

DOKUZ EYLÜL UNIVERSITY
GRADUATE SCHOOL OF NATURAL AND APPLIED SCIENCES

ACETONE, BUTANOL AND ETHANOL
PRODUCTION FROM WASTE SUBSTRATES



by
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October, 2020
İZMİR

ACETONE, BUTANOL AND ETHANOL PRODUCTION FROM WASTE SUBSTRATES

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**by
Gizem DOĞAN**

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İZMİR**

M.Sc THESIS EXAMINATION RESULT FORM

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ABSTRACT

ABE (Acetone, Butanol, and Ethanol) fermentation is a promising process for biofuel production. Batch and fed-batch experiments were conducted to produce solvent.

At the first batch experiments, *Clostridium acetobutylicum* and *Clostridium beijerinckii* as organisms and watermelon peel, tea waste, peach waste, wheat waste, and rice husk were used as waste substrates. *C. acetobutylicum* tended to ethanol production while *C. beijerinckii* produced acetone as the major solvent. The highest butanol concentration (6.97 g/ L) was obtained with hydrolyzed peach waste at *C. acetobutylicum* where different types of waste produced nearly the same amount of butanol. The highest butanol yield (0.139 g butanol/ g substrate), volumetric butanol production rate (0.00116 g butanol/ L. h) and volumetric ABE production rate (0.00235 g ABE/ L. h) were obtained with *C. acetobutylicum* and peach waste.

Fed-batch fermentation was subjected to hydrolyzed peach waste with *C. beijerinckii* at different dilution rates. Acetone was the major solvent produced where the highest acetone concentration (11.87 g/ L) and productivity (0.13 g/ L. h) was obtained with 0.0113 1/ h dilution rate. The highest acetone production yield (0.32 mol acetone/ mol glucose) was observed with 0.0056 1/ h dilution rate.

Keywords: ABE fermentation, *Clostridium acetobutylicum*, *Clostridium beijerinckii*, waste substrate, batch fermentation, fed-batch fermentation

ATIK HAMMADDELERDEN ASETON, BÜTANOL VE ETANOL ÜRETİMİ

ÖZ

ABE (Aseton, Bütanol ve Etanol) fermantasyonu biyoyakıt üretimi için umut verici bir prosestir. Kesikli ve yarı sürekli deneyler çözücü üretimini arttırmak için yürütülmüştür.

İlk kesikli deneylerde, organizmalar olarak *Clostridium acetobutylicum* ve *Clostridium beijerinckii* ve atık substratlar olarak karpuz kabuğu, çay atığı, şeftali atığı, buğday atığı, ve çeltik kavuzu kullanılmıştır. *C. acetobutylicum* ethanol üretimine eğilimli iken *C. beijerinckii* ana çözücü olarak aseton üretmiştir. En yüksek bütanol konsantrasyonu (6,97 g/ L), farklı türdeki atıkların hemen hemen aynı miktarda bütanol ürettiği hidrolizlenmiş şeftali atığı ile *C. acetobutylicum*' da elde edilmiştir. En yüksek bütanol verimi (0,139 g bütanol/ g substrat), hacimsel bütanol üretim hızı (0,00116 g bütanol/ L. sa) ve hacimsel ABE üretim hızı (0,00235 g ABE/ L. sa) *C. acetobutylicum* ve şeftali atığı ile elde edilmiştir.

Yarı sürekli fermantasyon, farklı seyrelme oranlarında *C. beijerinckii* ile hidrolize edilmiş şeftali atığına tabi tutulmuştur. En yüksek aseton konsantrasyonu (11,87 g/ L) ve verimliliği (0,13 g/ L. sa) 0,0113 l/ sa seyrelme oranı ile elde edildiğinde üretilen ana çözücü asetondur. En yüksek aseton üretim verimi (0,32 mol aseton/ mol glukoz) 0,0056 l/ sa seyrelme oranı ile elde edilmiştir.

Anahtar kelimeler: ABE fermantasyonu, *Clostridium acetobutylicum*, *Clostridium beijerinckii*, atık substrat, kesikli fermantasyon, yarı sürekli fermantasyon

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CHAPTER ONE

INTRODUCTION

1.1 The Problem Statement

Acetone, Butanol and Ethanol are important chemical commercial products and fuel alternative sources competing with fossil fuels. Butanol has the potential as a fuel, with no need for engine reconstruction due to its properties. Butanol was produced biologically in the industry at the beginning of the 20th century; however the invention of new technologies disfavored the biobutanol fermentation. The new technologies based on chemical butanol production are practical, efficient and have good production rates and yields when compared with butanol fermentation. For this reason, the production of biobutanol lost its preference in the 1960s (Lee et al., 2008). Today, the increasing prices and the lack of fossil fuels brought a need of alternative energy sources which one of it may be biobutanol (Nayak & Bhushan, 2019). Also, one of the main reasons, for choosing alternative renewable and clean energy sources, with an environmental point of view is reducing greenhouse gas emissions threatening climate change (Dahiya et al., 2018; Pugazhendhi et al., 2019). Renewable energy sources; can be obtained with biological fermentation; that are hydrogen, biogas, methanol, ethanol, biodiesel, biohythane, and butanol (Dahiya et al., 2018; Esteban & Ladero, 2018; Kumari & Singh, 2018; Nayak & Bhushan, 2019).

Butanol and ethanol can be used as a fuel source and a commercial chemical, acetone can be used as a commercial chemical (Hou, From, Angelidaki, Huijgen, & Bjerre, 2017; Vieira, F. M. Filho, R. M. Filho, & Mariano, 2019). Acetone (A), butanol (B) and ethanol (E) fermentation is known as ABE fermentation in the literature and widely has a ratio of 3:6:1 referring to A:B:E ratios (Abdehagh, Tezel, & Thibault, 2014; Brito & Martins, 2017; Green, 2011; Morone & Pandey, 2014; Nanda et al., 2017; Procentese et al., 2017c; Pugazhendhi et al., 2019; Qureshi, Saha, Hector, Hughes, & Cotta, 2008b; Xue, Zhao, Chen, Yang, & Bai, 2017). Acetone-butanol-ethanol (ABE) fermentation, compared with ethanol fermentation includes

some advantageous and disadvantageous. The positive aspects of ABE fermentation are; high heating value of butanol, usability at current engines of automobiles, low corrosivity, being safe fuel while the most important negative side is low solvent production (Ibrahim, Kim, & Abd-Aziz, 2018; Nanda et al., 2017).

Carbohydrate rich materials should be used for biofuel production. The feedstock that may be used for biofuel production is classified as first, second, third, fourth generation (Huzir et al., 2018; Kumari & Singh, 2018; Raud, Kikas, Sippula, & Shurpali, 2019; Wang et al., 2017). Waste food as a second generation feedstock provide advantageous for biofuel production. It is costless, provides recycling of waste materials and decreases the need of landfill areas where the waste is stored (Hegde, Lodge, & Trabold, 2018). The feedstock selection has a share of 60-80% of the total ABE production cost which makes it the most important issue in the process (Jang, Malaviya, Cho, J. Lee, & S. Y. Lee, 2012; Maiti et al., 2016; Pang et al., 2016). Studies continue to find suitable substrate which is inexpensive, easy to find and that does not decrease in quantity throughout the year and does not cause food-fuel conflicts for making butanol production feasible (Hegde et al., 2018).

Clostridium species are generally utilized for acetone-butanol-ethanol fermentation and exist in soil and decaying plants due to living anaerobic condition. Spore formation can occur in these microorganisms (Xin et al., 2018). ABE production process consists of four steps which are pretreatment, detoxification, fermentation and recovery (Brito & Martins, 2017; García, Pääkilä, Ojamo, Muurinen, & Keiski, 2011; Nanda et al., 2017). Biobutanol fermentation processes are designed including some of these steps, depending on the substrate, and feasibility of the process (Y. Jiang, Liu, W. Jiang, Y. Yang, & S. Yang, 2015).

In this study; different hydrolyzed waste foods were compared with two different *Clostridium* species (*Clostridium acetobutylicum*, *Clostridium beijerinckii*) in batch mode. For this purpose; tea waste, rice husk, peach waste, ground wheat, and watermelon peel were used and also experiments were tested with glucose and fructose as carbon source. At the second step of the study hydrolyzed peach waste

was subjected to fed-batch operation with different dilution rates varying between (0.0028-0.0169 1/ h).

1.2 Importance of Fermentable Solvents (ABE): Acetone, Butanol, Ethanol

Fermentable solvents are classified as acetone, butanol and ethanol. They are produced in proper conditions with mainly *Clostridium* species (Liberato et al., 2019).

Acetone is a solvent for synthetic fiber and plastic materials and can be used for production of medicine and pesticide (Li, Tang, Chen, Liu, & Lee, 2019). One of solvent products of ABE fermentation is acetone which fuel characteristic is quite low (Vieira et al., 2019). Also, acetone is inconvenient for rubber engine parts and causes to increase entire cost due to recovery of acetone (Hou et al., 2017; Vieira et al., 2019). Production of oil-based acetone is a byproduct of phenol production. The abundant amount of acetone as chemical and the disuse as fuel makes it unwilling due to demand in the industry. Therefore, decreasing, prevention of acetone, acetone free solvent produced microorganism (such as *Clostridium pasteurianum* and *Saccharomyces cerevisiae*), switch acetone to iso-propanol (IBE fermentation) and the studies have been continued (Vieira et al., 2019).

Butanol (C₄H₉OH) is an important liquid product due to utilization at different areas and can be used as solvent, extractant, chemical intermediate and fuel. Butanol has a significant place at plastic, paint, cosmetic industry and it can be used for production of butylacrylate, methacrylate, glycerol ethers, butyl acetate, butyl ethers (Gallego, Escobar, Peñuela, Peña, & Rios, 2015; Hegde et al., 2018; Ibrahim et al., 2018; Lee et al., 2008; Pang et al., 2016). Also, butanol usage as extractant includes recovery for antibiotics, hormones and vitamins (Hegde et al., 2018). The isomers of butanol are n-butanol, iso-butanol, sec-butanol and tertiary butanol. n-butanol is the most valuable form among them (Algayyim, Wandel, Yusaf, & Hamawand, 2018; Ndaba, Chiyanzu, & Marx, 2015; Trindade & Santos, 2017). It can be directly used

in engines which is also been approved by Environmental Energy Company (US) (Ndaba et al., 2015).

Ethanol (C_2H_5OH) can be used as solvent, disinfectant, cleaning; except for fuel additive. In addition application of ethanol includes chemicals, disinfectants, adhesives, pharmaceuticals, cosmetics, detergents, explosives, inks, hand cream, plastics and textile industries (Ozmihci, 2009).

Butanol, ethanol and methanol can be produced biologically and have 29.2, 19.6, 16 MJ/ L energy density, respectively. High carbon content of butanol makes it an attractive biofuel (Kolesinska et al., 2019; Kujawska, J. Kujawski, Bryjak, M., & W. Kujawski, 2015; Trindade & Santos, 2017). The energy density of butanol is close to gasoline (32 MJ/L) and diesel (≈ 36 MJ/L) (Kolesinska et al., 2019; Trindade & Santos, 2017). Energy consumption of 35% in the world was derived from crude oil (Amiri & Karimi, 2018). There is no sulfur and nitrogen oxide emissions with biobutanol combustion and the usage of biobutanol as fuel additive can reduce carbon emissions (Kujawska et al., 2015; Pugazhendhi et al., 2019; Vivek et al., 2019). So, utilization of butanol in current engines of cars as either fuel or mixing gasoline or diesel contributes to prevent global warming (Kamiński, Tomczak, & Górak, 2011; Vivek et al., 2019). As another positive advantage for butanol as being fuel in cars, corrosivity of butanol is lower than ethanol (Abdehagh et al., 2014; Ibrahim et al., 2018; Nanda et al., 2017). The butanol is named as a safe fuel due to its higher flash point ($35^\circ C$) and boiling point ($117.7^\circ C$), when compared with ethanol (flash point: $8^\circ C$, boiling point: $78.4^\circ C$) (Trindade & Santos, 2017). Ethanol can be mixed with gasoline at 85% ratio, while butanol can be mixed completely due to its high hydrophobicity (Avcı & Dönmez, 2011; Nanda et al., 2017). Mixture of over 10% ethanol and gasoline needs modification of car engines, beside butanol without gasoline as fuel in internal combustion engine does not need alteration in engines (Avcı & Dönmez, 2011). Ethanol-diesel blend is unsuitable due to solubility and miscibility problems. This can be solved with surfactants or co-solvents addition (Algayyim et al., 2018). Also, ethanol is soluble in water completely while butanol solubility in water is 7% (Kolesinska et al., 2019). The ignition difficulties are lower

than ethanol but octane number of butanol is lower than ethanol. Although fuels with high octane number can be preferred due to causing less knock during work, octane number of butanol is not too different from gasoline (Trindade & Santos, 2017). Air fuel ratio (A/F) of butanol (11.2) is another positive property due to the proximity to gasoline (14.6) while ethanol's is 9 (Kujawska et al., 2015; Lee et al., 2008). The properties of butanol make it a promising fuel, however it should be taken into account that the fuel consumption is higher than gasoline due to its energy density (Trindade & Santos, 2017).

1.3 ABE Production

Louis Pasteur (1861) explored ABE fermentation (Lee et al., 2008; Ndaba et al., 2015). Weizman (1916) isolated *C. acetobutylicum* which is capable of producing acetone. Acetone was an important product as cordite gunpowder throughout World War I (Algayyim et al., 2018; Pugazhendhi et al., 2019). In 1945, ABE fermentation became a common industrial biofuel production process. Japan used butanol as airplane fuel in the World War II (Maiti et al., 2016; Ndaba et al., 2015). Oxo-synthesis of butanol as a chemical butanol production way arised in 1950s (Maiti et al., 2016). In addition, expensive feedstock's caused to decline ABE fermentation. In 1960s, only two countries (Russia, South Africa) kept on ABE fermentation (Lee et al., 2008). Several reasons such as oil crisis, expensive oil prices and environmental issues; favored ABE fermentation again (Maiti et al., 2016). Corn, starch, molasses were previously used as substrate to produce ABE. Currently, lignocellulosic feedstock's have been significant due to renewable and abundant substrates (Gallego et al., 2015). ButamaxTM, Advanced Technologies (USA), Gevo (USA), Cathay Industrial Biotech (China), Cobalt Technologies (USA), Green Biologics Ltd. (UK), Butalco (Switzerland) are current butanol producers (Morone & Pandey, 2014; Nanda et al., 2017).

As mentioned earlier, the production of biological butanol is not the only way to produce butanol. Oxo synthesis, Reppe synthesis, crotonaldehyde hydrogenation or butanol production from ethanol are petrochemical ways for butanol production

(Huzir et al., 2018; Kolesinska et al., 2019; Lee et al., 2008). Oxo synthesis is a common method to produce butanol in petrochemical butanol producing industries (Brito & Martins, 2017; Huzir et al., 2018). Propylene hydroformylation results in aldehydes, and then hydrogenation of these causes butanol production by catalysts (Co, Rh or Ru) (Brito & Martins, 2017; Green, 2011; Huzir et al., 2018; Lee et al., 2008). Reppe synthesis includes propylene, carbon monoxide, and water reactions by catalysts (Lee et al., 2008). In third method, ethanol is been converted into acetaldehydes by dehydrogenation, then followed by aldol condensation of acetaldehyde's, finally butanol is been produced via hydrogenation (Ndaba et al., 2015). Crude oil is the most important factor in the cost of petrochemical butanol production, while the selected raw material affects the whole process in the production of biobutanol (Green, 2011; Karimi, Tabatabaei, Horváth, & Kumar, 2015).

1.3.1 Biobutanol Production

Clostridium acetobutylicum ATCC 824 and *Clostridium beijerinckii* NCIMB 8052 which belong to the *Clostridiaceae* family have been used widely in studies of ABE production (Dahiya et al., 2018; García et al., 2011; Pugazhendhi et al., 2019; Xin et al., 2018). Also, studies over these microorganisms are continued to get high solvent concentrations (García et al., 2011). Butanol production consists of upstream processes, fermentation and downstream processes. Upstream processes include pretreatment of substrate, hydrolysis and detoxification of formed inhibitors (salts or compounds derived from the substrate). Downstream processes include recovery and purification of produced solvents with ABE fermentation (García et al., 2011; Nanda et al., 2017). Indeed, this production process needs microorganism with having the ability of high butanol titers, cheap and renewable feedstock with high sugar content for a profitable fermentation technology and to perform these steps (Qureshi, Saha, & Cotta, 2008a).

The maximum stoichiometric butanol yield is 0.411 g butanol / g glucose (García et al., 2011). In an ABE fermentation batch experiment a yield of 0.3 g butanol / g

glucose, with 60 g/ L of glucose by *Clostridium* species was found (Avcı & Dönmez, 2011; Staggs & Nielsen, 2015; Wang et al., 2017). In another study, 40-60 g/ L glucose generated 9-12 g/ L of butanol concentration (Pugazhendhi et al., 2019). It is been seen that over 100 g/ L of sugar concentration caused substrate inhibition and another study revealed over 89.06 g/ L of sugar caused inhibition with *C. acetobutylicum* GX01 in batch fermentation (Ibrahim et al., 2018; Pang et al., 2016). Besides, below 40 g/ L of sugar induced high acid formation and affected organisms negatively after 2 days of ABE production (Ibrahim et al., 2018). Wang et al., (2017) obtained yield of 0.25-0.33 g/ g, productivity of <0.3 g/ L h and butanol concentration of below 20 g/ L in their study (Wang et al., 2017).

ABE fermentation results solvents, remained acids (acetic, butyric acid) in the liquid phase, and H₂ and CO₂ in the gas phase (Borah, Roy, Goyal, & Moholkar 2019; Raganati et al., 2013). In ABE fermentation the solvents after fermentation period result conventionally with a ratio of 3:6:1 which are acetone, butanol and ethanol, respectively (Abdehagh et al., 2014; Brito & Martins, 2017; Green, 2011; Kamiński et al., 2011; Morone & Pandey, 2014; Nanda et al., 2017; Procentese et al., 2017c; Pugazhendhi et al., 2019; Qureshi et al., 2008b; Xue et al., 2017). Anaerobic and mesophilic (35-37°C) conditions, suitable pH value and other necessary nutrients except for carbohydrate are required to get good solvent concentrations (Bruto & Martins, 2017; Kamiński et al., 2011). Controlled pH at 6.0 was the optimal value for *C. acetobutylicum* B18 while batch fermentation of *C. acetobutylicum* YM1 at 6.0±0.2 of pH as uncontrolled was the best initial pH value with the highest butanol concentration (13.5±1.42 g/ L), butanol yield (0.27g/ g) and productivity (0.188 g/ L h). It is clear that strain and other parameters of fermentation conditions affect optimal pH value (Al-Shorgani, Kalil, Yusoff, & Hamid, 2018). Fermentation media's components are important issue (Kamiński et al., 2011; Kujawska et al., 2015). For example, in a study the solvent concentration produced by *C. beijerinckii* BA101 was between 27-29 g/ L and the addition of sodium acetate was increased solvent concentrations (ABE) to 33 g/ L (Kujawska et al., 2015). Another study revealed that 0.001 g/ L ZnSO₄.7H₂O shortens time of conversion from acids to

solvents and enhances butanol production (Brenda, Comprehensive enzyme information system, 2020; Karimi et al., 2015).

Batch, fed-batch, continuous fermentations can be applied to ABE fermentation (Brito & Martins, 2017). In batch ABE fermentation 12-20 g/ L of solvents can be obtained at 2-6 days and generally distillation is used as recovery method (Avcı & Dönmez, 2011; Lee et al., 2008). Fed-batch fermentation can be selected to overcome inhibition of high substrate concentrations to the microorganisms causing low solvent formation (Gottumukkala, Haigh, & Görgens, 2017). However, in fed batch mode low solvent formation may occur and the high energy demand of recovery of these produced solvents may become a disadvantage in this method (Pugazhendhi et al., 2019). To overcome butanol toxicity and retrench from time, continuous fermentation can be selected but cell wash-out and two stage of ABE fermentation affect negatively this fermentation. Two-stage continuous fermentation can be used to supply of distinction of acid formation and solvent formation steps. Immobilization and recycling of cells are solutions to prevent other obstacles of continuous fermentation (Gottumukkala et al., 2017; Ibrahim et al., 2018; Jang et al., 2012; Pugazhendhi et al., 2019). In separate hydrolysis and fermentation (SHF), saccharification and fermentation occurs separately, while simultaneous saccharification and fermentation (SSF) occur in the same reactor providing shorter time but unsuitable conditions depending on the requirement of different conditions. The temperature in fermentation may be in the range of 35-37°C and 45-50°C in enzymatic hydrolysis, for instance. Also, strain improvement supplies all steps in same reactor via consolidated bioprocessing (CBP) (Gottumukkala et al., 2017; Ibrahim et al., 2018). In situ product recovery prevents solvent toxicity (Jang et al., 2012; Xue et al., 2017).

Pentose and hexose sugars can be utilized to produce ABE when compared to ethanol production. Some of ethanol producing organisms can not use pentose sugars (Nayak & Bhushan, 2019; Raud et al., 2019). On the other hand, low butanol concentrations with 2% (w/ v) cause high recovery costs ethanol fermentation can result with 10% (w/ v) of ethanol (Maiti et al., 2016).

1.4 Microorganisms Used in Butanol Production

Substantiation of ABE fermentation needs successful microorganisms to obtain high yield, productivity and solvent concentrations. *Clostridium acetobutylicum* and *Clostridium beijerinckii* are well-known and the most used *Clostridium* species to produce ABE. They can ferment sugars to solvents at anaerobic conditions in mesophilic conditions at 35-37°C. Also, *C. aurantibutyricum*, *C. butyricum*, *C. saccharobutylicum*, *C. saccharoperbutylacetonicum*, *C. ljungdahlii*, *C. sporogenes*, *C. chauvoei*, *C. roseum*, *C. pasteurianum* and *C. tetanomorphum* can be used with cheap and renewable substrates for ABE fermentation (Abd-Alla, Zohri, El-Enany, & Ali, 2017; Avcı & Dönmez, 2011; Karimi et al., 2015; Kolesinska et al., 2019; Li et al., 2019).

12-13 g/ L butanol concentrations can be produced via ABE fermentation with batch cultures. *Clostridium* is negatively affected from butanol, when the butanol concentration is over 20 g/ L (García et al., 2011; Gottumukkala et al., 2017; Karimi et al., 2015). This concentration is toxic for the microorganisms and the cell membranes which gain a fluid-like nature can be slit due to hydrophobic effect of butanol (Pugazhendhi et al., 2019). Mutagenesis, evolutionary and molecular engineering studies have been continued to obtain suitable strains for industrial applicable ABE fermentation (Li et al., 2019). Genes encode enzymes and some of genes at ABE metabolic pathway can be ejected, added, or overexpressed (Pugazhendhi et al., 2019). Studies for strains focus on genes subtraction for prevention of acid by-products formation, development of high butanol tolerable microorganism, exploration of microorganism having capable of producing butanol without acetone, exploration of strain having ability with conversion from acetone to iso-propanol and development of non-spore forming microorganisms (Gottumukkala et al., 2017; Pugazhendhi et al., 2019). Among the genetically modified microorganisms; *Clostridium acetobutylicum* JB200 is known to have the capability to produce over 20 g/ L of butanol concentration and other strains like *Lactobacillus* sp, *Lactococcus* sp, *Pseudomonas* sp, and *Escherichia coli* have relatively lower butanol production abilities. The ABE fermentation yields and rates, with genetically

modified strains, fluctuate due to non stability of the strains properties (Ibrahim et al., 2018; Li et al., 2019).

1.5 ABE Fermentation Metabolism

ABE fermentation actualizes in two stages. These are acidogenesis and solventogenesis. Organic acids production occurs when *Clostridium* species are in exponential phase. Drop in pH is observed due to formation of acetic and butyric acids. In addition, CO₂ and H₂ are produced when pH is approximately 4.5. These organic acids are essential for solvent production using as substrate during end of exponential phase. Also, spore formation is observed during solvent production and pH value of fermentation medium becomes approximately 5.5 (Abdehagh et al., 2014; García et al., 2011; Green, 2011; Kamiński et al., 2011; Kolesinska et al., 2019; Lee et al., 2008; Raganati et al., 2013). High butanol is an obstacle for ABE production because lipophilic characteristic of butanol causes membrane fluidity (Kamiński et al., 2011; Kolesinska et al., 2019; Xin et al., 2018).

Pentose sugars are metabolized first at Pentose Phosphate Pathway (PPP) and then at Embden-Meyerhof-Parnas (EMP) pathway; and hexose sugars direct to EMP pathway to produce pyruvate (Avcı & Dönmez, 2011; Xin et al., 2018). Figure 1.1 depicts the metabolic pathway of ABE production (García et al., 2011; Gottumukkala et al., 2017; Li et al., 2019; Zhao, Kasbi, Chen, Peres, & Jolicoeur, 2017). After production of pyruvate, direct conversion of butanol includes formation of 2 moles acetyl-CoA, one mol of acetoacetyl-CoA, β -hydroxybutyryl-CoA, crotonyl-CoA, butyryl-CoA, butyraldehyde and butanol via pyruvate ferredoxin oxidoreductase, thiolase, 3-OH-butyryl-CoA dehydrogenase, crotonase, butyryl-CoA dehydrogenase, butyraldehyde dehydrogenase and butanol dehydrogenase respectively (García et al., 2011; Li et al., 2019). This channel can be named as hot channel (Gottumukkala et al., 2017). Also, 2 moles of acetyl-CoA convert 2 moles of acetaldehyde and ethanol, respectively. On the other hand, 2 moles of acetyl-CoA convert 2 moles of acetyl-P and acetate via phosphotrans-acetylase and acetate kinase, respectively. Acetoacetyl-CoA converts acetoacetate and acetone by CoA-transferase and acetoacetate

decarboxylase respectively while butyryl-CoA converts butyryl-P and butyrate by phosphotrans-butyrylase and butyrate kinase, respectively (García et al., 2011; Li et al., 2019). Pathway resulting with acetate, acetone and butyrate is named as cold channel (Gottumukkala et al., 2017).

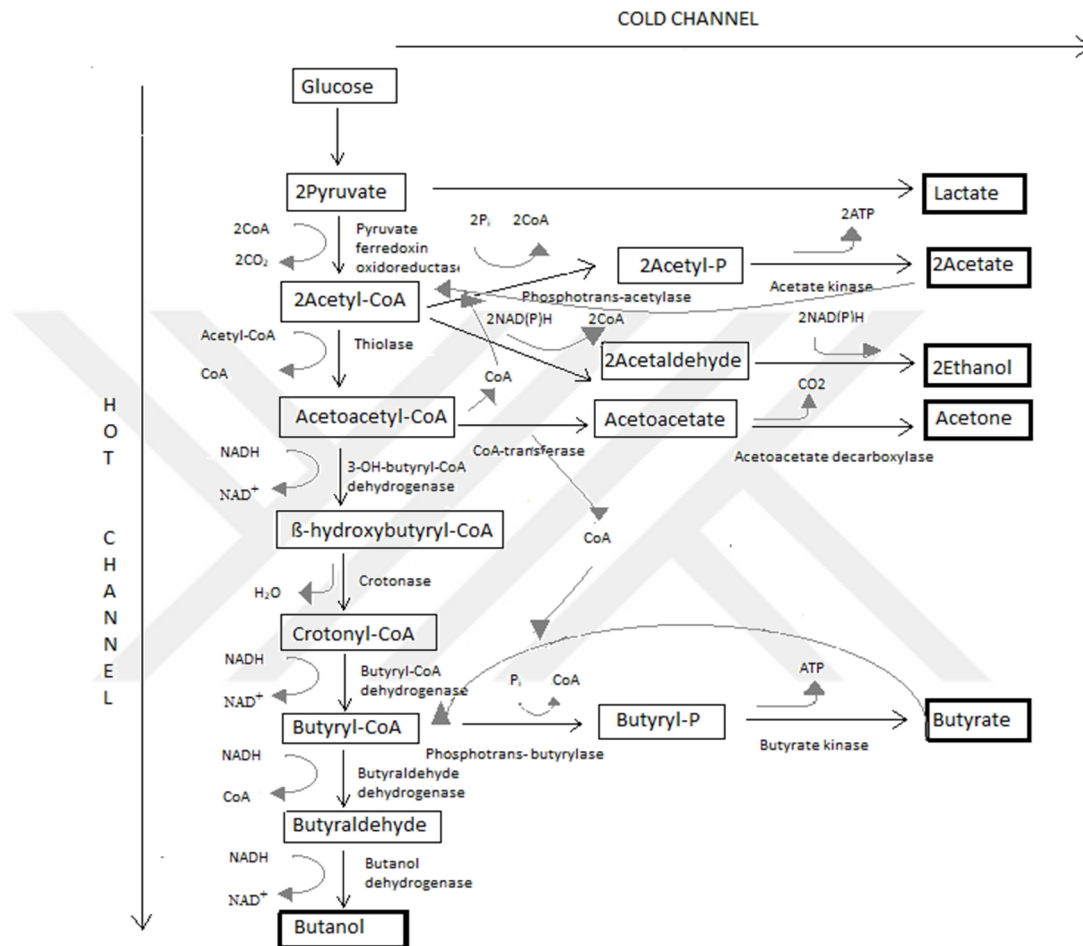


Figure 1.1 ABE fermentation metabolic pathway (García et al., 2011; Gottumukkala et al., 2017; Li et al., 2019; Zhao, Kasbi, Chen, Peres, & Jolicœur, 2017)

1.6 Substrates Used in Butanol Production

C. acetobutylicum can utilize different sugars (simple and complex) to produce solvents (acetone, butanol, ethanol). The simple sugar preference in order of this microorganism is glucose, mannose, arabinose, xylose (Maiti et al., 2016). Availability of xylose and glucose at the same time in the fermentation media may

cause inhibition due to xylose usage. This situation can be explained as carbon catabolite repression (Xin et al., 2018).

Clostridium or genetically engineered microorganisms need one or more substrate for solvent fermentation. These raw materials can be from first generation, second generation, third generation and fourth generation feedstock (Huzir et al., 2018). First generation feedstocks are rich in sucrose and starch. These are potato, corn, wheat, whey, sugarcane, sugar beet etc.. Several obstacles for utilization of the first generation feedstock are expensive and conflict between food and fuel (Ibrahim et al., 2018; Kolesinska et al., 2019). However, amylase which can be excreted from *Clostridium*, degrades starch biomass (Nanda et al., 2017). Second generation feedstocks are lignocellulosic biomass and they can be agricultural, municipal, industrial or forest waste (Kolesinska et al., 2019; Morone & Pandey, 2014). They are not edible and expensive, which help to overcome first generation feedstock's disadvantages (Morone & Pandey, 2014). Lignocellulosic biomass is a quite plentiful carbohydrate (Ibrahim et al., 2018; Jiang et al., 2015). However, some pretreatments of second generation raw material supply usability as substrate for ABE fermentation (Ibrahim et al., 2018; Kolesinska et al., 2019). For instance, the second generation materials are rice, wheat, barley straw, corn cobs, corn stover, sugar cane bagasse, peel of fruits, wood etc. (Green, 2011; Huzir et al., 2018). Algae can be used as third generation feedstock. They are rich in carbohydrates and do not need pretreatment because they do not contain lignin. Studies for algae focus on biodiesel, biogas and bioethanol production (Ibrahim et al., 2018). Classification of microalgae, carbohydrate-rich and lipid-rich microalgae, differs in end product production while, biodiesel can be produced from microalgae's lipid and carbohydrates of micro algae can be utilized for production of biobutanol or bioethanol. Even though fast growing, no requirement of cultivation area due to growing in wastewater or sea water are benefits, high capital cost, fluctuation depending on season are weaknesses of using third generation feedstock (Wang et al., 2017). Photobiological solar fuels and electrofuels are utilized as fourth generation feedstock (Huzir et al., 2018). Studies of converting carbon dioxide into fuel have been continued by photosynthesis ability of algae (genetically modified or engineered) (Raud et al., 2019; Wang et al., 2017).

Biomass selection is significant to comprise of 60-80% of total production cost (Jang et al., 2012; Maiti et al., 2016; Pang et al., 2016). Also, raw materials chosen for biofuel production should be abundant, cheap, renewable, with high carbohydrate content and including low inhibitory compounds (Maiti et al., 2016).

1.6.1 Waste Food as Raw Material

Food wastes comprise huge amounts (approximately 45-55%) of municipal solid wastes (Esteban & Ladero, 2018). Every year, 1.3 billion tonnes of food become waste (Dahiya et al., 2018). Food processing waste can be used for production of ABE (Hegde et al., 2018). One of the alternative way is incineration where food waste is destroyed. This causes environmental problem because dioxin formation can occur depending on high moisture content of wastes (Raganati, Procentese, Olivieri, Russo, & Marzocchella, 2016). Other options of utilizing food wastes can be composting, usage as animal feed, biogas production via anaerobic digestion, bioethanol, biohydrogen (Kolesinska et al., 2019; Ozmihci & Kargi, 2008; Ozmihci, 2017). As value added components, polyphenol, pectin and protein can be extracted from food wastes. Bio-polymers, enzymes, organic acids, single cell protein are obtained from food wastes. Also, food wastes can be used as adsorbents, such as bran from rice, wheat, husk from rice, wheat, shells from coconut, hazelnut, walnut, groundnut, hulls from cotton seed, soybean, peels from apple, banana, orange, stalks from cotton, sunflower, grape etc. (Nayak & Bhushan, 2019). Currently, butanol production with food waste has appeared as environmental friendly biofuel production. They comprise suspended solids, organic matter, nitrogen, oils, and inorganic materials. Characterization of used substrate contributes system optimization which the substrate composition can be stable without seasonal and regional fluctuations (Hegde et al., 2018).

In the literature, *Saccharomyces cerevisiae* is known to be used in ethanol fermentation from wastes. This yeast can merely utilize hexoses. However, *Zymomonasmobilis*, *Pichia rhodanensis* can use pentoses. In a study, 0.98 g ethanol/g substrate was obtained with rice husk by *S. cerevisiae*. Yield of glucose was 49 g/

100 g rice husk. In another study, coffee waste was used and the ethanol yield was 0.74 g/ g by *S. cerevisiae*. In a different study, food waste (23.4 g glucose/ 100 g food waste) was used to produce bioethanol. This study revealed 0.12 g/ g yield of ethanol by *S. cerevisiae* (Nayak & Bhushan, 2019).

Waste materials are utilized to produce biohydrogen as a green fuel. In a study municipal food waste was utilized for biohydrogen production and 370 mL H₂/ g VS was obtained by anaerobic activated sludge. In another study, biohydrogen production was conducted with vegetable wastes resulting with 85.65 mL H₂/ g VS by anaerobic activated sludge (Dahiya et al., 2018). In a continuous stirred-tank reactor (CSTR) biohydrogen production was subjected with 150 days duration and 1.3 days hydraulic retention time and the hydrogen production yield was 283 mL/ g VS with food waste (Nayak & Bhushan, 2019).

There are different biogas producing studies in the literature with fruit and vegetable wastes. Qiao et al. (2011) used thermal pretreated and untreated kitchen waste for biogas production and found that pretreatment enhances biogas production with 18.5% and Lin et al. (2012) found a 0.3 m³ CH₄/ kg dw of methane with mixed fruit-vegetable wastes (Lin et al., 2012; Qiao et al., 2011). High-solids anaerobic digestion with mixture of tomato or grape pomace, dry bovine manure and mature green waste compost at a total solids content of 28% was subjected to fed batch cycles. A methane yield of 201.61 mL/ g dry pomace in mesophilic and 132 mL/ g dry pomace in both thermophilic and mesophilic conditions with a 5% feedstock loading level was evaluated (Achmon et al., 2019). Vinnase is been utilized for biogas production in many studies. The energy consumed from vinnase was used in yeast drying and ethanol distillation. Recently, Parsaee, Kiani, & Karimi (2019) mentioned that 22.4 gigaliter of vinnase are produced throughout the world with a potential of 407.68 gigalitres biogas production (Parsaee, Kiani, & Karimi, 2019). Date palm was evaluated for biomethane production and untreated and diluted (3/ 20) dates resulted a 203 L CH₄/ kg dw of biomethane with recycle of digestate (Lattieff, 2016).

Decrement of fruits and vegetables is 30-40% from farmer to consumer. This amount is quite high (Kolesinska et al., 2019). Non-edible agro industrial waste which are peels, seeds and pulps, consists of 50% of a fruit (Raganati et al., 2016).

1.6.2 Some Food Wastes Used as Substrate in the Thesis

Rice straw is the third most grown crop and mainly in Asia, have a production of annually 731 million tons in the world which is disposed by commonly burning in these countries (Ranjan & Moholkar, 2013; Sen, Chou, Wu, & Liu, 2016). In Japan, 13 million tons of rice straw is produced annually, where it is been utilized as feed for animal or manure (30%), and others of them is remained useless (Kogo et al., 2017). Rice straw is consisted of 37% cellulose, 24% hemicelluloses and 14% lignin. In the literature, rice straw is used abundantly as substrate for biobutanol production. Also, nitrogen, phosphorus, potassium, sulfur content of rice straw makes it a suitable substrate for ABE fermentation (Ranjan & Moholkar, 2013). Rice straw was not only utilized for ABE production, but also for ethanol and hydrogen fermentation (Sen et al., 2016). Pretreatment requirement derives from high lignin percent of rice straw (Ranjan, Khanna, & Moholkar, 2013). Among steam explosion, acid hydrolyzation and enzymatic hydrolyzation after acid pretreated rice straw; the maximum ABE productivity and yield was achieved with enzymatic hydrolyzation after acid pretreated rice straw by *C. acetobutylicum* MTCC 481 (Ranjan & Moholkar, 2013). *Clostridium sporogenes* BE01 has the ability to produce butanol and ethanol without acetone production and Gottumukkala et al. (2013) used this microorganism for butanol production from rice straw. Addition and no-addition mineral experiments were applied to acid pretreated and enzymatic hydrolyzed rice straw. Butanol concentration (3.43 g/ L) in the studies without mineral addition was higher than the studies obtained with addition of minerals to fermentation medium (2.79 g/ L). This result could be derived from excess nutrition because some of minerals in rice straw composition could exist (Gottumukkala et al., 2013).

One of the important crops is wheat and the amount of wheat straw depends on wheat production. Approximately 529 million tons of wheat straw is produced

annually in the world. Wheat straw includes 30–40% of cellulose, 20–25% of hemicellulose, and 15–22% of lignin. Different pretreatment methods were applied for biofuel production from wheat straw. One of them as a new pretreatment method is deep eutectic solvents. Zhao et al. (2018) studied some deep eutectic solvents and they found that pretreatment of strongly basic ethanolamine based deep eutectic solvents more successful than neutral or weakly basic deep eutectic solvents. Among the used deep eutectic solvents, choline chloride: monoethanolamine (C:M) achieved 71.4% of lignin removal and 93.7% of cellulose hold and 89.8% of cellulose conversion and 62% of xylose conversion were achieved (Zhao et al., 2018). In another study, ethanol production conducted with organosolv pretreated and enzymatic hydrolyzed wheat straw by *Saccharomyces cerevisiae*. Ethanol, methanol, butanol, acetone and diethylene glycol was utilized for organosolv pretreatment. Cellulose conversion, ethanol concentration and theoretical yield was 89%, 32.59 g/L and 67.24%, respectively. Ethanol (at 180°C and 40 min) was used as solvent for pretreatment and ended up as the best results. Another good result (29.75 g/L and 65% yield of ethanol) was obtained with diethylene glycol (at 160°C and 40 min) due to nearly same result with lower temperature (Salapa, Katsimpouras, Topakas, & Sidiras, 2017). Bellido et al. (2014) pretreated wheat straw with steam explosion and ozonolysis to compare them and then enzymatic hydrolysis was applied. ABE fermentation of *C. beijerinckii* DSM 6422 was conducted with 6.2 initial pH and without pH control at 35°C and 175 rpm, during 120 hours. Steam explosion of all of hydrolysate (without removal of liquid part of hydrolysate) resulted in 7.21 g/L butanol and 11.44 g/L ABE with 0.36 g/g butanol yield, 0.40 g/g ABE yield as the highest concentrations and yields of ABE, butanol (Bellido, Pinto, Coca, González-Benito, & García-Cubero, 2014). Also, ground wheat residue was used for hydrogen production. In one of the hydrogen production studies, suitable moisture content was found with batch solid state dark fermentation by *C. acetobutylicum* (B-527). 80% of moisture content as the best result ended up with 1222 mL of cumulative hydrogen formation, hydrogen yield of 63 mL H₂/g starch, formation rate of 10.64 mL H₂/g starch/h and specific hydrogen formation rate of 0.28 mL H₂/g biomass/h (Ozmihci, 2017).

Tea production was 5.06 million metric tons in 2013 and tea production consisted of 0.07% of world's agricultural lands (Food and Agriculture Organization of the United Nations). Tea powder waste composition consists of carbon, hydrogen, nitrogen, oxygen with approximately 72.34, 8.23, 4.54, 31.54 (%Total Solids). Tea powder waste was used for biogas production as a co-substrate with fruit, food and vegetable waste. When fruit, food and vegetable waste: tea powder waste: Methanogens in activated sludge ratio was 1:2:1 and experiment in this ratio resulted in high methane yield (0.536 L/ day) at mesophilic conditions and 60 days. High nitrogen ratio of tea powder waste with using as co-substrate showed positive effect on methane production (Thanarasu et al., 2018). Jain et al. (2020) was used waste tea residue as adsorbent for adsorption of anionic dye with batch and continuous experiments. Among obtained results, 127.14 mg/ g was the best dye uptake (Jain et al., 2020).

In 2013, peach [*Prunus persica* (L.) Batsch] production was 21.63 million tons in the world. China, Italy and Spain have produced most of peach and USA and France have been included last years. Rise of peach production in different countries has been continued. Peach consists of vitamins, minerals (K, Ca, Mg, Fe etc.), and some photochemical compounds (phenolic acids, flavonoids, anthocyanins) (Saidani et al., 2017). A large part of produced peach is used for peach juice production. Waste of peach juice includes some part of peach, seeds, skin. Valorization of peach wastes has problems depending on high moisture content. Drying or freezing are solutions for valorization of this waste. Extraction of vitamins and polyphenols is applied as bioactive compounds to valorize peach waste (Plazzotta, Ibarz, Manzocco, & Martín-Belloso, 2020). There are not abundant usage of fruits as substrate for biofuel production (Sanguanchaipaiwong & Leksawasdi, 2018).

Watermelon (*Citrullus lanatus* sp.) production is 3.97 million tons in Turkey. This production consists of 16.32% of total vegetable production according to Turkish Government Statistical Record. Watermelon is rich in vitamins and includes lycopene depending on red colour. Also, phenolics and flavonoids exist in

watermelon juice (Altaş, G. Kızıl, M. Kızıl, Ketani, & Haris, 2011). There are no studies in the literature using watermelon for ABE fermentation.

1.7 Pretreatment Methods

Lignocellulosic biomass can be used as substrate for ABE fermentation due to availability, sustainability, being economical and producing lower emission (Ndaba et al., 2015; Procentese et al., 2017b). Pretreatment cost with different substrates composes between 18-20% of total cost (Morone & Pandey, 2014). Lignocellulose consists of cellulose (35-50%), hemicelluloses (20-35%) and lignin (15-20%) (García et al., 2011; Kumari & Singh, 2018; Morone & Pandey, 2014). Cellulose comprises 10,000 glucose units while hemicellulose includes 50-300 sugar units and is a heteropolymer (Kumari & Singh, 2018). The crystallinity of cellulose increases when the glucose groups are over 15,000 (Huzir et al., 2018). Cellulose (homopolymer) includes glucose oligomers and they can be obtained with hydrolysis of enzymes (endoglucanases, exoglucanases and β -glucosidases) (Amiri & Karimi, 2018). Hemicellulose includes glucose, galactose, mannose, xylose, acids such as acetic, glucuronic, galacturonic acids (Karimi et al., 2015; Kumari & Singh, 2018). Various enzymes are required to attain all sugar types (Kumari & Singh, 2018). *Clostridium*s can excrete enzymes (α -amylase, β -amylase, glucoamylase, α -glucosidase, β -glucosidase, pullulanase, amylopullulanase, saccharase), however their activity does not meet complete hydrolysis to obtain glucose syrup (Amiri & Karimi, 2018; Kolesinska et al., 2019; Nanda et al., 2017). Agricultural wastes, soft woods and hardwoods are lignocellulosic biomass. Dominant sugar in lignocellulosic substrate can vary; such as hexoses are abundant in agricultural wastes and soft woods while xylose is dominant in hardwoods (Amiri & Karimi, 2018; Karimi et al., 2015). Complicated structure of lignocellulosic biomass needs pretreatment of these substrates and it depends on the preferred substrates (Trindade & Santos, 2017). Also, lignin does not include sugar and prevents to reach hemicelluloses and cellulose. There are a lot of pretreatment methods (physical, chemical, physicochemical, biological and combined pretreatment) in order to get simple sugars (pentose,

hexose) for ABE fermentation (Gallego et al., 2015; Jang et al., 2012; Kumari & Singh, 2018).

Mechanical extrusion, milling, ultrasound and microwave can be used as physical pretreatment methods. Milling is usually the first step for processing of the substrate. Milling reduces the particle size, however very fine particle size (0.2 mm) does not provide raise of hydrolysis efficiency (Kumari & Singh, 2018; Vivek et al., 2019). Extrusion consists of blending, heating and shearing for higher than 15-20% humidity of the substrate (Kumari & Singh, 2018). Microwave is another method. Compared to heating, heat distribution for the material is not equal with heating because outside heat is higher than inside. This drawback can be prevented with microwave and it is a simple and energy efficient pretreatment method (López-Linares, García-Cubero, Lucas, González-Benito, & Coca, 2019).

Acid, alkaline, organosolvent, ozonolysis, ionic liquids pretreatments can be used as chemical pretreatment methods. In acid pretreatment, organic and inorganic acids can be used and they can be concentrated or diluted. Dilute sulfuric acid pretreatment is generally preferred in industrial plants for ABE production. However, while acid pretreatment is an important method for reaching simple sugars this method can cause formation of unwilling inhibitory compounds (5-hydroxymethyl furfural (HMF), furfural). Detoxification can be applied in order to remove these compounds. Also, this method needs non-corrosive equipment and neutralization before fermentation. All these negative ways of this method make it expensive. Low temperature can prevent composition of inhibitors (Karimi et al., 2015; Kumari & Singh, 2018; Maiti et al., 2016; Vivek et al., 2019). Alkaline pretreatment removes lignin and provides solubilization of hemicelluloses. Hydrolysis of hemicellulose is not successful in alkali pretreatment while acid pretreatment hydrolyzes 80-90% of hemicellulose (Gallego et al., 2015; Morone & Pandey, 2014; Qureshi et al., 2008b). In this method, sodium hydroxide, calcium hydroxide, ammonium hydroxide, potassium hydroxide may be applied. As well as acid pretreatment, alkaline pretreatment continues with neutralization, but reaction time of this method is longer than acid treatment. Organic solvents such as ethanol, methanol, acetone, ethylene

glycol can be used for lignocellulosic material pretreatment. Accendibility of this solvent is dangerous when this pretreatment is carried out at high temperature (Kumari & Singh, 2018; Vivek et al., 2019). Therefore combustibility, volatility, recovery and price of solvents are negative ways of this method (Raud et al., 2019). Ozonolysis is an effective and an expensive method (Kumari & Singh, 2018; Vivek et al., 2019). Cation and anion liquids can be used for pretreatment process. Notice requirement issues are corrosivity, toxicity and recyclability of ionic liquids (García et al., 2011). As an alternative method for ionic liquids, deep eutectic solvent (DES) which includes two or three ionic compounds can be used due to being low price and environmental friendly (Procentese et al., 2017c).

Steam explosion, liquid hot water, Ammonia Fiber Explosion (AFEX), supercritical CO₂ explosion, wet air oxidation which are physicochemical pretreatment methods, need high temperature and pressure. This process causes high energy consumption. Furthermore; steam, water, ammonia, CO₂ requirement in these methods make them expensive (Morone & Pandey, 2014).

Biological pretreatment occurs with bacteria, consortia of bacterium or fungi (white, brown or soft-rot) (Huzir et al., 2018; Morone & Pandey, 2014). Secretion enzymes from white and soft-rot fungi lead to lignin while brown-rot fungi leads to cellulose (Raud et al., 2019). One or more methods of pretreatment as combined can be selected to get high reachable sugar by organism during ABE fermentation. A successful pretreatment process should ease cost effective ABE fermentation with obtaining high amount of usable sugars for the culture, spending low energy, using low amount of chemicals, supplying low or any amount of composition of inhibitory compounds (HMF, furfural) (Karimi et al., 2015; Kumari & Singh, 2018).

1.8 Detoxification Methods

After pretreatment of lignocellulosic material, furan compounds, aliphatic acids and phenolic compounds may be formed. Furan compounds are furfural, HMF while aliphatic acids are acetic, formic, levulinic acids. Also, phenolic compounds are

ferulic acid, syringaldehyde, p-coumaric acid due to lignin origin and they can harm membrane fluidity due to inhibition effect to the microorganism (García et al., 2011; Huzir et al., 2018; Maiti et al., 2016). 0.04 g/ L of formic acid affects the growth of microorganism and leads to fermentation formation of acid by-products (acid crash) (Amiri & Karimi, 2018; Maiti et al., 2016). However, acetic acid formation (below 10 g/ L) positively affects ABE fermentation (Amiri & Karimi, 2018). 0.5-2 g/ L HMF and furfural is good for microorganisms while over 2 g/ L causes inhibition (Amiri & Karimi, 2018; Maiti et al., 2016). One of upstream process is detoxification. Detoxification causes to be complex, expensive and high usage of water for ABE production. Depending on the selected substrate detoxification method may not be used. This creates positive effect for industrial applicability for ABE fermentation (Jiang et al., 2015). In addition to substrate types, used pretreatment methods affect inhibitor formation (Algayyim et al., 2018). Biological, physical or chemical methods can be applied. Some of them are overliming, adsorption, ion exchange, evaporation, dilution with water, using peroxidase enzymes (García et al., 2011; Gottumukkala et al., 2017).

1.9 Recovery Methods

Recovery and purification of solvents are named as downstream processes (Pugazhendhi et al., 2019; Xue et al., 2017). Produced low concentrations of butanol give rise to increase in cost (Xue et al., 2017). Recovery methods need to be feasible in ABE fermentation, chosen materials or chemicals should be non-toxic to microorganisms, and the method should be simple with high selectivity. Reusability of the chemicals or materials affects positively the cost of the entire process (García et al., 2011; Staggs & Nielsen, 2015). Recovery methods are distillation, adsorption, gas stripping, liquid-liquid extraction, perstraction, pervaporation, reverse osmosis, vacuum stripping, supercritical water and supercritical carbon dioxide (Abdehagh et al., 2014; Kujawska et al., 2015; Nanda et al., 2017, Staggs & Nielsen, 2015).

Distillation is a common method for recovery of solvents. Butanol has a higher boiling point than water (Kujawska et al., 2015; Nanda et al., 2017). In distillation of butanol, 12 t steam is necessary for 1 t of solvent and the distillation cost which consists of 14% of the entire ABE process makes this method not cost-effective (Green, 2011). Also, the low selectivity of distillation causes to search effective and cheap recovery methods (Kujawska et al., 2015).

Adsorption can substantiate with having strength, high capacity, affinity, selectivity, biocompatible adsorbent (Abdehagh et al., 2014; Staggs & Nielsen, 2015; Xue et al., 2017). Some adsorbents used for butanol separation are activated carbon, silicalite, polyvinylpyridine, polymer resins, zeolites (Abdehagh et al., 2014; Gottumukkala et al., 2017; Pugazhendhi et al., 2019). Heat treatment, gas or steam stripping, vacuum application, solvent usage as displacer can be performed for desorption of solvents (Abdehagh et al., 2014; Gottumukkala et al., 2017; Nanda et al., 2017; Pugazhendhi et al., 2019; Staggs & Nielsen, 2015). Other solvents (acetone, ethanol), by-products (acetic acid, butyric acid), nutrients, bacteria can affect butanol adsorption due to competitive for the adsorbents (Nanda et al., 2017; Staggs & Nielsen, 2015; Xue et al., 2017). When compared to distillation, adsorption, gas stripping, extraction/ perstraction, pervaporation, the most energy efficient recovery method is adsorption (Staggs & Nielsen, 2015). Direct addition of adsorbent, packed or fixed bed adsorption or expanded bed adsorption can be applied (Gottumukkala et al., 2017; Staggs & Nielsen, 2015).

Gas stripping consists of utilization of H_2 , CO_2 , N_2 which may be one or mixture of their combinations (Abdehagh et al., 2014; Kujawska et al., 2015; Nanda et al., 2017; Staggs & Nielsen, 2015). N_2 usage is favorable because of creating anaerobic condition for ABE producing microorganisms (Kujawska et al., 2015; Nanda et al., 2017). They can provide formation of bubbles in fermentation media and captured solvents in bubbles occur due to volatility of the solvent. Finally, condensation is applied for recovery of butanol. Advantages of this method are simplicity, non-fouling, ineffective for microorganism (Karimi et al., 2015; Kujawska et al., 2015; Nanda et al., 2017; Staggs & Nielsen, 2015; Xue et al., 2017). Nutrients and formed

acetic acid, butyric acid during acidogenic phase do not affect to recovery of solvents (Kujawska et al., 2015; Xue et al., 2017). Excessive gas usage causes negative situations so, the diameter of bubbles should be between 0.5 and 5.0 mm (Kujawska et al., 2015). Selectivity, energy consumption, antifoam utilization owing to toxic possibility are disadvantages of this process (Abdehagh et al., 2014; Kujawska et al., 2015).

Liquid-liquid extraction can be applied as direct solvent addition or external extraction (Staggs & Nielsen, 2015). Oleyl alcohol, 2-ethyl-1-hexanol, 1-heptanol, 1-octanol, n-decanol, methyl tert-butyl ether, ethyl tert-butyl ether, tert-amyl methyl ether, tert-amyl ethyl ether, 4-n-butylphenol, room temperature ionic liquids, soybean-derived biodiesel, methylated crude palm oil can be utilized as extractant (Abdehagh et al., 2014; Kujawska et al., 2015; Xue et al., 2017). A successful extractant for direct utilization can not be toxic for fermentation broth, expensive and emulsion forming. Also, the partition coefficient can be high (Abdehagh et al., 2014; Kujawska et al., 2015; Nanda et al., 2017; Staggs & Nielsen, 2015; Xue et al., 2017). As toxic solvent, n-decanol should not be utilized alone, it can be mixed with oleyl alcohol owing to being nontoxic (Kujawska et al., 2015).

Perstraction is one of the solvent based recovery methods and includes membrane permeability (Staggs & Nielsen, 2015). Toxicity and emulsion issues which are observable with using liquid-liquid extraction don't arise from perstraction (Abdehagh et al., 2014; Kujawska et al., 2015; Nanda et al., 2017; Staggs & Nielsen, 2015). Depending on membrane usage, fouling can affect recovery negatively (Abdehagh et al., 2014). Polydimethylsiloxane (PDMS) is generally used as membrane while oleyl alcohol, 2-ethyl-1-hexanol, 1-octanol, 1-dodecanol, polypropylene glycol, tributyrin can be used as solvent (Abdehagh et al., 2014; Staggs & Nielsen, 2015).

Pervaporation (integration of pervaporation and evaporation) is based on the permeability of a membrane and thanks to vacuum or gas sweeping that enhances recovery rates. After feed passes through the membrane, the solvent transmits into

the gas state named as permeate. Condensation of the permeate provides to obtain the liquid phase biofuels. When vacuum pervaporation is applied, pressure is low in feed side, high in permeate side (Abdehagh et al., 2014; Brito & Martins, 2017; Gottumukkala et al., 2017; Kamiński et al., 2011; Kujawska et al., 2015; Nanda et al., 2017; Staggs & Nielsen, 2015). The limiting factor of the process to be cost – effective is to obtain low pressure in feed side (Pugazhendhi et al., 2019). This recovery method indicates low energy consumption for the entire process (García et al., 2011; Staggs & Nielsen, 2015). Essential parameters for pervaporation are separation factor and the flux. The separation factor over 30 is good for a successful pervaporation process (Staggs & Nielsen, 2015). Hydrophobic membranes are generally preferred and there are membranes such as; PDMS, polyether block amide (PEBA) and poly(1-trimethylsilyl-1-propyne) (PTMSP), polytetrafluoroethylene (PTFE), silicone, zeolites, composite membranes such as PDMS-silicalite, PDMS-zeolite that are used for butanol recovery in the literature (Nanda et al., 2017; Staggs & Nielsen, 2015). As well as membrane material, thickness of membrane, temperature, permeate side pressure affect achievement of pervaporation. This method does not influence butanol producing microorganisms; however the main problem in this method is fouling of the membrane. Reverse osmosis can be applied aiming recovery of butanol by membrane. Reverse osmosis studies for recovery of solvents are quite limited (Abdehagh et al., 2014). Vacuum stripping is another method for butanol recovery. In this method, pressure is declined above bioreactor headspace. However, this method is not preferable (Staggs & Nielsen, 2015). Also, supercritical water and supercritical carbon dioxide can be used for recovery of solvents (Nanda et al., 2017). All of recovery methods prevents occurrence of butanol toxicity to the microorganisms and aims to achieve high solvent concentrations (Dürre, 2011).

CHAPTER TWO

LITERATURE SURVEY

Important factors in ABE fermentation are medium components, pH, temperature, agitation etc.. Abd-Alla & El-Enany (2012) studied butanol production with using spoilage date palm as substrate. Co-culture of *C. acetobutylicum* ATCC 824 and *Bacillus subtilis* DSM 4451 were used. *Bacillus subtilis* provided anaerobic conditions without N₂ gas requirement and reducing agent. The co-culture produced 21.56 g/ L ABE, 0.42 of ABE yield and 0.30 g/ L h of ABE productivity from 75 g/ L substrate. Usage of NH₄NO₃ or yeast extract increased solvent concentration, ABE yield and productivity. When yeast extract and NH₄NO₃ were added to fermentation medium, ABE concentration increased from 21.56 g/ L to 31.73g/ L with 54.30 g/ L initial glucose (Abd-Alla & El-Enany, 2012). Statistical experimental designs were applied to find the best medium composition for high butanol production. Factors were corn steep liquor, CH₃COONH₄, K₂HPO₄-KH₂PO₄, MnSO₄.H₂O, MgSO₄.7H₂O and FeSO₄.7H₂O. Significant factors were corn steep liquor, CH₃COONH₄ and after other statistical studies, optimized conditions with corn cob hydrolysate produced 11.65 g/ L butanol and 19.22 g/ L ABE by *C. beijerinckii* SE-2. Also, butanol yield and productivity were 0.29 g/ g sugar and 0.16 g/ L h. Scale up of fermentation resulted in similar results (11.92 g/ L butanol, 20.29 g/ L ABE, 0.30 g/ g sugar of butanol yield and 0.17 g/ L h of butanol productivity) (Zhang & Jia, 2018). In another study, electron receptors (KNO₃, Na₂SO₄, and CaSO₄) addition to fermentation broth was applied in small scale with a working volume of 100 mL. Fermentation of *Clostridium acetobutylicum* ATCC 824 with added 2 g/ L of Na₂SO₄ and CaSO₄ resulted in 13.72 and 13.70 g/ L butanol, respectively. Also, these concentrations were higher than the control (10.27 g/ L butanol). They scaled up the fermentor to 7 L and the best butanol concentration was between 12.94 and 12.96 g/ L with addition of 2 g/ L Na₂SO₄ (Ding et al., 2018).

When glucose was utilized as a substrate for ABE fermentation different results were achieved. Batch fermentation of 56.7 g/ L glucose produced 3.9 g/ L acetone, 9.2 g/ L butanol, 1.1 g/ L ethanol, 14.2 g/ L ABE with *C. beijerinckii* P260. ABE

production yield was 0.35 g/ g and ABE productivity was 0.22 g/ L h (Huang, Singh, & Qureshi, 2015). Another study resulted in nearly 9.9 g/ L ABE and 6.5 g/ L butanol from 60 g/ L glucose by *C. acetobutylicum* ATCC 824 (Gallego et al., 2015). Qureshi et al. (2007) studied ABE fermentation in batch mode using 62.0 g/ L glucose. 6.7 g/ L of acetone, 12.7 g/ L of butanol, 0.6 g/ L of ethanol and 20.1 g/L ABE were produced by *C. beijerinckii* P260. 48.9 g/ L glucose was utilized, resulting with an ABE production yield of 0.41 and a productivity of 0.28 g/ L h. Also 100 g/ L glucose (Sugar utilization: 56%) was tried in this study achieving 23.5 g/ L ABE, 0.36 g/ L h ABE productivity and 0.42 ABE production yield (Qureshi, Saha, & Cotta, 2007). Batch fermentations of immobilized *C. acetobutylicum* DSM 1731 shortened fermentation time according to free-cell fermentation when glucose was used as substrate for ABE production. Also, immobilized continuous and fed-batch fermentations were applied in repeated batch experiments. The best butanol and solvent productivity were 1.21 and 1.91 g/ L h with combination of batch and fed-batch immobilized fermentation. In the fermentation of butanol, ABE concentration was 9.12 and 14.53 g/ L, respectively (Dolejš, Krasňan, Stloukal, Rosenberg, & Rebroš, 2014).

Selection of pretreatment method is a critical issue due to effect of total cost of ABE fermentation. In addition to pretreatment method, conditions of selected method affect ABE fermentation. Sulfuric acid hydrolysis was applied to 50 g/ L palm empty fruit bunches with different sulfuric acid concentrations. Yield of reducing sugars were 0.15, 0.90, 0.94, 0.99, 0.99 with 0, 0.5, 1.0, 1.5, and 2.0% of sulfuric acid, respectively. 0.5% sulfuric acid hydrolyzed substrate was selected considering economic and environmental factors. Firstly hydrothermal, acid, base and base with acid treatment were applied. Then cellulose hydrolyzation of pretreated palm empty fruit bunches resulted in maximum reducing sugar with base pretreated and cellulase hydrolysed substrate (Noomtim & Cheirsilp, 2011). Rice straw without pretreatment produced 2.2 ± 0.2 g/ L butanol by co-culture of *C. thermocellum* NBRC 1034000 and *C. saccharoperbutylacetonicum* N1-4. Butanol concentration did not affect cellulase addition. However, alkali (NaOH) pretreated rice straw produced 5.5 ± 0.4 g/ L. Among the added commercial enzymes (cellulase,

β -glucosidase), the highest butanol concentration (6.9 ± 0.3 g/ L) was obtained with cellulase (100U/ g biomass) (Kiyoshi et al., 2015). Municipal solid wastes were used as substrate for ABE fermentation by *C. acetobutylicum* NRRL B-591 and dilute acid or hot water treatment was applied. However, tannin content of municipal solid wastes caused low solvent production. To increase solvent production, acetone, butanol, ethanol and water were utilized as extractant and the most suitable of extractants was ethanol. Ethanol extracted and dilute acid treated liquor of the substrate produced 17 g/ L ABE at the end of the fermentation period (Farmanbodar, Karimi, & Amiri, 2018).

Detoxification for some raw materials is necessary and studies conducted clearly it is due to low concentrations of butanol. In a study, ABE fermentation of barley straw hydrolysate was applied with nearly 60 g/ L total sugar by *C. beijerinckii* P260. Different experiments conducted to show detoxification effect on barley straw hydrolysate (BSH). These studies include ABE fermentation of undiluted/untreated BSH, diluted BSH with water (1:1 ratio), diluted with wheat straw hydrolysate (1:1) and overlimed BSH. In this order, 7.09, 17.42, 17.18, 26.64 g/ L ABE was produced. Same initial concentration of glucose produced 21.06 g/ L ABE. So, overliming was a good detoxification method for production of high solvent concentrations (Qureshi, Saha, Dien, Hector, & Cotta 2010). Alkaline extracted and acid hydrolyzed wood pulp was used for ABE production by *C. beijerinckii* CC101. The most suitable detoxification method was investigated among overliming, activated charcoal adsorption and resin adsorption. Also, some dilution rates were applied to decrease inhibitor effects. Wood pulping hydrolysate of batch fermentation without dilution or detoxification resulted in 4.48 g/ L butanol and 6.73 g/ L ABE while the highest butanol and ABE (9.14 and 11.35 g/ L) was obtained with resin adsorption. When in situ gas stripping as recovery method was applied, obtained butanol and ABE concentration was 13.46 and 17.73 g/ L, respectively (Lu, Dong, & Yang, 2013). Microwave assisted hydrothermal pretreatment was used to brewer's spent grain to produce butanol. Pretreatment liquid produced 1 g/ L butanol by *C. beijerinckii* DSM 6422. While 0.10 g ABE/ g consumed sugar, of ABE yield obtained with pretreatment liquid, 0.20 g ABE/ g consumed sugar, of ABE yield was achieved with

detoxified (activated charcoal) pretreatment liquid. 8.3 g/ L butanol produced with enzymatic hydrolysate of pretreated brewer's spent grain. It is been suggested to produce ABE with higher solid loadings; while in this study the initial sugar concentration was below 26 g/ L (López-Linares et al., 2019).

In ABE fermentation, microorganism is significant because it should be tolerable to high concentration of butanol. *C. acetobutylicum* and *C. beijerinckii* have been used for solvent production (Jin, Qureshi, Wang, & Huang, 2019). Different strains were used in literature to increase produced butanol amount. In one of these studies, the optimum conditions were found as 7.0 of pH, 37.1°C and 1.23 g/ L of butyrate. Obtained butanol concentration was 7.88 g/ L and butanol productivity was 0.244 g/ L h with high butanol tolerant *C. beijerinckii* F-6. This microorganism can tolerate 2.5% (w/ v) butanol (Sheng et al., 2019). In another study, cellulolytic strains are generally used with solvent produced organisms as co-culture. However, *Clostridium cellulovorans* was used in this study after metabolic engineered of this organism. Heterologous bifunctional acetaldehyde/ alcohol dehydrogenase (adhE2) was introduced. Different factors (pH, carbon source, pretreatment method, medium) which affected butanol production were studied. The best result (3.37 g/ L butanol, 0.147 g/ g glucose of butanol yield, 0.046 g/ L h) was obtained with consolidated bioprocessing (pH at 6.5, NaOH pretreated corn cob, DSMZ basal media) (Ou et al., 2017).

ABE fermentation can be produced in different operational modes including batch, fed-batch, continuous with immobilized cells or free cells (Table 2.1). Lipovsky et al. (2016) contrasted different fermentation types which were batch, fed-batch and continuous experiments. Continuous ABE fermentation was applied with *C. pasteurianum* NRRL B-598. Dilution rate was changed during continuous fermentation with packed-bed bioreactor using corncob support material. In this continuous experiment, 0.48 g/ L h butanol productivity which was the best productivity obtained at 0.12 1/ h dilution rate. However, fed-batch experiment with suspended solid resulted in 8.3 g/ L of butanol concentration as the best butanol concentration (Lipovsky et al., 2016). ABE production can be applied with, separate

hydrolysis and fermentation, simultaneous hydrolysis and fermentation, and consolidated bioprocessing. One of study applied alkaline pretreated and enzymatic hydrolyzed (cellulases) King Grass to ABE fermentation. In this study, separated hydrolysis and fermentation (SHF) was compared with simultaneous saccharification and fermentation using *C. acetobutylicum* ATCC 824. SHF of solid hydrolsate of King Grass (78 g/ L sugar) without pH control produced the highest solvent concentration (ABE:18 g/ L) including 10.4 g/ L of butanol (Gallego et al., 2015). Immobilized cell ABE fermentation was used to get high butanol production by *C. acetobutylicum* ATCC 824. Different generation process (solvent casting (plate-like structure), wet jet, electrospinning (fibrous structure)) was applied for poly (3-hydroxybutyrate) (PHB) immobilization material. Electrospinning used PHB had the highest specific surface area (22.45 m²/ g) however the highest butanol yield was 0.28±0.01 g butanol/ g glucose with batch fermentation of wet jet used PHB. In addition to specific surface area, pore size was an important parameter to increase solvent concentration (He, Lee, Kuo, Wu, & Li, 2017). Continuous fermentations with suspended and surface immobilized cells were applied by *Clostridium pasteurianum* DSM 525. Corn stover was used as the immobilization material. Two different dilution rates were 0.13 and 0.44 1/ h for immobilized ABE fermentation while dilution rate was 0.01 h⁻¹ for suspended cells. Continuous suspended cells in ABE fermentation did not reach to the steady state. The highest butanol productivity was 4.2 g/ L h with 0.44 1/ h dilution rate of immobilized cells and 35 g/ L feed glycerol concentration (Gallazzi, Branska, Marinelli, & Patakova, 2015). In a different study, a two-stage consolidated process including hydrogen production and ABE production was applied to wheat straw. The first stage performed with three-species synthetic microbial consortia integrated by epiphytic species of *Enterococcus* in wheat straw xylose showed a maximum hydrogen production yield of 79.5 mL H₂/ g wheat straw xylose. At the second stage the fermented wheat straw xylose enriched with cellulose was utilized to produce ABE; using a co-culture of *Clostridium beijerinckii* 10132 and *Clostridium cellulovorans* 35296 (T: 37°C, 10% TS of fermented wheat straw, pH: 5.5, Fermentation time: 10d). In the first stage the hemicellulose of wheat straw and in the second stage the remaining cellulose was consumed by the consortia. Solvent concentrations at 120 hours were 5.4 g/ L

acetone, 14.2 g/ L butanol, 3.7 g/ L ethanol and 23.3 g/ L ABE with an ABE production yield of 0.39 g/ g (Valdez-Vazquez et al., 2015).

Different recovery methods have been used in the literature to prevent solvent inhibition. Adsorption is one of butanol recovery method which was used to compare adsorbents. Also, immobilized and free-cell experiments, with adsorption and without adsorption were applied. Batch ABE fermentation of *C. beijerinckii* CC101 produced 21.9 g/ L total butanol (adsorbed and remained in the fermentation broth) as the highest result with in situ activated carbon (Norit ROW 0.8) adsorption. Fed-batch, free cell ABE fermentation of *C. acetobutylicum* JB200 produced 31.6 g/ L total butanol while fed-batch immobilized cell ABE fermentation resulted in 54.6 g/ L total butanol. However, butanol yield was same as approximately 0.22 g/ g, butanol productivity of free cell and immobilized cell was nearly 0.30 and 0.45 g/ L h, respectively (Xue et al., 2016). Gas stripping was applied abundantly in the literature. Some studies used this solvent recovery technology is shown in Table 2.1.

Alternative fermentation to ABE fermentation is IB (Isopropanol-butanol) or IBE (Isopropanol-butanol-ethanol) fermentation to obtain all of products having fuel characteristics. Butanol and isopropanol were produced with *C. beijerinckii* under batch conditions by Kim & Day (2010). They found solvent yields of 0.42 and 0.36 g/ g sugar utilized with sugarcane juice, having a sugar content of 5.9%, and molasses, including 4.9% of sugar; respectively (Kim & Day, 2010). In another study, *C. beijerinckii* DSM 6423 which can produce butanol and isopropanol was used in batch experiments. A mixture of sugarcane-sweet sorghum industrial juices (68.1 g/ L total initial sugar) with P2 solution, commercial vitamin complex and yeast extract was used. In situ gas stripping recovery resulted in 10.9 g/ L isopropanol and 7.8 g/ L butanol (Rochón, Cebreiros, Ferrari, & Lareo, 2019). Liquid-liquid extraction was used for IB fermentation. Dodecanoic acid (35%) and oleyl alcohol (24.75%) extractant mixture resulted in 0.53 g/ g IB yield and 0.35 g/ L h IB productivity as the best result among used extractants (Zhang et al., 2017).

Table 2.1 Different ABE fermentation process examples

Substrate	Organism	Pretreatment/ Detoxification	Ezymatic hydrolysis	Operation mode	Recovery	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent productivity (g/ L h)		Initial sugar	References
						A*	B*	E*	ABE*	B	ABE	B	ABE		
Inedible dough	<i>C. beijerinckii</i> NCIMB 8052			Batch		2.78± 0.34	9.26± 0.75	2.44± 0.75	14.44 ±1.37	0.19± 0.01	0.37± 0.02	0.22	0.24± 0.02	nearly 50 g/ L (starch)	Ujor, Bharathidasan, Cornish, & Ezeji, 2014
Breadings						2.78± 0.17	10.47± 0.59	1.56± 0.18	14.80 ±1.03	0.22± 0.01	0.36± 0.02	0.19	0.20± 0.01		
Batter liquid						2.95± 0.52	10.00± 0.34	2.46± 0.15	15.11 ±1.30	0.21± 0.01	0.37± 0.02	0.22	0.31± 0.01		
Wheat straw	<i>C. beijerinckii</i> P260	Acid-(Sulfuric acid)	Cellulase, β- glucosidase, xylanase	Batch	Gas stripping				25.0		0.42		0.60	60.2 g/ L (total)	Qureshi et al., 2007
Wheat straw with 35 g/L glucose									28.2		0.42		0.63	92.9 g/ L (total)	
Wheat straw with 60 g/L glucose									47.6		0.37		0.36	128.3 g/ L (total)	
Wheat straw	<i>C. beijerinckii</i> P260	Alkaline peroxide	Cellulase, β- glucosidase and xylanase	Batch		0.98	1.26	0.35	2.59		0.32		0.04	60.1 g/ L (total)	Qureshi, Saha, Hector, & Cotta, 2008
		Alkaline peroxide Twice dilution as detoxification													
						2.97	8.69	1.14	12.80		0.37		0.18	60.3 g/ L (total)	
		Alkaline peroxide Electrodialysis detoxification				9.34	12.33	0.5	22.17		0.42		0.55	59.6 g/ L (total)	

A* : Acetone, B* : Butanol, E* : Ethanol, ABE* : Acetone+Butanol+Ethanol

Table 2.1 continues

Substrate	Organism	Pretreatment/ Detoxification	Ezymatic hydrolysis	Operation mode	Recovery	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent productivity (g/ L h)		Initial sugar	References
						A	B	E	ABE	B	ABE	B	ABE		
Food waste	<i>C. beijerinckii</i> P260			Batch					18.8- 20.9		0.36- 0.38		0.38- 0.46	81-228 g/ L (food waste)	Huang et al., 2015
					Integrated vacuum stripping				27.2		0.36		0.49	129 g/ L (food waste)	
Wheat straw	<i>C. beijerinckii</i> P260	Acid-(dilute sulfuric acid)	Cellulase, β - glucosidase and xylanase	Fed-batch	Gas stripping	10.57	5.80	0.22	16.59		0.44		0.36	Initially 86 g /L wheat straw Feed I 150 g/L sugar Feed II 206.4 g/ L sugar to keep culture 1.6- 42.5 g/ L sugar (preferable < 35 g/ L)	Qureshi et al., 2008a

Table 2.1. continues

Substrate	Organism	Pretreatment/ Detoxification	Enzymatic hydrolysis	Operation mode	Recovery	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent productivity (g/ L h)		Initial sugar	References
						A	B	E	ABE	B	ABE	B	ABE		
Wheat straw	<i>C. beijerinckii</i> P260	Acid-(dilute sulfuric acid)	Cellulase, β - glucosidase and xylanase	Batch – SHF* with removing sediment		2.72	6.05	0.59	9.36		0.37		0.19	25.4 g/ L (total)	Qureshi et al., 2008b
				Batch- SHF without removing sediment		4.52	8.09	0.77	13.38		0.32		0.14	41.9 g/ L (total)	
				Batch- SSF* without agitation		3.74	7.40	0.79	11.93		0.42		0.27	25.6 g/ L (total)	
				Batch-SSF with additional sugar supplemen tation					17.92		0.40		0.19	51.7 g/ L (total)	
				Batch-SSF	Gas stripping				21.42		0.41		0.31	26.1 g/ L (total)	

SHF*:separate hydrolysis and fermentation, SSF*; simultaneous hydrolysis and fermentation

Table 2.1 continues

Substrate	Organism	Pretreatment/ Detoxification	Enzymatic hydrolysis	Operation mode	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent yield (g/ g raw biomass)	Solvent productivity (g/ L h)	Initial sugar	References
					A	B	E	ABE	B	ABE	ABE	ABE		
Lettuce residues	<i>C. acetobutylicum</i> DSMZ 792	Alkaline- (NaOH)	Cellic CTec2	Batch		1.1		1.44			0.02		19.5 g/ L (total)	Procentese, Raganati, Olivieri, Russo, & Marzocchella, 2017a
Apple peel extracts (peels to water ratio 1/6)	<i>C. acetobutylicum</i> DSMZ 792	Boiling for 30 min. and extraction		Batch		≈14		≈20	0.20	0.28			41 g/ L glucose 36 g/ L fructose 30 g/ L sucrose	Raganati et al., 2016
Substandard and surplus dates	<i>C. beijerinckii</i> ASU10 (KF372577)			Batch	7.40	16.16	0.51	24.07					3.6%	Abd-Alla, Zohri, El- Enany, & Ali, 2015
	<i>C. chauvoei</i> ASU55 (KF372580)				2.95	9.76	1.08	13.79						
	<i>C. roseum</i> ASU58 (KF372581)				5.69	13.87	0.64	20.20						
	<i>C. acetobutylicum</i> ATCC 824				4.62	9.71	0.67	15.01						
Palm kernel cake	<i>C. acetobutylicum</i> YM1	Acid- (Sulfuric acid)		Batch	1.97± 0.1	3.55± 0.03	0.20± 0.008	5.72±0.01		0.24		0.08	30±0.13 g/ L (total)	Al-Tabib, Al- Shorgani, Hasan, Hamid, & Kalil, 2018
		Acid- (Sulfuric acid) Overliming			0.92± 0.0	3.26± 0.05	0.19± 0.01	4.37±0.04		0.17		0.06		
		Acid- (Sulfuric acid) Activated charcoal			1.63± 0.04	4.27± 0.24	0.25± 0.01	6.15±0.26		0.26		0.09		

Table 2.1 continues

Substrate	Organism	Pretreatment/ Detoxification	Enzymatic hydrolysis	Operation mode	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent yield (g/ g raw biomass)	Solvent productivity (g/ L h)	Initial sugar	References
					A	B	E	ABE	B	ABE	ABE	ABE		
Pineapple waste juice	<i>C. beijerinckii</i>			Batch	1.03± 0.07	3.14± 0.16		4.17±0.23	0.08	0.098		0.022	39.11± 0.68 g/ L total sugar of substrate and glucose added to obtain 60 g/ L sugar	Sanguanchaipaiwong & Leksawasdi, 2018
Hydrolyzed apple pomace	<i>C. beijerinckii</i> P260	Acid- (Sulfuric acid)	Cellic CTec 2 and pectinase	Batch				9.6		0.32	0.042	0.10		Jin et al., 2019
		Alkaline (Sodium hydroxide)						9.4		0.34	0.041	0.13		
Hydrolyzed + soluble sugars of apple pomace		Acid- (Sulfuric acid)				7.1		10.7		0.34	0.260	0.22	32.1 g/ L (total)	
		Alkaline (Sodium hydroxide)				7.1		10.8		0.36	0.262	0.23	30.1 g/ L (total)	
Soluble sugars of apple pomace						7.5		10.1		0.33	0.203	0.21	30.6 g/ L (total)	

Table 2.1 continues

Substrate	Organism	Pretreatment/ Detoxification	Ezymatic hydrolysis	Operation mode/ Recovery	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent yield (g/ g raw biomass)		Solvent productivity (g/ L h)		Initial sugar	References
					A	B	E	ABE	B	ABE	B	ABE	B	ABE		
Sweet sorghum bagasse	<i>C. acetobutylicum</i> NRRL B-591	Organosolv- (Acetone) (180°C,60 min)	Cellic CTec2 and HTec2	Batch	3.2	7.1	1.1	11.4		0.31				0.25	36.3 g/ L (total)	Jafari, Amiri, & Karimi, 2016
Rice straw	<i>C. acetobutylicum</i> NRRL B-591	Organosolv- (Ethanol) (180°C,30 min)	Cellulase, β- glucosidase at %8 solid loading from rice straw	Batch	1.9	7.1	1.5	10.5			0.080	0.124		0.20	nearly 23 g/ L (total)	Amiri, Karimi, & Zilouei, 2014
Rice straw	<i>C. acetobutylicum</i> NCIM 2337	Dilute sulphuric acid with shear stress		Batch	6.24	13.5	0.82	20.05	0.33	0.5					23.29 g/ L glucose 39.88 g/ L (total)	Ranjan et al., 2013
Rice straw	<i>C. acetobutylicum</i> NRRL B-591	Acid- (Phosphoric acid)	Cellulase, β- glucosidase	Batch	0.60	1.40	<0.1				0.044				192.3 g glucose/ kg straw	Moradi, Amiri, Soleimanian- Zad, Ehsani, & Karimi, 2013
		Alkaline- (NaOH)			0.80	2.00	0.05	2.85			0.045	0.063			163.5 g glucose/ kg straw	
		Untreated				0.16					0.009	0.064			101.8 g glucose/ kg straw	
Corncoobs	<i>C. saccharobutylicum</i> DSM 13864	Alkaline- (NaOH) (washed and without sediment removal)	Cellic CTec2	Batch	6.86	12.27	0.32	19.44		0.35				0.54	55.22 g/ L (total)	Gao & Rehmann, 2014

Table 2.1 continues

Substrate	Organism	Pretreatment/ Detoxification	Ezymatic hydrolysis	Operation mode/ Recovery	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent yield (g/ g raw biomass)		Solvent productivity (g/ L h)		Initial sugar	References
					A	B	E	ABE	B	ABE	B	ABE	B	ABE		
Brown seaweed <i>Laminaria digitata</i>	<i>C. beijerinckii</i> DSM-6422		Cellulases	Batch	0.85	7.16	0.12	8.13	0.42		0.27				47.8% glucose (the equivalent from glucans)	Hou et al., 2017
Wood	<i>C. acetobutylicum</i> ATCC 824	Steam explosion combined with hot water	Hydrolysis with <i>Paenibacillus campinasensis</i> DSM 21989			6.0		10.3					0.052	0.0887	60 g/L xylose (added xylose to existence xylose)	Theiri, Chadjaa, Marinova, & Jolicoeur, 2020
		Steam explosion combined with hot water -Chemical detoxification using ferric sulfate -Biological detoxification using co- culture of <i>Ureibacillus thermosphaeri</i> cus NCIMB 13819 and <i>Cupriavidus taiwanensis</i> DSM 17343		Separate bio- detoxification and biohydrolysis		1.1							0.009	0.0173		
				Simultaneous bio- detoxification and biohydrolysis	4.2	6.8	0.8	11.8					0.058	0.1020		

Table 2.1 continues

Substrate	Organism	Pretreatment/ Detoxification	Ezymatic hydrolysis	Operation mode/ Recovery	Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent yield (g/ g raw biomass)		Solvent productivity (g/ L h)		Initial sugar	References
					A	B	E	ABE	B	ABE	B	ABE	B	ABE		
Mixed feedstock of invasive weeds	<i>C. acetobutylicum</i> MTCC 11,274	Dilute acid and alkaline delignification	Cellulase, β - glucosidase	Batch-control mechanical shaking	0.76 $\pm$ 0.02	5.81 $\pm$ 0.36	3.04 $\pm$ 0.04	9.61 $\pm$ 0.38	0.15	0.376		0.168		0.08	50 g/ L (total) including 22.7 g/L pentose sugars and 27.7 g/ L hexose sugars	Borah et al., 2019
				Batch-test Intermittent sonication (%10 duty cycle) + mechanical shaking	4.56 $\pm$ 1.01	9.10 $\pm$ 1.3	2.82 $\pm$ 0.29	16.5 $\pm$ 2.6	0.23	0.645		0.288		0.18		
Sugarcane- sweet sorghum juices	<i>C. acetobutylicum</i> DSM 792			Batch	5.9 $\pm$ 0.6	10.5 $\pm$ 1.0	1.20 $\pm$ 0.1	17.6 $\pm$ 1.8	0.18 $\pm$ 0.01				0.10 $\pm$ 0.01	0.17 $\pm$ 0.02	76 $\pm$ 1 (total)	Rochón, Ferrari, & Lareo, 2017
				Fed-batch In-situ gas stripping	10.0 $\pm$ 1.0	18.6 $\pm$ 1.8	3.30 $\pm$ 0.4	31.8 $\pm$ 3.2	0.16 $\pm$ 0.01				0.13 $\pm$ 0.01	0.22 $\pm$ 0.02	59 $\pm$ 1 g/ L total sugar and total sugar added during fed-batch mode 39g/ L	

Table 2.1 continues

					Solvent concentration (g/ L)				Solvent yield (g/ g consumed substrate)		Solvent yield (g/ g raw biomass)		Solvent productivity (g/ L h)			
Substrate	Organism	Pretreatment/ Detoxification	Ezymatic hydrolysis	Operation mode/ Recovery	A	B	E	ABE	B	ABE	B	ABE	B	ABE	Initial sugar	References
Cassava bagasse	<i>C. acetobutylicum</i> JB200		Glucoamylase, cellulase	Batch	4.33	9.71	1.37	15.41	0.22	0.34			0.24	0.39	44.8 g/ L glucose	Lu, Zhao, Yang, & Wei, 2012
Cassava bagasse + glucose					10.23	20.32	3.32	33.87	0.24	0.39			0.37	0.62	86.2 g/ L glucose	
Cassava bagasse				Fed-batch fibrous bed bioreactor with simultaneous gas stripping	25.72	59.81	4.78	90.31	0.25	0.37			0.35	0.53	244.6 g/ L glucose utilized	
				26.98	76.44	5.09	108.5	0.23	0.32			0.29	0.41	336.6 g/ L glucose utilized		
Corn stover bagasse	<i>C. acetobutylicum</i> ABE-P 1201	Alkaline- (NaOH)	Cellulase	Fed-batch Immobilization with in situ gas stripping	7.3	18.6	2.4	28.3		0.29	0.055	0.083		0.13	97.6 g/L total sugar	Cai et al., 2017

CHAPTER THREE

MATERIAL AND METHODS

3.1 Experimental Set-Up and Procedure

3.1.1 Batch Experiments

In the first batch experiments tea waste, watermelon peel and glucose were used as substrate. *Clostridium acetobutylicum* NRRL 527 and *Clostridium beijerinckii* DSM 791 were used as organisms. There were two repetitions in the batch experiments. Fermentations were applied in 500 mL erlenmeyer flasks with 200 mL working volume and including 20 mL of inoculums (OD: 0.4). The substrates containing moisture were acid hydrolyzed (121°C, 15 min.) to achieve a constant initial sugar concentration around 3000 mg/L. Table 3.1 shows fermentation media 1 components which was used in this batch experiment. Fermentations were carried out at 30°C with an agitation speed of 75 rpm and the initial pH was set to nearly 7.2. The pH was adjusted to over 6 if needed with 10 M NaOH or 10% H₂SO₄ solution.

In the second batch experiments, tea waste, peach waste, non-hydrolyzed peach waste (peach waste without hydrolysis), wheat waste, rice husk were utilized for batch experiments. 5 g dry weight substrate was used in the experiments. Also, as control experiments glucose with approximately 50 g/ L total sugar concentrations and fructose with 5 g/ L total sugar concentrations were used. Table 3.1 shows a modified fermentation media called as fermentation media 2 components which was used in this batch experiment. *Clostridium acetobutylicum* NRRL 527 and *Clostridium beijerinckii* DSM 791 were used to produce butanol. Fermentation was carried out at 37°C without agitation. Working volumes were 125 mL including 25 mL organism at 250 mL serum bottles. Table 3.2 shows fermentation ambient conditions differences. The protein concentrations were 5214.79 mg/ L for *C. acetobutylicum* and 5236.03 mg/ L for *C. beijerinckii* at the tested substrates of tea waste, peach waste, wheat waste, rice husk, and glucose. The protein concentrations

were 6266.61 mg/ L for *C. acetobutylicum* and 6408.10 mg/ L for *C. beijerinckii* at ABE fermentation of fructose and non-hydrolyzed peach waste.

For all batch experiments, anaerobic conditions were supplied with nitrogen gas passing through fermentation bottles for 10 min. In the second experiments after the fermentation media was sterilized, 0.1 % butanol and 0.1 % ethanol were added to the fermentation medium to trigger the metabolic pathway to solvent production. The experimental results were justified with the added ethanol and butanol concentrations. The fermentations were carried out for 7 days. Samples were taken from fermentation medium daily. Samples were centrifuged at 7000 rpm and 15 min. Samples were kept at 4°C for further experiments. Total sugar, protein/(Suspended solid matter -610 nm Abs-Protein), pH, ORP, simple sugars, volatile fatty acids, solvents were measured. Also, furfural, 5-hydroxymethyl furfural were measured in the second batch fermentation.

Table 3.1 Fermentation media 1 and media 2 components which were used in batch experiments

Fermentation Media 1 (Ozmihci & Kargi, 2011)	Fermentation Media 2 (Hou et al., 2017; Lu et al., 2013; Procentese et al., 2017a, Ozmihci & Kargi, 2011).
2 g/ L nutrient broth	10 g/ L peptone
0.5 g/ L yeast extract	3 g/ L yeast extract
2 g/ L (NH ₄) ₂ SO ₄	3 g/ L sodium acetate
3.9 g/ L KH ₂ PO ₄	1 g/ L NaCl
2.8 g/ L K ₂ HPO ₄	2 g/ L (NH ₄) ₂ SO ₄
0.25 g/ L MgSO ₄ .7H ₂ O	3.9 g/ L KH ₂ PO ₄
0.2 g/ L L-Cysteine	2.8 g/ L K ₂ HPO ₄
0.1 g/ L FeSO ₄ .7H ₂ O	0.25 g/ L MgSO ₄ .7 H ₂ O
	0.1 g/ L FeSO ₄ .7 H ₂ O
	0.01 g/ L MnSO ₄ .H ₂ O
	0.2 g/ L L-cysteine
	4mL/ L of % 0.025 resazurin
	0.1 % butanol
	0.1 % ethanol

Table 3.2 Fermentation ambient conditions differences

Batch experiments	
Ambient conditions of 1. experiment (Gao & Rehmann, 2014; Kiyoshi et al., 2015; Zhang & Jia, 2018)	Ambient conditions of 2. experiment (Pang et al., 2016; Sheng et al., 2019)
30°C with 75 rpm agitation speed initial pH 7.2 10% inoculum volume	37°C without agitation initial pH 7 (adjusted to pH 6 in the experiments) 20% inoculum volume

3.1.2 Fed-Batch Experiments

Fed-batch experiments were conducted with different dilution rates (0.0028, 0.0056, 0.0084, 0.0113, 0.0141, 0.0169 1/ h) to produce ABE. Fermentations were applied in a 10 L serum bottle with 3 L working volume and 1 L of inoculums. Figure 3.1 shows schematic diagram of fermentor used in fed-batch experiments. *C.beijerinckii* -DSMZ-791 was used for production of solvents. Acid hydrolysis was applied to peach waste (60g/ L) at 90°C and 30 minutes at pH 2. Supernatant of hydrolyzed peach waste was used as substrate at fed-batch experiments. The feed medium included hydrolyzed peach waste supernatant and 3 g/ L yeast extract , 3 g/ L sodium acetate, 1 g/ L NaCl, 2 g/ L (NH₄)₂SO₄, 3.9 g/ L KH₂PO₄, 2.8 g/ L K₂HPO₄, 0.25 g/ L MgSO₄.7H₂O, 0.1 g/ L FeSO₄.7H₂O, 0.01 g/ L MnSO₄.H₂O, 0.2 g/ L L-cysteine. The feed was autoclaved at 121°C for 15 min. Nitrogen gas was passed through the reactor for two hours. Fed-batch experiments were carried out at 37°C with an agitation of 50-100 rpm. Initial pH of fed-batch fermentations was 6.5 and the pH of the feed was 7. The pH was adjusted to 6 periodically with 10M NaOH. Samples were taken from fermentation medium daily. The samples were centrifuged at 7000 rpm for 15 min and the supernatant was kept at 4°C for further analysis. Total sugar, pH, simple sugars, volatile fatty acid, solvent were measured. Also, another sample was taken for protein analysis daily.

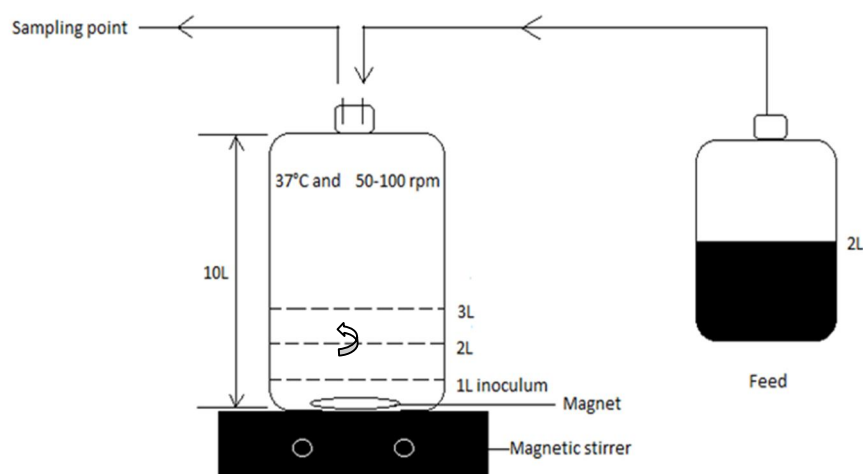


Figure 3.1 Schematic diagram of fermentor used in fed-batch experiments

3.2 Organisms

C. acetobutylicum-NRRL 527 and *C. beijerinckii* -DSMZ-791 were achieved from Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH (DSMZ) in lyophilized form.

For the first batch experiments, microorganisms growing medium were consisted of fermentation media 1 containing 7.5 g/ L glucose. The mediums were sterilized at 121°C for 15 minutes. The cultures were grown at 75 rpm and 30°C with an initial pH of 7 for approximately 60 h. Inoculum volume was 10% v/ v. Figure 3.2 shows calibration of suspended solid and protein concentration with optic density (OD) at abs (610 nm) for *C. acetobutylicum*. Figure 3.3 shows calibration of suspended solid and protein concentration with OD at abs (610 nm) for *C. beijerinckii*.

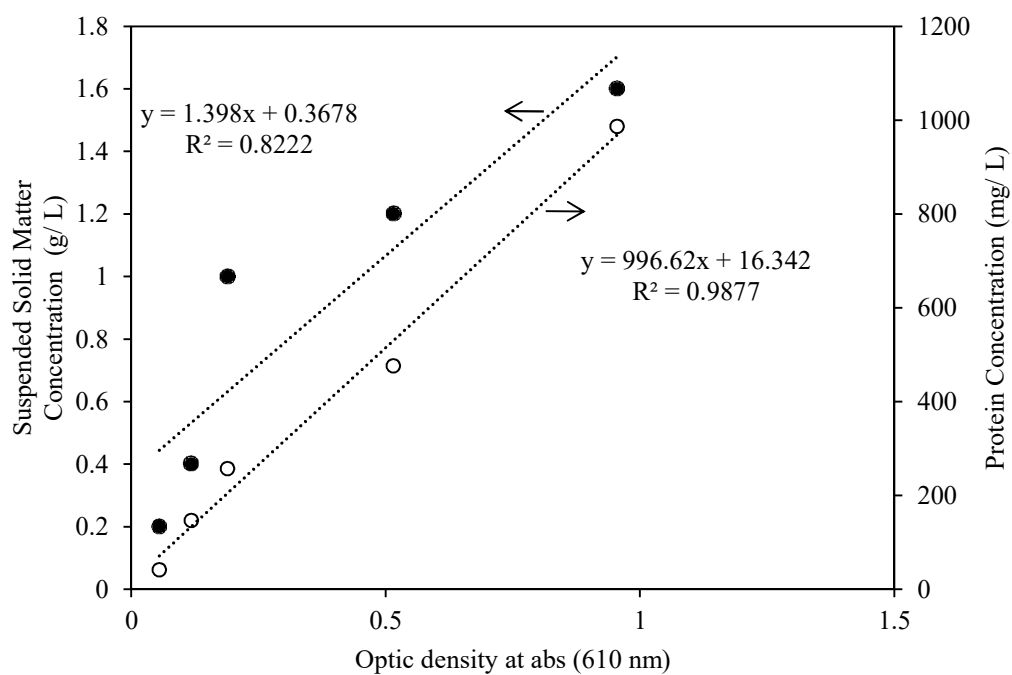


Figure 3.2 Calibration of suspended solid and protein concentration with abs (610 nm) for *C. acetobutylicum*

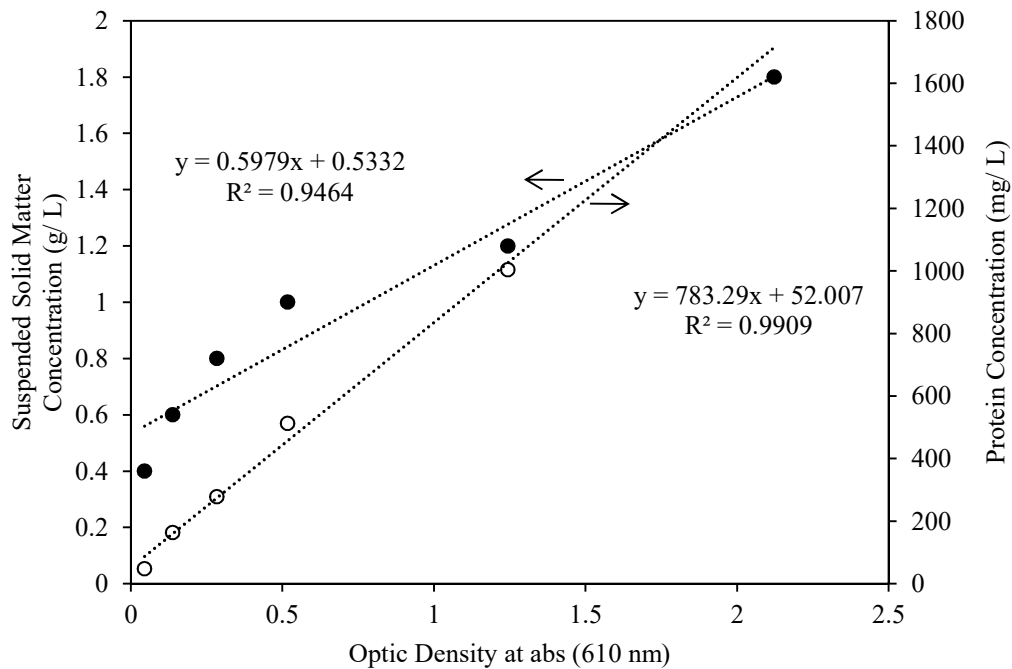


Figure 3.3 Calibration of suspended solid and protein concentration with OD at abs (610 nm) for *C. beijerinckii*

For other batch experiments and fed batch experiments, the microorganisms were cultivated on a medium containing fermentation media 2 with 12.5 g/ L glucose. After sterilization, the cultures were grown anaerobically at 37°C with an initial pH of 7. Inoculum volume was 20% and the culture was grown approximately 60 h. The cultivated cultures were used as inoculums in the experimental set-up.

3.3 Substrate Preparation

In the first batch experiments, tea waste and watermelon peel were used without drying. The moisture content of tea waste and watermelon peels were approximately $82.48 \pm 0.20\%$ and $92.60 \pm 1.25\%$, respectively. The moisture content results of Precise XM50 humidity device were verified with suspended solid method. Watermelon peels were chopped into small pieces. 5 g/ 200 mL of raw substrate including moisture was acid hydrolyzed with H_2SO_4 at pH 2 in an autoclave at 121°C for 15 min. Then different substrate concentrations were used to obtain approximately 3000 mg/ L constant soluble total sugar in the fermentation media. 180 mL supernatant of the hydrolyzed substrate was used in the experiments.

In the second set of batch experiment, tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk, glucose, fructose were the substrates. Acid hydrolysis (Hydrolysis conditions: pH=2, 121°C ,15 min, 100 mL) was applied for 5 g dry weights of tea waste, peach waste, wheat waste, rice husk. All the substrates were measured in dried form.

In the fed-batch experiments, peach waste was grinded (74-300 μm) to obtain high amount of fermentable sugar for ABE fermentation. 60 g/ L of peach waste was acid hydrolyzed with sulphuric acid and pH was adjusted to 2. Then, it was autoclaved at 90°C and 30 minutes. After 2 L of supernatant of hydrolyzed peach waste was obtained, other chemicals were added to the feed.

3.4 Analytical Methods

Samples were taken from fermentation medium periodically and were centrifuged at 7000 rpm for 15 minutes. Then analyzes were conducted to determine total sugar, protein, saccharides, volatile fatty acids, solvents concentrations and the pH, ORP values of the samples were measured instantly (WTW). Total sugar concentration (TS) of samples was determined by using acid-phenol method. 1 mL sample, 1 mL of 5% phenol solution and 5 mL pure H₂SO₄ were added into glass tube and mixture of them stayed at 25-30 °C. After 20 minutes, absorbance value of samples were read at 480 nm (Dubois, Gilles, Hamilton, Rebers, & Smith, 1956). ABE (acetone-butanol-ethanol), total volatile fatty acids (TVFA) and saccharides concentrations of samples were determined by using a high performance liquid chromatography (HPLC, Agilent 1100) with a mobile phase of 5 mM H₂SO₄ with a flow rate of 0.6 mL/ min, organic acid analysis column (Aminex HPX-87H Ion Exclusion Column) 300 mm x 7.8 mm and a UV detector of 210 nm. Column temperature was 40°C for ABE analyzes and 50°C for TVFA and saccharide analyzes. RID was used for ABE and saccharides analysis and DAD was used for TVFA analysis (Islam, Cicek, Sparling, & Levin, 2009). Also, furfural-HMF analysis were conducted with the same high performance liquid chromatograph (HPLC, Agilent 1100), 150 mm x 4.6 mm C18 column and a UV detector of 284 nm was used with a mobile phase of %5 acetonitril and a 0.5 mL/ min of flow rate (García-Villanova, Guerra-Hernández, Martínez-Gómez, & Montilla, 1993; Gomis, Alvarez, Naredo, & Alonso, 1991; Rufian-Henares, Garcia-Villanova, & Guerra-Hernandez, 2001; Theobald, Müller, & Anklam, 1998). Modified Lowry Protein assay kit (Thermo Scientific) was used to determine protein concentrations of fermentation medium. At suspended solid analysis, filter paper was dried at 105°C for two hours in the drying oven. The sample was filtered by vacuum pump. After it was dried in the oven, it was kept in the desiccators for 30 minutes. The biomass concentration was obtained from weight difference depending on volume of the filter paper owning the biomass and the tarred filter paper (Greenberg, Trusell, & Clesceri, 1989). Humidity for watermelon peel and tea waste were measured with Precise XM50 and with suspended solid method.

3.5 Theoretical Background

- Biomass concentration

$$X = \frac{A-B}{N} * 1000 \quad (3.1)$$

X=Biomass concentration (g/ L)

A=Weight of filter paper with sample (g)

B=Weight of filter paper (g)

N=Volume of sample (mL)

- Solvent (ABE, acetone, butanol or ethanol) Production Yield (Y)

$$Y_{P/S} = (\Delta P) / (S_o - S_e) \quad (3.2)$$

Y= ABE, acetone, butanol or ethanol / initial substrate (g/ L)

ΔP = ABE, acetone, butanol or ethanol production (g/ L)

S_o = Initial substrate/ total sugar concentration (g/ L)

S_e = Final substrate/ total sugar concentration (g /L)

- Volumetric ABE Production Rate (VABEPR)

$$VABEPR = \frac{C_{ABE, \text{ acetone, butanol and ethanol}}}{\Delta t} \quad (3.3)$$

VABEPR= Volumetric ABE, acetone, butanol and ethanol production rate (g/ L h)

$C_{ABE, \text{ acetone, butanol and ethanol}}$ = Solvent (acetone+ butanol+ ethanol) concentration (g/ L)

Δt = Fermentation time (h)

- Volumetric Acetone Production Rate (VAPR)

$$\text{VAPR} = \frac{C_{\text{acetone}}}{\Delta t} \quad (3.4)$$

VAPR=Volumetric acetone production rate (g/ L h)

- Volumetric Butanol Production Rate (VBPR)

$$\text{VBPR} = \frac{C_{\text{butanol}}}{\Delta t} \quad (3.5)$$

VBPR= Volumetric butanol production rate (g/ L h)

Liquid volume (V) increased throughout fermentation due to no removal of effluent and fed-batch operation conditions. The initial liquid (V_0) including a dense biomass for fermentation started with 1L and a constant flow rate (Q) was added to the fermentation media in time (t) to obtain a final liquid volume of 3 L. Theory of fed-batch operation (Echegaray et al., 2000) and equations are shown below:

$$V = V_0 + Qt \quad (3.6)$$

By controlled addition of feed, the substrate concentration remains at a low level in the fermentor named ‘Quasi-Steady-State’ at which approximately $dS/dt = 0$, $dX/dt = 0$ and $dP/dt=0$. At quasi-steady-state:

$$\mu = D = \frac{1}{Q_H} \quad (3.7)$$

D = Dilution rate ($Q/V = \Theta_H$) (1/h)

CHAPTER FOUR

RESULTS AND DISCUSSION

4.1 Batch Experiments

4.1.1 ABE Fermentation with Tea Waste, Watermelon Peel and Glucose as Substrate with Fermentation Media 1

In the first batch experiment, three substrates were subjected to two different ABE producing bacteria, *C. acetobutylicum* NRRL 527 and *C. beijerinckii*-DSMZ-791 to investigate the ABE production capacity. Fermentation media 1 was used in this experiments which is explained in Table 3.1. Humidity percents of tea waste and watermelon peel were $82.48 \pm 0.20\%$ and $92.60 \pm 1.25\%$, respectively. ABE fermentation was applied with humid substrates to consider a feasible process. A constant total sugar concentration after pretreatment was applied which was approximately 3000 mg/ L. 26.28 g/ L dw tea waste (TS: 2300-3400 mg/ L, 150 g/ L tea waste) and 15.17 g/ L dw watermelon peel (TS: 2800-3700 mg/ L, 205 g/ L watermelon peel) were used to obtain this sugar concentrations. The ABE fermentation was conducted for 168 hours at 30°C with 75 rpm in an incubator shaker with an inoculum volume of 10% at 200 mL working volume where 500 mL erlenmeyer flasks were sealed with silicone rubbers. The initial pH at the experiments was 7.2.

4.1.1.1 Clostridium acetobutylicum

The initial sugar concentration in this experiment was approximately 3000 mg/ L. The sugar concentration decreased with time in all tested substrates. Figure 4.1 shows the sugar consumption for tea waste, watermelon peel and glucose at the end of the fermentation time with *C. acetobutylicum*. The highest sugar consumption (85.53%) was obtained with glucose while the lowest was 31.41% at tea waste. 54% sugar consumption in watermelon peel was observed. Figure 4.2 depicts variation of pH with time for tea waste, watermelon peel and glucose with *C. acetobutylicum*. At

the first 48 hours, pH of fermentation mediums decreased. The decrease of pH at the first hours is due to acidogenic phase where organic acid formation occurs. At 72 hours of fermentation, pH of medium slightly increased and was nearly stable till the end of the fermentation period.

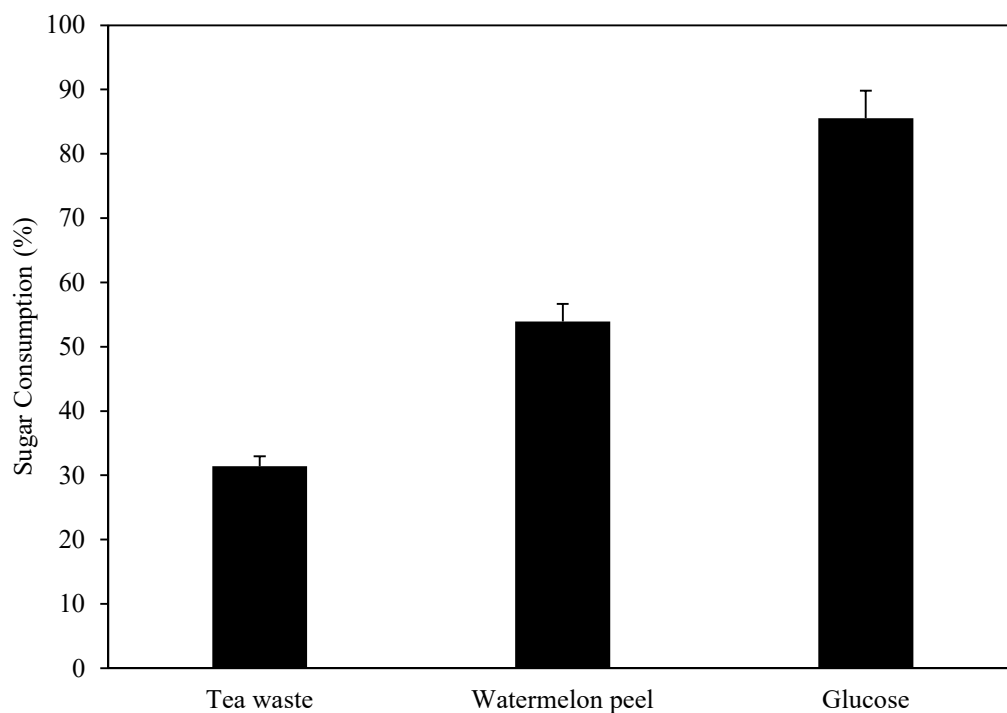


Figure 4.1 Sugar consumption for tea waste, watermelon peel and glucose with *C. acetobutylicum*

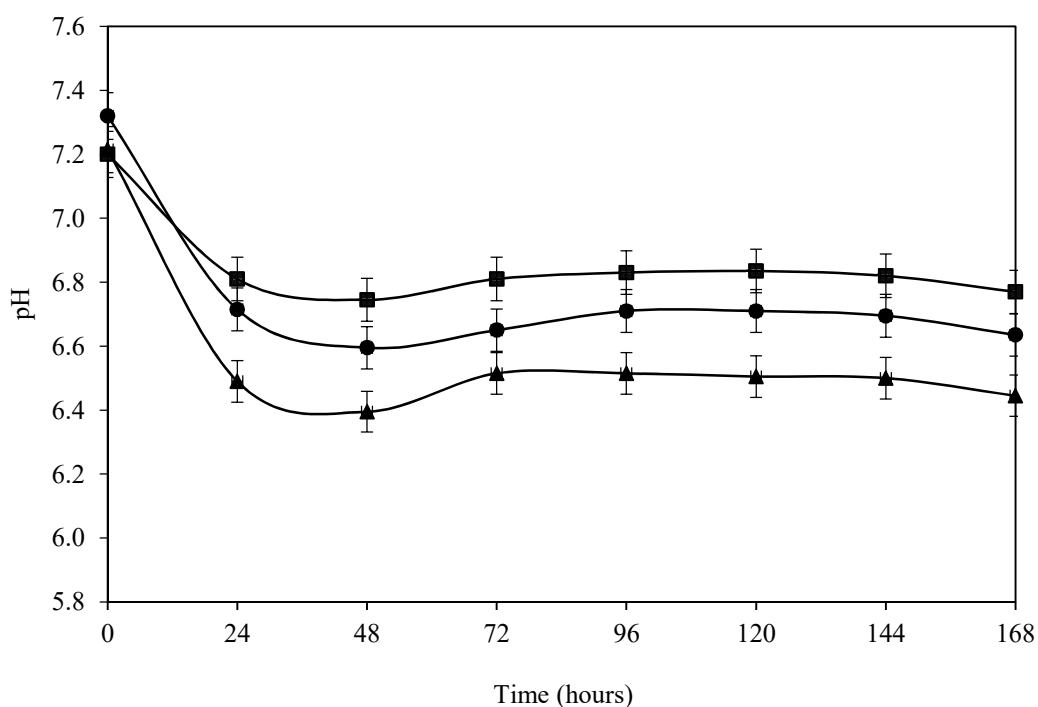


Figure 4.2 Variation of pH with time for tea waste, watermelon peel and glucose with *C. acetobutylicum* (● Tea waste, ■ Watermelon peel, ▲ Glucose)

Variation of suspended solid concentration with time for tea waste, watermelon peel and glucose with *C. acetobutylicum* is given in Figure 4.3. The highest suspended solid concentration was 9.45 g/ L with tea waste and watermelon peel (3.64 g/ L) and glucose (2.75 g/ L) followed it, respectively. These results were observed with OD measurements which were calibrated with protein concentrations. The highest protein concentrations in tea waste, watermelon peel and glucose were 4299 mg/ L, 2351 mg/ L and 1711 mg/ L, respectively.

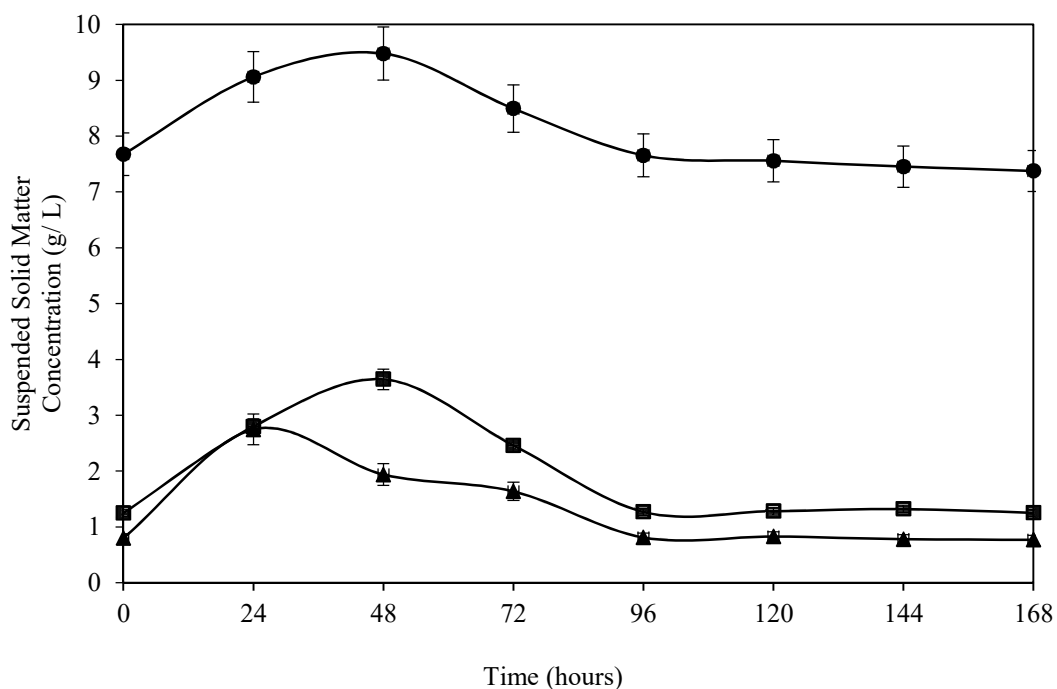


Figure 4.3 Variation of suspended solid concentration with time for tea waste, watermelon peel and glucose with *C. acetobutylicum* (● Tea waste, ■ Watermelon peel, ▲ Glucose)

Solvent production starts with acidogenic phase where the volatile fatty acids are formed. Acetic acid, butyric acid, propionic acid and lactic acid are the major organic acids that can be metabolized. Stoichiometrically the organic acids produced from one mol glucose are shown below (Seppälä, Puhakka, Yli-Harja, Karp, & Santala, 2011; Xu et al., 2018):

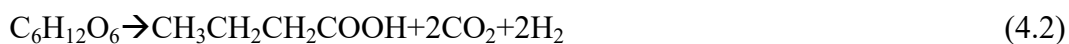
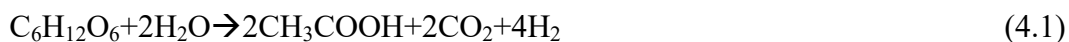


Figure 4.4 shows volatile fatty acid concentration for tea waste, watermelon peel and glucose at the end of fermentation with *C. acetobutylicum*. Butyric acid formation did not occur in tea waste, watermelon peel and glucose when *C.*

acetobutylicum was used. Acetic acid formation was seen in the fermentation media. The highest acetic acid concentration was 736.40 mg/ L with watermelon peel. The result showed that acetic acid was converted into ethanol after some time and the ethanol concentration in watermelon peel was 283 mg/ L. The remaining acetic acid concentration in the fermentation media was high cause of low conversion to solvents. The tea waste showed the same trend with watermelon peel. Some of the acetic acid was converted into ethanol and butyric acid was not produced. 487 mg/ L ethanol was produced due to acetic acid formation which was 417 mg/ L at the end of the fermentation period with glucose. Figure 4.5 shows solvent concentrations for tea waste, watermelon peel and glucose with *C. acetobutylicum* in this experiment. ABE fermentation of tea waste, watermelon peel and glucose by *C. acetobutylicum* resulted with 0.34 g/ L, 0.28 g/ L, and 0.49 g/ L, respectively. Among the solvents, ethanol was the dominant solvent obtained in the fermentation media. Acetone and butanol production were not observed, all the produced solvent was ethanol.

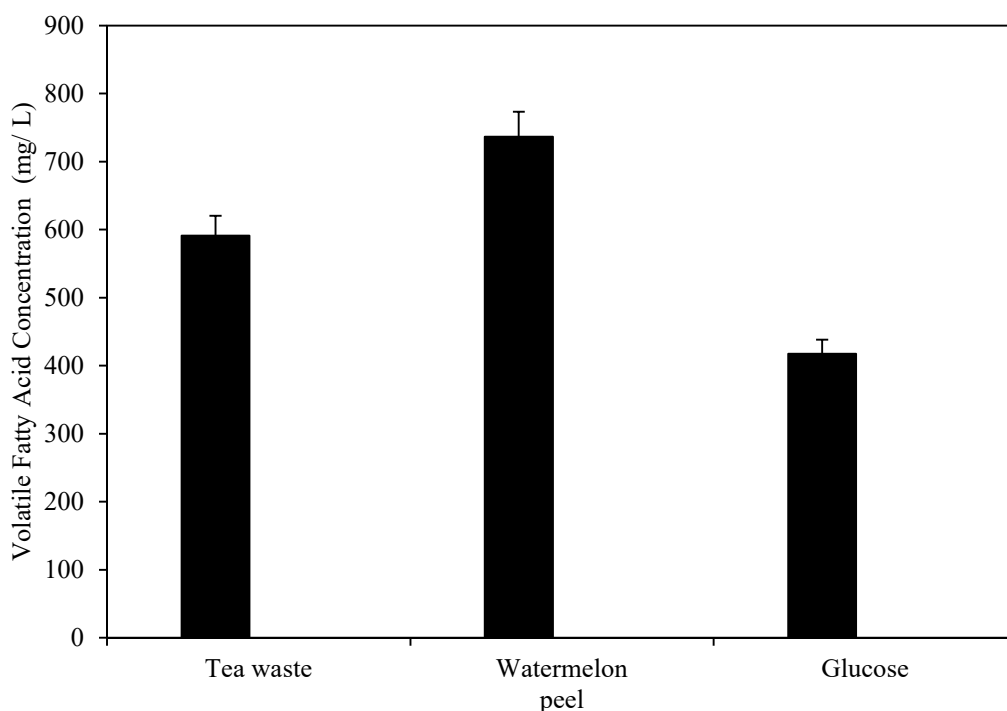


Figure 4.4 Volatile fatty acid concentration for tea waste, watermelon peel and glucose at the end of fermentation with *C. acetobutylicum* (■ Acetic acid, ■ Butyric acid)

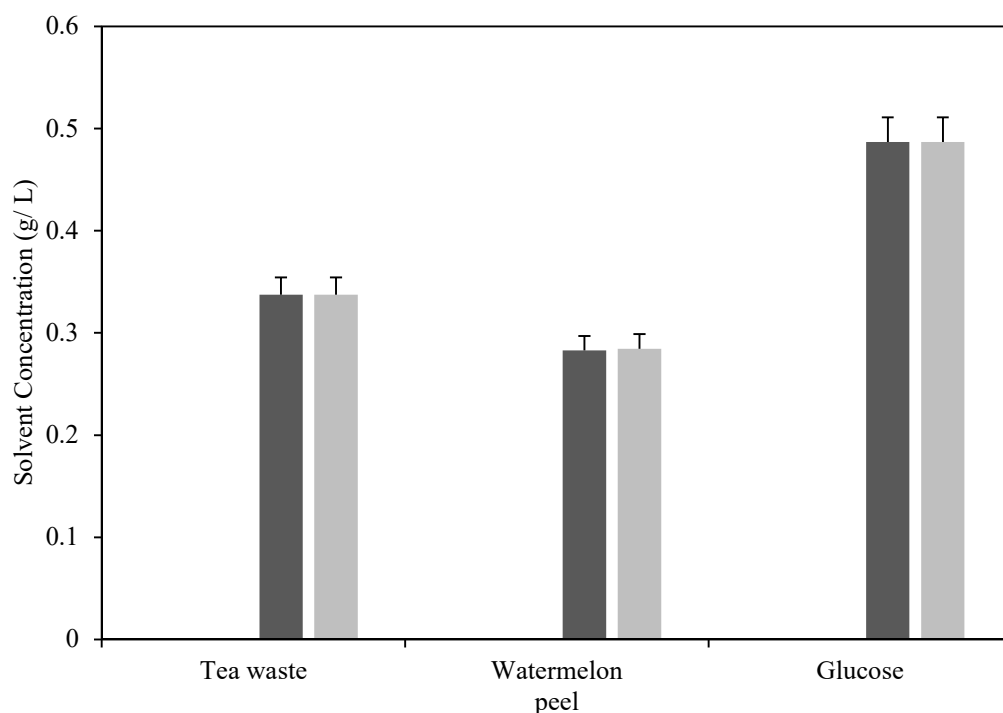


Figure 4.5 Solvent concentrations for tea waste, watermelon peel and glucose with *C. acetobutylicum* (■ Acetone, ■ Butanol, ■ Ethanol, ■ ABE)

4.1.1.2 *Clostridium beijerinckii*

The same fermentation conditions and substrates were used with *C. beijerinckii*. The sugar consumption for tea waste, watermelon peel and glucose with *C. beijerinckii* is given in Figure 4.6. Sugar consumption was 83.15, 87.52 and 93.98% for tea waste, watermelon peel and glucose, respectively. *C. beijerinckii* was able to maintain the sugar in the wastes. Figure 4.7 shows variation of pH with time for tea waste, watermelon peel and glucose with *C. beijerinckii*. First step of ABE fermentation is acidogenic phase and pH declines depending on acid formation. In all tested flasks, pH declined from the initial pH of 7.2 and after 48 hours of fermentation it increased and then remained approximately same at a pH range of 6.5-6.7. Figure 4.8 shows variation of suspended solid concentration with time for tea waste, watermelon peel and glucose with *C. beijerinckii*. The highest suspended solid concentration was 8.47 g/ L with glucose. At the end of the fermentation time tea waste had nearly the same biomass concentration with glucose. In watermelon peel

the suspended solid matter concentration reached to 7 g/ L. These results were observed with OD measurements which were calibrated with protein concentrations. The highest protein concentrations in tea waste, watermelon peel and glucose were 3927 mg/ L, 2757 mg/ L and 4632 mg/ L, respectively.

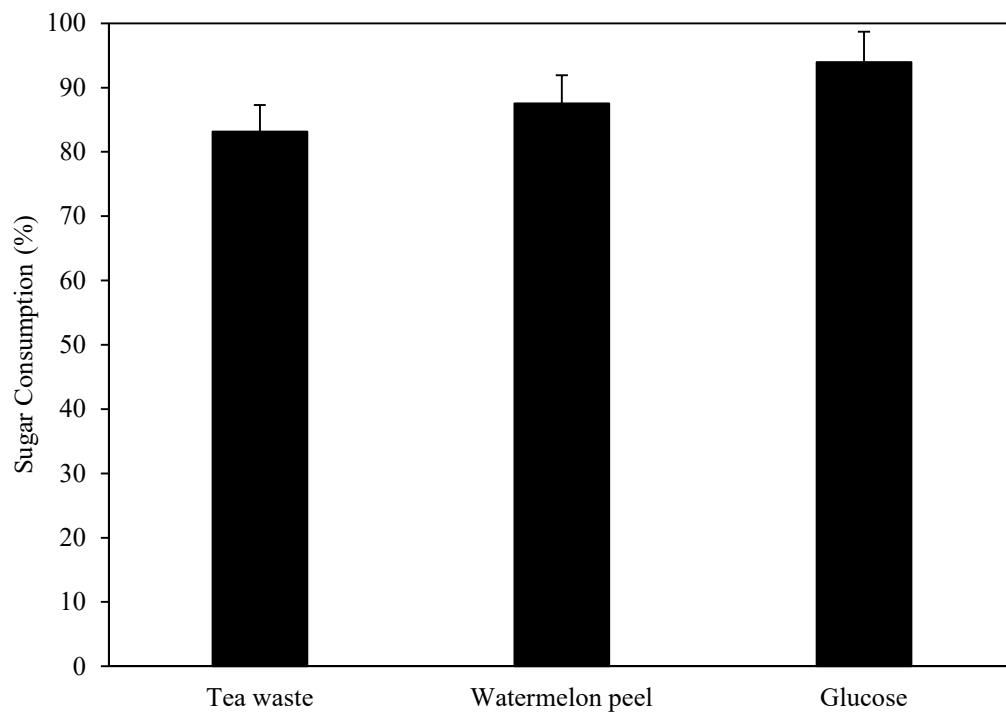


Figure 4.6 Sugar consumption for tea waste, watermelon peel and glucose with *C. beijerinckii*

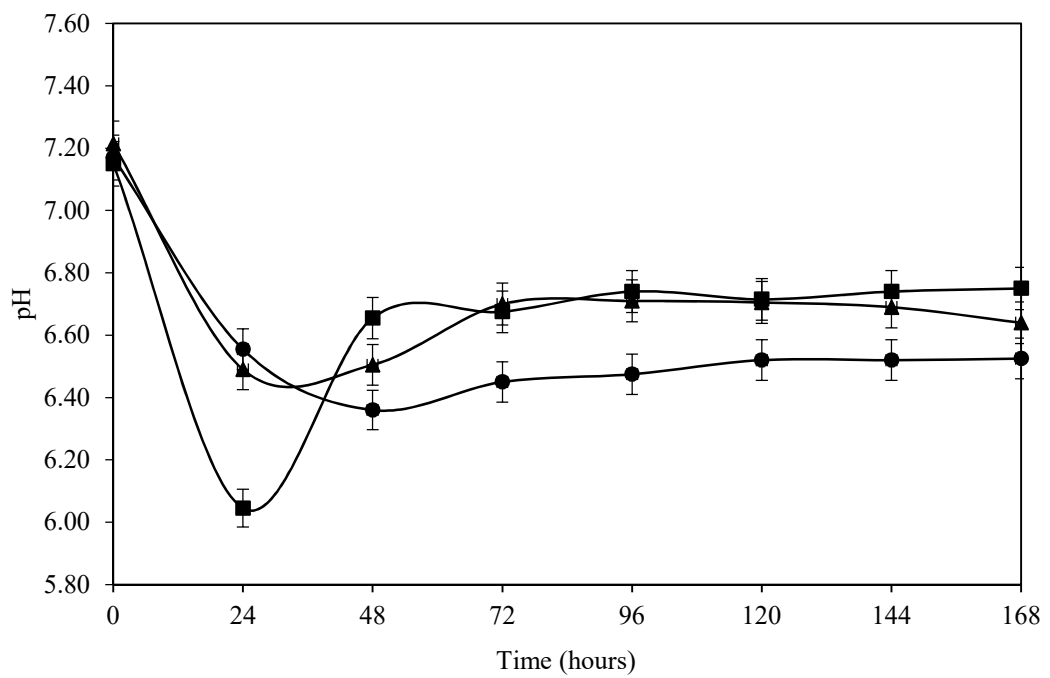


Figure 4.7 Variation of pH with time for tea waste, watermelon peel and glucose with *C. beijerinckii* (● Tea waste, ■ Watermelon peel, ▲ Glucose)

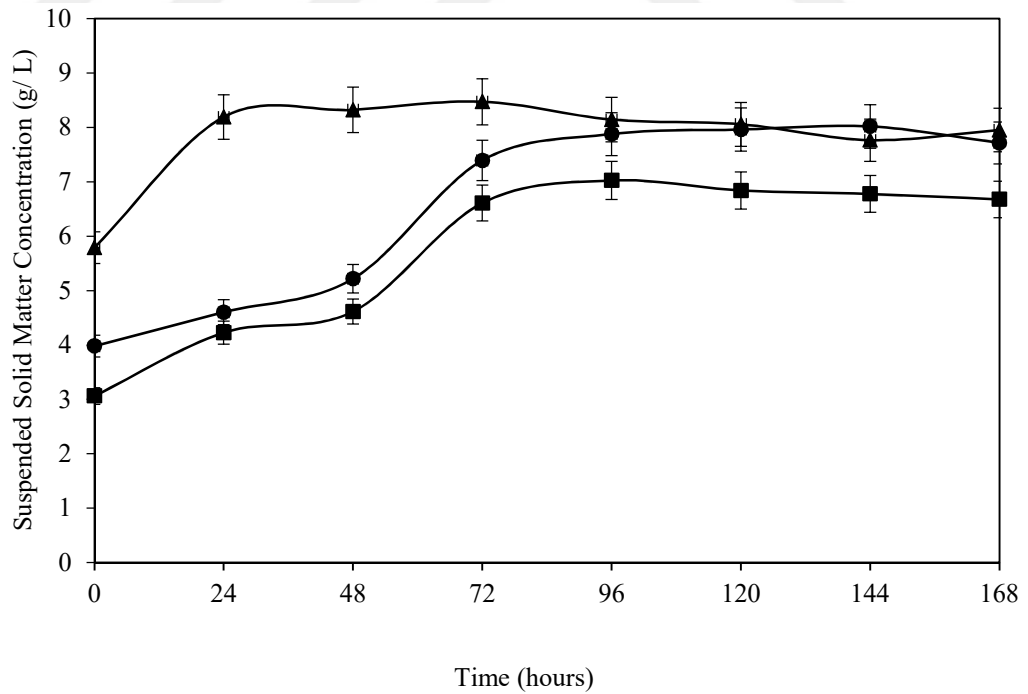


Figure 4.8 Variation of suspended solid concentration with time for tea waste, watermelon peel and glucose with *C. beijerinckii* (● Tea waste, ■ Watermelon peel, ▲ Glucose)

Figure 4.9 shows volatile fatty acid concentration at the end of the fermentation for tea waste, watermelon peel and glucose with *C. beijerinckii*. Acetic acid, butyric acid were measured and the highest acetic acid concentration was found at the fermentation media with watermelon peel (1513.52 mg/ L). In all tested flasks, butyric acid formed with all substrates. The highest butyric acid concentration was 1173.62 mg/ L with glucose at the end of the fermentation. Figure 4.10 shows solvent concentrations for tea waste, watermelon peel and glucose with *C. beijerinckii*. The highest concentration was acetone for all substrates. The highest butanol concentration was obtained with watermelon peel 0.37 g/ L butanol, along with 3.48 g/ L acetone and 0.84 g/ L ethanol was produced. Also, the highest solvent production (4.69 g/ L) was obtained with watermelon peel. 0.25 g/ L butanol was produced with tea waste. ABE concentration was 3.74 g/ L at the end of fermentation time with tea waste. Butanol concentration (0.31 g/ L) with glucose was higher than tea waste however ABE concentration (1.58 g/ L) at this time is lower than tea waste. If the butyric acid converted to butanol, butanol concentration for medium including glucose would be higher. The ABE concentration with *C.beijerinckii* was higher than *C. acetobutylicum* in the tested substrates. Ethanol was the only solvent when *C. acetobutylicum* was used. However, acetone and butanol were produced along with ethanol when *C. beijerinckii* was the organism chosen for ABE fermentation.

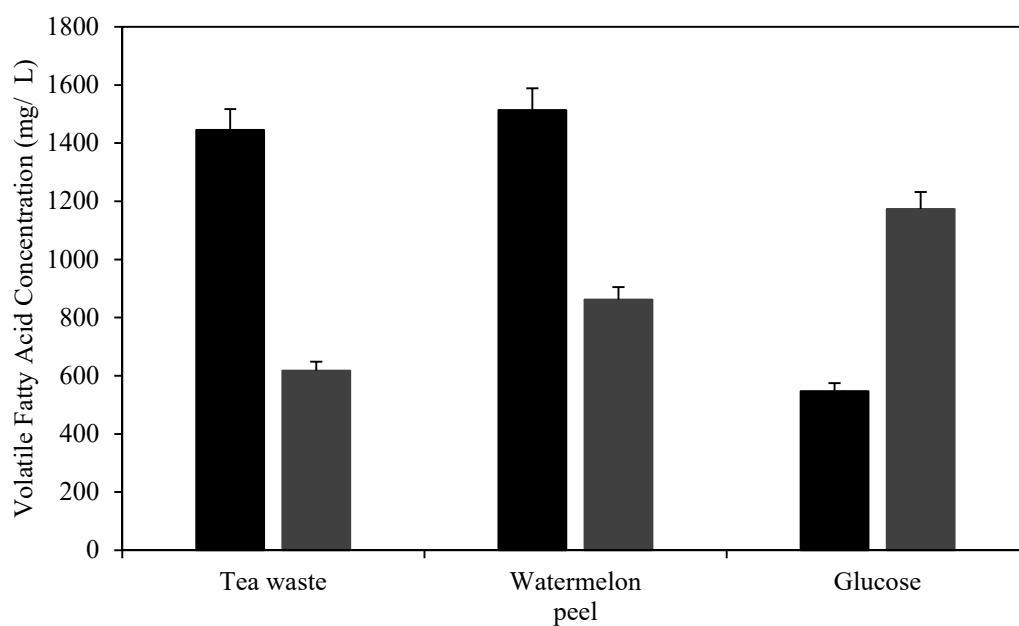


Figure 4.9 Volatile fatty acid concentration at the end of fermentation for tea waste, watermelon peel and glucose with *C. beijerinckii* (■ Acetic acid, ■ Butyric acid)

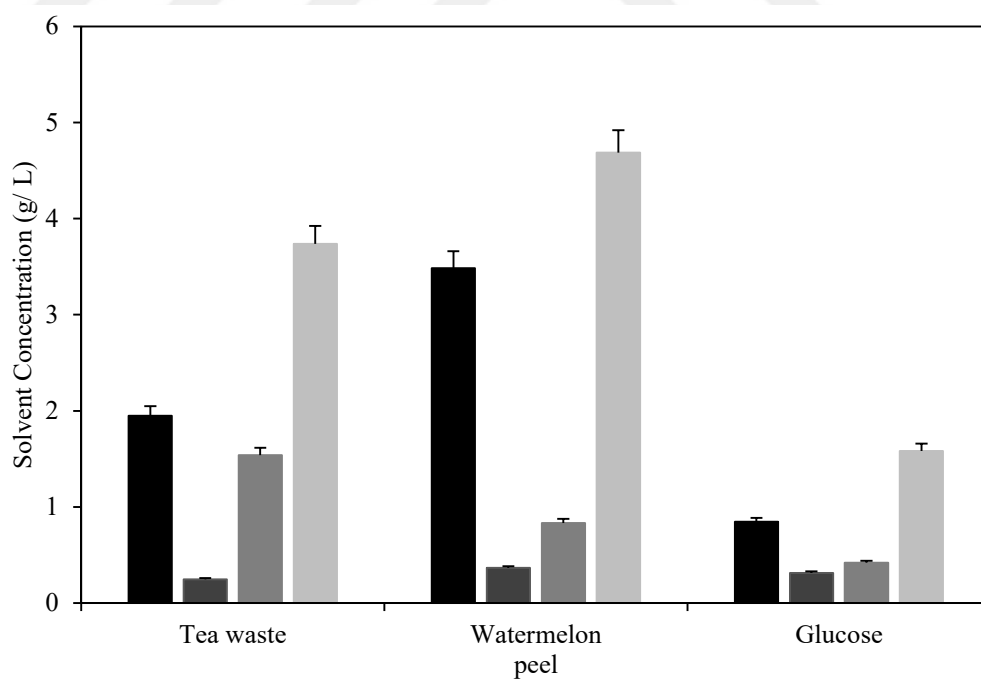


Figure 4.10 Solvent concentrations for tea waste, watermelon peel and glucose with *C. beijerinckii* (■ Acetone, ■ Butanol, ■ Ethanol, ■ ABE)

4.1.2 ABE Fermentation Using Different Substrates with Fermentation Media 2

Seven different substrates were tried for production of butanol for 168 hours without agitation with *Clostridium acetobutylicum* NRRL 527 and *Clostridium beijerinckii* DSM 791. Initially, 50 g/ L acid hydrolyzed tea waste, acid hydrolyzed peach waste, non-hydrolyzed peach waste, acid hydrolyzed wheat waste and rice husk were used. 50 g/ L glucose was used as positive controls. Also, 5 g/ L fructose was tested for ABE fermentation as another positive control. The initial pH of the experiments were 7. The pH was adjusted to 6 throughout the experiment with 10M NaOH. After acid hydrolysis of substrates, there was no evidence of furfural and 5-HMF.

In this set of experiments, a modified fermentation media (Fermentation media 2) was used and peptone, sodium acetate, sodium chloride, manganese sulphate were added to the fermentation media (Hou et al., 2017; Lu et al., 2013; Procentese et al., 2017a). The most important difference was the temperature which was chosen as 37°C to enhance ABE fermentation and the inoculum was used as 20% at 125 mL total fermentation volume.

4.1.2.1 Utilization of Different Substrates for ABE Fermentation with *Clostridium acetobutylicum*

In this set of experiments tea waste, non-hydrolyzed peach waste, peach waste, wheat waste, rice husk, glucose, fructose, was subjected to *C. acetobutylicum* for ABE fermentation. Substrate consumption for different substrates with *C. acetobutylicum* is given in Figure 4.11. The initial substrate concentrations varied after acid hydrolysis and had a wide range. The initial substrate concentrations for tea waste, peach waste, wheat waste, rice husk, glucose, and non-hydrolyzed peach waste (without hydrolysis) were 3.2 g/ L, 10.1 g/ L, 14.5g/ L, 2.9 g/ L, 44.1 g/ L and 7.4 g/ L, respectively. The initial fructose concentration was used as 5.7 g/ L. The highest sugar consumption was 82.98% with non-hydrolyzed peach waste. Fructose followed it with 75.06% sugar consumption. However, the lowest sugar consumption

was obtained with glucose as 25.94%. It is been understood that in batch fermentation high sugar concentration caused substrate inhibition to the organism.

Variation of pH with time with *C. acetobutylicum* is given in Figure 4.12. At fructose and peach waste without hydrolysis, the pH decreased to approximately 5.6 at the first 24 hours and then stabilized at nearly 5.8 till end of the fermentation time. At peach waste and glucose, the pH dropped to 5.25 at the 72 hours. The pH of the peach waste then increased to 6 at the end of the fermentation time and glucose stabilized at pH 5.4 after 72 hours. At tea waste, wheat waste and rice husk the pH decreased to approximately 6 and stood at 6 till the end of the fermentation time. At the first stage of the fermentation acid formation caused to decline pH.

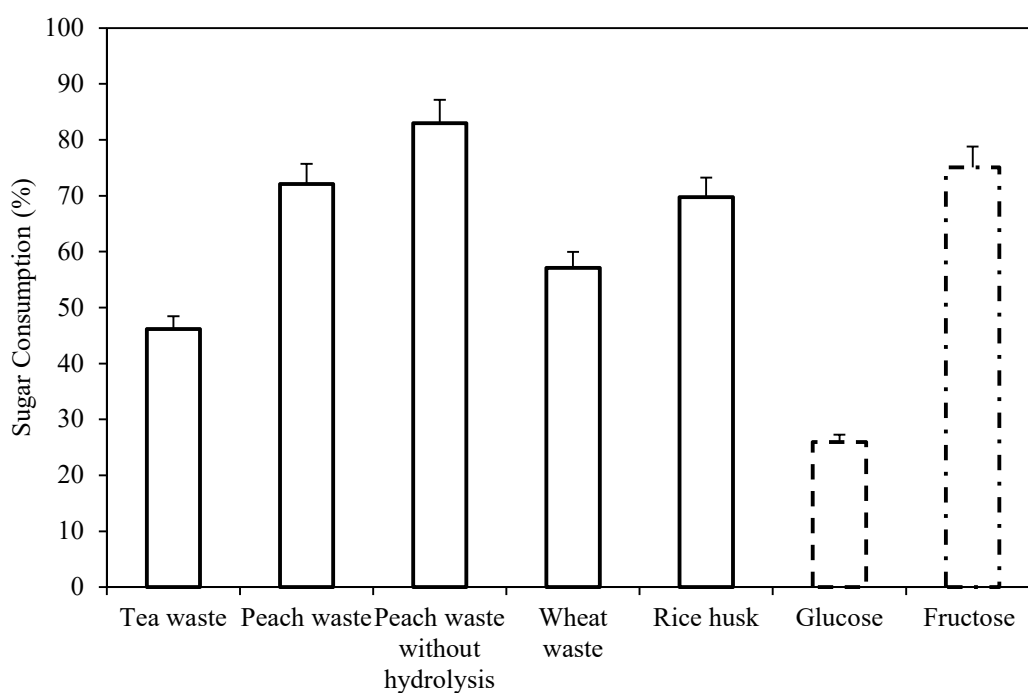


Figure 4.11 Sugar consumption for different substrates with *C. acetobutylicum*

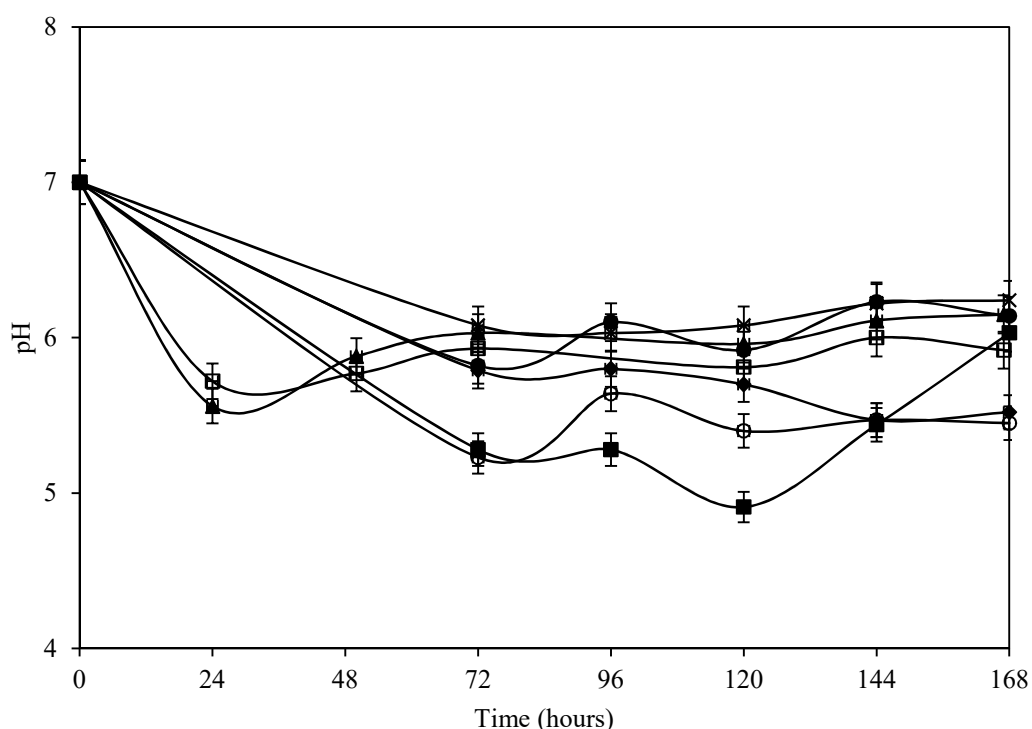


Figure 4.12 Variation of pH with time with *C. acetobutylicum* (● Tea waste, ■ Peach waste, ▲ Peach waste without hydrolysis, ◆ Wheat waste, × Rice husk, ○ Glucose, □ Fructose)

Figure 4.13 shows volatile fatty acid concentration (acetic acid, butyric acid) at the end of the fermentation with *C. acetobutylicum*. Acetic acid is an important by-product for solvent production. The highest acetic acid was 2312.65 mg/ L with non-hydrolyzed peach waste at the end of the fermentation. The acetic acid at this substrate was utilized at the first 24 hours and then increased. At hydrolyzed peach waste the acetic acid concentration increased at the first 96 hours and then decreased to a concentration of 1453.90 mg/ L. At the other substrates acetic acid concentrations were stable. There was also lactic acid in the tested bottles (not shown in the graph) which does not contributed to solvent production. In all tested flasks, butyric acid could not be observed however, the solvent concentrations for different substrates with *C. acetobutylicum* (Figure 4.14) showed that the butyric acid produced may be converted into butanol simultaneously in the fermentation media except fructose and non-hydrolyzed peach waste.

In Figure 4.14, the highest butanol (6.97 g/ L) was obtained with peach waste and ABE concentration was 14.11 g/ L. Butanol concentration for tea waste, wheat

waste, rice husk, and glucose, was 6.56, 6.23, 6.68, and 6.26 g/ L, respectively. Among the substrates that were tested the highest ABE concentration was obtained with wheat waste (15.27 g/ L). The literature reveals that A:B:E ratio is 3:6:1 at ABE fermentation. In our experiments at tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk the A:B:E ratios were 0.02:0.52:1, 0.03:0.63:1, 0:0.30:1, 0.02:0.44:1, 0.02:0.54:1, respectively with *C. acetobutylicum*. At glucose and fructose the A:B:E ratios were 0.03:0.53:1, 0:0.33:1, respectively. These results are quite different from the literature due to chosen environmental conditions and estimated inhibition effects.

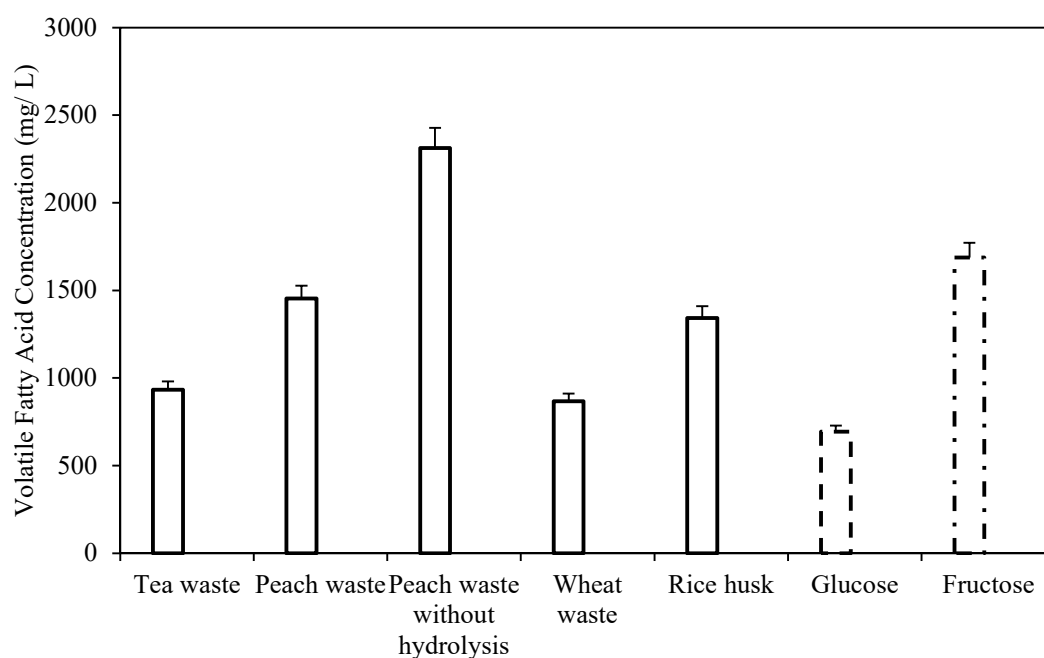


Figure 4.13 Volatile fatty acid concentration at the end of fermentation with *C. acetobutylicum* (□ Acetic acid, □ Butyric acid)

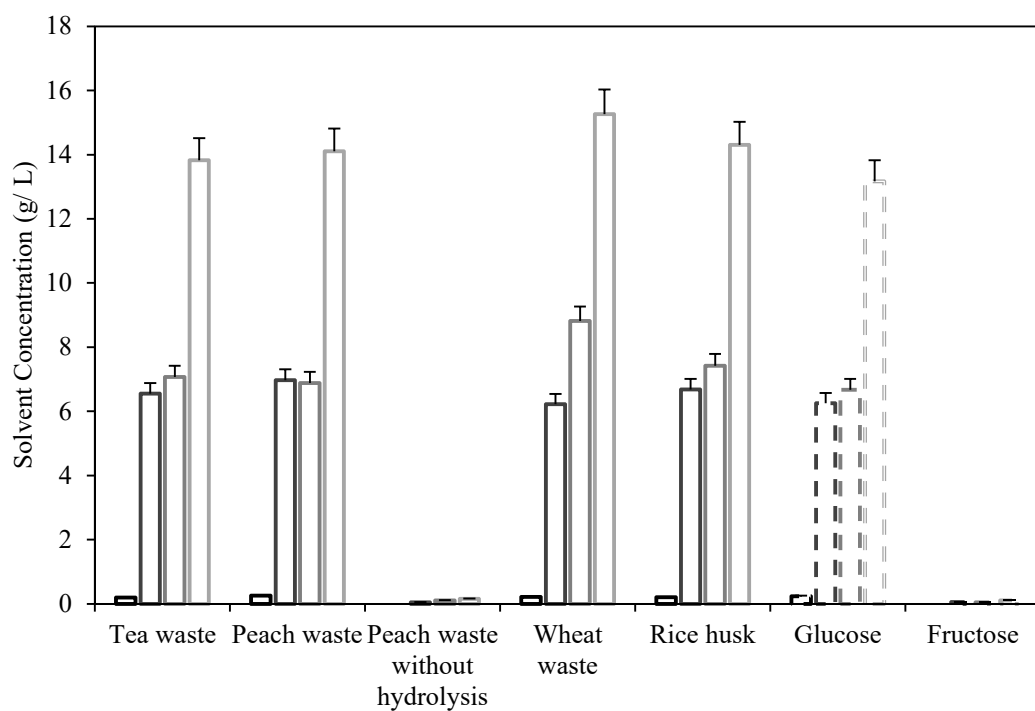


Figure 4.14 Solvent concentration for different substrates with *C. acetobutylicum* (□ Acetone, □ Butanol, □ Ethanol, □ ABE)

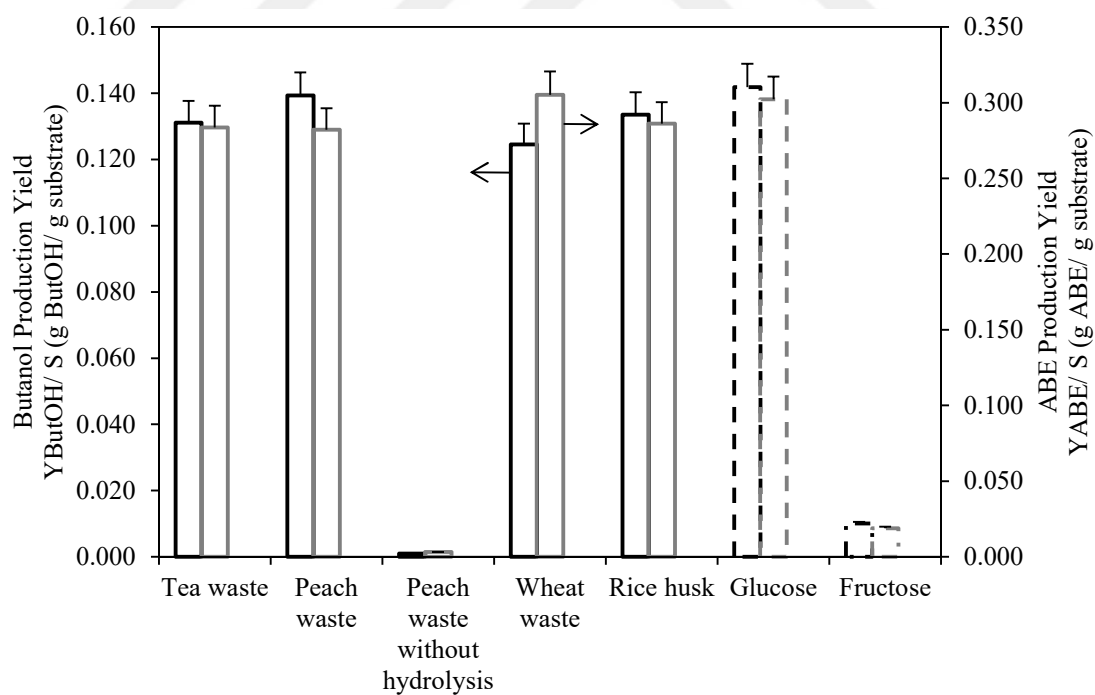


Figure 4.15 Butanol and ABE production yield at different substrates with *C. acetobutylicum* (□ Butanol Production Yield, □ ABE Production Yield)

Figure 4.15 shows butanol and ABE production yield at different substrates with *C. acetobutylicum*. The highest butanol production yield, among the wastes that were tested, was 0.139 g butanol / g substrate with peach waste. Butanol production yield for tea waste, wheat waste, rice husk was 0.131, 0.125, 0.134 g butanol/ g substrate, respectively. The lowest butanol production yield was obtained with non-hydrolyzed peach waste (0.001 g butanol/ g substrate). At this substrate high sugar consumption and high volatile fatty acids were obtained, showing that the organism was able to metabolize sugars into at acidogenic phase. However, low solvent concentrations and butanol yield showed that the organism was not able to convert this TVFA into solvents in this substrate. As expected the highest butanol yield (0.142 g butanol/ g substrate) was seen with glucose. Fructose as another control showed butanol yield of 0.010 g butanol/ g substrate. As well as non-hydrolyzed peach waste, high sugar consumption and TVFA production were observed but solvent production was quite low with fructose due to not being able to metabolize the produced TVFA into solvents in the solventogenic phase. ABE production yield of tea waste, peach waste, peach waste without hydrolysis, wheat waste and rice husk were 0.284, 0.282, 0.003, 0.305, 0.286, g ABE/ g substrate. ABE yield with wheat waste was higher than glucose (0.302 g ABE/ g substrate) and the ABE production yield with fructose was 0.019 g ABE/ g substrate.

Figure 4.16 shows volumetric butanol and ABE production rate for different substrates with *C. acetobutylicum*. Among the wastes, the highest volumetric butanol (0.0012 g butanol/ g substrate. h) and ABE production rate (0.0024 g ABE/ g substrate. h) was obtained with peach waste. Tea waste, wheat waste and rice husk followed peach waste. The highest ABE production rate was obtained with glucose (0.0025 g ABE/ g substrate. h).

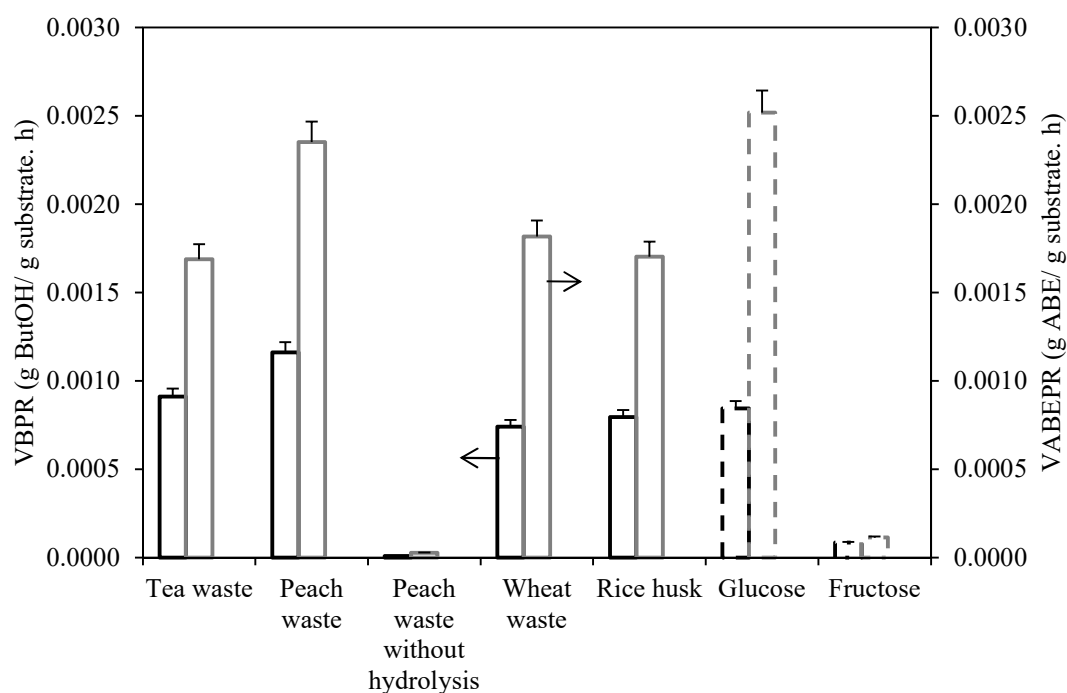


Figure 4.16 Volumetric butanol and ABE production rate for different substrates with *C. acetobutylicum* (▀ Volumetric Butanol Production Rate, ▀ Volmetric ABE Production Rate)

4.1.2.2 Utilization of Different Substrates for ABE Fermentation with *Clostridium beijerinckii*

In this set of experiments tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk, glucose, fructose was subjected to *C. beijerinckii* for ABE fermentation. The same environmental conditions and procedure were used in this experiment as the bottles where *C. acetobutylicum* was tested. Sugar consumption for different substrates with *C. beijerinckii* is given in Figure 4.17. Non-hydrolyzed peach waste and peach waste resulted in high sugar consumption 83.75, 70.11%, respectively. The sugar consumption with tea waste, wheat waste and rice husk was 51.22%, 16.39% and 64.09%, respectively. In these substrates a hydrolysis step was applied giving furfurals and 5-HMF that can be eliminated. However, the inhibitors after hydrolysis may cause negative effect on sugar consumption. The sugar consumption when glucose was used as the sole substrates was 35.25%. This sugar consumption is low due to high initial soluble sugar concentration which may caused substrate inhibition. The highest sugar consumption (89.92%) was obtained with the positive control of fructose with an initial concentration of 6.3 g/ L.

Figure 4.18 shows pH variation with time with *C. beijerinckii*. The pH dropped to a range of 5.62- 4.69 within 72 hours at substrates of tea waste, peach waste, wheat waste, rice husk, and glucose. This decrease showed that the first stage acidogenesis occurred in solvent metabolism. Then, the pH increased to 6.14- 5.65 and stood nearly stable depicting the solventogenesis phase of the solvent metabolism till the end of the fermentation time. Fructose and non-hydrolyzed peach waste pH decreased to 5.45 and 5.71, respectively. Then pH of these substrates reached to 6.55. After 96 hours of fermentation, pH was nearly constant.

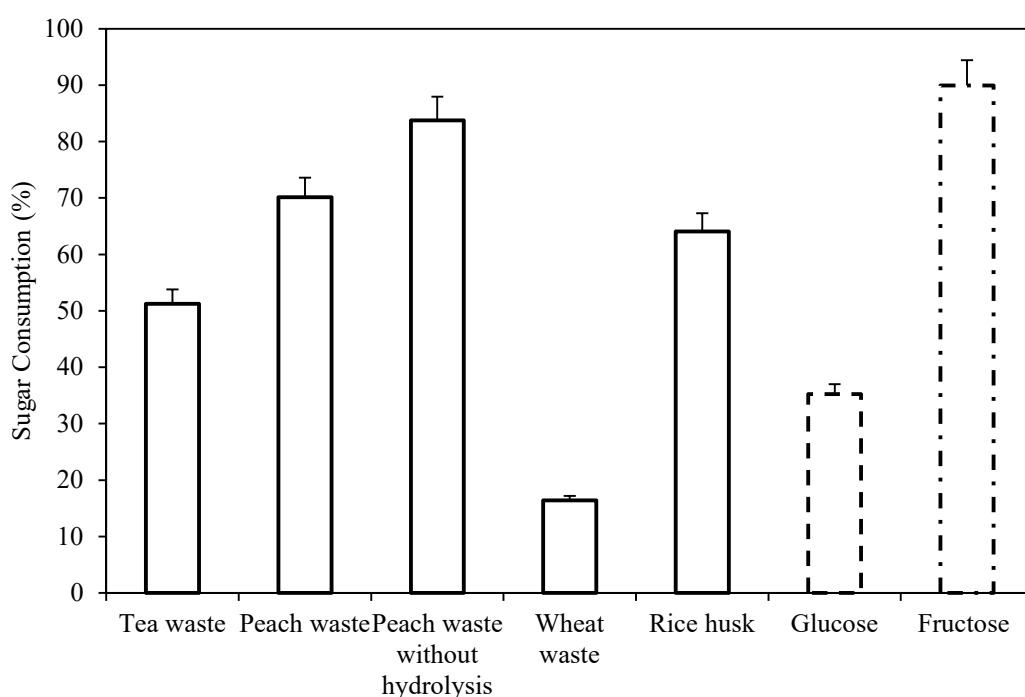


Figure 4.17 Sugar consumption for different substrates with *C. beijerinckii*

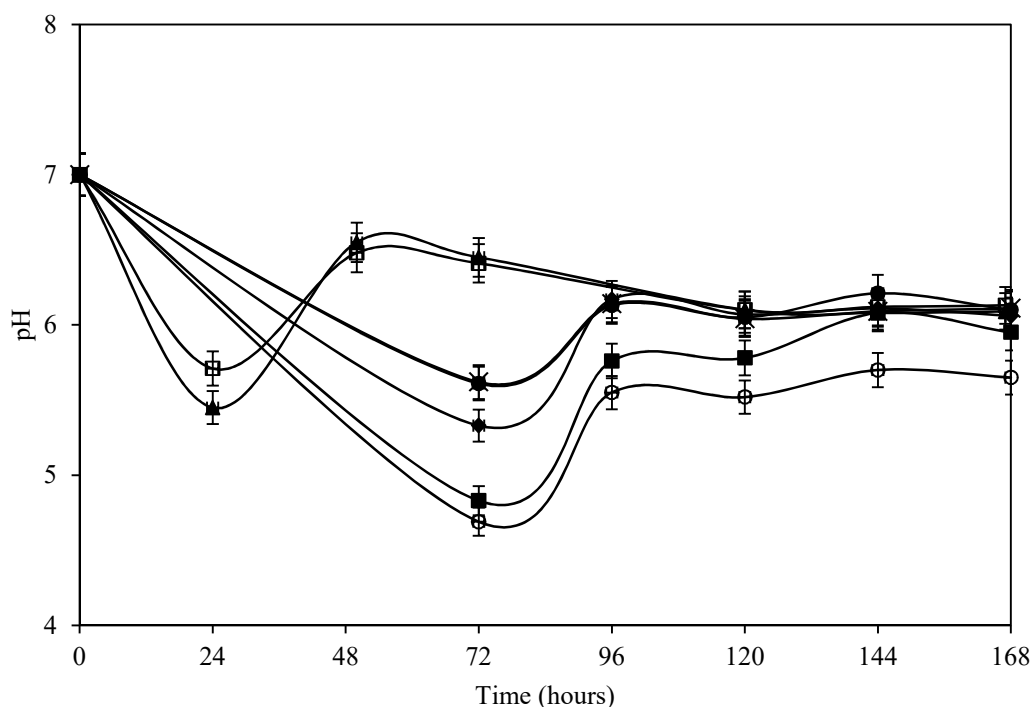


Figure 4.18 Variation of pH with time with *C. beijerinckii* (● Tea waste, ■ Peach waste, ▲ Peach waste without hydrolysis, ◆ Wheat waste, × Rice husk, ○ Glucose, □ Fructose)

Figure 4.19 shows volatile fatty acid concentration at the end of fermentation with *C. beijerinckii*. 2145.65 mg/ L acetic acid remained in the fermentation medium including hydrolyzed peach waste. At non-hydrolyzed peach waste 2006.80 mg/ L acetic acid and 3907.55 mg/ L butyric acid were produced. This high TVFA concentration obtained with non-hydrolyzed peach waste depicted that the organism was able to hydrolyze the substrate into simple sugars and convert them into TVFA successfully. Tea waste, wheat waste and rice husk the only TVFA was acetic acid with a concentration range of 1200-1400 mg/ L. In the tested bottles containing glucose, the sole organic acid was acetic acid with a concentration of 2304 mg/ L and with fructose acetic acid (1516 mg/ L) and butyric acid (3145 mg/ L) were produced.

Solvent concentrations for different substrates with *C. beijerinckii* are given in Figure 4.20. Butanol concentration was nearly same except for non-hydrolyzed peach waste and fructose. The highest butanol concentration (6.73 g/ L) was obtained with tea waste. The highest ABE concentration was 13.90 g/ L with tea waste at the

time period when the highest butanol concentration was obtained. It was expected that tea waste could include toxic matter. It is known that acid hydrolysis of substrates may cause some inhibitory compounds such as furfurals and 5-HMF etc. (Brito & Martins, 2017). Furfurals and 5-HMF were not observed in the tested bottles for all substrates. Butanol production with non-hydrolyzed peach waste and fructose was lower however, acetone concentration was higher than the other substrates. The highest acetone concentration (6.48 g/ L) was produced with non-hydrolyzed peach waste. The same calculations of A:B:E as well as *C. acetobutylicum* were done for *C. beijerinckii*. At tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk the A:B:E ratios were 0.02:0.54:1, 0.03:0.59:1, 19.34:0.62:1, 0.03:0.57:1, 0.03:0.55:1, respectively with *C. beijerinckii*. At glucose and fructose the A:B:E ratios were 0.07:0.56:1, 17.03:0.58:1, respectively. These results are quite different from the literature due to chosen environmental conditions and estimated inhibition effects. There are very different A:B:E ratios in the literature too. There are also examples from the literature close to the common ratio of 3:6:1. Liberato et al., (2019) mentions at their review that in 1980's an A:B:E ratio of 7:2:1 with *C. acetobutylicum* (Liberato et al., 2019). Zhang & Jia, (2018) obtained 3.6:6.4:1 in vessel scale fermentation and 2.9:5.9:1 in scale up fermentation by *C. beijerinckii* SE-2 (Zhang & Jia, 2018). The comparison of solvent production capability of *C. acetobutylicum* and *C. beijerinckii* at different substrates showed that at non-hydrolyzed peach waste and fructose *C. acetobutylicum* could not produce acetone however *C. beijerinckii* shifted to acetone with high ratios. At tea waste, wheat waste and rice husk the A:B:E ratios were close in both organisms. When glucose was tested, *C. beijerinckii* produced with more butanol than *C. acetobutylicum* showing a higher A:B:E ratio.

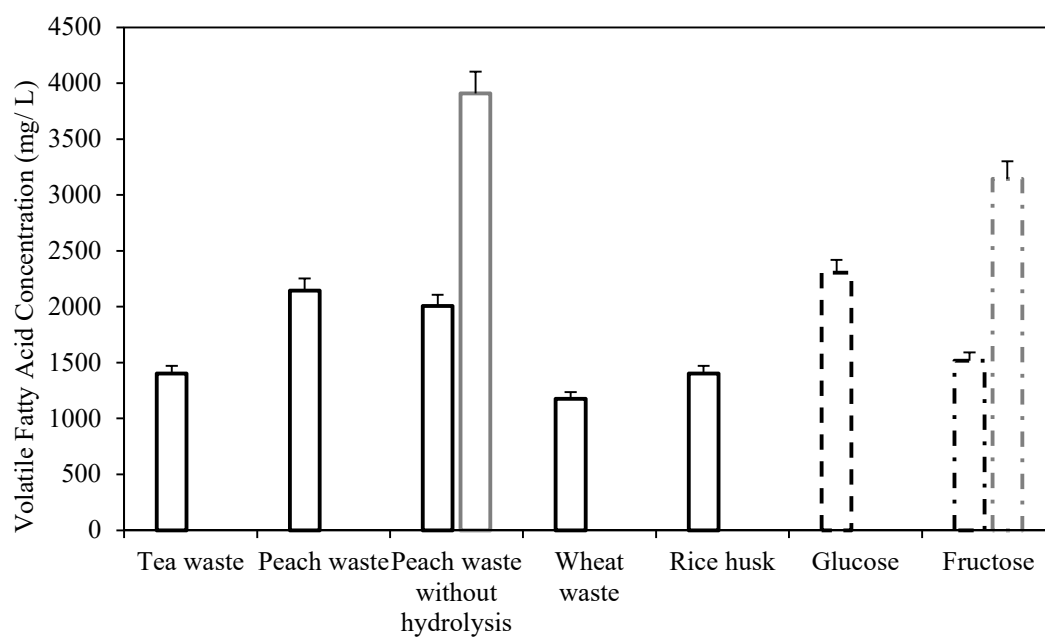


Figure 4.19 Volatile fatty acid concentration at the end of fermentation with *C. beijerinckii* (□ Acetic acid, □ Butyric acid)

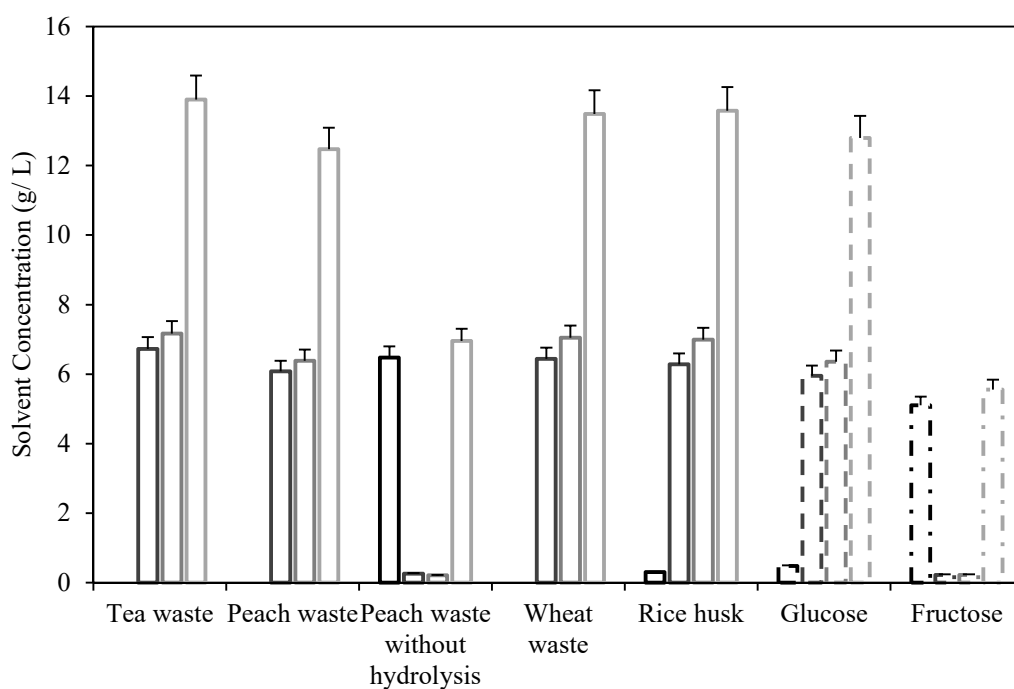


Figure 4.20 Solvent concentration for different substrates with *C. beijerinckii* (□ Acetone, □ Butanol, □ Ethanol, □ ABE)

Figure 4.21 shows butanol and ABE production yield at different substrates with *C. beijerinckii*. Among the wastes, the butanol yield (0.135 g butanol/ g substrate) and ABE yield (0.282 g ABE/ g substrate) were obtained with tea waste. Butanol production yield was 0.122, 0.005, 0.125, 0.126 g butanol / g substrate with peach waste, non-hydrolyzed peach waste, wheat waste, and rice husk, respectively following tea waste. Butanol and ABE yield for glucose were 0.162 g butanol / g substrate and 0.354 g ABE/ g substrate, respectively. As another positive control, butanol yield (0.036 g butanol / g substrate) and ABE yield (0.885 g ABE/ g substrate) were calculated for fructose. As different from other substrates, the highest produced solvent was acetone with peach waste without hydrolysis.

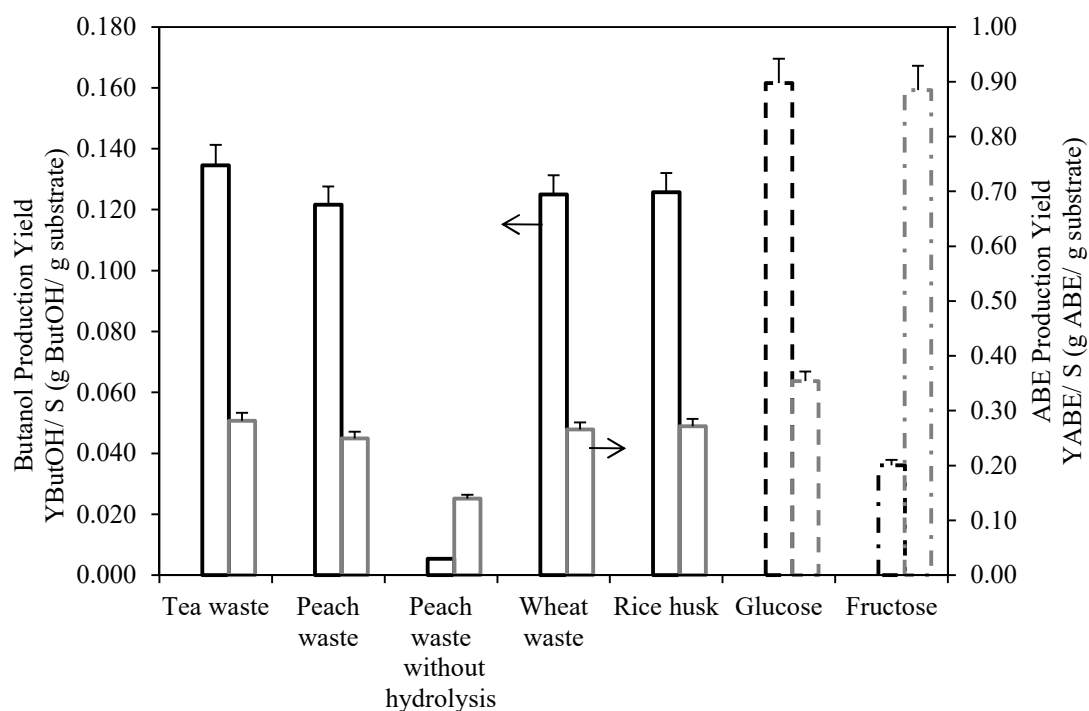


Figure 4.21 Butanol and ABE production yield at different substrates with *C. beijerinckii* (□ Butanol Production Yield, □ ABE Production Yield)

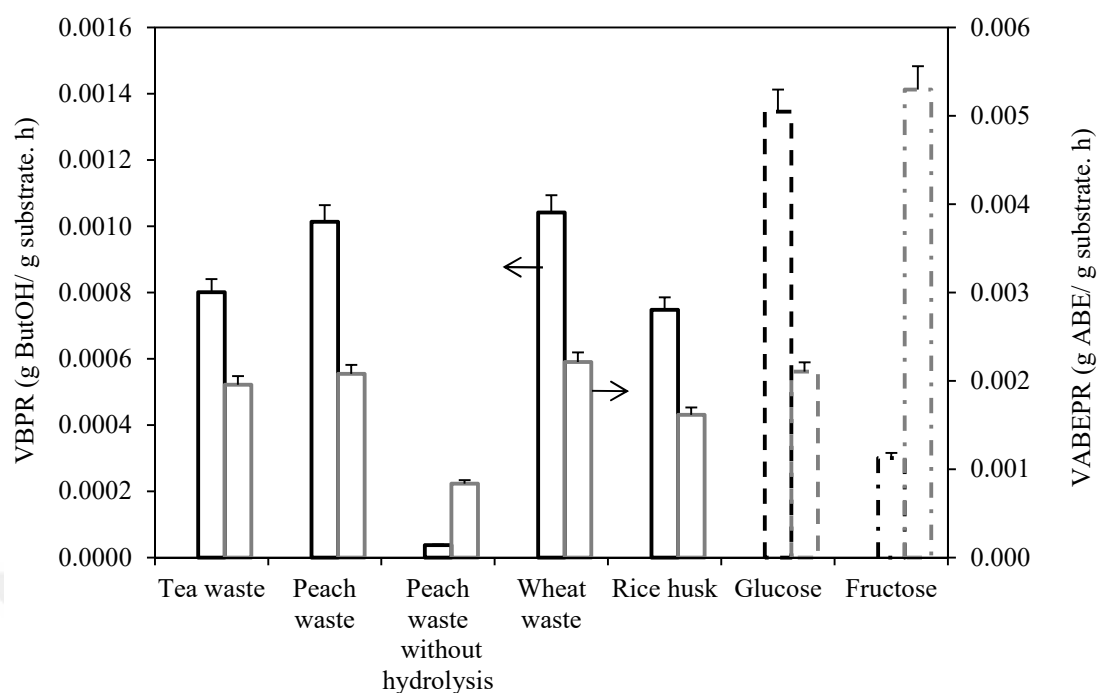


Figure 4.22 Volumetric butanol and ABE production rate for different substrates with *C. beijerinckii* (□ Volumetric Butanol Production Rate, □ Volumetric ABE Production Rate)

Figure 4.22 shows volumetric butanol and ABE production rate for different substrates with *C. beijerinckii*. Among the used wastes, the highest volumetric butanol production rate (0.00104 g butanol/ g substrate. h) and ABE production rate (0.0022 g ABE/ g substrate. h) were obtained with wheat waste. The volumetric butanol and ABE production rate of peach waste were very close to wheat waste. Non-hydrolyzed peach waste production rates were low because this substrate needed time for hydrolysis. Tea waste and rice husk had a volumetric butanol production rate of 0.00080 and 0.00075 g butanol/ g substrate. h, respectively. The volumetric ABE production rates of tea waste and rice husk were 0.00196 and 0.00162 g ABE/ g substrate. h, respectively. The highest volumetric butanol production rate was obtained with glucose (0.00135 g butanol/ g substrate. h). There was a hydrolysis step which may produce inhibitors that affect fermentation. There was no evidence of furfurals and 5-HMFs in hydrolyzed wastes however, the high volumetric production rate of glucose may give a hind of inhibition effects due to hydrolysis in the tested bottles including wastes.

In these experiments tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk were tested to produce ABE. Table 4.1 gives the comparison of two different organisms with different substrates at ABE fermentation. The highest sugar consumption was found in non-hydrolyzed peach waste (82.98% and 83.75%) with *C. acetobutylicum* and *C. beijerinckii*, respectively. Volatile fatty acids of the same waste resulted as acetic acid (2313 mg/ L) in *C. acetobutylicum* while acetic (2007 mg/ L) and butyric acid (3907 mg/ L) were observed with *C. beijerinckii*. However, at non-hydrolyzed peach waste both organisms were not able to convert TVFAs into solvents. Also, *C. beijerinckii* produced acetone (6.48 g/ L) as major product while *C. acetobutylicum* produced a quite low ABE concentration (0.16 g/ L).

The highest solvent concentration was obtained with wheat waste (15.27 g/ L) at *C. acetobutylicum* however the highest butanol concentration (6.97 g/ L) was produced with peach waste. At these substrates *C. beijerinckii* produced approximately the same ABE and butanol concentrations at tea waste, wheat waste and rice husk, meanwhile butanol concentration of tea waste was higher than the others. Sugar consumption of peach waste was 72.09% at *C. acetobutylicum* however, wheat waste (57.08%) ended up with a quite low sugar consumption. Sugar consumption of tea waste was 51.22% while in peach waste it ended up as 70.11% sugar consumption at *C. beijerinckii*. Sugar consumption of peach waste by both organisms was successful according to other wastes.

The highest butanol yield (0.139 g ButOH/ g substrate) was obtained with peach waste at *C. acetobutylicum* and ABE yield (0.305 g ABE/ g substrate) was obtained with wheat waste. ABE yield of other wastes was approximately same. The highest butanol (0.135 g ButOH/ g substrate) and ABE yield (0.282 g ABE/ g substrate) was obtained with tea waste at *C. beijerinckii*.

The best volumetric butanol and ABE production rate at *C. acetobutylicum* were 0.00116 g ButOH/ g substrate. h and 0.00235 g ABE/ g substrate. h at peach waste. The highest volumetric butanol production rate at *C. beijerinckii* was obtained with wheat waste (0.00104 g ButOH/ g substrate. h) and peach waste followed it with

0.00101 g ButOH/ g. h substrate. Volumetric ABE production rates of peach waste (0.00208 g ABE/ g substrate. h) and wheat waste (0.00221 g ABE/ g substrate. h). were similar. Peach waste was selected to use as substrate for fed-batch experiments due to high butanol yield and volumetric butanol production rates.

Table 4.1 Comparison of two different organisms with different substrates at ABE fermentation

<i>C. acetobutylicum</i>						
Substrate	Butanol (g/ L)	ABE (g/ L)	Y _{ButOH/ S} (g ButOH/ g substrate)	Y _{ABE/ S} (g ABE/ g substrate)	VBPR (g ButOH/ g substrate. h)	VABEPR (g ABE/ g substrate. h)
Tea waste	6.56	14.19	0.131	0.284	0.00091	0.00169
Peach waste	6.97	14.11	0.139	0.282	0.00116	0.00235
Peach waste without hydrolysis	0.05	0.16	0.001	0.003	0.00001	0.00003
Wheat waste	6.23	15.27	0.125	0.305	0.00074	0.00182
Rice husk	6.68	14.30	0.134	0.286	0.00080	0.00170
Glucose	6.26	13.33	0.142	0.302	0.00084	0.00252
Fructose	0.06	0.11	0.010	0.019	0.00008	0.00011
<i>C. beijerinckii</i>						
Substrate	Butanol (g/ L)	ABE (g/ L)	Y _{ButOH/ S} (g ButOH/ g substrate)	Y _{ABE/ S} (g ABE/ g substrate)	VBPR (g ButOH/ g substrate. h)	VABEPR (g ABE/ g substrate. h)
Tea waste	6.73	14.09	0.135	0.282	0.00080	0.00196
Peach waste	6.08	12.47	0.122	0.249	0.00101	0.00208
Peach waste without hydrolysis	0.27	6.97	0.005	0.139	0.00004	0.00083
Wheat waste	6.44	13.58	0.125	0.266	0.00104	0.00221
Rice husk	6.29	13.58	0.126	0.272	0.00075	0.00162
Glucose	5.95	13.03	0.162	0.354	0.00135	0.00210
Fructose	0.23	5.59	0.036	0.885	0.00030	0.00530

4.2 Fed-Batch Experiments

In fed-batch experiments, peach waste as substrate and *C. beijerinckii* as the organism was used. In fed-batch experiments, 60 g/ L peach waste was used for feed after acid hydrolysis of substrate. The major simple sugars in peach waste were fructose and glucose: it is been known that glucose can be converted very easily into

butanol. Fructose can be converted into end products after glucose via diauxic growth (Velioğlu Tosuner, Taylan, Özmihçi, 2019). Acid hydrolyzed peach waste with 23725 ± 2500 mg/ L total sugar and a pH of 7 was used in the experiments along with *C. beijerinckii* at different dilution rates (0.0028, 0.0056, 0.0084, 0.0113, 0.0141, 0.0169 1/ h). The inoculum volume of fed-batch experiments started with pH 7 and then due to the acidogenic phase the pH decreased and was adjusted to 6.5. Fed batch experiments started with 1 L densed culture and after feeding continued at 3 L.

Figure 4.23 shows feed and effluent simple sugar concentrations of fed-batch experiments at different dilution rates. Total sugar concentration of the feed was between 21747.15 and 26272.42 mg/ L. Sugar analysis with HPLC showed that the highest glucose and fructose of feed in fed-batch experiment with 0.0113 1/ h dilution rate were 11376.16 and 20947.86 mg/ L, respectively. The HPLC results gave a higher total sugar concentration due to being able to separate different simple sugars. Effluent glucose concentrations increased with increasing dilution rates. Effluent fructose concentrations decreased with increasing dilution rates till 0.0084 1/ h and then increased with further increasing dilution rates.

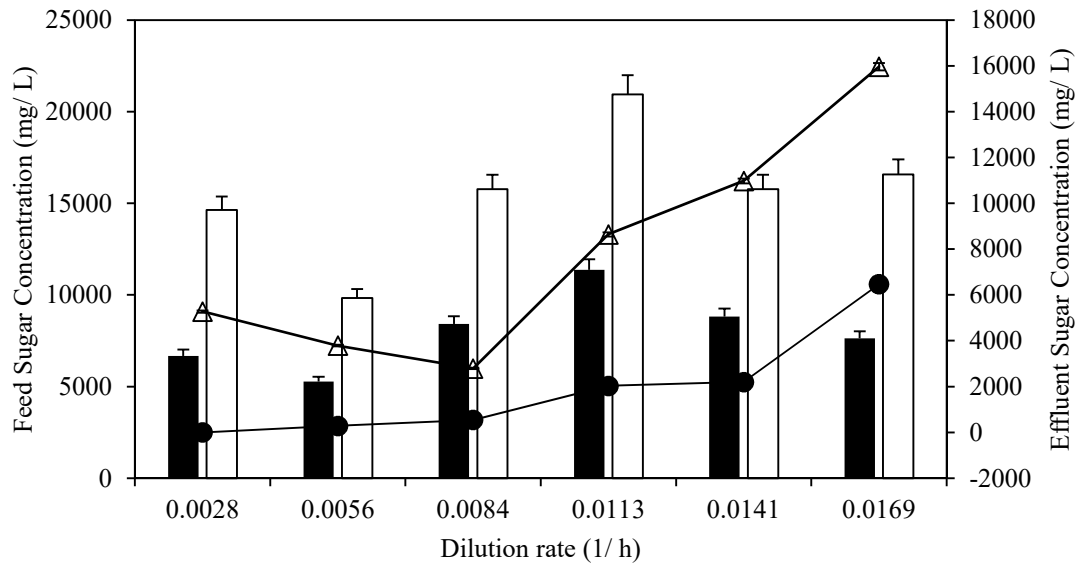


Figure 4.23 Feed and effluent simple sugar concentrations of fed-batch experiments at different dilution rates (■ Glucose of feed, □ Fructose of feed, ● Glucose of effluent, Δ Fructose of effluent)

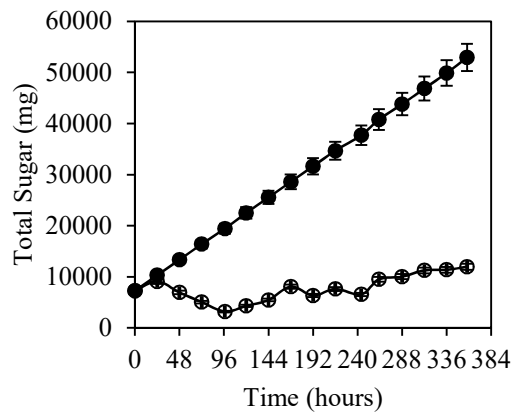
After pretreatment of substrate, inhibitor formation is an obstacle for successful ABE fermentation. In addition to furan derivatives (5-HMF, furfural), phenolic compounds and aliphatic acid such as acetic, formic, levulinic acids can appear due to pretreatment method conditions (García et al., 2011; Huzir et al., 2018; Maiti et al., 2016). At previous batch experiment with peach waste hydrolysate, 5-HMF and furfurals were not detected. In fed-batch experiments, acetic acid at hydrolyzed peach waste was between 1288.56 and 1771.47 mg/ L.

Figure 4.24 shows variation of sugar amount with time at different dilution rates. At the control reactor, the sugar amounts increased with time and varied between 54 ± 3 g throughout the dilution rates. At Figure 4.24 a depicts the sugar amount with time at dilution rate of 0.0028 1/ h. Sugar amount decreased until the first 97 hours of fermentation and then reached to quasi-steady state giving a sugar concentration of 12 g at the end of the fermentation time. The highest sugar consumption (84%) was seen with this dilution rate. Figure 4.24 b gives the results of 0.0056 1/ h dilution rate. Sugar amount increased during the first 72 hours of fermentation and could not reach to a quasi-steady state. After 24 hours, the sugar concentration decreased till 128 hours. The highest sugar consumption was obtained at 128 hours of fermentation which was higher than 0.0028 1/ h dilution rate. However could not reach to quasi-steady state conditions. The sugar concentration increased at the following time. Figure 4.24 c, d, e, f) gives the sugar amounts of 0.0084 1/ h, 0.0113 1/ h, 0.0141 1/ h, 0.0169 1/ h dilution rates with time resulting with 22.3 g, 28.5 g 33.2 g, 36.9 g sugar at the end of the fermentation time, respectively. At dilution rate of 0.0084 1/ h sugar concentration decreased till 48 hours and then increased till the end of the fermentation time (Figure 4.24 c). At dilution rates of 0.0113, 0.0141 1/ h, the sugar concentration increased linearly at the fed-batch reactors (Figure 4.24 d, e). At the highest dilution rate the sugar concentration increased with time however after 48 hours a decrease was observed (Figure 4.24 f).

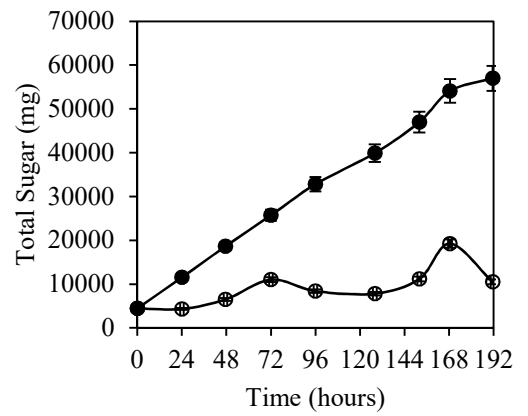
Figure 4.25 shows variation of simple sugar concentration with time at different dilution rates. Glucose content of feed was consumed by *C. beijerinckii* throughout the fermentation at 0.0028 1/ h, 0.0056 1/ h and 0.0084 1/ h dilution rates (Figure

4.25 a, b, c) however, glucose was over 2 g/ L in the fermentor at the end of the fermentation with 0.0113 1/ h, 0.0141 1/ h and 0.0169 1/ h dilution rates (Figure 4.25 d, e, f). These results show that glucose concentration decreased with decreasing dilution rates. Fructose concentration was nearly stable at low dilution rates till 0.0084 1/ h, however it increased with further dilution rates. The results showed that *C. beijerinckii* prefers glucose instead of fructose and can not consume fructose at a desired level.

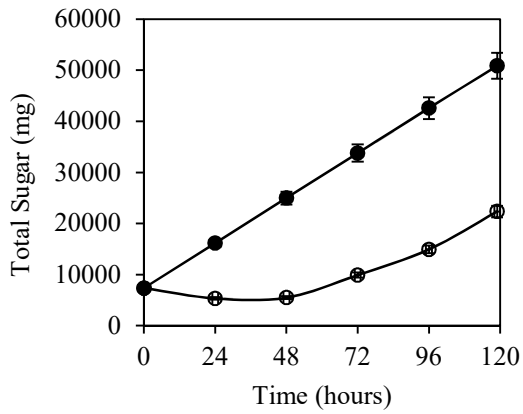
It should be taken into consideration that all the glucose in the fermentation media was consumed in all tested dilution rates however, the fructose could not be consumed totally. *C. beijerinckii* showed a diauxic growth where the glucose was preferred firstly and after glucose utilization, fructose began to be used. Fructose concentration was stable with 0.0028, 0.0056, 0.0084 1/ h dilution rates however the fructose concentration increased with the other dilution rates.



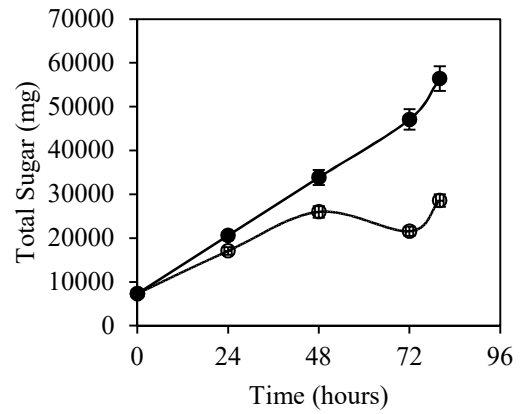
(a)



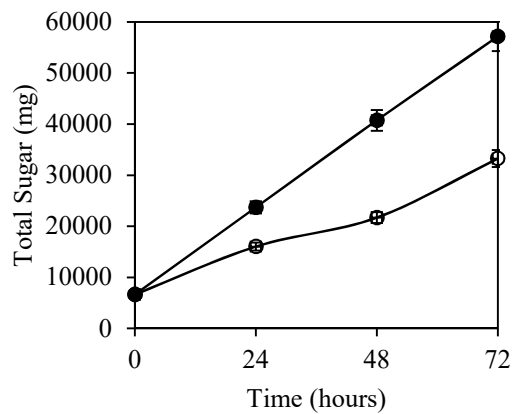
(b)



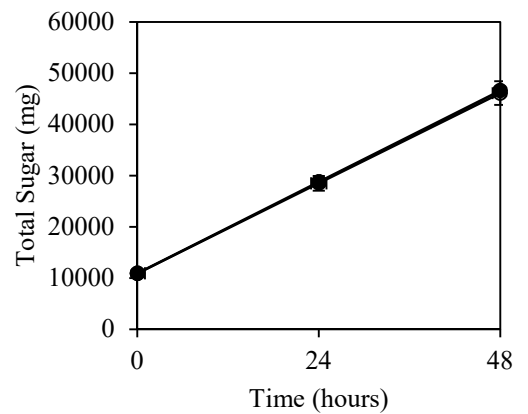
(c)



(d)



(e)



(f)

Figure 4.24 Variation of sugar amount with time at different dilution rates (● Control, ○ Experimental) a) 0.0028 1/h, b) 0.0056 1/h, c) 0.0084 1/h, d) 0.0113 1/h, e) 0.0141 1/h, f) 0.0169 1/h

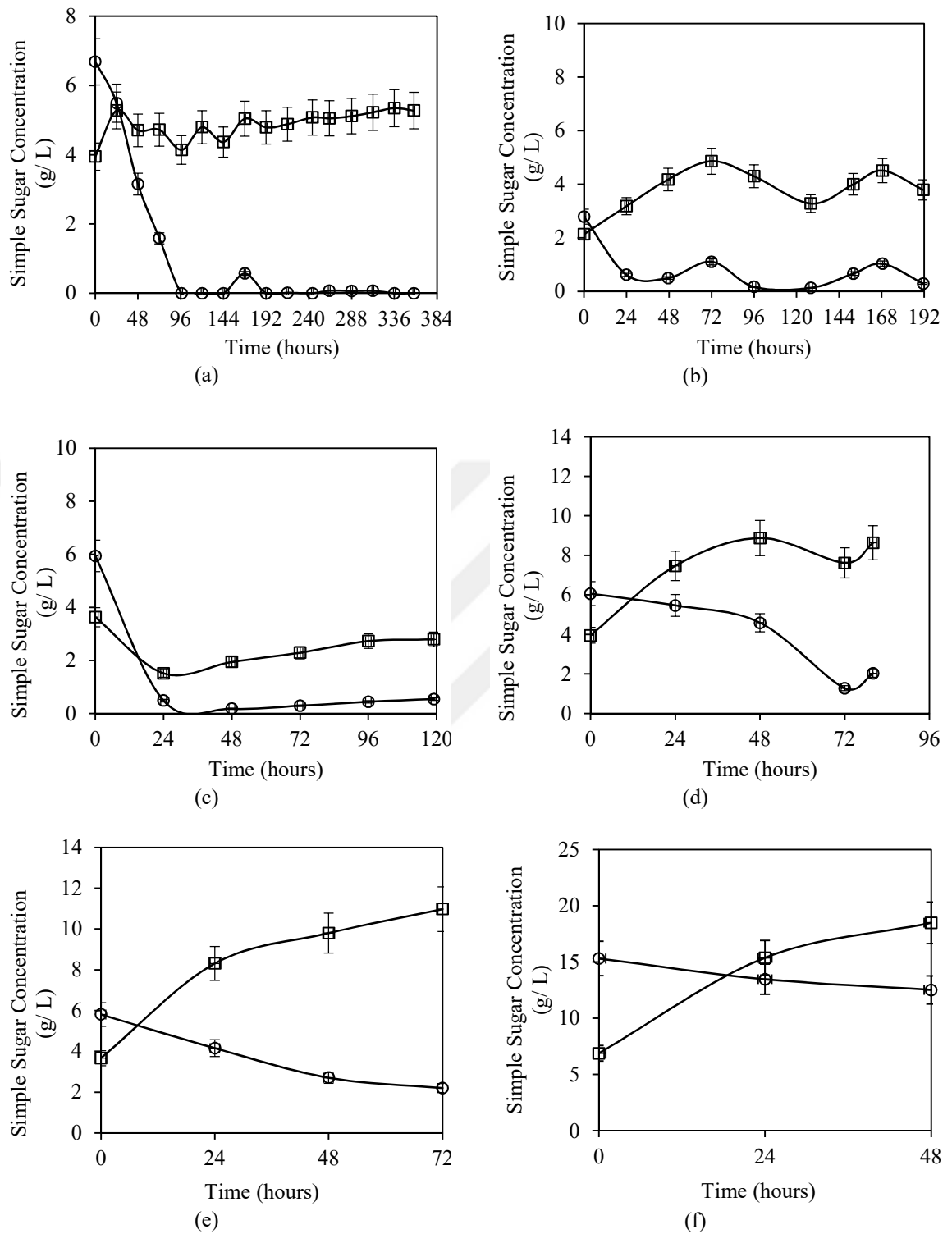
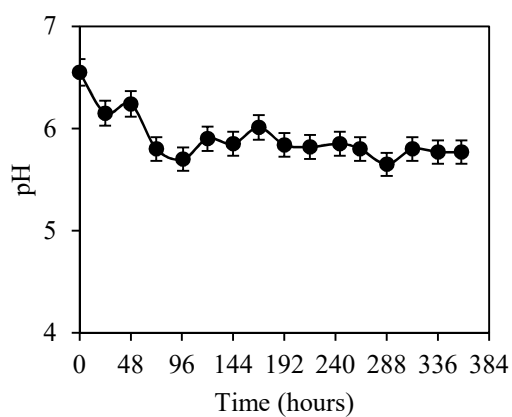


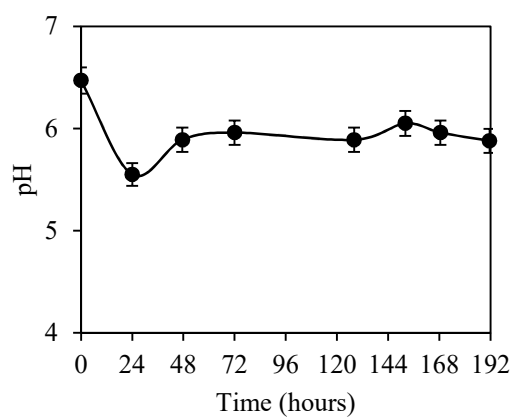
Figure 4.25 Variation of simple sugar concentration with time at different dilution rates ($\circ$ Glucose, $\square$ Fructose) a) 0.0028 1/h, b) 0.0056 1/h, c) 0.0084 1/h, d) 0.0113 1/h, e) 0.0141 1/h, f) 0.0169 1/h

Figure 4.26 shows variation of pH with time at different dilution rates. ABE fermentation includes acidogenic and solventogenic phases. After pH of 1L inoculum was adjusted to 6.5 at the beginning of fermentation, feed (pH 7) was added to the fermentation medium. The pH declined in all tested dilution rates due to acidogenic phase. At dilution rate of 0.0028 1/ h (Figure 4.26 a), the pH reached to a quasi-steady state condition with an approximate pH of 5.8. 0.0056 1/ h dilution rate showed nearly same trend with 0.0028 1/ h. Quasi-steady state condition with this dilution rate were obtained at pH 6 (Figure 4.26 b). At the dilution rate of 0.0084 1/ h, quasi-steady state conditions was observed at 72 hours (Figure 4.26 c). After 72 hours of the fermentation with 0.0113 1/ h dilution rate, quasi-steady state conditions were nearly obtained, however at other dilution rates pH varied between 5-6 (Figure 4.26 d, e, f).

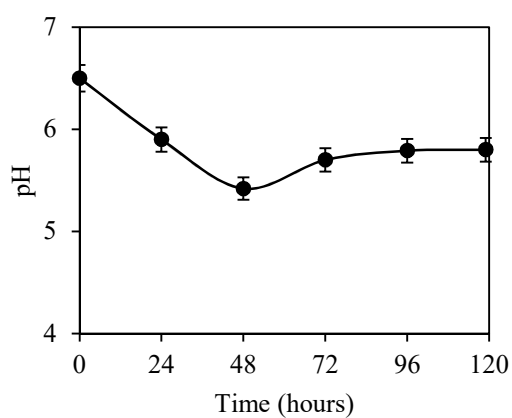
Figure 4.27 shows variation of protein concentration (X_e) and productivity ($D.X_e$) with dilution rate. Protein concentration was nearly stable throughout all fed-batch experiments. The protein concentrations were approximately 7.5 g/ L at dilution rates of 0.0028, 0.0056, 0.0084 and 0.0141 1/ h. At dilution rates of 0.0113 1/ h and 0.0169 1/h, the protein concentrations were nearly stable at 8.7 g/ L and 6.5 g/ L, respectively. Biomass productivity increased with increasing dilution rates.



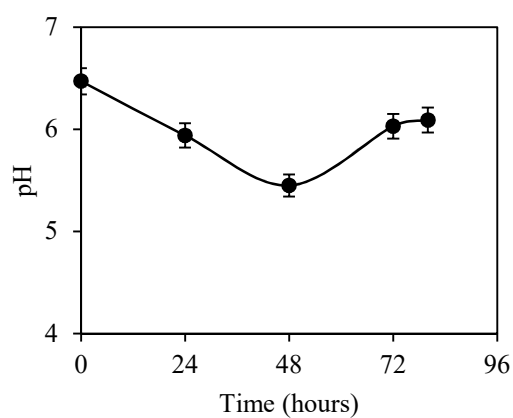
(a)



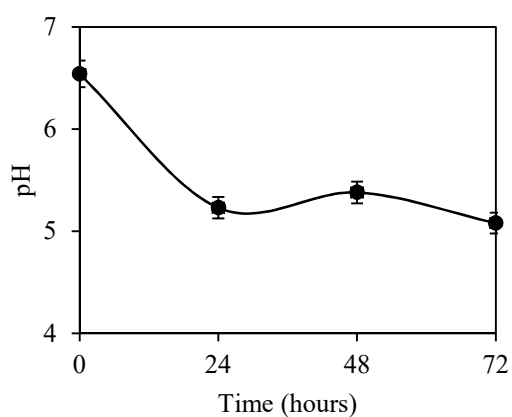
(b)



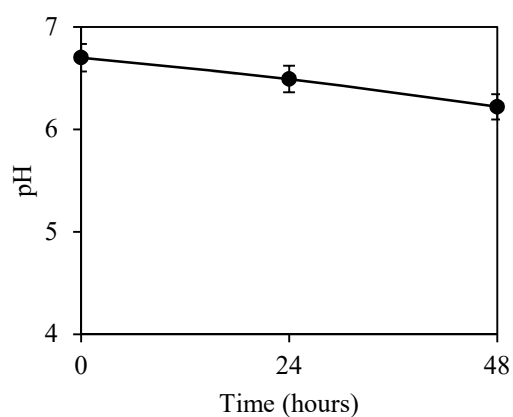
(c)



(d)



(e)



(f)

Figure 4.26 Variation of pH with time at different dilution rates a) 0.0028 1/ h, b) 0.0056 1/ h, c) 0.0084 1/ h, d) 0.0113 1/ h, e) 0.0141 1/ h, f) 0.0169 1/ h

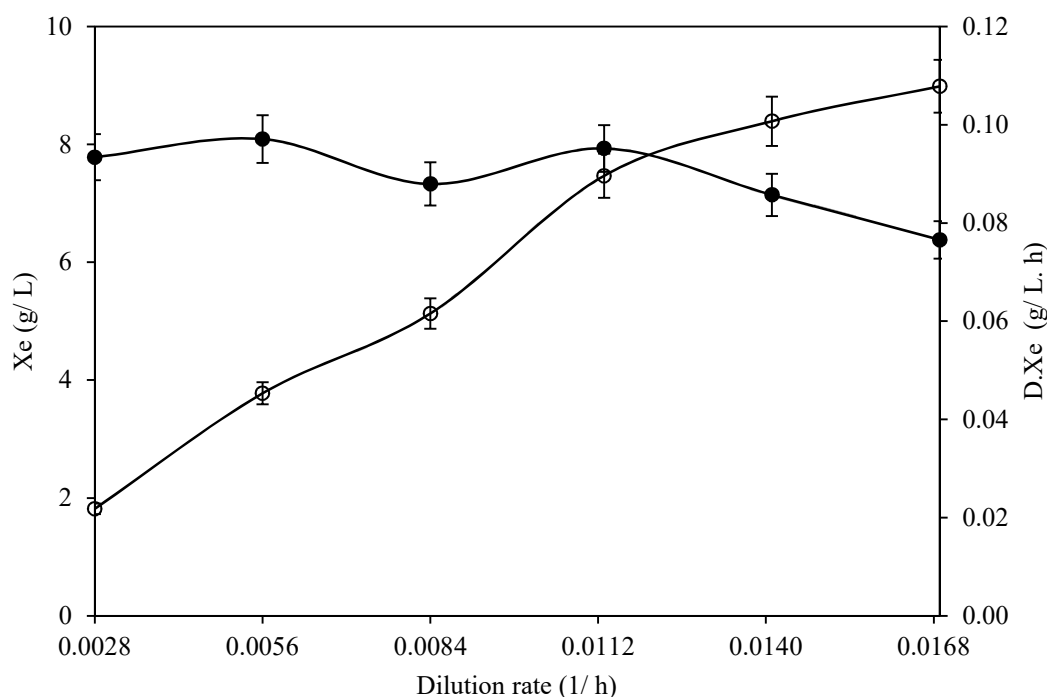


Figure 4.27 Variation of protein concentration (Xe) and productivity (D.Xe) with dilution rate (● Protein concentration, ○ Productivity)

Figure 4.28 shows variation of volatile fatty acid concentration with time at different dilution rates. The major volatile fatty acids (VFA) produced at fed-batch fermentation were acetic acid, butyric acid, propionic acid and lactic acid. At initial conditions of fed-batch fermentation, there were organic acids produced in the inoculum due to 60 hours batch start up procedure. The aim of the batch stage was to obtain a dense culture and consume the glucose used as substrate in the inoculum. At dilution rate of 0.0028 1/h (Figure 4.28 a), the highest produced organic acid was acetic acid with a concentration of 6.6 g/L and was followed by butyric acid with a concentration of 5.58 g/L. The butyric acid produced in fed-batch fermentation had a nearly stable concentration after 72 hours. The acetic acid concentration stabilized after 168 hours giving a higher concentration than butyric acid, however began to increase once more after 336 hours. This concentration was obtained at a quasi-steady state where the pH was 5.8. This demonstrates condition of acidogenic phase which is the first stage of solvent production. The initial lactic acid concentration decreased depending on dilution however increased after 216 hours. Propionic acid was not observed throughout the fermentation. At 0.0056 1/h dilution rate, acetic

acid concentration started to increase after 48 hours of the fermentation (Figure 4.28 b). Initial butyric acid concentration was quite high in the fermentation media and butyric acid reached to highest level (5 g/ L) at 128 hours of the fermentation. Propionic acid was stable throughout the fermentation at this dilution rate and lactic acid was not detected in the media. At 0.0084 1/ h dilution rate (Figure 4.28 c), acetic acid concentration was 1.06 g/ L at the beginning of the fermentation. At the first 24 hours of the fermentation, acetic acid concentration decreased to 0.3 g/ L. Then, the acetic acid increased to 0.7 g/ L at 72 hours. The highest butyric acid concentration was 4.7 g/ L at 24 hours. Then a decline was observed which was derived from dilution. At 0.0113 1/ h dilution rate, the highest total volatile fatty acid concentration was obtained at 72 hours of the fermentation (Figure 4.28 d). Acetic acid declined and butyric acid increased to the highest concentration (5.05 g/ L) at this time. At 0.0141 1/ h and 0.0169 1/h dilution rates (Figure 4.28 e, f), acetic acid concentration increased throughout the fermentation and butyric acid did not formed. Propionic acid concentration was nearly 1 g/ L during the fermentation.

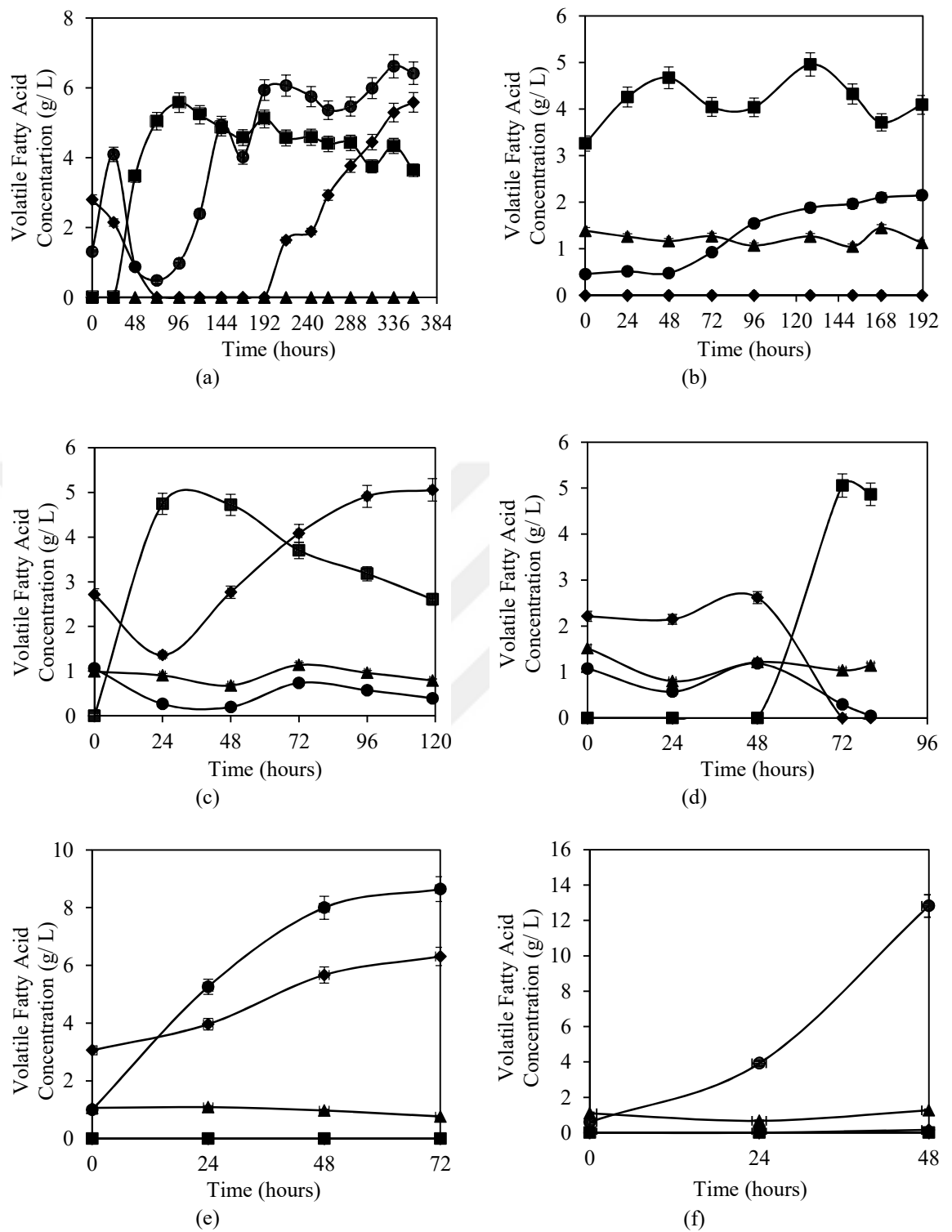


Figure 4.28 Variation of volatile fatty acid concentration with time at different dilution rates (● Acetic acid, ■ Butyric acid, ▲ Propionic acid, ◆ Lactic acid) a) 0.0028 1/h, b) 0.0056 1/h, c) 0.0084 1/h, d) 0.0113 1/h, e) 0.0141 1/h, f) 0.0169 1/h

Variation of solvent concentration with time at different dilution rates are given in Figure 4.29. *C. beijerinckii* has the ability to produce butanol via ABE fermentation. In our experiments acetone was the dominant solvent produced with hydrolyzed peach waste. Butanol formation metabolic pathway is complicated. In this metabolic pathway acetone and ethanol named as cold line is shorter than butanol production. The hot line referring to butanol formation needs special enzymes and conditions (Gottumukkala et al., 2017). At 0.0028 1/ h dilution rate, acetic acid was (4.1 g/ L) formed at a pH of 6.15 at 24 hours and butyric acid was produced after 97 hours at a concentration of 5.6 g/ L at a pH of 5.7. The organic acid concentration produced due to acidogenic phase was converted into acetone (6 g/ L) due to solventogenic phase at 72 hours and stabilized throughout fed-batch fermentation. There were no ethanol and butanol observed in this dilution rate. This may be due to inhibitory effects of hydrolysis. Another reason may be due to high organic acid formation which may inhibit solvent production (Figure 4.29 a). At 0.0056 (1/ h) dilution rate, the highest acetone was 7.31 g/ L and obtained at 128 hours of fermentation (Figure 4.29 b). There was just a little ethanol and butanol observed at the beginning of the fermentation due to addition of trace amount of ethanol (0.1%) and butanol (0.1%) to trigger the metabolic pathway to produce ethanol and butanol. After dilution rate of 0.0056 1/ h, acetone concentration increased with increasing dilution rates. At 0.0084 and 0.0113 1/ h dilution rates, the highest acetone concentration was 7.82 and 11.87 g/ L, respectively (Figure 4.29 c, d). At the beginning of fermentation acetone, butanol, ethanol and ABE concentration was 0.52, 0.29, 0.05, 0.86 g/ L, respectively with 0.0141 1/ h dilution rate (Figure 4.29 e). Solvent concentrations reduced depending on dilution during fermentation time. At 0.0169 1/ h dilution rate, initial acetone, butanol, ethanol, and ABE concentration were 0.26, 0.17, 0.05 and 0.48 g/ L, respectively (Figure 4.29 f). At dilution rates of 0.00141 and 0.00169 1/ h did not produce any solvent. At the tested dilution rates ethanol and butanol were not produced, this may be due to substrate inhibition of organic acids produced. Also, in the literature it is been known that some activators and inhibitors should be added into the fermentation media to enhance butanol production. For instance, some minerals (Zn, Mn, Mg), some enzymes, NADH^+ , acetone etc. are added to increase

butanol production (Brenda, Comprehensive enzyme information system, 2020). These type of activators and inhibitors were not added to the fermentation media.

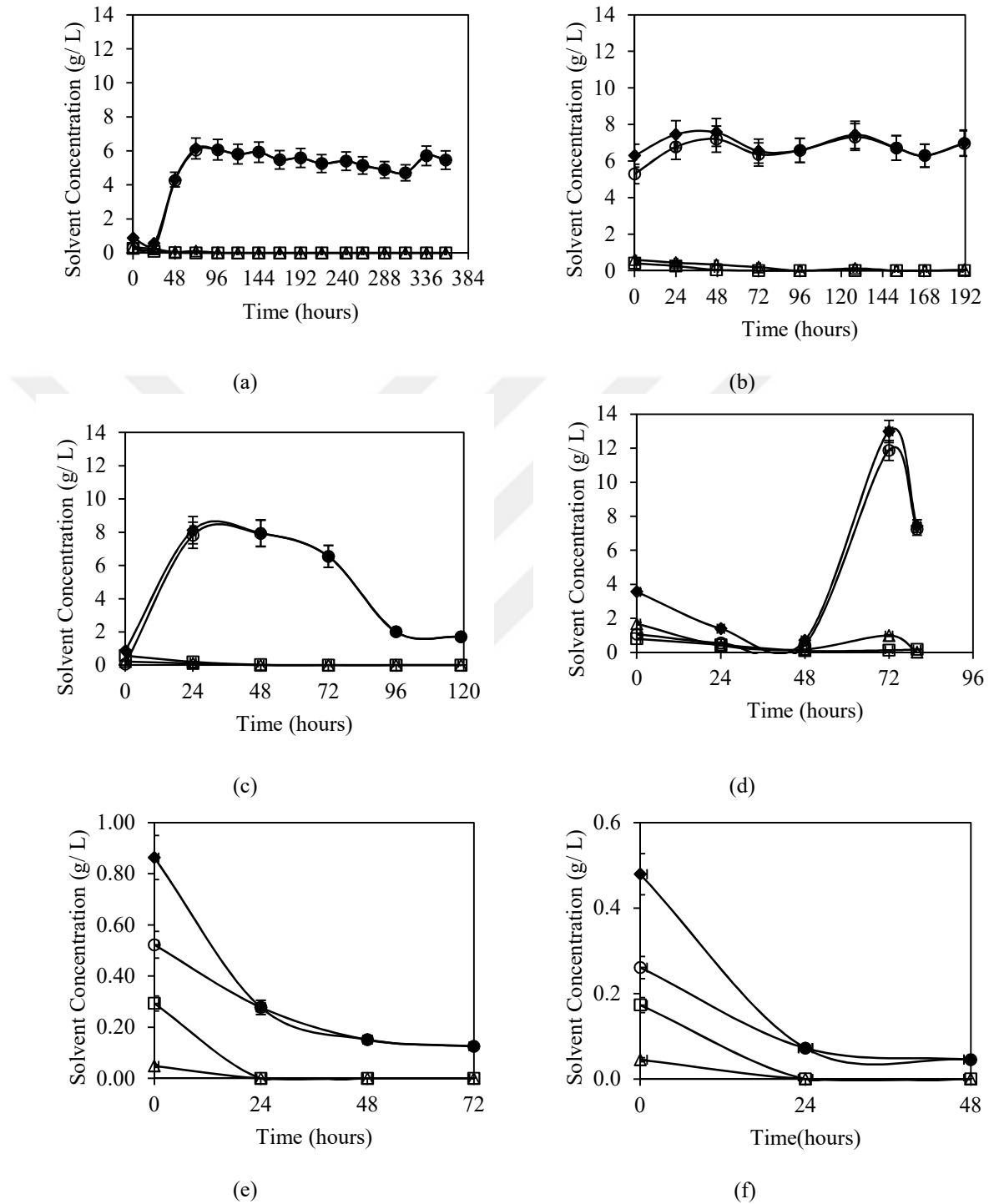


Figure 4.29 Variation of solvent concentration with time at different dilution rates (○ Acetone, □ Butanol, △ Ethanol, ◆ ABE) a) 0.0028 1/ h, b) 0.0056 1/ h, c) 0.0084 1/ h, d) 0.0113 1/ h, e) 0.0141 1/ h, f) 0.0169 1/ h

Variation of percent sugar consumption and solvent productivity (D.P) with dilution rate is depicted in Figure 4.30. Sugar consumption decreased with increasing dilution rates. The highest sugar consumption was seen at 0.0028 1/ h dilution rate however the highest solvent productivity was observed at dilution rate of 0.0113 1/ h dilution rate. Solvent productivity increased with increasing dilution rates till 0.0113 1/ h dilution rate and decreased with further increases. At 0.0141 1/ h dilution rate, the high TVFA concentrations produced with 47% sugar consumption caused substrate inhibition where the organism could not transmit solventogenic phase.

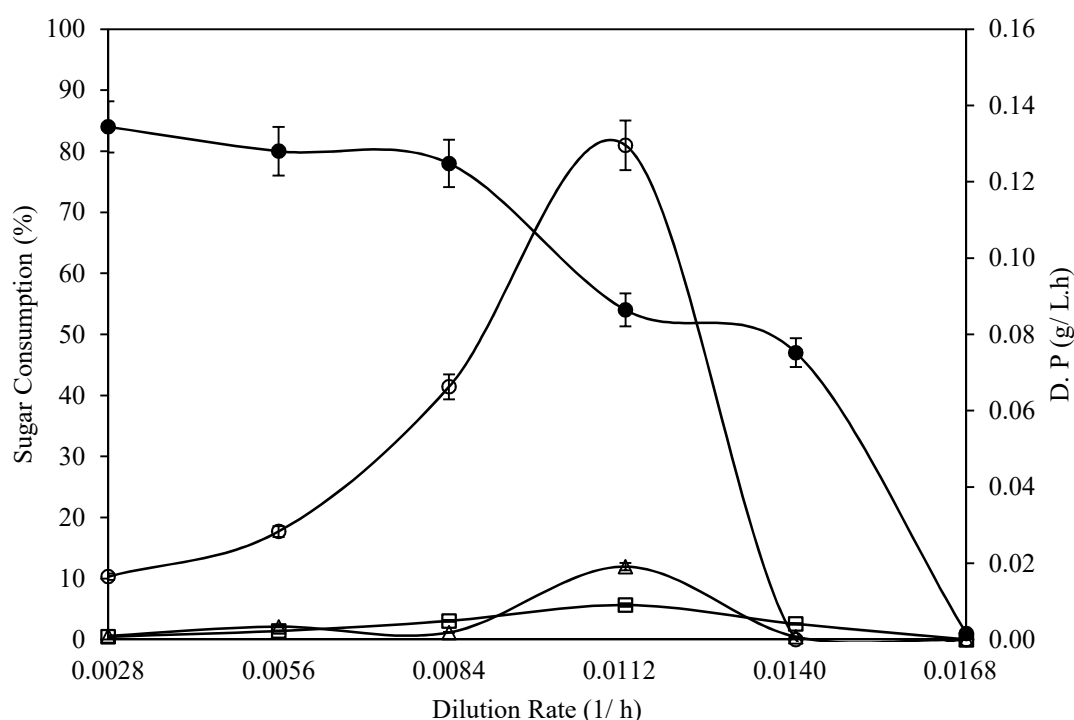


Figure 4.30 Variation of percent sugar consumption and solvent productivity (D.P) with dilution rate (● Sugar consumption, ○ Acetone productivity, □ Butanol productivity, Δ Ethanol Productivity)

Inhibitor formation after hydrolysis, or salts depending on neutralization step could affect butanol formation. Qureshi et al. (2008a) conducted fed-batch simultaneous saccharification and fermentation with *C. beijerinckii* P260. The highest ABE concentration was 16.59 g/ L which included 10.57 g/ L acetone, 5.80 g/ L butanol and 0.22 g/ L ethanol. The major solvent was acetone as well as our fed-batch experiments (Qureshi et al., 2008a).

Hydrolyzed peach waste was subjected to fed-batch mode with ABE fermentation at different dilution rates and the yield variation is shown in Figure 4.31. Acetone was the only solvent that was produced in all tested dilution rates and the yield calculations are based on just acetone production. The highest acetone production yield was observed with 0.0056 1/ h. The production yield increased with increasing dilution rates till 0.0056 1/ h and then decreased with further increases. There was no acetone and other solvents produced at the dilution rate of 0.0141 1/ h and 0.0169 1/ h.

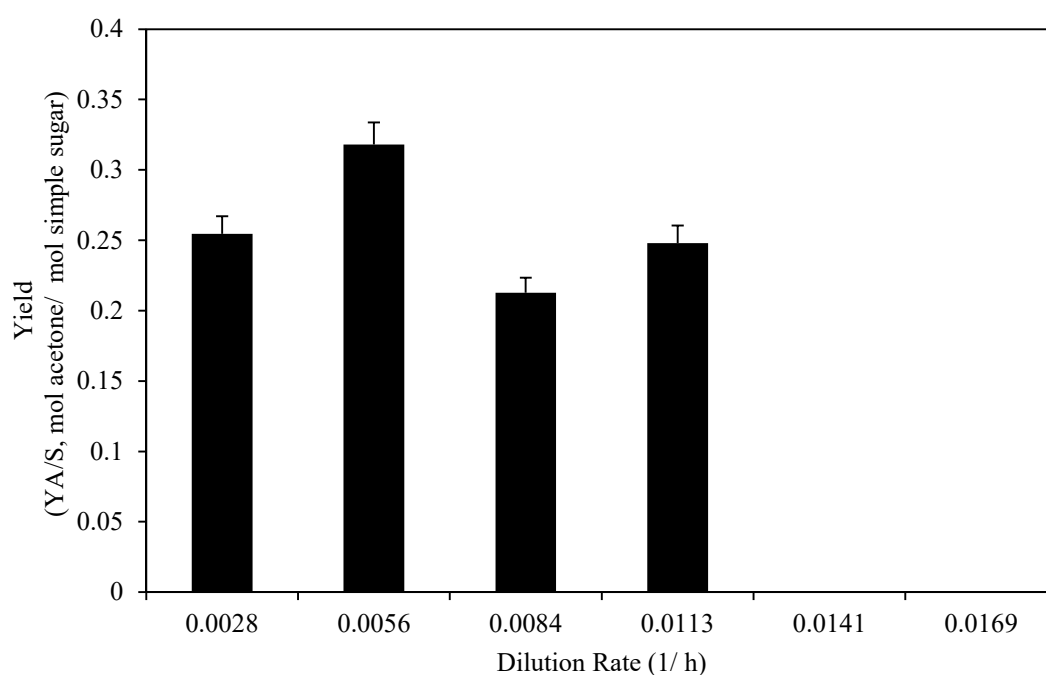


Figure 4.31 Acetone production yield for fed-batch experiments with different dilution rates

CHAPTER FIVE

CONCLUSION

At the first stage of the experiments, two different ABE producing bacteria, *C. acetobutylicum* NRRL 527 and *C. beijerinckii*-DSMZ-791 was subjected to different substrates to provide high solvent production. Batch experiments were conducted to select suitable substrate and organism for ABE fermentation. Batch experiments with fermentation media 1 resulted low or no butanol concentration so modified fermentation media (Fermentation media 2) was used in the second batch experiments.

In the first batch experiment, acid hydrolyzed substrates (tea waste, watermelon peel) and glucose were used with two different ABE producing bacteria, *C. acetobutylicum* and *C. beijerinckii* to investigate the ABE production capacity. Fermentation media 1 was used in this experiment. A constant total sugar concentration after pretreatment was applied which was approximately 3000 mg/ L. *C. beijerinckii* was succesful than *C. acetobutylicum* according to sugar consumption percents. The highest acetic acid concentration at the end of the fermentation obtained with watermelon peel with *C. acetobutylicum* (736.40 mg/ L) and *C. beijerinckii* (1513.52 mg/ L). Butyric acid was not observed in all substrates at *C. acetobutylicum* at the end of the fermentation while butyric acid concentrations for tea waste, watermelon peel and glucose was 617.12, 861.70, 1173.62 mg/ L, respectively at *C. beijerinckii*. The highest solvent at *C. acetobutylicum* was ethanol and at *C. beijerinckii* was acetone.

In second batch experiments, tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk were tested to produce ABE. The highest sugar consumption was found in non-hydrolyzed peach waste (82.98% and 83.75%) with *C. acetobutylicum* and *C. beijerinckii*, respectively. Volatile fatty acids of the same waste resulted as acetic acid (2313 mg/ L) in *C. acetobutylicum* while acetic (2007 mg/ L) and butyric acid (3907 mg/ L) were observed with *C. beijerinckii*. However, at non-hydrolyzed peach waste both organisms were not able to convert TVFAs into

solvents. The highest solvent concentration was obtained with wheat waste (15.27 g/ L) at *C. acetobutylicum* however the highest butanol concentration (6.97 g/ L) was produced with peach waste. At these substrates *C. beijerinckii* produced approximately the same ABE and butanol concentrations at tea waste, wheat waste and rice husk, meanwhile butanol concentration of tea waste was higher than the others. Sugar consumption of peach waste was 72.09% at *C. acetobutylicum* however, wheat waste (57.08%) ended up with a quite low sugar consumption. Sugar consumption of tea waste was 51.22% while in peach waste it ended up as 70.11% sugar consumption at *C. beijerinckii*. Sugar consumption of peach waste by both organisms was successful according to other wastes. The highest butanol yield (0.139 g ButOH/ g substrate) was obtained with peach waste and ABE yield (0.305 g ABE/ g substrate) was obtained with wheat waste at *C. acetobutylicum*. ABE yields of other wastes were approximately same. The highest butanol (0.135 g ButOH/ g substrate) and ABE yield (0.282 g ABE/ g substrate) was obtained with tea waste at *C. beijerinckii*. The best volumetric butanol and ABE production rate at *C. acetobutylicum* were 0.00116 g ButOH/ g substrate. h and 0.00235 g ABE/ g substrate. h at peach waste. The highest volumetric butanol production rate at *C. beijerinckii* was obtained with wheat waste (0.00104 g ButOH/ g substrate. h) and peach waste followed it with 0.00101 g ButOH/ g. h substrate. Volumetric ABE production rates of peach waste (0.00208 g ABE/ g substrate. h) and wheat waste (0.00221 g ABE/ g substrate. h) were similar. Peach waste was selected to use as substrate for fed-batch experiments due to high butanol yield and volumetric butanol production rates.

In our experiments at tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk the A:B:E ratios were 0.02:0.52:1, 0.03:0.63:1, 0:0.30:1, 0.02:0.44:1, 0.02:0.54:1, respectively with *C. acetobutylicum*. At glucose and fructose the A:B:E ratios were 0.03:0.53:1, 0:0.33:1, respectively. At tea waste, peach waste, non-hydrolyzed peach waste, wheat waste, rice husk the A:B:E ratios were 0.02:0.54:1, 0.03:0.59:1, 19.34:0.62:1, 0.03:0.57:1, 0.03:0.55:1, respectively with *C. beijerinckii*. At glucose and fructose the A:B:E ratios were 0.07:0.56:1, 17.03:0.58:1, respectively.

In fed-batch experiments, peach waste as substrate and *C. beijerinckii* as the organism was used. In fed-batch experiments, 60 g/ L peach waste was used for feed after acid hydrolysis of substrate at different dilution rates (0.0028, 0.0056, 0.0084, 0.0113, 0.0141, 0.0169 1/ h). The major simple sugars in peach waste were fructose and glucose where fructose concentration was almost twice as the glucose concentration. Sugar consumption decreased with increasing dilution rates. Besides, effluent glucose concentrations increased with increasing dilution rates however, effluent fructose concentrations decreased with increasing dilution rates till 0.0084 1/ h and then increased with further increasing dilution rates. The pH declined in all tested dilution rates due to acidogenic phase. Then, rise of the pH was observed with 0.0028, 0.0056, 0.0084 and 0.0113 1/ h and quasi-steady state conditions were obtained. The protein productivity increased with increasing dilution rates. The protein concentration reached to quasi-steady state in all tested dilution rates and varied between 6.3 -8.1 g/ L. Final acetic acid concentrations decreased with increasing dilution rates till 0.0113 1/ h and increased with further increasing dilution rates. Acetone was the major produced solvent at these fed-batch experiments. The highest acetone concentrations (11.87 g/ L) was observed with 0.0113 1/ h dilution rates. The highest acetone production yield (0.32 mol acetone/ mol glucose) was observed with 0.0056 1/ h. The production yield increased with increasing dilution rates till 0.0056 1/ h and then decreased with further increases. Literature reveals that, below 40 g/ L of sugar induced high acid formation and affects organisms negatively. As a result of the fed-batch studies, ABE fermentation of hydrolyzed peach waste using *C. beijerinckii* is successful at acetone production.

RECOMMENDATION FOR FUTURE STUDIES:

Some recommendations for the future studies are listed bellow:

- Different pretreatment methods can be applied to the substrate to prevent substrate inhibition due to by-products produced after hydrolysis.
- In order to make the production of biobutanol even more efficient, more resistant species may be applied to ABE fermentation.
- Different types of activators and inhibitors can be added to the fermentation media to enhance solvent production.
- IBE fermentation can be preferred instead of ABE fermentation to produce end products that can all be used as biofuels.
- Simultaneous recovery methods such as distillation, adsorption etc. may be applied to prevent solvent accumulation and product inhibition.

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APPENDICES

A.1 Raw Data of Batch Experiments

Table A.1.1 Raw data of tea waste with *C. acetobutylicum* at fermentation media 1

Tea waste with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar (mg/ L)	pH	Protein (mg/ L)	Suspended solid matter (g/ L)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Acetone (g/ L)	Butanol (g/ L)	Ethanol (g/ L)
0	3360.87	7.32	3013.75	7.68	294.07	0	1269.57	0	0	0.277
24	2085.48	6.72	4000.41	9.06	640.77	0	1137.88	0	0	0.181
48	1715.85	6.60	4299.39	9.48	550.65	0	1104.26	0	0.003	0.175
72	2052.18	6.65	3596.78	8.49	454.08	0	1046.25	0	0.002	0.231
96	1978.92	6.71	2998.80	7.66	505.27	0	852.16	0	0	0.276
120	2328.57	6.71	2929.04	7.56	281.50	0	303.10	0	0.001	0.298
144	1899.00	6.70	2854.29	7.45	483.68	0	391.99	0	0	0.286
168	2305.26	6.64	2799.48	7.38	590.98	0	197.77	0	0	0.337

Table A.1.2 Raw data of watermelon peel with *C. acetobutylicum* at fermentation media 1

Watermelon peel with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar (mg/ L)	pH	Protein (mg/ L)	Suspended solid matter (g/ L)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Acetone (g/ L)	Butanol (g/ L)	Ethanol (g/ L)
0	3494.07	7.20	643.22	1.25	1910.89	0	1567.16	0	0	0.563
24	1526.04	6.81	1748.47	2.80	1037.38	0	1185.13	0	0	0.201
48	1642.59	6.75	2350.92	3.64	935.58	0	1123.49	0	0.005	0.226
72	1446.12	6.81	1507.78	2.46	1046.94	0	1116.52	0	0.001	0.273
96	1093.14	6.83	658.17	1.27	923.28	0	833.87	0	0	0.218
120	2062.17	6.84	667.63	1.28	490.59	0	308.35	0	0.001	0.227
144	1486.08	6.82	694.54	1.32	977.49	0	385.96	0	0.002	0.218
168	1609.29	6.77	646.21	1.25	736.40	0	194.93	0	0.002	0.283

Table A.1.3 Raw data of glucose with *C. acetobutylicum* at fermentation media 1

Glucose with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar (mg/ L)	pH	Protein (mg/ L)	Suspended solid matter (g/ L)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Acetone (g/ L)	Butanol (g/ L)	Ethanol (g/ L)
0	3201.03	7.22	323.30	0.80	280.85	0	1264.93	0	0	0.649
24	800.10	6.49	1711.09	2.75	593.09	0	1168.72	0	0.001	0.174
48	646.92	6.40	1135.55	1.94	43.46	0	1123.33	0	0.005	0.218
72	417.76	6.52	919.78	1.64	529.43	0	1098.77	0	0.001	0.292
96	506.34	6.52	331.27	0.81	394.30	0	797.44	0	0	0.371
120	488.36	6.51	343.23	0.83	808.90	0	536.88	0	0	0.394
144	530.32	6.50	309.85	0.78	149.52	0	315.83	0	0.001	0.372
168	463.05	6.45	302.87	0.77	417.32	0	139.79	0	0	0.487

Table A.1.4 Raw data of tea waste with *C. beijerinckii* at fermentation media 1

Tea waste with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	Protein concentration (mg/ L)	Suspended solid matter (g/ L)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Acetone (g/ L)	Butanol (g/ L)	Ethanol (g/ L)
0	2515.05	7.17	1981.31	3.98	621.51	0	3183.36	0	0	0
24	1506.06	6.56	2795.94	4.60	941.78	232.03	3336.63	0.816	0	0.308
48	856.71	6.36	3602.73	5.22	976.01	635.10	3344.94	1.270	0.001	0.389
72	575.78	6.45	3218.50	7.39	1122.53	664.91	3277.65	1.850	0.208	0.749
96	492.35	6.48	3743.31	7.88	1212.73	659.34	3398.95	1.440	0.239	0.851
120	412.43	6.52	3852.97	7.96	1300.20	757.49	3257.29	1.537	0.204	0.996
144	369.81	6.52	3927.38	8.02	1376.81	619.63	3368.63	1.856	0.183	1.129
168	423.76	6.53	3531.82	7.71	1445.19	617.12	3311.40	1.950	0.248	1.538

Table A.1.5 Raw data of watermelon peel with *C. beijerinckii* at fermentation media 1

Watermelon peel with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	Protein concentration (mg/ L)	Suspended solid matter (g/ L)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Acetone (g/ L)	Butanol (g/ L)	Ethanol (g/ L)
0	2861.37	7.15	726.09	3.06	1040.44	0	3606.42	0	0	0
24	1432.80	6.05	2249.59	4.23	466.81	0	3655.11	0	0.001	0.274
48	770.13	6.66	2756.77	4.61	855.81	994.20	3352.71	3.409	0.000	0.421
72	457.24	6.68	2082.73	6.61	1014.40	863.70	3425.53	3.311	0.065	0.626
96	407.11	6.74	2627.12	7.02	1256.41	969.55	3570.93	3.020	0.239	1.094
120	364.48	6.72	2384.30	6.84	1360.38	927.14	3618.99	3.280	0.281	1.073
144	340.51	6.74	2302.06	6.78	1371.31	867.99	3649.17	3.460	0.355	1.320
168	357.16	6.75	2168.90	6.67	1513.52	861.70	3548.49	3.485	0.366	0.835

Table A.1.6 Raw data of glucose with *C. beijerinckii* at fermentation media 1

Glucose with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	Protein concentration (mg/ L)	Suspended solid matter (g/ L)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Acetone (g/ L)	Butanol (g/ L)	Ethanol (g/ L)
0	2584.98	7.22	1119.29	5.79	402.49	0	1254.65	0	0	0
24	876.69	6.49	4264.20	8.19	668.94	0	1179.44	0	0.001	0.196
48	806.76	6.51	4436.52	8.32	366.00	367.79	1112.77	0	0.003	0.100
72	145.37	6.70	4632.34	8.47	357.19	958.80	1088.75	0.801	0.163	0.165
96	169.25	6.71	4201.53	8.14	447.19	902.77	721.70	0.831	0.240	0.222
120	187.90	6.71	4087.96	8.06	496.05	979.07	508.65	0.849	0.313	0.230
144	193.23	6.69	3708.06	7.77	547.61	1143.54	265.66	0.846	0.314	0.421
168	155.60	6.64	3950.88	7.95	547.49	1173.62	115.82	0.758	0.293	0.153

Table A.1.7 Raw data of tea waste with *C. acetobutylicum* at fermentation media 2

Tea waste with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	3164.52	7.00		865.29	0	949.26	0	0	0	0
72	2334.45	5.82	50.6	823.64	0	19.92	0	0	0	0.019
96	2006.57	6.10	86.4	833.85	0	0	0	0	0.009	0.030
120	1736.56	5.92	59.0	871.72	0	0	0	0	0.744	0.956
144	1820.13	6.23	35.5	936.17	0	0	0	0.025	0.817	0.896
168	1704.41	6.14	-16.1	932.95	0	0	0	0.027	0.762	0.997

Table A.1.8 Raw data of peach waste with *C. acetobutylicum* at fermentation media 2

Peach waste with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	10094.98	7.00		1016.16	0	3957.38	6925.95	0	0	0
72	9477.80	5.28	25.7	2112.86	0	3450.55	6431.94	0	0	0.013
96	7163.36	5.28	-12.5	3731.80	0	2324.18	5957.39	0	0.009	0.025
120	6096.14	4.91	-26.0	3976.88	0	493.02	3393.97	0.033	0.869	0.872
144	4398.89	5.44	-22.0	2829.23	0	0	2568.34	0.032	0.775	0.850
168	2817.35	6.03	-125.0	1453.90	0	0	1914.10	0.033	0.790	0.839

Table A.1.9 Raw data of peach waste without hydrolysis with *C. acetobutylicum* at fermentation media 2

Peach waste without hydrolysis with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	7386.94	7.00		3325.57	0	921.26	6238.35	0	0	0
24	4597.39	5.56	-80.0	1465.37	0	1114.85	4611.52	0	0	0
50	2849.27	5.88	-63.5	1329.77	0	555.84	4038.66	0	0.001	0.009
72	1894.63	6.03	-95.0	1438.57	0	353.79	4187.18	0	0.003	0.008
120	1244.87	5.96	-40.0	1577.16	0		4152.17	0	0.006	0.014
144	1269.66	6.11	-33.3	2082.17	0	0	4346.63	0	0.006	0.011
167	1257.26	6.15	-55.0	2312.65	0	0	4349.91	0	0.006	0.009

Table A.1.10 Raw data of wheat waste with *C. acetobutylicum* at fermentation media 2

Wheat waste with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	14506.72	7.00		1006.35	0	1047.25	2142.92	0	0	0
72	14120.98	5.79	-44.5	999.35	0	598.98	2100.32	0	0	0.005
96	10469.31	5.80	-67.4	844.96	0	155.52	2140.27	0	0.008	0.027
120	9054.93	5.70	-32.8	766.33	0	149.91	2031.80	0	0.773	0.970
144	7872.00	5.47	-57.0	801.78	0	128.48	2076.23	0	0.776	1.059
168	6226.17	5.52	-56.8	866.83	0	121.32	2185.37	0.027	0.777	1.118

Table A.1.11 Raw data of rice husk with *C. acetobutylicum* at fermentation media 2

Rice husk with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	2855.93	7.00		896.51	0	1012.23	0	0	0	0
72	1479.40	6.08	-20.5	1004.94	0	36.87	0	0	0	0.007
96	920.07	6.03	-68.8	997.16	0	77.47	0	0	0.005	0.028
120	877.21	6.08	12.4	1127.78	0	72.85	0	0	0.785	0.946
144	964.64	6.22	-50.9	1212.26	0	42.06	0	0	0.811	0.975
168	864.35	6.24	-35.9	1341.93	0	65.74	0	0.026	0.833	0.941

Table A.1.12 Raw data of glucose with *C. acetobutylicum* at fermentation media 2

Glucose with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	44110.19	7.00		596.32	0	28576.92	0	0	0	0
72		5.23	-29.4	570.36	0	27604.46	0	0	0	0.002
96	31380.77	5.64	-35.0	566.38	0	24776.44	0	0.026	0.002	0.025
120	34916.72	5.40	-25.0	577.08	0	24779.40	3478.40	0	0.743	0.934
144	34723.85	5.47	-48.0	498.14	0	23004.76	2668.20	0.026	0.713	0.900
168	32666.57	5.45	-92.7	694.28	0	21470.30	2867.20	0.031	0.780	0.846

Table A.1.13 Raw data of fructose with *C. acetobutylicum* at fermentation media 2

Fructose with <i>C. acetobutylicum</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	5738.01	7.00		1415.24	0	558.24	7287.24	0	0	0
24	3965.09	5.72	-63.0	1427.56	0	226.91	6014.57	0	0	0
50	3035.24	5.77	-48.0	1343.28	0	193.90	5533.94	0	0.001	0.002
72	2204.58	5.93	-82.0	1246.57	0	265.95	5213.02	0	0.003	0.005
120	1666.40	5.81	-52.0	1345.41	0		4557.65	0	0.007	0.006
144	1666.40	6.00	-66.0	1496.75	0	249.56	4759.82	0	0	0
167	1430.84	5.92	-80.0	1687.88	0	206.66	4457.39	0	0	0.014

Table A.1.14 Raw data of tea waste with *C. beijerinckii* at fermentation media 2

Tea waste with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	3691.70	7.00		1164.85	0	776.48	0	0	0	0
72	2141.58	5.61	-68.9	1300.02	0	0	0	0	0	0
96	1974.43	6.13	-101.6	1266.04	0	0	0	0	0.006	0.014
120	1968.00	6.05	-34.0	1260.05	0	0	0	0	0.799	0.870
144	1890.85	6.21	-20.0	1295.78	0	0	0	0.026	0.764	0.984
168	1800.85	6.10	-64.7	1401.67	0	0	0	0	0.839	0.909

Table A.1.15 Raw data of peach waste with *C. beijerinckii* at fermentation media 2

Peach waste with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	9683.52	7.00		1236.56	0	3656.10	6700.00	0	0	0
72	4694.62	4.83	-62.0	1814.13	0	1872.56	1669.64	0	0	0
96	4231.73	5.76	-106.2	1732.40	0	1301.15	1589.68	0	0	0.008
120	3820.28	5.78	-98.4	2011.24	0	726.04	2322.51	0	0.758	0.810
144	2688.77	6.08	-120.4	2060.21	0	92.27	2278.29	0.034	0.729	0.737
168	2894.50	5.95	-109.7	2145.65	0	0	2332.03	0.034	0.757	0.762

Table A.1.16 Raw data of peach waste without hydrolysis with *C. beijerinckii* at fermentation media 2

Peach waste without hydrolysis with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	8118.42	7.00		1754.34	0	3003.04	6405.82	0	0	0
24	5849.59	5.45	-63.0	3741.32	0	860.75	6900.13	0	0	0.020
50	4969.33	6.55	-252.0	1159.56	1960.68	721.68	6325.02	0.431	0.006	0.033
72	3022.84	6.45	-176.0	1171.71	2908.74	481.33	5919.25	0.606	0.009	0.034
120	1375.04	6.10	-95.0	1047.88	3730.89	0	4152.64	0.823	0.017	0.034
144	1375.04	6.08	-36.0	1276.28	3923.00	0	4039.68	0.826	0.033	0.027
167	1319.25	6.09	10.0	2006.79	3907.55	0	3957.61	0.829	0.031	0.028

Table A.1.17 Raw data of wheat waste with *C. beijerinckii* at fermentation media 2

Wheat waste with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	15843.96	7.00		1174.68	0	1013.83	2291.90	0	0	0
72	12680.89	5.33	-91.2	1126.28	0	52.24	2110.59	0.026	0	0.001
96	13040.91	6.17	-86.0	1023.87	0	141.75	2041.61	0	0.002	0.012
120	13838.11	6.07	-46.9	1176.68	0	52.72	2070.08	0	0.779	0.891
144	13735.24	6.11	-94.7	962.66	0	50.82	2011.37	0	0.803	0.893
168	13246.64	6.06	-49.0	1176.66	0	56.22	2059.63	0.035	0.781	0.893

Table A.1.18 Raw data of rice husk with *C. beijerinckii* at fermentation media 2

Rice husk with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	2457.33	7.00		1208.03	0	816.42	0	0	0	0
72	1132.23	5.62	-109.0	1269.60	0	31.08	0	0.026	0	0
96	1106.51	6.14	-91.0	1382.21	0	31.60	0	0.026	0	0.012
120	887.49	6.04	-21.8	1386.01	0	30.86	0	0.028	0.777	0.867
144	967.21	6.09	-12.0	1320.66	0	30.87	0	0.028	0.736	0.898
168	882.35	6.11	-27.7	1401.74	0	31.42	0	0.039	0.784	0.886

Table A.1.19 Raw data of glucose with *C. beijerinckii* at fermentation media 2

Glucose with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Xylose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	36845.42	7.00		882.96	0	27564.36	3052.60	0	0	0
72	29580.65	4.69	-60.0	1034.78	0	22108.94	1308.20	0	0	0
96	24501.74	5.55	-99.0	1721.36	0	24029.26	854.60	0.025	0	0.008
120	27523.37	5.52	-119.7	1695.36	0	20130.58	835.60	0.061	0.742	0.806
144	26044.70	5.70	-118.0	2742.98	0	19271.34	1003.80	0.032	0.711	0.800
168	23858.84	5.65	-111.9	2304.08	0	15162.68	715.80	0.073	0.735	0.833

Table A.1.20 Raw data of fructose with *C. beijerinckii* at fermentation media 2

Fructose with <i>C. beijerinckii</i>										
Time (hours)	Total sugar concentration (mg/ L)	pH	ORP	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	6320.71	7.00		1462.90	0	1054.54	7292.17	0	0	0
24	4051.88	5.71	-78.0	1740.46	0	0	7541.03	0.002	0.004	0.019
50	3122.03	6.48	-144.0	1725.27	1349.15	0	6992.38	0.307	0	0.031
72	1993.81	6.41	-165.0	1712.95	2254.20	0	5554.07	0.458	0.003	0.029
120	600.17	6.10	-40.0	1317.85	2863.63	0	3949.80	0.651	0.028	0.029
144	649.76	6.12	-31.8	1521.37	3118.96	0	3898.23	0.647	0.022	0.023
167	637.36	6.13	28.5	1516.46	3144.07	0	4100.13	0.666	0.021	0.026

A.2 Raw Data of Fed-Batch Experiments

Table A.2.1 Raw data of feed at different dilution rates

Peach waste hydrolysate							
Dilution rate (1/ h)	Total sugar concentration (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Propionic acid (mg/ L)	Lactic acid (mg/ L)
0.0028	22553.02	6677.62	14638.52	1599.65	0	0	0
0.0056	26272.42	5270.53	9826.69	1771.47	0	1585.34	0
0.0084	21747.15	8415.75	15774.68	1708.54	0	0	0
0.0113	24536.70	11376.16	20947.86	1770.57	0	0	0
0.0141	25249.59	8816.48	15765.57	1522.86	0	0	0
0.0169	21995.11	7630.21	16573.31	1288.56	0	1573.06	0

Table A.2.2 Raw data of fed-batch fermentation at 0.0028 1/ h dilution rate

Dilution rate: 0.0028 (1/ h)							
Time (hours)	Cumulative volume (L)	Total sugar (mg/ L)	pH	Protein (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	1.000	7266.63	6.55	7402.71	0.04	0.03	0.04
24	1.135	8013.61	6.15	7402.71	0.03	0.01	0.03
48	1.270	5468.92	6.24	7304.28	0.54	0	0.01
72	1.405	3606.12	5.80	7771.81	0.77	0	0.01
97	1.540	2078.63	5.70	7980.97	0.77	0	0
120	1.675	2573.98	5.90	8116.30	0.74	0	0
144	1.810	3022.84	5.85	7550.35	0.76	0	0
168	1.945	4160.93	6.01	7045.91	0.70	0	0
192	2.080	3051.31	5.84	6947.48	0.71	0	0
216	2.215	3444.38	5.82	7710.29	0.67	0	0
244	2.350	2787.28	5.85	7267.37	0.69	0	0
263	2.485	3828.71	5.80	7636.47	0.66	0	0
288	2.620	3828.71	5.65	7980.97	0.62	0	0
312	2.755	4089.07	5.80	7956.36	0.60	0	0
336	2.890	3940.30	5.77	7821.02	0.73	0	0
358	3.000	3989.89	5.77	7784.11	0.70	0	0

Table A.2.2 continues

Dilution rate: 0.0028 (1/ h)						
Time (hours)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Propionic acid (mg/ L)	Lactic acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)
0	1307.64	0	0	2798.62	6684.00	3947.40
24	4095.30	0	0	2146.01	5487.20	5279.04
48	875.99	3475.27	0	878.93	3151.29	4705.66
72	483.47	5049.38	0	0	1588.22	4721.24
97	972.72	5580.09	0	0	0	4140.36
120	2396.07	5231.69	0	0	0	4795.20
144	4936.30	4866.41	0	0	0	4365.75
168	4018.90	4574.57	0	0	569.45	5040.97
192	5939.72	5113.75	0	0	0	4789.84
216	6068.08	4567.94	0	1640.20	14.10	4883.10
244	5752.56	4586.81	0	1884.62	0	5074.87
263	5360.61	4395.89	0	2930.08	75.14	5051.61
288	5465.80	4427.04	0	3768.08	63.77	5114.63
312	5994.30	3751.99	0	4447.69	70.67	5224.98
336	6620.21	4336.93	0	5294.64	0	5346.10
358	6420.57	3642.79	0	5586.25	0	5273.21

Table A.2.3 Raw data of fed-batch fermentation at 0.0056 1/ h dilution rate

Dilution rate: 0.0056 (1/ h)								
Time (hours)	Cumulative volume (L)	Total sugar (mg/ L)	pH	ORP	Protein (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	1.000	4439.88	6.47	-400	6812.14	0.68	0.05	0.08
24	1.270	3410.85	5.55	-440	7218.16	0.86	0.03	0.06
48	1.540	4226.02	5.89	-389	7501.13	0.92	0.01	0.04
72	1.810	6085.15	5.96	-440	7316.58	0.81	0	0.02
96	2.080	4039.48	5.61	-420	7353.49	0.84	0	0
128	2.350	3332.79	5.89	-389	7624.17	0.93	0	0.02
152	2.620	4262.64	6.05	-404		0.86	0	0
169	2.890	6655.46	5.96	-448	7661.08	0.80	0	0
192	3.000	3506.37	5.88	-459	8091.70	0.89	0	0.01

Table A.2.3 continues

Dilution rate: 0.0056 (1/ h)						
Time (hours)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Propionic acid (mg/ L)	Lactic acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)
0	452.88	3259.31	1386.93	0	2784.95	2128.33
24	511.30	4256.27	1259.29	0	615.38	3178.75
48	476.02	4669.87	1162.95	0	494.08	4174.74
72	926.63	4041.61	1266.77	0	1100.87	4857.94
96	1543.60	4035.55	1067.87	0	162.81	4296.42
128	1878.04	4956.14	1262.99	0	126.19	3276.36
152	1964.92	4321.46	1046.40	0	660.86	3996.42
169	2098.55	3710.23	1444.84	0	1027.57	4508.00
192	2147.07	4089.07	1126.19	0	285.81	3787.71

Table A.2.4 Raw data of fed-batch fermentation at 0.0084 1/ h dilution rate

Dilution rate: 0.0084 (1/ h)								
Time (hours)	Cumulative volume (L)	Total sugar (mg/ L)	pH	ORP	Protein (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	1.000	7357.08	6.50	-30	7624.17	0.01	0.07	0.03
24	1.405	3803.92	5.90	-460	7501.13	1.00	0.02	0.01
48	1.810	3035.24	5.42	-305	7341.19	1.01	0	0
72	2.215	4473.41	5.70	-217	7378.10	0.83	0	0
96	2.620	5700.81	5.79	-310	7144.34	0.26	0	0
119	3.000	7448.93	5.80	-345	7328.89	0.22	0	0

Table A.2.4 continues

Dilution rate: 0.0084 (1/ h)						
Time (hours)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Propionic acid (mg/ L)	Lactic acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)
0	1061.62	0	990.94	2710.35	5933.72	3628.75
24	266.53	4744.61	902.68	1360.28	482.78	1511.62
48	198.24	4720.16	678.93	2765.81	175.30	1939.11
72	736.73	3704.46	1137.69	4083.57	289.40	2287.48
96	573.16	3179.79	962.16	4911.36	441.46	2724.89
119	390.81	2607.02	788.81	5056.42	541.25	2790.51

Table A.2.5 Raw data of fed-batch fermentation at 0.0113 1/ h dilution rate

Dilution rate: 0.0113 (1/ h)							
Time (hours)	Cumulative volume (L)	Total sugar (mg/ L)	pH	Protein (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	1.000	7332.28	6.47	8608.44	0.14	0.10	0.21
24	1.540	11081.54	5.94	8620.74	0.07	0.06	0.05
48	2.080	12494.92	5.45	8792.99	0.06	0.01	0.02
72	2.620	8230.00	6.03	8792.99	1.51	0.02	0.13
80	3.000	9519.40	6.09	7931.75	0.92	0.02	0

Table A.2.5 continues

Dilution rate: 0.0113 (1/ h)						
Time (hours)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Propionic acid (mg/ L)	Lactic acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)
0	1075.86	0	1517.72	2209.91	6063.31	3956.48
24	571.95	0	801.20	2146.93	5467.26	7468.12
48	1186.89	0	1216.78	2616.78	4588.95	8876.47
72	296.31	5054.10	1034.07	0	1273.54	7624.52
80	45.29	4864.56	1139.36	0	2029.93	8639.81

Table A.2.6 Raw data of fed-batch fermentation at 0.0141 1/ h dilution rate

Dilution rate: 0.0141 (1/ h)							
Time (hours)	Cumulative volume (L)	Total sugar (mg/ L)	pH	Protein (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	1.000	6655.46	6.54	7857.93	0.07	0.04	0.01
24	1.675	9568.99	5.23	7193.55	0.04	0	0
48	2.350	9234.24	5.38	7599.56	0.02	0	0
72	3.000	11081.54	5.08	7144.34	0.02	0	0

Table A.2.6 continues

Dilution rate: 0.0141 (1/ h)						
Time (hours)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Propionic acid (mg/ L)	Lactic acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)
0	992.81	0	1059.12	3057.42	5808.53	3666.67
24	5257.68	0	1086.06	3961.23	4155.14	8308.49
48	7997.38	0	970.31	5666.45	2704.82	9798.65
72	8641.55	0	760.08	6308.75	2197.99	10974.83

Table A.2.7 Raw data of fed-batch fermentation at 0.0169 1/ h dilution rate

Dilution rate: 0.0169 (1/ h)							
Time (hours)	Cumulative volume (L)	Total sugar (mg/ L)	pH	Protein (mg/ L)	Acetone (%)	Butanol (%)	Ethanol (%)
0	1.000	10927.14	6.70	6553.77	0.03	0.02	0.01
24	1.810	15743.19	6.49	6959.78	0.01	0	0
48	2.620	17590.49	6.22	6516.86	0.01	0	0

Table A.2.7 continues

Dilution rate: 0.0169 (1/ h)						
Time (hours)	Acetic acid (mg/ L)	Butyric acid (mg/ L)	Propionic acid (mg/ L)	Lactic acid (mg/ L)	Glucose (mg/ L)	Fructose (mg/ L)
0	600.30	0	1095.12	0	15303.65	6893.704
24	3921.48	0	670.78	0	13469.48	15375.11
48	12820.66	0	1262.55	155.87	12517.49	18483.55