


Fermentation of Dried Distillers' Grains and Solubles (DDGS) hydrolysates to solvents and value-added products by solventogenic clostridia

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Midwest Consortium for Biobased Products & Bioenergy


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.
- Approximately 9 and over 1 million metric tons of DDGS were produced in the North America and EU, respectively in 2005
- 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)


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


Liquid Fuel Characteristics

Characteristic	Gasoline	Diesel	Methanol	Ethanol	Butanol
Formula	C4-C12 hydrocarbons	C14-C20 hydrocarbons	CH_3OH	CH_3CH_2OH	**$CH_3(CH_2)_3OH$**
Boiling Point					
^oC	32-210	204-343	65	78	**118**
^oF	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**

Objectives:

- Screen various solventogenic clostridia for their ability to ferment mixed hexose and pentose sugar streams for acetone butanol ethanol (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

Objectives (cont.)

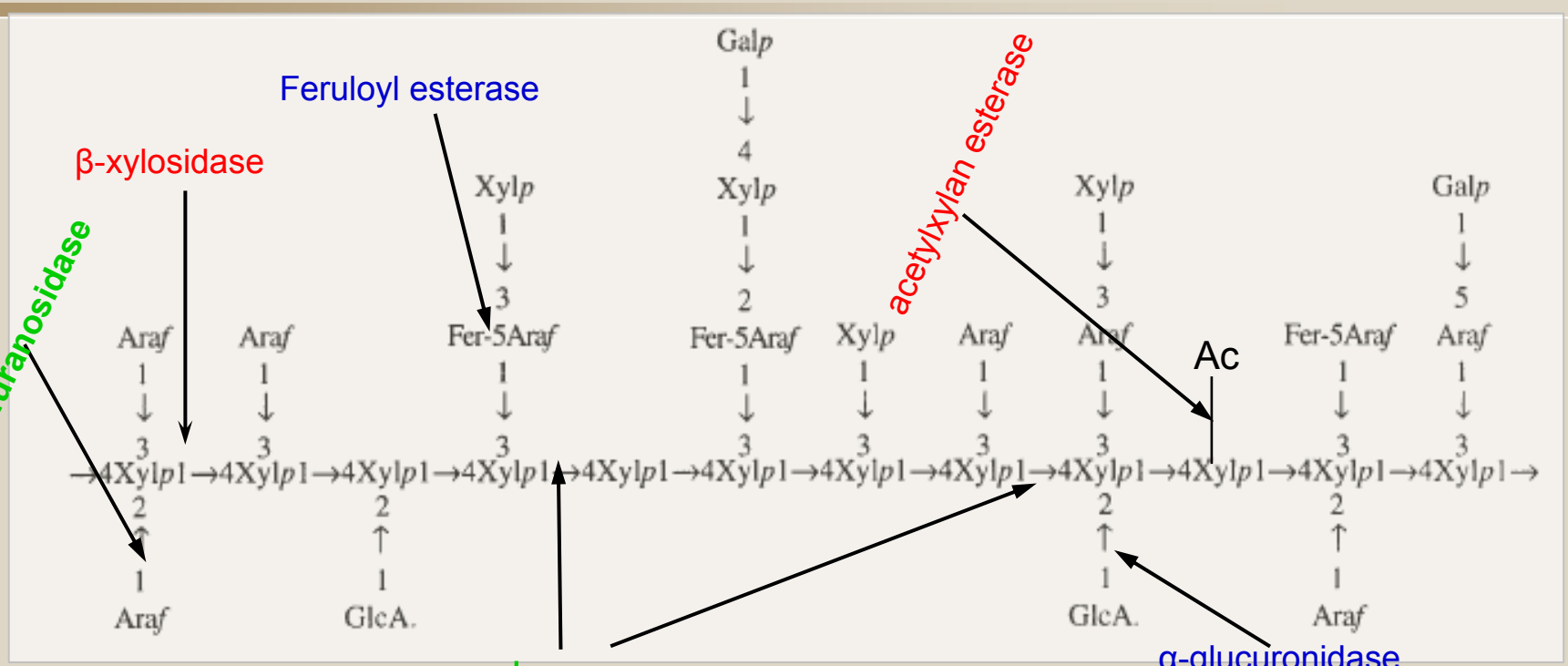
- Test for the ability of DDGS which has been subjected to different pre-treatment regimes to be fermented into ABE

Composition of DDGS

		Average (95% confidence intervals)
Gross Matter Composition	Moisture Content (% total)	11.2 ± 0.6%
	Dry Matter Content (% total)	88.8 ± 0.6%
Dry Matter Composition	Glucan (total) (% dry matter)	20.9 ± 7.1%
	Cellulose (% dry matter)	16.0 ± 6.6%
	Starch (% dry matter)	5.2 ± 1.0%
	Xylan (% dry matter)	8.2 ± 3.3%
	Arabinan (% dry matter)	5.3 ± 0.7%
	Protein (% dry matter)	26.4%
	Total Dry Matter Mass Closure	92.7%

Based on analysis at U. Illinois, USDA NCAUR, Purdue

Schematic structure of maize bran heteroxylan



Araf: Arabinofuranose, Xylp: Xylopyranose, Galp: Galactopyranose, GlcA: Glucuronic acid, Fer: Ferulic acid, AC: acetylxylan

Source: Saulnier et al, 1999

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Pretreatment and Hydrolysis of DDGS

- Dilute acid
- Alkali
- Hot water/and SO_2 impregnation
- Steam explosion/and SO_2 catalyzed steam explosion
- Ammonia fiber explosion (AFEX)
- Enzymatic

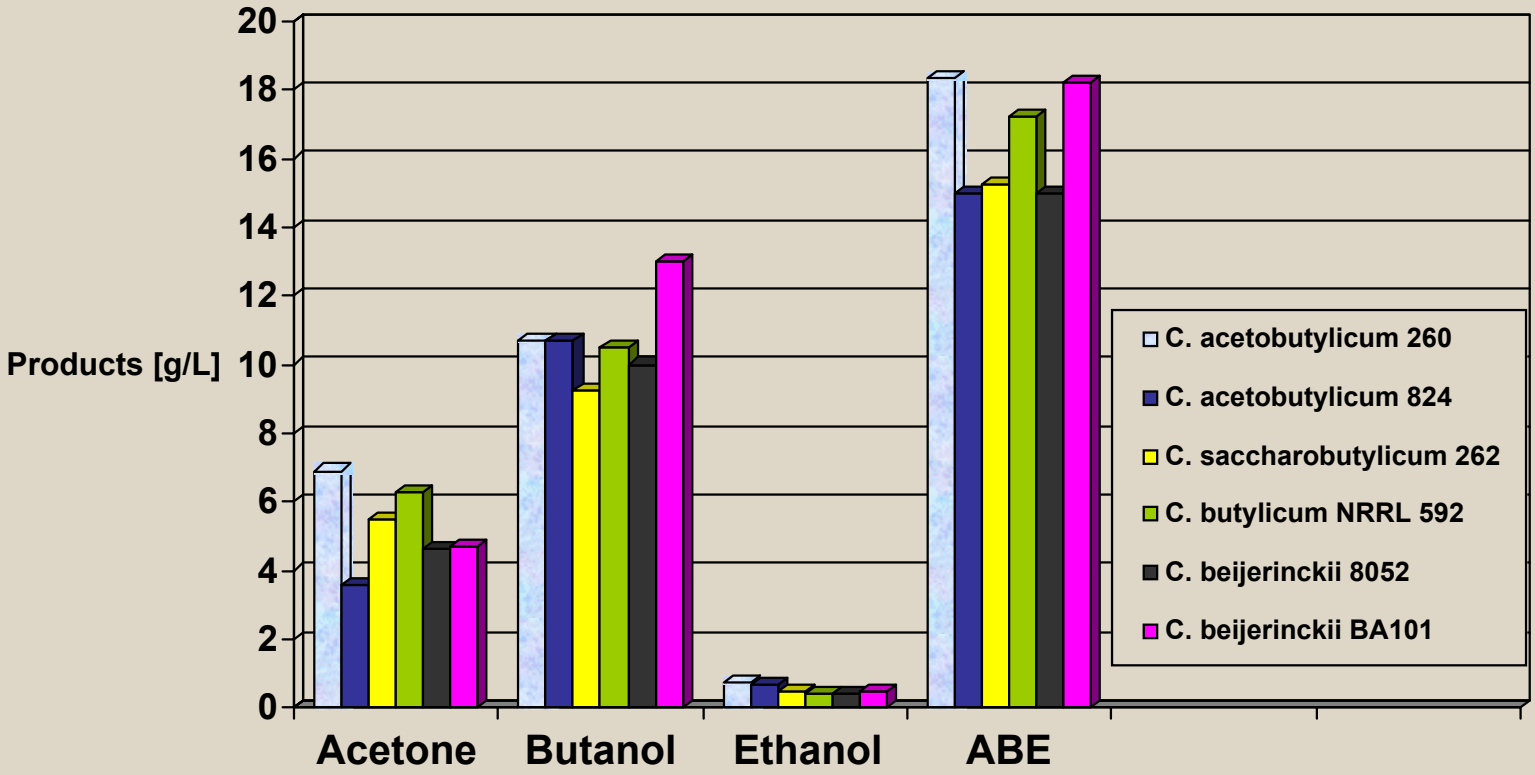
Composition of DDGS hydrolysates

- Hexose sugars: glucose, mannose, galactose, fructose
- Pentose sugars: xylose and arabinose
- Ferulic and glucuronic acids
- Acetate
- Proteins, Fat and oils

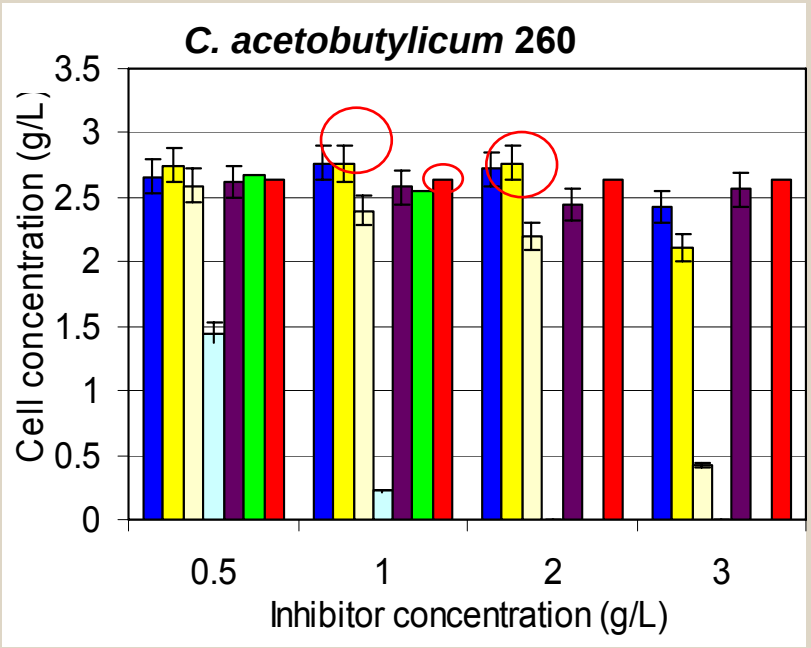
Composition of DDGS hydrolysates: potential degradation products

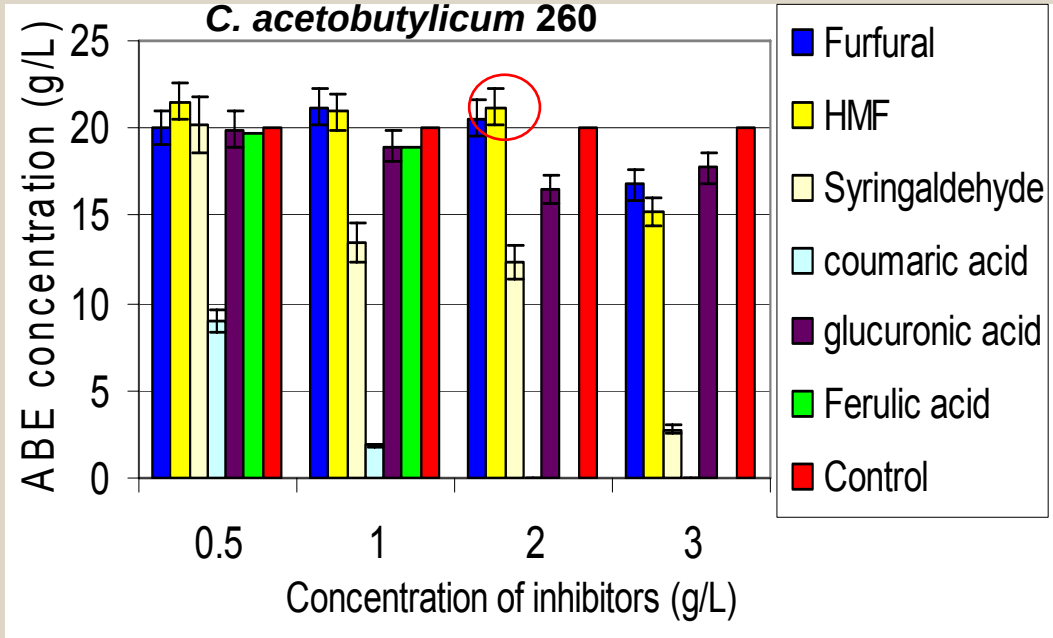
- Glucose → Hydroxymethylfurfural → Formic acid
  Hydroxymethylfurfural → Levulinic acid
- Xylose → Furfural → Tars
  Furfural → Other degradation products
- Lignin →
  - Syringaldehyde
  - p-hydroxybenzaldehyde
  - Cinnamaldehyde
  - P-coumaric acid
  - Other products

Fermentation of glucose to ABE by solventogenic clostridia

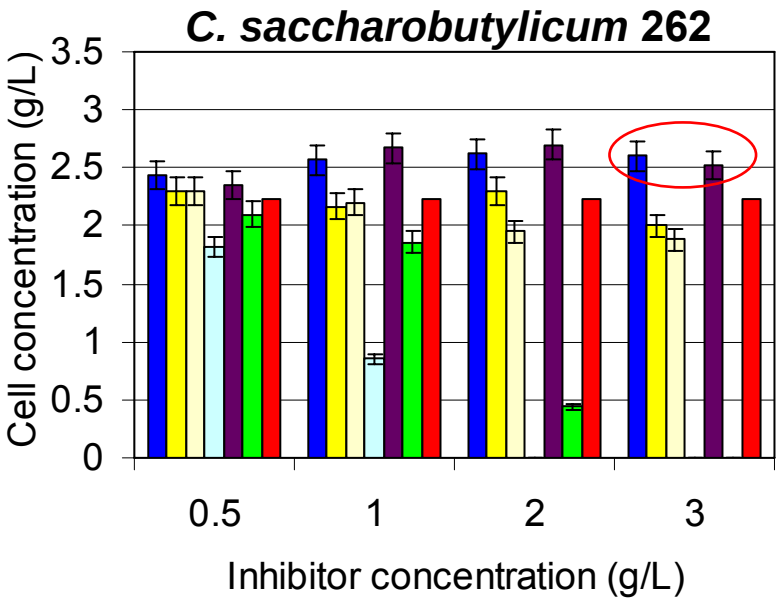


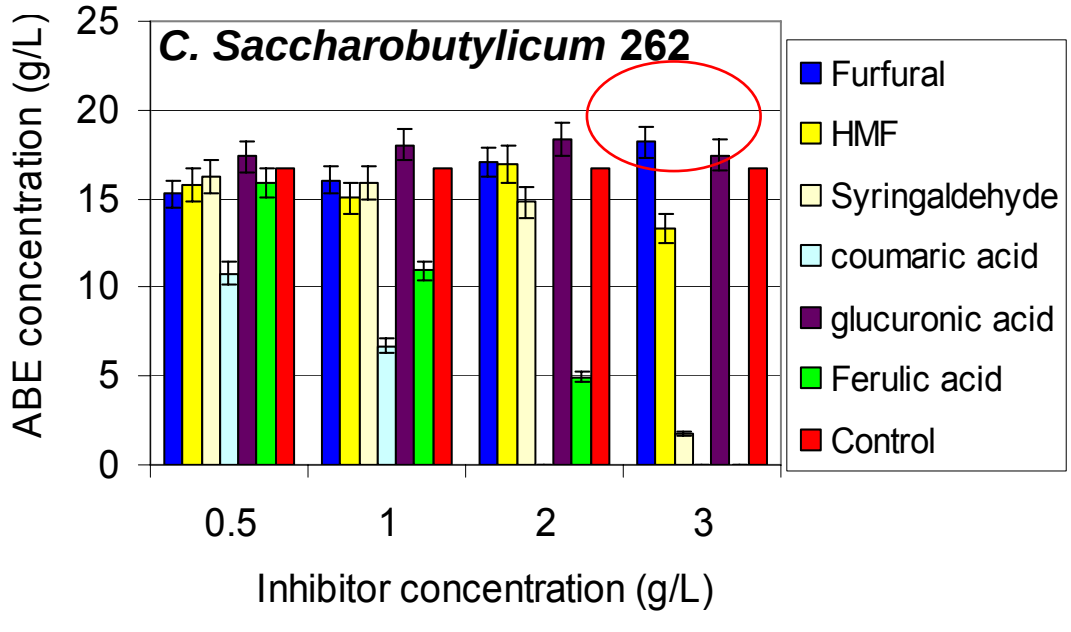
Effect of representative degradation and hydrolysis products on growth and ABE production by Clostridia



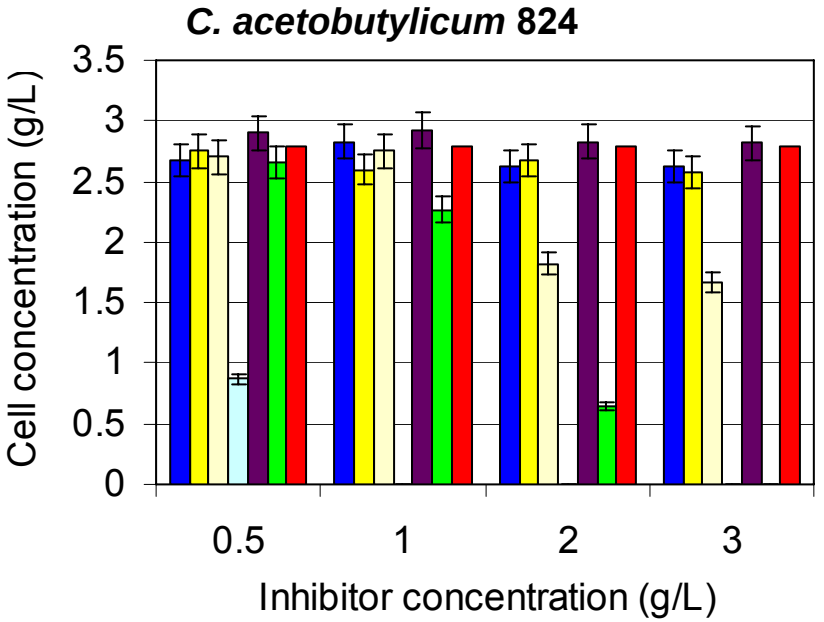


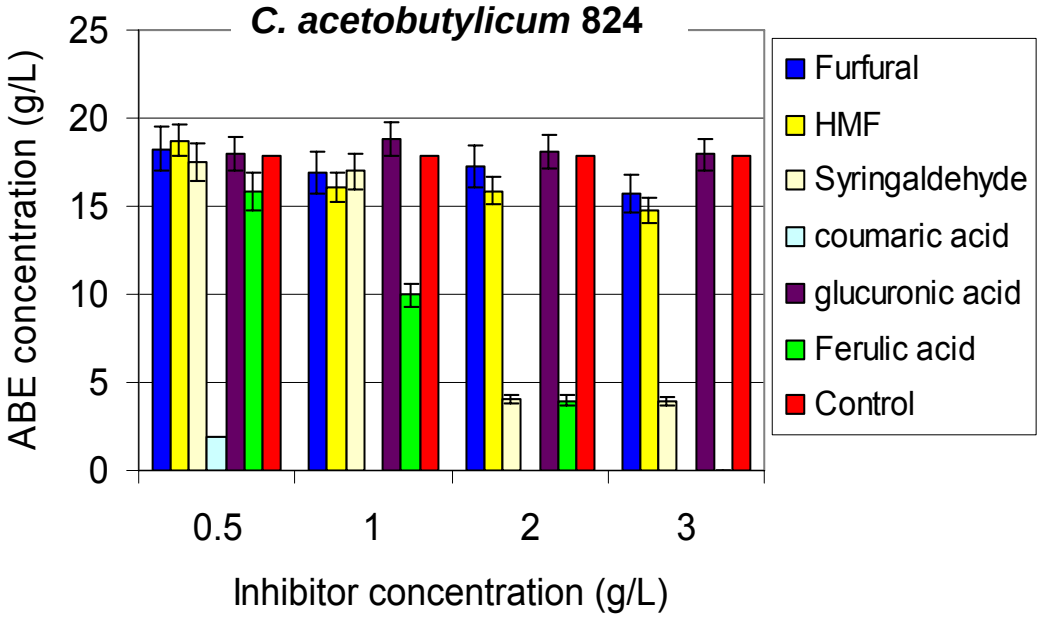
Effect of representative degradation and hydrolysis products on growth and ABE production by Clostridia Contd.



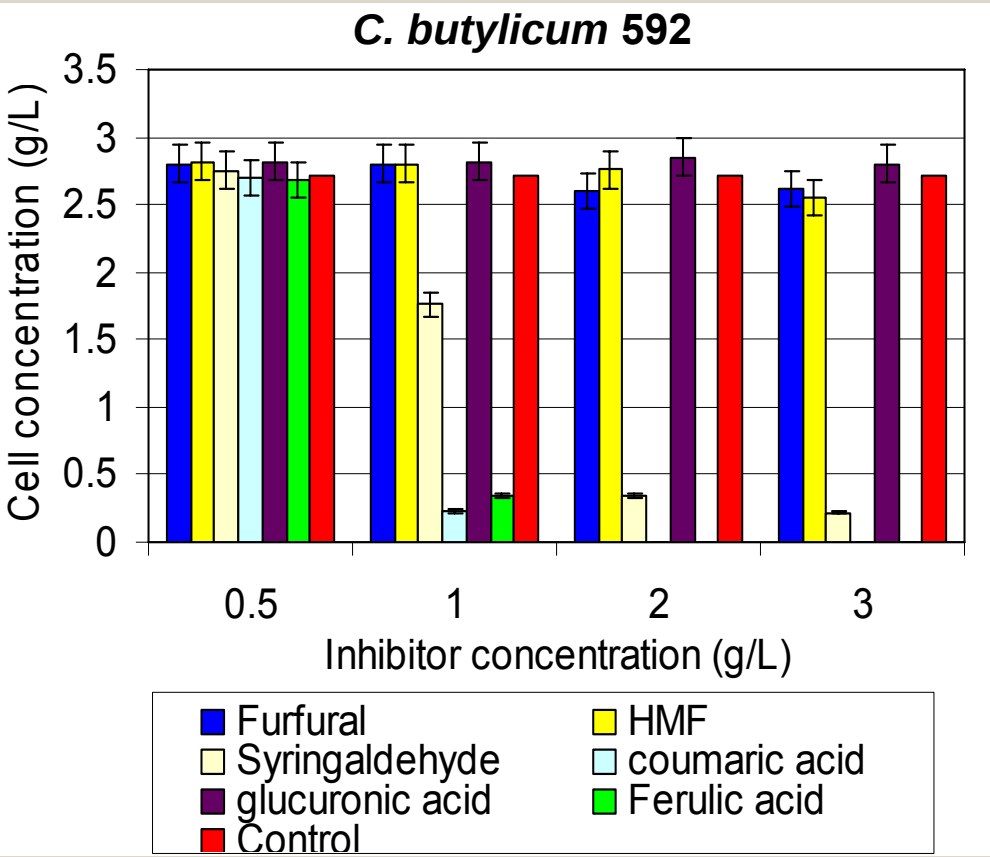


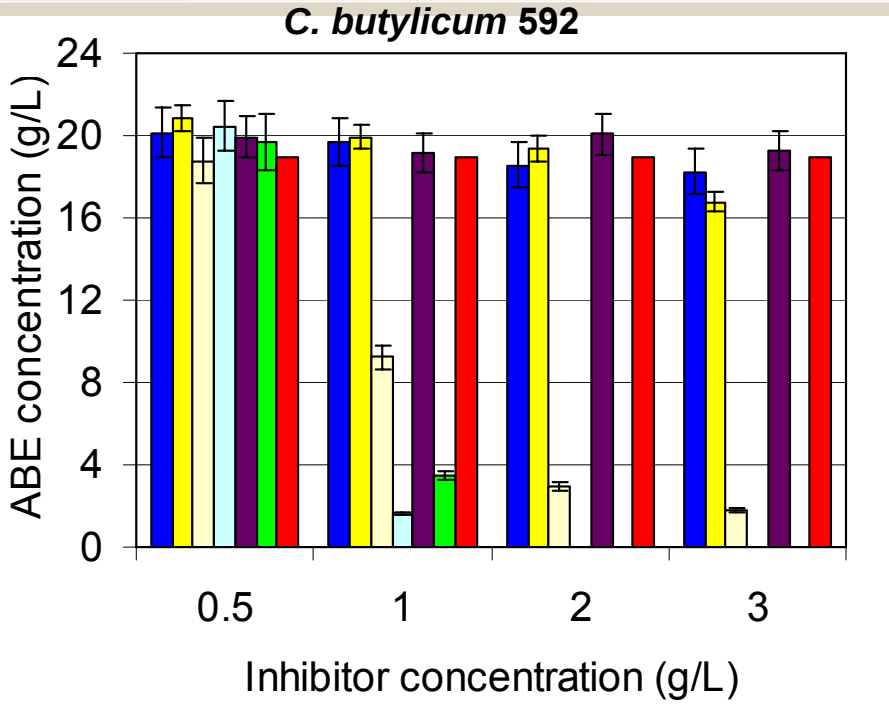
Effect of representative degradation and hydrolysis products on growth and ABE production by Clostridia Contd.





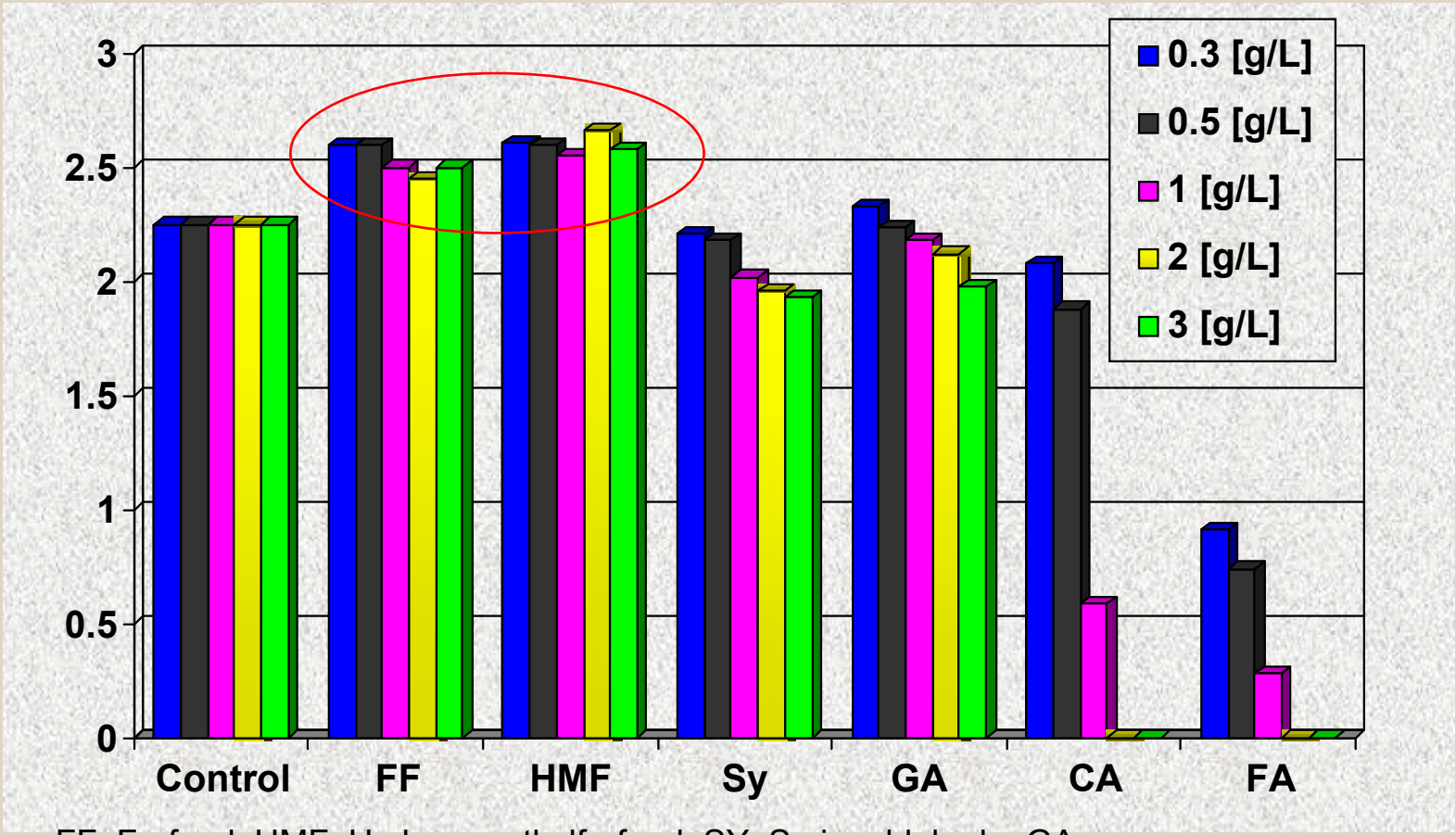
Effect of representative degradation and hydrolysis products on growth and ABE production by Clostridia Contd.





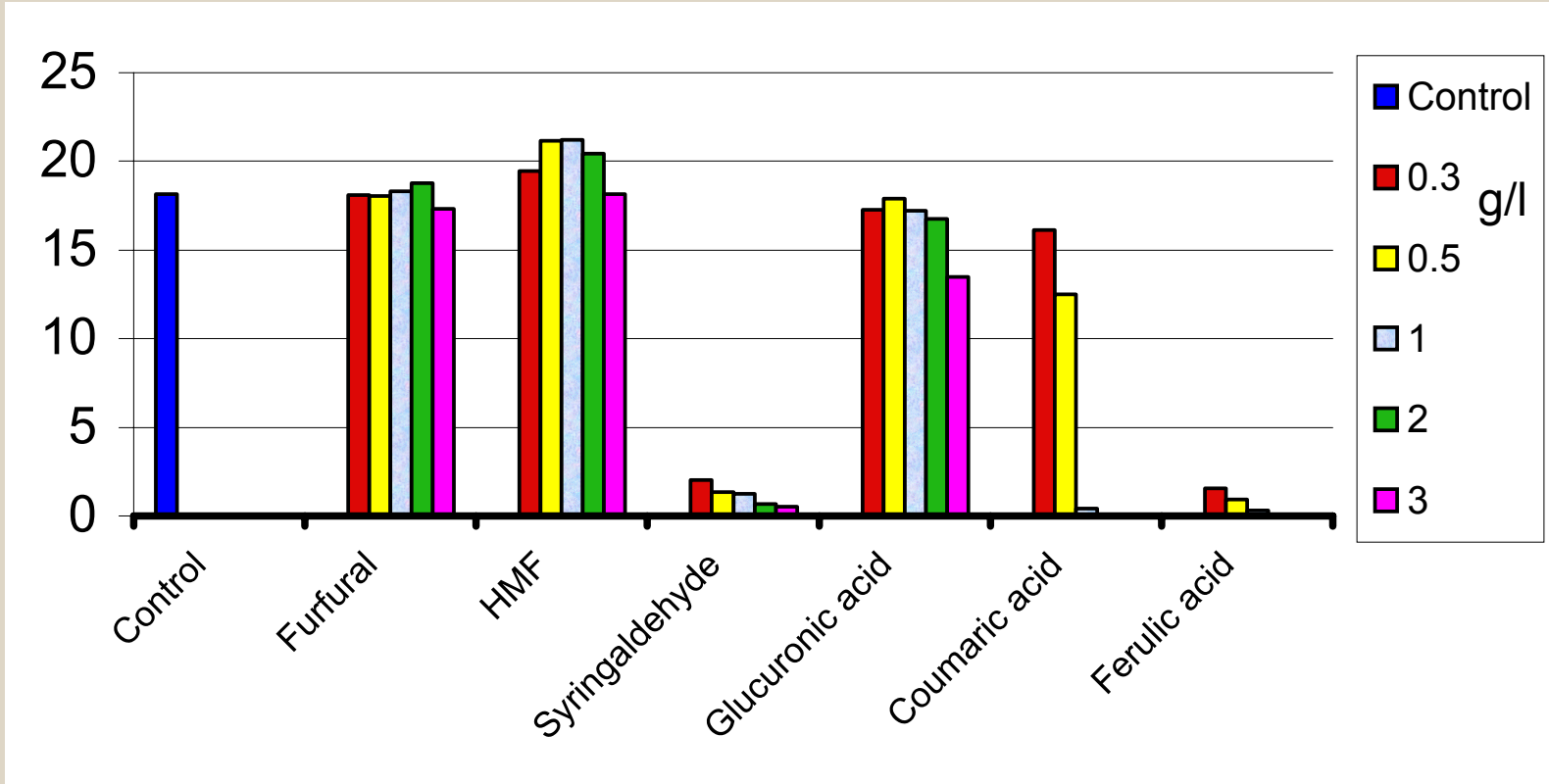
Effect of representative degradation and hydrolysis products on the growth of *Clostridium beijerinckii* BA101

Cell Conc. g/l

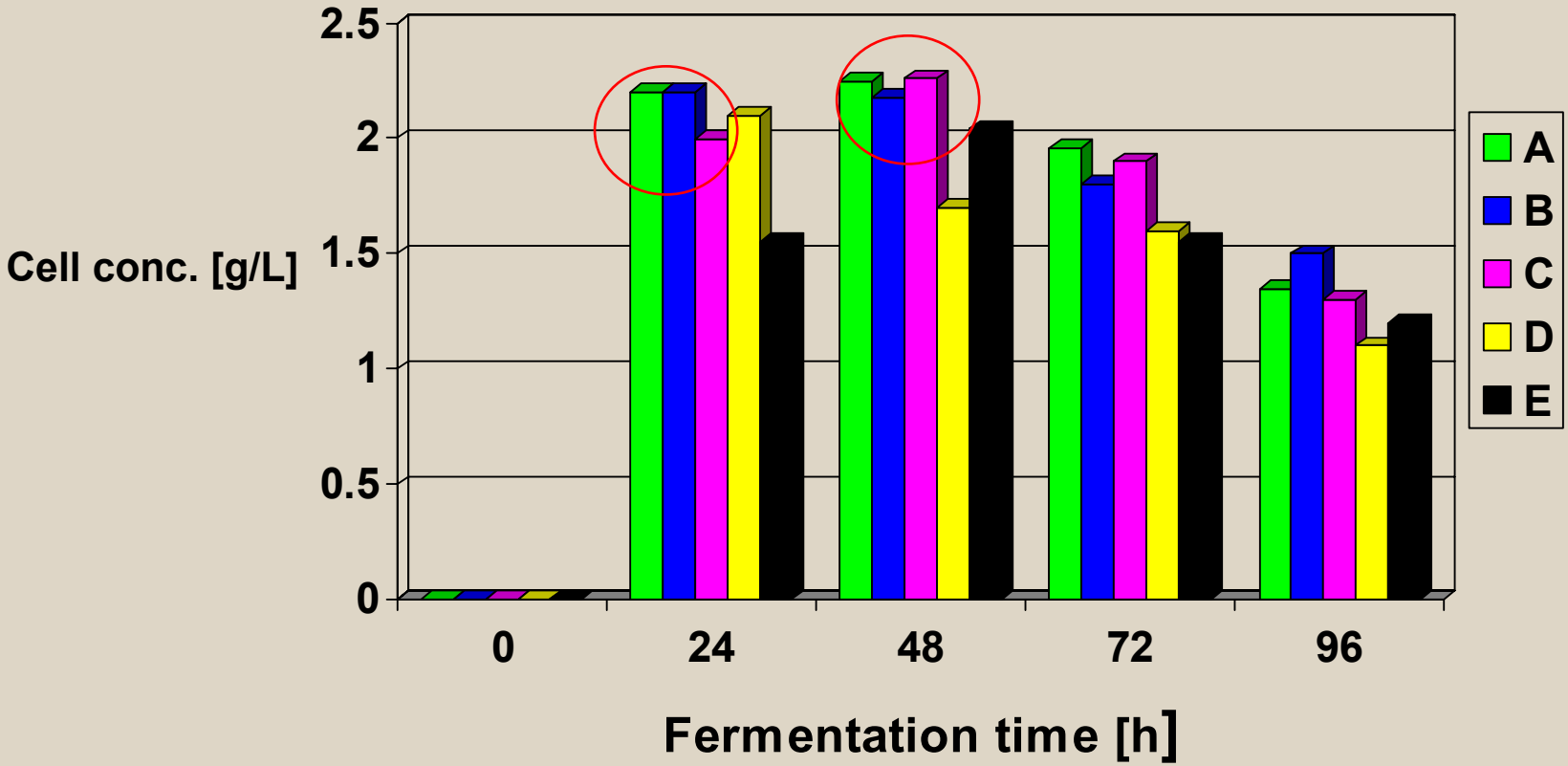


FF: Furfural; HMF: Hydroxymethylfurfural; SY: Syringaldehyde; GA: Glucuronic acid; CA: Coumaric acid; FA: Ferulic acid

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

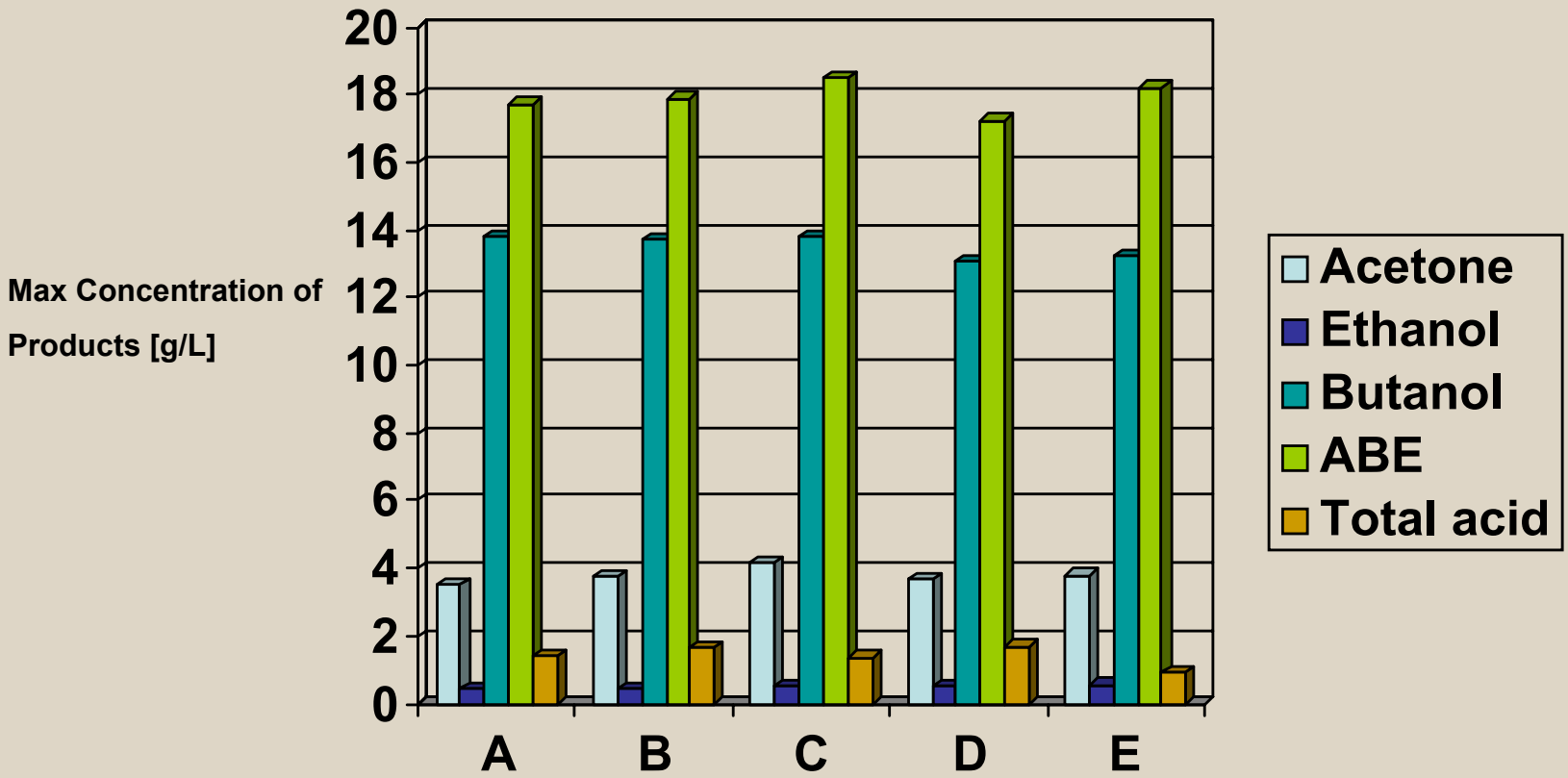


Effect of degradation and hydrolysis products on cell concentration and ABE production by *C. beijerinckii* BA101



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) .

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



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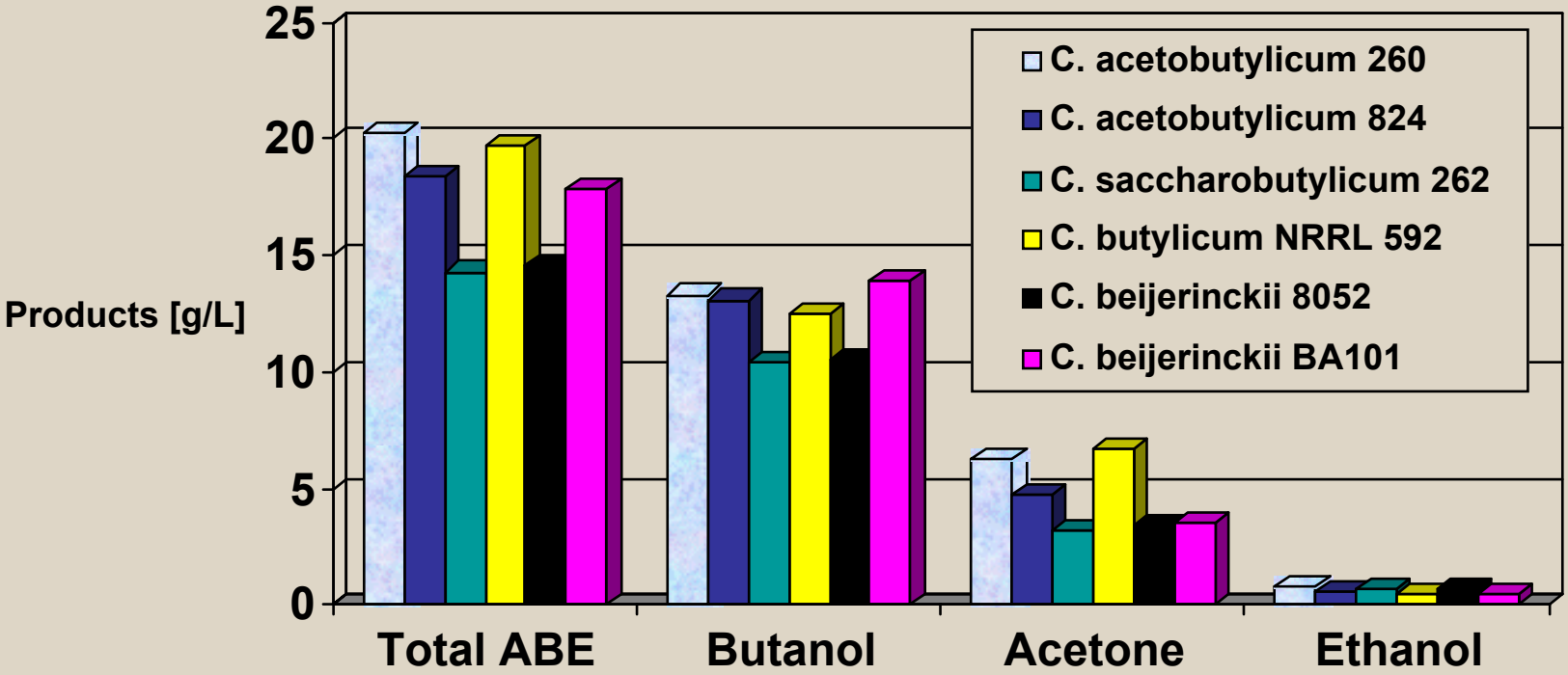
Utilization of hexose and pentose sugars by solventogenic clostridia

Organisms	**Sugar preference***
C. acetobutylicum 260	**Glucose> cellobiose > mannose > arabinose>galactose >xylose**
C. acetobutylicum 824	**Glucose>arabinose xylose> cellobiose > galactose >mannose**
C. saccharobutylicum 262	**Glucose> arabinose > galactose > cellobiose > xylose> mannose**
C. butylicum NRRL 592	**Glucose> cellobiose > mannose > arabinose > galactose > xylose**
C. beijerinckii BA101	**Cellobiose > glucose> xylose > arabinose > mannose > galactose**

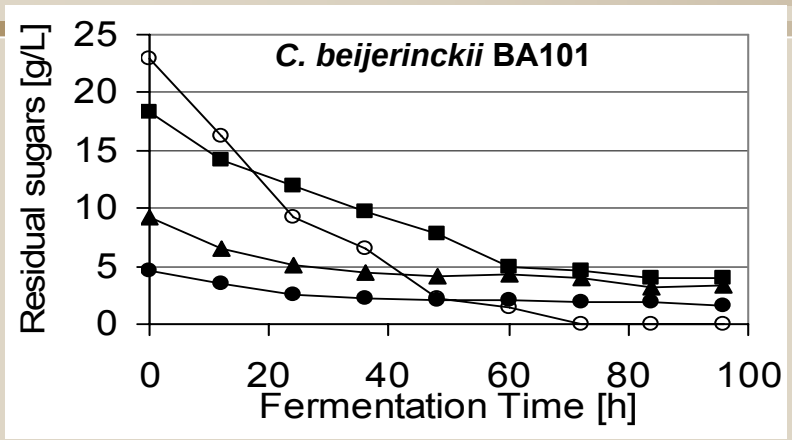
*Preference rated according to maximum ABE produced

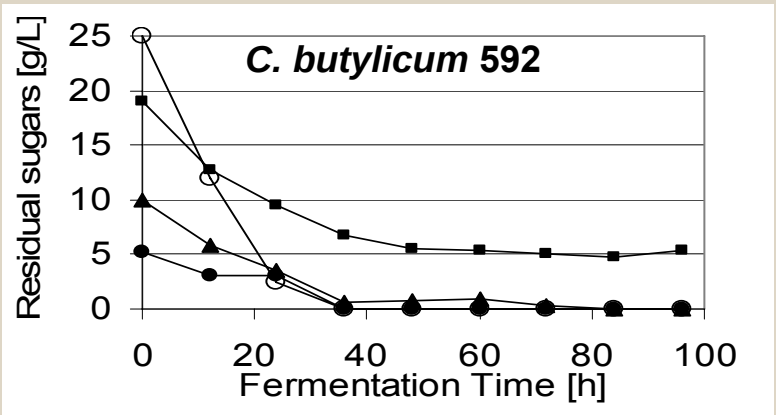
Fermentation of Hexose and Pentose sugars for ABE production by solventogenic clostridia

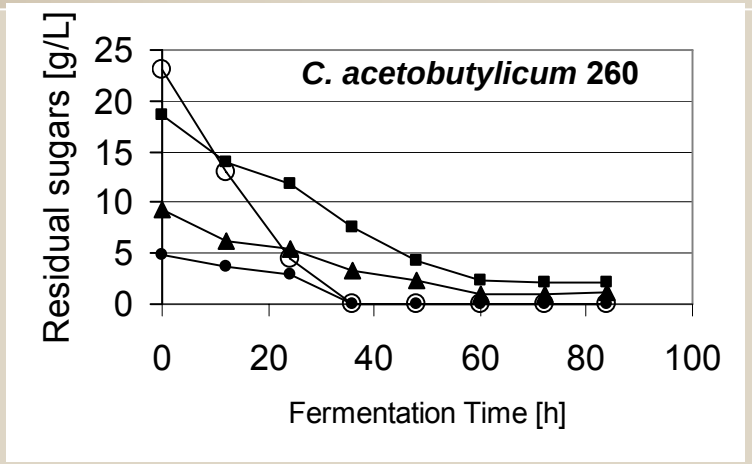
Glucose-mannose-arabinose-xylose composite (g/L: 25:5:10:20)

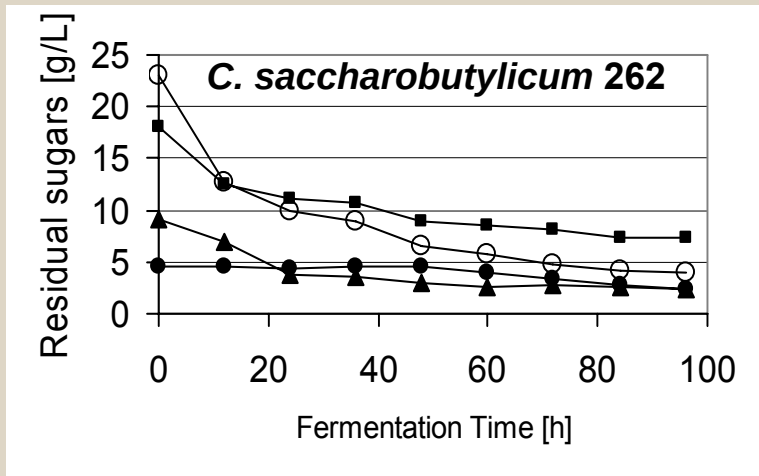


Utilization of hexose and pentose sugars by various solventogenic Clostridia









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

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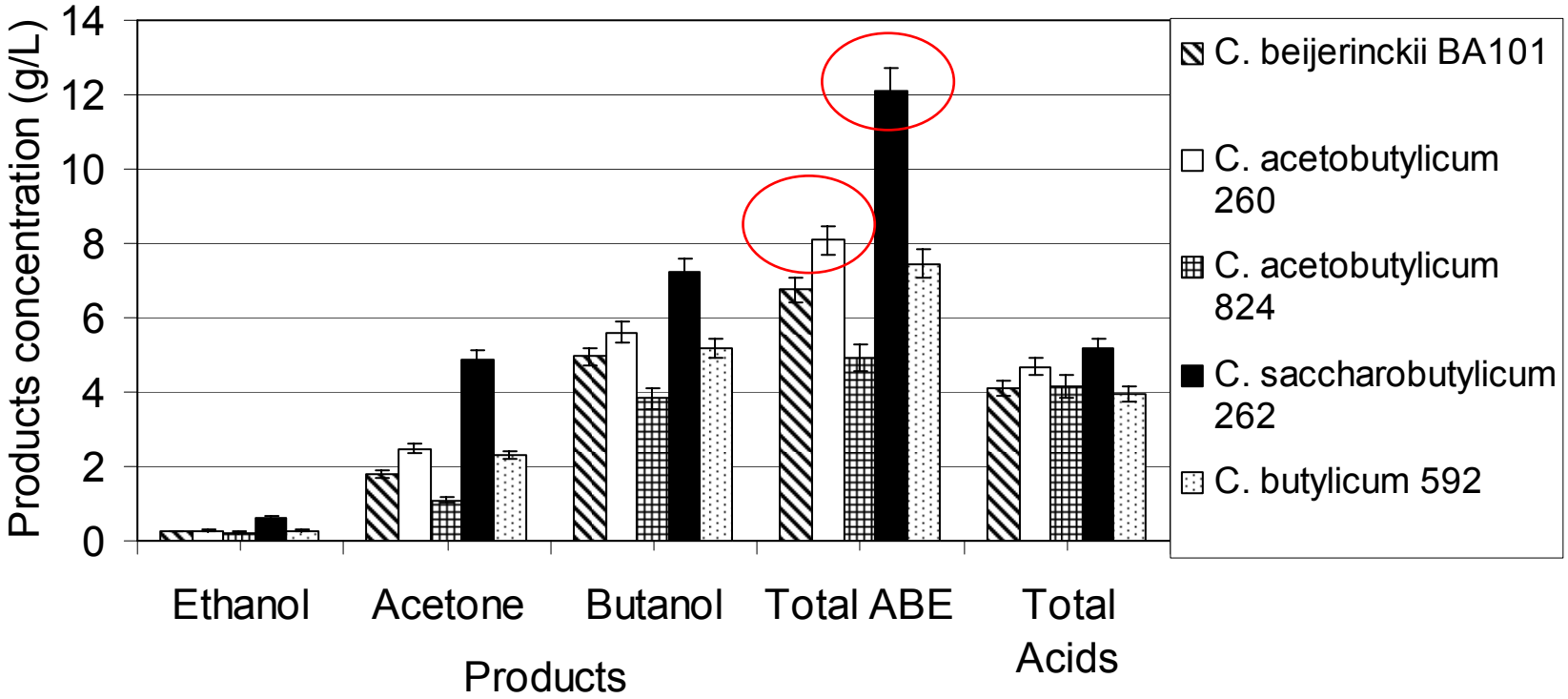
Sugar preference during batch fermentation of mixed sugar streams (grams: glucose 25, mannose 5, arabinose 10, xylose 20) by solventogenic clostridia

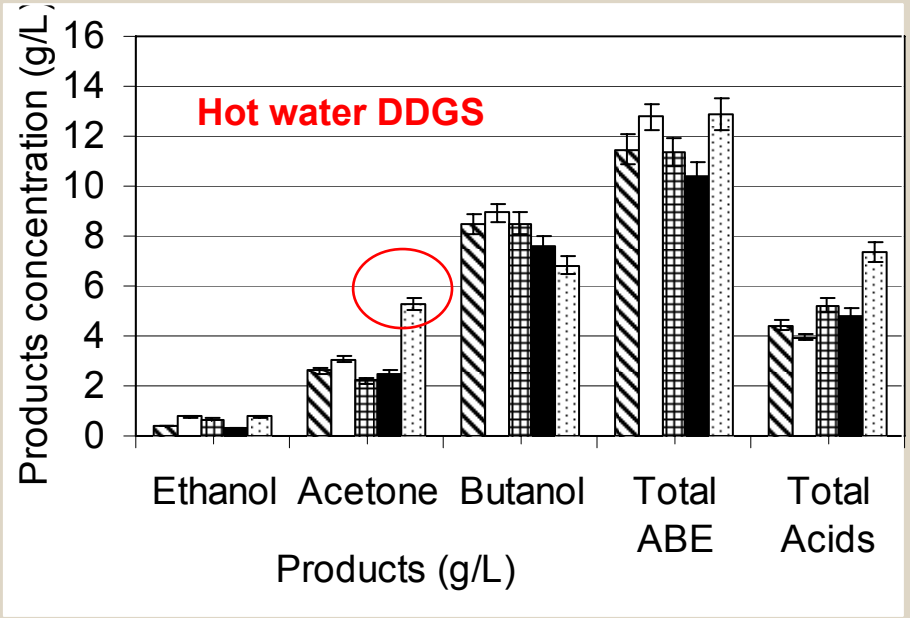
Organisms	Fermentation Time [h]*	**Sugar preference**
C. acetobutylicum 260	60	**Glucose> mannose > arabinose>xylose**
C. saccharobutylicum 262	72	**Glucose > arabinose > xylose> mannose**
C. butylicum NRRL 592	60	**Glucose > mannose > arabinose > xylose**
C. beijerinckii BA101	72	**Glucose > xylose > arabinose > mannose**

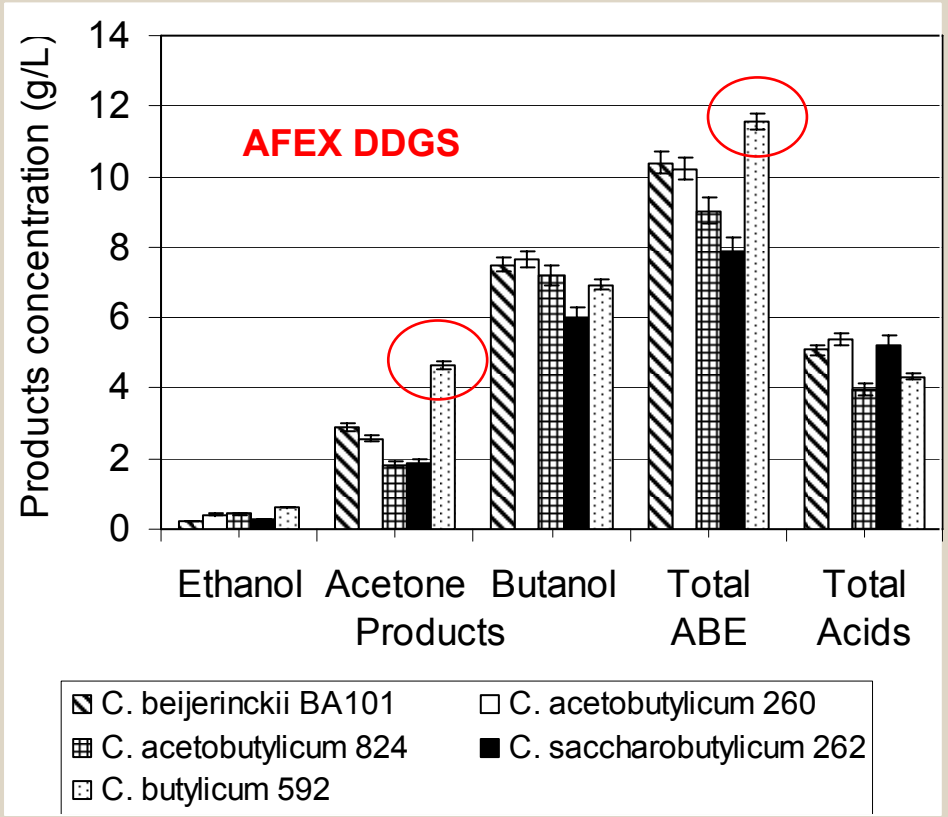
*time required to achieve maximal ABE concentration.

ABE production by solventogenic clostridia using dilute acid pretreated DDGS



ABE production by solventogenic clostridia using dilute Hot water and AFEX pretreated DDGS





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Summary

- All the solventogenic clostridia 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.
- Furfural and HMF (3g/L) are not inhibitory to *C. beijerinckii* BA101, rather they are stimulatory and the mixture of the two affects the culture negatively.
- Presence of furfural and HMF (2.0 g/L) in the fermentation broth was not inhibitory to other solventogenic clostridia; rather they are stimulatory to some of the clostridia.
- Syringaldehyde, ferulic and p-coumaric acids were potent inhibitors of ABE production by solventogenic clostridia.
- Ferulic acid being the most abundant phenolic acid in maize bran may be the limiting factor in efficient dilute acid treated DDGS and corn fiber fermentation.
- Fermentation of hot water and AFEX pretreated DDGS was successful.

Acknowledgements

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- Midwest Consortium