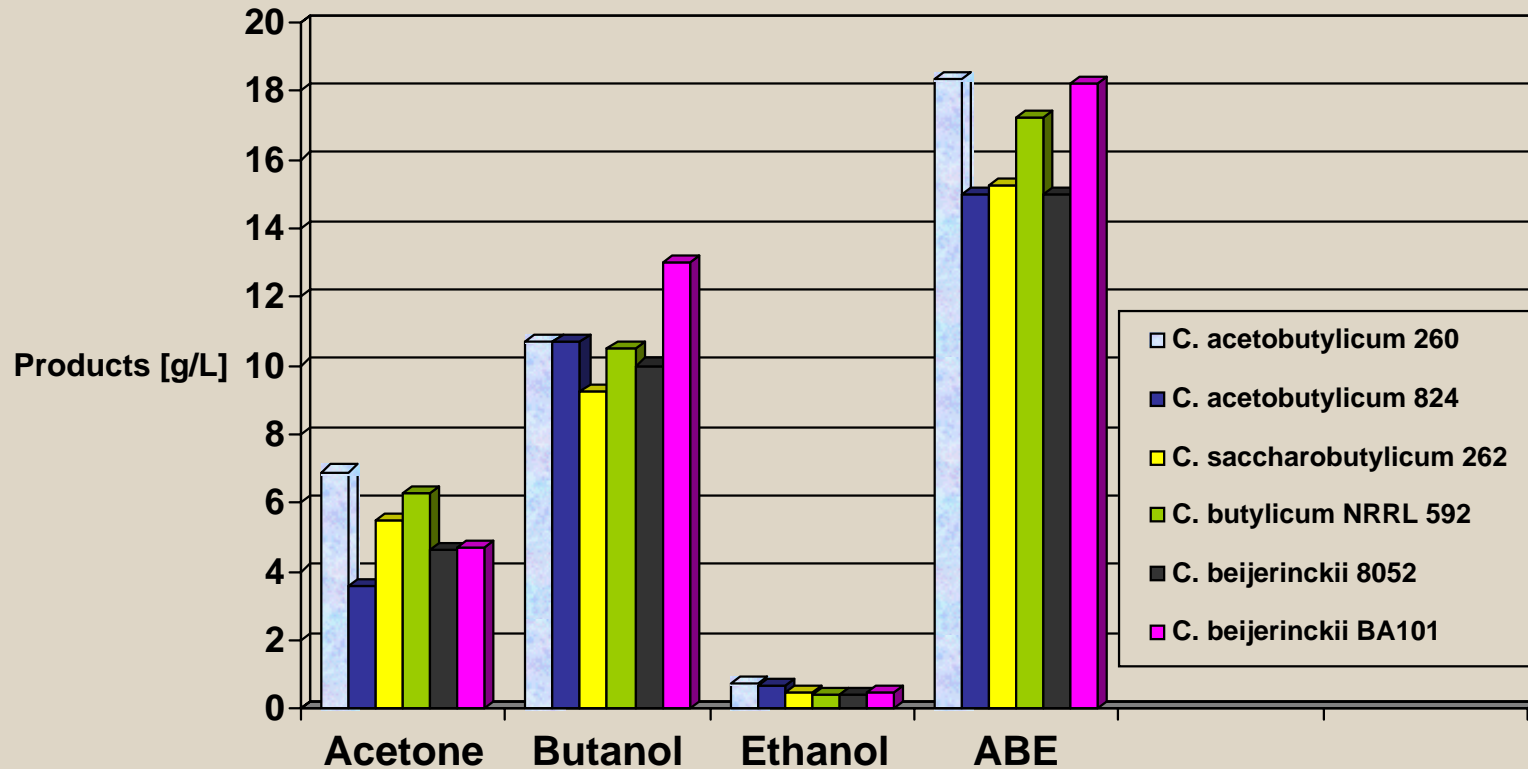
- **Glucose** \longrightarrow Hydroxymethylfurfural
 - Formic acid
 - Levulinic acid
- **Xylose** \longrightarrow Furfural
 - Tars
 - Other degradation products
- **Lignin** \longrightarrow
 - Syringaldehyde
 - p-hydroxybenzaldehyde
 - Cinnamaldehyde
 - Other products



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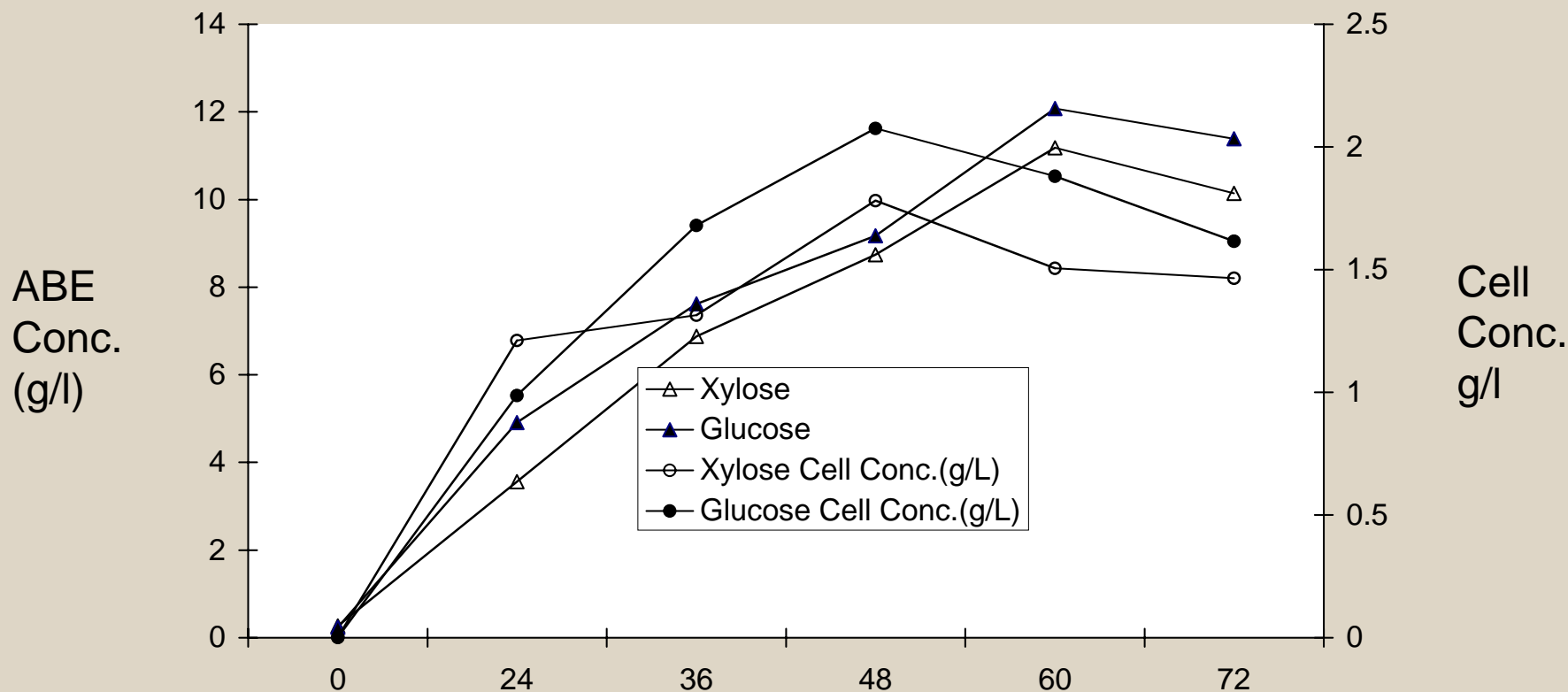
Fermentation of glucose to ABE by solventogenic clostridia



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ABE Fermentation by *Clostridium beijerinckii* using xylose or glucose (30 g/L) as substrate

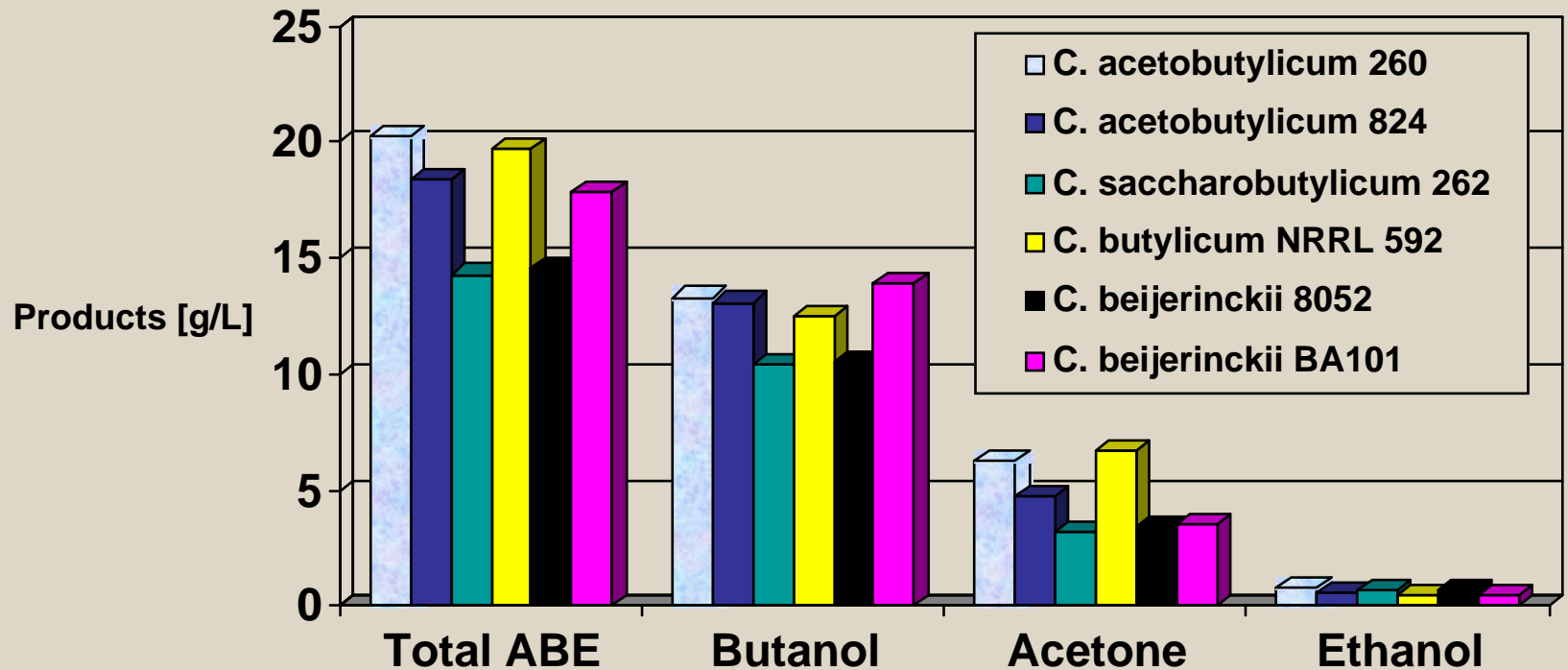


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Fermentation of Hexose and Pentose sugars for ABE production by solventogenic clostridia

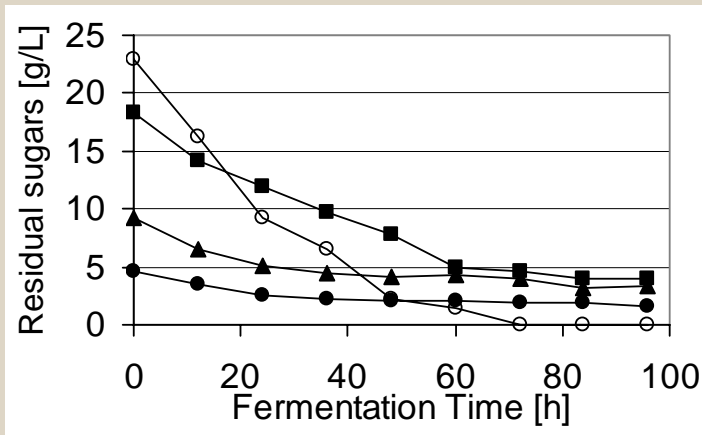
Glucose-mannose-arabinose-xylose composite (g/L: 25:5:10:20)



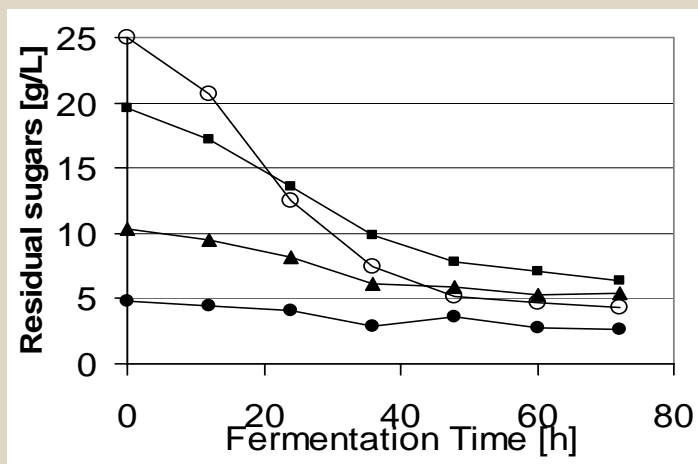
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Utilization of hexose and pentose sugars by various solventogenic Clostridia



C. beijerinckii BA101



C. beijerinckii 8052

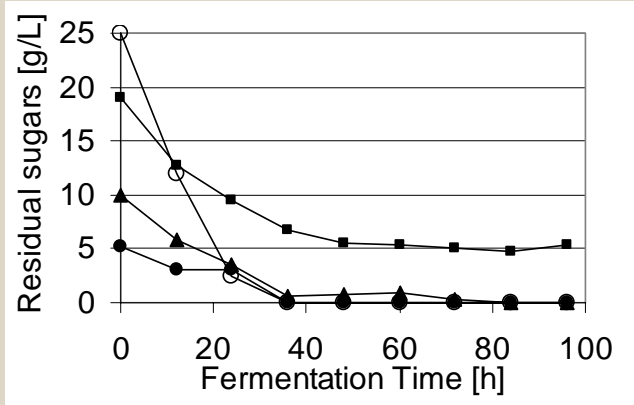
Symbols: O , glucose; ■ , xylose; ▲ , arabinose; ● mannose.



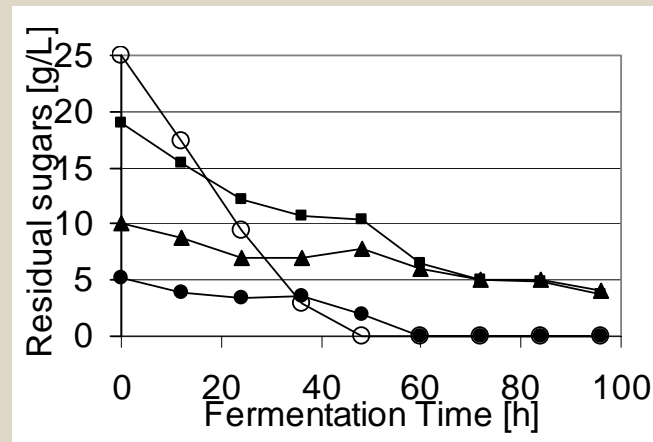
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Utilization of hexose and pentose sugars by solventogenic clostridia



C. acetobutylicum 260



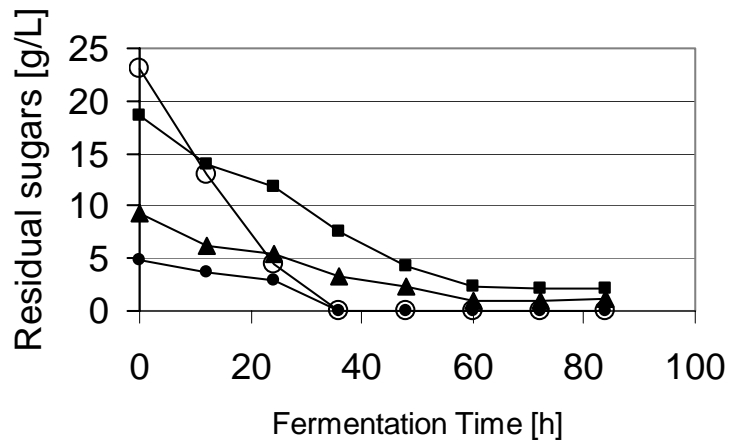
C. acetobutylicum 824



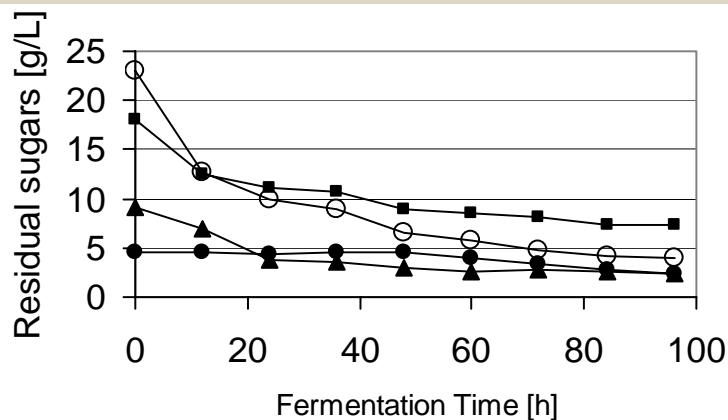
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Utilization of hexose and pentose sugars by solventogenic clostridia



C. butylicum 592



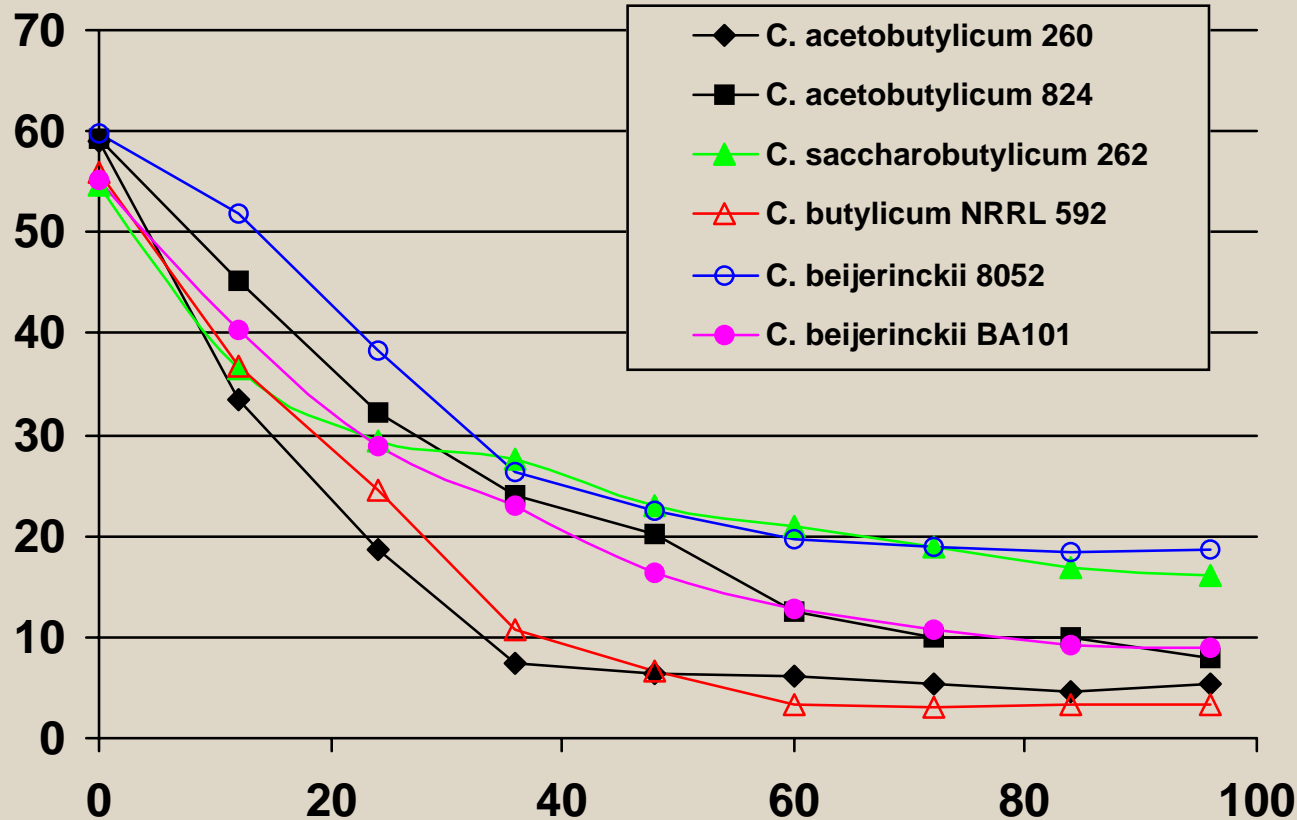
C. saccharobutylicum 262



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Residual total sugar concentrations during growth and ABE production by solventogenic Clostridia



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Sugar preference during batch fermentation of mixed sugar streams (glucose, mannose, arabinose, xylose) by solventogenic clostridia

Organisms	Fermentation Time [h]*	ABE produced [g/L]	Glucose: mannose: arabinose: xylose (%) utilized	Sugar preference
<i>C. acetobutylicum</i> 260	48	20.3	100: 100: 92: 71	Glucose> mannose arabinose>xylose
<i>C. acetobutylicum</i> 824	96	18.4	100: 100: 60: 80	Glucose> mannose> xylose>arabinose
<i>C. saccharobutyl- icum</i> 262	72	14.3	74: 38: 63: 42	Glucose> arabinose > xylose> mannose
<i>C. butylicum</i> NRRL 592	60	19.7	100: 100: 89: 86	Glucose> mannose > arabinose>xylose
<i>C. beijerinckii</i> 8052	60	14.6	81: 42: 49: 64	Glucose> xylose > arabinose >mannose
<i>C. beijerinckii</i> BA101	84	18.0	100: 59: 65: 78	Glucose> xylose > arabinose >mannose

*time required to achieve maximal ABE concentration.

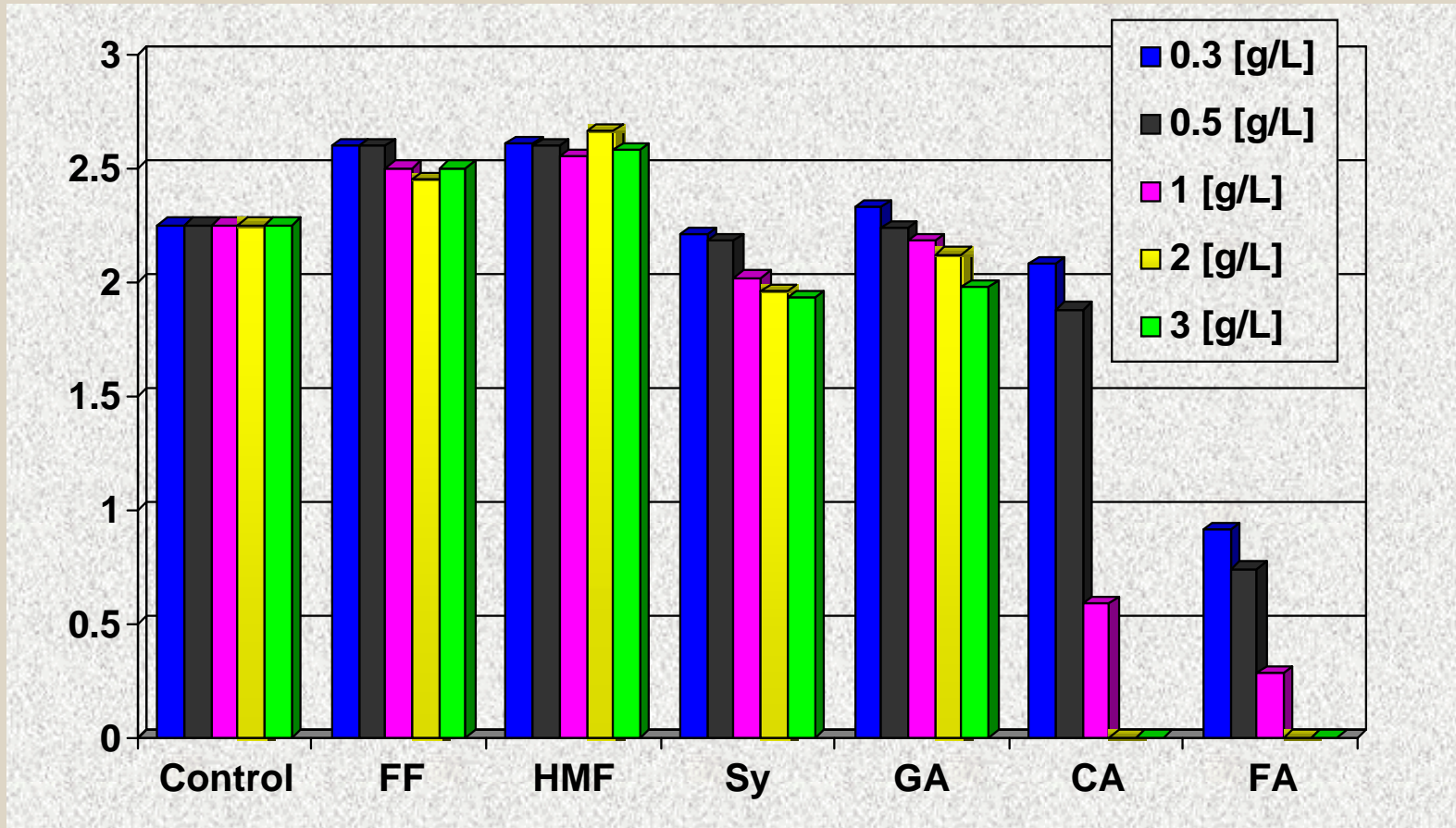


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Effect of representative degradation and hydrolysis products on the growth of *Clostridium beijerinckii* BA101

Cell
Conc.
g/l



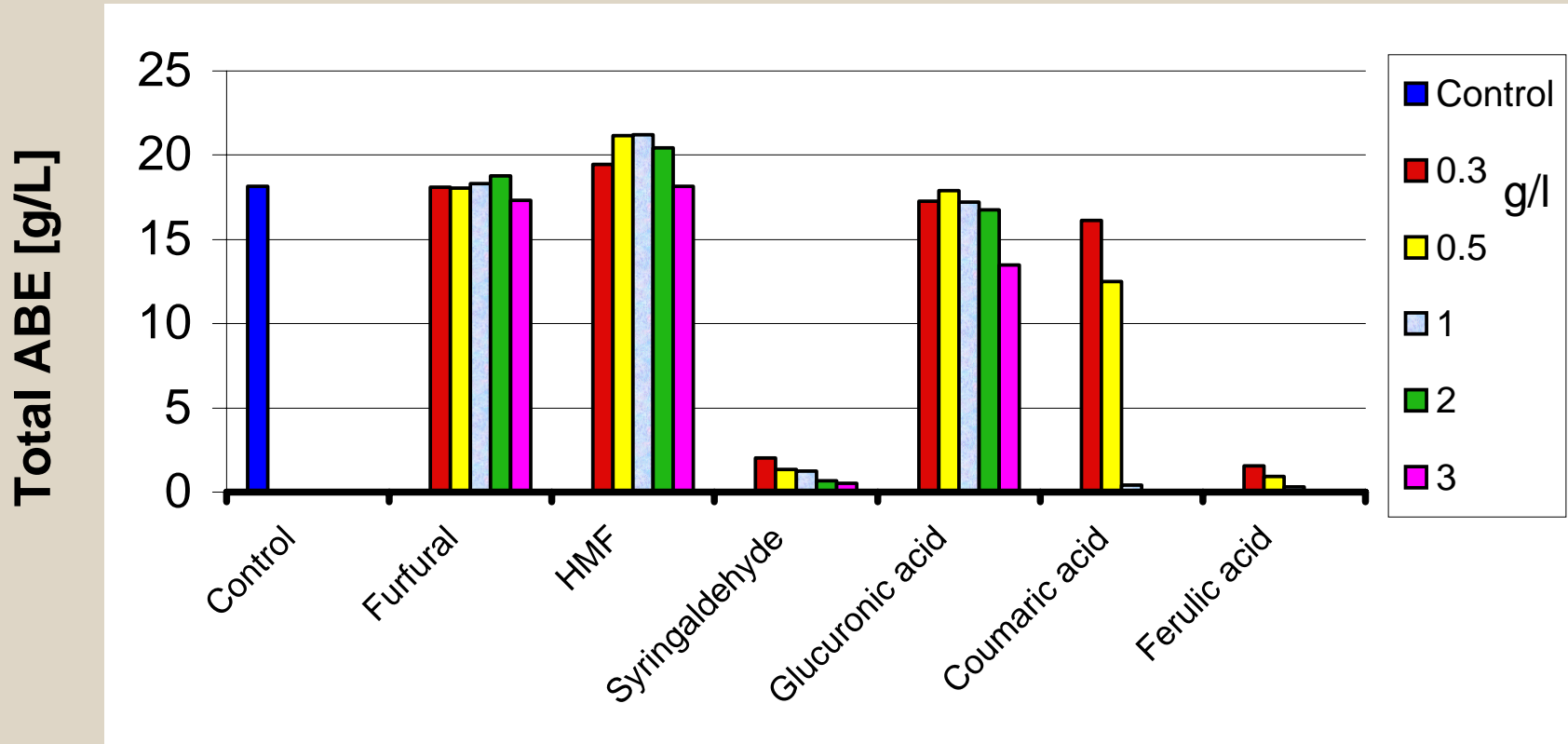
FF: Furfural; HMF: Hydroxymethylfurfural; SY: Syringaldehyde; GA: Glucuronic acid; CA: Coumaric acid; FA: Ferulic acid



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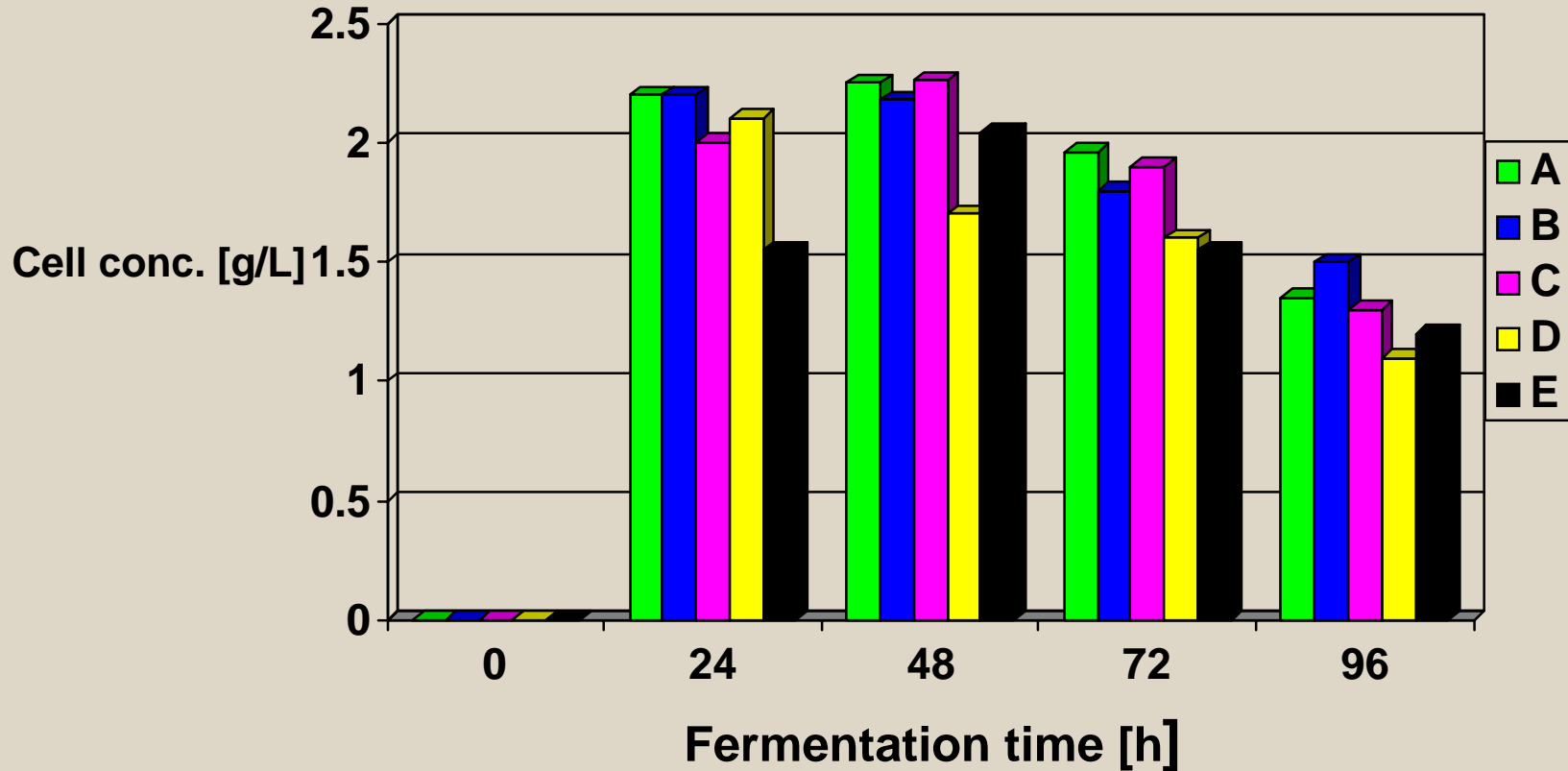
Effect of potential degradation and hydrolysis products on the ABE fermentation by *Clostridium beijerinckii* BA101



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Effect of degradation and hydrolysis products on cell concentration and ABE production by *C. beijerinckii* BA101



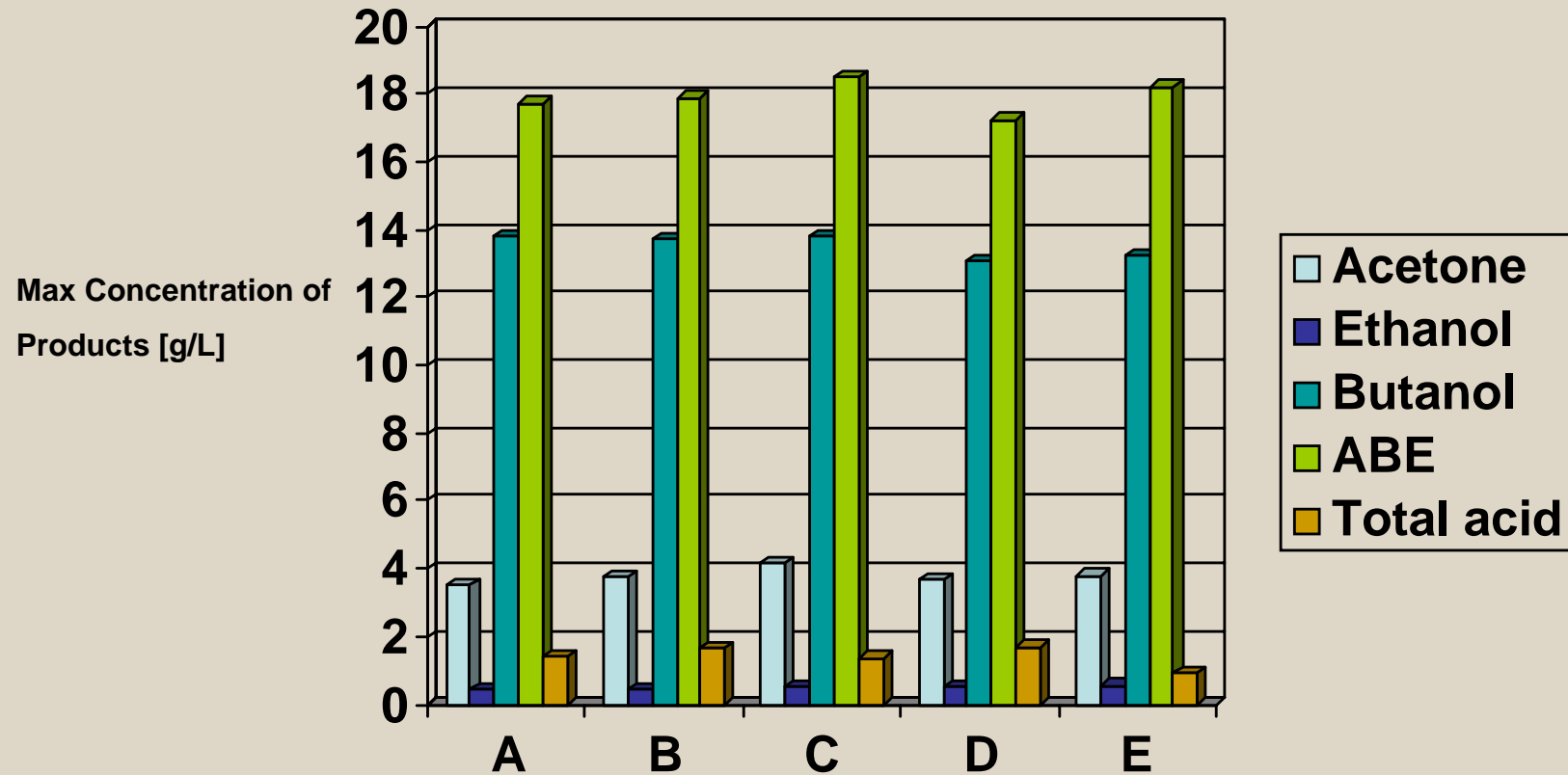
A = Control; B = Furfural + HMF (1.0 g each); C = Furfural + HMF (1.5 g each);
D = Furfural + HMF + Glucuronic acid (0.67 g each); E = Furfural + HMF + Glucuronic acid (1.0 g each) .



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Effect of degradation and hydrolysis products on cell concentration and ABE production by *C. beijerinckii* BA101



A = Control; B = Furfural + HMF (1.0 g each); C = Furfural + HMF (1.5 g each);
D = Furfural + HMF+ Glucuronic acid (0.67 g each); E = Furfural + HMF + Glucuronic acid (1.0 g each). Solvent concentration results reported are the maximum produced



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Summary

- *C. beijerinckii* BA101, *C. acetobutylicum* 260, and *C. butylicum* 592 utilized a mixed sugar stream for ABE production. Although glucose is the preferred carbon source, these microbes have the ability to concurrently utilize different (hexose and pentose) sugars for ABE production.
- At the concentration tested, furfural and HMF have no inhibitory effects on *C. beijerinckii* BA101. No negative synergistic effect was observed when these were combined.
- Syringaldehyde, ferulic and p-coumaric acids were potent inhibitors of ABE production by *C. beijerinckii* BA101.



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Future work

- Hydrolysates of DDGS produced by different pretreatment methods will be analyzed for the presence and concentration of microbial inhibitors.
- Hydrolysates of DDGS produced via different pretreatment methods will be used for ABE fermentation using *C. beijerinckii* BA101, *C. acetobutylicum* 260, and *C. butylicum* 592.
- Adaptation experiments that include degradation and hemicellulose by-products generated during acid, alkali or thermal pretreatment of DDGS may be carried out.



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