BUTANOL PRODUCTION FROM SUGARCANE JUICES

By

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Abstract

BUTANOL is an aliphatic saturated alcohol with the molecular formula of C_4H_9OH, which can be used as a transportation fuel, an intermediate and a solvent for a wide variety of chemical applications. The acetone-butanol fermentation was the standard for industrial production of solvents until the 1950s. Modern microbiological techniques have improved the original organism such that it produces high levels of butanol rather than mixed solvents. Butanol has many advantages as an alternative fuel source; 1) a higher energy content, 2) usable in existing pipelines, 3) easy to blend with gasoline. Butanol can be produced from sugarcane juice, molasses or sugars from bagasse hydrolysates using a strain of *Clostridium beijerinckii.* Sugarcane juice and molasses ferment directly to butanol. The yield of butanol was 0.30 g/g sugar from molasses and 0.34 g/g sugar from juice whereas equivalent sucrose concentrations produced 0.27g butanol per g sugar. Details of the economics for a viable production of butanol from sugarcane products are presented.

Introduction

The acetone butanol ethanol (ABE) fermentation by *Clostridium acetobutylicum* is one of the oldest known industrial fermentations. It was ranked second only to ethanol fermentation by yeast in its scale of production, and is one of the largest biotechnological processes ever known. Prior to the 1950s, the industrial solvents acetone, n-butanol and isopropanol (generically referred to as butanol and propanol in this discussion) were produced by fermentation (Figure 1).

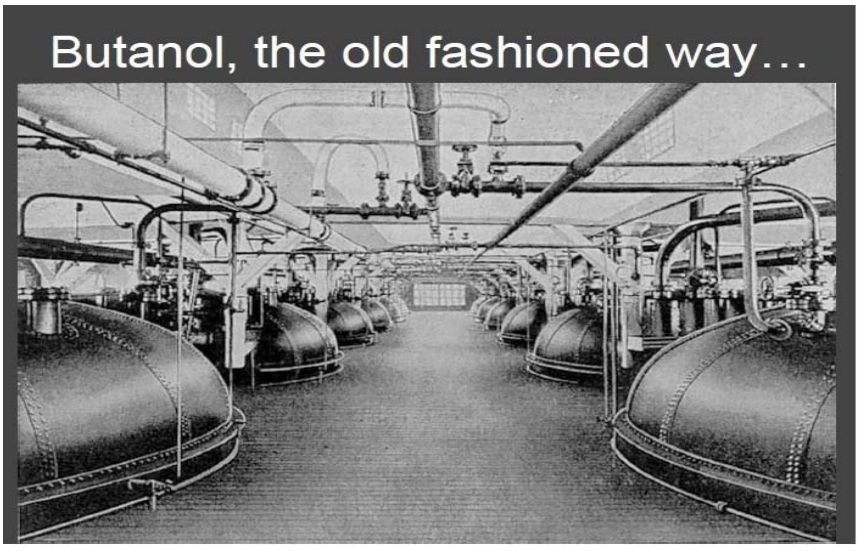

Fig. 1—Acetone-butanol plant, pre 1950, 600 000 gallon fermentation capacity, Terre Haute, Ind.

Historically, two clostridial species, *Clostridium acetobutylicum* or *Clostridium butylicum* were the fermentation organisms of choice. In a typical ABE fermentation by *C. acetobutylicum*, butyric, propionic, lactic and acetic acids are produced first, the culture pH drops, and the culture undergoes a metabolic 'butterfly' shift, then butanol, acetone, isopropanol and ethanol are formed (Zheng *et al.*, 2009).

The butanol yield from glucose is low, typically around 15 percent and rarely exceeds 25%. The production of butanol is limited by severe product inhibition. Butanol at a concentration of 1 percent inhibits cell growth and fermentation by 20% while 1.6% inhibits growth 100%. The butanol concentration in the broth in conventional ABE fermentations is usually less than 1.3 percent (Jones and Woods, 1986).

The push for renewable fuels has re-ignited the interest in butanol production by fermentation. Butanol is both an important industrial solvent and potentially a better fuel than ethanol (Table 1).

Table 1—Properties of Alternative Fuels (Ramey, 2007).

	Ethanol	Butanol	Gasoline
BTU/ gal(gross caloric value)	84k	110k	115k
Octane(RON)	92	94	96
Air fuel ratio(mass of air to mass of fuel)	9	11–12	12–15
Vapour pressure psi@100F	2	0.33	4.5

BTU=1.05 x10^3 J: psi= 6.9 x 10^3 Pa

Butanol has many advantages as an alternative fuel source; 1) a higher energy content, 2) usable in existing pipelines, 3) easy to blend with gasoline. Because of its low solubility, butanol is less likely to separate from a fuel blend in the presence of water than is ethanol.

The production of butanol from sugars goes by the biochemical route where acetoacetyl-CoA is produced from glucose and then simultaneously split to acetone and butyrate followed by the conversion of butyrate to butanol and, depending on the organism strain, the conversion of the acetone to propanol. There have been numerous attempts to manipulate the genetics of these organisms to separate acetone production from butanol production. This has not been completely successful as both acetone and butanol share a common intermediate (Figure 2). Attempts to block acetone production to favour butanol production usually result in a loss of butanol yield.

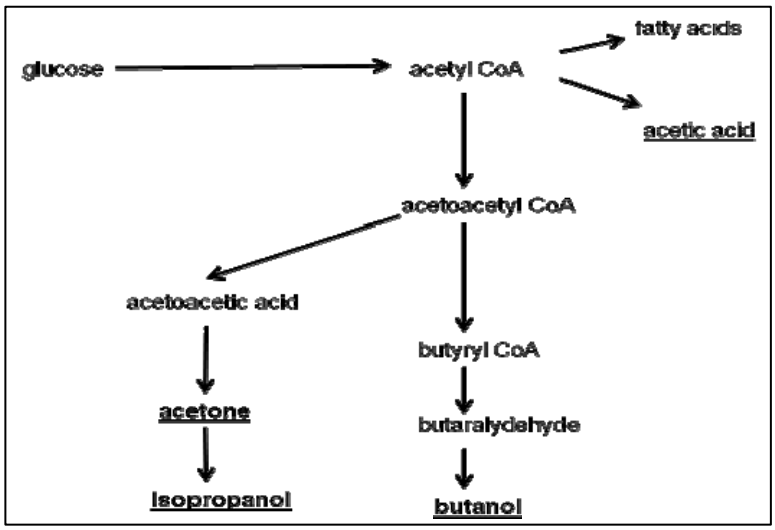

Fig. 2—Biochemical pathway leading to butanol and acetone (Stanier *et al*., 1970)

In the past twenty or so years, there have been numerous engineering attempts to improve butanol production in the ABE fermentation, including cell recycling and cell immobilisation to increase cell density and reactor productivity, and use of extractive fermentation to minimise product inhibition. The yields for ABE fermentations are still less than 2 percent in butanol concentration, 4.46 g/L/h productivity, and a yield of around 25 percent from glucose (Ezeji *et al*., 2007).

Optimisation of the ABE fermentation process has long been a goal of industry. As with any biofuel, the viability of the ABE fermentation will depend on feedstock cost and microbial productivity. The market demand for butanol is expected to increase dramatically if green butanol can be produced economically from low cost sugars (i.e. biomass derived). Recent research has focused on utilisation of sugars from lignocellulose conversion, with butanol being an alternative to ethanol production (Qureshi *et al*., 2007).

There is little published information on the conversion of 'traditional' feedstocks available from the cane sugar industry to butanol. This study was conducted due to our interest in bringing biofuel production to the Louisiana sugar industry.

Materials and methods

Microorganism and inoculum preparation

A strain of *Clostridium beijerinckii* (obtained from the Centraalbureaus voor Scimmelcultures in the Netherlands) was used in this research. Bacterial spores (200 µL) were heat shocked for 10 min at 80°C followed by cooling in ice, then inoculated into 27 mL tryptone-glucose-yeast extract (TGY) medium in 30 mL screw capped Pyrex tubes and incubated anaerobically for 12–16 h at 34°C (Qureshi et al., 2008).

Carbon source

Different carbon sources were used: sucrose (laboratory grade sucrose, Sigma Chemical co., St. Louis Mo.), blackstrap molasses (containing of 33.4% sucrose, 5.4% glucose, 7.3% fructose, 16.5% ash [conductivity ash] as % total solids 41.9% true purity), raw juice (12.35% sucrose, 0.38% glucose, 0.74% fructose, ash content not determined, values on juice volume), and glucose derived from corn starch for comparison. Glucose was prepared by hydrolysis of corn starch derived malto-oligosaccharide syrups using amyloglucosidase. Enzymatic conversion was allowed to proceed until in excess of 95% of the starch was glucose.

Batch fermentations

Fermentation studies were conducted initially in 125 mL screw capped bottles containing 120 mL of medium, at 34°C without agitation, gas flow, or pH control. The medium contained either sucrose (5.0% in a total medium), molasses (5.9% sugars), juice (4.9% sugars), or 5% glucose for carbon source, supplemented with 1 g/L yeast extract. After sterilisation, filter-sterilised stock solutions containing (buffer: KH_2PO_4, 50 g/L; K_2HPO_4, 50 g/L; ammonium acetate, 220 g/L), (vitamins: para-amino-benzoic acid, 0.1g/L; thiamin, 0.1 g/L; biotin, 0.001 g/L), and (minerals: $MgSO_4 \cdot 7H_2O$, 20 g/L; $MnSO_4 \cdot H_2O$, 1 g/L; $FeSO_4 \cdot 7H_2O$, 1 g/L; NaCl, 1 g/L)] (Qureshi and Blaschek, 1999) were added (1 mL each). The media was then inoculated with highly motile cells of *C. beijerinckii* (6 mL cell suspension in 114 mL medium). During the course of fermentations, 4 mL samples were collected at intervals for absorbance, pH, solvents, organic acid, and sugar analysis.

Analytical procedures

Cell concentration was estimated from the absorbance at 600 nm. The solvents, butanol, acetone, ethanol were measured using gas chromatography on a wax column (Phenomenex ZP Wax plus). Organic acids, acetic and butyric were determined by high performance liquid chromatography (HPLC) using an anion exchange column (Dionex Ionpac AS-11). Sugars were analysed by HPLC using a K column (Biorad HPS87K).

Results and discussion

The growth curves for *C. beijerinckii* were similar for all carbon sources, achieving late exponential phase of growth around 30 h and maximum growth after 49.5 h (data not shown). The media pH in either sucrose or glucose media decreased until late exponential phase, and then rebounded (data not shown). This is a typical growth pattern for *C. beijerinckii* (Parekh *et al*., 1998; Jones and Woods, 1986) and reflects the production and then utilisation of organic acids. In most ABE fermentations, butanol production starts after the stationary phase begins. Throughout our fermentations, low levels of acetone and ethanol were continuously present and, except for the molasses fermentations, butanol concentrations increased throughout the growth phase. This pattern is indicative of continuous, efficient butanol production by *C. beijerinckii* (Jones and Woods, 1986) (Figure 3). Cultures containing molasses were acidogenic because molasses feed has high levels of acetic acid (0.65% on Brix), which slowed the start of butanol production compared to the other carbon sources. Butanol production increased rapidly after the stationary phase was achieved. Sugar juices showed rapid increases in butanol production between 12 and 49.5 h of fermentation.

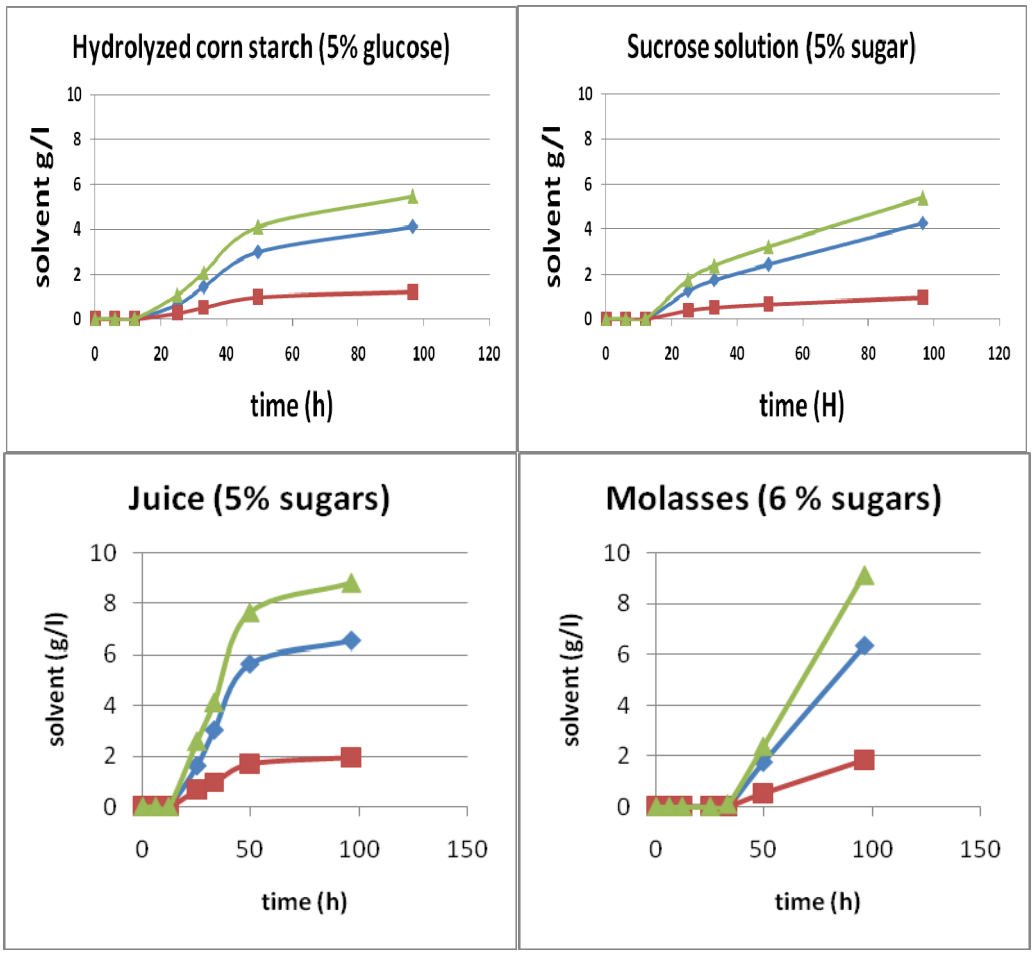

Fig. 3—Production of acetone-butanol, from the individual carbon substrates by *C. beijerinckii*. Propanol is indicated by the (■) symbol, butanol by (◆) symbol and total solvent by the (▲) symbol. The glucose was that obtained from corn starch hydrolysis.

Juice (containing 4.9% sugars) and molasses (5.9% sugars) were the preferred substrates for butanol production by this strain (Figure 4). Higher concentrations of molasses repressed growth, probably due to osmotic effects (data not shown). In all cases 2-propanol was the largest secondary product. This product pattern is very similar to that produced by *C. butylicum*. Small amounts of ethanol were found in all samples. No differences were observed between glucose and sucrose fed

cultures in the production of butanol. These fermentations were not optimised for carbon utilisation. In all cases, residual sugars remained at the conclusion of the fermentations.

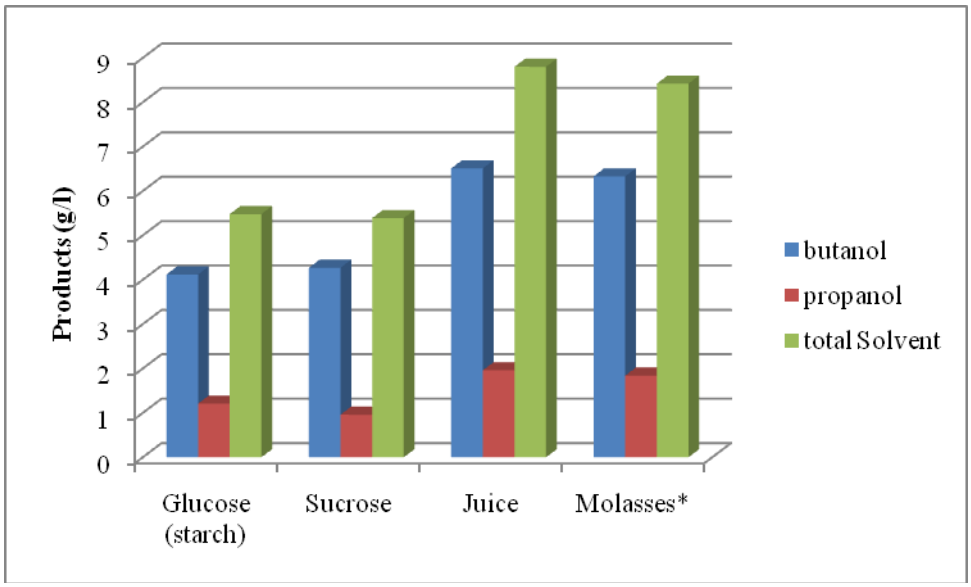

Fig. 4—Production of n-butanol and isopropanol and total solvents in g/L from individual fermentations, at 5% carbon source levels (*molasses was 5.9% carbon source) samples by *C. beijerinckii* at the end of the fermentation (96.5 hours).

The productivity of this culture was higher on juice and molasses than on sucrose or glucose. In these, non-optimised, fermentations there were 1.6 to 1.7 times more solvent in the fermentation broth with molasses or juice as feedstock than with sugars.

On a carbon source utilised, yield to product produced (Table 2), there was about 1.3 times more solvent produced using sugarcane feedstocks than with either pure sugar or corn hydrolysate.

Table 2—Solvent* Production by carbon source.

Carbon source	Solvent yield (g/g sugar utilised)
Sucrose or glucose	0.32
Juice	0..42
Molasses	0.36

*n-butanol and isopropanol.
The glucose was obtained from corn starch.

The solvent produced in all cases was primarily butanol with a significant portion of isopropanol. Acetone and ethanol were either absent or present as trace components (Table 3).

Table 3—Solvent yield %* by carbon source.

Carbon source	n-butanol	Isopropanol	Total solvent
Sucrose or glucose	25	5	32
Juice	32	9	42
Molasses	27	7	36

*weight % of product on carbon source utilised

The differences in solvent yields, as a function of feedstock, is significant. In comparing butanol production with the alternative (ethanol), it should be kept in mind that ethanolic (yeast) fermentations have a theoretical maximum yield of 51% and take 1.65 kg of sugar to produce a litre of ethanol. The amount of sugar used and relative feedstock costs (in the US) to produce butanol are given in Table 4.

Table 4—Estimated feedstock cost to produce butanol.

Feedstock	Kg sugars/litre solvent	Feedstock price* (estimated)/kg ($)	Feedstock cost/litre product ($)
Pure sugar	3.90	0.44	1.72
Cane juice	2.97	0.44	1.31
Molasses**	3.47	0.071	0.25
Corn (at $4.80/bu)	3.90	0.335	1.31

*Price in US dollars for American market
** Molasses estimated at 49% fermentable sugars, $70.00/tonne.

Even though the conversion of sugar(s) to butanol/propanol is lower than conversion to ethanol, the higher BTU values for butanol and reduced fuel transport handling problems make this fermentation worth considering for fuel production. (Qureshi and Blaschek, 2000). Butanol fuel made directly from sugar juice is probably competitive with corn ethanol and that from molasses is cheaper. Improvements in yields can be expected as further efforts are made in strain improvement and as the technology matures to the equivalent of fuel ethanol production technology.

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PRODUCTION DE BUTANOL A PARTIR DE JUS DE CANNE A SUCRE

Par

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MOTS-CLÉS: Butanol, Fermentation, Jus de Sucre, Biomasse.

Résumé

LE BUTANOL est un alcool aliphatique saturé avec la formule moléculaire de C_4H_9OH, qui peut être utilisé comme un combustible de transport, un intermédiaire et un solvant pour une grande variété d'applications chimiques. La fermentation acétone-butanol était la norme pour la production industrielle de solvants jusqu'aux années 1950. Les techniques microbiologiques modernes ont amélioré le micro-organisme d'origine pour qu'il produise des niveaux élevés de butanol plutôt que de solvants mixtes. Le butanol présente de nombreux avantages comme une source de carburant de remplacement. 1) Un contenu énergétique plus élevé; 2) Une utilisation dans les pipelines existants; 3) Un mélange plus évident avec l'essence. Le butanol peut être produit à partir de jus de canne à sucre, de mélasse ou des sucres issus des hydrolysats bagasse à l'aide d'une souche de *Clostridium beijerinckii*. Le jus de canne et la mélasse fermentent directement en butanol. Le rendement de butanol était 0.30 g/g sucre à partir de mélasse et de 0.34 g/g à partir de jus que canne alors qu'une concentration équivalent en saccharose produit 0.27 g butanol par g de sucre. Les détails d'ordre économique pour une production viable de butanol à partir de produits émanant de la canne à sucre sont présentés.

PRODUCCIÓN DE BUTANOL DE JUGOS DE CANA DE AZÚCAR

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PALABRAS CLAVE: **Butanol, Fermentación, Jugos Azucarados, Biomasa.**

Resumen

EL BUTANOL es un alcohol alifático saturado con la fórmula molecular de C4 H2 OH, que puede ser usado como combustible de transporte, como intermediario y como solvente para una amplia variedad de aplicaciones químicas. La fermentación acetona-butanol fue el estándar para la producción industrial de solventes hasta los años 1950. Modernas técnicas microbiológicas han mejorado el microorganismo original en un modo tal que produce elevadas cantidades de butanol en lugar de la mezcla de solventes. El butanol tiene ventajas como fuente alternativa de combustible: 1) mayor contenido energético, 2) posible de emplear en instalaciones actuales, 3) fácil de mezclar con gasolina. El butanol puede producirse del jugo de caña de azúcar, de melazas ó de azúcares de los hidrolizados de bagazo, empleando una cepa de *Clostridium beijerinckii.* Los jugos de caña y las melazas se fermentan directamente a butanol. El rendimiento de butanol fue de 0.30 g/g de azúcar de melazas y 0.34 g/g de azúcares de jugos, mientras concentraciones equivalentes de sacarosa produjeron 0.27 g de butanol por gramo de azúcar. Se presentan detalles acerca de la economía para una producción viable de butanol.