Acetone, Butanol, and Ethanol (ABE) Production from Food Waste via *Clostridium beijerinckii*

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ABSTRACT

Annually, approximately 150 million metric tonnes of food goes to waste in the U.S., potentially causing economic loss and environmental pollution. Fermentation of food waste to produce acetone, butanol and ethanol (ABE) via fermentation has the potential to valorize food waste by producing value-added chemicals. However, the composition of food wastes from different sources vary, which affects ABE fermentation performance and hinders the commercialization of food waste fermentation. The objective of this study is to investigate the compositional variation of food waste collected weekly for 16 weeks (a total of sixteen samples) and determine how this variation affects ABE fermentation performance. Samples collected from Southgate Center, a food processing facility operated by Virginia Tech Dining Services, was characterized for use as a feedstock for ABE fermentation. ~~Variation amongst each samples w~~Water, sugar, starch, fiber, protein, fat and ash concentrations in each of food waste samples ~~was~~ were determined. ABE fermentation of these wastes was performed using *Clostridium beijerinckii* via batch fermentations. Correlations of ABE and butanol yields with the individual components of food waste composition were performed to better understand which components are key to ABE fermentation.

Overall, this study demonstrated the feasibility of using food waste as a viable feedstock for ABE fermentation and investigated the effect of variation of food waste composition on the ABE fermentation performance. ~~During the 16 week collection period~~In the 16 collected samples, each major compositional attribute exhibited high variability. The concentration of total soluble sugar, defined as glucose, fructose, sucrose for the purposes of this experiment, ranged from 0.5 to 53.5% (dry basis) among different food waste samples. The concentration ranges of total starch, neutral detergent fiber (NDF), crude protein, crude fat and ash were 0 to 23.4% (dry basis), 0.6 to 25.8%, 5.5 to 21.2%, 0.1 to 37.9%, 1.4 to 13.7%, respectively. The high variation of food waste composition resulted in a high variation of ABE yield when these food wastes were subjected to fermentation by *C. beijerinckii*. The total ABE concentration following

fermentation ranged between 6.9 to 17.0 g/L with an average value of 13.2 g/L. ABE and butanol concentrations are positively correlated with starch and equivalent glucose, i.e., the ~~theoretical~~ sum of initial free glucose and glucose ~~which~~ that ~~should be made available by~~could be theoretically ~~hydrolysis of~~hydrolyzed from starch and sucrose during fermentation, but is negatively correlated with NDF concentrations.

GENERAL AUDIENCE ABSTRACT

Nearly 40% of food in the U.S. goes to waste, causing a huge amount of economic loss and environmental pollution. Use of microorganisms to ferment food waste is a viable way to mitigate many of the issues associated with food waste. Put simply, fermentation is a biological process in which an organic substrate, such as food waste, is consumed and a more valuable product is produced. In this study, different food wastes were collected from the campus food processing center weekly for 16 weeks. Water, sugars, starch, fiber, protein, fat and ash contents of the collected food wastes were determined. Fermentation of these food wastes were conducted using a microorganism called *Clostridium beijerinckii*. The results showed that there was a high variation amongst the composition of the food waste samples. The concentration of total soluble sugar (glucose, fructose, sucrose) ranged from 0.5 to 53.5% (dry basis) among different food waste samples. The concentration ranges of total starch, neutral detergent fiber (NDF), crude protein, crude fat and ash were 0 to 23.4% (dry basis), 0.6 to 25.8%, 5.5 to 21.2%, 0.1 to 37.9%, 1.4 to 13.7%, respectively. The variation of food waste composition also led to different fermentation yields. It was also found that a higher glucose ~~concentration~~ content in food waste results in a higher fermentation product yield; however, a higher fiber ~~concentration~~ content in food waste results in a lower fermentation product yield~~s~~.

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CHAPTER 1. INTRODUCTION

Food wastes have become a major cause for concern in recent history. The Food and Agricultural Organization (FAO) stated that 1.3 billion metric tonnes (MT) of food waste is generated annually (FAO, 2019). In the United States, 150 million ~~metric tonnes (~~MT~~)~~ of food exits the supply chain as food waste each year, which accounts for almost 40% of total U.S. food production. Unfortunately, 70 million MT of that waste was edible food when it was discarded (Dou et al., 2016). Growing populations have given rise to the need for greater food production, and the United Nations has predicted that the world population will spike to 9.3 billion people by 2050. This population would require a 70% increase in global food supply. As or more important than the capacity to produce more food is the ability to more efficiently and responsibly utilize existing resources, as some level of waste generation is an inevitable outcome of food production, processing, distribution and consumption. Efforts to mitigate unnecessary production of waste may be valuable, but could never completely eliminate the issue.

Though inedible to humans, these wastes represent an enormous sum of energy which could potentially be utilized in some industrial process. Incineration of food wastes as a means of energy production is inefficient due to its high moisture content and the relatively high proportion of organic matter in most food wastes. This can also result in unsteady burning and the production of harmful dioxins (Wang et al., 2014). Using food wastes as animal feed can be an effective way to avoid landfilling, but regulatory hurdles can sometimes be difficult to overcome. Composting, while definitely a better option than landfilling, is logistically challenging. The Environmental Protection Agency estimates that only 2.10 ~~million tons~~MT, or 5.3% of total food waste was composted in 2015 (EPA, 2016). Therefore, most food waste produced in the United States and the world is landfilled. Unfortunately, biodegradation of food

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waste in landfills inevitably produces methane, a powerful greenhouse gas which contributes to global warming (Evans & Nagele, 2018). Along with the production of greenhouse gasses, significant leaching of contaminants into the environment and the production of disagreeable odors are also common outcomes of landfilling food wastes improperly (Capson-Tojo et al., 2018).

Converting food waste to value-added chemicals and biofuels via fermentation has proven to be a viable strategy (Magyar et al., 2017). Food wastes are primarily comprised of three organic components: carbohydrates, proteins, and lipids. These components also represent the bulk of volatile solids, or the proportion of total soluble solids which are volatilized by combustion at 550 °C. Further, the ratio of volatile solids to total solids generally ranges from 80 to 97% (Li et al., 2017). Generally, 50% to 70% of these volatile solids are easily degradable carbohydrates (Capson-Tojo et al., 2018). Solventogenic *Clostridium spp.* are capable of utilizing both carbohydrates and proteins to produce the value-added compounds, acetone, butanol and ethanol (ABE) (Li et al., 2013). Also, food wastes contain relatively high concentrations of other compounds, including proteins, fatty acids, and minerals, which are highly nutritive and support fermentation (Huang et al., 2015). Fermentative processes utilizing food waste as a feedstock have been successfully performed to produce lactic acid, hydrogen gas, biogas, ethanol, and butanol (Capson-Tojo et al., 2018; Huang et al., 2015; Magyar et al., 2017; Wang et al., 2014).

Despite the invaluable and positive attributes mentioned above, food wastes also possess one incessant and consequential drawback. Food wastes collected at different times, even from the same ~~facility or~~ location, can display drastically different compositional attributes, which affects fermentation characteristics and product yields. Some types of food waste include

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relatively high concentrations of readily fermentable sugars and starches, such as bakery wastes, while other types include higher concentrations of unfermentable structural fibers, such as trimmings from non-starchy vegetables. This property and the challenge presented by food waste collection logistics are the main two hurdles inhibiting the commercialization of conversion of food wastes to value-added products (Magyar et al., 2017). Therefore, it is important to investigate the compositional variability of food wastes and determine how this variation affects the fermentation performance and yield. This information could be used as a foundation for the development of a strategy which mitigates the effect of these variations on food waste fermentation.

The overall goal of this study is to develop a practical process to convert food wastes collected from Virginia Tech's Southgate Center into butanol via ABE fermentation using *Clostridium beijerinckii* P260 and investigate the compositional variability of food waste on ABE fermentation yield. Hypothesis: Food waste is a superior feedstock for ABE fermentation to produce butanol due to its high concentrations of carbohydrates (starch and free sugars) and other nutrients (e.g., proteins, vitamins, minerals), and the variation of composition of food waste affects the characteristics and yield of ABE fermentation. The specific objectives of the study are

Objective 1: To determine the feasibility of using food waste as feedstock to produce butanol via ABE fermentation using *C. beijerinckii* P260. Working hypothesis 1: ABE fermentation using food waste as feedstock will have a higher final ABE concentration than the control ABE fermentation using glucose as feedstock.

Objective 2: To determine the variation of compositional attributes among different food wastes collected in Virginia Tech's Southgate Center. Working hypothesis 2: Food waste contains significant amounts of fermentable carbohydrates (starch and sugars), but there is a high

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variation of composition of food wastes collected at different times.

Objective 3: To determine the variation of ABE fermentation yields from different food wastes and to determine the significance and strength of correlations between food waste composition attributes and ABE fermentation yield. Working hypothesis 3: a) High variations among food waste composition leads to high variation among ABE yields in ABE fermentation; b) The ABE fermentation yield is highly correlated with food wastes composition.

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Chapter 2. REVIEW OF LITERATURE

2.1. Biofuels Production

A biofuel, less commonly referred to as an agrofuel, is a fuel derived from biomass, which can be a solid, liquid, or gas. One useful division when considering biofuels is by type of biomass, or feedstock, used to produce them. First generation biofuels are produced using tried and true methods which have defined the industry for centuries. These biofuels are produced from sugar or starch-based commodities such as corn, sugar beets, molasses, or sorghum. A defining characteristic of first-generation biofuels is the use of feedstocks which divert food away from human consumption (Dou et al., 2016). This issue and cost are the primary reasons why first-generation methods have fallen into disfavor when producing acetone, butanol, and ethanol via fermentation (Evans & Nagele, 2018).

Second generation biofuels are produced from feedstocks that do not compete with the production of food for humans. These include agricultural wastes, such as corn stover, rice and wheat straw, and sugarcane bagasse. The factors which define these materials are the relatively low concentrations of simple sugars and starches, as well as relatively high concentrations of lignocellulosic materials. Lignocellulose is an energy dense yet recalcitrant complex composed of cellulose, hemicellulose, and lignin. Cellulose and hemicellulose are polymers composed of glucose and various monosaccharides, respectively. Lignin, on the other hand, is composed of crosslinked phenolic polymers. Though the composition of each type of plant is different, the average proportions (w/w) of lignocellulose are 35-50% cellulose, 20-40% hemicellulose, and 5-30% lignin (Spychaj, 2018). To utilize the energy contained within these polymers, steps must be taken to remove lignin and depolymerize cellulose and hemicellulose. Chemical and biological processes have been tested on a lab scale with varying degrees of success (Wang et al., 2014).

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However, the high costs of chemical pretreatment and enzymatic hydrolysis to convert structural cellulose and hemicellulose to ~~free~~ monomer sugars ~~still act as~~is a barrier for the commercialization of the second-generation biofuels.

Use of microalgae and cyanobacteria as feedstocks for biofuel production is an innovative and burgeoning technology, which many are referring to as third-generation feedstocks (Li et al., 2013). While both second and third-generation feedstocks are promising and technically challenging, use of second-generation feedstocks is associated with several advantages unrelated to the production of biofuels. Namely, use of second-generation feedstocks presents the unique opportunity to valorize low value by-products and waste materials from the food processing, food retail, and agriculture industries. Lignocellulose is ubiquitous, being the most abundant renewable resource on earth. Currently, food waste is most commonly disposed of via landfilling, which is costly to manufacturers and the environment. Landfilling can cost as much as $100/MT, while approximately 60% of the 13.5 million MT of greenhouse gas emissions associated with Australian food waste originate from landfills (Capson-Tojo et al., 2018; Li et al., 2017). Utilizing these wastes as feedstock for ABE fermentation would sequester some of that carbon in the organic solvents produced.

Traditionally, research efforts have been funneled towards the use of ethanol as a biofuel. The base of knowledge and existing infrastructure makes improving ethanol fermentation technologies a simpler proposition. The US Environmental Protection Agency published regulations regarding the use of renewable fuels in 2010, requiring 36 billion gallons of renewable fuels be blended with petroleum derived fuels by 2022. Fifteen billion gallons of the 36 are expected to be first generation biofuels, such as corn starch derived ethanol. 16 billion gallons are required to be advanced biofuels, or biofuels which reduce greenhouse gas emissions

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by a minimum of 50%. Examples of advanced biofuels are cellulosic ethanol, or more importantly to this research, biologically produced butanol (Cascone, 2008; Tao et al., 2014). Compared to ethanol, butanol has several significant advantages (Tao et al., 2014). Butanol can be easily handled using the current petroleum infrastructure and butanol is far less miscible with water than ethanol, meaning it is less likely that butanol blended gasoline will be tainted with water (Cascone, 2008). Further, butanol has a heating value similar to gasoline, while ethanol's heating value is much lower. This means vehicles utilizing butanol blended fuel will experience a lower drop in fuel economy (Cascone, 2008; Maiti et al., 2016). Finally, unlike ethanol, butanol can be blended with gasoline at any ratio, including the use of unblended butanol, to fuel Otto cycle engines (Buehler & Mesbah, 2016; Maiti et al., 2016).

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2.2. Acetone, Butanol and Ethanol (ABE) Fermentation

The ABE fermentation of food waste is of particular interest due to the relatively high value of butanol, the major constituent of the solvents produced. The ABE fermentation pathway is found in several species of the *Clostridium* genus, notably, *C. acetobutylicum*, *C. saccharoperbutylacetonicum, C. saccharobutylicum,* and the species used in this study, *C. beijerinckii* (Spychaj, 2018). It is a biphasic metabolic pathway, in which, first, acetic acid, butyric acid, and some ethanol are produced in the acidogenic phase. Next, after a minimal pH is reached, these organic acids are reduced to butanol and ethanol in the solventogenic phase (Buehler & Mesbah, 2016). Sporulation and a shift in pH are associated with the onset of solventogenesis, but the exact mechanism is not well understood (Buehler & Mesbah, 2016). Though it has become common parlance, it is actually false to say that the acidogenic phase concludes and the solventogenic phase begins. During acidogenesis, substrate level phosphorylation of ADP using acetyl-P and butyryl-P yields ATP, acetic acid, and butyric acid.

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The solventogenic phase consumes reduced coenzymes to convert these organic acids to acetone and butanol. Meanwhile, ethanol is still being produced. This stage consumes energy, but it benefits the bacteria by increasing the pH of the fermentation media (Buehler & Mesbah, 2016). Four moles of NADH are consumed to produce one mole of butanol and two moles of NADH are consumed to produce one mole of ethanol during solventogenesis (Jiang et al., 2014). The evolutionary purpose of solventogenesis is to increase and stabilize the pH of the cells' external environment by reducing the organic acids produced during acidogenesis. ABE fermentation was used commonly on an industrial scale to produce solvents for decades, but was supplanted by the use of petroleum (Qureshi et al., 2012). Presently, companies such as BP, DuPont, Cobalt, Chevron Oronite, and Green Biologics are involved in efforts to commercialize biobutanol for the sake of sustainability (Tao et al., 2014).

~~There are s~~Several major extrinsic and intrinsic factors ~~which~~ affect *Clostridium* spp. and ABE fermentation. The main extrinsic factors are temperature and gas composition (i.e. dissolved oxygen). Some major intrinsic factors are nutrient availability, pH, redox potential, and the concentration of inhibitory substances in and of the feedstock (Buehler & Mesbah, 2016; Li et al., 2013). Temperature is a critical factor for the efficient growth of all microorganisms. Solventogenic *Clostridium spp.* are mesophilic, with the optimal temperature for the growth of *C. beijerinckii* being 35 °C. *Clostridium spp.* are obligate anaerobes, meaning that these microbes cannot grow in the presence of oxygen. Initially, oxygen free gas, such as nitrogen, can be used to purge the vessel of oxygen. Later, during active fermentation, the evolution of CO_2 and H_2 gasses can maintain the anaerobic nature of the media (Ezeji et al., 2012). Solventogenic *Clostridium spp.* are known to utilize the phosphotransferase system (PTS) to uptake several carbon sources, including glucose, fructose, xylose, sucrose, and some sugar alcohols.

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Interestingly, several of these pathways are regulated by repressors, including sucrose, xylose, lactose, and maltodextrin (Duerre, 2005). During fermentation, glucose, then fructose, are preferentially metabolized before other available carbon sources. In terms of other, non-caloric nutrients, a minimal media has been described which includes potassium, sodium, magnesium, manganese, and iron salts or hydrates (Maiti et al., 2016).

A pH of 6.0 has been shown to best support cell growth and fermentation. At a pH of 6.0, Geng and Park achieved a glucose consumption rate of 4.37 g/L/h, a butanol productivity of 1.0 g/L/h, and a cell growth rate of 0.2 h^{-1} (Geng and Park, ~~2016~~1993). Further, pH appears to be the primary factor which causes the upregulation of over 100 genes and the onset of solventogenesis. When the accumulation of organic acids causes the pH to drop to approximately 4.5, several enzymes are expressed more rapidly, including acetoacetate decarboxylase, alcohol/aldehyde dehydrogenase, and butyrate-acetoacetate CoA-transferase (Buehler & Mesbah, 2016). Redox potential, although intimately associated with pH, is worth considering as a factor in and of itself. Reduced coenzymes are required for the reduction of aldehydes to ~~alcohols,~~alcohols; hence, production of solvents cannot be uncoupled from glycolysis and primary metabolism ~~(Xin et al., 2014)~~. Redox potential also affects the ratios in which ABE are produced, cell growth, and metabolism (Liu et al., 2016, 2018). A genetically modified strain of *C. acetobutylicum* was transformed to possess the 2,3-butanediol -fermentation pathway. Glycolysis yields two moles of NADH per mole of glucose; however, one mole of NADH is consumed to produce one mole of 2,3-butanediol-. This results in an excess of one mole of NADH per mole of 2,3-butanediol-, which dramatically increases the reducing power within the cell. The result was a 94% reduction in acetone production, an oxidized end product, a 78% reduction in H_2 evolution, and a 19% increase to total alcohol yield (0.44 g/g glucose) (Liu et al., 2018). Finally, many compounds are

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known to inhibit ABE fermentation. Furans, common by-products of the acid hydrolysis of cellulose, polyphenols, common by-products of the alkaline degradation of lignin, and low molecular weight organic acids, such as oxalic and formic acid, are all known inhibitors to ABE fermentation (Spychaj, 2018; Wang et al., 2015). Perhaps the most difficult inhibitory compound to overcome is butanol, the primary product of ABE fermentation. Butanol, acting as a chaotropic agent, disrupts the structure of the cell membrane and totally dissipates the **Δ**pH at alcohol concentrations as low as 2% (v/v) (Terracciano & Kashket, 1986).

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2.3 Food Waste

Nearly 40% of food is wasted in the U.S., making it the single largest component of U.S. municipal solid waste, resulting in a $165 billion economic loss when considering the food itself, the associated water, energy, and chemicals spent in the food supply chain (Gunders, 2016). Food production in the United States consumes as much as 16% of all energy produced, nearly half of all land in the U.S., and 67% of all freshwater used (Gunders, 2016). The EPA reported that in 2012, 303 million ~~tons~~ MT of food waste was generated, representing 2% of the United States' annual energy consumption (Huang et al., 2015).

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Food waste is a growing threat to the environment, when considering its production and its disposal. Decay of food waste in landfills produces vast quantities of methane, a more potent greenhouse gas than CO_2. ~~34%~~Thirty-four percent of human related methane emissions in the United States is emitted from landfills (Evans & Nagele, 2018). In fact, food waste is the source of 2.6% of the greenhouse gas emitted in the United States, most of which is associated with the growth of food ("Wasted," 2017). These greenhouse gas emissions could potentially contribute to global warming. Further, rising temperatures will increase the heat stress placed on crops and livestock, which will necessitate the use of more water to produce the same mass of food.

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Simultaneously, increasing losses of water resources to evaporation will reduce water availability. Along with the release of greenhouse gasses, other environmental costs include air pollution from machinery and trucks used for transportation, water pollution, and damage to fisheries from run-off (Evans & Nagele, 2018).

Unfortunately, another effect of the widespread waste of edible foodstuffs is the aggravation of world hunger. Approximately 25% of global food waste could feed all of the worlds hungry people (Garcia-Garcia et al., 2017). One option is the diversion of suitable wastes from landfills to those people in need of food. As an example, Rolling Harvest Food Rescue, a non-profit organization which serves several counties in Pennsylvania and New Jersey, collects food which would otherwise be wasted from farms and farmers markets and delivers it to 50 separate hunger relief sites to serve 14,000 needy families (Dou et al., 2016). Further, reduction of food wastes by 20% would result in the retention of 260 million MT of food suitable for human consumption in the United States (Dou et al., 2016). Often, produce is discarded because of aesthetic imperfections, overproduction, or lack of cold storage (Evans & Nagele, 2018). However, due to the fact that large fractions of food waste are unavoidable, complete prevention of ~~of the generation all~~ food waste generation~~s~~ is impossible.

To better understand which wastes may be useful or not in different situations, it is helpful to classify them based on multiple parameters. The first useful classification is by waste type. Wastes can originate from all types of foodstuffs, including fruits and vegetables, pasta/rice/flour/cereals, bread and baked goods, meat and fish, dairy, drinks, snacks and confectionery, mixed meals, and others. In many European nations, it is required that food wastes be disposed of separately from other types of municipal waste. For instance, in 2013, regulations banning the landfilling of food wastes and mandating their separate collection after

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April 2016 were introduced by the Assembly of Northern Ireland. The food waste streams from four European countries, the United Kingdom, Finland, Portugal, and Italy, were examined and quantified. The averaged results, as a percentage of wet weight, for fruit and vegetable wastes, pasta/rice/flour/cereals, bread and bakery, meat and fish, dairy, drinks, snacks and confectionary, mixed meals, and other foods are 58.4%, 3.6%, 4.7%, 6.1%, 1.4%, 8.7%, 1.0%, 12.2%, and 3.8%, respectively (Tao et al., 2014). All food waste is composed of the same basic components, though, in different proportions. These components are carbohydrates, including sugars, starches, and structural fibers, lignin, lipids, and proteins (Cascone, 2008). Agricultural commodities high in starch have ~~been~~ long been used in industrial fermentations. In 2018 alone, over 15 billion gallons of ethanol were produced from corn in the United States. That is equivalent to 10% of gasoline used to fuel transportation that year ~~(Huang et al., 2015)~~.

Another useful division which has been made in this study is that of waste origin. Pre-consumer wastes are wastes produced before the product comes in contact with the consumer. An example would be the inedible portions of vegetables discarded by a food processing facility. Annually, in the United States, 80 million MT of unavoidable food wastes are discarded by manufacturers (Magyar et al., 2017). Post-consumer wastes, also called kitchen wastes, are wastes produced after the consumer has interacted with the food product. These are wastes discarded from homes and restaurants. Food manufacturers resemble point sources of waste, while households resemble non-point sources (Magyar et al., 2017). Generally speaking, this means that collection of pre-consumer wastes has the potential to be logistically much more straightforward. In Australia, for instance, approximately 60% of edible food wastes are pre-consumer wastes, with 24% being discarded from farms and 35% being discarded during manufacturing and distribution. Due to the fact that food manufacturers often implement their

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own waste management solutions without governmental guidance, these solutions are often non-optimal. Prioritizing factors such as costs and resource requirements often leave other factors unconsidered (Garcia-Garcia et al., 2017). Also, a large proportion of industrial food wastes are unavoidable, i.e. by-products. This fact often necessitates the implementation of food waste management strategies rather than prevention (Maitan-Alfenas et al., 2015). Third party organizations have concluded that most industrial wastes are unavoidable, and around half of all food wastes produced in the UK are produced during the manufacturing stage (Garcia-Garcia et al., 2017). Approximately 41% of edible wastes are post-consumer wastes discarded from homes and restaurants (Capson-Tojo et al., 2018). A study conducted at the University of Pennsylvania monitored the waste stream produced at an all-you-can-eat dining facility. ~~84%~~Eighty-four percent of food wastes, at this stage, were not recoverable for human consumption (Dou et al., 2016).

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2.4. Biological Conversion of Food Waste to Value-added Chemicals

Recently, food waste has been used as feedstock to produce higher hydrocarbons such as L-lactic acid (Li et al. 2015), succinic acid (Zhang et al., 20134) 2,3-butanediol (Ji et al. 2011), medium-chain acids (Ge et al., 2015) and butanol (Huang et al., 2015). Among the higher hydrocarbon products, butanol is a particularly promising product for a number of reasons: (i) butanol is an important commodity chemical with current global bio-butanol market of over $6 billion per year, which is expected to increase significantly to $18 billion by 2022, owing to the rising need for bio-based chemicals (Market, 2016). Currently, butanol is used as an industrial solvent and as an intermediate in chemical synthesis; ii) butanol is a suitable and safe biofuel alternative to ethanol. Butanol can be mixed in various proportions with gasoline to act as replacement fuel and can be further upgraded to jet fuels; and iii) unlike other higher hydrocarbons,

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butanol has been historically produced from renewable biomass through reliable anaerobic fermentation processes which reduces application risk at commercial scales.

Compared to cellulosic biomass, food waste is a much more cost-effective feedstock because of its high content of fermentable sugars and starch that can be easily utilized by the butanol-producing microorganisms, such as *Clostridium*. Moreover, food waste is rich in nutrient content (e.g. proteins, amino acids, minerals) suitable to support culture growth and metabolism, so the cost of added nutrients may be avoided. Food wastes have been used as feedstock for butanol production by several studies. Ujor et al. (2014) evaluated the feasibility of using industrial starchy food wastes as feedstocks for ~~the production of butanol~~butanol production. The results demonstrated that starchy food waste is a viable feedstock for butanol production via anaerobic fermentation. Huang et al. (2015) used food waste from grocery stores to produce butanol using *C. beijerinckii* and showed that ABE fermentation of food waste has advantages of higher fermentation rate and better sugar utilization efficiency when compared with expensive glucose. Although both of these studies demonstrate that food wastes are viable feedstocks for ABE fermentation, the variations of food waste composition on fermentation performance were overlooked.

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2.5. Pretreatment Methods to Improve Fermentation

Before lignocellulosic wastes can be most efficiently used in ABE fermentation, they must be pretreated to increase the effect of further biological processes (Pellera & Gidarakos, 2018). Further, utilization of food waste as a fermentation feedstock can be enhanced with pretreatment technologies (Hafid et al., 2017). Wastes high in starch or sugar can be utilized directly. Solventogenic *Clostridium* species are known to produce amylases, enzymes which allow them to hydrolyze starches, releasing glucose monomers (Scott & Hedrick, 1952). However, wastes

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high in structural polymers, such as cellulose, hemicellulose, and lignin, cannot be efficiently catabolized by these solventogenic species (Huang et al., 2015). These polymers can be hydrolyzed in a separate step, however, to release these monomer sugars before fermentation (Qureshi et al., 2008). Different pretreatment methods have been utilized to convert cellulose and hemicellulose to monomer sugars (Maitan-Alfenas et al., 2015). These techniques fall into several categories, including physical, chemical, and biological (Spychaj, 2018).

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Chemical pretreatments are very commonly used to pretreat lignocellulosic materials due to their ability to degrade structurally complex substrates (Pellera & Gidarakos, 2018). Some of these methods include acid, base, ozonolysis, oxidative delignification, and organosolv pretreatment methods. Most common among these methods are acid and base pretreatments. When considering acidic pretreatments, there are multiple options which can be implemented. Both dilute and concentrated inorganic acids can be used. Acidic pretreatments have been shown to result in a significantly enhanced enzymatic hydrolysis, which, in turn, results in the release of higher concentrations of fermentable sugars. Though the corrosive nature of acids is what make them such a powerful agent for the improvement of the hydrolysis of cellulose, it can also make its implementation more difficult. Reactors resistant to acids must be used which increases capital costs. Further, when concentrated acids are used, downstream processing to recover acids should be implemented to maintain economic feasibility.

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Basic pretreatment has a narrower range of effective use than acidic pretreatments do. While acidic pretreatments can be utilized more broadly, basic pretreatments are extremely effective at improving the enzymatic hydrolysis of materials high in lignin, such as woody biomass. Further, basic pretreatments are generally less expensive than other methods, and are effective at lower temperatures and pressures, also. Due to acidic pretreatments more general

activity, it tends to increase sugar degradation, potentially inhibiting fermentation. Also, often, the caustic salts can be recovered to improve economic feasibility. Sodium hydroxide has been most widely researched, but calcium hydroxide is less expensive and has shown promising results. One disadvantage of basic pretreatments is their propensity to require hours or days to be effective, which is significantly longer than other methods.

Chemical pretreatments, though advantageous in many ways, also have disadvantages. Acid and base pretreatments, especially, generate inhibitory compounds to enzymatic hydrolysis and fermentation. Some of these compounds are formic acid, oxalic acid, furfural, 5-hydroxymethylfurfural (5-HMF), and sodium sulfate. Furfural and 5-HMF seem to have drawn particular attention in the literature. These compounds are generated by the degradation of hexoses and pentoses, respectively, during acid pretreatments. These furans, and other inhibitory compounds, inhibit ABE fermentation by disrupting bacterial cell membranes, damaging polynucleotides, inhibiting the function of various proteins, inhibiting RNA synthesis, altering cytoplasmic pH, and the induction of general oxidative stress (Wang et al., 2015). *C. acetobutulicum* can ferment in the presence of low concentrations of furfural and 5-HMF. The concentrations of these two compounds range from 1.06-2.6 g/L and 1.99-2.3 g/L, respectively. (Yao et al., 2017) Although chemical pretreatments cause the generation of inhibitory compounds, because they are so much more cost effective to implement in comparison to enzymatic hydrolysis, they should be considered. For instance, microalgal biomass, a relatively new feedstock being considered, contains no lignin. Therefore, dilute acid can be used to efficiently saccharify micro-algal carbohydrates. The decrease in final ABE concentrations, a negative impact caused by the use of chemical pretreatment, can potentially be negated by the savings associated with the eschewing of enzymatic hydrolysis.

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Enzymes are catalysts of biological origin. Generally speaking, enzymes are of high molecular weight and catalyze a specific biochemical reaction with specific reactants. As a catalyst, enzymes serve to decrease the activation energy of biochemical reactions and increase the rate of reaction. Monosaccharides, such as glucose, fructose, xylose, arabinose, mannose, galactose, and rhamnose, are the preferential energy and carbon source for ABE fermentation. Unfortunately, although there are often significant concentrations of these sugars in food waste, they are most often sequestered in structural polymers. Cellulose, for instance, is a highly stable and recalcitrant structural polymer common to plant cell walls. Cellulose is composed of hundreds or thousands of D-Glucose monomers bound via β-1,4-~~Glycosidic~~ glycosidic bonds, giving it a linear, crystalline structure. Cellulases are a class of enzyme which catalyze cellulolysis. Alternatively, hemicellulose, though also common in plant cell walls, is classified as a copolymer consisting of two or more of the following monomers: glucose, xylose, arabinose, galactose, or several uronic acids. Due to hemicellulose's random and amorphous structure, it can be depolymerized with dilute acid or base. However, the use of a hemicellulase, a cocktail of multiple hemicellulolytic enzymes which catalyze the hydrolysis of the various possible glycosidic bonds, is another option. In general, however, the commercial use of hydrolytic enzymes is limited by their relatively high cost (Yao et al., 2017).

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Compared with chemical pretreatment, enzymatic pretreatment does not produce some inhibitory by-products, such as furfurals, whereas chemical pretreatments can. Due to the specificity of enzymes, these enzymes are unable to metabolize any other polymers. Another option is the use of a carbohydrase. This is a multi-enzyme complex, including enzymes which hydrolyze cellulose and hemicellulose. Synergism between cellulases and hemicellulases is a widely accepted phenomenon in biomass hydrolysis. One main factor which determines if this

synergistic effect will occur is the biological origin of the enzymes being used.

CHAPTER 3. MATERIALS AND METHODS

3.1. Waste Collection and Processing

The food waste used in this study was obtained from Virginia Tech Dining Services. Southgate Center, a small-scale food processing facility operated by Virginia Tech Dining Services, houses the pre-prep and bakery operations, cold, frozen, and dry storage of foodstuffs and supplies, as well as shipping products to other dine-in facilities on campus and receiving of wholesale deliveries from various suppliers. Due to the nature of the products Southgate Center produces, the vast majority of food wastes it discards are fruit, vegetable, and bakery wastes. These food wastes are produced on the scale of thousands of pounds per week. Wastes are stored in large garbage containers in a refrigerated warehouse to be later sent for composting.

Food wastes were collected on a weekly basis for sixteen weeks; therefore, there were a total of sixteeen food waste samples. No effort was made to collect certain types of waste. A waste container was chosen at random, and approximately 2 kg of waste were collected from the top of the container and stored in a Ziploc® bag. The date of collection and recognizable waste contents were recorded (Table 1). A Vitamix 7500 high-speed blender was used to slurry each sample, individually. Once processed, samples were stored in a Ziploc® bag at -20 °C for later use.

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Table 1. Collected food waste samples and most predominant food waste in each sample.

Sample #	Predominant food waste	Sample #	Predominant food waste
1	Green Cabbage	9	Butternut Squash
2	Red Onion Skins	10	Red Onion Skins
3	Strawberries (Bakery)	11	Green Bell Peppers
4	Red/Green Bell Peppers	12	Red Bell Peppers
5	Yellow Squash, Green Bell Peppers	13	Whole Pastries
6	Rotten Potatoes	14	Chocolate Pastry Dough
7	Tomatoes	15	White Pastry Dough
8	Potatoes	16	Yellow Pastry Dough

3.2. Food Waste Composition Analysis

The composition of the collected food waste samples was determined using standard methods. Moisture content and total solids were determined by drying food waste samples at 135 ℃ for 2 hr in a convection oven (AOAC 930.15, 2005a). The ash content was determined by incinerating samples in a muffle furnace at 550 ℃ for 12 hrs and weighing the difference before and after incineration (AOAC 942.05, 2005b). The crude fat content was determined by Soxhlet extraction using petroleum ether as an extraction solvent (AOAC, 2003.05, 20005c). For the measurement of crude protein content, the total nitrogen content was quantified using the Kjeldahl method, and the crude protein content as determined by multiplying the total nitrogen content by a factor of 6.25 (AOAC 2005d). The neutral detergent fiber (NDF) content was measured using the ANKOM200 fiber analyzer (ANKOM Technologies, Macedon NY). The starch content of food waste samples was measured by enzymatic hydrolysis of starch to glucose with alpha-amylase and glucoamylase in the total starch assay kit from Megazyme (Megazyme, 2009). The hydrolyzed glucose was then quantified using High Pressure Liquid Chromatography (HPLC) (AOAC 996.11). Each compositional analysis was conducted in duplicate~~triplicate~~. Soluble sugars (glucose, fructose, and sucrose) were analyzed by HPLC using the Luna Omega Sugar Column (Phenomenex, Torrance, CA). In brief, 0.15 grams of dry food waste samples were mixed with 1.5 mL of deionized water. The mixture was shaken at room temperature for 2 hr followed by centrifugation at 12,000 rpm for 10 min to collect the supernatant. One mL of supernatant was then mixed with 1 mL of acetonitrile and shaken for 10 min at room temperature for purification. Finally, the sample was centrifuged again at 15,000 rpm for 15 min to collect supernatant for HPLC analysis.

3.3. Culture and Cell Propagation

C. beijerinckii P260 was generously provided by the USDA Agricultural Research Station in Peoria, Illinois. Spores were maintained in distilled water at 4 °C. The spores (0.1 mL) were heat shocked at 75 °C for 2 min and transferred to cooked meat medium (CMM; Difco Laboratories, Detroit, MI, USA) for spore germination. In order to prepare liquid CMM, 3.5 g of CMM and 0.6 g of glucose were mixed with 35 mL distilled water in a 50 mL screw-capped Pyrex™ bottle. The mixture was autoclaved at 121 °C for 15 min followed by cooling to room temperature. The heat shocked spores were incubated at 35 °C for 16–18h when it was ready for inoculum development. Eight milliliters of actively growing cells (from liquid CMM) were inoculated into 100 mL of P2 medium, prepared in a 250 mL flask. The P2 medium was prepared by adding 3 g of glucose, 0.1 g of yeast extract, and 1 mL of stock solutions (buffer, mineral, and vitamin) in 100 mL of DI water. The stock buffer solution contains 0.5 g/L KH_2PO_4, 0.5 g/L K_2HPO_4, and 2.2 g/L ammonium acetate. The stock vitamin solution contains 0.001 g/L para-amino-benzoic acid, 0.001 g/L thiamine, and 0.00001 g/L biotin. The stock mineral solution contains 0.2 g/L $MgSO_4 \cdot 7H_2O$, 0.01 g/L $MnSO_4 \cdot 7H_2O$, 0.01 g/L $FeSO_4 \cdot 7H_2O$, and 0.01 g/L NaCl (Qureshi & Blaschek, 1999). The P2 media was sterilized at 121 °C for 15 min followed by cooling to room temperature. The culture (inoculum) was allowed to grow or 6 – 8 h at 35 °C when it was ready to be inoculated into the ABE production medium.

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3.4. Food Waste Fermentation

The batch fermentation was conducted in 150 mL Corning™ Milk Dilution Bottles containing 50 mL food waste medium. For the food waste medium preparation, pulverized wet food waste was adjusted to 10% solids with deionized water. Previous experimentation has shown that 10% solids forms a slurry with a low enough viscosity for pH adjustment and later

fermentation. Food waste medium were supplemented with 1 g/L Difco™ Yeast Extract (Difco Laboratories, Detroit, MI, USA) and then sterilized at 121 °C for 15 min. After cooling to room temperature, 0.5 mL of each stock solution (vitamin, buffer, and mineral) was added to provide minimum nutrients to the culture. The medium was then inoculated with 4 mL of stage-two culture developed in P2 medium. Fermentation was conducted at 35 °C in an anaerobic chamber for 72 hr, at which time, 1 mL samples were collected for sugar, acetone, butanol, and ethanol quantification. Each experiment was conducted in duplicate.

3.5. Fermentation Product Analyses

Fermentation products (acetone, butanol, and ethanol) in the broth were analyzed by HPLC (1260, Agilent Technologies, Santa Clara, CA) with a refractive index detector (RID) and diode array detector (DAD). Sample was centrifuged at 15,871 x g for 5 min and then filtered through 0.45 um filters (Waters Corporation, Milford, MA) before being analyzed via HPLC. An Aminex HPX HPX-87P column (Bio-Rad Laboratories, Hercules, CA) was used with 5 mM sulfuric acid solution as the mobile phase at 50 °C. The flow rate was set at 0.6 mL/min. Ethanol and butanol were detected by the RID and acetone was detected by DAD at 265 nm. The injection volume was 5 µL.

The performance of ABE fermentation was evaluated by the fermentation butanol (ABE) yield and butanol (ABE) productivities, which were calculated using the following equations:

$$Fermentationbutanol(ABE)yield = \frac{Totalproducedbutanol(ABE)(g)}{Totalsugar\wedge starchutilized(g)} \quad (1)$$

And the butanol and total ABE productivities will be calculated with the following equations:

$$Butanol(ABE)productivity(gL^{-1}) = \frac{Finalbutanol(ABE)concentrationbroth(g/L)}{Fermentationtime(h)} \quad (2)$$

Fermentation time, as utilized in the calculation describing butanol (ABE) productivity is defined as the active fermentation time, and not necessarily the full 72 hours allowed for each treatment. For the purposes of this experiment, the active fermentation is considered complete when increase in ABE concentration halts.

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3.6. Statistical Analysis

Composition analysis of food wastes was conducted in ~~triplicate~~duplicate. That is to say, that each component analysis was conducted ~~three~~ two times per sample. Sampling replication was not conducted due to the suspected, and later confirmed, compositional variability among food wastes. Fermentation of each food waste sample was performed in duplicate. Data is presented as mean ± standard error. Standard one-way Analysis of Variance (ANOVA) was used to identify any statistical difference between food waste composition and mean solvent concentrations. Pearson's correlation analysis was conducted to develop the correlations between the fermentation yields (final butanol and ABE concentrations) and each component concentration of food wastes. Statistical significance was set to $P < 0.05$ and all statistical tests were performed using JMP (Version 14, SAS Institute Inc., Cary, North Carolina).

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CHAPTER 4. RESULTS AND DISCUSSION

4.1 Feasibility of Using Food Waste as Feedstock for ABE Fermentation

To determine if food waste can serve as feedstock for ABE fermentation, three food waste samples whose compositional attributes should theoretically support ABE fermentation were selected. Peels from honeydew melons and cantaloupes, as well as the peels and cores from pineapples were selected as representative food waste samples. Wastes of fruit origin were predicted to have relatively high concentrations of soluble sugars and micronutrients (e.g. vitamins and minerals) which, hypothetically, should support ABE fermentation and were considered the "low-hanging fruit" of feedstocks of food waste origin. For use as a control, a standard fermentation medium composed of glucose, yeast extract, and several stock vitamins and minerals was ~~generated~~prepared. This represents the most basic minimal medium commonly used to propagate *C. beijerinckii* and produce ABE via fermentation.

Figure 1 shows the fermentation characteristics of ABE fermentation using control and food wastes as feedstocks. Fermentations using food waste as feedstock fermented more rapidly than the control (glucose--based media). All food waste fermentations were complete after 24 hours, while the control continued to ferment for 48 hours. This result aligns well with the one reported by Huang et al. (2015), who also found that ABE fermentation of food waste has a higher productivity compared to the ABE fermentation of glucose. One of the possible reasons for the noticed improvements of fermentation attributes is that food waste contains some essential nutrient or nutrients that stimulate *Clostridium* metabolism. However, this needs to be confirmed with further investigations.

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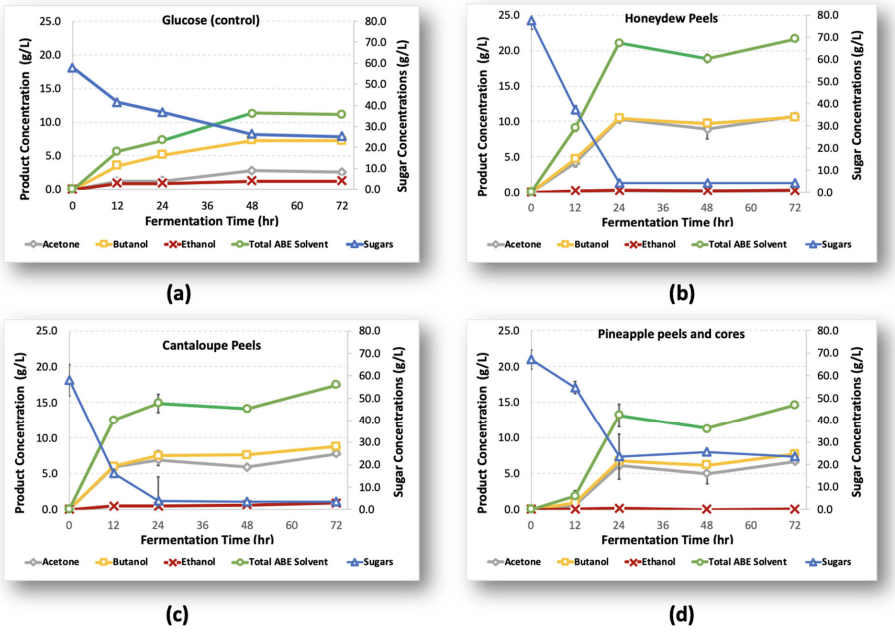

Figure 1. Fermentation of food waste using *Clostridium beijerinckii* P260. (a) Glucose control; (b) Honeydew peels; (c) cantaloupe peels; (d) Pineapple peels and cores.

The summarized results of this experiment are shown in Table 2. While 32.6 g/L glucose was utilized in the control fermentation, 73.5, 54.5, and 43.5 g/L of soluble sugars was utilized in honeydew, cantaloupe, and pineapple-based fermentations. This result is significant because while 56.4% of initial sugar was utilized by the control, 94.7%, 93.8%, and 64.7% of initial sugar was utilized by honeydew, cantaloupe, and pineapple based experimental fermentations, respectively. -This indicates that *C. beijerinckii* could convert more glucose to chemicals when food wastes were used as fermentation media. A total of 11.1, 21.6, 17.4, and 14.6 g/L of ABE was produced by the control, honeydew, cantaloupe, and pineapple-based fermentations,

respectively, with yields of 0.34, 0.29, 0.32, and 0.34. The most telling comparison is that of the control fermentations productivity and those of the experimental fermentations. The control fermentations ABE productivity was 0.23 g/L/h, while those of honeydew, cantaloupe, and pineapple-based fermentations were 0.90, 0.72, and 0.61 g/L/h, respectively. These results prove that ABE fermentation using food waste as a feedstock can exhibit ABE yields similar to the control and can potentially exhibit significantly higher ABE productivities. Not only is it feasible that food wastes could serve as feedstock for ABE fermentation, but use of food waste as fermentation feedstock has the potential to improve the fermentation characteristics in terms of final ABE concentration, ABE yield, and ABE productivity, when compared to the standard minimal media utilized as control in this experiment. This coupled with the comparatively negligible cost and ubiquitous nature of food waste could make it an ideal ABE fermentation feedstock.

Table 2. Summary of batch fermentation after 72 hr using glucose and food wastes.

	Initial sugars (g/L)	Sugar consumed (g/L)	Acetone (g/L)	Butanol (g/L)	Ethanol (g/L)	Total ABE (g/L)	ABE Yield (g/L)	ABE Productivity (g/L/h)
Pure glucose	57.8	32.6	2.6	7.3	1.3	11.1	0.34	0.23
Honeydew	77.6	73.5	10.7	10.6	0.3	21.6	0.29	0.90
Cantaloupe	58.1	54.5	7.8	8.8	0.9	17.4	0.32	0.72
Pineapple	67.2	43.5	6.7	7.7	0.2	14.6	0.34	0.61

Note: ABE productivity was calculated based on the total ABE yield and fermentation ending time. The fermentation ending time was 48hr for glucose, and 24 hr for honeydew peels, cantaloupe peels, and pineapple peels and cores.

Also, worthy of note are the ratios of solvents produced by the food waste-based fermentations in comparison to the control fermentation. Generally, a ratio of three parts acetone, six parts butanol, and one part ethanol (3/6/1 ABE) can be observed (Huang et al., 20105; Qureshi et al., 2012). The control fermentation exhibited a ratio of 2.0/5.6/1.0 ABE. Ratios of 35.7/35.3/1.0 ABE, 8.7/9.8/1.0 ABE, and 33.5/38.5/1.0 ABE were exhibited by honeydew, cantaloupe, and pineapple-based fermentations. The results of the control fermentation are approximately consistent with the expected outcome of a 3/6/1 ABE ratio. However, the food waste-based fermentations exhibited an acetone to butanol ratio of approximately one to one. Similar results were obtained by Liu et al. from ABE fermentation of lignocellulosic hydrolysates; namely an acetone/butanol ratio of 0.95 (Liu et al., 2018). The authors concluded that the effect of solubilized lignin compounds on redox potential caused this modulation. This outcome is disadvantageous, as acetone is much less valuable than butanol. Acetone is an oxidized end-product, while butanol and ethanol are reduced end-products, and the final ratio of these compounds is thought to be determined by the redox potential of the fermentation media. Lower concentrations of reduced coenzymes in food waste-based fermentations would result in higher final acetone concentrations and is likely the cause of this result. Use of some method to improve the redox potential of food waste feedstocks could improve butanol yield. However, even considering the comparatively high concentrations of acetone, the low cost of food waste could potentially negate this issue.

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4.2. Variation of Composition of Different Food Wastes Collected from The Campus Food Processing Center

Determining the concentrations of water, free sugars, starch, neutral detergent fiber, crude protein, crude fat, and ash was vital to this experiment for two reasons. First, this data will be used to determine variance of these attributes among the collected samples. Second, this data was

used to determine if any statistically significant correlations exist with the production of ABE or butanol. These results will help determine which basic attributes can be used to define a potential ABE fermentation feedstock as higher or lower quality or more or less desirable. In this study, compositional attributes are represented as percentages on a dry weight basis, except for moisture content with is given on a wet weight basis.

The first compositional attribute to be determined was moisture content. The initial moisture content is very important for the fermentation process control, because ABE fermentations were conducted at a moisture content of 90%, or total solids content of 10% by adding additional water to or removing excessive water from food waste. This data is interesting, however, because of the sixteen samples collected, seven had moisture contents higher than 90%, necessitating oven drying. In a large-scale operation, drying of waste samples would utilize a significant amount of energy, drastically increasing the cost of operation. Moisture content of the sixteen food waste samples exhibited a standard deviation of 22.73%, high and low values of 95.65% and 29.23%, and an average moisture content of 76.97% (Figure 2).

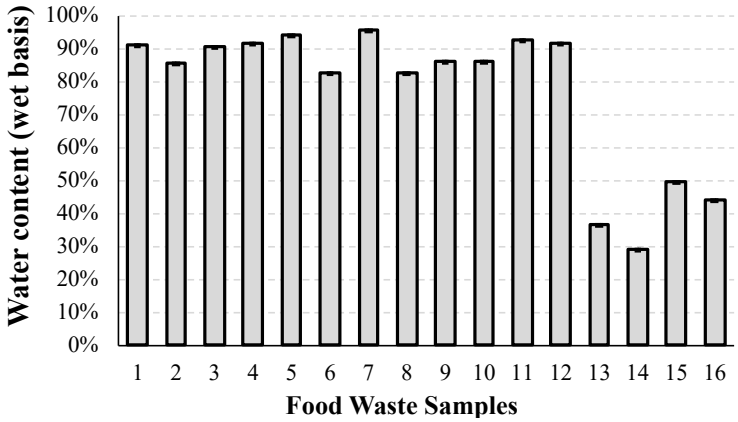

Figure 2. Moisture contents (wet basis) of food waste samples. Data are presented as means ± standard errors (n = 2).

Concentrations of glucose, fructose, and sucrose were determined and their sum was defined as free sugars. As these components and starch make up the total fermentable carbohydrates, this is considered one of the more important attributes of food waste. As one might expect from such drastically different samples, a high variability was found with a standard deviation of 15.4% for total free sugars. Of the sixteen food waste samples collected, the highest and lowest total free sugars exhibited were 53.5% and 0.5% with an average of 23.0% (Figure 3). More specifically, the highest and lowest concentrations of free glucose were 26.5% and 0.3% with an average of 10.0% (Figure 3). The highest and lowest concentrations of free fructose were 21.9% and 0.1% with an average of 10.4% (Figure 3). Finally, the highest and lowest concentrations of free sucrose were 15.3% and 0.0% with an average of 2.7% (Figure 3).

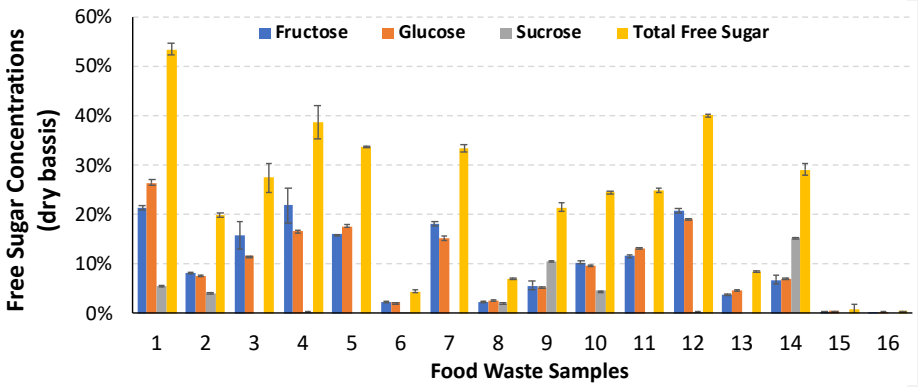

Figure 3. Fructose, glucose, sucrose and total sugar concentrations (dry basis) of different food waste samples collected during the sixteen weeks period.

Besides sugars, starch is another carbon source that can be utilized by *Clostridia*. Previous studies

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have indicated that *Clostridia* secrete amylolytic enzymes for efficient hydrolysis of starch to glucose for subsequent ABE fermentation (Annous and Blaschek 1991; Huang et al., ~~, Singh, and Qureshi~~ 2015~~a~~). Samples 6, 8, 15 and 16 had relatively high starch contents above 16% which can be explained by their predominance of starchy food wastes, such as potatoes and pastry dough (Figure 4). Relatively low starch contents (less than 0.5%) were detected in vegetable or fruit-based food wastes samples, such as samples 1, 2, 4, 5. Overall, the starch contents in the sixteen food waste samples ranged between 0.0 and 25.2%, with an average value of 6.7%. Moreover, the total fermentable carbohydrates, including both sugars and starch, in food waste samples ranged from 16.8 and 53.6%, with an average of 29.7% and a standard deviation of 9.7% (Table 2). In summary, food waste samples contained significant concentrations of fermentable carbohydrates, making them potential feedstock for ABE fermentation; however, high variations in sugar and starch contents were observed among the sixteen food waste samples, which would probably lead to a high variation in ABE yield during fermentation.

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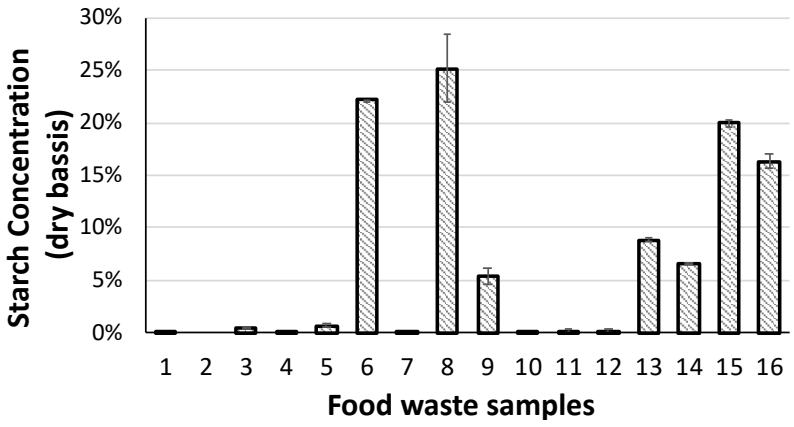

Figure 4. Starch concentrations (dry basis) of different food waste samples collected during the sixteen weeks period.

Neutral detergent fiber is determined through a process which utilized a detergent to remove all components of plant cells except cellulose, hemicellulose, and lignin. While cellulose and hemicellulose are carbohydrates, because of their recalcitrant physical structure, *C. beijerinckii* is incapable of depolymerizing them. Due to this fact, in ABE fermentations that do not include pretreatment and hydrolysis step to hydrolyze fibers to monomer sugars, fibrous material will go unconsumed. Among the sixteen food waste samples, a standard deviation of 10.0% was exhibited. High and low values of 26.9% and 0.6%, as well as an average neutral detergent fiber concentration of 14.0% was found (Figure 5).

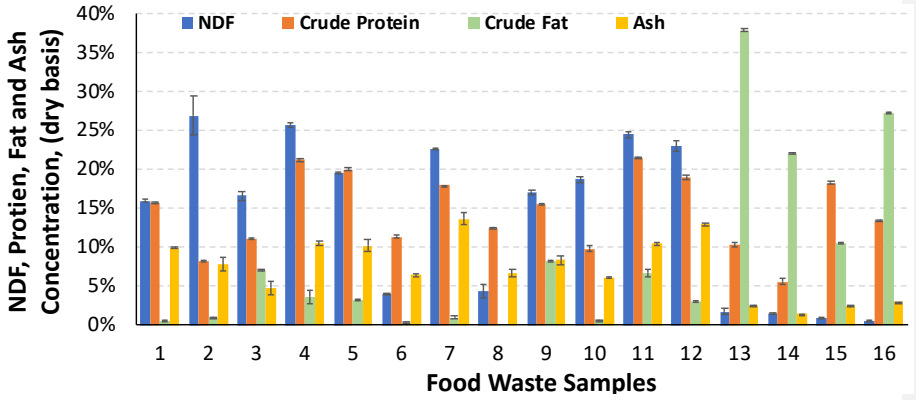

Figure 5. Neutral detergent fiber (NDF), crude protein, crude fat, and ash concentrations (d.b.) of different food waste samples collected during the sixteen weeks period.

Crude protein was determined because a source of nitrogen is vital to microbial growth. Further, it has been shown that solventogenic *Clostridia* are capable of utilizing protein for ABE fermentation. A somewhat lower variance was discovered amongst the sixteen samples crude protein concentrations, with a standard deviation of 4.9%. High and low crude protein concentrations of 21.5% and 5.51%, with an average of 14.5%, were exhibited (Figure 5).

Crude fat was determined because lipids represent one of the main classes of macronutrients which microbes can utilize as a source of carbon and energy. Amongst the sixteen food waste samples, a standard deviation of 11.2% was exhibited. Further, high and low values of 37.9% and 0.06% were found, with an average of 8.3% crude fat (Figure 5). This variance is due to the simple fact that bakery wastes have a much higher proportion of fat than wastes of fruit or vegetable origin are likely to.

Finally, ash generally represents the smallest proportion of foodstuffs composition. While this is true, it does not negate the importance of ash to ABE fermentation, or fermentations in general. The minerals which constitute ash are vitally important in their role as co-enzymes. Amendment of anaerobic digestion media with mineral wastes from municipal solid waste incineration and construction demolition was shown to increase methane production as much as 45% without affecting microbial community compositions (Shamurad et al., 2019). A standard deviation of 3.8% was found amongst ash concentrations. High and low values of 13.7% and 1.4% were exhibited, with an average of 7.3%. A summarized food waste composition is shown in Table 3.

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Table 3. A summary of compositional attributes of the sixteen food waste samples.

	Total sugars (%, d.b.)	Starch (%, d.b.)	Crude protein (%, d.b.)	Crude fat (%, d.b.)	NDF (%, d.b.)	Ash (%, d.b.)
Highest value	53.5	25.2	21.5	37.9	26.9	13.7
Lowest value	0.5	0.5	5.5	0.1	0.6	1.4
Average	23.0	6.7	14.5	8.3	14.0	7.3
Standard error	10.9	6.4	3.5	7.9	7.0	2.7

4.3. Variation of ABE Fermentation of Different Food Wastes and The Correlations of ABE Yields with Food Waste Composition.

The acetone, butanol, ethanol and total ABE concentrations after 72 hr fermentation of food wastes are shown in Figure 7. All food waste samples were successfully fermented to ABE by *C. beijerinckii* except for the food waste samples collected in week two (food waste sample 2).

Records revealed that the primary constituent of food waste 2 was red onions. Previous studies have revealed that onions possess strong antimicrobial and antifungal properties due to their high concentrations of phenolic compounds (Santas et al., 2010; Lee et al., 2011). Therefore, the *Clostridium* growth in food waste 2 was inhibited and ABE fermentation was not successful.

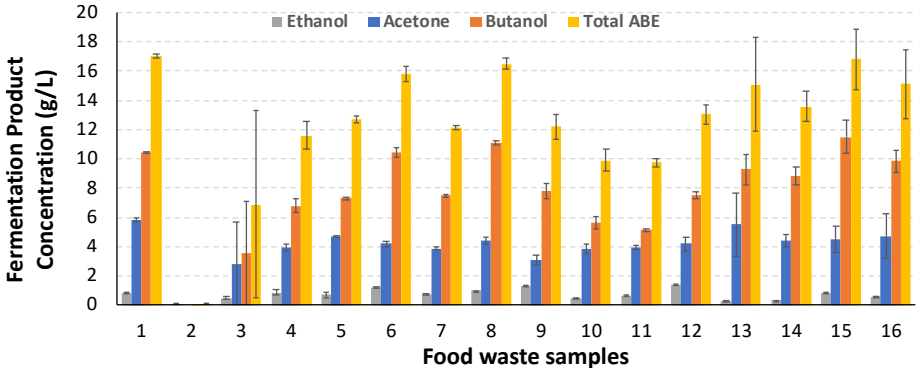

Figure 6. Acetone, butanol and ethanol (ABE) concentration in fermentation after 72 hr fermentation of different food waste samples at a solids content of 10%.

A high variation of acetone, butanol and ethanol concentrations was observed for the fermentations of different food wastes. Except for the fermentation of food waste 2, the butanol concentrations of the fermentation of other food waste samples ranged from 3.5 to 11.5 g/L, with an average value of 8.2 g/L. The total ABE concentrations ranged from 6.9 to 17.0 g/L, with the average value of 13.2 g/L. The high variation amongst butanol and ABE concentrations is clearly attributed to the high variation of food waste composition as discussed in section of 4.2. The fermentation industry would prefer to have a consistent and predictable yield from each batch of ABE fermentation from the standard operation (e.g., downstream distillation) and uniform product quality perspective. Therefore, the high variation of food waste fermentation might

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create problems when the process is scaled up to a commercial level. One mitigation strategy would be the homogenization of multiple batches of food waste based on their chemical composition to reduce the effect of variable composition on food waste fermentation.

The butanol concentration was capped at 11.5 g/L, which is probably due to the strong inhibitory nature of butanol itself. Butanol disrupts the cell membrane structures via a chaotropic effect (Terracciano & Kashket, 1986); thus, most of the previous studies showed the final butanol concentrations ranged between 8 and 15 g/L (Qureshi et al., 2010; Gao et al., 2014).

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Considering the compositional variation amongst food waste samples, it is useful to know which attributes are beneficial to ABE fermentation. To this end, Pearson's correlation analysis was conducted for total ABE and butanol versus compositional attributes (Table 2). This study defines fermentable carbohydrates as the sum of all starch and free sugars, including glucose, fructose, and sucrose. Equivalent glucose is defined as the sum of all free glucose and the glucose which would be theoretically yielded by the hydrolysis of starch and sucrose. The results revealed that the equivalent glucose and starch concentrations are significantly and positively correlated with total ABE and butanol concentrations. The correlations between equivalent glucose and total ABE and butanol concentrations were strong, with a Pearson's correlation coefficient of higher than 0.8. This result makes sense because glucose is the primary carbon source which *Clostridium* metabolizes to produce ABE.- Therefore, a higher equivalent glucose concentration in food waste led to a higher ABE yield during fermentation.

Table 4. Pearson's correlation coefficient analysis of final ABE and butanol concentration with food waste components.

		Equivalent glucose	Starch	Free Sugars	Fermentable Carbohydrates	NDF	Crude Protein	Crude Fat	Ash
ABE (g/L)	Pearson Corr.	**0.808**	**0.673**	-0.328	0.068	**-0.639**	-0.081	0.183	-0.234
	P-value	**< 0.001**	**0.006**	0.233	0.811	**0.010**	0.774	0.513	0.401
Butanol (g/L)	Pearson Corr.	**0.801**	**0.761**	-0.429	-0.019	**-0.712**	-0.150	0.192	-0.319
	P-value	**< 0.001**	**0.001**	0.110	0.947	**0.003**	0.594	0.493	0.246

Note: Equivalent glucose is defined as the sum of free glucose and hydrolyzed glucose from starch and sucrose. Fermentable carbohydrates are defined as the sum of starch and free sugars.

Unintuitively, fermentable carbohydrates and free sugars are not significantly correlated with total ABE and butanol concentrations (Table 4). One possible reason for this difference of significance could be the inevitability of product inhibition due to accumulation of butanol in batch fermentation media. The processes of cell growth and fermentation were totally halted in *C. beijerinckii* by 15 g/L butanol and severely inhibited by 10 g/L butanol. *C. beijerinckii's* preferential metabolism of glucose and the relatively low inhibitory concentration of butanol could mean that other fermentable compounds, namely fructose and proteins, were not consumed. Therefore, the correlations between the ABE yield and the total fermentable sugar or free sugars were not significant.

Neutral detergent fiber, on the other hand, is negatively correlated with total ABE and butanol concentrations (Table 4). Unlike those it produces to hydrolyze sucrose and starch, *C. beijerinckii* does not produce any enzymes enabling it to hydrolyze cellulose or hemicellulose. Therefore, even though fiber contains a large store of potential energy, it is all inaccessible for the purposes of non-pretreated ABE fermentation. The significant and negative correlation of total ABE and butanol concentrations with neutral detergent fiber is likely due to the fact that

increasing concentrations of fiber in a feedstock proportionally decreases the concentration of initial equivalent glucose and other nutritive compounds. In the same way that fiber is considered bulk, or indigestible material that has little utility, in a human diet, fiber could be considered the bulk of an ABE fermentation feedstock.

Crude fat, crude protein, and ash are not significantly correlated with either total ABE or butanol concentrations (Table 4). Although they are insignificant, it is odd to note that while the correlations of total ABE and butanol concentration with crude fat are positive, their correlations with protein and ash are negative.

Figures 8 and 9 show the linear relationship between the total ABE and butanol and food waste compositional attributes. Similar to the results of the Pearson's correlation analysis, the ABE and butanol concentrations are positively related with equivalent glucose and starch, but negatively related with NDF. Moreover, no linear relationships were found between the ABE and butanol concentration and the other compositional attributes (including total fermentable sugars, free sugars, crude protein, crude fat and ash).

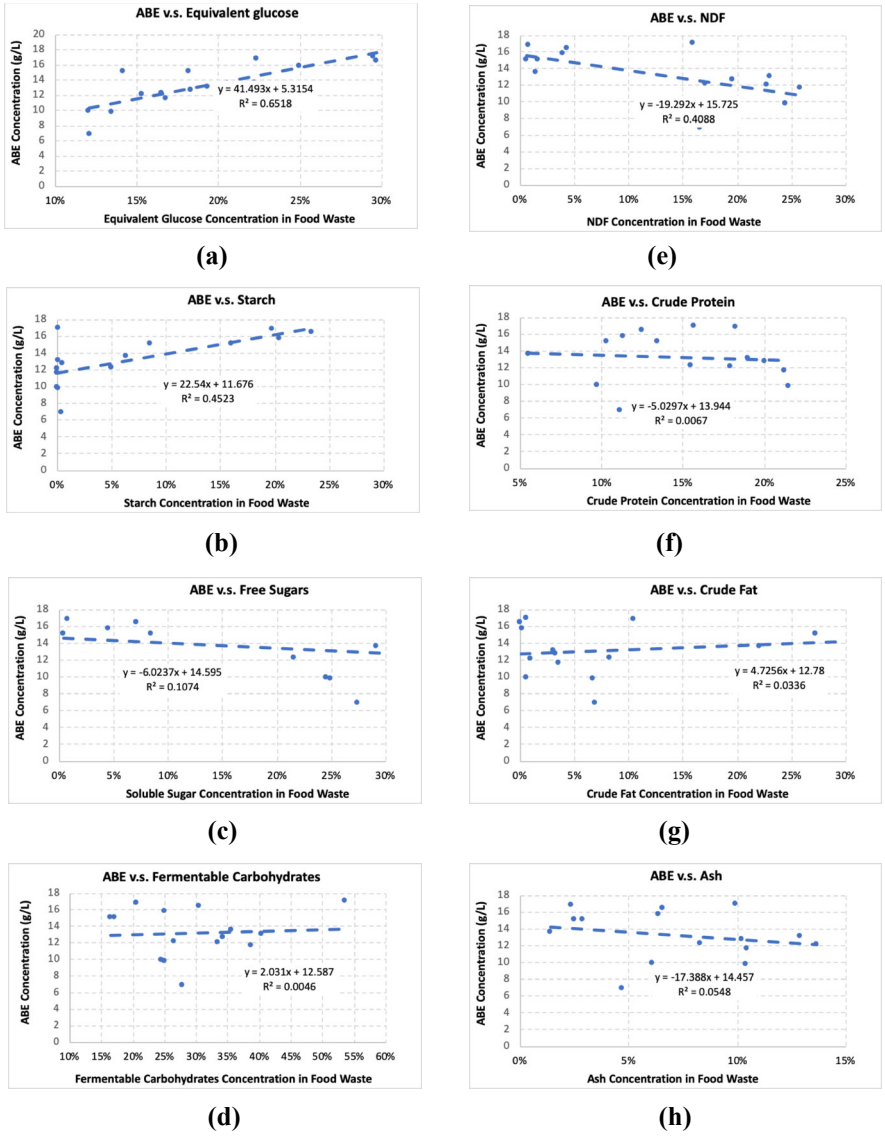

Figure 7. The correlations between final ABE concentration and each of the food waste components.

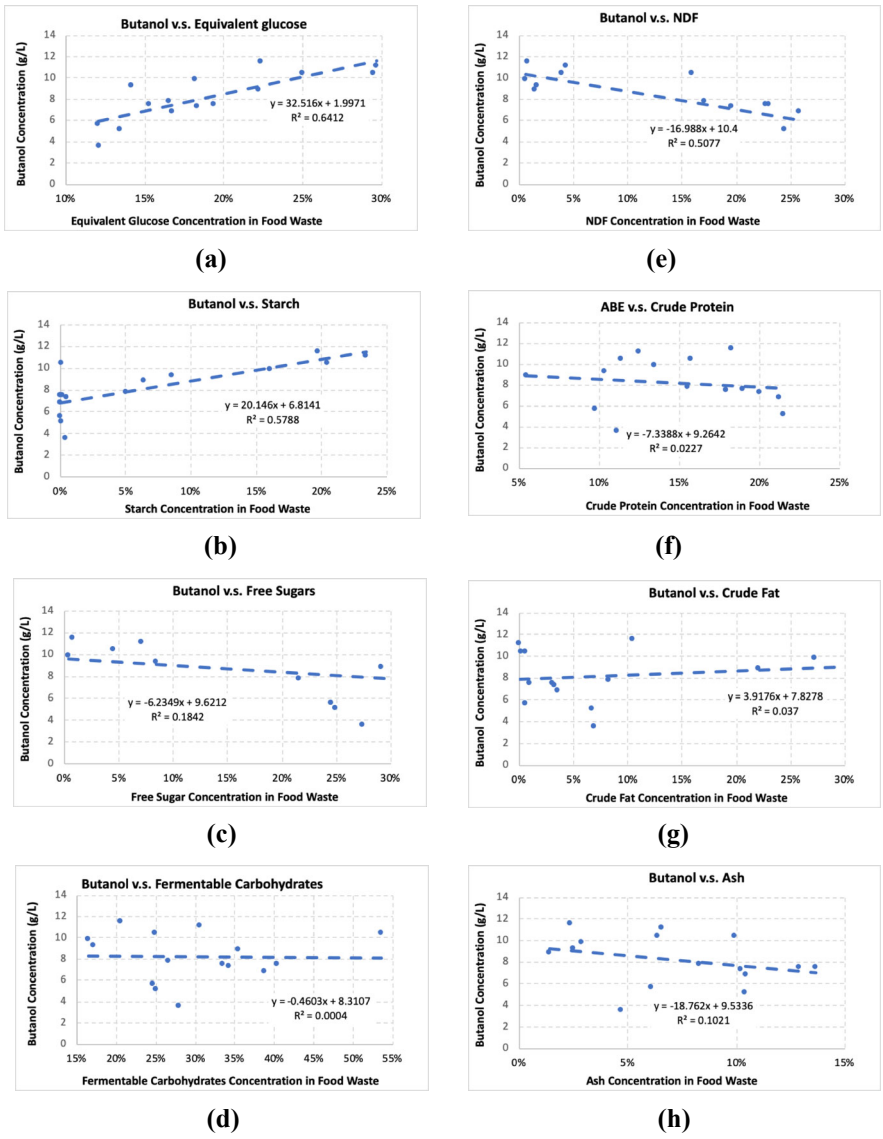

Figure 8. The correlations between final butanol concentration and each of the food waste components.

CHAPTER 5. CONCLUSIONS AND FUTURE WORK

First, this study demonstrated that the ABE fermentation of food waste by *C. beijerinckii* is, in fact, viable. Further, use of food waste in place of a glucose-based media increased fermentation rate and ABE productivity. This is likely due to the relatively high concentrations of some macronutrients and micronutrients in comparison to the control. Some examples could be equivalent glucose, vitamins, and minerals. The variability among the concentrations of fermentation products could lead one to conclude, however, that not all food wastes are equally positioned to serve as an ABE fermentation feedstock.

Second, compositional analysis of ~~16~~ sixteen food waste samples showed significant variability among all those compositional attributes quantified.~~-~~ During the 16-week collection period, no major changes were made by Southgate Center which would be likely to affect waste composition (i.e. processing methods, storage parameters, etc.). One could conclude, then, that the compositional variation discovered in this experiment is an artifact of compositional variation which was likely present in the unprocessed produce from which the wastes were rejected. Further, if it is the case that produce, for instance, can have significantly variable composition, wastes rejected from a rigorously defined and consistent industrial process will also exhibit compositional variation. To better understand this finding, it is important to remember the drastically different types of wastes collected for use in this experiment. Without conducting any analysis, most people would likely surmise in the affirmative that cake batter and bell peppers vary greatly in composition. It follows, then, that wasted cake batter and bell pepper cores and seeds would vary in composition. It is possible that this finding would remain true for wastes rejected from a facility processing only one specific type of food, but variability among their

inputs would likely be smaller and further experimentation would be necessary to make any determination.

Third, the results of this study showed that a high variability of ABE fermentation yields from different food waste samples and found that the variable composition of food waste is correlated with the concentrations of total ABE and butanol produced by *C. beijerinckii*. It is important to determine which compositional attributes can be used to determine if a batch of food waste is of high or low quality for use as an ABE fermentation feedstock. Until now, it has been assumed that higher concentrations of free sugars and starch improve the quality of a feedstock. -These results, however, allow for improved specificity. Due to the significant and positive correlation of equivalent glucose with total ABE and butanol, it can be concluded that the quality of an ABE fermentation feedstock improves as the concentration of equivalent glucose increases. Further, due to the significant and negative correlation of neutral detergent fiber with total ABE and butanol, it can be concluded that the quality of an ABE feedstock improves as the concentration of neutral detergent fiber decreases. No significant correlations were discovered between total ABE or butanol with crude protein, crude fat, or ash. Therefore, it can be concluded that increasing or decreasing the concentrations of crude protein, crude fat, or ash will not affect the quality of an ABE fermentation feedstock, as long as they remain in the range of concentrations likely to be found in a food waste sample. However, crude protein and ash are likely to have a minimum concentration required for ABE fermentation.

Studies which would serve to further illuminate the use of food waste as an ABE fermentation feedstock have the potential to be very useful. Firstly, development of a reliable method to modulate the ratio of acetone and butanol concentrations. This would likely involve the improvement of the feedstock redox potential. Secondly, determination of minimal

concentrations of each compositional attribute would improve the capacity of fermentation operators to reject or better utilize comparatively less suitable food waste samples. Thirdly, a study which correlates micronutrient (e.g. vitamin and mineral) concentrations with total ABE and butanol could further elucidate the burgeoning definition of a "high quality" ABE fermentation feedstock. Finally, this study was able to show that some food wastes are more suitable than others for use as an ABE fermentation feedstock. This is extremely valuable information, however, much more valuable would be the development of some pretreatment strategy which would allow initially less suitable food waste samples to be used. To this end, an enzymatic pretreatment strategy making use of some cellulolytic enzyme could be extremely beneficial. Theoretically, this pretreatment strategy would decrease the variability among initial equivalent glucose concentrations while simultaneously decreasing the concentrations of neutral detergent fiber. In turn, this process should decrease the variability of total ABE and butanol yielded by the eventual fermentation.

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