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TWO STAGE ABE FERMENTATION WITH *IN SITU* PERVAPORATION AND HIGH CELL DENSITY

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TWO STAGE ABE FERMENTATION WITH IN SITU PERVAPORATION AND HIGH CELL DENSITY

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ABSTRACT

Solvent recovery via in situ pervaporation directly coupled to two stage ABE (Acetone-Butanol-Ethanol) fermentation was studied. Organophilic pervaporation decreased the inhibitory effect of butanol successfully. The permeate enriched to 55-214 $g \cdot L^{-1}$ total solvents depending on solvent concentrations in the fermenter. The maximum flux reported was 719 $g \cdot h^{-1} \cdot m^{-2}$ with a total ABE flux of 148 $g \cdot h^{-1} \cdot m^{-2}$. The continuous fermentation was performed for 500 hours with a xylose/glucose mixture (100/50 $g \cdot L^{-1}$) feedstock and the maximum overall productivity calculated as 0.7 $g \cdot L^{-1} \cdot h^{-1}$ with lowest dilution rate, 0.017 h^{-1}.

To obtain higher productivities and to be able to deal with high flow rates cell recycling via ultrafiltration was applied to two staged fermentation. Dry cell weight (DCW) in the solventogenic reactor increased to a maximum of 43 $g \cdot L^{-1}$ after 44 hours of cultivation, while the average DCW value over 72 hours of operation was 23 $g \cdot L^{-1}$. Overall productivity was 1 $g \cdot L^{-1} \cdot h^{-1}$ eventhough the glucose conversion was lower that expected.

Keywords: In situ product recovery (Organophilic pervaporation), Two-stage continuous fermentation, *Clostridium acetobutylicum* ATCC 824, High cell density fermentation, Ultrafiltration

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ABBREVIATIONS

Symbol	Explanation	Unit
A	Membrane Area	m^2
AF	Acidogenic Fermenter	
BC	Biochemical	
C_i	Concentration	$kg.L^{-1}$
D	Dilution Rate	h^{-1}
DDGS	Distiller's dried grains with solubles	
F_i	Volumetric flow	$L.h^{-1}$
GREET	Greenhouse Gases Regulated Emissions and Energy Use in Transportation	
HTR	The hydraulic retention time	h
ILs	Ionic Liquids	
J	Flux	$kg.h^{-1}.m^{-2}$
LCA	Life cycle assessment	
LHV	Lower Heating Value	
M	Mass	kg
NA	Natural Gas	
P ov	Overall productivity	$g.h^{-1}.\ L^{-1}$
PABA	p-aminobenzoic acid	
PDMS	polydimethylsiloxane	
Pi	pressure	bar
P_M	Permeability of Membrane	$mol.m^{-1}.s^{-1}.bar^{-1}$
PTMSP	Poly[1-(trimethylsilyl)-1-propyne]	
PVDF	polyvinylidene fluoride	
Q	Mass Flow	$kg.h^{-1}$
$\dot{Q}$	molar heat	$J.mol^{-1}$
r_A	Reaction rate	$kg.h^{-1}.L^{-1}$
Rp	component production rate	$kg.h^{-1}.L^{-1}$
Rx	Cell growth rate	$kg.h^{-1}.L^{-1}$
S	Substrate concentration	$kg.L^{-1}$
SF	Solventogenic Fermenter	
SILMs	Supported ionic liquid membranes	
T	Time	h
T	residence time	h
TMP	Trans Membrane Pressure	
V	Volume	L
VFA	Volatile fatty acids	
VOC	Volatile Organic Compounds	
W	Weight of permeate	kg
w	width of unit	m
X	Biomass concentration	$kg.L^{-1}$
x_i	molar concentration of a selectively permeating component in the feed	$mol.L^{-1}$
$Y_{p/s}$	yield coefficient for product formation on substrate	

$Y_{x/s}$	yield coefficient for biomass formation on substrate	
y_i	molar concentration of a selectively permeating component in permeate	$mol.L^{-1}$
ρ	Density	$kg.L^{-1}$
θ	The effective fraction of water om the feed flow	
β	Enrichment Factor	
α	Selectivity	
j	Molar Flux	$mol.h^{-1}.m^{-2}$
S	Solubility Coefficient	
D	Diffision Coefficient	
$\dot{\epsilon}_\iota$	Mass Fraction	
$\dot{\varpi}_i$	Molar Fraction	
$\dot{\rho}$	Molar Density	$mol.L^{-1}$
$\dot{\kappa}_R$	Membrane resistance	$s.m^{-2}$
C_P	Heat Capacity of the feed	$J.C^{-1}.kg^{-1}$
Δh_V	Heat of Evaporation of Permeate	$J.mol^{-1}$

1. INTRODUCTION

BACKGROUND AND MOTIVATION

The production processes of second generation bio butanol has been taken great attention due to increased energy needs of world population. Butanol is a superior fuel especially with high LHV and transport friendly behaviours. We studied continuous two staged *Clostridium Acetobutylicum* fermentation with in-situ product recovery via pervaporation and high cell density. These different phenomenons were studied with double combinations in literature but no publication is available up to now for all three applications at same time.

In situ removal is a process that removes the products from fermentation broth while fermentation goes on. Reduced inhibition of solvents allows the higher productivities. Meanwhile high cell density is another approach to increase the productivity. The grown cells are recycled back to fermenter and higher cell/substrate ratio is obtained.

These are the two new approaches to improve butanol fermentation energy balance to positive scale.

OBJECTIVES

- Xylose/Glucose mixture as a feed stock will be subject to trial. The conversion of xylose is important since for trials of second generation biofuel production this mixture presents a good simulation especially for lignocellulosic biomass and waste biomass fermentation.
- Cell recycling with ultrafiltration membranes (UF) will applied to the system. UF performance, trans membrane pressures will be calculated.
- Filtration (UF) to increase cell density in fermenter and allow higher pervaporation temperatures.
- An overview on economic input of these new approaches will be studied.

2. LITERATURE REVIEW

2.1. HISTORY OF BIOBUTANOL AND CURRENT STIUATION

The conversion of plant biomass into solvents for fuel and chemical industry is in principle an old technology. In fact, the fermentation of sugar and starch to ethanol can be regarded as the oldest and largest biotechnological process (for beer and wine production). Butanol is a fermentation product of anaerobic bacteria. In 1862, one of the most famous French microbiologists Louis Pasteur was the first to describe the synthesis of this C4-alcohol by his “Vibrion butyrique” probably by mixed culture fermentation. Nowadays, biobutanol has the economic and technological potential to replace petrochemical derived butanol for the production of fuels from renewable resources. In addition to its usefulness for the biofuel sector, butanol is a valuable C-4 compound for chemical synthesis for which it is presently chemically synthesized from fossil-oil-derived ethylene, propylene, and triethyl-aluminum or carbon monoxide and hydrogen (Dürre, 1998; Zverlov et al., 2006).

Butanol became famous in both biotechnology and politics at the beginning of the last century with the large acetone demand in World War I. Respective patents were granted to Fernbach and Strange in 1911 and 1912, and the process was started in the United Kingdom under supervision of Strange & Graham, Ltd. (Fernbach and Strange, 1912). This company also had collaboration with the chemists Perkins and Weizmann, based at the University of Manchester. The latter ended the cooperation in 1912, but still continued the project. Weizmann succeeded in isolating an organism that produced significantly larger amounts of acetone and butanol than the strains of Fernbach and Strange. This bacterium and the respective fermentation process and production methods were patented in between the years 1915-1920 (Weismann and Hamlyn, 1290; Weismann, 1919; Weismann and Alliston, 1922). At that time, Great Britain was in urgent need for acetone as an essential chemical for production of cordite (smokeless ammunition). Butanol was an unnecessary by-product during the war. Due to its superior product formation, Weizmann’s *Clostridium Acetobutylicum* became the organism of choice for acetone synthesis and was used in all fermentation plants throughout the former British Empire and later also in Britain’s ally, the United States. The constant supply of acetone was certainly a decisive factor in winning World War I. Weizmann refused to accept any financial or official acknowledgments by the government, but made clear that he was in favor of a Jewish homeland in Palestine. There is no doubt that this attitude affected the Balfour declaration of 1917, leading eventually to the foundation of the State of Israel. Chaim Weizmann became its first president. With the increasing needs for butanol after the war, the first large-scale industrial plants were erected in Canada and USA. After 1930s, large production facilities were erected in USA, Japan (Kyowa Hakko), and South Africa (National Chemical Products in Germiston), among others. One of the best production strains isolated was NCP262, which is now the type strain of *Clostridium saccharobutylicum* (Jones and Keis, 1995; Dürre, 2008). In addition, butanol is a potential fuel and fuel

extender for airplanes, as was demonstrated during the World War 2 when Japan converted its sugar refineries into plants to produce butanol as aviation fuel (Ezeji et al., 2010).

However, the glorious period of ABE fermentation encountered a heavy blow by the end of 20th century, because of the rapid emergence of petrochemical synthetic processes. After a peak in the 1950s, the capacity of the AB fermentation plants in the Western industrialized countries declined constantly due to persistent problems with fermentation reliability because of frequent bacteriophage infections and decreasing quality of molasses by improved sugar processing technology (Zverlov et al., 2006; Chiao and Sun, 2007).

It is only recently that the whole situation has come to a turning point. Coupled with economic reasons more critical problems occurred due to petrochemical based production. An accelerated release of fossil-based CO_2 due to human activity is now generally accepted as a major factor contributing to the greenhouse effect. Approximately 28% of the energy available for consumption in the 25 EU countries is attributed to transportation, of which, more than 80% is due to road transport. Worldwide, about 27% of primary energy is used for transportation, which is also the fastest growing sector (Eurostat 2012).

In current situation it has been estimated that 10–12 billion pounds of butanol is produced annually, which accounts for 7–8.4 billion dollar market at current price. Butanol has a projected market expansion of 3% per year. Half of the butanol production is used in the form of butyl acrylate and methacrylate esters used in latex surface coating, enamels and lacquers (Lee et al., 2008a).

Under the Directive 2009/28/EC on the promotion of the use of energy from renewable sources this share rises to a minimum 10% in every Member State in 2020. Regarding the expand of biofuels use in the EU, the Directive aims to ensure the use of sustainable biofuels only, which generate a clear and net Green House Gas (GHG) saving without negative impact on biodiversity and land use.

The total energy demand in transport (public road transport, private cars and motorcycles and trucks) in the EU was 12 EJ in 2005, with an estimated increase to 15 EJ in 2020 (European Commission, 2009). This means that in order to meet the target of 10% renewable energy in transport, 1.2-1.5 EJ biofuel could be needed. If all wood, paper and cardboard waste not already recovered or recycled is used for biofuel production, it could cover 3-4% of the total 2005 fuel demand. If also all waste already used is assumed available for conversion into biofuels, a total of 5-8% of fossil transport fuels could be replaced (European Commission, 2007).

In the United States, the policies about minimum usage of biofuels requirement are known as the Renewable Fuel Standard (RFS). Due to yearly regulation changes the minimum volume of biofuels is to be used in the national transportation is modified. The expanded RFS (referred to as RFS2) required the annual use of 9 billion US gallons (34,000,000 m^3) of biofuels in 2008 and expanded the mandate to 36 billion gallons annually in 2022. The Act included the provision that of the 36 billion US gallons (140,000,000 m^3) used, no more than 15 billion US gallons (57,000,000 m^3)

could be corn-based ethanol. In addition, of the 36 billion US gallons (140,000,000 m^3), no less than 16 billion must be from cellulosic biofuels. The act also contained provisions setting usage requirements for biodiesel (Agency Environmental Protection, 2013).

Some old ABE plants have started to restore the fermentation production again and biotechnological enterprises are planning to establish new plants. The goals are not only to reduce carbon dioxide emissions -as it is agreed in Kyoto and Rio protocols - but also nationally to help reverse stagnation of rural agricultural communities and resulting unemployment and mass migration from rural to urban areas.

The first-generation renewable products have provided a good start on the way of decreasing energy dependence, but may not provide an optimal economic solution across the value chain since the feedstock is not sustainable. Second-generation biofuels are produced from sustainable feedstock. Sustainability of a feedstock is defined by availability of the feedstock, impact on greenhouse gas emissions, and impact on biodiversity and land use. Today Sovert (UK), CATHAY (CN), Cobalt (USA), Green Biologics (UK), Butyl Fuel LLC (USA), Tetravitae Bioscience on behalf of Eastman Chemicals (USA), ABENGOA (ES) can be considered the companies which are most associated with the development of n-butanol as an advanced biofuel and which aim to market.

Despite the production limitations of biobutanol production, like toxicity to the bacteria, low yield and concentration; there is an accelerated growing movement for commercialization of the process. Up to now, generally in industry batch reactor designs are being used. Eventhough it is the most conventional way of biobutanol production, long lag phases and butanol inhibition decrease the economic value of the production.

Overall advantages of butanol over other biofuels can be listed as follows:

- Butanol has higher energy content than ethanol and can be blended with gasoline at higher concentrations (11.5% in the United States, with the potential to increase to 16%).
- Suitable for transport in pipelines, can be introduced into gasoline easily and without additional supply infrastructure. The current method for transporting the ethanol gasoline mix is by tanker trucks directly to refilling stations, which further increases the cost for bioethanol production.
- Butanol/gasoline mixtures are less susceptible to separate in the presence of water than ethanol/gasoline blends, demanding no essential modifications to blending facilities, storage tanks, or retail station pumps.
- Butanol has lower vapour pressure than ethanol.
- Butanol is less corrosive rather than the other solvents.
- Production routes from conventional agricultural feedstocks are all possible, supporting global implementation. Lignocelluloses from fast-growing energy crops (e.g., grasses) or agricultural “wastes” (e.g., corn stover) are also feasible feedstock.

Not just n-butanol, the isomer of butanol, isobutanol has a great attention on market since the golden age of ethanol as a fuel substituent was suspended by the low performance of ethanol.

Compared with conventional unleaded gasoline, ethanol is a particulate-free burning fuel source that combusts with oxygen to form carbon dioxide, water and aldehydes. Gasoline produces 2.44 CO_2 equivalent kg·L-^{1} and ethanol 1.94 kg·L^{-1}. However ethanol contains 2/3 of the energy per volume as gasoline, ethanol produces 19% more CO_2 than gasoline for the same energy. The Clean Air Act requires the addition of oxygenates to reduce carbon monoxide emissions in the United States (Jacobson, 2007). These insufficient results lead butanol investments to grow faster. Butanol contributes to clean air by reducing emissions and unburned hydrocarbons in the tail pipe exhaust. Butanol has research and motor octane numbers of 113 and 94 compared to 111 and 92 for ethanol (Ladish, 1991) . Although 100% butanol fuel has a slightly lower energy density than gasoline, combustion occurs at a uniform temperature and pressure, as it is a single component fuel. This is different from gasoline as gasoline ignites over a broader temperature and pressure, resulting in incomplete combustion as it is made of many different kinds of molecules. This incomplete combustion results in lower efficiency for internal combustion engines. (Biobutanol.com, 2012)

Since 2006, BP and DuPont have undertaken an extensive program of technology development and fuel testing for biobutanol (isobutanol) to prove the benefits of the molecule over alternative biofuel molecules. Under this trials, Dyson Racing won a American Le Mans Series (ALMS) race in August 2010 using biobutanol as the fuel in a real race car engine. (BP and DuPont, 2012)

By 2010, State-owned Russian Technologies Corporation started to build the country's first biofuel plant. June 2012, in USA seven ethanol plants had expressed an interest in retrofitting isobutanol production technology. Butanol offers a renewable alternative to petro-based chemical-grade butanol - a $10 billion global market. n-Butanol is a key building block chemical in the $85 billion coatings, adhesives and inks market and an intermediate in the $700 billion global polymers market. (European BiofuelsTechnology Platform, 2012)

Abengoa, a Spanish company that produces biosolvents in Brazil, EU and, USA have been engaged in technology development for the production of n-butanol using bio-based ethanol. The company’s bioenergy business has been very active in developing commercial market for cellulosic ethanol, and its 0.1 m m^{3}·year $^{-1}$ Kansas biorefinery plant is expected to start production in the fourth quarter of 2013. (Abengoa, 2013)

The history and current market position of biobutanol were covered for general overview of reader. The literature review will continue with fundamental research in next chapter.

2.2. FEEDSTOCK

Glucose, xylose and glycerol mixtures can be used depending on the microorganism type for ABE fermentation. Cane molasses, corn, millet, wheat, rice, tapioca, soy molasses, and potatoes have been used as a substrate successfully. Although the successful usage and remarkable fraction of usage of these substrates, other carbon sources will be required if renewable biofuels are to make

more significant inroads into the world's energy portfolio. Lignocellulosic biomass and waste products of agricultural processes can be alternative for feedstock. (Qureshi et al., 2008) Lignocellulosic biomass consists of a variety of agro-industrial residues (e.g. corn fiber, corn stover, wheat straw, barley straw and sugarcane bagasse), energy crops (e.g. switchgrass), forestry products (wood chips), and municipal solid wastes. Several lignocellulosic materials such as corn fiber, dried distiller grains and soluble, wheat straw, and switchgrass have been reported and successfully applied in ABE fermentation as substrates to produce butanol. In general, cellulose and hemicellulose present in the lignocellulosic feedstocks are not directly accessible to the microorganisms. Butanol producing *Clostridia* do not express enzymes with cellulolytic activity. Therefore, lignocellulose has to be pretreated and hydrolyzed to release all the sugars that can be utilized by the microorganisms in the subsequent fermentation process (Howard and Abotsi, 2004; Reddy and Yang, 2005; Kumar and Gayen, 2011).

Two lignocellulosic waste source available – wood waste and paper and cardboard waste. Leduc and Wetterlund, 2010; studied lignocellulosic waste production based on Eurostat statistics with the date of 2010. Figure 2.1. shows the waste production of EU. Wood waste defined as waste from the forest industry and from construction and demolition of buildings. Paper and cardboard waste includes for example collected waste as well as waste from pulp, paper and cardboard production.

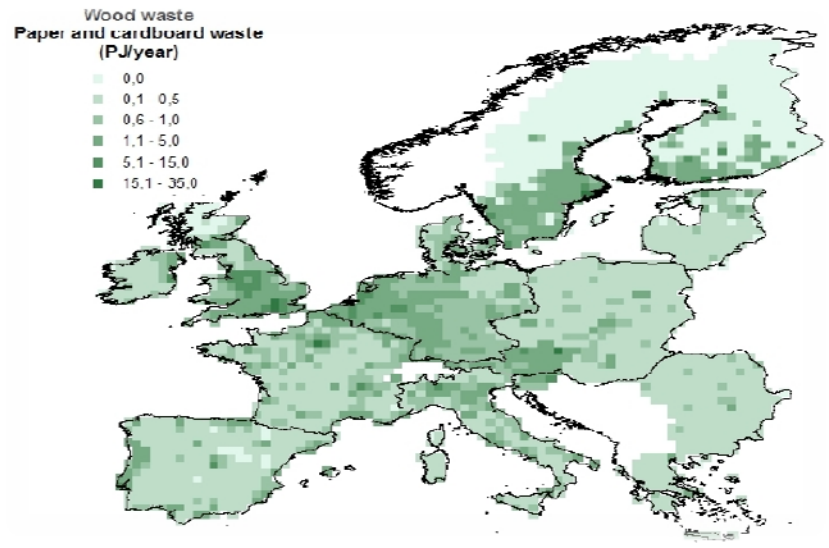


Figure 2.1: Amounts of wood waste and paper and cardboard waste available for biofuel production (PJ/year), 2006 (Eurostat, 2010).(Leduc and Wetterlund, 2010)

Low-cost substrates, such as algal biomass, excess sludge, palm oil, soy molasses, etc., have been investigated for biobutanol production; however, further research into how to utilize these substrates more effectively is needed. There is no doubt that lignocellulose is potentially the best substrate for butanol production, and more efficient bioconversion of cellulose and hemicellulose is crucial to the economic success of the industrial production of butanol (Howard and Abotsi, 2004).

Zheng et al. (2013) and Kumar et al. (2011); reviewed the carbon source and fermentation type effects on continuous ABE fermentation. Table 2.1. represents the results of different continuous culture systems for butanol production using various carbon sources.

Table 2.1. Comparison of continuous butanol production using different carbon sources with free cells, cell recycling, or immobilization

Fermentation Mode	Carbon Source	Butanol Concentration $g.L^{-1}$	Butanol Productivity $g.L^{-1}.h^{-1}$	Ref.
Free Cells	Xylose	2.03	0.529	1
Free Cells	Glucose	6.21	1.24	1
Free Cells	Glucose+glycerol	8.6	0.42	1
Free Cells	Starch	9.8[a]	0.2[b]	1
Free Cells	Sago Starch	9.1[a]	0.85	2
Free Cells	Degermed corn	14.28[a]	0.3	2
Free Cells	Satrch+Glucose	9.9[a]	0.42	2
Cell Recycling	Xylose	4.26	3.32	1
Cell Recycling	Glucose	12.9[a]	7.34	1
Cell Recycling	Lactose	4.9	2.01	1
Cell Recycling	Starch	15[a]	3.3[b]	1
Cell Recycling	Syntehetic Medium	8.8[a]	11	2
Immobiliziation	Glucose	7.19[a]	13.66[c]	1
Immobiliziation	Lactose	2.1	2.3	1
Immobiliziation	Whey Permeate	8.6[a]	0.36-1.1	2
Immobiliziation	Lactose+yeast extact	1.43[a]	0.78	2
Immobiliziation	Corn	12.5	4.60	2
Immobiliziation	Sweet potato slurry	7.73[a]	1.00	2
Immobiliziation	Starch	5.52	0.71	1

a ABE concentration

b ABE productivity

1 (Zheng et al., 2013)

2 (Kumar and Gayen, 2011)

Utilization of alternative feedstock can be done by physical pretreatment, steam explosion, chemical treatment and vacuum treatment. The goal is to optimize the particle size, reduce inhibitors and partially or completely hydrolyze hemicellulose, break down the lignin structure and disrupt the cellulose crystallinity for further enzymatic digestion to release fermentable sugars. Under the extreme conditions employed in pretreatment processes, many toxic compounds that are severe fermentation inhibitors are inevitably generated (Hendriks and Zeeman, 2009). Some commercial pretreatment methods for feedstocks are represented on Table 2.2. Represented information obtained from Tao and Aden, (2009) and Meyer and Papoutsakis, (1989).

Table 2.2: Commercialized pretreatment routes for corn-sugar cane, whey permeate and corn stover. (Meyer and Papoutsakis, 1989; Tao and Aden, 2009)

Feed Stock	Pretreatment Steps				
	1	2	3	4	5
Corn	Milling	Saccharification	Dehydration		
Corn	Steeping	De-germentation	De-fiberation	Enzyme Hydrolysis	
Sugar Cane	Milling	Boling	Solid/Liquid Separation		
Whey permeate	Spray Drying				
Corn Stover	Steam & Acid Treatment	Solid/Liquid Separation	Lime & Gypsium Treatment	Enzyme Hydrolysis	CO_2 Removal

Investigations on simultaneous hydrolysis and fermentation with barley straw and wheat straw were performed. Elimination of separate pretreatment step can increase the economic value of ABE fermentation in two ways. First of all cheap feedstock will be available for commercial use and additional pretreatment equipment will not be necessary.

The lime treated barley straw resulted in successful batch fermentation with *Clostridium beijerinckii* 260 and ABE concentration of 26.64 $g \cdot L^{-1}$ was achieved. Lime was used against inhibitors that released during hydrolysis. This was superior to both glucose and untreated barley straw (initial sugar 60 $g \cdot L^{-1}$) fermentations (Qureshi et al., 2010). Same with barley straw experiments wheat straw run in simultaneous hydrolysis and fermentation with agitation by gas stripping mode resulted with productivity 0.31 $g \cdot L^{-1} \cdot h^{-1}$ and yield 0.41 (Qureshi, Saha, Hector, et al., 2008). When it has been concluded that simultaneous hydrolysis of wheat straw to achieve 100% hydrolysis to simple sugars and fermentation to butanol is possible. Another fed-batch fermentation performed for 533 hours. In addition to wheat straw, the reactor was fed with a sugar solution containing glucose, xylose, arabinose, galactose, and mannose. The culture utilized all of the above sugars. It was noticed that near the end of fermentation (286–533h), the culture had difficulties utilizing xylose. As a result of supplemental sugar feed to the reactor, increase of ABE productivity was observed by 16% with productivity of 0.36 $g \cdot L^{-1} \cdot h^{-1}$ (Qureshi, Saha, and Cotta, 2008).

Low-grade glycerol has also been used to produce butanol in a chemostat culture of *C. Acetobutylicum*, resulting in the high yield and productivity of 0.34 $mol.mol^{-1}$ and 0.42 $g \cdot L^{-1} \cdot h^{-1}$, respectively (Vasconcelos et al., 2004). Underneath these promising results usage of alternative feedstock for ABE fermentation getting closer to commercialize.

Alternative feedstock investigation is the matter of another research. Pretreatment pathways and sub metabolic products appearing during fermentation can cause problems for the glucose-optimized processes. However once process is optimized for conventional feedstock like glucose or glucose/xylose mixture, modification for alternative feedstock can be applied easily. In these experiment sets we will use glucose and glucose/xylose feedstock.

2.3. MICROORGANISM

Butanol (and acetone, ethanol, and isopropanol) can be naturally formed by a number of clostridia. In addition, clostridia can produce chiral products which are difficult to make by chemical synthesis and degrade a number of toxic chemicals (Lee et al., 2008b). Clostridia is rod-shaped, spore-forming gram-positive bacteria and typically strict anaerobes (Monot et al., 1982). Gram-positive bacteria, such as solventogenic clostridia, differ from gram-negative bacteria with respect to physiology, cell structure, and pathology in that they do not have an outer cell membrane and, consequently, lack a periplasmic space.

Solventogenic clostridia can utilize a large variety of substrates from monosaccharides including many pentoses and hexoses to polysaccharides (Jones and Woods, 1986). Complex nitrogen sources such as yeast extract are generally required for good growth and solvent production, but otherwise the nutrient requirements for the growth of clostridia are rather simple (Monot et al., 1982).

A great advantage of clostridial strains is that they can accept pentoses and hexoses. Wild type yeasts can normally only utilize hexoses leaving the pentoses from the hemicellulose unconverted. This is the most significant advantage of butanol in comparison with ethanol plants. To extract as much energy content as possible from the biomass during combustion, the transformation into fuels has to reduce the number of oxygen atoms per carbon. This is achieved in a disproportionation reaction, a balanced redox reaction, during the anaerobic metabolism of microorganisms. During the process CO_2 is extracted from carbohydrates. Glucose ($C_6H_{12}O_6$), a single sugar molecule, would for example be converted to two molecules CO_2 and a molecule of butanol.

$$C_6H_{12}O_6 \rightarrow C_3H_7COOH + 2\,CO_2 + 2\,H_2$$

$$2\,C_3H_7COOH \rightarrow C_4H_9OH + CH_3COCH_3 + CO_2$$

Butanol-producing Clostridia include a variety of species, including *acetobutylicum*, *saccharoacetobutylicum*, *beijerinckii, aurantibutyricum*, *pasteurianum, sporogenes*, and *tetanomorphum*. (Kumar and Gayen, 2011) Among these species, *C. acetobutylicum*, *C. beijerinckii, C. saccharoacetobutylicum,* and *C. saccaroperbutylacetonicum* are the primary producers with good butanol production and yields (Lee et al., 2008c).

The substrate utilization ability among naturally solventogenic Clostridia is very different from each other, as well as their optimal pH, temperature, and product profiles. Most of the species produce butanol as the main product, although some also produce significant amounts of 1,3-propanediol and isopropanol. *C. acetobutylicum* was the main species employed in industrial ABE fermentation until more detailed taxonomy was developed and some strains of *C. acetobutylicum* were re-classified as *C. beijerinckii* based on the product type (Dürre, 1998). Many different strains of these two species have been extensively studied, including *C. acetobutylicum* ATCC 824, P262, P260 and DSM 1731,

and *C. beijerinckii* ATCC 55025, NCIMB 8052, and BA101 (Ennis et al., 1989; Qureshi and Blaschek, 2000; Zheng et al., 2009).

It is generally accepted that sporulation happens when solvents are produced and endospores function as a defence against the harsh environment, which however also results in unstable solvent production in ABE fermentation. A very distinctive feature of Clostridia is the biphasic fermentation. During the first phase, which is known as acidogenesis, acids (acetate and butyrate) and carbon dioxide are produced as the main products during the exponential growth phase, lowering the pH of the medium. Then, through a series of regulations, signals and change in gene expression, the second phase, which is known as the solventogenesis, is triggered and acids are reassimilated and converted to solvents (acetone, butanol and ethanol). The transition from acidogenic to solventogenic phase is the result of a dramatic change in gene expression pattern (Dürre, 1998). A typical ABE fermentation using *C. acetobutylicum* yields acetone, butanol and ethanol in the ratio of 3:6:1.

Figure 2.2. shows the metabolic pathway of *C. acetobutylicum* for ABE fermentation. As it can be seen from the figure clostridia requires high redox potential to produce butanol (and ethanol) and the supply of additional reducing power results in increased butanol and ethanol formation with reduced acetone formation. The formation of butyric acid during the acidogenic phase is important for maintenance of the redox equilibrium because nicotinamide adenine dinucleotides (NADHs) produced during glycolysis are only oxidized in the butyric acid formation pathway, not in the acetic acid formation pathway, resulting in the regeneration of NAD+. It seems that acetyl-CoA is mostly used to form butyryl-CoA, based on the fact that the conversion of acetyl-CoA to butyryl-CoA exhibits enhanced thermodynamic stability (Zheng et al., 2009). Butyryl phosphate appears to be important for butanol production. When the *buk* gene (which encode butyrate kinase) was inactivated, more butanol was produced (Lee et al., 2008b). It was reported that the initiation of butanol formation corresponded to the time when butyryl-P concentration reached its peak. It was suggested that the concentration of butyryl-P should be higher than 60 – 70 pmol·gDCW^{-1} for butanol production (Zhao et al., 2005; Ezeji et al., 2010).

Groot and Luyben, 1987; studied glucose and xylose conversion kinetics with immobilized continuous fermentation. They concluded that xylose is only converted when glucose is completely consumed and the biocatalyst is not fully inhibited by butanol. Another study investigated the inhibition of *C. acetobutylicum* metabolism of glucose and xylose by end products of fermentation and concluded that *C. acetobutylicum*, when grown in xylose, is inhibited to a greater extent by ABE than is *C. acetobutylicum* grown on glucose. Among the end products of fermentation, butanol is the most potent inhibitor, and results obtained by Ounine et al. (1985) indicates that when glucose and xylose are used as substrates in the *C. acetobutylicum* fermentation, growth inhibition on both substrates is correlated with the inhibitory effects of butanol on sugar transport. This phenomenon is most pronounced when growth occurs on xylose rather than on glucose (Ezeji et al., 2010).

Meyer and Papoutsakis, (1989) investigated morphological forms and changes during the continuous fermentations. A schematic representation of this morphological cycle is given in Figure 2.3. The rate of conversion, or differentiation, of one morphological form to another is designated by a reaction rate K. These non-reversible changes occur as a result of complex interactive triggering mechanisms. For example, the morphological change from vegetative cells to clostridial cells at rate K_1, involves the acid concentration, pH, and residual sugar and nitrogen concentrations.

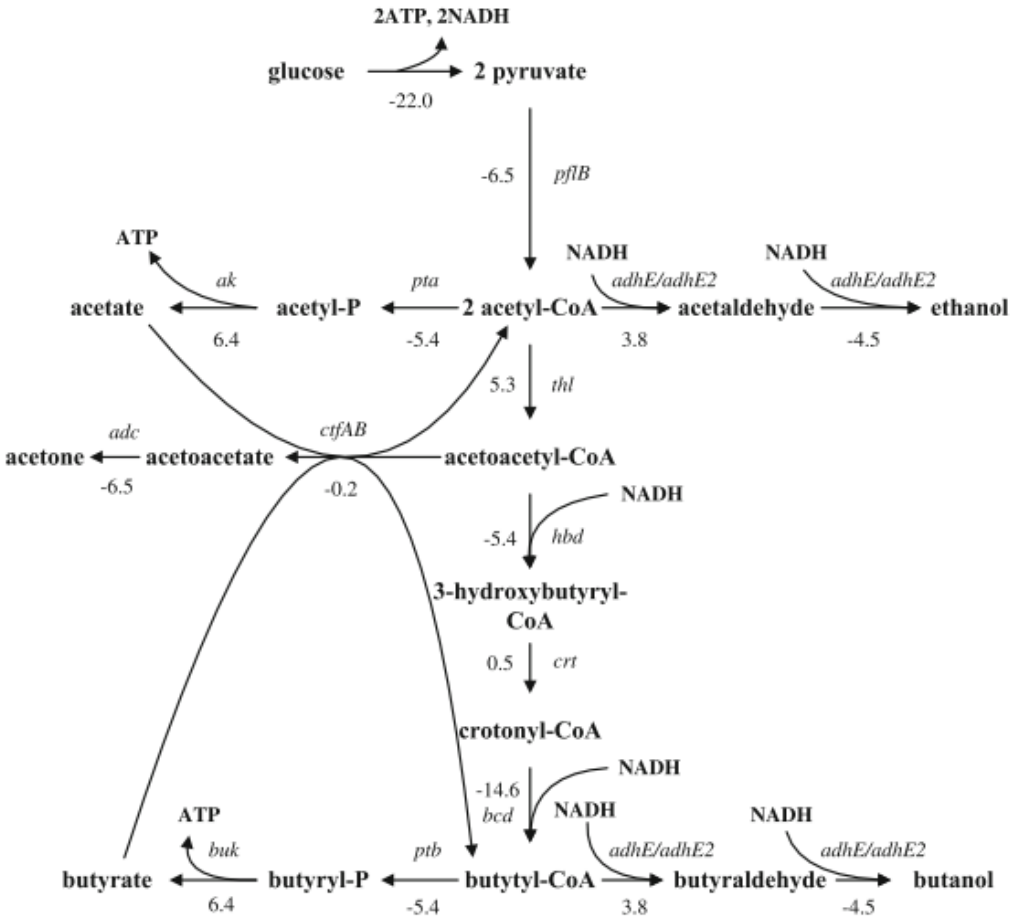


Figure 2.2: Metabolic pathways of C. acetobutylicum. The numbers shown in the figure represent the standard Gibbs energy changes $\Delta_r G_m^{\theta}$ of the corresponding reactions. The genes are shown in italics, and their corresponding enzymes are as follows: pflB, pyruvate ferredoxin oxi- doreductase; thl, thiolase; hbd, 3-hydroxybutyryl-CoA dehydrogenase; crt, crotonase; bcd, butyryl-CoA dehydrogenase; pta, phosphotransa-cetylase; ak, acetate kinase; ptb, phosphotransbutyrylase; buk, butyrate kinase; ctfAB, acetoacetyl-CoA:acetate/butyrate:CoA transferase; adc, acetoacetate decarboxylase; adhE/adhE2, aldehyde/alcohol dehydrogenase (Zheng et al., 2009).

Conceptually, for the maintenance of continuous stable solvent production, the conditions must be such that the clostridial cells are maintained in a stable non-growing solvent production phase, with vegetative growth, sporulation, and cell lysis inhibited or closely controlled, i.e. a balance

of culture morphology must be maintained. If the biomass removal rate were maintained at a rate equal to the differentiation rate K_1, (assuming no sporulation or cell lysis) then, theoretically, control over the solvent-producing clostridial population would be possible. Whether this remained stable would depend on the balancing of environmental conditions, e.g. avoidance of toxic product concentrations. In this situation however, vegetative cells could still accumulate and the differentiation rate K_1 would be expected to change. Therefore, the maintenance of the growth rate (μ) and spore germination rate, K_4, equal to the differentiation rate of vegetative cells to clostridial cells, K_1, minus the clostridial cell lysis rate, Kz, and sporulation rate, K_3, ($K_4 + \mu = K_1 - K_2 - K_3$) is a controlling index for stable solvent production.

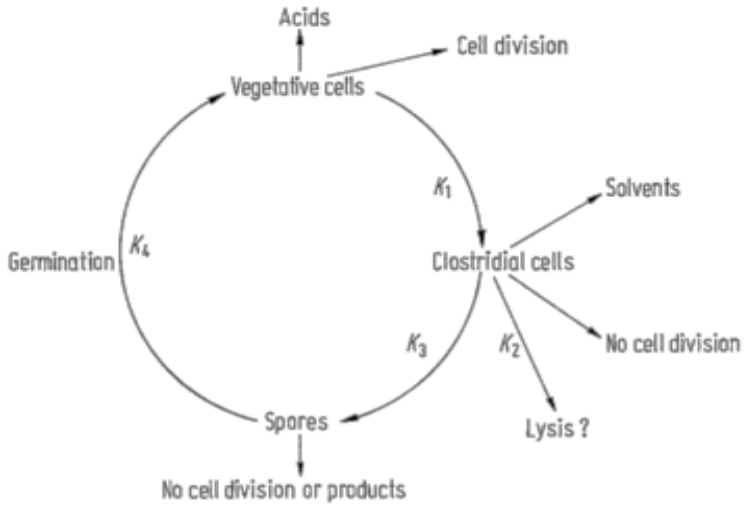


Figure 2.3: A schematic diagram of the morphological changes observed during continuous fermentation by C. acetobutylicum, P262, fermenting whey permeate medium. K1 designates the rate of conversion (or differentiation) of vegetative cells to clostridial forms. K2 designates the rate of loss of clostridial forms due to lysis. K3 designates the rate of sporulation of clostridial forms. K4 designates the rate of spore germination to vegetative cells (Meyer and Papoutsakis, 1989)

Metabolic Engineering of cultures

We can list the objectives of metabolic engineering clostridia as it follows:

- Enhanced butanol production with respect to the final concentration and productivity,
- Increased butanol (solvent) tolerance,
- Extended substrate utilization range,
- Increased butanol yield on carbon source,
- Selective production of butanol instead of mixed acids/solvents production.

For successful metabolic engineering of *C. acetobutylicum*, it is necessary to have efficient genetic engineering tools for metabolic pathway manipulation. Several *B. subtilis* to *C. acetobutylicum* and *E. coli* to *C. acetobutylicum* shuttle vectors were developed in the early 1990s (Lee et al., 2008b).

Several examples of metabolic engineering of *C. acetobutylicum* have been reported over the years, mainly by the research groups of E. Terry Papoutsakis and George Bennett. The first successful metabolic engineering example was the amplification of the acetone formation pathway in *C. acetobutylicum*. This resulted in increased final concentrations of acetone, butanol, and ethanol by 95%, 37%, and 90%, respectively, compared to its parental strain (Mermelstein et al., 1993).

One of the earliest developed butanol-tolerant strain was a mutant of *C. acetobutylicum* ATCC 824 called initially as SA-1. This strain was developed using serial transfer, a procedure where samples of *C. acetobutylicum* culture at OD_{585} 0.8 (or the highest attainable) are transferred into fresh media containing increasing concentrations of n-butanol. SA-1 had a 121% increase in tolerance over wild type when grown on 6% extruded corn broth (Lin and Blaschek, 1983). Similar experiments later led to strain SA-2, grown on brain heart infusion and P2 (minimal) medium. SA-2 had a 27% increase in butanol tolerance over wild type and was hypothesized to have adjusted its lipid membrane content in order to maintain a stable environment for cellular functions (Baer et al., 1987). In both SA-1 and SA-2, increased tolerance did not result in a greater overall butanol yield, suggesting that tolerance is not the only variable limiting butanol yield. A new method for strain development in C. acetobutylicum was introduced in 1994 by, Mermelstein et al., employing a plasmid vector system for introducing foreign DNA into ATCC 824 by electrotransformation. This genetic transfer technique has been the most widely used to investigate the effect of overexpressing or inactivating various genes on ABE tolerance in solventogenic clostridia (Ezeji et al., 2010).

As it is mentioned previously toxic effect of butanol limits the fermentation performance on first hand. The accepted dogma is that toxicity in the ABE fermentation is due to chaotropic effects of butanol on the cell membranes of the fermenting microorganisms, which poses a challenge for the biotechnological whole-cell bio-production of butanol. Development of solvent-tolerant strains to ameliorate solvent toxicity has typically followed one of two approaches: (1) enhancement of butanol toxicity defences in solventogenic clostridia and (2) metabolic engineering of well-characterized microorganisms (*E. coli* and *S. cerevisiae*) for ABE production (Kharkwal et al., 2009, Tomas et al., 2003).

A recently published international patent by DuPont describes the production of butanol in *E. coli*, *Bacillus subtilis* and *S. cerevisiae*. By overexpressing the *thl, hbd, crt, bcd, ald* and *bdhAB* genes of *C. acetobutylicum* involved in butanol biosynthesis, 0.8 mM, 0.19mM and 0.01mM butanol could be produced by engineered *E. coli, B. subtilis* and *S. cerevisiae,* respectively (Donaldson et al., 2007).

Genomic library screening has been used to identify individual genes capable of improving solvent tolerance. Before in vivo experiments, in silico genome-scale metabolic networks constructed based on the complete genome sequence can be used for virtual experiments under various genotypic and environmental conditions. The results can provide strategies for metabolic engineering, including genes to knock-out and amplify, to achieve desired metabolic performance. Considering the greater difficulty of engineering clostridia compared with *E. coli*, in silico simulation before actual metabolic engineering experiments will be beneficial.

Ezeji et al. (2004) developed a hyper amylolytic *C. beijerinckii*, designated BA101. Although many butanol-producing microorganisms secrete starch-hydrolyzing enzymes, *C. beijerinckii* BA101 has an enhanced capability to utilize starch and accumulate higher concentrations of butanol (17–21 $g \cdot L^{-1}$) in the fermentation medium. In addition to the use of corn, liquefied corn meal and corn steep liquor (a byproduct of corn wet milling process that contains nutrients leached out of corn during soaking) were tested for acetone–butanol production. In the batch process with recovery, 60 $g \cdot L^{-1}$of liquefied corn meal and corn steep liquor yielded ca. 26 $g \cdot L^{-1}$of solvent.

Attempts have been made to develop a *C. acetobutylicum* strain that can utilize cellulose directly. There is evidence that *C. acetobutylicum* ATCC 824 can produce a cellulosome, that is, a cellulose-degrading multienzyme complex consisting of several catalytic components surrounding a scaffold protein. *C. acetobutylicum* ATCC 824, however, has no cellulolytic activity suggesting that some element of the cellulosome is missing or not expressed. In an effort to make *C. acetobutylicum* utilize cellulose directly, the cellulase gene from *C. cellulovorans* or the gene encoding the scaffold protein from *C. cellulolyticum* and *C. thermocellum* were introduced into *C. acetobutylicum*. However up to now no sufficient results were obtained. More studies are needed to characterize the existing cellulase gene cluster in *C. acetobutylicum* before further metabolic engineering. (Lee et al., 2008b)

We have chosen two-step fermentation because of the advantageous biphasic behaviour of *C.acetobutylicum.* First reactor is kept in the conditions to support acidogenesis phase. Grown culture is fed to second solventogenic reactor where conditions are optimized for solvent production. This layout has several advantages, which will be discussed further. For all experiments *C. acetobutylicum* culture had been used.

2.4. FERMENTER CONFIGURATIONS

In this section, fermenter behaviours will be studied for three different modes of operation; batch, fed-batch and continuous mode.

Both physical and biological information are required for the design and estimation of reactor performances. Physical factors are generally effecting hydrodynamic environment of the bioreactor. Liquid flow patterns and circulation time, and intensity of mixing and the effects of shear can be listed as physical parameters. These factors generally depend on bioreactor geometry. Also some physicochemical parameters such as liquid viscosity and interfacial tension highly depended on agitator speed, effect of baffles. Agitator properties also have a significant effect on gas bubble size and a relatively effecting both liquid and gas phase hydrodynamics. The biokinetic input involves such factors as cell growth rate, cell productivity and substrate uptake rate.

For all 3 kinds of reactors, conversion of mass will be main equation as a starting point. For well defined, time depended ABE fermentation reactors, we can express component mass balance;

$$\left\{\begin{matrix}Rate\ of\\ accumulation\\ of\ mass\\ of\ component\\ in\ the\ system\end{matrix}\right\} = \left\{\begin{matrix}Mass\ flow\\ of\ the\\ component\\ into\\ the\ system\end{matrix}\right\} - \left\{\begin{matrix}Mass\ flow\\ of\ the\\ component\\ out\ of\\ the\ system\end{matrix}\right\} + \left\{\begin{matrix}Rate\ of\\ production\\ of\ the\\ component\\ by\ the\ reaction\end{matrix}\right\}$$

Rate of accumulation:

This term express the derivation of mass in the system boundaries according to one species. If we consider the total mass balance derivative term will be equal to zero. But as in the generative fermentation, the behaviour of single specie aqueous solutions mass can be written in terms of concentration-C_i and volume- V.

$$\left(\frac{dM_i}{dt}\right) = \left(\frac{d(C_i V)}{dt}\right) kg \cdot h^{-1} \qquad [1]$$

Since volume change during fermentation can be negligible, accumulation of component during time can be written as it follows:

$$\left(\frac{dM_i}{dt}\right) = \left(V\frac{dC_i}{dt}\right)\ kg \cdot h^{-1} \qquad [2]$$

Convective flow terms

Total mass flows - Q ($kg \cdot h^{-1}$) into and out of the system are given as volumetric flow - F ($L \cdot h^{-1}$) multiplied by density – ρ ($kg \cdot L^{-1}$). On the other hand, when component balance is being created, component flows must be expressed. Instead of density, concentration - C_i with respect to mass ($kg \cdot L^{-1}$) can be used to express component mass flow over/in the system. Concentration can be also be expressed by mass fraction –ε_i in case of total mass flow. ε_i is a dimensionless number that shows the ratio of component over total species and can be formulate as;

$$\varepsilon_i = \frac{C_i}{C_T} \qquad [3]$$

We can conclude that, for component balance two starting point can be created;

$$Q\ \varepsilon_i = F\ C_i \qquad [4]$$

The models are being subject to represent here, considered as ideally mixed. The compositions inside the reactors are assumed as homogenous. Leaving stream has the same properties as the reaction volume.

Production rate

This term in the balance equation represents the production or consumption of component by reaction in the system boundaries. r_A is defined as rate of production of the component for per reaction volume – $(kg \cdot h^{-1} \cdot L^{-1})$. The product formation for single component can be defined as;

$$\left(\frac{dM_A}{dt}\right) = r_A V \ (kg \cdot h^{-1}) \qquad [5]$$

r_A is positive with the case of generation and counted as negative for consumption. The term r_A is valid for single component balance, hence if we want to observe the overall change in the system we have to consider overall production rate. Since a chemostat is being subject to modeling, for example for substrate balance, *S* $(kg \cdot L^{-1})$, two main consumptions should be taken into account: Cell growth and component production. The relationship between r_X, r_P and r_S can be expressed as a constant yield coefficients, yield coefficient for product formation on substrate $(Y_{P/S})$, yield coefficient for biomass formation on substrate $(Y_{X/S})$ and yield coefficient for product formation $(Y_{P/X})$. $r_X(kg \cdot h^{-1} \cdot L^{-1})$ indicates positive values of biomass production in the system, meanwhile r_P $(kg \cdot h^{-1} \cdot L^{-1})$ stands for product formation rate. In chemostats, yield can be defined as the amount of product that was obtained during fermentation. Since there is component accumulation through the reaction yield value should indicate positive values. Correlations for biomass/substrate and product/substrate can be expressed as it follows.

$$Y_{P/X} = \frac{mass\ of\ the\ product}{mass\ of\ the\ biomass\ produced} = -\frac{r_P}{r_x} \qquad [6]$$

$$Y_{P/S} = \frac{mass\ of\ the\ product}{mass\ of\ the\ substrate\ consumed} = -\frac{r_P}{r_S} \qquad [7]$$

$$Y_{X/S} = \frac{mass\ of\ the\ biomass\ produced}{mass\ of\ the\ substrate\ consumed} = -\frac{r_X}{r_S} \qquad [8]$$

Since r_S is depended value with 2 different variables, r_S should be written in terms of r_P and r_X. If the equations 6 to 8 rearranged two different expression of r_S can be obtained.

$$r_S = \frac{r_P}{Y_{P/X} Y_{X/s}} \quad or \quad r_S = \frac{Y_{P/X}\, r_X}{Y_{P/S}} \qquad [9,10]$$

Now we can define the equation for the profile of S as;

$$\frac{dS}{dt} = \frac{r_P}{Y_{P/X} Y_{X/s}} \cdot V \qquad or \qquad \frac{dS}{dt} = \frac{Y_{P/x}\, r_X}{Y_{P/s}} \cdot V \qquad [11,12]$$

2.4.1. Batch – Fed Batch Fermentation

Batch reactors are the most common reactor type in industry. The reliability is highly favored by industry, and therefore has sometimes preference over fed-batch and continuous processes. The general procedure for batch production: the bioreactor is first charged with medium, inoculated with cells, and the cells are allowed to grow for a sufficient time, such that the cells achieve the required cell density or optimum product concentrations.

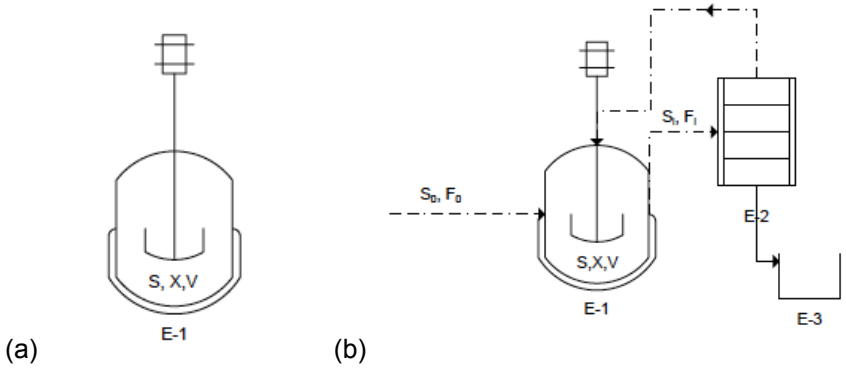


Figure 2.4: (a) Simplified batch reactor. E-1: autoclaved fermenter (b) Simplified fed-batch reactor E-2 recovery unit (optional), E-3 product collection tank

Figure 2.4-a shows a simplified batch reactor. Since there is no flow in or out of the bioreactor, during normal operation, the biomass and substrate balances both take the form,

$$\left(\frac{dM_i}{dt}\right) = \left(\frac{dS}{dt}\right) kg \cdot h^{-1} \qquad [13]$$

The highest butanol concentration achieved in batch fermentation with MP2 medium and 0.60 M sodium acetate and 8% glucose, *C. beijerinckii* BA101 produced 20.9 g·L^{-1} butanol (32.6 g·L^{-1} total solvents). MP2 is chemically defined medium has following components per liter. 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01g $MnSO_4$ H_2O, 0.01g $FeSO_4$, 0.01g NaCl, 1.0 g PABA, 0.01g Biotin, 0.1 g thiamin, 0.5 g KH_2PO_4, 0.5 g K_2HPO_4, 2.0 g $(NH_4)_2SO_4$ and various amounts of CH_3COONa. In all batch cultures glucose conversion was limited below about 80 g · L^{-1} due to accumulation of butanol in the broth and also substrate inhibition. In a batch fermentation and exceeds the threshold concentration of 22∓28 g·L^{-1} that is estimated to be required for the ABE fermentation to become economically competitive once again. (Chen and Blaschek, 1999)

In the figure 2.4-b a simple fed-batch layout can be seen. The important characteristics of fed-batch operation are therefore as follows:

1. Extension of batch growth or product production by additional substrate feeding.

2. Possibility of operating with separate conditions for growth and production phases.

3. Control possibilities on feeding policies.

4. Development of high biomass and product concentration.

For a case study, parallel batch and fed-batch fermentations were carried under same initial conditions. Sugar supply and online product recovery; oleyl alcohol extraction applied to fed batch system with defined time sequences. At the end of fermentation, the organic phase contained over 30 $g{\cdot}L^{-1}$ butanol and 5 $g{\cdot}L^{-1}$ acetone. This is over twice the concentration of butanol that can be obtained in conventional batch fermentation (Roffler et al., 1987). This translates into not just increased sugar conversion but also reduced wastewater and maintenance costs.

Volume change due to sugar addition or solvent removal in fed batch fermenters can be expressed as;

$$V(t) = V_i + \theta \int_0^t F(t) - \sum_{i=0}^{n} V_{si} \qquad [14]$$

with V_i being the initial broth volume, F(t) the volumetric flowrate of feed into the fermenter, θ the effective fraction of water in the feed, V_{si} the volume of the i-th aqueous phase sample, and n being the number of samples taken up to time t. The effective fraction of water in the feed is the sum of the water actually in the feed and the amount of water produced from the fermentation of the glucose contained in the feed.

2.4.2. Continuous Fermentation and Cell Recycling

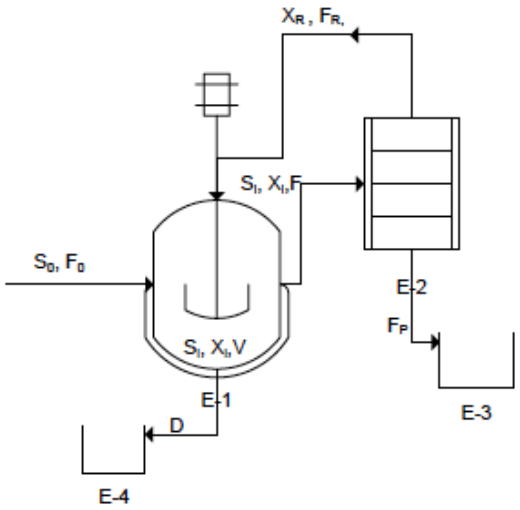


Figure 2.5: Simplified continuous reactor. E-1: autoclaved fermenter, E-2: recovery unit, E-3: product collection tank, E-4 bleed tank

Continuous operation (Figure 2.5.) is the third option and has several advantages over batch and fed-batch operations, including minimizing equipment downtime and time loss due to the lag phase of the microbial culture. Also, the dilution effect during the addition of the substrate solution may also solve the problem of catabolite toxicity. Dilution rate *(D, h^{-1})* is defined as the ratio of the volumetric feed rate of medium (F, $L \cdot h^{-1}$) to the volume of fermentation culture solution (V, L).

$$D_{ov} = \frac{F}{V} \quad h^{-1} \qquad [15]$$

The hydraulic retention time (HRT), residence time (τ, h) is a measure of the average length of time that a soluble compound remains in a constructed bioreactor. It can also be defined as the inverse of dilution rate.

$$\tau = \frac{V}{F} = \frac{1}{D} \quad h \qquad [16]$$

Another necessary data to discuss about feasibility of reactors is productivity. We can define overall productivity as; rate of product per time per volume.

$$P_{ov} = D_{ov} C_{ABE} = \mathrm{g} \cdot h^{-1} \cdot L^{-1} \qquad [17]$$

The rate of accumulation equals to zero for steady state continuous reactors. The composition inside the reactor assumed constant from beginning of reaction until the end. For sugar consumption, S_{ov}, we can rearrange the equation with respect to initial and final sugar concentration, $C_0 - C_T$, and dilution rates.

$$\frac{dS_{ov}}{dt} = D_{ov}(C_0 - C_T) \qquad [18]$$

Productivity of ABE fermentation has been subject to development via continuous culture usage. Increased productivity affects the capital costs in first hand. Butanol production, however, can degrade over time when *Clostridium acetobutylicum* is grown in continuous culture. Studies have therefore been carried out, in which the concentrations of glucose, phosphate, nitrogen, sulphate, or other nutrients have been purposely limited to prevent decreasing butanol yields in continuous culture. (Roffler et al., 1987)

High concentrations of butanol are desirable, because product recovery costs are strongly dependent on butanol concentration in the product stream. High concentrations of butanol can be produced in continuous culture, but the productivity of the fermentation is highly reduced. For continuous culture, a high butanol concentration continuously limits productivity.

For further discussion of ABE fermentation two stage continuous lay out must be investigated more deeply. The two-stage process was advantageous not only to dampen out the oscillations frequently observed but, also to permit greater experimental freedom with the second stage than

possible in a single stage system (Gapes, 2000) . When a two-stage continuous process is used the inhibitory effect of butanol can be decreased. The acidogenic and solventogenic phases are separated in two consecutive fermenters (Bahl et al., 1982; Afschar and Schaller, 1991). The first stage is then maintained at a relatively high dilution rate (D_1) and the second at a low one. Only the bacteria in the second stage are exposed to high levels of butanol and there is a continuous supply of non- inhibited cells from the first fermenter. (Godin and Engasser, 1990)

The main problems with continuous operation that must be dealt with is that while a higher process flow rate typically results in higher reactor productivities, it also leads to lower substrate conversion and lower product concentrations. And also most production systems exhibited a very low volumetric ABE productivity due to low cell concentration; it has generally been observed that the cell concentration in anaerobic cultures is lower than that in aerobic cultures. Biomass recycle appears to be one possible means of overcoming this problem. By promoting a higher biomass concentration, cell recycle reactors may achieve high product concentrations and substrate conversion, even at high throughput rates (Meyer and Papoutsakis, 1989; Tashiro et al., 2005).

High cell density can be obtained via adsorption, entrapment, covalent bond formation and membrane filtration. For first three cases, the culture may experience substrate, nutrient, and product diffusion limitations. In substrate diffusion limitations, the culture may not get carbon source for its energy needs thus resulting in death of the innermost cell layers due to starvation or formation of spores. This will reduce the amount of active cells that take part in the reaction. Due to reduced amount of cells will lead to reduced reactor productivities. At the same time, product diffusion limitation may not allow diffusion of toxic products from the cell surroundings thus affecting cell viability.(Nasib Qureshi & Thaddeus Ezeji 2008) Compared with bioreactors that use cell-immobilizing, bioreactors with cell-recycling are advantageous due to the homogeneity of the broth that facilitates diffusion in the bioreactor as well as the total recycling of microorganisms (Ferras et al., 1986). Table 2.3 shows the fermentation performances of ABE production in bioreactors coupled with membrane cell recycle systems.

Tashiro et al. (2005) could obtain a high cell density by concentrating the solventogenic cells of the broth 10 times. Hollow fiber ultra-filtration modules were used for recycling. 20 $g.L^{-1}$ of active cells were obtained after only 12 h of cultivation and after 200 h continuous operation the overall volumetric ABE productivity was 7.55 $g{\cdot}L^{-1}{\cdot}h^{-1}$. Maximum cell concentration increased gradually through cultivation to an average final value greater than 100 $g{\cdot}L^{-1}$.

Meyer et al. (1989) reached to the steady state with the high recycle ratio at D = 0.23 h^{-1}, the butanol concentration was over 12.30 $g{\cdot}L^{-1}$, which is in the range in which butanol is toxic to most strains of *C. acetobutylicum*. The total solvent concentration 20 $g{\cdot}L^{-1}$ obtained in steady state condition, that can be considered as the maximum concentration that was typically achieved in industrial batch fermentations.

Pierrot et al. (1986) used hollow-fiber ultrafiltration to separate and recycle cells in a continuous fermentation of *C. acetobutylicum*. Under partial cell recycling with and at a dilution rate of 0.5 hr^{-1}, active cellular concentration of 20 $g \cdot L^{-1}$ and a solvent productivity of 6.5 $g \cdot L^{-1} \cdot h^{-1}$ is maintained for several days at a total solvent concentration of 13 $g \cdot L^{-1}$. With partial cell recycling, it is the bleed dilution rate which at steady-state equals the specific growth rate. Thus the growth of cells in the recycling fermenter is simply controlled through the bleed flow rate. The total dilution rate, on the other hand, mainly determines the extent of substrate conversion in the continuous fermenter.

Ennis et al. (1989) was investigated for the production of ABE from whey permeate using *Clostridium acetobutylicum P262*. Reported overall productivity was 1.32 $g \cdot L^{-1} \cdot h^{-1}$ with overall dilution rate 0.3 h^{-1}.

Schlote & Gottschalk (1986) performed phosphate imitated runs (1.34 $g \cdot L^{-1}$) and with dilution rate of D=0.10h^{-1} overall productivity reported as 1.41 $g \cdot L^{-1} \cdot h^{-1}$. Three kinds of membrane performances and biomass-membrane interactions were reported for recycling *Clostridium.* A cellulose-triacetate ultrafiltration membrane with a cut-off volume of 20000 MW was found to work best. The decrease of the flow rate of the filtrate with time is depicted in Figure 2.6. It can be seen that the polysulfone filter with a cut-off volume of 100000 MW was clogged by 50% already after 24 h, since the cellulose acetate filter was clogged by 50% after 90 h.

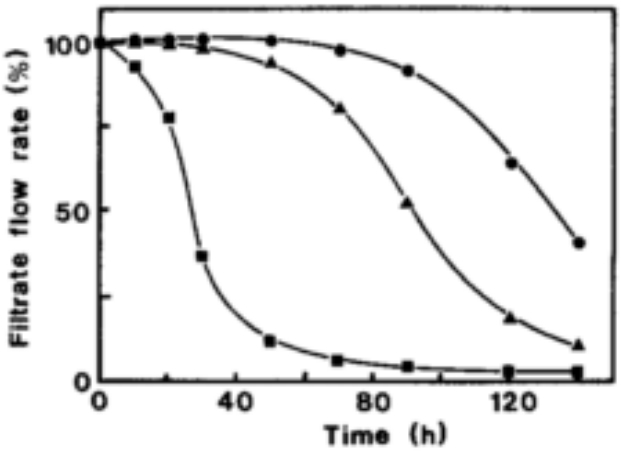


Figure 2.6: Decrease of the filtrate flow rate of different filter membranes with time. Suspensions of inactive cells of C. aecetobutylicum were used. Overflow rate, approximately 101/h. Cellulose-acetate filter membrane, pore-diameter 0.2 μm, Δ; polysulfone ultrafilter, cut-of volume 100000 MW,■ ; cellulose-triacetate ultrafilter, cut-off volume 20000 MW, • (Schlote and Gottschalk, 1986)

Afschar et al. (1985) used two-stage cascade with cell recycling and concentration control turned out to be the best solution, the first stage of which was kept at relatively low cell and product concentrations. A solvent productivity of 3 and 2.3 $g \cdot L^{-1} \cdot h^{-1}$, respectively, at related solvent concentrations of 12 and 15 $g \cdot L^{-1}$.

Ferras et al. (1986) investigated ceramic membrane usage for cell recycle as sterilization process of ceramic membranes are easier than polymeric membranes. The device developed was sterilizable by steam and permitted drastic cleaning of the ultrafiltration ceramic membrane without interrupting the continuous fermentation. It has been shown to be an easily operated and reliable experimental tool for studying high-cell-density cultures and inhibition phenomena. With total recycle of biomass, a dry weight concentration of 125 $g \cdot L^{-1}$ was attained, which greatly enhanced the volumetric solvent productivity of fermentation in averaging 4.5 $g \cdot L^{-1} \cdot h^{-1}$ for significant periods of time (>70 h) and maintaining solvent concentration and yield at acceptable levels.

Xylose conversion has been studied by J. Zheng et al. (2013). A continuous acetone–butanol–ethanol (ABE) production system with high cell density obtained by cell-recycling of *C. saccharoperbutylacetonicum N1-4*. To obtain a high cell density at a faster rate, the solventogenic cells of the broth were concentrated 10 times by membrane filtration and were able to obtain approximately 20 $g \cdot L^{-1}$ of active cells after only 12 h of cultivation. Cell concentration increased gradually through cultivation and the highest concentration was reported as 100 $g \cdot L^{-1}$. The maximum butanol productivity of 3.32 $g \cdot L^{-1} \cdot h^{-1}$ was obtained at a dilution rate of 0.78 h^{-1}.

The potential advantages associated with the use of membrane bioreactors are: productive cells are returned to the fermenter for re-use; greater biomass concentrations are achievable due to the availability of fresh nutrients in the feed medium, facilitating improved volumetric productivities; and inhibitory products can be removed from the cell- free filtrate stream, possibly more easily than when cells are present, prior to its return (partial) to the fermenter, giving rise to even greater productivities.

Potential disadvantages may include: the additional investment in membrane modules; risk of membrane fouling, reducing the operational period of continuous fermentation; the increase in biomass concentration may not give proportional increases in productivity due to diffusional limitations or retarded growth or metabolic activity; and the process may be complex and difficult to operate for extended periods. Additionally, comparatively little is known about the fermentative behaviour and kinetic parameters of concentrated cell suspensions in continuous culture for extended periods.

Table 2.3: A brief summary of ABE production in bioreactors coupled with membrane cell recycle systems

Reference	**Unit**	**Ferras et al. (1986)**			**Afschar et al. (1985)**
Configuration		SingleUF module	Parallel 2 UF module	Series 2 UF module	MF module
Fermentation		Single Stage			Single Stage
Bleed$_1$	h^{-1}	N/A			N/A
D $_{ov}$	h^{-1}	0.33		<0.33 not stable value	0.4
pH $_1$		5.4			4.4
T_1	^oC	35			37
Operation period	h	504	288	528	N/A
ABE Concentration	gL^{-1}	20.5-9.3	N/A	N/A	7
A	gL^{-1}	N/A	N/A	N/A	N/A
B	gL^{-1}	5.9-12.4	N/A	N/A	N/A
E	gL^{-1}	N/A	N/A	N/A	N/A
Productivity $_1$	$g\ L^{-1}h^{-1}$	1.3	3.5	4.5	N/A
Productivity$_2$	$g\ L^{-1}h^{-1}$	N/A	N/A	N/A	N/A
Productivity$_{overall}$	$g\ L^{-1}h^{-1}$	N/A	N/A	N/A	4.5
$C_{initial}$	gL^{-1}	70			60
$C_{residual\ sugar}$	gL^{-1}	5-60[a]	N/A	N/A	N/A
Organism		Clostridium acetobutylicum (ATCC 824)			Clostridium acetobutylicum ATCC 824 (DSM 792)
Manufacturer		Carbosep M1 SFEC, Bollene, France			(ENKA, Wuppertal)
Membrane Material		mineral			polypropylene
Avarege Pore size	µm	N/A			N/A
Mw cut-off	Dalton	N/A			N/A
Crosflow velocity	Lh^{-1}	0.97-2.27			N/A
ΔP across membrane	kPa	N/A			N/A
V_1	L	3.45			2
Feed Type		Glucose			Glucose
DCW	gL^{-1}	125		N/A	N/A
Cell Density	gL^{-1}	14	17	50	8

a: data is provided from table
b: butanol productivity
c: culture vessel
d: linear velocity is given in m/s but inner diameter of fibers was not given

Table 2.3 (continued): A brief summary of ABE production in bioreactors coupled with membrane cell recycle systems

Reference	Unit	Schlote et al. (1986)		Ennis et al. (1989)	
Configuration		UF module		MF module	
Fermentation Stage		Single Stage		Single Stage	
Bleed$_1$	h^{-1}	N/A		0.018	
D $_{ov}$	h^{-1}	0.1	0.4	0.24	0.3
pH $_1$		4.4		5.4-5.6	
T_1	^oC	37		34	33
Operation period	h	N/A		80	215
ABE Concentration	gL^{-1}	22.02	N/A	9.3	4.6
A	gL^{-1}	6.75	N/A	N/A	
B	gL^{-1}	14.08	N/A	6	
E	gL^{-1}	1.19	N/A	N/A	
Productivity $_1$	$g\ L^{-1}h^{-1}$	1.41[b]	4.1[b]	1.02[b]	
Productivity$_2$	$g\ L^{-1}h^{-1}$	N/A	N/A	N/A	
Productivity$_{overall}$	$g\ L^{-1}h^{-1}$	N/A	N/A	N/A	1.32
$C_{initial}$	gL^{-1}	60-90		N/A	
$C_{residual\ sugar}$	gL^{-1}	N/A	N/A	N/A	
Organism		Clostridium aeetobutylicum DSM 1731		Clostridium aeetobutylicum P262	
Manufacturer		Sartorius GmbH, Göttingen, FRG		Norton Company (Worcester, Massachusetts, USA)	
Membrane Material		cellulose- triacetate		Ceramic / S. Alumina	
Avarege Pore size	µm	N/A		45	
Mw cut-off	Dalton	20000		N/A	
Crosflow velocity	Lh^{-1}	N/A		0.75[d]	
ΔP across membrane	kPa	N/A		14-17	
V_1	L	0.3-0.7		1.2	
Feed Type		Glucose		Lactose	
DCW	gL^{-1}	13.1	23.3		
Cell Density	gL^{-1}	N/A		12.5	19.5

a: data is provided from table
b: butanol productivity
c: culture vessel
d: linear velocity is given in m/s but inner diameter of fibers was not given

Table 2.3 (continued): A brief summary of ABE production in bioreactors coupled with membrane cell recycle systems

Reference	Unit	Pierrot (1986)		Tashiro et al. (2005)		
Configuration		Hollowfiber Filtration		Hollowfiber Filtration		
Fermentation Stage		Single Stage		Single Stage		
Bleed$_1$	h^{-1}	0.027	0.065	N/A	0.09	0.11-0.16
D $_{ov}$	h^{-1}	0.3	0.565	0.85	0.76	0.71-0.74
pH $_1$		4.8		6.5		
T_1	^oC	N/A		30		
Operation period	h	150	150	48	60	207
ABE Concentration	gL^{-1}	16.00	13	12.9	11.5	8.58
A	gL^{-1}	6.40	N/A	N/A		
B	gL^{-1}	8.53	N/A	N/A		
E	gL^{-1}	1.07	N/A	N/A		
Productivity $_1$	g $L^{-1}h^{-1}$	4.5	6.5	N/A		
Productivity$_2$	g $L^{-1}h^{-1}$	N/A	N/A	N/A		
Productivity$_{overall}$	g $L^{-1}h^{-1}$	4.5	6.5	11	9.77	7.55
$C_{initial}$	gL^{-1}	45				
$C_{residual\ sugar}$	gL^{-1}	≈0		less than 20		
Organism		Clostridium aeetobutylicum ATCC 824		Clostridium saccharoperbutylacetonicum N1-4 ATCC 13564		
Manufacturer		Amicon hollow-fiber module (HIPIO0 20)		MICROZA PSP-102, Asahi Kasei Co. Ltd., Tokyo, Japan		
Membrane Material		N/A		Organic Polymer		
Avarege Pore size	µm	N/A		25		
Mw cut-off	Dalton	100000		N/A		
Crosflow velocity	Lh^{-1}	0.405		N/A		
ΔP across membrane	kPa	41.4		N/A		
V_1	L	2		0.4		
Feed Type		Glucose		Glucose		
DCW	gL^{-1}	N/A		N/A	33.1	17.2-13.6
Cell Density	gL^{-1}	20		N/A	N/A	106

a data is provided from table
b butanol productivity
c culture vessel
d linear velocity is given in m/s but inner diameter of fibers was not given

Table 2.4 (continued): A brief summary of ABE production in bioreactors coupled with membrane cell recycle systems

Reference	Unit	Meyer et al. (1989)				
Configuration		UF module				
Fermentation Stage		Single S. /non glucose limited			Single S. / glucose limited	
Bleed$_1$	h^{-1}	N/A	N/A	N/A	N/A	N/A
D $_{ov}$	h^{-1}	0.43	0.3	0.2	0.23	0.2
pH $_1$		4.5	4.8		4.5	
T_1	^oC	35				
Operation period	h	not mentioned but there is a cleaning break for fouling every 6 h				
ABE Concentration	gL^{-1}	10.13	8.92	14.39	20.04	6.18
A	gL^{-1}	2.17	0.69	2.26	4.93	0.22
B	gL^{-1}	5.23	3.57	6.94	12.30	0.82
E	gL^{-1}	0.33	0.22	0.52	0.99	0.08
Productivity $_1$	$g\ L^{-1}h^{-1}$	N/A	N/A	N/A	N/A	N/A
Productivity$_2$	$g\ L^{-1}h^{-1}$	N/A	N/A	N/A	N/A	N/A
Productivity$_{overall}$	$g\ L^{-1}h^{-1}$	N/A	N/A	N/A	N/A	N/A
$C_{initial}$	gL^{-1}	41.4	73.26		59.4	13.5
$C_{residual\ sugar}$	gL^{-1}	10.098	53.82	38.7	41.22	0.0126
Organism		Clostridium acetobutylicum ATCC 824				
Manufacturer		CECI Column Eluate Concentrator Amicon, Danvers, MA, USA				
Membrane Material						
Avarege Pore size	µm	N/A				
Mw cut-off	Dalton	100000				
Crosflow velocity	Lh^{-1}	0.6-1.2				
ΔP across membrane	kPa	N/A				
V_1	L	0.7				
Feed Type		Glucose				
DCW	gL^{-1}	N/A				
Cell Density	gL^{-1}	N/A				

a data is provided from table
b butanol productivity
c culture vessel
d linear velocity is given in m/s but inner diameter of fibers was not given

Table 2.4 (continued): A brief summary of ABE production in bioreactors coupled with membrane cell recycle systems

Reference	Unit	Zheng et al. (2013)
Configuration		Hollowfiber Filtration
Fermentation Stage		Single Stage
Bleed$_1$	h^{-1}	N/A
D $_{ov}$	h^{-1}	0.78
pH $_1$		> 5.6
T_1	^oC	30
Operation period	h	> 110
ABE Concentration	gL^{-1}	5.89
A	gL^{-1}	N/A
B	gL^{-1}	4.26
E	gL^{-1}	N/A
Productivity $_1$	$g\ L^{-1}h^{-1}$	3.32[b]
Productivity$_2$	$g\ L^{-1}h^{-1}$	N/A
Productivity$_{overall}$	$g\ L^{-1}h^{-1}$	N/A
$C_{initial}$	gL^{-1}	50
$C_{residual\ sugar}$	gL^{-1}	28
Organism		Clostridium saccharoperbutylacetonicum N1-4 ATCC 13564
Manufacturer		MICROZA PMP-102; Asahi Kasei, Tokyo, Japan
Membrane Material		Organic Polymer
Avarege Pore size	µm	25
Mw cut-off	Dalton	N/A
Crosflow velocity	Lh^{-1}	N/A
ΔP across membrane	kPa	N/A
V_1	L	0.4
Feed Type		Xylose
DCW	gL^{-1}	17.4
Cell Density	gL^{-1}	N/A

a data is provided from table
b butanol productivity
c culture vessel
d linear velocity is given in m/s but inner diameter of fibers was not given

2.5. ONLINE PRODUCT RECOVERY

The lipophilic solvent butanol is more toxic than others as it disrupts the phospholipid components of the cell membrane causing an increase in membrane fluidity. Increased membrane fluidity causes destabilization of the membrane and disruption of membrane-associated functions such as various transport processes, glucose uptake, and membrane-bound ATPase activity (Bowles and Ellefson, 1985).

Gas stripping is a simple but efficient way to recover butanol from the fermentation broth (Figure 2.7a). The production of ABE is associated with generation of H_2O and CO_2. The fermentation gas is bubbled through the fermentation broth then passed through a condenser for solvent recovery. The stripped gas is then recycled back to the fermenter and the process continues until all the sugar in the fermenter is utilized. Gas stripping enables the use of a concentrated sugar solution in the fermenter (Qureshi and Blaschek, 2001d) and a reduction in butanol inhibition and high sugar utilization (Maddox et al., 1995).

Liquid–liquid extraction is another efficient technique to remove solvents from the fermentation broth. This approach takes advantage of the differences in the distribution coefficients of the chemicals. Because butanol is more soluble in the extractant (organic phase) than in the fermentation broth (aqueous phase), it is selectively concentrated in the extractant, in this way necessary nutrients and substrates stays in the fermentation broth. Common extractants employed include decanol and oleyl alcohol, which are considered as relatively non-toxic (Evans and Wang, 1988). However, liquid–liquid extraction (Figure 2.7b) has critical problems, such as the toxicity of the extractant to the cell and emulsion formation. These problems can be overcome if the fermentation broth and the extractant are separated by a membrane contactor for butanol exchange between the two immiscible phases; this is termed ‘‘Perstraction’’ (Figure 2.7c) (Ezeji et al., 2007). The membrane contactor provides the surface area where two immiscible phases can exchange butanol. As there is no direct contact between the two phases, extractant toxicity, phase dispersion, emulsion and rag layer formation are drastically reduced or eliminated. In such a system, butanol would diffuse preferentially across the membrane, while other components and fermentation intermediates are retained in the aqueous phase. The total mass transport of butanol from the fermentation broth to the organic side depends on the rate of diffusion of butanol across the membrane. The membrane does, however, present a physical barrier that can limit the rate of butanol extraction. Liquid–liquid extraction has high capacity and selectivity, although it can be expensive to perform.

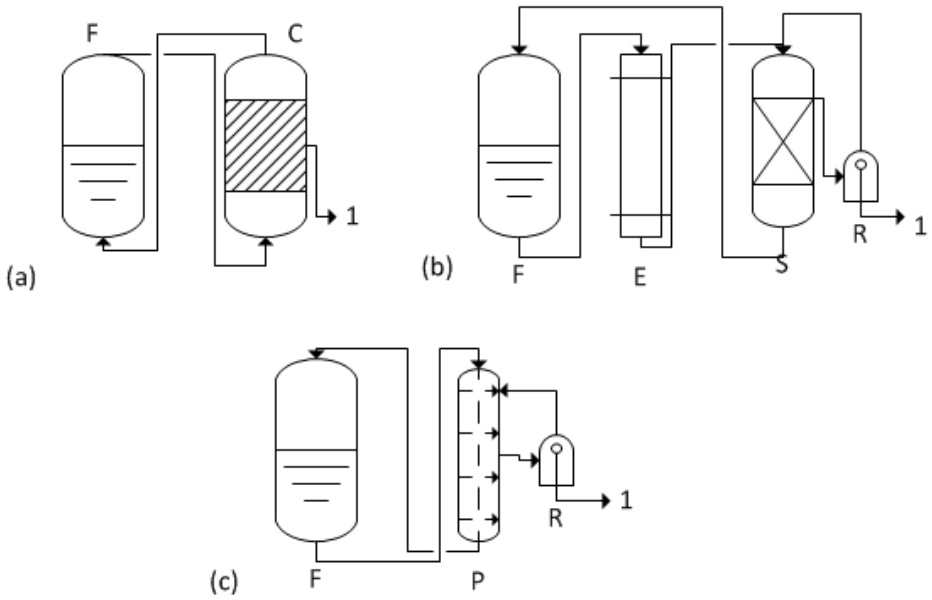


Figure 2.7: Removal Techniques (a) Gas Stripping (b) Liquid-Liquid Extraction (c) Perstraction F: Fermenter, C:Condenser, E:Extractor, S:Seperator, R:Extractant Recovery, P: Membrane Contactor Unit for Perstraction, 1: Product line (composition: water and ABE)

Qureshi et al. (1992); studied different product removal techniques that had been integrated into the identical fermentation process. The techniques that were investigate; liquid-liquid extraction, perstraction, gas-stripping, and pervaporation. Whey permeate was used as a substrate and *Clostridium acetobutylicum* P262 was used for all fermentations. The performance results are represented on Table 2.4.

Table 2.4: Total ABE and Acids Produced and ABE Productivity during Integrated Fermentation/Product Removal Experiments

	Total ABE g	Total Acid g	Productivity $g.L^{-1}\ h^{-1}$	Total Lactose used g	Yield $g.g^{-1}$
Control Batch	7a	0,6b	0.07	45,1c	0.32
Liq-Liq Extraction	28	1.51	0.15	68.6	0.35
Perstraction	57.8	2.1	0.24	157.5	0.37
Gas Stripping	69.1	0.7	0.26	182.5	0.38
Pervaporation	42	9.9	0.14	123.4	0.34

a: Final ABE concentration $g{\cdot}L^{-1}$

b: Final total acid concentration $g{\cdot}L^{-1}$

c: Lactose Utilization %

Results indicated that direct contact during liquid-liquid extraction between the culture and the oleyl alcohol has led to saturation of the aqueous phase with the extractant, leading to cell toxicity. Another possible disadvantage of extraction with oleyl alcohol is that it removes acetic and butyric acids from the culture, thus depleting some reaction intermediates. Other problems include loss of cells at the interface, formation of emulsions, and low concentrations of ABE in the extractant.

These problems mostly overcame with perstraction by placing membrane between the two phases, thereby minimizing passage of extractant into the culture. Any membrane used should have a high selectivity for the reaction products but not for nutrients or reaction intermediates. The membrane used in the present work allowed diffusion of butanol into the extractant, but diffusion of acetone was poor. Diffusion of acetic and butyric acids was also poor, but their retention contributed to a high product yield (0.37, Table 2.4) since these acids are reaction intermediates. Overall, the use of perstraction gave superior results to liquid-liquid extraction, suggesting that cell toxicity is a major problem for liquid-liquid extraction. Also controlled dispersion of liquids in the carrier phase avoided the formation of emulsions. However oleyl alcohol diffusion across membrane during process can be stated as a potential problem.

One advantage of gas-stripping over the other techniques studied is that it provides a more concentrated ABE solution to be presented to the distillation column for further purification. Also, recycling of the gases produced during the fermentation may be beneficial to solvent production by retaining a source of reducing power (hydrogen) within the culture. However, there were foaming problems, which necessitated control via antifoam addition.

Pervaporation is a technique by which volatile chemicals pass across a membrane and are then removed using a sweep gas or a vacuum, followed by their recovery by condensation. In the mentioned study above, nitrogen was used as the sweep gas. A possible advantage of pervaporation is that the technique can be used as a replacement for distillation in the purification of ABE. Although acetic and butyric acids do diffuse across the membrane, they do so only at high concentrations, which are not normally reached in the ABE fermentation process. Pervaporation has the greatest potential to remove butanol from fermentation broth and has received more attention when compared to other techniques it can accomplish separation and concentration in a single step without alcohol recovery from extractants.

The comparative table pervaporation energy consumption with other techniques like steam distillation, gas stripping, extraction and adsorption represented in Table 2.5.

Table 2.5: Comparison of selectivity and energy requirement for butanol recovery by different separation methods.

		Energy Requirements (MJ/kg)[b]			
		Butanol	Butanol	ABE	ABE
Separation Method	Selectivity [a]	1	2	3	4
Steam Distillation	72	>50	24		91[c]
Gas Striping	4-22	14-31	22	21	
Pervaporation	2-209	2-145	14	9	
Extraction/ perstraction	1.2-4100	7.7	9	14	26[c]
Adsorption	130-630	1.3-33	8	33	

a: Selectivity (against water): butanol concentration in the recovered stream/butanol concentration in the feed stream. Data from: Oudshoorn et al. (2009)
b:Data for energy requirements are from (1) Oudshoorn et al. (2009), (2) Qureshi et al.(2005), (3) Groot et al, (1992), (4) Roffler et al. 1987.
c:Based on total steam usages estimated for an ABE plant with molasses as the feedstock using conventional-batch fermentation with steam stripping for butanol recovery or extractive fed batch fermentation with continuous butanol by high-boiling solvent in a counter current Karr column. The steam or energy consumption in the extractive fermentation process is only 0.28 percent of that required for the batch process, largely because of reduced water usage and increased reactor productivity.

It should be emphasized that the recovery and purification processes are directly affected by the performance of fermentation, which in turn is affected by the strain characteristics. For example, when a strain is metabolically engineered to produce butanol without or much less acetone and ethanol, the purification process will be considerably simplified. When the butanol tolerance of a strain is increased by metabolic engineering, this will also facilitate the recovery process as higher butanol concentration can be achieved during the fermentation. Thus, the overall process needs to be optimized from strain development to fermentation to downstream processes. This will lead to the reduction in overall production costs. For example, an engineered strain capable of producing butanol to a high concentration with high productivity will result in significant reduction in the direct fixed capital costs (e.g., costs of fermenter and recovery units) and associated depreciation costs because smaller fermenter can be used to produce a desired amount of butanol.

2.5.1. Organophilic pervaporation

Pervaporation is a process in which a liquid stream containing two or more miscible components is placed in contact with one side of a non-porous polymeric membrane or molecularly porous inorganic membrane (such as a zeolite membrane) while a vacuum or gas purge is applied to the other side (Vane, 2005). Industrial applications of pervaporation began in the 1970s. This was made possible by the development of highly selective polyvinyl alcohol composite membranes. The process is clearly discussed by Sander and Soukup. They provide engineering performance curves for removing water from an ethanol/water mixture. The curves showed how the temperature and ethanol concentration of the feed affect the flux of water through the membrane and the composition of the

permeate. Sander and Soukup make the statement that “The product concentration is simply controlled by adjusting the alcohol feed rate”. In most chemical plants, throughput is set by product demand and therefore cannot be used as a manipulated variable in a process control structure. (Luyben, 2009)

Pervaporation research has continued in various parts of the world without any significant breakthrough in commercialization. In 1982 G.F.T., a German company commercialized a pervaporation plant for alcohol dehydration. This plant could produce 1300 L of ethanol per day of 99.2% purity from pre-distilled ethanol. In 1987 G.F.T. was taken over by a French company, Carbone Lorraine. In 1994 Carbone Lorraine sold its pervaporation technology to Sulzer Chemtech. In Japan during the same time Mitsui, Sasakura Engineering, and Asahi Chemicals put a lot of effort into research and development to commercialize the pervaporation technology. In 1999, based on 10 years of research by membrane scientists of Petro Sep Membrane Technologies Inc. with the Industrial Membrane Research Institute (IMRI) of the University of Ottawa, Petro Sep Membrane Technologies Inc. of Oakville, Canada, introduced a new type of pervaporation membranes. These membranes are very robust and chemically resistant and are available in hollow-fiber as well as flat-sheet configuration. The design considered as user friendly and very economical. Today, more than 40 industrial pervaporation plants built by Sulzer Chemtech Membran- technik AG (former GFT) are in operation worldwide. They are used for the dehydration of different solvents and/or solvent mixtures. (Kujawski, 2000; Baig, 2008)

There are three kinds of pervaporation membranes: (a) hydrophilic membranes, (b) hydrophobic membranes, and (c) organophilic membranes. Hydrophilic membranes can be used to dehydrate organic solvents or organic mixtures. Hydrophobic membranes can be used to extract organic solvents or volatile organic compounds (VOCs) from water. (Jonquières et al., 2002) Organophilic pervaporation, hydrophobic solutes with a limited solubility in aqueous media sorb very favourably to the dense, hydrophobic non- porous membrane, diffuse across it if an adequate driving force is applied and desorb in the downstream side under vacuum (Brazinha et al., 2011). A schematic diagram of pervaporation is shown in Figure 2.8.

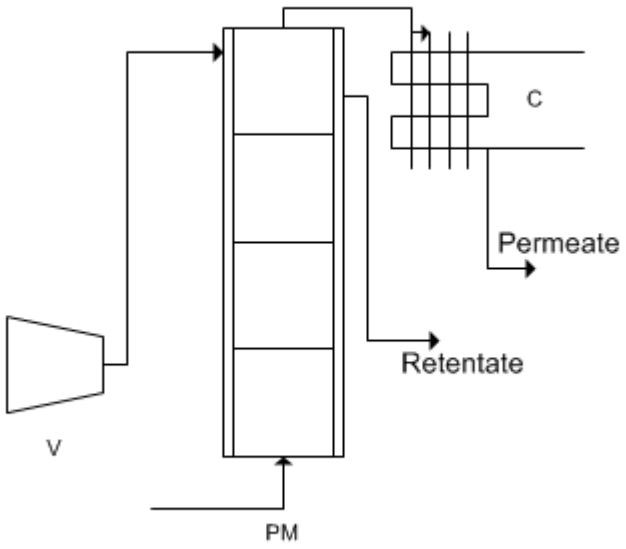


Figure 2.8: Pervaporation Unit
V: Vacuum Pump, PM: Pervaporation Unit, C: Condenser

In this process (this case ABE fermentation broth) liquid feed mixture circulates in contact with the active nonporous side of the membrane while a vacuum is applied on the other side of the membrane. A phase change of membrane-selective permeate takes place on the membrane. The membrane-selective permeate diffuses through the membrane and desorbs on the posterior side of the membrane. Later, it evaporates with the help of a vacuum from the posterior side of the active nonporous membrane. These organics later condense in a condenser.

When a vacuum is applied to the permeate side of the membrane, a driving force and activity gradient can be created across the membrane thickness. The resistance to transport across the membrane includes diffusion in the stagnant feed liquid to the membrane, diffusion through the membrane, and diffusion in the permeate vapor. Selective permeation takes place, and then feed flow rate as well as feed composition change through the differential volume. Since the heat of evaporation is supplied from the feed side, the feed temperature falls constantly, and so the flux through the membrane decreases. Therefore, three different balances over the differential volume are taken into account as follows:

Mass Balance:

$$\frac{d}{dz}F = -J(2w_{unit}) \qquad [19]$$

Mass balance in terms of concentration:

$$\frac{d}{dz}Fx_i = -Jy_i(2w_{unit}) \quad [20]$$

Heat Balance:

$$\frac{d}{dz}Fh_f = -J\Delta h_v(2w_{unit}) \quad [21]$$

Where F denotes feed flow rate ($L \cdot h^{-1}$), J is total flux ($L \cdot h^{-1} \cdot m^{-2}$) and w_{unit} is the width of unit (m), x_i is the molar concentration of a selectively permeating component in the feed, y_i is the molar concentration of a selectively permeating component in permeate, h_f is the enthalpy of feed flow ($J \cdot mol^{-1}$), and Δh_v is the heat of the evaporation of permeate ($J \cdot mol^{-1}$). Equations can be rewritten as follows, respectively:

$$dF = -2\,J\,w_{unit}dz \quad [22]$$

$$x_i dF + F dx_i = -2\,J\,y_i\,w_{unit}dz \quad [23]$$

$$FC_p dT = -2\,J\,\Delta h_v\,w_{unit}dz \quad [24]$$

Where C_P is the heat capacity of the feed liquid. When we rearrange the equations 19 and 20 concentration, the following equation can be obtained:

$$dx_i = \frac{2\,J\,w_{unit}}{F}\,(x_i - y_i)\,dz \quad [25]$$

It has been concluded that the primary factors affecting the separation by pervaporation are membrane materials and feed species, whereas feed temperature, composition and permeate pressure are only secondary factors (Vane, 2005). In the case of butanol separation from water by pervaporation, a hydrophobic membrane is needed in order to get butanol-rich condensate on the permeate side. Figure 2.9 represents the flows through a differential element of volume (*dz*) and temperature gradient along feed flow.

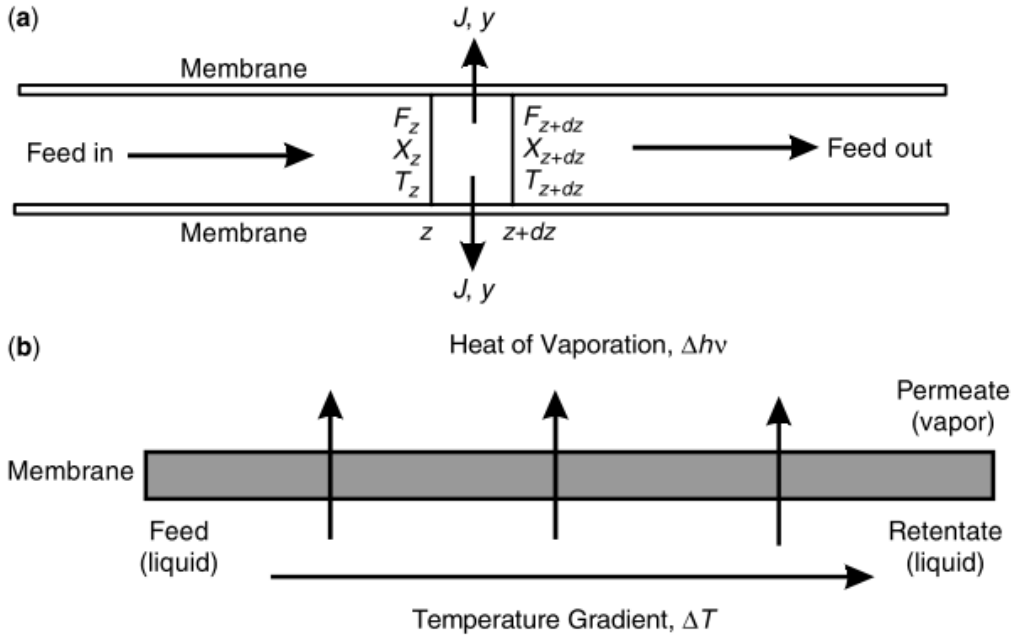


Figure 2.9: (a) Feed channel and flows through a differential element of volume (dz) and (b) temperature gradient along feed flow.

Due to the selective nature of the membrane and diffusion rates of different components, the concentration ratio of one component in permeate to feed can range from single digit to over a 1000 (Vane, 2004). Temperature dependence is of the Arrhenius type, usually with doubling of flux at temperature increase of 10-12 K^0 (Kujawski, 2000). According to equation (26), the maximum driving force, relatedly maximum flow, is achieved at the maximum feed concentration, the minimum permeate partial pressure of a given species, and the maximum feed liquid temperature. Obviously, there are limits to each of these. For example, the feed concentration (Equation 27) is dictated by the upstream process and is generally not independently controlled. The changes in feed flow rate, feed composition, and feed temperature along the z direction can be calculated if the flux and permeate composition are expressed as a function of both feed composition and feed temperature:

$$J = f(x_i, T) \qquad [26]$$

$$y = g(x_i, T) \qquad [27]$$

The flux can also be expressed as;

$$\mathrm{J} = \frac{W}{A\,t} \qquad [28]$$

where, W is given as weight of permeate (g), A is Membrane area (m^2) and t is Time (h).

Diffusion through the membrane surface (y axis) is represented on Figure 2.10. Flux can be defined in terms of chemical activity difference of a species between the bulk feed liquid and the bulk permeate vapor. Flux through the membrane is inversely proportional to the overall resistance (κ_M) and proportional to the concentration gradient (as a representation of the activity difference)

between the bulk liquid and the bulk permeate vapor. We can derive the equations starting from the Fick's law.

$$\dot{J} = -\bar{D}_{\iota}\frac{\partial\, \dot{x}_i}{\partial\, y} \qquad [29]$$

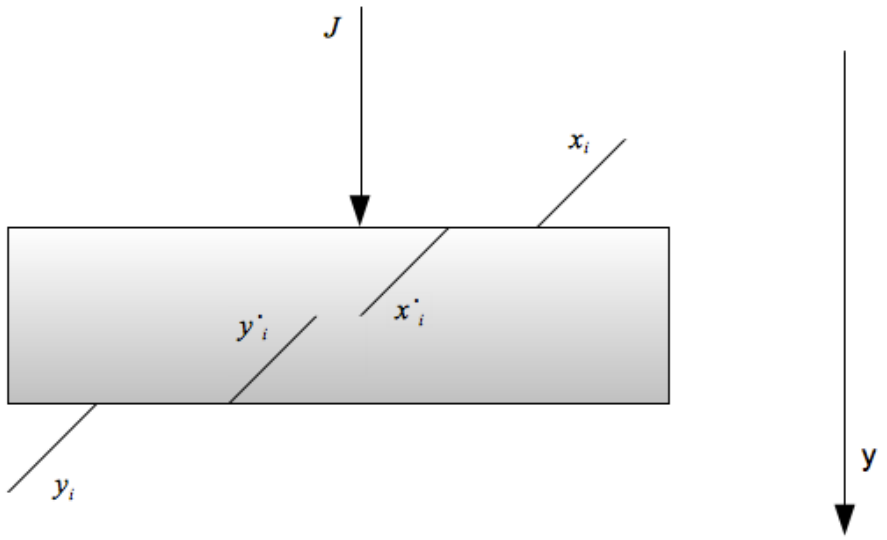


Figure 2.10: Diffusion through the membrane surface (y axis).

Previously we defined x_i molar concentration of component in bulk phase, but the concentration on the membrane surface is function of solubility coefficient - $\acute{S}$. The concentration of the selective specie can be expressed as;

$$\dot{x}_{\iota} = x_i \acute{S} \qquad [30]$$

Now, we can substitute bulk concentration with surface concentration:

$$\dot{J} = -\bar{D}_{\iota}\, \acute{S}\, \frac{\partial\, x_i}{\partial\, y} \qquad [31]$$

If we integrate the equation through the membrane thickness -δ_m:

$$\dot{J} = -\bar{D}_{\iota}\, \acute{S}\, \frac{x_i - y_i}{\delta_m} \qquad [32]$$

The concentrations in bulk phase and permeate can be expressed alternatively in terms of molar fraction of species in bulk phase ϖ_i, bulk partial pressure of component i -$P_i^V\ (bar)$, $\dot{\rho}_p$ the total molar density of the feed liquid $(\mathrm{mol}\cdot L^{-1})$, γ_i- the activity coefficient of i in the feed liquid, and P_i^{sat} is the saturated vapor pressure of component i (bar).

$$x_i = x_T \varpi_i \,;\; y_i = \dot{\rho}_p \frac{P_i^V}{\gamma_i P_i^{sat}} \qquad [33]$$

We can use an equation relating permeability- $P_M\,(mol \cdot m^{-1} \cdot s^{-1} \cdot bar^{-1})$ of membrane to the properties of the diffusing molecule to see how well permeability correlates with the dissolvability of molecules in membrane material.

$$P_M = \frac{\bar{D}\delta_M \dot{\rho}_p}{\gamma_i P_i^{sat}} \qquad [34]$$

The selectivity of membrane for selected component is calculated as:

$$\alpha = \frac{y_i/(1-y_i)}{x_i/(1-x_i)} \qquad [35]$$

According to this equation, a membrane with a separation factor of 1 yields with the permeate, that is identical composition as feed. A membrane with a separation factor that approaches to infinity would permeate only singe component and would be the ideal membrane. The truly ideal pervaporation membrane would exhibit both a high flux and a high separation factor. In reality, even this 'ideal' membrane is not appropriate for all scenarios (for example, if the permeate partial pressure of the preferential permeating species limits transport). Thus, the properties of the membrane must be matched to the desired separation performance and the physical limitations of the process. (Vane, 2005)

Molecules which end up in the permeate experience a phase change from liquid to vapor which requires energy to fuel the evaporation. Therefore, one result of pervaporation is a cooling of the feed liquid as it traverses the membrane module. Fortunately, only the fraction of material, which permeates the membrane, is evaporated. In the case of an infinite separation factor, heating and cooling would only be used to evaporate and condense the desired permeate product species. Since selectivity is never infinite, heat must be applied to evaporate not only the desired permeate species, but the undesired permeating species as well.

Another factor for membrane characterization defined as enrichment factor (β):

$$\beta = \frac{y_i}{x_i} \qquad [36]$$

Unfortunately, neither the separation factor nor the enrichment factors are constant. Both parameters are the strong function of the feed composition. Condition base comparisons must be done between multiple data.

If the temperature change of the feed liquid is excessive due to evaporation, then inter-stage heating may be required to maintain the desired liquid temperature. If batch or semi-batch operation is feasible, then an alternative to inter-stage heating is to increase the recirculation flow rate through the membrane modules. The effect of increased flow rate is a decrease in the single-pass temperature

drop so that the per-stage temperature drop is decreased. The higher flow rate will also result in a lower per pass alcohol removal.

In laboratory scale experiments, a vacuum pump is usually used to draw a vacuum on the permeate side of the system. Industrially, the permeate vacuum is most economically generated by cooling the permeate vapor, causing it to condense; condensation spontaneously creates a partial vacuum. The permeate partial pressure is theoretically limited to absolute ‘‘0’’ pressure, but practically limited by the capital and operating costs associated with producing a given vacuum level.

Membrane materials for butanol recovery by pervaporation:

Many membrane materials have been studied for the purpose of in-situ recovery of butanol from fermentation broth. It is difficult to compare different authors' work on butanol separation because of the number of variables involved.

The current benchmark hydrophobic pervaporation membrane material is poly-dimethyl siloxane [PDMS], often referred to as ‘silicone rubber’. The rapid chain segment motion in the silicone and other rubbery polymers leads to a large free volume that favours the diffusion of the permeating molecules. PDMS is an elastomeric material, which can be used to fabricate hollow fiber, tubular, unsupported sheet, or thin layer supported sheet membranes. While homogeneous membranes may be adequate in basic permeability studies, composite membranes comprising of a thin active skin layer and a microporous substrate are desired for practical applications in order to enhance the mass transfer rate without compromising their mechanical stabilities. The substrate should be highly porous to minimize its resistance to mass transfer of the permeating components. Otherwise, the overall permselectivity of the composite membrane will be lowered. On the other hand, the pores on the substrate membrane should be small enough so as to prevent intrusion and filling of the pores with the top layer material during surface coating, which is commonly used in membrane formation. Several companies have manufactured thin PDMS supported membranes over the years. At present, Membrane Technology and Research Inc. (MTR) of Menlo Park, CA is the leading supplier, manufacturing spiral wound modules out of their supported silicone rubber membranes.(Qureshi and Blaschek, 1999; Fouad and Feng, 2009)

Liu et al. (2011); investigated the PDMS/ceramic composite membrane behaviours directly coupled with ABE fermentation. Fermentation medium or broth, were maintained at 37 ^{o}C by the water-bath. The flow rate was fixed at 15 L·h^{-1} during the pervaporation experiment. The permeate vapor was collected in liquid nitrogen trap. Permeate pressure was below 0.004 bar during collections. Although a fluctuation of membrane performance was observed due to the occurrence of membrane fouling in the fermentation–PV coupled process, the PDMS/ceramic composite membrane exhibited a high flux of 0.670 kg· m^{-2} ·h^{-1} and applicable ABE separation factor of 16.7 (See Table 2.7).

The silicalite–silicone composite membrane (306μm thick and 0.022m^2 total area) was tried for removal acetone and butanol from all the fermentation broths. It was found that the silicalite–silicone composite membrane was not fouled by the fermentation broth after 120 h of operation. Selectivities of

butanol and acetone were unaffected. Reported selectivities are 38 and 97 for acetone and butanol relatively. Acetic acid and ethanol did not diffuse through the membrane at feed concentrations <0.4 $g^2 \cdot L^{-1}$. (Qureshi and Blaschek, 2001)

Table 2.6:Performance of ABE fermentations used for ABE separation

Membrane Type	T C	Total Flux g m^{-2} h^{-1}	Separation Factor A	B	E	Notes	Ref.
PDMS/ceramic	37	951	21.1	16.2	6.8	Cell free broth $A_{effective}$ =48.9 cm^2	1
PDMS/ceramic	37	670	20.6	15.1	6.7	ABE broth $A_{effective}$ =48.9 cm^2	1
Silicalite–Silicone	78	88.91	38.14	97.3	N/A	UF applied to system to work on higher temperatures	2
Supported ionic liquid membranes	25	560	N/A	23.2	N/A	Model solution less than 5%(w/w) butanol concentration.	3

1 (Liu et al., 2011)
2 (Qureshi and Blaschek, 2001)
3 (Heitmann et al., 2012)

Supported ionic liquid membranes (SILMs) for continuous ABE removal has been tried for removal of solvents from fermentation broth. Future promising applications of SILMs in technical separation processes can only be possible if SILMs are able to compete with conventional membranes in terms of stability, flux and separation efficiency the pervaporation performance of SILMs with tetracyanoborate and tris(pentafluoro-ethyl)trifluorophosphate ionic liquids (ILs) had been investigating. Pervaporation was carried out at 37 ℃ using binary mixtures of n-butanol and water with n-butanol concentrations lower than 5% (w/w). Two concepts for immobilisation of ILs were tested using nylon or polypropylene as support material. ILs were immobilised by inclusion between silicone layers or by dissolution in poly(ether block amide). It was observed that a higher affinity of the IL for n-butanol increases the permeability of the membrane for more than three times, whereas no changes in the selectivity occurred. Furthermore it was shown, that fluxes increased with an increasing IL content in the membrane. The maximum permeate flux achieved was 560 $g \cdot m^2 \cdot h^{-1}$, and the highest concentrations of n-butanol in the permeate was found to be 55% (w/w). In future thickness of SILMs needs to be reduced to make these membranes competitive with respect to conventional pervaporation membrane (Heitmann et al., 2012)

Effect of fermentation broth components on pervaporation membranes and modules:

Vane 2005; summarized the fermentation broth effect on pervaporation modules. Viable and dead whole cells and suspended solids have the potential to accumulate in modules. This can lead to blockage on the flow path and membrane surface. Cell components (such as proteins, cell wall, etc) showed lipid adsorption especially for PTMSP membranes if materials precipitate when heated. Glucose above 100 $g \cdot L^{-1}$, Xylose above 50 $g \cdot L^{-1}$ and lactose showed decreasing influence on water and alcohol flux however alcohol-water selectivity increased. Organic acids such as acetic acid, butyric acid shows no major impact on pervaporation performances. Fermentation broth components may also alter the thermodynamic behaviour of alcohols. For example, simply adding more salt to the broth will increase the activity coefficient of the alcohol such that the alcohols prefer to partition out of the

water phase and into the membrane. While the concentrations of main fermentation products ethanol, acetone and n-butanol increase in the main broth, especially for PDMS membranes, permeabilites tend to decreased over water. pH as no direct effect with the range 3 to 6, but has secondary effect through the fraction of organic acids which are protonated in aqueous phase.

Energy considerations

The energy required to evaporate permeate in a pervaporation process, normalized per unit of butanol permeated, $-\dot{Q}_{butanol}$ $(J \cdot mol^{-1})$ is calculated as follows:

$$\dot{Q}_{butanol} = \frac{\sum_i \Delta h_{vi} J_i}{J_{butanol}} \qquad [37]$$

Since butanol, acetone and water dominate the feed and the permeate, equation (37) can be rewritten in terms of the butanol–water and acetone-water separation factor ($\alpha_{b/w}, \alpha_{a/w}$) as:

$$\dot{Q}_{evaporation} = \Delta h_{vwater} + \Delta h_{vbutanol} \left(\frac{\dot{x_b}}{\alpha_{b/w}\, \dot{x_w}} \right) + \Delta h_{vacetone} \left(\frac{\dot{x_a}}{\alpha_{a/w}\, \dot{x_w}} \right) \qquad [38]$$

The heat which must be removed in order to condense the permeate vapor is approximately the same as the heat required for the evaporation step ($\dot{Q}_{evaporation} = -\dot{Q}_{condensation}$). For ideal process design the heat that released during condensation should be used for evaporation. Due to heat transfer resistances and the difference between the temperatures of the feed liquid and the permeate condensate necessary to maintain a permeate pressure driving force, the heat released during condensation cannot be directly used to heat the feed liquid. When pervaporation is operated at an elevated feed temperature, the temperature of the permeate condenser may also be elevated relative to ambient temperatures. Thus, the heat released during condensation can be removed with a simple forced air heat exchanger, requiring little energy input. Under these circumstances, the heat of evaporation is the dominant energy sink in the pervaporation process

We can conclude that:

- Pervaporation is an emerging technology with significant potential to efficiently recover alcohols and other biofuels from fermentation broths.
- Pervaporation to be economically viable (Vane, 2004):
    1. Increased energy efficiency - improved ethanol-water separation factor and heat integration.
    2. Reduction of capital cost for pervaporation systems - reduction in the membrane/module cost per unit area and increase in membrane flux to reduce required area.
    3. Longer term trials with actual fermentation broths to assess membrane and module stability and fouling behaviour.
    4. Optimized integration of pervaporation with fermenter - filtration to increase cell density in fermenter and allow higher pervaporation temperatures, removal/avoidance

of inhibitors

5. Synergy of performing both alcohol recovery and dehydration by pervaporation

3. ECONOMIC EVALUATION OF BIOBUTANOL PRODUCTION

3.1. OVERALL VIEW

Economic evaluation of ABE fermentation is highly important to go to large scales from pilot plants. Although pilot scale fermentations ends up with promising results, in reality more complicated supply chain analyses must be done to prove the superiority of biobutanol over its competitive fuels. Nowadays bioethanol is the market leader of biofuels due to stabile history of fermentation. However this picture is might change in favor of butanol due to listed advantages of biobutanol in Section 1. The principal questions of interest about economic evaluation are: (a) Is the energy balance for the process positive? (b) What impacts can various agricultural products have on satisfying our energy demand?

The full Life cycle assessment (LCA) of ABE production is done by Wu et al. (2007). The study employs a well-to-wheels analysis tool — the Greenhouse Gases, Regulated Emissions and Energy Use in Transportation (GREET) model developed at Argonne National Laboratory — and the Aspen Plus® model developed by AspenTech.

The GREET model separately calculates:

• Consumption of total energy (energy in non-renewable and renewable sources), fossil fuels (petroleum, natural gas, and coal combined), petroleum, natural gas, and coal;

• Emissions of carbon-dioxide (CO_2) -equivalent GHGs — primarily CO_2, methane (CH_4), and nitrous oxide (N_2O); and emissions of six criteria pollutants: Volatile organic components (VOCs), carbon monoxide (CO), nitrogen oxides (NO_X), particulate matter measuring 10 micrometers or less (PM_{10}), particulate matter measuring 2.5 micrometers or less ($PM_{2.5}$), and sulfur oxides (SO_X).

The system boundaries for GREET are listed as;

1. Corn farming,

2. Corn transportation,

3. Bio-butanol production,

4. Bio-butanol transportation and distribution, and

5. Bio-butanol use in gasoline vehicles.

The bio-butanol life cycle begins with the manufacture of fertilizer and farming machinery. Corn farming operations include irrigation, tillage, application of fertilizer, lime, herbicides, and pesticides, and corn harvest. Harvested corn grain is transported via barge, rail, and truck to fuel production facilities, where it undergoes biochemical (BC) processing for fuel production. The demand for heat and power (steam and electricity) from the BC processing is met by grid electricity and natural

gas (NG). Liquid fuel is then transported to refueling stations via rail, barge, and truck. It is assumed that bio-butanol would be used in unblended form in Gas Vehicles.

The study analysed a facility that produces 150,000 metric tons of bio-butanol per year, requiring 33 million bushels of corn. The operation runs 315 days per year. Product and byproduct specifications were as follows: butanol purity, 99.5% (w/w); acetone purity, 99.5% (w/w); and ethanol purity, 99.5 % (w/w). They established different cases:

1. Bio-butanol with natural gas as the process fuel, where bio-acetone is regarded as a chemical to displace petroleum-based acetone and is therefore credited by product displacement. Distiller's dried grains with solubles (DDGS) and ethanol displace animal feed and gasoline, respectively.

2. Bio-butanol with natural gas as the process fuel, where acetone and DDGS are regarded as energy products and are thus credited on the basis of the energy allocation method among butanol, acetone, ethanol, and DDGS.

3. Corn ethanol from dry milling with natural gas as the process fuel, where DDGS is credited by product displacement to displace animal feed (GREET default).

The product energy allocation method to co-products, in which emission and energy burdens are allocated among products according to their energy output shares from the bio-butanol plant. In the energy allocation method, shares of output product energy for each product are determined according to the heating value. Energy use and associated emissions from bio-butanol production and from upstream feedstock production and transportation activities are partitioned among acetone, butanol, ethanol, and DDGS on the basis of their corresponding energy shares. This approach treats all energy products from the production process as equal, regardless of the form and quality differences among them. It also implies that all four products are energy products. The energy allocation method is applicable to this case because, of the ABE products, both butanol and ethanol are liquid fuels. Although acetone is normally regarded as a chemical solvent and feedstock, its energy content (in LHV) is, in fact, in between that of butanol and ethanol (Table 3.1). Furthermore, DDGS has a LHV of 20.24 $KJ \cdot kg^{-1}$ and can be used as a solid fuel for plant operations. Although the fuel quality of DDGS is lower than that of the liquid fuels, considering its large quantity, use of DDGS for process heat brings energy savings and yet relaxes pressure on the already stagnant DDGS market.

Table 3.1: Properties of ABE Products

	LHV	Density
Product	($KJ.L^{-1}$)	($g.L^{-1}$)
Acetone	23.2	0.784
Butanol	27.6	0.811
Ethanol	21.2	0.79
Gasoline	32.4	0.746

Description of process

Acetone, butanol, and ethanol (ABE) are produced by *C. beijerinckii* BA101. Corn is fed into a conventional corn dry mill for conversion to glucose through liquefaction and saccharification. The glucose is fermented to ABE through a fed-batch system. During fermentation, the ABE compounds are removed by means of *in situ* gas stripping. ABE products are recovered through molecular sieve adsorption and a three-stage distillation that separates the acetone, butanol, and ethanol. Solids and biomass that are removed from grain processing and fermentation undergo centrifugation and proceed to drying, along with syrup from distillation; DDGS generated from drying is used as animal feed.

Corn feed rate to fermenter is set to 1.7 $kg \cdot min^{-1}$. Glucose utilization is 95.1% (i.e., 4.9% glucose is converted into biomass solids as a result of microbial growth). Butanol, acetone, ethanol, acetic acid, and butyric acid yields are 0.303, 0.155, 0.0068, 0.0086, 0.0084 $g \cdot g^{-1}$ glucose, respectively.

During bio-butanol production, several co-products are generated along with butanol — these include acetone, DDGS, a small amount of ethanol, fatty acids (butyric acid and acetic acid), and H_2 gas. On the scale that was analysed, acetone is the major co-product of the biobutanol plant, with 82,000 metric tons produced per year. Acetone contributes to more than one- half (by weight) of the butanol production. The bio-butanol plant also generates 253,600 metric tons of DDGS (11% moisture) per year — the second largest co-product. The yield of ethanol is relatively small. Ethanol yield from ABE fermentation accounts for 1.5% of total ABE (acetone, butanol, and ethanol) by weight and only 1.2% by energy content. The fatty acids were not separated and purified, and so they were not treated as co-products. Significant amounts of H_2 and CO_2 gas were produced from fermentation; these gases were used internally for gas stripping and then for maintaining anaerobic conditions for ABE fermentation. The H_2 could be separated as a fuel product if high-purity nitrogen gas is used in place of fermentation gas. However, using such nitrogen gas to maintain anaerobic conditions and for stripping in large-scale operation could be cost prohibitive. Further studies to evaluate the engineering economics of alternative approaches for process operation while economically producing bio-butanol and hydrogen would be beneficial. In this initial attempt to address the life cycle of bio-butanol, H_2 was not considered as a co-product.

Net energy balance

Corn-derived butanol achieves substantial fossil energy savings (39–56%) compared with gasoline for case 1 and 2. In Cases 1 and 2, bio-butanol production contributes to a positive energy balance (defined as KJ of a unit of biofuel minus KJ of fossil fuel used to produce the amount of biofuel).

A life-cycle fossil energy breakdown (Figure 3.1.) indicates that about three-fourths of the fossil fuel is spent in the butanol production plant (73%). Corn cultivation accounts for a total of 23% of WTW fossil energy (12% for agricultural chemical and fertilizer manufacturing and 11% for farming operations). Vehicles fuelled by bio-butanol achieve small to moderate reductions in GHG emissions

relative to gasoline vehicle on a WTW basis in Cases 1 (8%, displacement) and 2 (32%, energy allocation). For every million kJ of bio-butanol used in place of one million KJ of gasoline, 7.5–30 kg of GHG emissions could be avoided (Cases 1 and 2 relatively).

Because acetone is not used as a fuel at present, net fuel production (butanol and ethanol) is small. A typical dry mill can deliver 0.36 $L \cdot L^{-1}$ of fuel ethanol, or 0.33 $L \cdot L^{-1}$ of undenatured ethanol, which is ~5 L more than the butanol and ethanol yield together from the ABE process (0.2 $L \cdot L^{-1}$). On the other hand, butanol contains 30% more energy (LHV) than ethanol (Table 3.1) by volume. When we take fuel energy yield into consideration, each bushel of corn could generate nearly 160,000 KJ of liquid fuels from the ABE process (butanol and ethanol together), while the same bushel delivers 209,000 KJ of liquid fuels (undenatured ethanol) from the conventional ethanol dry mill. Hence, from a liquid fuel production perspective, the ABE process option does not offer an increase in renewable fuel production, in comparison with conventional corn ethanol production (case 3).

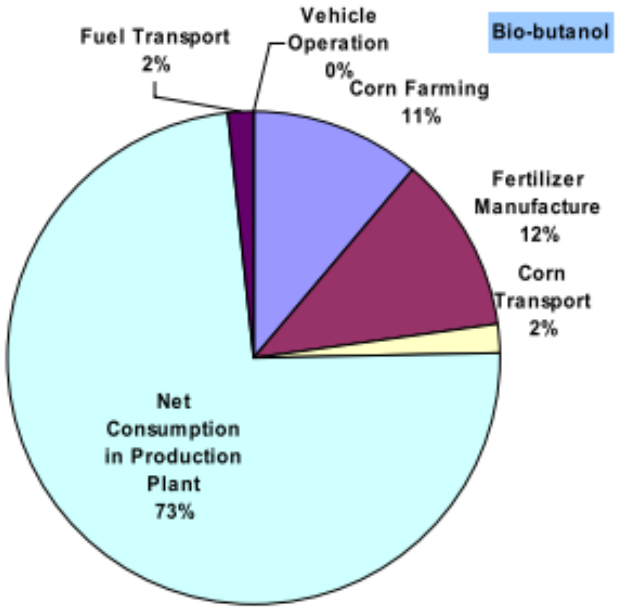


Figure 3.1:Breakdown of Fossil Energy Use in Various Stages of Fuel Life Cycle for Corn-Based Butanol (Case 3) (Wu et al., 2007)

3.2. BIOBUTANOL FUTURE

The ‘’zero’’ net energy balance suggests that the principle ‘’benefit’’ of corn-based biofuel production is to produce a transportation-friendly liquid fuel using a transportation-nonfriendly fuel, e.g. coal, to run the process. This benefit is further diminished, because transportation-friendly petro-diesel and electricity are used to fuel the transport and agricultural components of these processes.

If we consider neutral energy balance of biobutanol process further improvements should be done to make biobutanol commercially compatible.

The biochemical conversion of celluloses is currently a technical bottleneck, but progress is being made to solve that problem. Even considering the differences due to different yield parameters reported in the literature, it appears from these estimates that growing switchgrass, or corn land will not be sufficient to replace the gasoline currently used annually in the US (Akinci et al., 2008). Ragauskas et al. (2006) sustainable biofuel production requires approximately two-fold increase of current biomass yield per unit area. In circumstances in which ‘‘free’’ energy is available, such as the lignin in switchgrass or the bagasse in sugar cane the butanol refinery’s energy needs can be met using the ‘‘free’’ energy, which in fact is just converted solar energy. Placing corn with lignocellulosic materials in bioethanol production can further increase the net energy to 15.90 $MJ{\cdot}L^{-1}$ for ethanol production (Swana et al., 2010). If we consider butanol has higher LHV than ethanol, the energy efficiency is expected to be higher. By sustainable harvest based on current yields, these materials can be converted to 31 billion liters of biobutanol replacing 29 billion liters of gasoline annually. To further expand the scale, significant crop yield increases.

The water requirement in biological fuel production drives the energy balance in the plant. A higher concentration of product in fermentation reduces energy needs for product concentration in subsequent process steps. As covered in the Section 2.3. metabolic engineering of ABE producing bacteria’s has crucial position on biobutanol future. If only we can decrease the energy required for butanol distillation by avoiding by products, this results in the greatest net energy return. The decrease in energy investment in butanol production compared with ABE is probably due to the fact that recovering butanol alone avoids dealing with the azeotrope formation between ethanol and water, which requires tremendous energy for separation. The best theory that been suggested instead of distillation using pervaporation both for recovery and dehydration of solvents. (Swana et al., 2011, Roy et al., 2012) In addition, the energy picture for the recovery of biofuels from ABE fermentations is much more attractive for pervaporation because of the high butanol–water separation factor and the liquid–liquid phase separation offered by the n-butanol/water system. Vane, (2005) compared n-butanol recovery by pervaporation using an oleyl alcohol liquid membrane followed by distillation to that of distillation alone. For a broth containing 0.5% (w/w) n-butanol, the pervaporation–distillation system required 7.4MJ per kilogram of n-butanol recovered while the distillation only system required ten times as much energy (79.5 $MJ{\cdot}kg\text{-}BuOH^{-1}$).

New technologies on cell recycle, online recovery and continuous process applications are on the promising way to increase productivity and decrease the energy costs. In this research a new approach to butanol production had been introduced. Firstly, two-stage fermentation with online butanol removal via pervaporation run at continuous mode with xylose/glucose feedstock. Xylose conversion had been observed. Afterwards two-stage fermentation with cell recycle set-up ran in a continuous mode. Although these different phenomenas; two stage fermentation, online removal and cell recycle; have been applied to butanol process separately no publication had been available about the set-up lay out that was subject to experimental yet.

4. MATERIALS AND METHODS

4.1. CULTURE AND INOCULATION PREPARATION

C. acetobutylicum ATCC 824 was used for all the experiments. The stock culture, which had been stored at -20°C in 20% glycerol solution, was inoculated for 28 hours identical 5 shaking flasks. The medium used for the inoculation of culture contained (for 1 L) 0.01 g NaCl, 2.2 g NH_4 acetate, 0.5 g K_2HPO_4, 0.5 g KH_2PO_4, 0.01 mg biotin, 1mg p-aminobenzoic acid (PABA), 0.2 g $MgSO_4 \cdot 7H_2O$, 0.01 g $MnSO_4 \cdot H_2O$, 11.1 mg NH_4Fe citrate, 40 g glucose and 20 g xylose. The ratio was defined as 0.1 mL culture/100 mL medium.

The carbohydrates were autoclaved separately from the nitrogen sources and salts while trace elements (biotin, PABA, $MgSO_4$, $MnSO_4$, NH_4Fecitrate) were sterile filtered and added afterwards.

4.2. TWO STAGE FERMENTATION WITH ONLINE BUTANOL REMOVAL

A two stage ABE fermentation setup that is represented in Figure 3.1. with an acidogenic fermenter of 3 L (working volume 1.1 L) and a solventogenic fermenter of 7 L (with working volumes 2.14 L, 2.97 L, and 3.94 L) (Infors, *Bottmingen*, Switzerland). Both fermenters are equipped with oxygen sensors and pH probes (Mettler-Toledo, Zaventem, Belgium) and both stirred at 150 rpm, respectively. The acidogenic fermenter was run at 35 ℃ while the solventogenic fermenter was run at 37℃. The optical density was monitored on-line in both bioreactors using an ASD19-N single channel absorption probe with an optical path length of 10mm (Optek-Danulet, Essen, Germany).

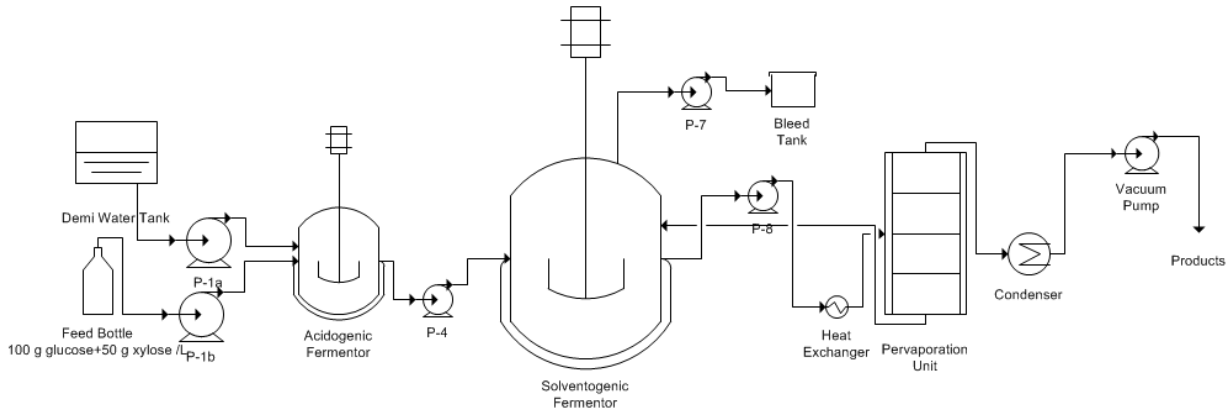


Figure 4.1: Two stage fermentation set-up with online butanol removal.

The shaken flask cultures were used to inoculate the fermenters (10% v/v). Peristaltic pumps (Watson-Marlow 520U, Cornwall, UK) were used to add concentrated feed medium to the acidogenic fermenter (Pump 1-a), to add de-ionized water (Pump 1-b) and to transfer fermentation broth from the acidogenic to the solventogenic fermenter (Pump 4). Nitrogen was sparged automatically in the reactors when the oxygen concentration rose above 0.1% O_2 saturation.

Feed solution has following components for 1 L: 0.02 g NaCl, 4.4 g NH_4 acetate, 1 g K_2HPO_4, 1 g KH_2PO_4, 0.02 mg biotin, 6 mg p-aminobenzoic acid (PABA), 0.4 g $MgSO_4{\cdot}7H_2O$, 0.02 g $MnSO_4{\cdot}H_2O$, 22.2 mg NH_4Fe citrate, 200 g glucose and 100 g xylose. Desired glucose/xylose concentration in the acidogenic fermenter is 100 $g{\cdot}L^{-1}$ and 50 $g{\cdot}L^{-1}$ respectively. This desired concentration is obtained through dilution with de-ionized water. Pump 1-a set to 0.999 $mL{\cdot}min^{-1}$and diluted with 0.983 $mL{\cdot}min^{-1}$ de-ionized water via pump 1-b. Final composition of sugars is calculated as 151.2 $g{\cdot}L^{-1}$. Level in the acidogenic fermenter was controlled by P&ID system. When the working volume is above 1.1 L broth transferred to solventogenic reactor by pump 4 for at the rate of 2.31 $mL{\cdot}min^{-1}$.

The laboratory-scale pervaporation unit consisted of three identical membrane modules (Pervatech, Enter, The Netherlands) connected in series. The total exchange surface is 0.027 m^2. Thin film composite membranes, consisting of a polydimethylsiloxane (PDMS) separating layer of approximately 1 µm thickness on top of a porous polyimide support (approximately 200 μm), were purchased from Pervatech.

Average permeate pressure of 10 mbar was established using a membrane vacuum pump (SC920, KNF Neuberger GmbH, Freiburg, Germany). A Watson-Marlow 620U peristaltic pump (Pump 8) was used to recirculate the fermentation broth from the solventogenic fermenter over the pervaporation modules with the rate of 2.54 $L{\cdot}min^{-1}$. The inner diameter is given as $5.6 \cdot 10^{-3}$ m thus cross-flow velocity is calculated as 1.69 $m{\cdot}s^{-1}$. The temperature set points of the heating bath (Hüber, Berching, Germany) in the recirculation coil and of the chiller (Unichiller, Hüber, Berching, Germany) for condensation of the permeate were set at 37 ℃, and -2℃, respectively.

4.3. SAMPLING AND ANALYSIS

Two samples per day were taken per fermenter. Analysis for pH, optical density (600 nm), dry cell weight, glucose/xylose, alcohols and volatile fatty acids were performed. Unfiltered culture was diluted 10 times for optical density measurements. For dry cell weight, 20 mL of fermentation broth was centrifuged for 15 min at 6500 rpm. The supernatant was decanted and the remaining pellet was washed with de-ionized water. A second centrifugation was applied for 15 min at 6500 rpm and pellet was frozen and lyophilized (24h). For component analyses the broth samples were filtrated with 0.45 µm syringe filters (GD/X 25 Syringe Filter, nylon, 0.45 µm, Whatman, Germany). Dilution rates for component analyses are given in the Table 4.1.

Table 4.1 Dilution rates for component analyses

	Fermenter Samples		Permeate Samples		
	Components	Sample-Water Ratio		Components	Sample-Water Ratio
Sugar Analysis	Glucose Xylose	1/149	Alcohols Analysis	Butanol Ethanol Acetone	1/59
Alcohols Analysis	Butanol Ethanol Acetone	1/9	Alcohols Analysis	Butanol Ethanol Acetone	1/999
Volatile Fatty Acids Analysis	Butric Acid Acetic Acid	1/11.5			

For all dilutions de-ionized water was used except for volatile fatty acids (VFA). For VFA sampling additional H_2SO_4 was added to lower down the pH of the sample below 2; so that all the volatile acids could be able to converted into undissociated molecular form. The rates are 0.2/1.8/0.5 relatively for sample, water and H_2SO_4.

Van Hecke et al. (2012) explained in details the analysis procedure. After filtration (0.45 μm, Acrodisc Syring Filter, Sartorius, Germany), 0.2 mL of sample from the cultures was added to 1.8 mL water and 500 μL H_2SO_4. Subsequently, 80 μL of an aqueous solution containing 6 $mg \cdot L^{-1}$ 2-methylhexanoic acid was added as internal standard. A small amount of NaCl was added together with 2 mL diethylether for extraction of the Volatile Fatty Acids (VFAs). The sample was vortexed during two min and subsequently centrifuged for three min at 1900 g (5810R centrifuge, Eppendorf, Hamburg, Germany). The supernatant was transferred to a vial for analysis by gas chromatography (Focus GC, Thermo Fischer Scientific, Waltham, Massachusetts) equipped with a AT^M-1000 capillary column (15 m x 0.53 mm; 1.20 μm film thickness) with flame ionization detection. The determination of acetic acid (retention time 4.46 min) and butyric acid (retention time 7.03 min) was carried out under the following conditions: injector temperature 145 ℃, detector temperature 200 ℃, column temperature linearly ramping from 40 to 100 ℃ at 3 ℃ per min. Helium at a flow rate of 3.3 $mL \cdot min^{-1}$ was used as the carrier gas. The determination of acetone (retention time 9.52 min), ethanol (retention time 13.54 min) and butanol (retention time 23.54 min) was performed by gas chromatography using an AT-WAX capillary column (60 m x 0.32 mm; 1.00 μm film thickness) with flame ionization detection. The analysis was carried out under the same temperature conditions as specified above: helium (carrier gas) flow rate, 1.6 $mL \cdot min^{-1}$; H_2 flow rate, 35 $mL \cdot min^{-1}$; airflow rate, 350 $mL \cdot min^{-1}$. D6-ethanol was used as an internal standard. The concentrations of glucose (retention time 11.8 min) were determined by high performance liquid chromatography (1200 Series, Agilent Technologies, Santa Clara, California) using a Prevail Carbohydrate ES 5u column (250 mm x 4.6 mm) with evaporative light scattering detector (Alltech 3300 ELSD, Grace, Maryland) for peak detection.

4.4. HIGH DENSITY FERMENTATION WITH CELL RECYCLING

To reach higher butanol productivity, first in-situ product recovery was applied to continuous fermentation. Product inhibition effect was eliminated. Next approach is defined as reaching high cell density by cell recycling to fermenters. Ultrafiltration units (UF) were coupled to fermenters. Whole process is controlled by control user interface Mefias, developed in-house by VITO. Detailed P&ID is displayed in Figure 4.2.

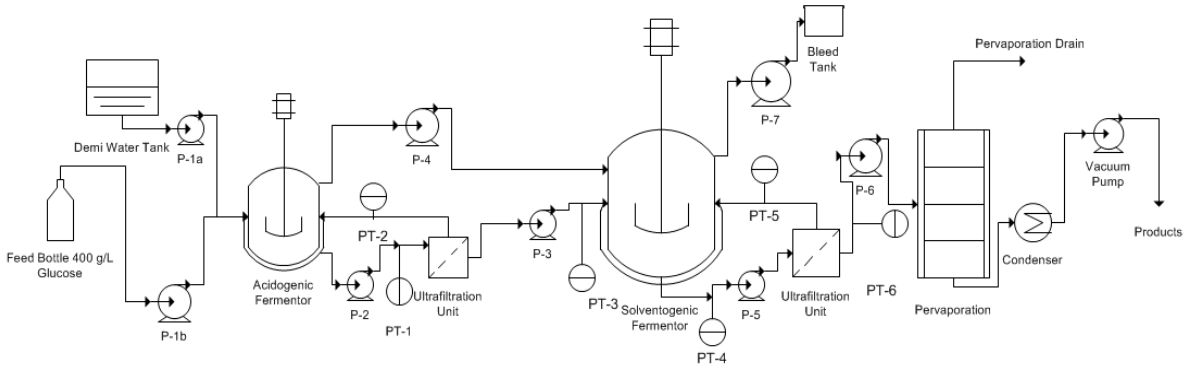


Figure 4.2: P&ID scheme of high density cell fermentation setup.

Culture and inoculation preparation were described detailed in previous section. Same reactors with working volume 0.66 L for acidogenic and 3.66 L for solventogenic were used for high cell density (HCD) two staged fermentation. The volumes of UF modules were 0.45 L and 0.42 L for acidogenic and solventogenic relatively and total working volumes are calculated as 1.1 L and 4.1 L.

Similar to previous experiment feed solution has following components for 1 L: 0.02 g NaCl, 4.4 g NH_4 acetate, 1 g K_2HPO_4, 1 g KH_2PO_4, 0.02 mg biotin, 6 mg p-aminobenzoic acid (PABA), 0.4 g $MgSO_4 \cdot 7H_2O$, 0.02 g $MnSO_4 \cdot H_2O$, 22.2 mg NH_4Fe citrate, 400 g glucose. Desired glucose concentration in the acidogenic fermenter is 60 $g \cdot L^{-1}$. Only glucose were used for first trial of HCD experiment. The desired concentration is obtained through dilution with de-ionized water. Pump 1-a was set to 3.8 $mL \cdot min^{-1}$and dilution was achieved by mixing with 21.2 $mL \cdot min^{-1}$ de-ionized water via pump 1-b. Final composition of sugars is calculated as 151.2 $g \cdot L^{-1}$. Level in the acidogenic fermenter was controlled by P&ID system like previously. When the working volume is above 1.1 L broth was transferred to solventogenic reactor by pump 4 for at the rate of 27.9 $mL \cdot min^{-1}$. Pump flows are listed in Table 4.2. No recovery system was used during experiment.

Table 4.2: Initial set point values of HCD fermentation

Pump ID	Flow Rate L h^{-1}
P1-a	0.228
P1-b	1.272
P2	152.4
P3	1.362
P4*	1.674
P5	152.4
P6	1.272
P7*	1.272

***Pumps are controlled by level sensor, flows are not constant*

Both acidogenic and solventogenic fermenters have side stream ultrafiltration units to increase the cell density in the fermenters. These units UF-1 for acidogenic and UF-2 for solventogenic have 5 polyvinylidene fluoride (PVDF), cross flow tubular modules that are connected in series. Shell material of the membrane modules was made from PVC and inner diameter was ID=8 mm, the length of the modules is L=1000 mm and $L_{effective}$ = 950 mm. Watson-Marlow 620U peristaltic pumps, P2 and P5 were set to 2.54 L·min^{-1} to obtain 2 m·s^{-1} crossflow through the membrane modules. The pressure difference between retentate and permeate side was created via Watson-Marlow 520U pumps, P3 and P6. Set value was 22.7 mL·min^{-1} for both reactors to obtain a flux of 17.54 L·m^{-2}·h^{-1} with the membrane area 0.077 m^{-2}.

Bleed from the solventogenic reactor was set at 5% of feed flow. Bleed is controlled by level sensor and P7 was activated automatically by control system with rate of 27.2 mL·min^{-1}. Clean water tests were performed per membrane module to determine the initial relation between TMP and water fluxes (*J*) since the water flux is defined as in the following equation.

$$J = TMP * P_M \qquad [38]$$

where TMP is given;

$$TMP = \frac{\left(P_{F,n} + P_{R,n}\right)}{2} - P_f \qquad [39]$$

P_f values were measured separately by moving the pressure sensor for each measurements. As feed and outlet pressure is measured from first and fifth module inlet and outlet pressures are calculated by average pressure drop.

$$P_{F,n} = P_{F,1} - \frac{(n-1) * \left(P_{F,1} - P_R\right)}{5} \qquad [40]$$

$$P_{R,n} = P_{F,n} - \frac{\left(P_{F,1} - P_R\right)}{5} \qquad [41]$$

And the correction factor for temperature;

$$P_M{}^{20°C} = P_M * e^{(-0.0239*(T-20))} \quad [42]$$

Where;

P_F: Feed pressure,

P_R: Retentate pressure,

P_f: Filtrate pressure,

$P_M{}^{20°C}$: Permeability at 20 ℃,

P_M: Permeability at experiment temperature.

Procedure:

- Membranes were wetted by de-ionized water.
- UF-1 is observed via the pressure sensors of PT1-PT2 and PT3c that belong relatively for feed, retentate and filtrate flows.
- The pressure sensors, PT4-PT5 and PT6c are observed relatively for feed, retentate and filtrate flows of UF-2.
- The filtrate pressure sensors, PT-3c and PT-6c were placed to each five modules to measure each filtrate pressure individually. During experiments filtrate pressure is measured from 3rd module, but to obtain individual permeabilities each filtrate pressure is necessary individually.
- Cross-flow velocity is defined as $v = 2\ m \cdot s^{-1}$.
- Cross-section area can be calculated $\frac{ID^2}{4} * \pi$ =2.123e-5 *m²*.
- Required cross-flow is $2\frac{m}{s} * 2.123\ e^{-5} m^2 * 3600\frac{s}{h} \cong 153\ L \cdot h^{-1} = 2.54\ L \cdot min^{-1}$
- Circulation pumps from fermenters to UF modules, **P-2** and **P-5** were set.
- Online pressure gathered from P&ID monitoring software Mefias.
- Fluxes were set at 100 and 300 $L.m^{-2}.h^{-1}$.
- The actual filtrate flow is measured and this value was used for calculation of permeabilities.

Clean water flux is reported to be higher than 1000 $L.m^{-2}.h^{-1}$. This corresponds to a flow of 77.6 $L.h^{-1}$. It is not necessary to reach these high fluxes, but it is important to determine the permeability of clean membranes at different fluxes. These values will be used to determine the degree of fouling of ultrafiltration units after use in the HCDF set-up.

5. RESULTS AND DISCUSSION

5.1. TWO STAGE FERMENTATION WITH ONLINE BUTANOL REMOVAL

Two stage fermentation was run for 503 hours. We can investigate the whole period in 5 different time zones. First zone was defined as batch fermentation. After 14 hours of batch fermentation the feed pumps were activated and continuous mode was started. The overall dilution rate of the fermentation was 0.037 h^{-1}. Third zone started when pervaporation was coupled to the system at 49th hour of fermentation. During the first three zones the working volume of solventogenic fermenter (SF) was 2142.2 mL. Acidogenic fermenter (AF) working volume was kept constant during all process and was measured as 1100 mL. Fourth zone started when residence time of SF was increased by increasing the level sensor at 144th hour of fermentation. The working volume during the fourth zone is calculated as 4284.4 mL. Last zone has the highest residence time with working volume of 5262.4 mL and time period is between 305 to 503 hours.

Temperatures were constant during experiment at 35 and 37 ℃ for acidogenic and solventogenic fermenter. The pH of the medium is very important to the biphasic acetone–butanol fermentation. In acidogenesis, rapid formation of acetic and butyric acids causes a decrease in pH. Solventogenesis starts when pH reaches a critical point, beyond which acids are reassimilated and butanol and acetone are produced. Therefore, low pH is a prerequisite for solvent production. However, if the pH decreases below 4.5 before enough acids are formed, solventogenesis will be brief and unproductive (Kim et al., 1984). pH was not controlled however it was measured online during experiment. For AF it varied between 4.3-4.83 with average value of 4.5. For SF the trend was more oscillating with range of 3.98 to 5.36 and average of 4.44. Like pH, optical density was observed constantly. The data for AF is available for all zones however SF data can be available after third zone when level is increased inside the SF. Online optical density (OD) data gave a good baseline during experiment about cell density however when we compare it with offline 600 nm measurements there was no trustable correlation between offline and online data. It can be explained due to dynamic system (foam, cell clusters inside the reactors and gas forming) during fermentation the value from online sensor could not be able to reflect the real OD as accurate as offline 600nm measurements. Figure 5.1 shows the online graph for pH and OD. Dry cell weight (DCW) was evaluated in function of OD, the correlations between OD and DCW can be seen at Figure 5.2.

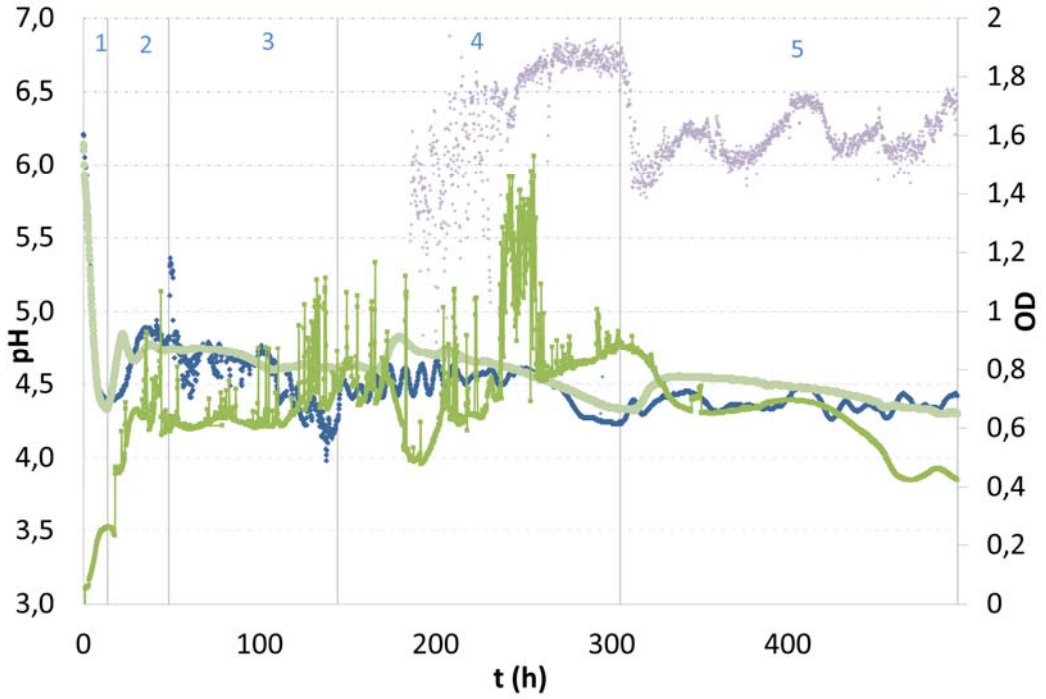


Figure 5.1: Online data for pH and OD for acidogenic and solventogenic fermenters. Symbols $pH\ SF$ (♦), $pH\ AF$ (•), $OD\ AF$ (■), $OD\ SF$ (+)

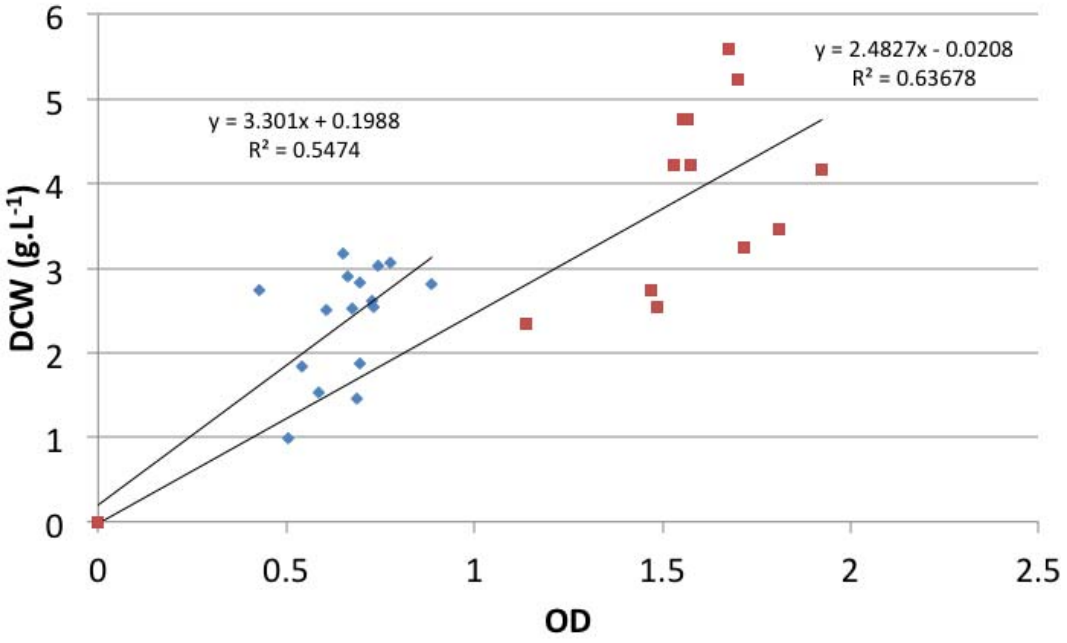


Figure 5.2: DCW (g· L-^{1}) function of online OD. Symbols: $DCW\ AF$ (♦), $DCW\ SF$ (■).

As it can be seen from Figure 5.3 the glucose and xylose concentration in AF decreased slightly as in SF glucose was completely consumed and final average xylose concentration for zone 5 is 19 g·L^{-1} . The average sugar utilization and the solvent productivity values are given in Table 5.1. Van Hecke et al. (2013) performed continuous fermentation directly coupled with pervaporation with

glucose feedstock. As a comparison data obtained from study with 150 $g \cdot L^{-1}$ glucose initial concentration and 0.027 h^{-1} overall dilution rate they reach the overall solvent productivity 1.13 $g \cdot L^{-1} \cdot h^{-1}$ with 0.8 glucose utilization. The corresponded values for this study presented in zone 3 as; for 0.028 h^{-1} dilution rate and 150 $g \cdot L^{-1}$ glucose/xylose concentration overall solvent productivity calculated as 0.3 $g \cdot L^{-1} \cdot h^{-1}$. This lower productivity is the result of lover xylose utilization rate reported as 0.1. However when we increased the residence time with $D_{overall}$=0.0195 and 0.0168 h^{-1} productivity increased to 0.5 and 0.7 $g \cdot L^{-1} \cdot h^{-1}$ respectively for zone 4 and 5.

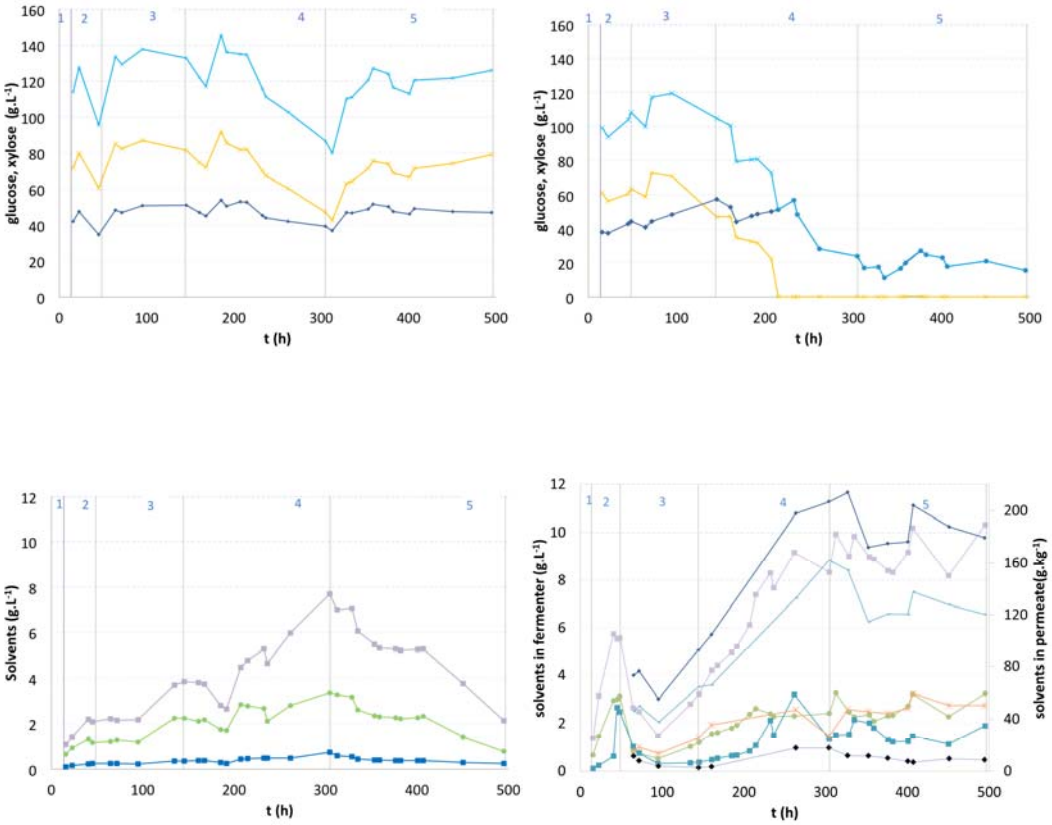
Solvent titres in AF remained relatively low to induce solvent production in second fermenter while acetic and butyric acid concentrations are higher. During first 4 zones total solvent concentration increased step by step and reached 12 $g \cdot L^{-1}$ with 8 $g \cdot L^{-1}$ butanol concentration. After the 4^{th} zone a significant drop at total solvent concentration is observed. Meanwhile acetic and butyric acid concentrations were increased.

For SF, during the first two zones the solvent concentration reached the level of 12 $g \cdot L^{-1}$. But when PDMS pervaporation modules with 0.027 m^2 total exchange surface were connected directly to the second fermenter, the solvent concentration dropped significantly with average of 4.2 $g \cdot L^{-1}$. The total solvent concentration in permeate reached the level 93 $g \cdot kg^{-1}$ with 65 $g \cdot kg^{-1}$ butanol concentration and maximum flux 0.618 $kg \cdot m^{-2} \cdot h^{-1}$. In the 4^{th} zone residence time were increased thus we reached higher productivity and solvent titres. When the dilution rate was set to D=0.0195 h^{-1} total solvent concentration in SF increased to 13 $g \cdot L^{-1}$ range and butanol concentration was in the range of 9 $g \cdot L^{-1}$. Permeate concentrations were 207 $g \cdot kg^{-1}$ for total solvents and for maximum butanol concentration was reported as 162 $g \cdot kg^{-1}$. During 4^{th} zone fluxes varied between 0.684 and 0.719 $kg \cdot m^{-2} \cdot h^{-1}$, also the maximum flux for total solvents was measured as 148 $g \cdot m^{-2} \cdot h^{-1}$. The final zone had the maximum total solvent concentration for both in the SF (15 $g \cdot L^{-1}$) and in the permeate (214 $g \cdot kg^{-1}$). The butanol concentration in the permeate decreased silently with 155 $g \cdot L^{-1}$. Overall fluxes in function of the solvent concentrations in SF can be seen at Figure 5.4. As it can be seen from Figure 5.3 acid concentrations in SF has reverse proportion with solvent concentration. In the SF more butyric acid is converted to butanol and overall concentration was lower than the AF.

DCW was observed daily as it was mentioned in Section 4. A constant increase of DCW in SF was observed related to its increasing residence time. But steady state conditions as it was aimed could not be reached. Figure 5.3 shows the dry cell weight evolution during time.

Table 5.1 represents all processed data. Calculations were done according to formulas that are given in Section 2.4. and 2.5. We have demonstrated the successful long term continuous fermentation with coupling of a pervaporation unit. The sugar utilization is investigated with glucose and xylose feedstock. As it is discussed before with same total sugar concentration and dilution rate, usage of only glucose feedstock showed better results. But when we increased the residence time productivities improved also to 0.7 $g.L^{-1}.h^{-1}$. Separation factors also improved while solvent titres increased. While total titres improved from 50 $g.L^{-1}$ to 204 $g.L^{-1}$ selectivity of butanol/water went from 15 to 22. Stabile results from different runs prove that PDMS membranes shows good performance

for *in-situ* recovery for ABE fermentation. The fermentation medium effect had been discussed in Section 2.5.1. PDMS performance with directly coupled fermentation reported previously by Van Hecke et al. (2012) and Van Hecke et al. (2013). For total solvent concentration 7.30 $g.L^{-1}$, they obtained 0.38 $kg.m^{-2}.h^{-1}$ flux and separation factors 35.1,15.7 and 8.7 respectively for acetone/water, butanol/water and ethanol/water (Hecke et al., 2012). The experiment run at 35 ^{o}C and crossflow was reported as 0.6 $m.s^{-1}$. For the similar experiment, but with 37 ^{o}C operation temperature the results were reported as it follows; Total concentration 11.20 $g.L^{-1}$, the total flux 0.61 $kg.m^{-2}.h^{-1}$ flux and separation factors 24.7, 16.1 and 7.5 respectively for acetone/water, butanol/water and ethanol/water. The average flux obtained during current work is higher and separation factors were displayed on Table 5.1 for each different zone. The slight differences on similar runs can be explained with xylose presence and higher residual sugar concentration in broth.



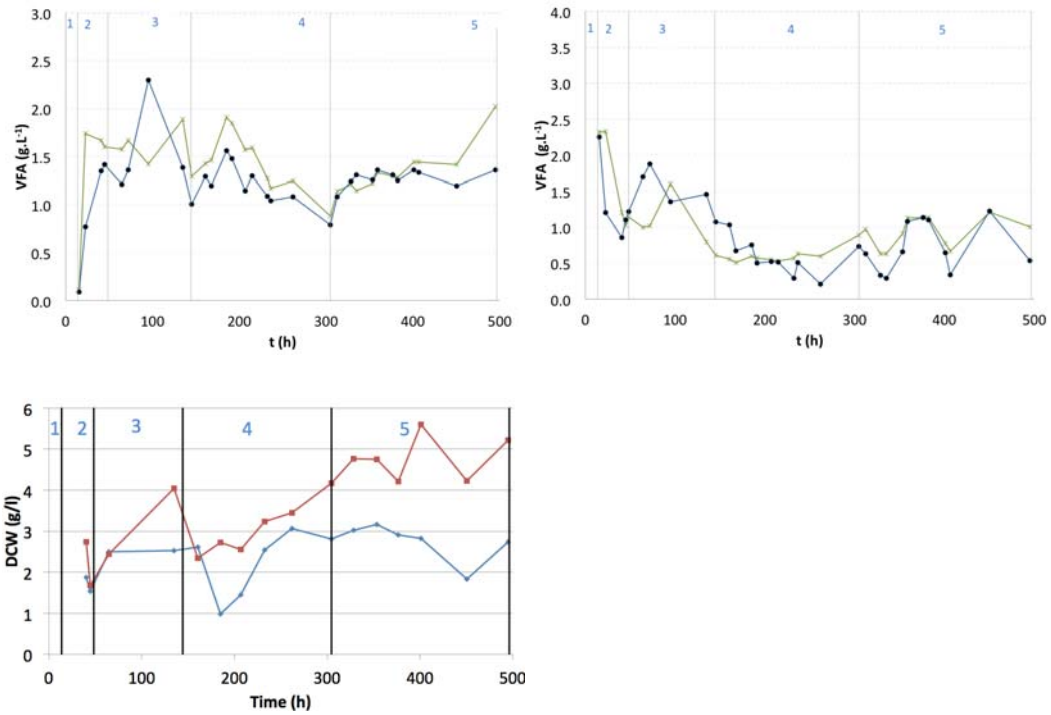


Figure 5.3: Evolution of concentrations of carbohydrates, VFAs and solvents in acidogenic (left) and solventogenic (right) fermenters. DCW evaluation during time for AF and SF. Symbols: (✳) glucose, (■) xylose, (×) total carbohydrates, (●) acetone, (■) butanol, (■) ethanol, (×) acetone in permeate, (-) butanol in permeate ,(◆) ethanol in permeate, (◆) total solvents in permeate, (✳) acetate, (●) butyrate, (◆) DCW AF (■) DCW SF

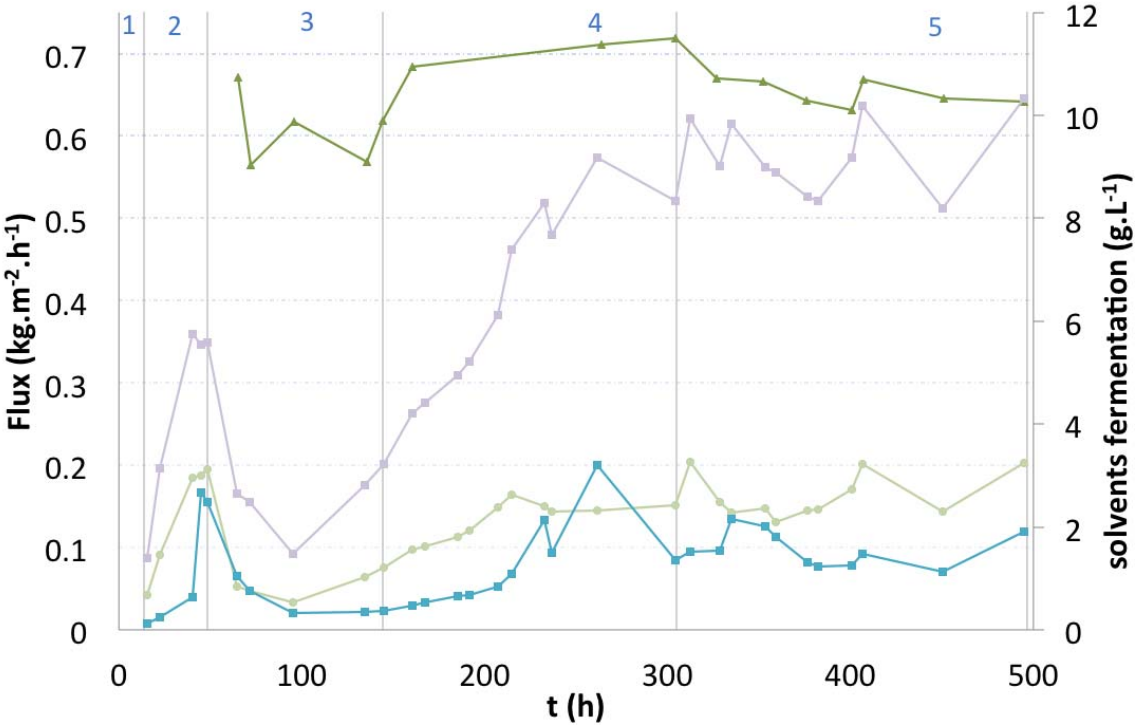


Figure 5.4: Overall flux values for PDMS pervaporation module. Symbols: (●) acetone, (■) butanol, (■) ethanol, total flux(▲)

Table 5.1: Fermentation profile of two stage continuous fermentation coupled with PDMS pervaporation.

	1	2	3	4	5
From start to end	0-13,75	13,75-48,53	48,53-144,33	144,33-304,85	304,85-496,55
Duration (h)	13.75	34.78	95.8	160.52	191.7
Q_F ($kg.h^{-1}$)	0.000	0.120	0.096	0.101	0.102
Q_P ($kg.h^{-1}$)	-	-	0.016	0.019	0.018
D_1 (h^{-1})	-	1.09E-01	1.02E-01	1.09E-01	1.09E-01
D_2 (h^{-1})	-	5.60E-02	3.85E-02	2.38E-02	1.99E-02
Overall dilution rate (h^{-1})	-	3.70E-02	2.80E-02	1.95E-02	1.68E-02
Solvents in acidogenic fermenter ($g.L^{-1}$)					
A	-	1.02	1.48	2.41	2.27
B	-	1.69	2.56	4.52	5.27
E	-	0.18	0.27	0.27	0.40
Total ($g.L^{-1}$)	**-**	**2.89**	**4.30**	**7.20**	**7.93**
Solvents in solventogenic fermenter ($g.L^{-1}$)					
A	-	2.25	0.79	2.05	2.60
B	-	4.28	2.36	6.28	9.21
E	-	1.23	0.62	1.17	1.58
Total ($g.L^{-1}$)	**-**	**7.76**	**3.76**	**9.49**	**13.39**
solvents in permeate ($g.kg^{-1}$)					
A	-	-	11.92	19.72	48.75
B	-	-	33.43	73.46	126.22
E	-	-	4.72	8.14	9.65
Total solvents in permeate ($g.kg^{-1}$)		**-**	**50.1**	**101.3**	**184.6**
Separation factor					
$\alpha_{Acetone/water}$	-	-	15.9	10.6	22.7
$\alpha_{BuOH/water}$	-	-	14.9	12.9	13.7
$\alpha_{EtOHwater}$	-	-	8.1	7.7	5.9
Flux ($g.m^{-2}.h^{-1}$)	**-**	**-**	**601**	**702**	**649**
Total solvents ($g.L^{-1}$)	-	7.76	10.47	23.99	38.39
Solvent yield (g.g-1)	-	0.159	0.277	0.286	0.294
Overall solvent productivity ($g.L^{-1}.h^{-1}$)	**-**	**0.29**	**0.29**	**0.47**	**0.65**
Solvent productivity in acidogenic fermenter($g.L^{-1}.h^{-1}$)	**-**	**0.31**	**0.44**	**0.78**	**0.86**
Solvent productivity in solventogenic fermenter ($g.L^{-1}.h^{-1}$)	**-**	**0.27**	**0.24**	**0.40**	**0.60**
Initial glucose ($g.L^{-1}$)	100	100	100	100	100
Initial xylose ($g.L^{-1}$)	50	50	50	50	50
Final glucose ($g.L^{-1}$)	-	60.44	67.43	19.69	0.08
Final xylose (g.L-1)	-	40.85	44.77	46.49	19.38

Table 5.1 (continued): Fermentation profile of two stage continuous fermentation coupled with PDMS pervaporation.

	1	2	3	4	5
Dry cell weight acidogenic fermenter ($g.L^{-1}$)	-	1.97	2.52	2.24	2.75
Dry cell weight solventogenic fermenter ($g.L^{-1}$)	-	2.20	3.24	3.08	4.80
Overall specific solvent productivity ($g.g^{-1}.h^{-1}$)	**-**		**0.08**	**0.21**	**0.22**
Specific solvent productivity in acidogenic fermenter ($g.g^{-1}.h^{-1}$)	**-**	**0.16**	**0.17**	**0.35**	**0.31**
Specific solvent productivity in solventogenic fermenter ($g.g^{-1}.h^{-1}$)	**-**	**0.01**	**0.07**	**0.13**	**0.13**
Glucose utilization	-	0.4	0.3	0.8	1.0
Xylose utilization	-	0.2	0.1	0.1	0.6
Glucose utilization rate ($g.L^{-1}.h^{-1}$)	-	1.46	0.91	1.57	1.68
Xylose utilization rate ($g.L^{-1}.h^{-1}$)	-	0.34	0.15	0.07	0.52
Carbohydrate utilization rate ($g.L^{-1}.h^{-1}$)	-	1.80	1.06	1.64	2.20

5.2. HIGH CELL DENSITY FERMENTATION WITH CELL RECYCLING

After performing successfully a two staged fermentation a high cell density fermentation was run in continuous mode for 71 hours. Before experiments were started, water tests were performed and Table 5.2 represents the permeability calculations of UF-2 for the flux value of100 $L.m^{-2}.h^{-1}$. As it can be noticed permeabilities varied a lot. The applied pressure difference was not enough to create driving force especially for first and last modules. Figure 5.6 shows the applied pressures for one module of unit. Fermentation broth flows from the lumen of the membrane and cell-free supernatant is collected from shell.

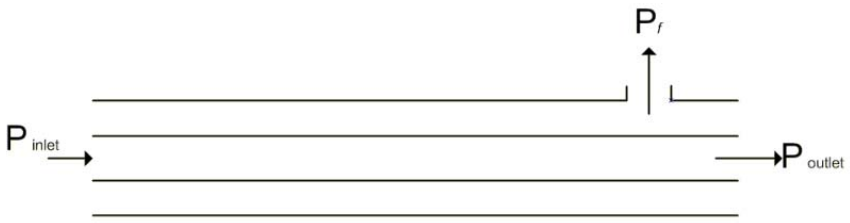


Figure 5.6: Applied pressures to UF modules.

Table 5.2: Calculated TMP and Permeability values for UF-2 module for filtrate flux 100 $L.m^{-2}.h^{-1}$

Membrane	Flux	Flow P6	PF PT-4	PR PT5	Pf PT-6c	Pn,inlet	Pn,outlet	TMP	P 20 C
	$Lm^{-2}h^{-1}$	$L\ h^{-1}$	bar	bar	bar	bar	bar	bar	$\mathbf{Lm^{-2}h^{-1}bar^{-1}}$
UF-2a	101.0	8.75	1.562	0.110	1.425	1.562	1.272	-0.008	**-11563.6**
UF-2b	101.1	8.77	1.597	0.112	1.051	1.300	1.003	0.100	**964.5**
UF-2c	100.9	8.75	1.595	0.111	0.751	1.001	0.705	0.102	**949.8**
UF-2d	101.8	8.83	1.577	0.107	0.414	0.695	0.401	0.134	**730.4**
UF-2e	101.9	8.83	1.513	0.104	0.249	0.385	0.104	-0.004	**-22554.8**
Water Temperature		21.8							

The same experiment repeated for UF-2 with 300 $L.m^{-2}.h^{-1}$ filtrate flux. The results are represented in Table 5.3.

Table 5.3: Calculated TMP and Permeability values for UF-2 module for filtrate flux 300 $L.m^{-2}.h^{-1}$

Membrane	Flux	Flow P6	PF PT-4	PR PT5	Pf PT-6c	Pn,inlet	Pn,outlet	TMP	P 20 C
	$Lm^{-2}h^{-1}$	$L\ h^{-1}$	bar	bar	bar	bar	bar	bar	$\mathbf{Lm^{-2}h^{-1}bar^{-1}}$
UF-2a	322.2	25.03	1.301	0.049	0.877	1.301	1.051	0.299	**1032.3**
UF-2b	322.6	25.06	1.332	0.055	0.605	1.076	0.821	0.343	**900.1**
UF-2c	322.9	25.08	1.336	0.057	0.369	0.824	0.568	0.327	**946.2**
UF-2d	324.9	25.24	1.340	0.054	0.102	0.568	0.311	0.338	**921.2**
UF-2e	325.5	25.28	1.346	0.059	0.065	0.317	0.059	0.122	**2545.9**
Water Temperature		21.8							
UF-2e	314.7	25.283	1.127	-0.054	-0.387	0.182	-0.054	0.451	**693.3**
Water Temperature		20.30							

For the module UF-2e membrane permeability is out of range 2545 $L.m^{-}2.h^{-}1.bar^{-1}$ and similar data reading between retentate and filtrate pressure can be considered as a sign of a membrane crack. UF2-e was replaced with new module and the performance test was repeated.

The clean water test results of UF-1 with 300 $L.m^{-2}.h^{-1}$ filtrate flux are represented in Table 5.4. The permeabilities of UF-1b and UF-1c were calculated as 811.2 and for 257.69 $Lm^{-2}h^{-1}bar^{-1}$. While the values for other permeabilities are between 1293-1469 $Lm^{-2}h^{-1}bar^{-1}$ these two values can be considered abnormal. Moreover during experiments on the UF-1c membrane bubble formation and visible air leak on the membrane surface had been observed. UF-1b and UF-1c were replaced and the test was repeated.

Table 5.4: Calculated TMP and Permeability values for UF-1 module for filtrate flux 300 L.m-2.h-1

Membrane	Flux	Flow P6	PF PT-1	PR PT2	Pf PT-3	Pn,inlet	Pn,outlet	TMP	P 20 C
	$Lm^{-2}h^{-1}$	$L\ h^{-1}$	bar	bar	bar	bar	bar	bar	$Lm^{-2}h^{-1}bar^{-1}$
UF-1a	318.5	19.11	1.372	0.052	1.026	1.372	1.108	0.215	**1469.8**
UF-1b	319.0	19.14	1.398	0.054	0.605	1.129	0.860	0.390	**811.2**
UF-1c	322.6	19.35	1.398	0.055	-0.514	0.861	0.592	1.240	**257.7**
UF-1d	320.2	19.21	1.403	0.057	0.221	0.596	0.326	0.240	**1322.0**
UF-1e	321.1	19.26	1.413	0.060	-0.051	0.331	0.060	0.246	**1293.6**
Water Temperature		21.4							
UF-1b	323.1	19.39	1.244	-0.076	0.591	0.980	0.716	0.257	**1279.9**
UF-1c	324.7	19.48	1.245	-0.078	0.293	0.716	0.451	0.290	**1137.9**
Water Temperature		20.4							

Despite the two UF modules have identical specifications, possible reasons for the different ranges of permeabilities was questioned. To calculate the error on pressure sensors P=0 bar values calculated and with corrected values permeability's recalculated. Corrected values were represented on Table 5.5. Recalculated range of the permeabilities shows better pattern. The signals that were obtained from pressure sensors were corrected according to zero signals. Correction was basically done by subtracting the zero signal from the read value. The errors in the first calculations were calculated as;

$$Error = \frac{P^{20\,C-1} - P^{20\,C-2}}{P^{20\,C-2}} * 100 \qquad [43]$$

Table 5.5: Recalculated permeability values for UF-1 and UF-2

Membrane	$P^{20\,C}$	Corrected $P^{20\,C}$	Error
	$Lm^{-2}h^{-1}bar^{-1}$	$Lm^{-2}h^{-1}bar^{-1}$	%
UF-1a	1469.8	1489.6	**1.3**
UF-1b2	1279.9	1244.5	**-2.8**
UF-1c2	1137.9	1111.8	**-2.3**
UF-1d	1322.0	1337.2	**1.1**
UF-1e	1293.6	1307.8	**1.1**
UF-2a	1032.3	1488.8	**30.7**
UF-2b	900.1	1223.5	**26.4**
UF-2c	946.2	1304.6	**27.5**
UF-2d	921.2	1250.1	**26.3**
UF-2e2	693.3	861.4	**19.5**

The online pH and OD for HCD fermentation represented on Figure 5.7. On Table 5.6 you can see the overall zones that are also represented performance graphs. Also, the performance data of the high cell density two stage fermentation is presented on Table 5.7. The solvent titres are lower than it

is expected. The productivity is calculated as 1 g L^{-1} h^{-1}. Total solvent concentration is 3.44 g L^{-1} with 2.03 g L^{-1} butanol. The most noticeable value is glucose utilization. Different from previous set-up 60 g L^{-1} glucose were used and total glucose utilization is calculated as 0.25. This value explains low titres and relatively productivity. Another reason may be that the strain lost its capacity to produce solvents. Oscillatory behaviour has been observed in long term continuous cultivations. VITO experience also learns that the viability and/or solvent producing property of stock cultures is limited in time, even when they are stored under proper conditions at -80°C.

Table 5.6: Time zones used on figures for HCD fermentation.

Zones	Time (h)	Explanations
Start Zone 1	0	Batch
Start zone 2	8.1	Start of continuous mode
Start zone 3	10.4	HCD - UF modules activated P3 acidogenic filtrate pump rate 22,7 mL.min^{-1}
Start zone 4	22.719	Decrease of P3 acidogenic filtrate pump rate 21.9 mL.min^{-1}
Start zone 5	27.052	Decrease of P3 acidogenic filtrate pump rate 21 mL.min^{-1}
Start zone 6	43.552	Decrease of P3 acidogenic filtrate pump rate 20 mL.min^{-1}
Start zone 7	50.85	Pervaporation coupling trial

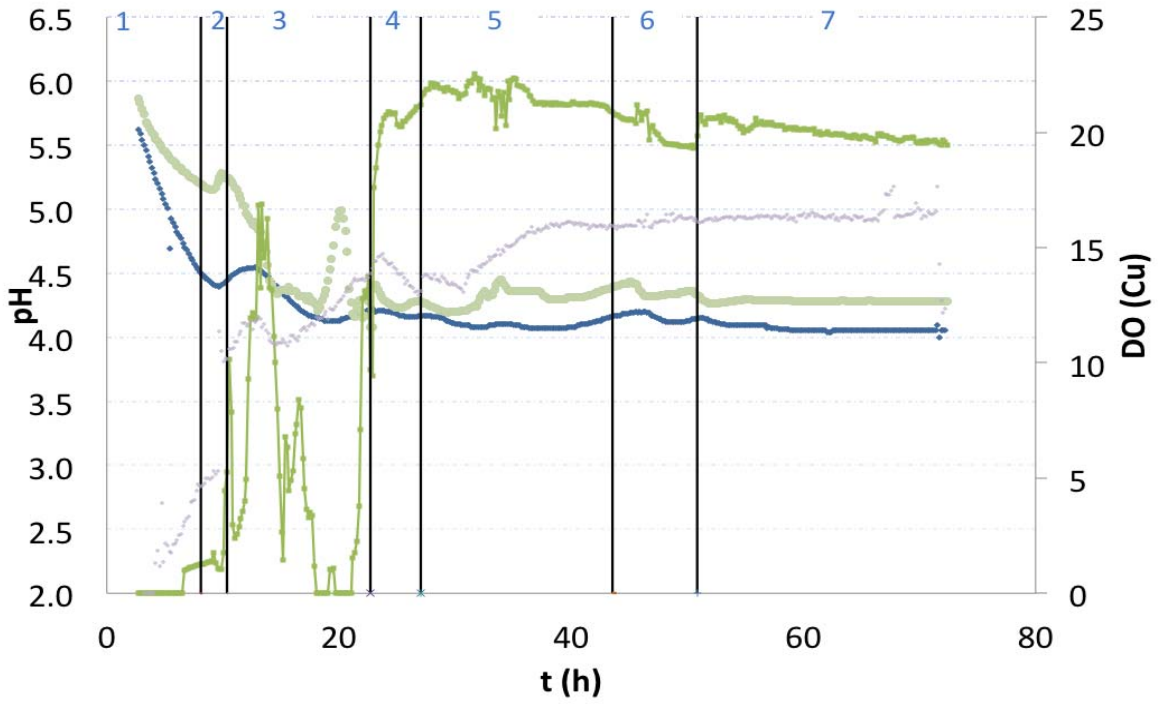


Figure 5.7: Online data for pH and OD for acidogenic and solventogenic fermenters. Symbols: $pH\ SF$ (♦), $pH\ AF$ (•), $OD\ AF$ (■), $OD\ SF$ (+).

For the calculations the different zones are not considered since they were relatively shorter zones to obtain an average value. On Table 5.7 we represented the zone 1 as the period first 10 hours of

operation until the UF units were coupled to the fermenter. No sampling had been done before the 13th hour of fermentation. Second zone stands for the HDC continuous fermentation.

Table 5.7: Performance profile for HCD fermentation.

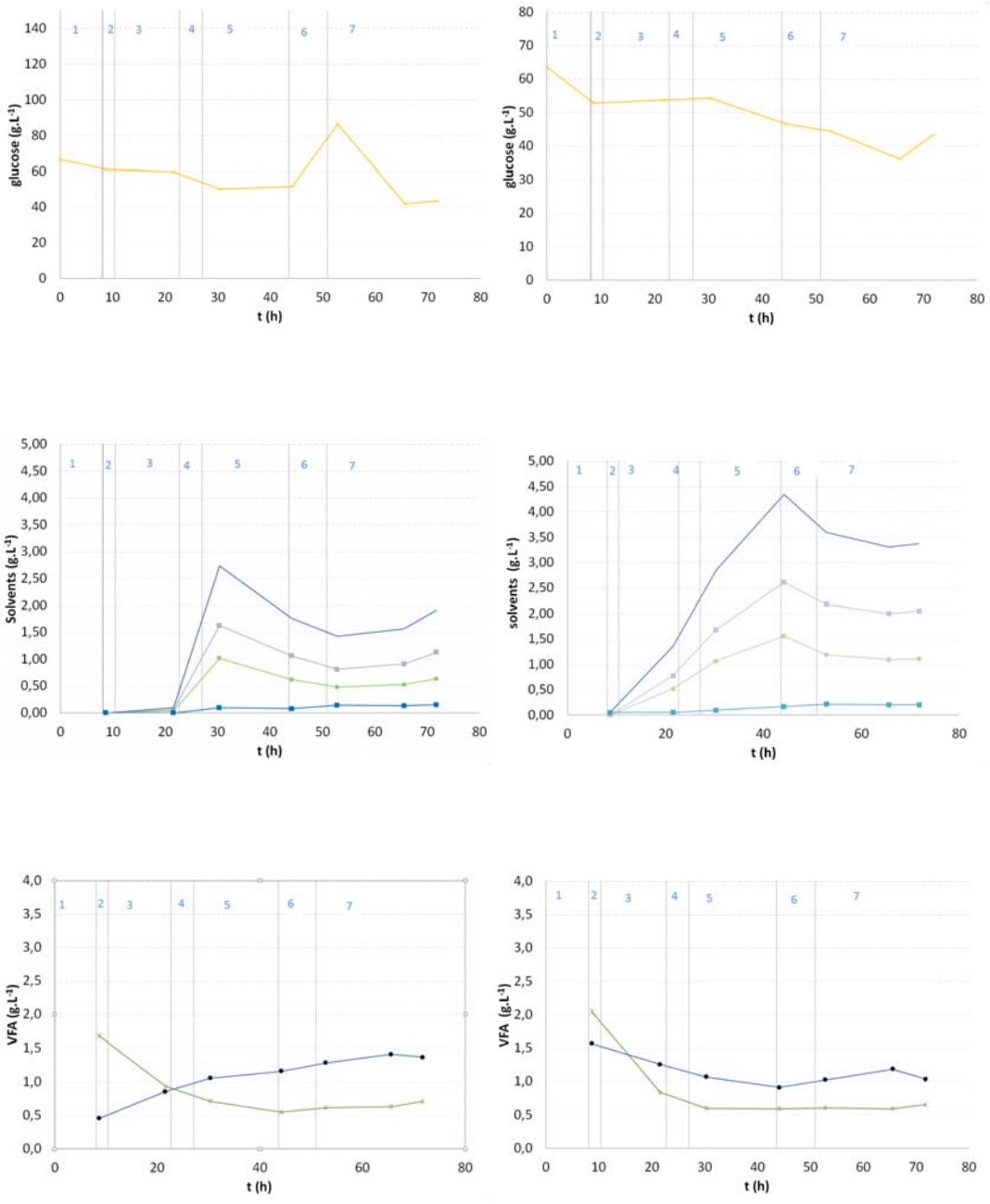
	1	2
From start to end	0-8,1	10,4-50,85
Duration (h)	8.1	40.45
QE (kg.h-$^{-1}$)	0	1.53
D1 (h^{-1})	-	1.35
D2 (h^{-1})	-	0.37
Overall dilution rate (h^{-1})	-	0.29
Solvents in acidogenic fermenter (g.L^{-1})		
A	-	0.65
B	-	0.95
E	-	0.14
Total (g.L^{-1})	-	1.74
Solvents in solventogenic fermenter (g.L^{-1})		
A	-	1.21
B	-	2.03
E	-	0.21
Total (g.L^{-1})	-	3.44
Solvent yield (g.g^{-1})		0.23
Overall solvent productivity (g.L^{-1}.h^{-1})		1.00
Solvent productivity in acidogenic fermenter(g.L^{-1}.h^{-1})		2.68
Solvent productivity in solventogenic fermenter (g.L^{-1}.h^{-1})		0.71
Initial glucose (g.L^{-1})		60.00
Final glucose (g.L^{-1})		44.97
Dry cell weight acidogenic fermenter (g.L^{-1})		9.14
Dry cell weight solventogenic fermenter (g.L^{-1})		22.84
Overall specific solvent productivity (g.g-$^{-1}$h^{-1})		0.05
Specific solvent productivity in acidogenic fermenter (g.g^{-1}h^{-1})		0.26
Specific solvent productivity in solventogenic fermenter (g.g^{-1}h^{-1})		0.03
Glucose utilization		0.25
Glucose utilization rate (g.L^{-1}h^{-1})		4.35

The start flow (pump 3) was set to 22.7 mL·min^{-1} corresponding to 17.54 L·m^{-2}·h^{-1}. But after 10 hours of HCD acidogenic reactor working volume dropped drastically. Filtrate flow was decreased to 21.9 mL min^{-1}. The fermenter run 16 hours but still volume decrease was observed. Finally 20 mL.min^{-1} was set as final filtration flow for acidogenic fermenter ultrafiltration unit.

Also second problem was detected as high bleed ratios. Real set point was 5% of feed flow. The pump that is responsible for bleed flow rate was controlled on-off when the broth level is above the set point. The measured data showed that bleed ratio varied between 13-26%, which causes lower residence times and more important cell washout. DCW reached a maximum for AF 10.20 g L^{-1} and

SF for 43.29 g L^{-1}. This values are nearly 5 and 11 times higher than in previous run for acidogenic and solventogenic fermenter relatively. These values are lower than expected and these values support our theory about washout due to high bleed rates.

On Figure 5.8 the performance graphs of HDC fermentation are displayed



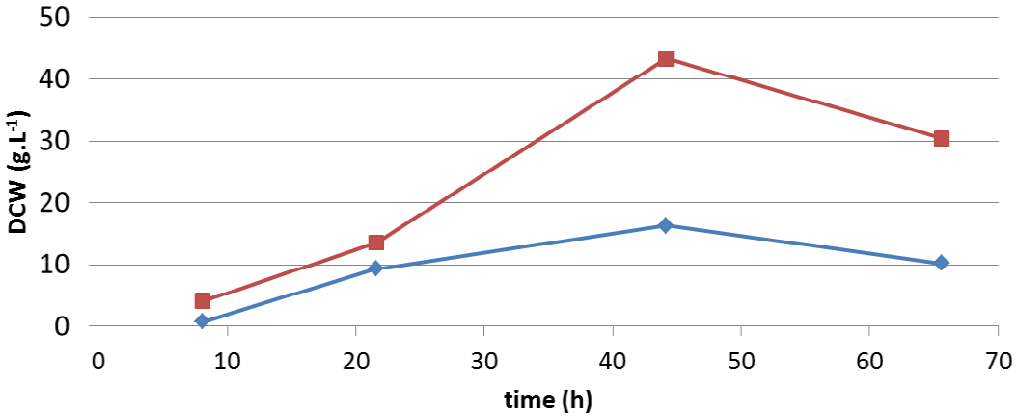


Figure 5.8: Evolution of concentrations of carbohydrates, VFAs and solvents in acidogenic (left) and solventogenic (right) fermenters. DCW evaluation during time for AF and SF. Symbols: (✳) glucose, (▰) xylose, (✕) total carbohydrates , (●) acetone, (▰) butanol, (▰) ethanol, (x) acetone in permeate, (-) butanol in permeate ,(◆) ethanol in permeate, (◆) total solvents in permeate, (✳) acetate, (●) butyrate, (◆) DCW AF (■) DCW SF

Li et al. 2011, indicates that the relative production of solvents and short fatty acids depends on the dilution rate. At a lower dilution rate, the fermentation culture mainly produced solvents while at a higher dilution rate, the fermentation culture mainly produced acids. As mentioned before ABE fermentation is known by its biphasic growth pattern: acidogenesis followed by solventogenesis. Therefore, at a higher dilution rate, i.e. a shorter residence time, the fermentation culture was dominated by cells in the acidogenic phase, where acetic and butyric acids were the main metabolites. At a lower dilution rate, i.e. a longer residence time, the fermentation culture had enough time to fully enter solventogenesis phase and hence increased solvent productivity was observed.

TMP's were calculated for UF modules (Figure 5.9). Filtrate pressure was measured from the middle third module. TMP remained fairly constant in the acidogenic fermenter and increased in the solventogenic fermenter. Once the TMP in the solventogenic fermenter UF module increased to over 2 bars pump -P5- shut down. The different TMP behaviour in the solventogenic fermentor may be related to the fact that the cell densities were a lot higher than in the acidogenic one and that the organisms are in a non-growing solvent production phase.

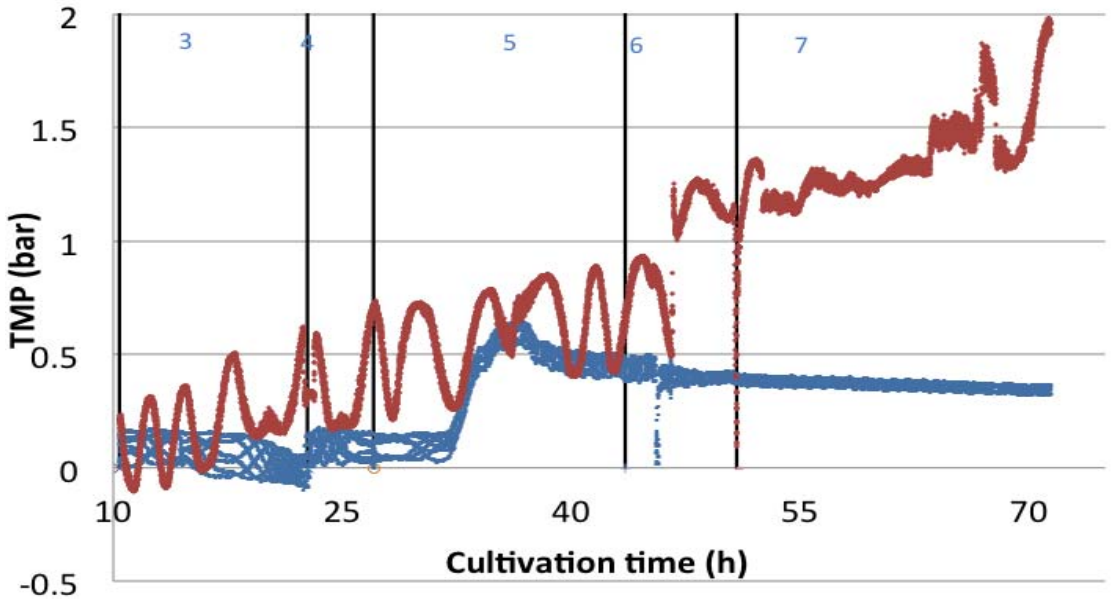


Figure 5.9: Calculated TMP values for UF modules during fermentation time. Symbols: $TMP\ AF-UF$ (♦), $TMP\ SF-UF$ (■).

Strong oscillations can be seen in the solventogenic ultrafiltration membrane. The feed, outlet and filtrate pressure signals of SF were investigated deeply. As it can be seen from figure 5.10, oscillations were due to variations in the feed pressure, but the reason for this could not be identified.

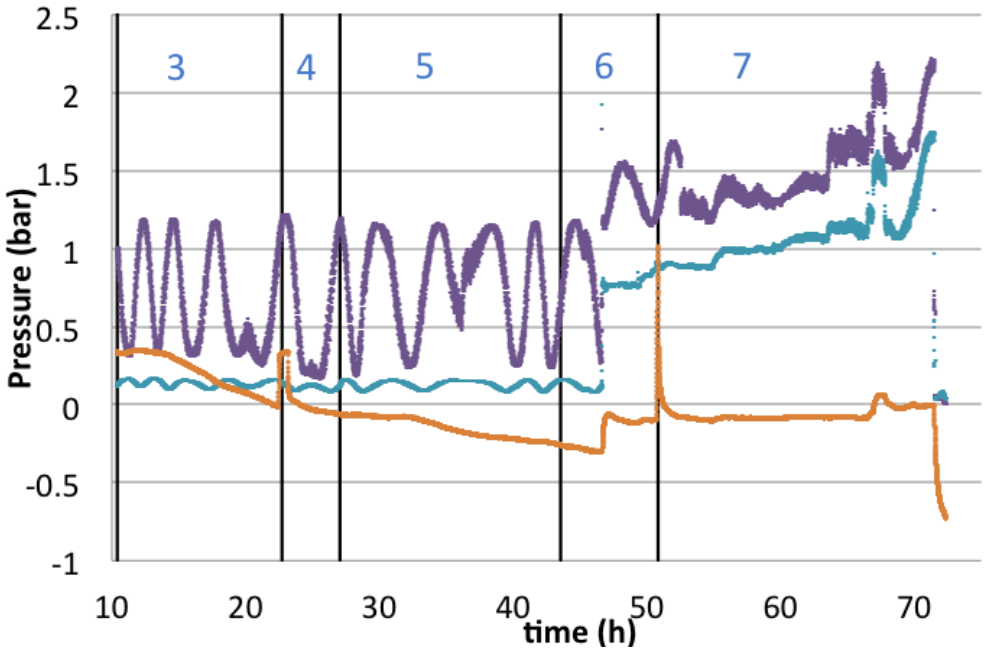


Figure 5.10: Feed, outlet and Filtrate pressure of SF- UF module. Symbols: Filtrate Pressure (•), Feed Pressure (•), Outlet Pressure (•).

In the present experiment, the bleed is turned out to be much higher than anticipated. Hence, the bleed pump needs to be recalibrated. For next trial, the control system of process could also be changed. In this run, the filtrate flow was set and the bleed varied according to the level control. Another approach could be to keep the bleed constant.

After the tests, the membranes were cleaned to recover the permeability levels to an acceptable range. To this end, 1% (w/w) of oxonia solution and 1%(w/w) ultrasil 141 was flushed for about an hour at 50°C. Then clean water permeability was determined. The same flow rates for filtrate and recycling were applied to get comparable results to the initial clean water permeability determination. Unfortunately the results were not satisfying (not reported). Therefore, in a second run, the same solution was backflushed through the UF units for 20 mins at 100 $L.m^{-2}.h^{-1}$. According to a next clean water test backflushing also did not provide efficient removal of the biofilm. Finally, a 1%(w/w) sodium hypochlorite solution was applied to the system at 50°C. Now, clean water permeabilities were higher, but for some membranes still significantly lower than the initial permeability. The calculated permeabilities are shown on Table 5.8. Serious fouling was especially observed on the solventogenic UF module. That is in line with the observed increase of TMP during operation. For UF-1a there is also a remarkable fouling problem. But it is not surprising that the first membrane in the series is the most heavily fouled.

Table 5.8: The permeabilities of UF units after cleaning

Membrane	P $^{20\,C}$	Initial P $^{20\,C}$	Difference
	$Lm^{-2}h^{-1}bar^{-1}$	$Lm^{-2}h^{-1}bar^{-1}$	Percent
UF-1a	521.4	1489.6	65.00%
UF-1b2	716.7	1244.5	42.41%
UF-1c2	950.6	1111.8	14.50%
UF-1d	1049.7	1337.2	21.50%
UF-1e	986.3	1307.8	24.58%
UF-2a	765.4	1488.8	48.59%
UF-2b	981.7	1223.5	19.76%
UF-2c	924.5	1304.6	29.14%
UF-2d	516	1250.1	58.72%
UF-2e2	517.3	861.4	39.95%

The results show that cleaning needs further optimization and that part of the fouling may be irrecoverable in nature. It remains to be confirmed whether similar fouling behaviour is observed during more stable operation of the fermentation.

6. CONCLUSION

In a first set-up, an ABE fermentation was performed on a glucose/xylose mixture and ABE were recovered through a directly coupled pervaporation unit with PDMS membranes. Glucose was completely consumed. The Clostridium strain was also capable of xylose consumption, though xylose conversion was not complete. For higher conversion of xylose, dilution rates should be investigated more deeply. The butanol concentration reached 10 $g \cdot L^{1}$ in the solventogenic reactor, with a corresponding productivity of 0.7 $g \cdot L^{-1} \cdot h^{-1}$. The pervaporation membrane showed a good performance as no fouling was observed after 500 hours of operation.

In a second set-up, high cell density fermentation was aimed for by coupling UF membranes to each fermentation step. Although some degree of cell density increase was obtained, sugar conversion was low. This was related to the fact that bleed rates were higher than planned. This can be resolved by applying a different control strategy. Another reason may be the occurrence of strain degeneration.

UF performance was stable in the acidogenic fermenter. In the solventogenic fermenter TMP increased steadily, probably due to higher cell densities and the different non-growing solvent producing conditions of the cells. Further optimizations are needed to obtain constant cell densities and stable operating conditions. Furthermore, regular cleaning procedures can be considered during fermentation to control the fouling.

For further work, HCD experiment with online pervaporation must be studied to investigate the decreasing effect of solvent inhibition on productivity.

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