A Study of Butanol Production in a Batch Oscillatory Baffled Bioreactor

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by

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Abstract

As with many bioprocesses, the acetone-butanol-ethanol (ABE) fermentation faces a number of economic drawbacks when compared to the petrochemical route for butanol production. In the 1920s biobutanol was the second largest biotechnology industry, after bioethanol production. However it became difficult to compete against the petrochemical route for reasons including the low product butanol concentration, because of product inhibition resulted in low butanol productivity and due to slow fermentation and low ABE yields. These lead to uneconomical butanol recovery by the conventional method, distillation, due to the high degree of dilution. Recent interest in biobutanol as a biofuel has led to re-examination of ABE fermentation with the aim of improving solvent yield, volumetric productivity and final solvent concentration to reduce the cost of production and thereby produce biobutanol that is cost-competitive with the chemical synthesis butanol.

ABE fermentations were carried out in an intensified plug flow reactor known as the batch oscillatory baffled bioreactor (BOBB). The "BOBB"s were designed and built for this project. The effect of oscillatory flow mixing on ABE fermentation was compared to that of conventional stirred tank reactors (STRs) at power densities in the range 0 to 1.14 Wm^{-3}. The maximum butanol concentration in this range in a BOBB was 34% higher than the STR. Some increase in butanol productivity was also observed: 0.13 $gL^{-1}h^{-1}$ in BOBBs, compared to 0.11 $gL^{-1}h^{-1}$ in the STRs. It can be concluded that at similar power densities, BOBB fermentation shifts to solventogenesis earlier than in STRs, resulting in higher solvent productivity. It is hypothesised that the reason for early solventogenesis in the BOBB was the higher solvent-producing cell concentration, due to the more uniform shear field in the BOBB, so the cell would be less exposed to high shear thereby reducing the risk of cell lysis.

Two-stage ABE fermentations in BOBB increased the butanol productivity by up to 37.5% over the one-stage fermentation. Butanol productivity was further increased by 97% when gas stripping was integrated to the two-stage ABE fermentation. While the one-stage fermentation integrated with gas stripping increased the butanol productivity by 69% to 0.12 $gL^{-1}h^{-1}$ (as opposed to 0.071 $gL^{-1}h^{-1}$ in a similar fermentation without gas stripping). A simple model to describe the one-stage at oscillatory Reynolds number (Re_o) 0 and 938, and the two-stage ABE fermentation in BOBB II was developed. The model summarizes the physiological aspects of growth and metabolite synthesis by *Clostridium* GBL1082. The prediction of the models were in good agreement with experimental results incorporating mixing (Re_o 938) and moderately agreed with results from Re_o 0 and the two-stage fermentation.

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Nomenclature and Abbreviations

Symbols		Unit
ω	angular frequency of the oscillation	rad s^{-1}
δ	baffle thickness	m
Z	column length	m
ρ	density	kg m^3
ν	liquid kinematic viscosity	m^2 s
f	oscillation frequency	Hz
μ	specific growth rate	h^{-1}
ε_v	power density	W m^{-3}
C_o	orifice coefficient	-
C_p	heat capacity of water	kJ kg^{-1} $^oC^{-1}$
D	column internal diameter	m
D_o	orifice diameter of the baffle plate	m
dT/dt	rate of temperature change	^oC h^{-1}
H	Overall reactor height	m
L	baffle spacing	m
m	mass of water	kg
N	impeller speed	rpm, rps
n	number of baffle plates	-
Q_{growth}	rate of heat generated by cell growth	kJ h^{-1}
Re_n	net Reynolds number	-
Re_o	oscillatory Reynolds number	-
S	fractional open cross-sectional area	-
St	Strouhal number	-
T	temperature	^oC
t	time	h
u	superficial net flow velocity	m s^{-1}
V_L	liquid volume	L
X	cell mass concentration	g L^{-1}
x_o	centre-to-peak amplitude	m

$1/Y_H$	metabolic heat evolved per gram of cell mass produced	kJ g^{-1}

Abbreviations

ABE	acetone, butanol and ethanol
ATP	adenosine triphosphate
BOBB	batch oscillatory baffled bioreactor
CDW	cell dry weight
DNA	deoxyribonucleic acid acid
DNS	dinitrosalicylic acid
GC	gas chromatography
HTR	heat transfer rate
NAD (P)	nicotinamide adenine dinucleotide phosphate
NAD (P)H	reduced form of NAD (P)
NADH	nicotinamide adenine dinucleotide
OBB	oscillatory baffled bioreactor
OBR	oscillatory baffled reactor
OD	optical density
OFμR	oscillatory flow micro reactor
OFR	oscillatory flow reactor
PHA	polyhydroxy alkanoate
pO_2	oxygen partial pressure
PTFE	polytetrafluoroethylene
PVC	polyvinyl chloride
RCA	reinforced clostridial agar
RCM	reinforced clostridial medium
rpm	revolutions per minute
rps	revolutions per second
RTD	retention time distribution
STR	stirred tank reactor

Chapter 1 INTRODUCTION

1.1 Research Background

More than a century ago, the production of acetone and butanol via fermentation using the "Weizmann Process" was commercially viable. There was a high demand for acetone, which was used in the manufacture of the explosive cordite during World War 1. At that time, butanol (as a by-product) was stored, as there was no ready use for it (Dürre, 2008). It was in the 1920s that butanol became an important chemical when the dramatic growth in the automobile industry created an urgent need for a solvent in the production of quick-drying lacquers for car manufacturing. Butanol proved to be an excellent solvent, and fermentation using the Weizmann Process became the method of choice for its production (Dürre, 2008). Butanol has also been used in the production of the rubber monomers, butadiene and dimethyl butadiene (Mollah and Stuckey, 1993). Until 1950, almost two-thirds of worldwide butanol demand were met from the fermentation process (Dürre, 2008). The largest plants were located in the United States: for example, Peoria, Illinois had 96 fermenter units with a total capacity of 21,821 m^3. This plant together with the Terre Haute plant (which had 52 fermenter units) produced over 100 tons of solvent per day (Jones and Woods, 1986).

In the 1950s, crude oil became much cheaper. Together with the increasing prices of biobutanol feedstock (mainly molasses) combined with lower sugar contents, this shifted butanol production routes away from biological processes to more efficient chemical processes. By 1960, acetone, butanol and ethanol (ABE) fermentation had virtually ceased in the UK, USA and Japan (Ni and Sun, 2009), followed by Africa and Germany in the 1980s, and finally in China and Russia in the 1990s (Jones and Woods, 1986; Lee *et al.*, 2008b). Since then, butanol has almost exclusively been produced from petrochemicals. The revival of ABE production by fermentation depends on economic conditions, principally the cost relative to the

petrochemical-based processes. The oil price crises in the 1970s and 1999, have revived interest in the ABE fermentation. As shown in Figure 1.1 below, research activities in academia and industry significantly increased in the early 1980s and again in 2000s as a response to the oil crises with effort distributed fairly evenly between various technical aspects fermentation, downstream processing, and research on physiology and genetics of solventogenic clostridia, from 1980 to 1990. In the last decade, scientific publications in clostridial research increased again, generally due to interests in biofuels (Lütke-Eversloh and Bahl, 2011).

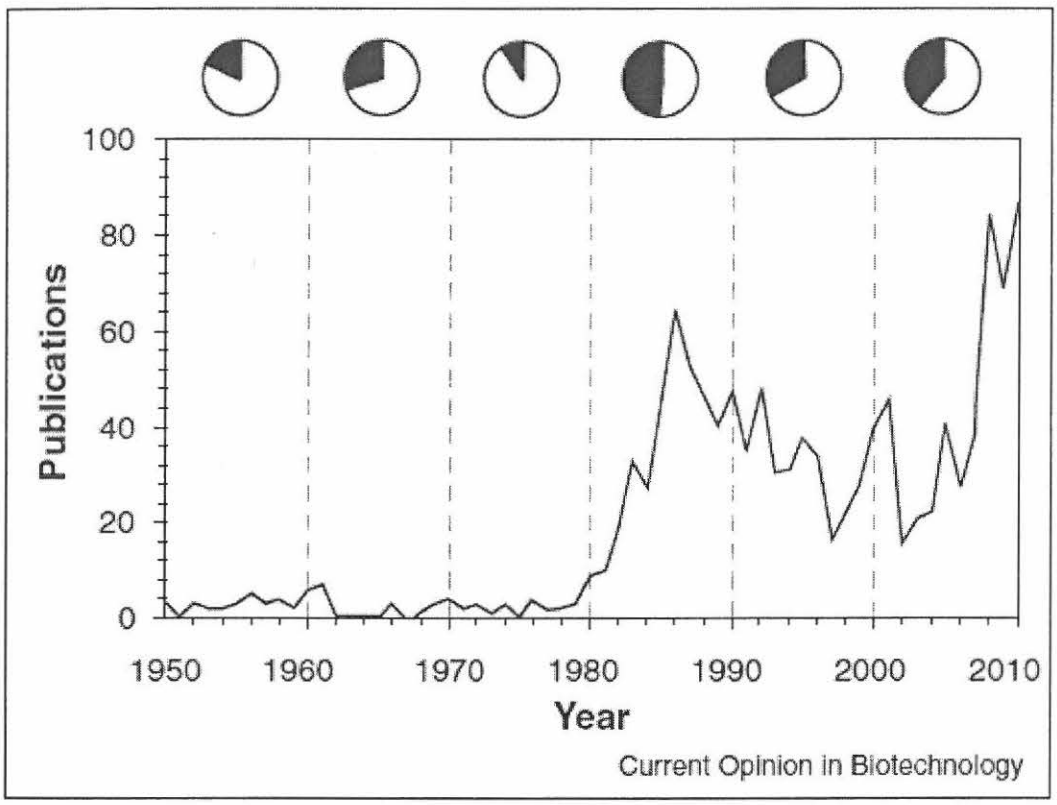

Figure 1.1 Scientific publications on solventogenic clostridia since 1950. The pie charts on top show the ratio between publications on physiology and genetics (white) and those covering topics of fermentation and downstream processing (black) for each decade. (Lütke-Eversloh and Bahl, 2011)

Researchers are working to improve the ABE fermentation process with the aim of reducing the cost of production, so that biobutanol is cost-competitive with chemically synthesised butanol. Recent developments in molecular techniques to

i. Mutant strain, *C beijerinckii* BA101 produce higher concentrations of butanol over its parent strain *C. beijerinckii* NCIMB 8052 (Qureshi and Blaschek, 2001a)

ii. Gene inactivation in *C. acetobutylicum* ATCC 824 (Green *et al.*, 1996) to disrupt metabolic pathways leading to acetate and butyrate production

iii. Mutant strains of *C. acetobutylicum* ATCC 824 (Matta-El-Ammouri *et al.*, 1986) *C. beijerinckii* NCIMB 8052 cloned with *Neocallimastix patriciacarum's* gene (Lopez-Contreras *et al.*, 2001)

iv. Alternative microbial hosts with artificial metabolic pathway of clostridia (Shen and Liao, 2008; Nielsen *et al.*, 2009)

Others have focussed their interest on media optimization using non-food feedstocks, such as wheat straw, corn stover, barley straw, switchgrass (Qureshi and Ezeji, 2008), and palm oil mill effluent (Somrutai *et al.*, 1996; Ngan *et al.*, 2004; Pang *et al.*, 2004; Hipolito *et al.*, 2008; Takriff *et al.*, 2009). ABE fermentation protocols have also been studied including: varying fermentation technique (batch, fed batch, continuous, continuous with cell recycling and bleeding, continuous with immobilized cell and co-culture fermentation) (Lienhardt *et al.*, 2002; Lee *et al.*, 2008a), novel downstream processing (Ezeji *et al.*, 2007) and integration of the processes *i.e.* pretreatment-fermentation-product recovery (Lienhardt *et al.*, 2002; Qureshi and Maddox, 2005; Qureshi *et al.*, 2007; Fischer *et al.*, 2008). These fermentation strategies had been carried out and resulted in differing degrees of success.

1.3 Research Objectives

The objectives of this research are:

i. Development of a batch oscillatory baffled bioreactor (OBB)

ii. Investigation of the ability of the oscillatory baffle reactor (OBR) as a bioreactor to perform ABE fermentation

iii. Comparison of the effect of different types of mixing (*i.e.* oscillating and stirring) by comparing stirred tank reactors (STRs) and OBBs

iv. Investigation of the effect of variation of oscillatory Reynolds number (Re_o) on batch ABE fermentation.

v. Investigation of the effect of mixing protocols in ABE fermentation on cell growth and solvent production with a view to maximising productivity.

vi. Evaluation of OBR ABE fermentation's integration with simultaneous product recovery (gas stripping) and the effect on the solvent yield and productivity

Chapter 2 LITERATURE SURVEY

2.1 Butanol

Butanol (IUPAC nomenclature 1-butanol) is a colourless, flammable liquid with a banana-like odour. It is an important bulk chemical in various industrial applications. Butanol is a major feedstock for the industrial manufacturing of various chemicals including butyl acrylate, butyl acetate, glycol ethers, and plasticizers (Mata *et al.*, 2010). These chemicals are widely used in water-based coatings, cosmetics, car care products, lacquers, pharmaceuticals, textiles, etc. Butanol is also used as a direct solvent in paints, dyes, varnishes, coatings and for other industrial purposes (Mata *et al.*, 2010). The latest application of butanol is as a transport fuel and it is expected to play a major role in the next generation of biofuels (Dürre, 2008). "Biobutanol", which is butanol derived from fermentation, has been claimed to be "superior biofuel", as it can be blended into standard gasoline similarly to ethanol but with several advantages. These include higher energy content and lower vapour pressure, which make storage and transportation easier. It is also immiscible with water, has a better blending ability with gasoline and diesel fuel, and can be used in conventional internal combustion engines without modification (Dürre, 2008).

Butanol can be produced from petrochemical or biotechnology routes. Chemically, butanol is derived from crude oil via three major routes: oxo synthesis, Reppee synthesis and crotonaldehyde hydrogenation, as shown in Figure 2.1. Oxo synthesis is the main process. It involves two main steps: hydroformylation, followed by hydrogenation. First, CO and H_2 are added to the carbon-carbon double bond of propylene using cobalt, rhodium or ruthenium as catalysts. This produces an aldehyde mixture, which undergoes hydrogenation to produce butanol (Figure 2.1a).

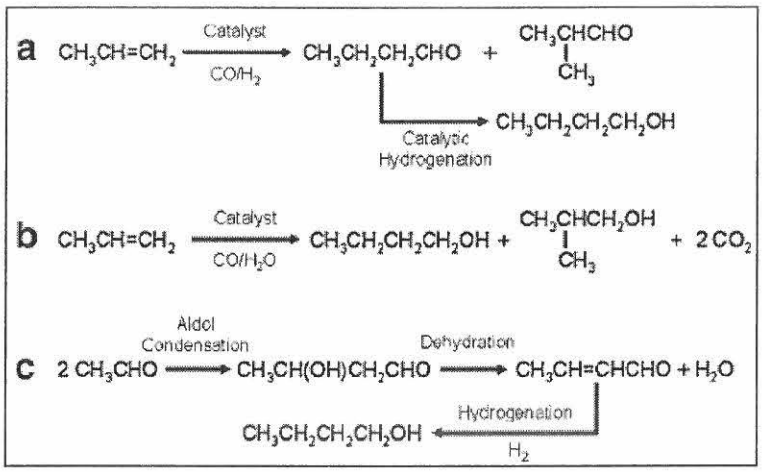

Figure 2.1 Chemical synthesis of butanol: (a) Oxo synthesis, (b) Reppe synthesis, (c) Crotonaldehyde hydrogenation (Lee *et al.*, 2008b)

Different isomeric ratios of butanol can be obtained, depending on reaction conditions of pressure and temperature, and the catalyst used. If hydrogen replaced by steam (as in Figure 2.1b), a mixture of primary butanols can be obtained directly. This is known as Reppe synthesis, which produces butanol directly from alkenes and operated at 100°C and 1.5 x 10^6 Pa absolute pressures, in the presence of pentacarbonyl iron, butylpyrrolidone and water. This process produces an 88% n-butanol and 12% isobutanol (Chauvel and Lefebvre, 1989). Crotonaldehyde hydrogenation (Figure 2.1c) used to be a common route for petrochemical-derived butanol a few decades ago. The process starts from acetaldehyde and consists of three-step reactions: aldol condensation, dehydration and hydrogenation. This process may again become important in the future as it provides an alternative route from ethanol which can be produced biologically from biomass (Machado, 2010). In this case, acetaldehyde is formed from the dehydrogenation of ethanol and the synthesis proceeds from there (Lee *et al.*, 2008b).

Biologically, butanol is produced from a fermentation known as the acetone, butanol and ethanol (ABE) fermentation. This ABE fermentation can use a wide range of biomass, as well as various sugars (glucose, sucrose and lactose) as substrates. Other than butanol, products include organic acid (lactate, acetate and

butyrate), solvents (acetone and ethanol) and gases (CO_2 and H_2). The wide range of products indicates that this fermentation has a complex metabolic pathway. Today's world demand for butanol has been met mainly via the oxo reaction from propylene. It is estimated that over 4.5 million tonnes of butanol are produced annually, which accounts for a market of 70 million GBP (1.1 billion USD). The market growth rate for butanol is estimated at 3.25% per year (Market Publishers, 2010).

2.2 ABE Fermentation

2.2.1 Microorganisms

The first microorganism used for ABE fermentation was a bacterium, *Clostridium acetobutylicum,* which was first isolated by Weizmann in the 1910s (Jones and Woods, 1986). Other than *C. acetobutylicum*, another three key species have been identified as butanol producers: *C. beijerinckii, C. saccharobutylicum* and *C. saccharoperbutylacetonicum.* It should be noted that the three species mentioned above were originally designated as *C. acetobutylicum* until the beginning of the 1990s (Dürre, 2008). All species follow anaerobic fermentation with minor differences, such as the type of substrate for optimum solvent production (Dürre, 2008).

Clostridia are rod-shaped, measuring 0.5 – 2 μm in width and up to 30 μm in length (Figure 2.2a). They are Gram-positive bacteria and typically strict anaerobes. Clostridia form robust endospores which are resistant to oxygen, heat, and alcohol. Spores either occur in central, terminal (Figure 2.2b) or subterminal positions, depending on the species. Most clostridia species are motile and have flagella projecting in all directions used for propulsion (Andreesen *et al.*, 1989).

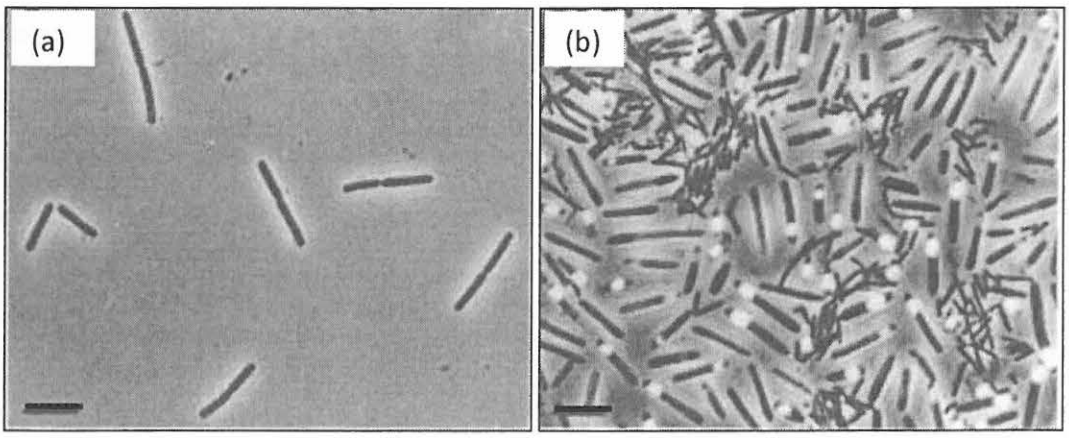

Figure 2.2 Photomicrographs of *C. acetobutylicum* during ABE fermentation. (a) Actively growing phase-dark vegetative rods. (b) Sporulating rods with terminal phase-bright spores. Bar, 10 ,um. (Jones *et al.*, 1982)