Food Waste Fermentation:

Valorization of Bread Waste into a Consumable Product

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Abstract

An alarming amount of food waste ends up in landfills, emitting harmful carbon emissions that contribute to climate change. This waste contains valuable nutrients and can be diverted to value-added production. While a good deal of the current research on food waste fermentation is focused on the production of biofuels, an interesting avenue is developing for the use of bread waste as a feedstock in spirit-making. With the collaboration of a local craft-distillery in Montreal, Cirka Distilleries, the sustainable production of a high-quality vodka from locally acquired bread processing waste is investigated. Alternative pre-treatment methods, such as heated enzymatic hydrolysis, microwave irradiation, sonification, and simultaneous saccharification and fermentation are discussed. Based on the energy consumption, material and operating costs, ease of operation and cleanup, safety, and ethanol conversion efficiency, a combined method of microwave irradiation followed by simultaneous saccharification and fermentation is selected for the design of the procedure. The effect of the particle size reduction method and solid loading on the ethanol produced is evaluated for the fermentation of waste bread via the selected design. The wet blended size reduction method with a high solid loading produced the most ethanol. The solid loading had a significant positive effect on the ethanol produced. Further experiments could optimize the temperature, enzyme use and time of fermentation to support the scale up of the selected design to a craft distillery operation. Analyses are conducted to examine the economic, social, and environmental aspects of the project, which influence the sustainability and feasibility of the project.

Keywords: Bread Waste, Fermentation Pre-treatment, Microwave Irradiation, Simultaneous Saccharification and Fermentation, Solid Loading

List of Abbreviations:

ABV: Alcohol by Volume
CLD: Causal Loop Diagram
GHG: Greenhouse Gas
IIR: Internal Rate of Return
LCA: Life Cycle Assessment
MC: Moisture Content
NPV: Net Present Value
PB: Payback Period
SSF: Simultaneous Saccharification and Fermentation
SWOT: Strengths, Weaknesses, Opportunities, Threats

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1. Introduction

Food insecurity affects two billion people globally and has been on the rise in the past 3 years (FAO, 2019). Meanwhile, a third of all food produced goes wasted (FAO, 2019). The use of energy, finite resources, human effort, transformation, packaging and distribution for food that is left unused has major environmental and economic impacts (Gooch, 2010). In Canada, almost 60% of all of the food produced is lost or wasted, representing 3% of the GDP (Gooch et al., 2019). The country is ranked 12th in the world with respect to the quantity of food waste per capita, with 123 kg of food wasted per person annually (Wang, 2017). Although a change in mentality is developing in organic waste management reducing the quantity of food in landfills through composting, the economic and environmental value of compost is still far less than the original product in its consumable state (Pandyaswargo, 2014). Interest in diverting this waste for potential use as feedstocks in biorefinery is growing. Such research is developing in the production of value-added products from food waste, such as biofuels (Chong et al., 2009; Kim et al., 2011; Leung et al., 2012; Hegde et al., 2018), functional chemicals (Bozell and Petersen, 2010; Yun et al., 2018), and bioplastics and biopolymers (Tsang et al., 2019; Xu et al., 2019). The team aims to look at the value-adding production of vodka by using bread waste as the raw material for fermentation. Food waste with high carbohydrate content, such as bread, is usually more valuable due to its high energy accessibility (Menezes, 2016). During storage, bread stales, losing its sensory qualities. Consumers perceive this state negatively even though the health of the product remains (Ribotta and Le Bail, 2007). Due to consumer preference for freshly baked food products, bakeries send copious amounts of bread to landfill every day. Rather than rotting away, contributing to the production of greenhouse gas (GHG) emissions, this waste can be upcycled. It gains a practical end use by contributing to the production of value-added products. (Tsang et al., 2019). Food industry waste, especially bakery waste, is also an appropriate and inexpensive input for distillation because of the increasing cost of crops suitable as raw material for ethanol production (Kawa-Rygielska et al., 2012).

Although most attempts at bread waste valorization are aimed towards the production of biofuels (Ebrahimi, 2008; Pietrzak, 2014; Pietrzak 2015; Hudečková, 2017), there is an opportunity for economic return in a high value consumer product such as a vodka. Sale values for spirits have been under a slow but steady climb in recent years and now represent a 5.5-billion-dollar industry in Canada, where vodka represents the highest share of all spirit sales at 22.64% (Bedford, 2019). According to the recent trend in spirit consumption, this share is bound to continue its increase (Pauley, 2017). Moreover, Montreal is a hub for craft brewing and increasingly for craft distillery products (Cirka, personal communication, November 9th, 2019).

With the support of Dr. Mark Lefsrud, associate professor at McGill University and Cirka distilleries, provincial leaders in grain to bottle distillation, the team aims to optimize the process of vodka-making using low-value waste bread as a feedstock to offer a sustainable alternative to craft-scale distillation. This report discusses relevant literature on bread waste characteristics, bread waste fermentation, and distillation, to analyze alternative pre-treatments and make the

best selection for the design. It presents the results of the experiments performed to determine the optimal particle size preparation, and solid loading for the selected pre-treatment of the fermentation process. In addition, it considers the economic, safety, environmental, and social aspects of the project and presents analyses of these factors.

1.1 Vision Statement

Valorization of food waste into a marketable product.

2. Background Information

In order to develop the project of valorizing bread waste into a consumable vodka, a literature review was conducted on each of the steps involved in the project. The following sections discuss: the use of bread waste as a fermentation feedstock; the fermentation process, with respect to yeast, pre-treatments, and fermentation parameters; and the distillation process. While the project's vision is the production of an alcoholic spirit from food waste, our team is designing the fermentation process. This includes the types of bread of the feedstock, the pre-treatment of the feed, the enzymes and yeast used, and the fermentation operating parameters. The main focus of the design is centered on the selection of an appropriate pre-treatment method for the optimization of the fermentation, and the appropriate levels of the parameters for the selected method.

2.1 Bread Waste Characteristics for Fermentation Feedstock

Bread exhibits many characteristics that identify it as a favorable food waste to use as a raw material in a fermentation feedstock. Since bread dry weight mainly consists of starch, and starch is a general feedstock for fermentation, bread can be a potential feed for a variety of fermentation applications (Ebrahimi et al., 2008). Studies on bread waste biorefinery report that 100 g of waste bread contains 45-60 g of starch, 22-29 g of water, and 8-10 g of protein (Kawa-Rygielska et al., 2012; Leung et al., 2012; Melikoglu et al., 2013). The types of bread ranged from sliced white bread to wheat-rye bread, yet all exhibited similar nutrient contents. Studies have shown that wheat bread (Ebrahimi et al., 2008) and wheat-rye bread (Kawa-Rygielska et al., 2012) can be efficient raw materials for ethanol fermentation. To produce any alcoholic beverage, sugar must be present for the yeast to ferment. Starch is a polysaccharide–numerous glucose molecules joined together by glycosidic bonds–that comes in a straight-chain form known as amylose, and a branched chain called amylopectin (Holliland, 2019). These large molecules must be broken down into usable forms for the microorganisms–also known as hydrolysis or saccharification of starch into simpler sugars (Okafor et al., 2019). Enzymatic hydrolysis can typically facilitate this process (Leung et al., 2012). The use of enzymes and other pre-treatment processes will be further discussed in section 2.2.2.

During bread making and storage, bread contents are subject to transformations that could affect its suitability as a raw material for fermentation feed. Processes such as

gelatinization, retrogradation (Ribotta and Le Bail, 2007), the formation of a gluten network (Singh, 2005), and Maillard reactions, transform the bread's contents and influence the availability of starch (Kawa- Rygielska et al., 2012). Starch gelatinization is defined as the endothermic process by which starch granules lose their crystallinity under specific temperature and moisture conditions (Kadam et al., 2015). In baking, part of the starch is gelatinized and partly depolymerized, making sugars more available. This can ease the future hydrolysis of the starch during mashing (Ebrahimi et al., 2008; Kawa-Rygielska et al., 2012). The physical changes of starch following gelatinization are referred to as retrogradation, which is defined as the reassociation of starch molecules (i.e. amylose and amylopectin) into an ordered structure. It is one of the main mechanisms behind staling and occurs most rapidly at 0-4°C (Kong and Singh, 2011; Wang et al., 2015). Gluten is a protein complex that serves as the main storage protein in wheat, rye and barley. It forms a continuous network of fine strands during kneading, is responsible for the viscoelastic properties of dough, and expands by trapping the carbon dioxide gas released during leavening (O'Sullivan, 2017). The gluten network may shield parts of the starch polymer, potentially reducing its availability to be broken down by the enzymes (Ebrahimi et al., 2008). Therefore, the variation in physical and chemical characteristics of the acquired bread waste can impact its suitability as a raw material for a fermentation feed.

A study of ethanol production from bread residues, by Ebrahimi et al. (2008), investigated the liquefaction and saccharification of bread residues into a suitable fermentation feed. They found that the bread residue feed behaved differently than typical starch feed with respect to liquefaction. However, the overall saccharification behaviour was similar to the established starch enzymatic saccharification. In addition, the conversion efficiencies of the bread residues conveyed the feasibility of fermentation from bread residues. It is important to note that the use of stale breads did not significantly impact the ethanol yield compared to fresh breads (Ebrahimi et al., 2008). Also, it is to be noted that the bread residues used by the authors originate as waste from food processing, which has a much greater consistency than heterogeneous waste bread from grocery stores and bakeries. Bread waste is also at risk of contamination from mold, which could adversely affect the fermentation. The contamination of raw material by mold is found to negatively affect the physiological condition of the yeast used in fermentation (Kawa-Rygielska et al., 2007). Ebrahimi et al. (2008) write that mold growth is associated with the consumption of valuable substrates and the introduction of heat-resistant mycotoxins that contaminate the feedstock. Furthermore, a review on the effects of mold encountered during malting and brewing identified that Fusarium spp. and other fungal pathogens can produce mycotoxins that survive the brewing process (Wolf-Hall, 2007). While further research is needed to identify the specific effects of heterogeneity caused by baking conditions, bread type, mold growth and presence of impurities (Ebrahimi et al., 2008), it is safe to assume that the timely use of the bread waste after retrieval as well as the disposal of mold contaminated pieces could help avoid adverse impacts on fermentation and the quality of the final product.

The waste bread must be first broken up into pieces to produce the fermentation feedstock. Pre-conditioning of the raw material via size reduction is an important step that is often left out of the major steps in ethanol production, namely pre-treatment, enzyme production, hydrolysis and fermentation. Size reduction is necessary to obtain high ethanol yields but must be balanced with reasonable energy requirements to ensure the economic feasibility of the fermentation (Cadoche and López, 1989). Particle sizes of the bread waste substrate of 20 mm dimensioned cubes were found to be optimal for microbial growth and product formation in a previous study on solid-state fermentation (Melikoglu et al., 2013). For example, larger particles in a fermentation feed result in a higher porosity, which generates better heat and mass transfer conditions and increases microbial growth and product yield (Kumar et al., 2003). While this may be the case for solid-state fermentation, particle sizes an order of magnitude smaller appear to yield higher ethanol production for submerged fermentation. A study investigating the effect of ground corn particle size on ethanol yield found that of five particle size distributions (0.5, 2, 3, 4, and 5 mm), the highest ethanol yield of beer was achieved using the 0.5 mm mill screen (Naidu et al., 2007). Dr. Orsat also advised that minimizing particle size supports higher ethanol yields (personal communication, February 12th, 2020).

Overall, the characteristics of the bread waste discussed above, are important to note for the selection of the appropriate pre-treatment process(es).The main challenges of using bread waste as a feedstock can be grouped into social, logistical, and physical concerns. First of all, the project must not compete with human food resources, but rather address the current disposal of breads that are not sold. Second, while it is reassuring that different types of breads do not seem to significantly differ in nutritional content, it may not be logistically possible to rely on grocery stores and bakeries for a consistent source of feedstock. Therefore, based on availability and storage concerns, sourcing the feedstock from bread processing waste is a potential alternative option. Finally, the physical concerns of the feedstock pertain to homogeneity, staling, and impurities. The lack of homogeneity may also be addressed by the choice of the feedstock source. While staling has been deemed inconsequential on the fermentation yield, careful attention must be placed to discard mold-contaminated breads.

2.2 Fermentation

2.2.1 The Role of Yeast and Yeast Strains

Yeast fermentation of sugars into ethyl alcohol is one of the oldest bioprocesses exploited by humans (Walker, 2018). While there are many types of alcohol-producing fungi and bacteria, the alcohol producing *Saccharomyces cerevisiae* is commonly recognized as the most utilized species of yeast for the production of wine, beer, spirits and biofuel production (Walker, 2018).

The process of fermentation by *S. cerevisiae*, occurring under anaerobic conditions, can be summarized as the glycolysis of sugar molecules into pyruvic acid which decarboxylates into acetaldehyde, which is then converted into ethanol through dehydrogenation, as shown in Eq.

(1)-(4) below. This process produces two molecules of adenosine triphosphate and two CO_2 molecules as by-products. (Walker, 2018).

Simplified alcoholic fermentation:

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \quad (1)$$

Glycolysis:

$$C_6H_{12}O_6 + 2\ ADP + 2\ P_i + 2\ NAD^+ \rightarrow 2\ CH_3COCOO^- + 2\ ATP + 2\ NADH + 2H_2O + 2H^+ \quad (2)$$

Decarboxylation:

$$CH_3COCOO^- + H^+ \rightarrow CH_3CHO + CO_2 \quad (3)$$

Dehydrogenation:

$$CH_3CHO + NADH + H^+ \rightarrow C_2H_5OH + 2\ NAD^+ \quad (4)$$

Yeast are not the only microorganisms that might be present in a fermentation mash. Microbial contamination must be avoided as it can reduce the alcohol content of the mash and introduce unwanted flavor (Pauley, 2017). The most common example of this flavour corruption is through *Lactobacillus*, which produces a sourness to the ferment through lactic acid production (Hittinger, 2018). This sourness is sought after in some cases to increase the complexity of the beverage, but it is undesirable in vodka.

Strains of *S. cerevisiae* are usually marketed by types of spirit (Newman, 2019). A yeast strain that minimally impacts the flavor of the liquor is preferable for neutral alcohol such as vodka and gin. For these spirits, any flavours and aroma produced by the yeast will be eliminated during the thorough distillation and filtration (Pauley, 2017). Therefore, the yeast strain will be chosen based on its fermentation efficiency. General desirable attributes of yeast include: rapid and continuous fermentation; high alcohol yield; acceptable character; low yeast biomass; tolerance to high gravity, high temperature, high ethanol and high fungal/bacterial competition; consistency of yield and consistency of taste (Walker, 2012). Prior to fermentation, there are no major differences in the performance of yeast whether they are pitched in dried or liquid form (Walker, 2018). Dry yeast is less expensive, has a longer shelf-life, and is easier to store and manage (Walker, 2018). Dry yeast is thus most appropriate for the scope of this project due to its low cost and consistent performance.

The final choice is based on availability and is consistent with our client's provider. Scott Labs' DistilaMAX DM will be used. It is optimized for use with starch based starch, does not induce a change in flavour and ships semi-locally from Ontario (Scott Labs, 2020).

2.2.2 Fermentation Pre-treatments for Using Bread as a Feedstock

Due to the novelty of using bread waste as an ethanol fermentation feedstock commercially, and the limited research available, our team must design the procedure for using

bread as a fermentation feedstock to produce consumable alcohol. Our preliminary approach is to alter an existing process from one that is used for a feedstock that is physio-chemically similar, or from one that is used to obtain a similar end product. When valorizing waste, the priority is placed on the time, money, and energy investment required to produce the end product of a certain quality. (Cirka, personal communication, November 9th, 2019). In our case, this product is a consumable vodka. In the context of fermentation, optimization is often centered around extracting the most amount of sugar from the feed and saving energy (Pietrzak, 2014). Pre-treatment is an essential step to optimize yield, but accounts for a high portion of production costs (Hegde et al., 2019). Therefore, pre-treatments must be optimized to reduce costs.

2.2.2.1 Pre-treatment Method 1: Enzyme Use

For starch fermentation, the most widely used pre-treatment is the use of bacteria derived enzymes to break down the polysaccharide structure into its glucose monomers for improved accessibility to the yeast (Pietrzak, 2014). This technique is commonly used in grain and tuber fermentation to efficiently use the feedstock. The limitation of this method is its high energy use. The ideal temperature for the efficient saccharification of starch to simpler sugars using alpha-amylase, which generally has to be maintained for more than an hour, can range from 50-75℃ (Hudečková, 2017). The energy needed to reach and maintain this temperature therefore represents a major expense for a large-scale production.

Gluco-amylase is a vastly different enzyme than alpha-amylase as it operates at the same range of temperature as most distiller's yeast, which is around 25-30℃ (Pietrzak, 2015). This temperature range enables the simultaneous saccharification and fermentation (SSF) of the feedstock solution. This enzyme appears preferable as it requires no additional energy input for heating, it saves time by eliminating a step from the fermentation process (Pietrzak, 2015) and it can hydrolyse around 60% waste bread starch into glucose (Leung et al., 2012). However, the use of alpha-amylase rather than gluco-amylase by our partner Cirka Distillery could mean it is most appropriate to their brewing conditions or preferences. There is an interest in the potential use of both enzymes for maximised sugar content (Pietrzak, 2015). Using enzymes is not technologically intensive. Although it requires an adapted methodology of work to make efficient use of them, they don't represent a major monetary investment or require specific installations in the factory to use (Scott Labs, 2020). Thus, they are very easy to combine with other pre-treatment methods to increase the efficiency of the fermentation process.

2.2.2.2 Pre-treatment Method 2: Sonification

Sonification is a novel method to convert plant starch into fermentable sugars (Pietrzak, 2014). It was originally developed as an alternative to the energy intensive process of jet cooking corn starch (Montalbo, 2010). The objective was to reduce the energy and equipment costs. The experiment achieved a 20% increase in sugar conversion compared to an enzyme-only base treatment (Montalbo, 2010). The biggest drawback of this technology for saccharification is the current state of the commercial sonification bath industry. Those available do not meet the volume requirement for the mash volume of a micro-distillery. They are mainly used to clean

equipment and material in industries such as dentistry, wet labs, and pharmaceuticals (Kohn, n.a.; Laval Lab, 2020; ITM Instruments Inc, 2020). Although forms of this technology exist other than the sonification bath, they are not commercially available for the scale of the project.

2.2.2.3 Pre-treatment Method 3: Microwave Irradiation

Microwave irradiation of a fermentation feedstock is an interesting low-cost pre-treatment method (Pietrzak, 2014). Some of the main advantages of this method are the low energy input and the omission of chemical alkaline or acidic catalysts. The latter benefit increases the environmental sustainability of this option with respect to the chemical additive alternatives (López-Linares, 2019). Microwave irradiation can either be done with the bread's original solid state or after hydrolysis. A study of its application to hydrolyzed brewer's spent grain achieved a recovery of 82% of available sugars when paired with enzymatic saccharification (López-Linares, 2019). Brewer's spent grain has limited sugar availability as most of the remaining carbohydrates are in cellulose form. This makes microwave irradiation an interesting avenue for the pre-treatment of a feedstock with more readily available sugars, such as starch in bread. In the context of this project however, an industrial sized microwave is an unusual cost for most brewers and our client, Cirka Distillery (Cirka, personal communication, 2019). The cost of this technology is relatively low but introducing a new technique to an established production line can be a complicated matter.

2.2.2.4 Pre-treatment Method 4: Phosphoric Acid Treatment

Another option to increase fermentation efficiency is acid hydrolysis (Alrumman, 2016; Pleissner, 2017; Rojas-Chamorro, 2018). Rojas-Chamorro et al. (2018), use dilute phosphoric acid in biofuel production from brewer's spent grain. They found that under optimal conditions, 92% of the sugar was recovered (Rojas-Chamorro, 2018). These results are obtained at 155℃, which represents a major energy expense. It is important to note that this temperature did not have to be maintained, only reached before cooldown (Rojas-Chamorro, 2018). Another issue with this method is the toxicity ensued from the treatment. For the purpose of *E. coli* fermentation in the context of the study, the toxicity was not an issue (Rojas-Chamorro, 2018). However, this assumption might not be applicable for yeast fermentation. The toxicity of the final product is unacceptable for a consumable final product, in contrast with the biofuel produced in the study, and the resulting toxicity in the waste product contradicts the sustainability of the solution to food waste.

2.2.3 Fermentation Parameters

Parameters of fermentation include temperature, pH, solid loading, time of fermentation, and filtration. These parameters have an enormous impact on fermentability and require extensive experimentation to optimize them. They are also influenced by topics previously discussed, such as yeast strain and pre-treatment.

2.2.3.1 Temperature

The ideal fermentation temperature is highly dependent on the yeast strain since heat stress can damage the cell membrane of the yeast. Since heat and ethanol are the two main stresses on yeast in fermentation, the technique of temperature staging allows brewers to operate at the maximal temperature at all times (Walker, 2018). Temperature staging is the reduction of temperature as the ethanol content increases towards its final maximal alcohol content (Walker, 2018). Each commercial strain of yeast has an indicated ideal temperature range. The main concern for fermentation temperature is to respect these specifications. For example, Scott Labs' DistilaMAX DS, a yeast strain selected for neutral spirits, has a range of 31-34°C (Scott Labs, 2019).

2.2.3.2 pH

Yeast is an acid tolerant organism that performs better under acidic conditions (Walker, 2018). A pH of 4.5 is optimal for most yeast used in studies on ethanol production from waste bread (Ebrahimi, 2008; Pietrzak, 2014; Pietrzak, 2015). The yeast will also further acidify its own environment (Walker, 2018). Similarly to temperature, the ideal pH range is specified for every commercial yeast strain and should be followed. For example, Scott Labs' DistilaMAX DS, a yeast strain selected for neutral spirits, has a range of 3.5 to 6 pH (Scott Labs, 2019).

2.2.3.3 Fermentation Parameter 3: Solid Loading

Solid loading is one of the biggest issues in waste bread fermentation because the gluten network of bread makes the texture of the mash very difficult to deal with at high loadings (Pietrzak, 2015). Bread also has a high volume to mass ratio compared to other grain products due to the leavening and baking process (Ebrahimi, 2008). The use of enzymes greatly alleviates solid loading issues, but they can only be added once the mash reaches a certain temperature (Hudečková, 2017). Most studies on the subject have achieved positive results with a solid loading of 100 to 150 g/kg of dry feedstock in aqueous solution (Hudečková, 2017; Pietrzak, 2014). A paper by Kawa-Rygielska and Pietrzak (2012) has studied the effect of fermentation of bread feedstock at a loading of 350 g/kg with good results.

2.2.3.4 Fermentation Parameter 4: Fermentation Time

Adequate fermentation time is frequently studied as it dictates the ferment output rate. For this parameter, qualitative observations of fermentation activity and the presence of CO_2 bubbles are representative of the fermentation state. Yeast strains used in spirit-making undergo fast fermentations; the majority of the fermentation process using waste bread is complete within 72 h (Ebrahimi, 2008). One study even found that the last 24 h of fermentation of waste bread did not significantly increase the ethanol content to justify a longer time period (Pietrzak, 2014).

2.2.3.5 Fermentation Parameter 5: Filtration

Filtration is not necessary in fruit juice fermentation for products such as wines, cider and many fruit-based spirits. When using starch however, it becomes an essential step to improve the quality of the texture of the finished product. In the case of distillation, it is even more important as the presence of solids can damage the equipment or add a lot of cleaning work through

burning (Cirka, personal communication, November 9th, 2019). Waste bread fermentation brings additional constraints since the solids are in a slurry state. Modeling the process after potato fermentation is a logical choice as it is the closest in mash texture to waste bread and it is also done commercially (Cirka, personal communication, November 9th, 2019). In the context of experimental research, filtration is often done through centrifugal force separation (Korhola, 2012; Menezes, 2016). However, this method is not appropriate for the micro-distillery scope of the project;the cost of an industrial centrifuge would be difficult to justify. Sieving is another common filtration method. However, the mesh size must be very small in order to keep the fine solids out of the liquid. This would increase the duration of the process (Cirka, personal communication, November 9th, 2019). During fermentation, the solids naturally separate from the liquids through sedimentation. This makes the liquid portion available to be retrieved by pumping (Menezes, 2016). However, in order to avoid the sediments, there would be a considerable loss in the amount of liquid obtained.

2.3 Distillation

2.3.1 The Distillation Process

Distillation is an ancient unit operation used to concentrate alcohol from alcoholic beverages. It is a process used to separate an alcoholic mixture into its volatile components through heating to their specific boiling points. The mixture is heated up to the point where the components vaporize. Alcohols have lower boiling points than water and therefore tend to vaporise first in the process of distillation. Vaporised components are then condensed back to their liquid form and harvested. Distillation is usually the most efficient way of separating components from a liquid mixture (Lea and Piggott, 2003). Among the various existing distillation processes, the following sections discuss the two most commonly used equipment in the alcohol industry: alembic distillation and column distillation.

2.3.2 Alembic Distillation

Alembic distillation is mainly used for producing flavoured spirits such as cognac, armagnac or whiskey (Bamforth and Cook, 2019; Léauté, 1990). An alembic apparatus is composed of three main sections: the boiler, the preheater and the condenser. During the boiling process, the alcohol mixture (or wash) is heated up to high temperatures. Volatile components of different boiling points vaporise from the boiler to the chapiteau, where they are condensed. The chapiteau's shape helps to determine the selection of volatile components during the distillation process. Some volatile components would condense quickly and fall back into the boiler to go through the boiling process again: this is known as reflux (Bamforth and Cook, 2019; Léauté, 1990). The heated vapours pass through the storage tank in order to heat up the wash before the boiling process. This step improves the overall efficiency of the distillation process (Léauté, 1990; Lukić et al., 2011). In the condenser, the vapours go through a spiralling tube, the

serpentin, immersed in a tank with flowing cold water (Bamforth and Cook, 2019; Léauté, 1990). The serpentin is made of copper which reacts with the liquid giving it its particular flavours (Léauté, 1990; Lukić et al., 2011). After condensation, a hydrometer helps the distiller assess if the targeted alcohol-by-volume (ABV) (usually around 80%) is reached or if another run through the alembic is needed (Léauté, 1990; Lea and Piggott, 2003).

2.3.3 Column Distillation

Column distillation is used to produce neutral spirit bases for vodka, gin or whiskey. It is divided into two different columns: the rectifier and the analyser (Bamforth and Cook, 2019; Watson and Hill, 2017). First, the wash goes through a spiralling tube in the rectifier before being injected in the first analyser from the top of the column (Bamforth and Cook, 2019; Lea and Piggott, 2003; Watson and Hill, 2017). Steam is injected at the bottom of the analyser, increasing the temperature of the device and helping to transport volatile compounds. The analyser is divided into different stages in order to increase the distillation time (Lea and Piggott, 2003). Steam strips out volatile compounds from the wash as it falls through the column by acting as a carrier for these compounds that are soluble at high temperatures. Alcohol vapours are then injected at the base of the rectifier, where they would be condensed and selected (Bamforth and Cook, 2019; Lea and Piggott, 2003; Watson and Hill, 2017). The flowing wash from the first step helps to cool down the vapours and condensation begins. Since vapours with higher boiling points condense faster, water and wash residues are collected first at the bottom of the column (Bamforth and Cook, 2019; Watson and Hill, 2017). These residues are recycled by being injected in the analyser for another distillation through the reflux process (Lea and Piggott, 2003). On the other hand, alcohol vapours are harvested on top of the rectifier. The distiller chooses them according to the characteristics they would bring to the final product, typically at, or greater than, 95% ABV (Lea and Piggott, 2003).

2.3.4 Heads and Tails: Selection of Distilled Compounds

At the end of the distillation process, different compounds with different boiling points can be selected regarding their characteristics. The heads are the most volatile compounds of the distillation process. They are composed of unwanted compounds that are either harmful, or simply do not represent an interest for the distiller (Balcerek et al., 2017; Douady et al., 2019). They often have low boiling points and are either re-distilled to extract some essential chemicals that are vaporised too early in the process, or they are discarded by the distiller (Balcerek et al., 2017; Léauté, 1990). The heads are usually composed of ethyl esters, acetone and methanol (Balcerek et al., 2017). Methanol is a very volatile compound that is highly monitored throughout the process as it can cause blindness, and even death (Balcerek et al., 2017; Tephly, 1991). After the heads, the hearts are the compounds that distillers look after during the process. The hearts are ultimately what becomes the finished product. They are composed of ethanol and other essential compounds that balance the chemical composition of the spirit (Douady et al.,

2019). The distiller decides when to collect the hearts for the end product. After the hearts, the tails, which are composed of chemicals with higher boiling points than the others, are distilled. They are mainly composed of long chains of alcohols such as fusel oils (Douady et al., 2019; Lea and Piggott, 2003). The fusel oils, such as butanol, propanol, hexanol, have a bitter taste and are often discarded during the process. In a still column distillation process, fusel oils tend to concentrate in the middle of the column, peaking at a high 130 proof (i.e. 65% ABV) (Lea and Piggott, 2003). Due to their high ABV, it is often laborious to discard fusel oils during the distillation process. Neutral spirits like vodka will tend to have less fusel oils than other liquors made from distillates collected at a lower proof. This is one of the reasons why column distillation is preferred for the making of vodka and other neutral spirits (Lea and Piggott, 2003).

2.3.5 Distillation Methods: Summary

Overall, column distillation is preferred to alembic distillation since the distillate produced is more neutral in taste (Lea and Piggott, 2003) and has a higher ABV (Bamforth and Cook, 2019; Lea and Piggott, 2003). However, according to Cirka (personal communication, November 9th, 2019), the column distillation process is more energy consumptive. Also, due to the volume of the project's experimental batches, a small-scale alembic distillation apparatus will be made available to the team. Therefore, the research will be performed using a simple alembic apparatus, while the potential scale-up of the project could use a column distillation apparatus.

3. Design Approach

3.1 Design Criteria

Upon consulting our client, Cirka distilleries, the following design criteria were established.

Quality: In order to create a high-quality product, the ABV of the distillate should be greater than 95%. Also, the end product must respect a threshold of impurities and be palatable. Otherwise, there has been no value added and it is not marketable.

Sustainability: The food waste to be used must otherwise have been destined to landfill or another means of disposal that is considered less sustainable. The feedstock should be provided from a single location, on the island of Montreal or within a 50 km radius of the brewing and distilling location, to limit transportation costs and emissions. The design must also aim to make the fermentation procedure the least energy intensive to increase sustainability and economic feasibility.

Efficiency: The ABV must be above 8% after fermentation to limit the volume of mash that has to be distilled and therefore reduce the energy cost. The preparation of the feedstock for fermentation should be able to be completed in a single workday (7 h) and optimize the

starch-to-sugar conversion, while the full process from acquiring the feedstock to bottling should be done in less than a week.

Safety: Laboratory safety procedures must be respected, and the final product must be safe for consumption. Mitigation techniques from the risk factor matrix must be followed.

Client satisfaction: The design project must respect Cirka Distilleries' vision statement: "Create unique and high-quality spirits that represent where we live and who we are." (Cirka, personal communication, November 9th, 2019)

3.2 Alternative Designs

The pre-treatments discussed in the literature were used to construct a Pugh chart, shown in Table 1, to facilitate our design selection. The evaluation criteria were established and assigned a weighting from 1 to 4 based on our design criteria and goals. The ratings spanned from -2 to 2, 2 being an exceptional rating. Both the independent and hybrid pre-treatment methods were compared to the baseline method of enzymatic hydrolysis with alpha-amylase which was set to a rating of 0 for all criteria. This method was chosen as it is used by our client presently. Our designed method will thus need to perform better to be worth implementing. A study on pre-treatment methods for waste bread conversion to bioethanol fuel, by Pietrzak and Kawa-Rygielska (2014), demonstrates that enzymatic and microwave treatments are the most promising by a small margin. Acid treatment was however not considered for the study for reasons presented in the literature review. The study was conducted on relatively small volume batches, with a feedstock of wheat and rye bread (Pietrzak and Kawa-Rygielska, 2014). There is a possibility that these findings would differ under large-scale conditions. Qualitative evaluation of the ease of operation and cleanup were based on discussions with our client (Cirka, personal communication, November 9th, 2019) and by an evaluation of the method described by Pietrzak and Kawa-Rygielska (2014). The detailed calculations comparing the energy use and cost of each pre-treatment, found in Appendix A, influenced the ratings in the Pugh chart. The irradiation followed by SSF method may be subject to improvements based on the experimental results.

It is important to note that although the distillation process is not part of the designed process, it influences the ratings given to the pre-treatment methods for the energy consumption criteria. A low ethanol production efficiency will produce a lower total ethanol content for the same volume of liquid to be distilled. Since the end product is a spirit at a fixed ABV of 40%, the output of consumable liquid volume will be lower. This represents a lower efficiency of ethanol production per energy unit used and thus higher expenses for power. Also, since distillation is the most energy intensive step in spirit making, this dynamic has a major influence.

To correctly assess the energy consumption criteria, each technology is assessed based on the recommendation in a paper by Pietrzak and Kawa-Rygielska (2014). The energy consumption is then translated to a cost based on Hydro-Quebec prices for industrial power use.

The evaluation is based on the volume of mash fermented in the baseline method experiment (section 3.3) and can be found in Appendix A.

		Independent methods								Hybrid methods					
		Enzymatic hydrolysis with alpha-amylase (baseline)		Heated gelatinization		Microwave irradiation		Sonification		Simultaneous saccharification and fermentation (SSF)		Microwave irradiation followed by alpha-amylase hydrolysis		Microwave irradiation followed by SSF	
Criteria	W	R	W	R	W	R	W	R	W	R	W	R	W	R	W
Initial cost	3	0	0	1	3	-1	-3	-2	-6	0	0	-1	-3	-1	-3
Operating cost	4	0	0	1	4	0	0	0	0	1	4	-1	-4	1	4
Energy Consumption	4	0	0	0	0	1	4	1	4	1	4	-1	-4	1	4
Duration of process	2	0	0	-1	-2	1	2	0	0	-1	-2	0	0	-1	-2
Ethanol production efficiency	4	0	0	-1	-4	-1	-4	0	0	-1	-4	2	8	1	4
Ease of operation	2	0	0	1	2	1	2	-1	-2	1	2	0	0	-1	-2
Safety	1	0	0	0	0	1	1	1	1	1	1	0	0	1	1
Ease of cleanup	2	0	0	0	0	0	0	-1	-2	0	0	-1	-2	0	0
Score			0		3		2		-5		5		-5		6

Table 1: Pugh Chart comparing independent and combined methods of saccharification to the baseline method (W - Weight of criteria; R - Rating for method)

Alpha-amylase hydrolysis requires a high temperature for activation, which translates to an increased energy cost. The influence of the ethanol production efficiency on distillation would not outweigh this expense. The high temperature heating of the high starch mash also makes cleanup very arduous, or requires a stirring mechanism at an added cost. Microwave irradiation is a more energy efficient way of breaking down the bread starch. It does however require the purchase of new equipment which is not usually found in distilleries. Although new purchases are hard to justify to an established enterprise, an industrial microwave is not a major expense at approximately $2 000 (Global Industrial, 2019). The purchase of an ultrasonic bath for sonification (approximately $4 000) is more expensive than a microwave, but less effective at converting starch to sugar (Pietrzak et al., 2014). As for the cost of operation based on the starch breakdown process, heating with a hot plate is far more expensive than all other options according to the calculations in Appendix A. The sonification method is minutely more expensive to operate than microwave irradiation. Although a certain level of heat will be applied to the feedstock regardless of the method chosen, the high working volume causes each increment in temperature to be costly.

An established distillery would be opposed to significant changes in their existing operations and scheduling. Therefore, options that significantly increase the time of work per

batch or the amount of cleanup needed are ranked lower. For this reason, methods that require a high temperature and long heating process, such as alpha-amylase hydrolysis are penalized in the Pugh chart. Methods that require the purchase of new equipment also receive a lower ranking because of the increased cost, required training for workers and space occupancy.

3.3 Baseline Method Experiment

A preliminary experiment was performed using the baseline method of enzymatic hydrolysis with alpha-amylase. The objective of this experiment was for the team to familiarize themselves with fermentation as a biochemical process with the method used by our client, while investigating the feasibility of a bread waste feedstock. Using an unconventional feedstock can generate many unexpected challenges that need to be addressed in the design of experiments. This preliminary experiment is the stepping stone of this experimental project and is used to adapt expectations and protocol to the realities of bread waste fermentation.

The challenges observed included the difficulty in manipulating a mash with high solid loading, and the energy expense associated with maintaining a high temperature. These challenges are addressed in the selected pre-treatment method.

3.4 Selected Design

Among the proposed alternatives, the selected design is the combination of microwave irradiation and simultaneous saccharification and fermentation (SSF). This selection was made using the Pugh chart in Table 1 assessing the criteria established to meet both Cirka Distilleries' and our team's objectives. It relies on two pre-treatment methods that require little energy but together will amount to a significant starch to sugar conversion. SSF efficiency is highly dependent on the temperature at which both the yeast and enzymes are most effective. This will translate into thoughtful purchasing decisions and experimental testing. The bread waste size, density and moisture content can also alter the efficiency of the process, making solid loading a critical part of the optimization. A high solid loading represents a high alcohol content in the mash and a lower energy cost for distillation. However, it also greatly increases the complexity of manipulations and cleanup time which is one of the main concerns of our client.

4. Design Implementation

In order to apply our selected design to a large-scale production, the fermentation parameters must be considered. After the optimization of the key fermentation factors, a final fermentation batch is to be conducted and distilled at Cirka Distilleries for a final assessment and comparison to the baseline method experiment. Unfortunately, this final batch and comparison was not possible given the extenuating circumstances of the global pandemic.

4.1 Experimental Fermentation Parameters

Based on the selected pre-treatment method, the fermentation parameters: temperature, pH, solid loading, fermentation time, and filtration, must be addressed. The fermentation temperature. depends on the enzyme selected for saccharification. One of the factors behind the selection of SSF is a shortened duration of the process and the use of an enzyme, such as gluco-amylase, at a lower temperature requirement. Therefore, temperature will reflect the suggested requirements of the enzyme and the yeast. Alpha-amylase is added to the enzyme selection to ease the manipulation of the mash as it dramatically reduces the viscosity. Although conventional use of this enzyme requires a prolonged, high heat, activation period for complete saccharification, preliminary experiments conducted by the team revealed its effectiveness in liquefaction. Even at low temperature, the enzyme considerably decreased the viscosity and eased the manipulation of the mash. Furthermore, a study on the ethanol fermentation of rye bread found that the use of additional enzymes during mashing significantly reduced the fermentation time (Kawa-Rygielska et al., 2012). In addition to the fermentation temperature, the pH, the amount of enzyme and yeast, as well as the time of the yeast pitching are fixed based on the guidelines and recommendations put in place by the manufacturer. This decision was made to narrow the focus of the optimization process to the parameters with the least amount of supporting literature. The time of fermentation is based on similar experiments found in the literature (Ebrahimi, 2008; Pietrzak and Kawa Rygielska, 2014; Pietrzak and Kawa Rygielska, 2015). It is also supported by qualitative observations of the rate of the carbon dioxide escaping via the airlock.

The solid loading and particle size are the determining factors of both the efficiency of ethanol production and the ease of manipulation, which are two important aspects to be optimized. The optimal solid loading is to be investigated for three different levels. Further research and discussion with Dr. Valerie Orsat (personal communication, February 12th, 2020), indicates that minimizing the particle size leads to higher ethanol yields. However, the method to do so affects both the ease of manipulation of the mash and the performance of the yeast. Two different methods to minimize the particle size are established and tested for comparison. The first method consists of first hydrating the pieces of bread, then using a handheld blender to obtain a homogeneous paste that is ready for liquefaction. It is not possible to accurately determine the particle size for this method. The second method consists of first passively drying the bread, then processing it in a food processor to obtain small particles. These dry-grinded particles are subsequently sieved to ensure a consistent size of less than 2 mm. The dry and consistent feedstock powder produced by this method addresses the logistical concerns of availability and storage of a raw bread waste feedstock.

4.3 Experimental Design

The design of experiments (DOE) aims to evaluate the effect of two input factors on the resulting ethanol yield response: the particle size reduction method, and the solid loading. Unlike

a “one factor at a time” approach, a DOE allows important interactions between input factors to be identified (ASQ, 2020). A full-factorial, blocking approach is chosen with three replicates. The low and high levels of the input factors are the wet-blended versus dry-grinded methods of size reduction, and 25% versus 35% by weight solid loading. The design matrix for the factors being investigated is shown in Table 2 below.

	Input Factor Level: Size Reduction Method	**Input Factor Level: Solid Loading**
Experiment #1	A	-1
Experiment #2	A	$^{+}1$
Experiment #3	B	-1
Experiment #4	B	$^{+}1$

Table 2: Experiment design matrix: Levels represent wet-blended (A) and dry-processed (B) size reduction methods; 25 wt% (-1) and 35 wt% (+1) solid loadings.

The particle size reduction methods represent two separate experiment blocks. This blocking method is selected over randomization to reduce time and complication of the experiments. The first experiment block is for the wet blending method (block A), and the second experiment block is for the dry grinding method (block B). The solid loading factor is in fact evaluated at three levels: 25%, 30% and 35% by weight, in order to also test the significance of the effect of solid loading on ethanol yield. It is hypothesized that solid loading has a significant effect on the ethanol yield of the fermentation. These chosen values are based upon methods and results presented in various studies of bread fermentation (Ebrahimi, 2008; Kawa Rygielska and Pietrzak, 2012; Pietrzak and Kawa Rygielska, 2014). Each treatment is replicated three times to increase accuracy and reliability of results, and to limit outlying data.

5. Materials and Methods

The following materials and methods describe the experiments that were conducted to test the effects of particle size reduction method and solid loading on the fermentation yield. Due to time constraints and unforeseen situational changes due to the global pandemic, additional parameters, such as the effectiveness of the microwave pre-treatment, the duration of microwave irradiation, fermentation time, and filtration methods, were not tested.

5.1 Procurement and Assessment of Bread

The feedstock was obtained from a local grocery store at no cost. There was no issue obtaining the quantity necessary as the daily waste was estimated at 6-9 kg (Bakery Manager, personal communication, February 15, 2020). It consisted of different types of bread, such as

baguettes, loafs, and multi-grain breads, to be thrown away because of their dry state. Breads with dried fruits, cheese or other non-conventional additions were avoided. Although none were encountered, bread showing any sign of mold would also be discarded to reduce sources of inaccuracy between tests.

The composition of the bread was evaluated solely on the nutritional fact sheets of the respective packaging to evaluate starch, fiber, sugar, fat, protein, and salt content. Moisture content (MC) of the bread was initially estimated from data in the literature. It was subsequently corrected after the experiments by using a representative sample weighed before and after drying. This resulted in a known MC of 21.4% for the wet-blended method and 0.6% for the dry-grinded method. It is important to note that carbohydrates not in the form of fiber or sugar were assumed to be starch. The equivalent sugar to starch content was evaluated stoichiometrically from the hydrolysis of starch reaction and all starch was assumed to be completely hydrolysed for the calculations.

5.2. Bread Fragmentation and Microwave Irradiation

Prior to saccharification, the bread feedstock was cut into manageable square pieces of approximately 5 cm × 5 cm in dimension. Pieces from every bread type were mixed into an even distribution. The pieces were then microwaved at a power of 900 W for 2 minutes. This differs from the values used by Pietrzak and Kawa-Rygielska (2014) due to the microwave ovens available to the team. This power level was also used in order to maximize saccharification. Unfortunately due to resource constraints, different microwaves were used between tests which may reduce comparability between tests. The microwaving resulted in a significant water loss equivalent to 10.9% of the weight of the bread.

For the dry processing method, the feedstock was then left to air dry for 48 hours before being processed into a fine powder using a Hamilton Beach food processor. The powder was then sieved to remove particles greater than 2 mm, which were recycled back into the food processor to reach the desired particle size distribution less than 2 mm. For the wet blending method, the bread fragments were used directly after the microwave irradiation. After adding the measured volume of water, the solution is blended into a homogenous paste using an Oster handheld immersion blender.

5.3 Solid Loading Treatment Preparation

The solid loadings of 25, 30 and 35% w/w were chosen based on the values evaluated in the available scientific literature (Ebrahimi, 2008; Pietrzak and Kawa Rygielska, 2014; Pietrzak and Kawa-Rygielska, 2015). However, during experiment A, the mash prepared with a 25% w/w solid loading and wet-blended method had a very high viscosity. It became clear that the subsequent loadings were not feasible for the necessary manipulation and that it would be challenging to extract a reasonable volume of alcohol from the solid paste. The evaluated solid loadings were thus modified to 15, 20 and 25% w/w for both blocks of the experiment.

For experiment block A, the bread was weighed and combined with the calculated amount of water to achieve the selected loading for a total solution mass of 2 kg. The weight of additives such as yeast, acid and enzymes were considered insignificant. Since the majority of the waste bread received was baguette bread, the MC was estimated at 26.1 % based on MC data from a baguette drying experiment performed by a bread manufacturer (Cauvain and Young, 2008). This value was applied to the bread prior to microwaving. After obtaining the actual MC from the dried sample, and knowing the moisture loss from the microwaving, the actual solid loadings were calculated to be 16.34, 21.86 and 27.62% w/w. These solid loadings from the first experiment block were targeted for the second experiment block B. The dry powder was first assumed to have a MC of 0%. After an adjustment from a dried sample data, the actual solid loadings evaluated were 16.24, 21.18 and 27.45% w/w. These small differences in solid loading are assumed to have minimal impact on the data analysis. The total weight of each solid loading treatment, for both experiment blocks was fixed at 2 kg. Each solid loading treatment batch was divided into three replicates subsequently to the mashing, and the enzyme and yeast additions.

5.4 Mashing process

All tools and equipment were cleaned and sanitized prior to use. The mashing process began at a boiling temperature in order to pasteurize the feedstock. The goal of the pasteurization was to eliminate wild yeast strains that could be found on the bread, as well as any pathogens, mold or bacteria that could interfere with the fermentation or the overall quality of the mash. To ensure pasteurization, the appropriate amount of water was brought to a rolling boil on a hotplate with a lid. The bread was then added and mixed in and the heat source was removed. In experiment A, the immersion blender was used at this point to create a homogeneous paste. In experiment B, the dried powder was simply mixed in with the water. A Brix reading of the solution was taken at this point as well as a sample to perform the iodine test. The iodine test consisted of adding 5 drops of 5% iodine solution to a 20 g sample of the solution. This iodine test is a well-established method to quantitatively assess the hydrolysis of starch into glucose (Fleischer, 2019). Iodine interacts with starch to give a blue-black complex. The test solution turns a certain shade of purple depending on the starch content; a dark blue-purple or a light brown color indicates a large or a small amount of starch remaining respectively. This solution was compared to a control sample of the iodine solution in 20 g of distilled water. Pictures were taken after 2 min for comparison.

Alpha-amylase enzyme in powder form was used for liquefaction: to reduce the viscosity of the bread solution and ease its manipulation. Following package instruction, 2 mL of the enzyme was stirred into the mash while it was still at a high temperature. The pH was then adjusted to 4.5 using a brewing acid blend consisting of malic, citric and tartaric acid. Another Brix measure and iodine test were performed at this point to evaluate the immediate impact of the enzyme. The mash was then left to cool down to 30-35°C.

5.5 Yeast pitching

The yeast was pitched when the mash temperature reached 35°C. 2 g of *Lallemand Inc.* DistilaMax DS active dry yeast was added to 20 g of distilled water at 30°C. The yeast was left to hydrate prior to inoculation for 15 min and kept at a temperature between 30-35°C using a heated water bath. After the hydration period, the temperature of both the mash and the yeast solution were verified to be within a 2°C differential. Approximately 20 g of mash was added to the yeast solution and left to acclimate for 1 minute to minimize the temperature difference. The contents were then poured into the mash and mixed throughout by stirring. The steps described were identical for experiments A and B.

5.6 Simultaneous saccharification and fermentation

30 min after the yeast inoculation, 2 mL of gluco-amylase enzyme in powder form was added to the mash for the simultaneous saccharification and fermentation in the fermentation vessel. The mash volume of each solid loading treatment was separated into 3 bottles, representing 3 replicates of approximately equal volume and weighed. 600 mL brown and opaque beer bottles were used and filled to 60% of their volume capacity. An airlock consisting of a distilled water filled plastic loop was fixed at the bottle entrances with duct tape (Figure 1). The full set of 9 bottles representing the 3 solid loading levels with 3 replicates each were put in a metal steam table pan filled with water. The tray was surrounded by a heating cord with the aim of keeping the bottle content at a constant temperature of 30°C during the 72 hours of SSF by using the water as a thermal buffer mass. Due to a lack of heat output by the heating cord, the bottle's average temperature was kept at a constant 25°C during the SSF process. Since this temperature was outside of the optimal range for both the yeast strain and the gluco-amylase enzyme, it was decided that the fermentation would be kept active until a noticeable drop in CO_2 production in the bottles. This occurred after 96 hours. The total fermentation period was extended to 15 days in order to obtain the absolute maximum ethanol content and due to the reading week. Changes in the methodology due to unexpected constraints were reproduced for the set of experiment B to maintain identical conditions and methodology.

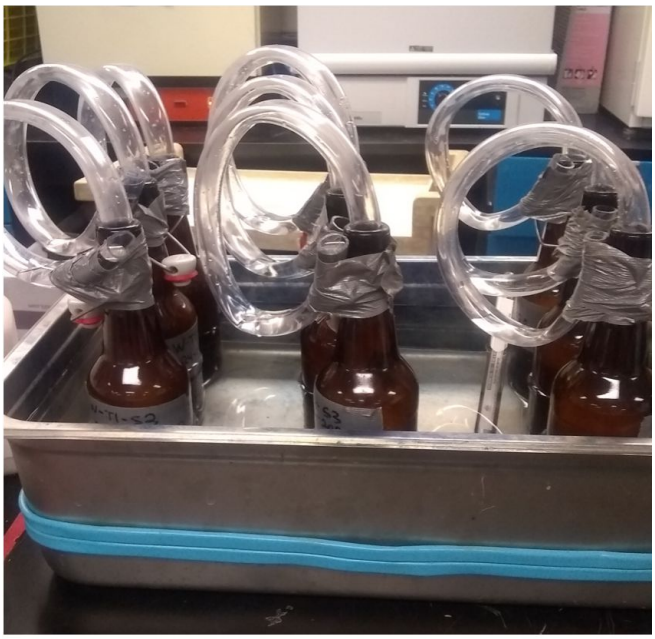


Figure 1. Experimental set-up for SSF: individual sample bottles fitted with plastic tubing airlocks, sitting in heated water bath

5.7 Monitoring and measurements

Daily observation of the experiment was done when possible. CO_2 production and temperature were the main observations as the team aimed for minimal disturbance of the fermentation environment. A water change was done at 24 h after noticing the initial drop of water temperature due to the insufficient heat of the chord. This action was not repeated as it was assumed that water changes would not have a significant impact given the rate at which they were possible for the team to execute. Samples were taken from each replicate for Brix measurement after 96 h. An iodine test was performed on the median replicate, with respect to Brix reading, for each solid loading level. The samples were thoroughly mixed before taking a sample. It was estimated that each Brix testing sample represented a loss of 2 g of solution and each iodine test contributed a loss of 20 g. The experiment was terminated after a total of 15 days, at which point final iodine and Brix tests were performed. The iodine test was done on the same sample previously evaluated. The fermented mash from each bottle was then weighed and the volume was measured. A sample of each test was kept in case future tests such as process waste characterization or direct alcohol by volume measurement would have been possible.

The experiment block B followed the same steps. The unplanned changes due to the challenges encountered, such as the low fermentation temperature and extended fermentation time, were maintained in order to compare methods. However, block B was disrupted by a forced change of location due to the closure of the lab partway through the fermentation, which may have impacted the environment of the yeast and the temperature of the mash.

6. Results and Discussion

The results of the experiments are based upon the Brix measurements taken throughout the fermentation for all treatments and samples. The instrument used is a Reichert *r*^{2} *mini* digital Brix refractometer with a resolution of 0.1% (Brix). Unfortunately, partway through experimentation, the team noticed a source of random error associated with the Brix readings. The team believes that the observed readings taken repeatedly for the same sample varied unpredictably as the sample cooled to the temperature of the device. Despite conscious efforts to allow the sample to cool before taking the measurement, the readings from the initially heated mash at the start of fermentation were considerably more temperamental than those taken in the middle or at the end of the fermentation (while the solution was slightly above ambient temperature). Since the variability was observed at the start of experiment block B, the same instrument was used for the remaining measurements. The team proceeded with precaution by only recording the reading once the variability in repeated readings of the same sample slowed. However, the team is unsure of the accuracy of the results, especially those recorded prior to the discovery, due to this random error.

The iodine test was used to qualitatively assess the breakdown of starch during the fermentation of the mash. All initial tests (before enzyme addition) displayed a dark blue-purple color, indicating a high starch content. Tests performed directly after the enzyme addition

displayed red-purple colors for experiment A and red-brown colors for experiment B. The 96-hour tests followed similar trends in decrease of the deep purple pigment, and thus the starch content. The final tests displayed light red-purple colors for experiment A and red-orange colors for experiment B, except for one sample (the low level of solid loading) that showed no sign of starch content based on color. The results of these tests are useful to identify anomalies, possible mistakes and causes of error. An unexpected result represents an error in the method or an issue in the enzyme's function. During testing, every iodine test gave predictable results and it can thus be assumed that the saccharification process occured as expected.

It is also important to compare the ease of manipulating the mash between the two experiment blocks as it is a criteria in our evaluation of methods. The dry-grind method is more time consuming because of the extra steps of drying, grinding and sieving the bread. It also requires planning to coordinate these steps, but saves time when incorporating the bread in the water for mashing. However, this time reduction was not as significant as anticipated, since the dry solids need to be incorporated slowly to avoid the formation of clusters. Although the immersion blending in the wet-blend method must be thorough, the bread can be incorporated at once since the blending homogenizes the solution. It is important to note that working with the dry-grind feedstock was considerably more pleasant than the wet-blend feedstock because it required less cleaning of the equipment and work area. The viscosity of the dry-grind solution was also lower and thus easier to work with. Despite the advantage in manipulating the mash, the low viscosity was a result of clusters of the solid particles suspended in the water, which is not preferred over a uniform mixture. These aggregates of particles can also interfere with the Brix instrument, which is meant for liquids.

The following subsections explain the calculations and analyses that were conducted and discuss the results.

6.1 Experimental Data and Calculations

The theoretical and actual ethanol yield are estimated using a mass balance approach, based on balanced stoichiometric equations. The theoretical ethanol produced for each sample is calculated from the nutritional information found on the bread packaging. This information is not highly precise and could be improved by testing the starch, sugar and fibre content in a laboratory. To get an estimation of the fermentable sugars, the balanced starch hydrolysis equation (5) is used to convert starch mass in the bread to equivalent glucose mass. It is assumed that fiber is not broken down into simple sugars. Given the solid loading, the fraction of sugar per kg of dry feedstock, and the weight of the ferment for each replicate, the total sugar content is calculated. From the sugar content, the theoretical maximum ethanol and CO_2 weight is determined using the balanced equation of alcoholic fermentation (6).

Starch hydrolysis:

$$C_6H_{10}O_5\ (162.14\ g) + H_2O\ (18.02\ g) \rightarrow C_6H_{12}O_6\ (180.16\ g) \quad (5)$$

Simplified alcoholic fermentation:

$$C_6H_{12}O_6\ (180.16\ g) \rightarrow 2\ C_2H_5OH\ (92.14\ g) + 2\ CO_2\ (88.02\ g) \quad (6)$$

The estimation of the actual alcohol yield is calculated from the differential of the Brix measurements at the start and end of fermentation. The measure of unit for Brix is the degree Brix which represents 1 gram of glucose per 100 mL of solution. Converting Brix readings to the reduction in sugar content of the solution represents the actual fermented sugar. This value can be adjusted to the weight of the solution per replicate to obtain the ethanol produced in grams. Knowing the volume of solution in the replicate, the ABV can be calculated. The actual ABV over the theoretical ABV corresponds to the efficiency of the fermentation process using the designed methodology and is depicted in Tables 3 and 4. Measurements from a hydrometer could not be used to calculate ABV in these experiments due to the consistency of the mash and the uneven separation of the liquid between samples. Also, a hydrometer requires a large amount of volume, which was not possible for our sample sizes. Instead, the samples were thoroughly mixed before taking a representative sample for the Brix reading.

Inaccuracies are inevitable due to the nature of the design and the technical limitations. Firstly, the use of Brix to measure sugar in solution during the SSF does not allow the tracking of the saccharification of starch since the glucose is simultaneously transformed into ethanol. Therefore, the data for total reduced sugar is not accurate. Ideally, each experiment would have a control sample with no yeast addition to solely track the saccharification. Secondly, since Brix is based on light refraction, the high viscosity of the liquid solution caused by other elements than simple sugars interferes with the reading for sugar content (IRMCO, 2020). Bread at the time of Brix evaluation still contains a high concentration of non-saccharified starch, gluten proteins and some fat particles (Martinez and Goméz, 2019). All of these components cause an increase in viscosity of non-glucose origins, which may affect the readings (Martinez and Goméz, 2019). Although clusters of particles in the mash sample may cause random sources of error by interfering with the light refraction, it is possible that the interference of other elements of the mash were the source of a systematic error. In such a case, it is possible that any source of systematic error associated with the Brix device would be eliminated when taking the Brix differential. Thirdly, there is also a source of error in the volume measurements. This is partly due to the difficulty in pouring out the total volume of the fermentation bottles due to the high viscosity of some samples. Additionally, the volume measurements for experiment B were taken with a kitchen measuring cup since the project was moved out of the lab. Therefore, the data of ethanol produced is evaluated in the statistical analyses rather than the ABV.

A sample of the ABV calculations described above for the first replicate of the second treatment of the method A (A-T2-S1) is shown below, where A, T2, and S1 represent the experiment block, the solid loading treatment, and the sample replicate number respectively. The

Brix measurement data, the Brix differential, and the corresponding sugar consumed and ethanol produced for both experimentation blocs can be found in the Tables B1 through B10 of Appendix B.

Nutritional values in solution (e.g. carbohydrates):

$$Carbohydrate\ content = Solid\ loading * replicate\ weight * \frac{carbohydrate\ per\ 100\ g}{100} \quad (7)$$

$$Carbohydrate\ content = 21.886\ \% * 420.6\ g * \frac{62.7}{100} = 57.678\ g$$

Fermentable sugar estimate:

$$Fermentable\ sugar = (Total\ carbohydrates - Sugar - Fiber) * \frac{glucose\ atomic\ mass}{starch\ atomic\ mass} + Sugar \quad (8)$$

$$Fermentable\ sugar = (57.678\ g - 1.99\ g - 2.543\ g) * 1.1111 + 1.99\ g = 61.039\ g$$

Theoretical ethanol content for replicate:

$$Ethanol\ content = sugar\ content * \frac{2\ ethanol\ atomic\ mass}{glucose\ atomic\ mass} \quad (9)$$

$$Ethanol\ content = 61.039\ g * 0.5114 = 31.215\ g$$

* CO_2 content represents the remaining unaccounted weight between glucose to ethanol.

Actual fermented sugar:

$$Fermented\ sugar = \frac{Brix\ differential}{100} * replicate\ weight \quad (10)$$

$$Fermented\ sugar = \frac{13}{100} * 420.6 = 54.678\ g$$

Actual ethanol produced:

$$Ethanol\ content = 54.678\ g * 0.5114 = 27.962\ g$$

Actual ABV:

$$ABV = \frac{Ethanol\ content}{ethanol\ density * volume} * 100 \quad (11)$$

$$ABV = \frac{27.962\ g}{0.78924 \frac{g}{ml} * 315.63\ ml} * 100 = 11.225\ \%\ ABV$$

The ethanol production by weight is calculated with respect to the weight of the pre-fermentation mash because it is dependent on the amount of sugar present at the start of the

fermentation. Since weight loss of the ferment is caused by CO_2 release, the ethanol weight is evaluated with respect to the initial input weight.

Test	Ethanol by weight (% w/w)	ABV % (theory)	ABV % (actual)	% fermentation efficiency
A-T1-S1	4,09	8,01	5,91	73,85
A-T1-S2	3,99	8,50	6,12	72,01
A-T1-S3	3,94	7,98	5,67	71,08
A-T2-S1	6,65	12,53	11,23	89,58
A-T2-S2	6,80	11,61	10,64	91,65
A-T2-S3	6,60	11,61	10,32	88,89
A-T3-S1	8,13	16,54	14,36	86,83
A-T3-S2	7,93	17,50	14,81	84,65
A-T3-S3	8,23	17,92	15,76	87,92

Table 3: Estimation of ethanol production and efficiency with the wet-blend method (A)

Test	Ethanol by weight (% w/w)	ABV % (theory)	ABV % (actual)	% fermentation efficiency
B-T1-S1	4,70	6,87	7,45	108,41
B-T1-S2	4,45	6,23	6,39	102,52
B-T1-S3	4,70	6,32	6,85	108,41
B-T2-S1	5,27	10,20	9,50	93,07
B-T2-S2	5,32	9,48	8,91	93,98
B-T2-S3	5,37	9,73	9,23	94,88
B-T3-S1	6,19	12,78	10,78	84,35
B-T3-S2	6,44	12,51	10,99	87,83
B-T3-S3	6,19	13,60	11,47	84,35

Table 4: Estimation of ethanol production and efficiency with the dry-grind method (B)

From the data presented in Tables 3 and 4, it is observed that the treatment involving a wet blend has achieved the highest alcohol content out of both methods. This could be explained by an observation made during experimentation that the dry powder solution did not appear as homogeneous as the wet blend. This could indicate that some of the starch was unavailable for saccharification due to the formation of clusters via particle aggregation. Interestingly, the fermentation efficiency was superior for the dry-grind method at low solid loading. This could be caused by the tendency of the particles to aggregate together, rendering particles unavailable to the enzymes and yeast, which may be accentuated at a higher level of solid loading. This effect could not be controlled during the fermentation period and would require constant agitation. Although feasible to implement, this solution would require an adaptation of the equipement. It

is also important to note that the ABV values at the highest range seem very high compared to previous studies. In the Pietrzak and Kawa-Rygielska (2015) paper, similar ABV values were obtained but with a solid loading of 30% rather than 27.6%. This potential inaccuracy corresponds however with the hypothesis that the high viscosity of the liquid and the solid particles it contained caused an overestimation of the sugar content during the Brix readings. It could also be caused by a loss of fermentation efficiency at the high solid loading used in the study (Pietrzak and Kawa-Rygielska, 2015). Even though these results are high, they are possible both based on the mass transfer analysis and the maximum ethanol concentration achievable by the yeast strain used (Scott Labs, 2020). This is not true for the treatment 1 of the experiment block 2 which will be discussed shortly.

The fermentation efficiency is observed to be greatest at the middle level of solid loading (21.9 wt%), as opposed to the highest solid loading. Again this could be explained by the increased tendency of particles to aggregate at a higher solid content. It is encouraging that the resulting fermentation efficiencies closely match the range found in the consulted studies (Pietrzak, 2014; Pietrzak and Kawa-Rygielska, 2015). However the possible sources of error previously discussed are noticeable in the resulting fermentation efficiencies. The calculated fermentation efficiencies of the low solid loading samples of experiment B are unrealistically greater than 100%. Although this result could potentially be explained by inaccurate nutritional information on the bread packaging, which would impact the theoretical maximal ABV, it is most likely an effect of error in Brix results. Unfortunately, it is not possible to determine if this magnitude of error is unique to this treatment only or if it is generalized.

6.2 Effects and Interaction of Input Factors

The analysis of the design of experiments was performed using the final ethanol yield by weight. Two factors were investigated in the design of experiments. The results of each experiment involved in the design of experiments, as well as the high and low values of the interaction of the two factors are shown in Table 5. The level of interaction of an experiment is found by multiplying the levels of its factors (ASQ, 2020). In this case, the wet blended method (A) and the dry grinded method (B) are taken as the low and high levels of size reduction method respectively. The high (+1) and low (-1) values of the solid loading correspond to 16 wt% and 28 wt% respectively.

	Input Factor Level			Ethanol Produced (wt%)			
	Size Reduction Method	Solid Loading	**Interaction**	Sample 1	Sample 2	Sample 3	**Average**
Experiment #1	A	-1	$^{+}1$	4.1	4.0	3.9	4.0
Experiment #2	A	$^{+}1$	-1	8.1	7.9	8.2	8.1
Experiment #3	B	-1	-1	4.7	4.5	4.7	4.6
Experiment #4	B	$^{+}1$	$^{+}1$	6.2	6.4	6.2	6.3

Table 5. Design matrix and results of the experiments; -1 and +1 indicate solid loadings of 16 wt% and 28 wt%.

The effects of a factor can be calculated by subtracting the average response at the low levels (A, -1) from the average response at the high levels (B, +1). The effect of the interaction between two factors can be calculated in the same way (ASQ, 2020). These effects are calculated in Eq. (12)-(14) and illustrated in a 3D-Column chart of Figure 2 below.

Effect of size reduction method on ethanol produced:

$$(4.6+6.3)/2 - (4.0+8.1)/2 = -0.6\text{wt\%} \quad (12)$$

Effect of solid loading on ethanol produced:

$$(8.1+6.3)/2 - (4.0+4.6)/2 = 2.9\text{wt\%} \quad (13)$$

Effect of interaction on ethanol produced:

$$(4.0+6.3)/2 - (8.1+4.6)/2 = -1.2\text{wt\%} \quad (14)$$

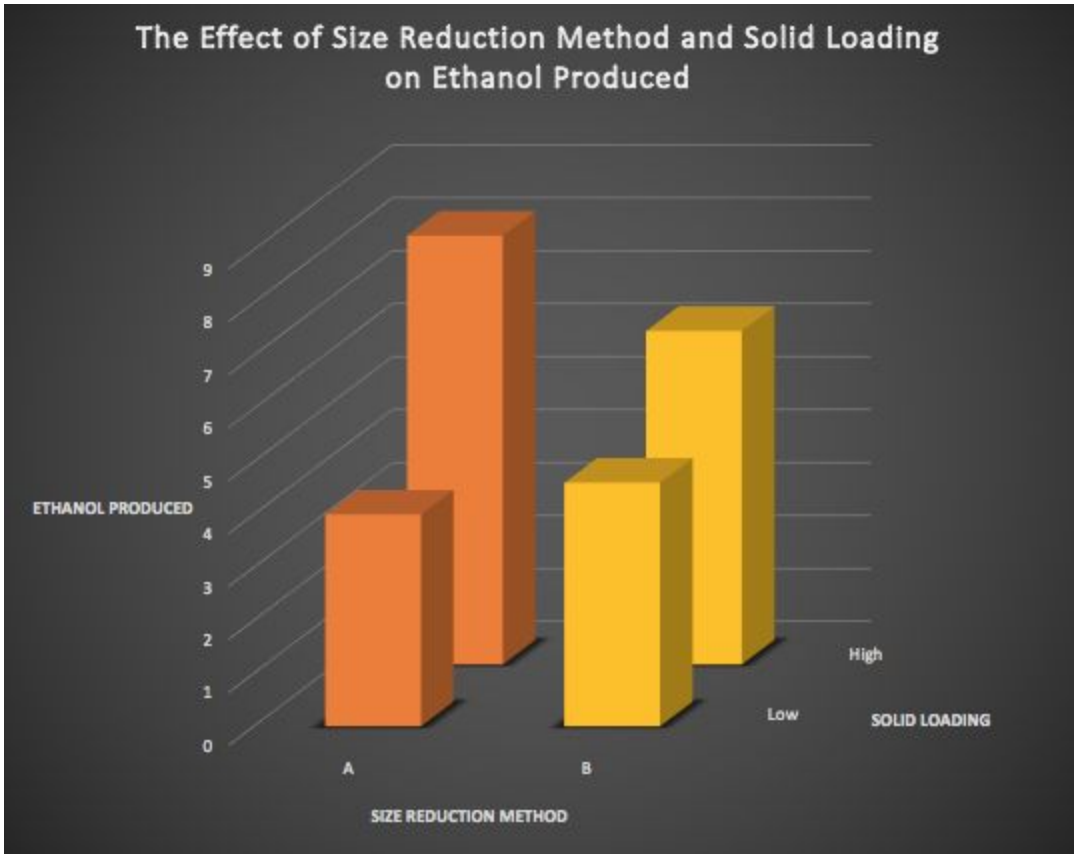


Figure 2. Column graph illustrating the effects of size reduction method and solid loading on ethanol produced during fermentation

According to results of the design of experiments, the size reduction method has a negligible effect (-0.6 wt%) on the amount of ethanol produced during fermentation. Meanwhile,

the solid loading has the greatest effect (2.9 wt%) on the ethanol produced in the design of experiments. This effect is investigated in more detail in section 6.3. The negative effect of the interaction is most easily seen when the wet blended method is used with a solid loading of 16 wt%. While the small effect of the first input factor makes it difficult to evaluate the optimal size reduction method, the wet blended method at 28 wt% solid loading achieved the highest overall average yield of ethanol. These results may be explained by the previous comparison of the size reduction methods. Since the mash prepared with the dried powder feedstock experienced greater clumping, the effect of a higher solid loading on ethanol produced for method B is less than that of method A. Furthermore, the effect of solid loading on ethanol produced under method A highlights the ability of a mash prepared by wet blending to efficiently ferment under a high solid loading. This is perhaps a benefit of thorough blending that made a greater amount of starch and sugars available for saccharification and conversion.

6.3 Linear Regression Model

Statistical analysis, using *R* statistical language and software, was performed on the data collected to evaluate how solid loading affects ethanol production. A linear regression model was used to determine if there is a significant linear relationship between these two variables for both experiment block A and experiment block B. The complete *R* code can be found in Appendix C.

First, for experiment A, while the median value of the residual is very close to 0 (Median = 0.00176), the residuals of the model are not normally distributed. This could be attributed to the small amount of data points tested. The results indicate that 99% of the variation in the data is explained by the model (R^2=.9884, F(1, 7)=685.6, p<3.03e-08). It was found that there is a significant positive relationship between solid loading and ethanol produced (β_1=0.36558, p<3.03e-08). Therefore, the results met the prediction that ethanol yield would increase with solid loading. The linear model for experiment A is displayed in orange in Figure 3.

Next, the effect of solid loading on ethanol yield was investigated for experiment B. The median value of the residuals is close to zero (Median = -0.01773), however the residuals do not follow a normal distribution. The results indicate that almost 98% of the data is explained by the model (R^2=.9758, F(1, 7)=323.4, p<4.06e-07). There is a significant positive relationship between solid loading and ethanol produced (β_1=0.147972, p<4.06e-07), but to a lesser magnitude than that of experiment A. These results support the hypothesis that solid loading significantly affects ethanol production. The linear model for experiment B is shown in yellow in Figure 3.

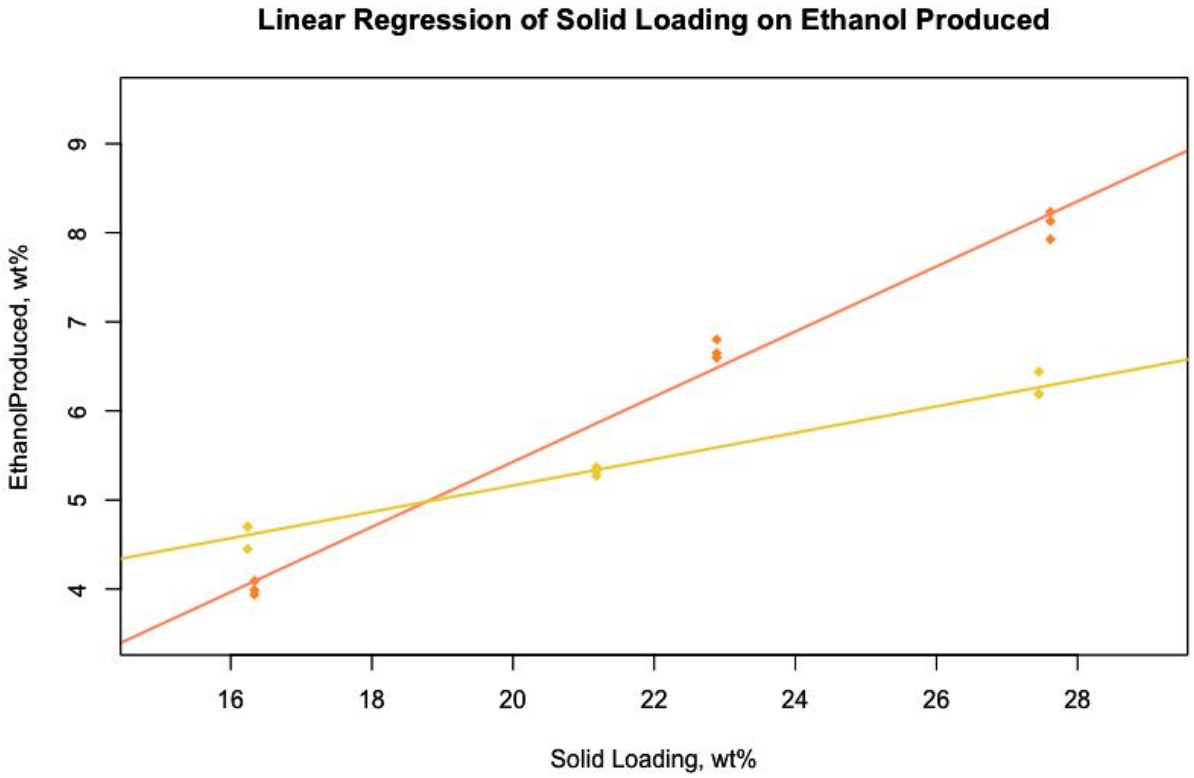


Figure 3. Linear regression model of the effect of solid loading on ethanol produced. Orange and yellow points indicate experiments A and B respectively. The results for experiment A show a significant relationship between solid loading and ethanol yield (β_1=0.36558, p<3.03e-08), and that 99% of the variation in the data is explained by the model (R^2=.9884, F(1, 7)=685.6, p<3.03e-08). The results for experiment B also show that there is a significant relationship between solid loading and ethanol yield (β_1=0.147972, p<4.06e-07). Nearly 98% of the variation of the ethanol produced can be predicted by the solid loading (R^2=.9758, F(1, 7)=323.4, p<4.06e-07), indicating that the data adequately fits the model.

The results of the linear regression support previous discussions of the size reduction methods. The difference in slope highlights the greater effect that solid loading exerts on fermentation yield under the wet blending method. While the data adequately fits the linear model, the solid loading is ultimately limited by the ability to manipulate the mash. This limit may be extended with the use of proper equipment in the scale-up of the project.

7. Future Recommendations

This section includes recommendations for the improvement of the fermentation experiments and future testing, as well as the application of the selected design at a craft distillery scale, noting the improvements for the entire design system.

7.1 Fermentation Testing Recommendations

The conversion of food waste to value-added fermentation products requires precise control and optimization of conditions such as pretreatment, pH, temperature, and microbes (Waqas et al., 2019). The time and resource constraints limited the team's experiments to evaluating the effects of size reduction method, solid loading and pre-treatment. However, the effect of the selected pre-treatment could not be compared to the baseline after all. Additional effects of parameters such as liquefaction temperature, microwave irradiation operation, fermentation time, and filtration methods could be tested, with improved experimental methods.

First of all, a randomization of experiments, given ample time, could help eliminate possible biases. A consistent source of bread feedstock from processing waste would improve the compatibility of the results. A proper analysis of the nutrient content of the feedstock, as well as carbohydrate profiles throughout the fermentation would greatly improve the accuracy compared to Brix measurements. Carbohydrate profiles can assess the rate and extent of sugar consumption by the yeast through the use of high performance liquid chromatography (Kawa-Rygielska et al., 2012). Furthermore, the fermentation dynamics could be assessed by the amount of carbon dioxide released, to determine fermentation activity of the yeast and predict other aspects of fermentation (Kawa-Rygielska et al., 2012; MacIntosh, 2013). Finally, performing the distillation after the optimized fermentation would be the final test of the selected pre-treatment design.

7.2 Application of Selected Design and Design Improvements

The designed methodology is to be applied on a craft distillery scale to respond to the project's vision statement, as well as our client's needs. The overall process, from feedstock procurement to vodka production, including the selected pre-treatment design, is illustrated in Figure 4. First of all, the origin of the feedstock must be reliable, consistent, low cost and local. Compared to a partnership with multiple small-scale bakeries, partnering with a bread factory greatly simplifies the feedstock procurement and meets the aforementioned criterias. This business model is inspired and adapted from a Quebec gin company, known as *Loop*, that sources its feedstock from the waste of the *Yum Yum* chips factory (Cliche, personal communication, March 7th, 2020). Furthermore, a consistent feedstock reduces the time needed for the triage step which can be adapted overtime to correspond to the usual level of quality.

Based on the results of the data and the observations during the experiments, a combination of both size reduction methods will be used. The bread pieces will be dried to aid in storage and availability logistics. However, the dried feedstock will not be processed into a powder, as this method did not have a significant effect on the ethanol yield and resulted in particle aggregation. The mash will therefore be blended once the bread pieces are combined with water, as this method produced the highest ethanol yield at a high solid loading. While the experimental comparison of the selected pre-treatment design to the baseline was not possible,

the fermentation process is established under the assumption that microwave irradiation combined with SSF meets the design criteria.

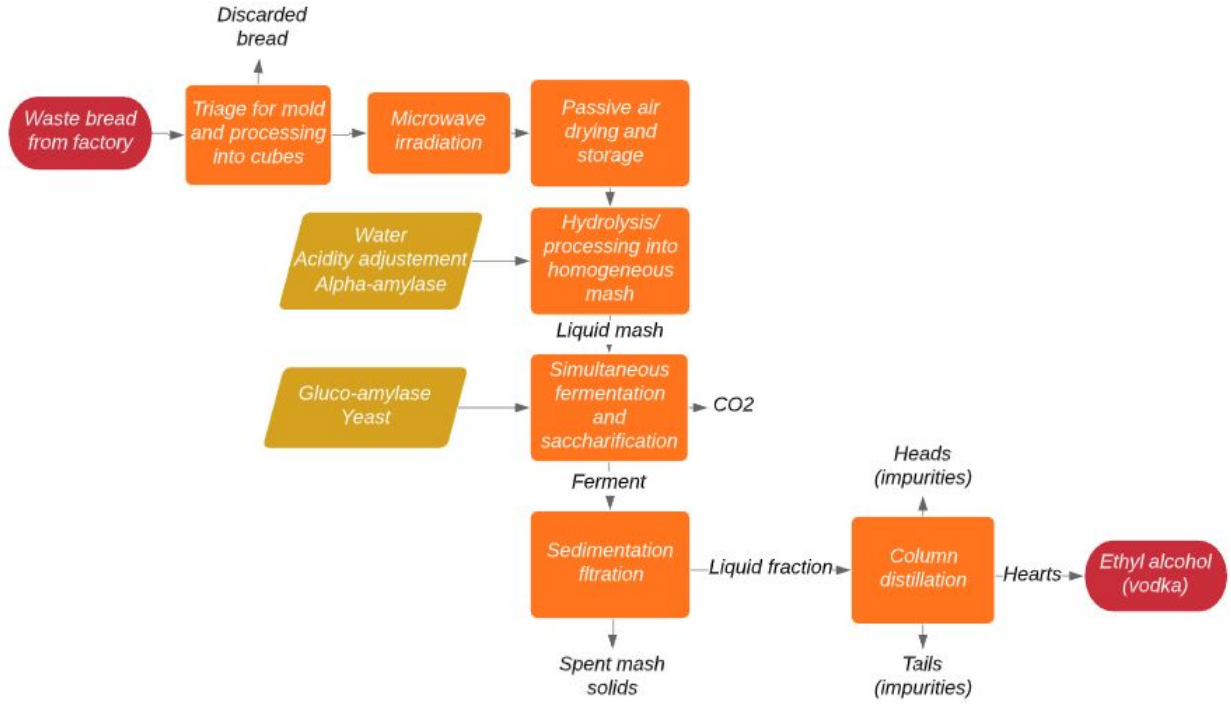


Figure 4. Flowchart illustrating the complete process from bread processing waste to vodka.

The specifications of the process are as follows: Processing of the bread will be done with the Omcan SB-CN-0025 industrial bread slicer and microwaving will make use of the Amana RFS12TS Heavy Duty industrial microwave which has a load capacity of 34 L and a maximal power of 1200 W (iFoodEquipment, 2020; Global Industrial, 2020). Passive air drying is chosen to increase the shelf life of our feedstock and its capacity for longer storage periods than fresh bread. It requires a storage area in a controlled environment with good aeration and possibly a dehumidifier.

For the fermentation method, the design process is closely followed but at a larger scale. The Speidel 625 L stainless steel fermentation tanks are used for the mashing and fermentation to limit transferring the high volume of mash from one container to another (Canadian Home Brew Supplies, 2020). The blending of the solids for homogenization will be done through Dynamic TB002 Heavy Duty Mobile Immersion Blender which has the capacity to blend high volumes while protecting the worker's physical health since it is free standing (iFoodEquipment, 2020). All operations of acidity adjustment, enzyme addition and yeast addition are made directly in the tank. Temperature is kept constant during the 72 h fermentation and saccharification with the FermFlex temperature regulating system for brewing (Canadian Home Brew Supplies, 2020). The separation of the solid fraction happens in the fermentation vessel. Since the sedimentation of solids is time consuming, a multi-layered metal mesh will be used to

press the solids to the bottom of the vessel. The liquid fraction over the mesh can be siphoned out and distilled using a distillation column, which is the most efficient way to produce a neutral product with little impurities. The resulting distillate is then diluted to the right alcohol concentration and flavored to produce the high quality vodka for the market.

7.3 Evaluation of the Final Fermentation Process

The process described in section 6.4 differs from the original chosen fermentation process due to the inclusion of alpha-amylase.. The ability of alpha-amylase as a liquefaction agent was made obvious during the baseline method experiment. The method can thus be described as the microwave irradiation followed by SSF with low temperature alpha-amylase liquefaction. This modification to the original selection of microwave irradiation followed by SSF adds an extra input cost. However, it increases ethanol production while reducing the processing time through the diversification of enzymes. It also drastically eases both the operation and cleanup through the significant decrease in viscosity. This modified method is preferred and the addition of alpha-amylase does not alter the overall Pugh chart score. A re-examination of the weighting and criteria after experimentation reveals that the ease of operation can be given a higher weighting.

Criteria	Initial cost	Operating cost	Energy Consumption	Duration of process	Ethanol production efficiency	Ease of operation	Safety	Ease of cleanup	Total
Weight	3	4	4	2	4	2	1	2	-
Rating	-1	-1	1	-1	2	0	1	1	-
Score	-3	-4	4	-2	8	0	1	2	6

Table 6. Evaluation of the modified pre-treatment process by the established Pugh chart criteria

8. Design Considerations

8.1 Economic Analysis

While alcohol production is a potentially efficient end use option for food waste, the economic evaluation and optimization of the process is an important consideration before scale-up and commercialisation (Hegde et al., 2018). In order to estimate the economic feasibility of ta craft distillery, such as Cirka, adopting our project, four analyses were conducted. Firstly, a strengths, weaknesses, opportunities, threats (SWOT) analysis investigates the potential market of the finalized product, supported by a market analysis that was conducted in the community. Next, a cost-benefit analysis estimates the costs, expenses and revenues of the project. Finally, a statistical model evaluates the economic risk and the probability of the project's success.

8.1.1 SWOT and Market Analysis

In order to analyse our potential market, we first need to assess the different variables that could impact our project, and to orient our marketing strategies accordingly. Our strategic planning technique relies on a SWOT analysis. This analysis specifies internal and external factors that could be favourable or unfavourable to our business strategies. Strengths are internal factors that could impact our potential business in a favourable way, whereas weaknesses would be unfavourable. On the same note, opportunities are external factors that would have a positive impact on our business plan, whereas threats would have a negative impact (Helms and Nixon, 2010; Catron et al., 2013). The results of the SWOT analysis are summarized in Table 7 below.

Strengths:

- *Food Waste reduction:* reducing the amount of food waste going to landfill.
- *Experienced client:* our client, Cirka Distilleries, has years of experience in the domain.
- *Inexpensive raw material*: We are relying on a readily available, free or low-cost feedstock.

Weaknesses:

- *Visibility:* Challenges associated with propelling a new product on the market.
- *Target Audience:* Our audience is limited to those of legal age who consume alcohol. It is important to note that our product must aim to encourage responsible alcohol consumption.
- *Energy Use:* The column distillation process is energy intensive. The cost of powering the operation, as well as the environmental consequences could determine the feasibility of the operation.

Opportunities:

- *Environmental Awareness:* There is an increasing publicity, awareness and discussion centered around environmental issues today. Our product can help raise awareness of the impacts of food waste.
- *Student promotion:* A student- led project could capture the interest of the general population and initiate a client base of supportive peers.
- *Blooming market:* Spirit sales in Canada have risen by 19% from 2011 to 2018 (Statista, 2018).

Threats:

- *Narrow market:* There could be a rivalry with existing micro-distilleries and other small-scale producers.
- *Product Acceptance:* The product's message, sustainability efforts or quality could be misinterpreted or badly received. For example, the project addresses the consequences of food waste rather than tackling the issue directly. Ultimately if the product is not appealing to customers, then it would fail on the market.
- *Dependence on food waste*: Our product depends on food waste availability. If this issue is solved, the project is no longer viable.

Table 7. A summary of the Strengths, Weaknesses, Opportunities, and Threats of the project.

Locally sourcing the feedstock input from food waste, not only helps reduce the amount of food heading to landfill but presents an opportunity to spread awareness of food waste. A new business or product may face challenges of propelling themselves in an existing market. The team's partnership with Cirka provides useful expertise to ensure the quality of the product and can also aid the promotion to existing customers. However, there is a certain degree of unpredictability with how the product will be received by consumers. Furthermore, the project depends on the availability of bread waste. If a solution to this waste is presented, another feedstock input would need to be found. Overall, the project could be a success if marketing and economic resources are well-managed, taking into account the existing micro-distillery market in Montreal.

Finally, a survey was developed in order to understand the potential market audience of the project. The survey was conducted over a period of 30 days through social media platforms. The survey reached 169 applicants from various backgrounds. A large majority (79%) of participants were between 18 and 25 years old at the time of the survey. Participants between 25 and 35 years old represented 9.5% of the total candidates. The remaining was shared amongst the 35 to 50 years old age group (5.0%) and the 50 years old and more with 6.5%. More than two thirds of the applicants from Quebec (67%), while those remaining were either from the rest of Canada (6.0%), or another country (27%). The drinking habits amongst participants were also surveyed. A little more than 1.0% of the participants never drink spirits, 19% rarely, 37% on a monthly basis, 36% weekly, and almost 7.0% regularly. The most preferred types of alcohol consumed were almost equally shared between vodka, gin, rum and whiskey (20%, 32%, 20%, and 16% respectively). Overall, the responses indicated a great interest in the product; when asked if they were interested in purchasing a vodka or gin made from waste bread, 63% said they were definitely interested, 34% were unsure, and the remaining 3% did not show any particular interest. The results of the survey demonstrated that the target audience for the market of this product is young adults. However, there is a bias in the average participant age since the survey was made available on the social media platform of the team members. The drinking habits amongst the participants show that the majority of applicants are not regular consumers, but tend to drink occasionally. Furthermore, it seems that there is an interest for neutral base spirits (i.e. vodka, gin), over flavoured liqueurs (i.e. whiskey, tequila). Therefore, the responses indicate a general interest in the product. The positive results indicate that younger people are inclined to drink sustainable products that drive a powerful message: which is to reduce food waste. Understanding this existing market is the key to the development of the project and further research could specifically target that category of consumers.

8.1.2 Cost-Benefit Analysis

In order to determine the economic feasibility of the project adopted on a craft-distillery by our client Cirka, a risk analysis is developed. Firstly, as cost-benefit analysis of the project is conducted. Operational costs, fixed costs, as well as revenues are gathered from different

sources. The cost breakdown of the project helps determine its payback period (PB), net present value (NPV), and internal rate of return (IRR). Assuming a constant production, facilitated by the ability to store the dry bread feedstock, there is no need to consider marginal cost in the objective of scaling up the operations. Finally, a risk model is generated using a statistical method, namely the Monte Carlo analysis. The simulation indicates the probability of losing money (i.e. NPV<0) if the project was undertaken at a large scale.

In order to develop the accounting analysis, the following assumptions were made:
The monthly cost of running the distillery is $23 000 (Cirka, personal communication, November 24th, 2019). This accounts for one full-time employee, utilities and rent. However, it does not account for variable costs of the products used during fermentation, such as yeast and cleaning solution. The distillery reports a profit of $11.00 from the sale of a 750 mL Vodka bottle sold at $43.50 in SAQ stores (Cirka, personal communication, November 24th, 2019). This corresponds to an interest of approximately 25% (or a tax rate of 75%), which will be used in further calculations for all bottle types. From the results of the survey, 30% of the applicants showed interest in buying 375 mL bottles, while the remaining 70% showed interest for 750 mL bottles. Therefore, these proportions will be used in further calculations. Furthermore, on average, survey participants were more likely to spend $20 for a bottle of 375 mL, and $30 for a bottle of 750 mL. We assumed these values for the retail prices at SAQ stores. Therefore, the net profit per bottle is $5 and $7.5 for bottles of 375 mL and 750 mL respectively. The bread feedstock is considered to be localized and supplied at a constant rate. This assumption implies that the shipment costs remain constant throughout the year. Furthermore, it is assumed that one employee would be in charge of the feedstock shipment. It is assumed that the worker would be paid minimum wage, for 10 hours per week. Supply costs are assumed to be negligible, assuming that processing waste would be donated from a local bread making facility.

When purchased in bulk, the equivalent unit cost of a 750 mL glass bottle with its cap is $2.64 (Bottlestore, *750 mL (25.4 oz) Flint Nordic Spirits Bar Top Glass Bottle*, 2019), and $1.89 for a 375 mL bottle (Bottlestore, *375 mL (12.7 oz) Flint Nordic Spirits Round Glass Bottle*, 2019). The retail price of Amana RFS12TS Heavy Duty Commercial Microwave is $2 000 (Global Industrial, 2020). The retail price of Dynamic TB002 Heavy Duty Mobile Immersion Blender is $11 088 (iFoodEquipment, 2020). These are the only equipements that would need to be purchased to adapt Cirka's operations to our method. Based on the ABV calculated from the experiment results and the use of 6 fermentation tanks, the maximum spirit production rate in the factory is calculated to be 238 L/day. The production rate is assumed at 128 L/day representing approximately 55% of the actual distillery capacity, in order to accommodate market fluctuations. This margin allows to adjust the production according to the market's demand, as well as repair works in the facility. The discount rate for the economic analysis is assumed to be 12%. It also assumed that 100% of the yearly production is sold. Finally, the project starts at year 0, and finishes at year 9, where utilities are sold at a discounted price.

Costs			
	Value	Units	Notes
Indus. Microwave	2 000,00	$	
Indus. Food proces	11 088,00	$	
Sum NPV	13 088,00	$	
Cirka Operation Costs	23 000,00	$/month	Cirka
Shipment Wage	541,67	$/month	10h/week min wage
Monthly OP costs	23 541,67	$/month	combined
Operational costs	298 567,30	$/yr	rent, utilities, equipment and 1 employee
Bottles	177 107,58	$/yr	Empty bottles cost

Benefits			
	Value	Units	Notes
Bottles 1 sold	22000,9	units/year	Expected # bottles of 375 mL sold
Price per bottle	5,00	$/unit	Net revnue per bottle
Change in bottle price	1,5%	/yr	Inflation rate
Bottles 2 sold	51335,5	units/year	Expected # bottles of 750 mL sold
Price per bottle	7,50	$/unit	Net revnue per bottle
Change in bottle price	1,5%	/yr	Inflation rate
Residual price of utilities	1 308,80	$	With an interest of 10%, assume all utilities sold at year 9.

Table 8. Cost and benefit breakdown of the project.

As described in Table 8, if the daily production of spirit is 128 L per day, the maximum number of bottles sold would be 22 000 and 51 335 bottles of 375 mL and 750 mL respectively. Furthermore, it is assumed that the net revenues from selling the bottles would fluctuate according to the inflation. As of today, the yearly inflation in Canada is 1.5% (Worldwide Inflation Data, 2020), and the project's net revenues are assumed to fluctuate accordingly. As shown in Table 8, in order to simplify the calculations, the monthly operation costs provided by Cirka were compounded yearly. Assuming a monthly interest rate of 1%, $23 541.67 compounded monthly corresponds to $298 567.04 compounded yearly. Finally, the cost of buying empty bottles in bulk can be obtained by multiplying the unit bottle prices to the corresponding number of bottles sold. As shown in Table D1 of Appendix D, the cost-benefit analysis indicates an NPV of $202 309.77, which means that the project is economically feasible in the stated conditions. However, the accuracy of the model depends on the assumptions made.

8.1.3 Economic Risk Analysis

Although the cost-benefit analysis indicates the project's feasibility, the accuracy of this model can be affected by various factors that can influence the outcome of the project. For example, the net benefits are obtained from the number of bottles sold to the public. Market laws, such as the demand and the offer can influence sales, and thus the number of bottles sold may vary accordingly. Furthermore, the operational costs may also be influenced by different factors such as: the overall production rate, the feedstock quality, inflation, etc. Therefore, it is important to assess those changes in order to build a more realistic and accurate model. In order to do so, the Monte Carlo analysis was used. In brief, this statistical method consists of solving deterministic issues (i.e. the feasibility of a project) using randomness principles (Rubinstein and Kroese, 2016).

8.1.3.1 Determining factors

The first step of the analysis is to determine which inputs most affect the outcome of the project. Through a "What if?" analysis, the value to which cost and benefit factors need to be modified to achieve an NPV of $0 was determined. The change from the original value to this new value determines if the factor has a significant influence on the project's outcome. The smaller the change, the more significant the factor is. As shown in Table 9, the two factors that influence the outcome of the project the most are the operational costs and the number of litres produced per year. The latter directly influences the annual income of the project. Other inputs like the present cost of utilities did not significantly affect the NPV of the project.

	Original Value	When NPV=0	%change	Actual Change
Cirka OP costs	23 000,00	26 250,76	14,13%	#N/A
Shipment	541,67	3 792,43	600,14%	#N/A
Op costs (monthly)	23 541,67	26 792,43	13,81%	3 250,76
Op costs (yearly)	298 567,30	332 925,34		
Bottles 1 unit price	1,89	3,17	67,72%	1,28
Bottles 2 unit price	2,64	3,19	20,83%	0,55
Litres prod per year	46 752	53 190	13,77%	6 438

Table 9. Change in a factor's value when the NPV is equal to 0.

8.1.3.2 Assigning weights

The second step of the analysis is to assign random weights to the chosen factors. Each weight influences the NPV of the project. In order to obtain a significant model, the weights must be realistic. They should represent the market's offer and demand, as well as existing criteria from the industry. The simplest way to do so is to generate intervals from which the weights will then be selected randomly. For this analysis, the intervals were determined using results from the market analysis survey, as well as consultations with our client. The different interests amongst the applicants of the survey provided a better perspective on the potential demand for such a product. Furthermore, our client helped us estimate the potential variations in operational costs. The following weights and their intervals can be found in Table 10.

The economic conditions represent the fluctuations in demand by the consumers, whereas the operation efficiency accounts for the variation in operation costs. The weight interval for the economic conditions is smaller than that for the operation efficiency since the production can be easily adjusted to the market's demand. Moreover, the distillery should maintain a constant production rate, thus not significantly affecting its net benefits. However, the operation costs may vary significantly as they depend on various external factors, such as transportation, bread

feedstock, bread quality, and failures. The maximum, minimum, average as well as the standard deviation of each interval are described in Table 10. From these, weight values can be randomized to follow a normal distribution. In order to determine the weights, the excel function NORM.INV was used where: the probability p of the normal distribution is a random number between 0 and 1; and the average and standard deviation are the corresponding values in the table. The weight values are randomized and therefore will be different each time the user refreshes the program. The weights displayed in this report are kept constant for demonstration purposes.

	Max.	Min.	Avg.	Standard Deviation	Random Weight
Economic conditions	1.05	0.85	0.95	0.0333	0.980363989
Operation efficiency	1.15	0.75	0.95	0.0667	1.007648952

Table 10. Factors and their corresponding weights.

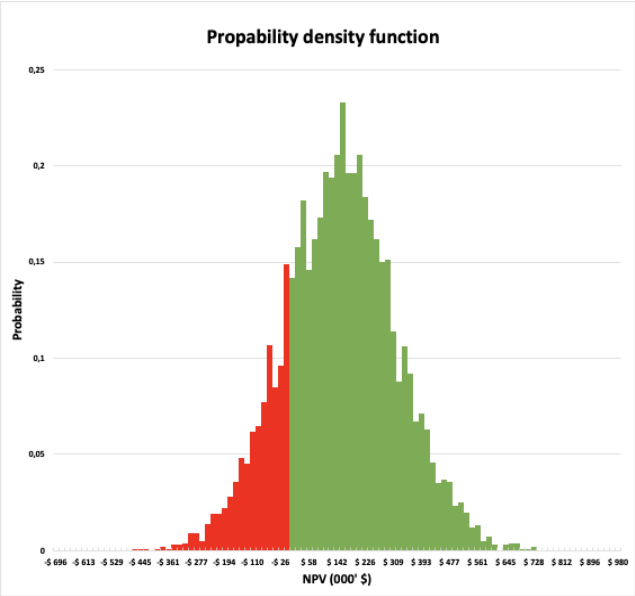
8.1.3.3 Creating the model

Each weight computed in Table 10 is then assigned to its corresponding factors. From the cost-benefit analysis, the economic conditions affect the net benefits, while the operation efficiency affects the operational costs. Each weight generates a unique scenario (i.e. NPV). Using excel's "What if?" analysis, 5 000 unique scenarios were generated from the model. Each scenario is unique since weights are generated randomly. A histogram of the NPVs, seen in Figure 5, follows a normal probability distribution. A colour code differentiates the NPVs that are greater than zero (green) from those that are less than zero (red).

The model follows a normal distribution $N(\mu, \sigma^2)$, with a mean, μ, and standard deviation, σ. A cumulative distribution function (Figure 6) can be obtained from the results of the study. This type of graphical representation is arguably easier to interpret when proceeding to an economic risk analysis.

8.1.3.4 Results and Discussion

As seen in Figures 5 and 6, the model shows that the project has an 80% chance of being economically feasible. In other words, there is a 20% chance of losing money according to the results of the analysis. In contrast, there is a 60% chance that the project will generate \$0 to \$300k and a 20% chance that the project will generate more than \$300k.



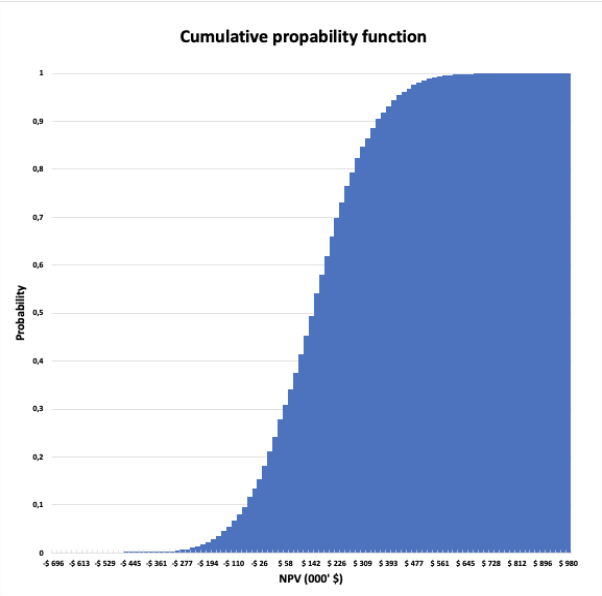


Figure 5. (left) Probability density function of the economic risk analysis model. **Figure 6. (right)** Cumulative probability function of the economic risk analysis model.

At first glance, this project seems lucrative. However, the large standard deviation indicates that the project still has a considerable risk involved. Indeed, NPV values generated through random scenarios are notably "spread out". For this project, the expected minimum NPV is -$484 907.95, and the expected maximum $841 442.97. The standard deviation of this project is $165 053.07. In order to narrow down the standard deviation of the model, and therefore, reduce the economic risk of the project, further market analyses should be conducted. Furthermore, additional research would help reduce the variations in operational costs. For example, surveying existing distilleries and their methods would likely give a better estimation of operational costs in the industry. Also, further research could assess the distillation process in order to optimize the production rate and reduce variations in operational costs. In summary, there is a considerable risk to pursue such an enterprise. This may be justified by the lack of resources and information available to increase the accuracy of the model. The main goal in improving the existing model would be to reduce its standard deviation. This could be done through further research, as well as collaboration with experts, to provide a more statistically meaningful outcome of the model.

8.2 Risk and Safety Considerations

8.2.1 Risk Factor Matrix

In fermentation and distillation procedures, it is important to take precautions to ensure the safety of those involved. A risk factor analysis was developed using a risk factor matrix

(Government of Canada, 2019b; ISO, 2018) and by consulting guidelines concerning fermentation and distillation (WorksafeBC, 2018). Each risk factor was ranked according to its severity and likelihood of occurrence and assigned a number. For example, a "very low risk" (rated 1-3) is a risk factor that is very unlikely and of insignificant severity, while an "immediate danger" (rated 16-25) is one that is almost certain to occur and could result in death. The risk contributors are analyzed for each risk factor and control methods are suggested. The risk factor matrix, color-coding table, and risk analysis Tables are located in Appendix E.

The two most dangerous risks involved are flammable products and by-products, and confined spaces. Flammable product hazards could lead to serious burns, fire hazards and explosions, whereas confined spaces could lead to asphyxia and death. Mitigation measures, namely labelling hazardous areas, installing physical barriers on platforms, following governmental safety guidelines and many others are to be undertaken at all costs to ensure the health and safety of workers. A complete list of all the mitigation measures can be found in Table E3 of Appendix E.

8.2.2 Product Safety Standards

Additionally, the final product must be safe for consumption. The most common impurity in spirit-making is methanol (Lea and Piggott, 2003; Paine and Davan, 2001). The maximum methanol threshold for an adult consuming 100 mL of an alcoholic beverage containing 40% ABV over the span of two hours is 2% methanol by volume. In contrast, the European Union legislation indicates that a threshold of 0.4% methanol by volume provides a larger safety margin (Paine and Davan, 2001). In order to prevent methanol level from reaching that particular threshold, the distiller must monitor its concentration throughout the duration of the distillation process. Since methanol (i.e. the heads) is distilled at the beginning of the process, the distiller must only yield the distillate when the concentration of methanol is safe for consumption (Cirka, personal communications, November 24th, 2019; Lea and Piggott, 2003).

8.2.3 Labelling Standards

Finally, the spirit produced must meet regulations before being sold to the general public. The Government of Canada's (2015) standards indicate that the product shall be a potable alcoholic beverage. For vodka, the distillate must be filtered with charcoal, or other means, in order to remove any impurities in the final product. The spirit must be produced from material of agricultural origin (i.e. grains, potatoes, grain products, etc.). The ingredients must be clearly listed on the product, following the statement "produced from:". Furthermore, in accordance to the Food and Drug Regulations (FDR) (Government of Canada, 2019a), sulphites added a level superior to 10 ppm must be declared and clearly shown on the product's label.

8.3 Environmental and Social Considerations

Food waste valorization can not only offer economic benefits but can address social and environmental impacts of food waste as well. It can offer a solution to problems associated with the degradation of food waste in the environment that largely contributes to GHG emissions (Hegde et al., 2018). The Food and Agricultural Organization of the United Nations (2013) estimated that wasted food has a carbon footprint of 3.3 Gt of CO_2 equivalents. The project intercepts bread waste before it is disposed of in a landfill. Locally sourcing the bread within a 50 km radius, as well as the product's anticipated local distribution to SAQ stores, will help minimize GHG emissions from transportation. However, one of the main concerns is the energy consumption of the entire manufacturing process. The criteria on energy use guided the design selection for the optimization of the procedure. The energy expense for the fermentation process of 625 L of mash was estimated at 82.33 kWh through heat transfer calculations found in Appendix F. Finally, the waste stream of the fermentation must be properly disposed of. The team was unable to experimentally characterize the waste stream since the final batch could not be produced under the circumstances. The intention is to follow the notion of a circular economy in both sourcing our feedstock from a waste source, and properly dealing with the waste created. Waste disposal options will be discussed in more detail in section 8.3.3.

From a social perspective, the main concern is that the project is dependent on the availability of the bread waste. If an alternative sustainable solution to the disposal of bread processing waste is found, then the project is no longer viable. Also, the project's success is determined by the reception of the product by consumers of legal drinking age. The expertise and guidance from our client can help increase the quality of the final product, and potentially introduce the product to their existing clientele in the craft-distillery market. The responses from the market survey showed positive interest by consumers, but a large-scale formal market analysis should be conducted.

8.3.1 Causal-Loop Diagram

A systems-thinking exercise was conducted by the team members to create a Causal-Loop Diagram (CLD) surrounding the issue of food waste in Montreal, in order to better understand the issue and explore where our project could fit into the overall system. A CLD can be an efficient framework in understanding the complex interactions between key variables of a particular issue (Lannon, 2016). CLDs, and other system analysis tools, can be helpful in communicating insight into complex engineering problems (Haraldsson et al., 2006). Sources were consulted before the exercise to identify possible causes (Abdulla et al., 2013; Bhattacharyya et al., 2019; Dahl et al., 2019; Rinkesh, 2017) and consequences (Bhattacharyya et al., 2019; Rinkesh, 2017; "The Environmental Impact of Food Waste," 2015) of food waste. Following a discussion of the causes and consequences, the CLD illustrated in Figure 7 was constructed. Next, the polarity of the causal arrows connecting each variable was established as

either positive or negative. Finally, notable feedback loops were identified and labeled as reinforcing or balancing.

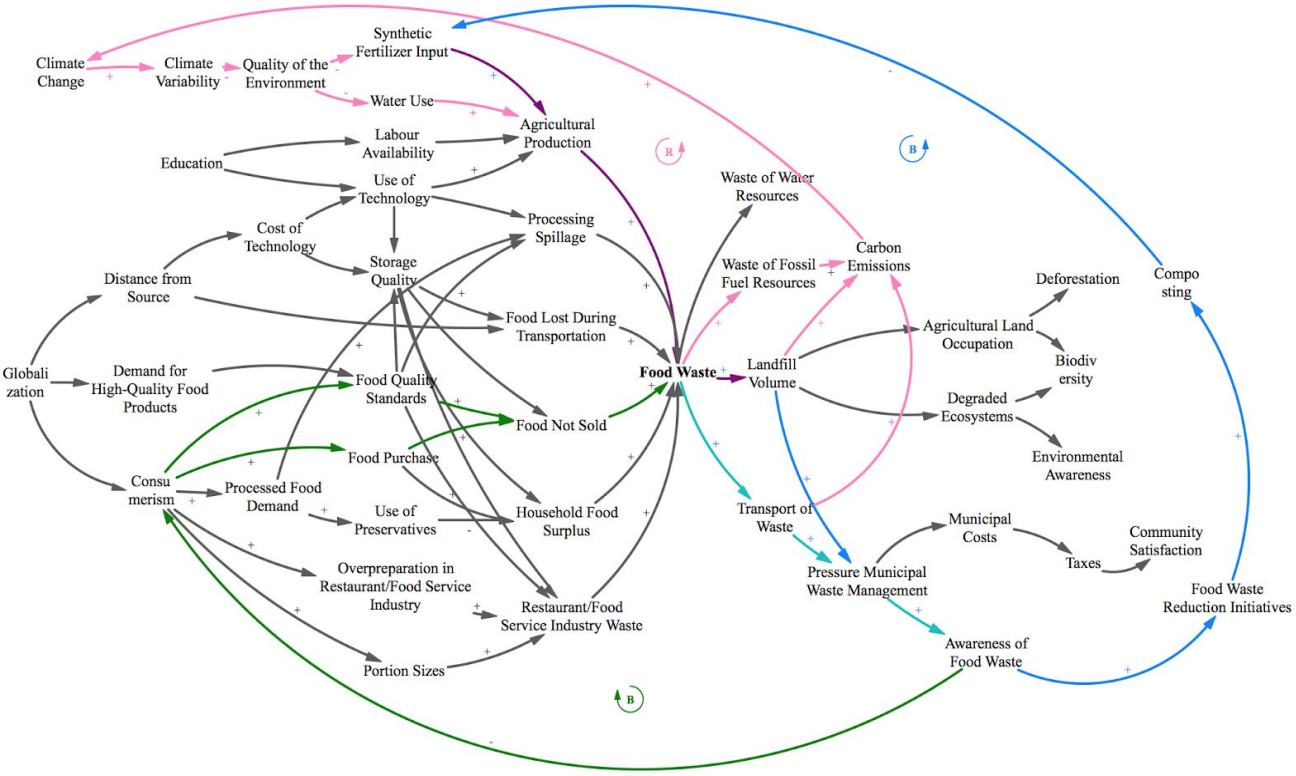


Figure 7. CLD analyzing the causes and consequences of food waste in Montreal: general connectors between variables are in grey; (+) indicates a direct proportional relationship; (-) indicates an inversely proportional relationship; connectors of notable balancing loops are blue or green; connectors of a notable reinforcing loop are pink.

Food is lost or wasted along every step of the food supply chain, from harvesting and processing to retail and household food levels (Lundqvist et al., 2008). Initially, our design targeted the food that is not sold in grocery stores and bakeries, falling under the category indicated by the variable labeled "Food Not Sold" in Figure 7. However, in order to have a consistent and reliable feedstock, as well as a single source location, the design targets bread processing waste, falling under the variable "Processing Spillage". Among the notable loops outlined in the CLD, the two balancing feedback loops, colored blue and green, represent the tendency of the effects of food waste to balance out over time in those respective loops of this particular model. This is due to the assumption that increased awareness to the issue of food waste would result in greater actions taken, such as increased composting efforts, and decreased consumer food purchases. While balancing loops can tend to be reassuring, in this context they represent the stubborn nature of the main issue of food waste to persist. With respect to bread waste fermentation, the green balancing loop accentuates a stable availability of the project's

initial feedstock source. According to this loop of the CLD model, the availability of the raw material depends on the level that the unsold food stabilizes to. Meanwhile, there are no loops identified that directly incorporate processing waste, which supports the decision to rely on this source for a dependable source of feedstock. Processing waste depends on bread production, which is not anticipated to slow down in the near future. The reinforcing feedback loop, in pink, corresponds to the compounding effects between food waste, GHG emissions and the climate. Roughly 20% of Canada's methane emissions come from landfills (David Suzuki Foundation, 2019). Although well-run landfills can recover, contain, or combust methane, Environment and Climate Change Canada (2017) reports that in 2015, of the 30 Mt of carbon dioxide equivalent that were generated at Canadian landfills, 19 Mt were eventually emitted into the atmosphere. In this pink loop, the potential reduction in the quantity of food waste, through the design's value-added production of vodka, could help mitigate the severity of the effects of food waste on carbon emissions and ultimately, climate change.

8.3.2 Life Cycle Assessment

Life cycle assessment (LCA) is a theoretical approach for assessing the environmental impacts throughout all stages of a product's life, from raw material extraction to waste disposal (Muralikrishna and Manickam, 2017). It is a useful and widely used method in environmental engineering and environmental management (Burnley et al., 2019). It has a fixed structure and is practiced according to international standards (ISO) 14040 (Muralikrishna and Manickam, 2017). The International Organization of Standardization identifies four phases of the LCA: 1) the goal and scope definition; 2) the inventory analysis; 3) the impact assessment; and 4) the interpretation (ISO, 2016).

A recent study performed a comparative life cycle assessment of vodka production from locally sourced expired bakery waste and virgin wheat (Bhattacharyya et al., 2019). The purpose of the LCA was to determine if other environmental consequences from the use of this feedstock would outweigh the benefits of food waste reduction. The comparative technique emphasizes the differences between the two productions. Human toxicity, freshwater eutrophication, and ecotoxicity showed the greatest consequences after normalization. Meanwhile, climate change and agricultural land occupation were the most sensitive categories to the controllable aspects of vodka production–the choice of feedstock and transportation. They found that small-scale vodka production from locally sourced bakery waste (Case A) had significantly lower environmental impacts than small-scale (Case B) and large-scale (Case C) production from virgin wheat across all categories, as shown in Figure 8. The differences with respect to the first three categories stem from the use of food waste as a feedstock, since it requires no extra agricultural land occupation. In the same way as the team's project using bread processing waste, the bakery waste used in the study would have otherwise been sent to landfill. Therefore, they found that raw material extraction, related to wheat and glass bottle production, is the most dominant life cycle phase in the LCA (Bhattacharyya et al., 2019).

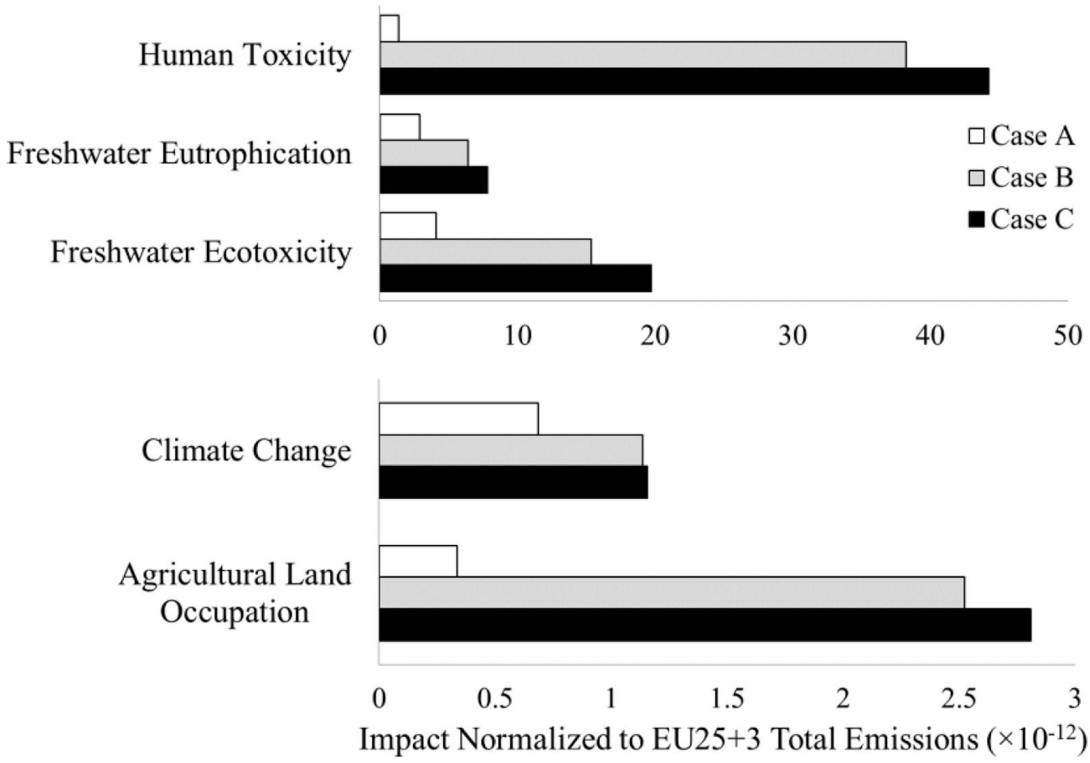


Figure 8. Normalized LCA impact categories for three cases of vodka production: craft-scale vodka production from locally sourced bakery waste (Case A); craft-scale vodka production from locally sourced virgin wheat (Case B); and large-scale mass vodka production from locally sourced virgin wheat, shipped internationally (Case C).

While there are important deviations between the team's project and the studied case, the main differences would further reduce the environmental impact of the vodka production. For example, being based in the UK, the study is based on energy from natural gas. In addition, the analysis is based on heating the mash from room temperature to 85°C and maintaining it there for one hour to promote sugar extraction (Bhattacharyya et al., 2019). Quebec's hydroelectric energy source as well as the reduced heating requirements of the selected design would potentially decrease the environmental impact of the project compared to the studied case. Based on the energy consumption calculations found in Appendix F the estimated CO_2 release equivalent for the fermentation of 625 L of mash is of 493.98 g CO_2 eq. (Hydro-Québec, 2017). Furthermore, the solid waste produced in all three cases of the study was used locally for secondary purposes, such as livestock feed or wet composting. However, the authors conclude that investigating other waste streams may reveal more sustainable options (Bhattacharyya et al., 2019). These options of waste disposal will be discussed in the following section.

8.3.3 Waste Stream

Without the experimental characterization of the project's waste stream, the team is relying on recent studies to properly dispose of the ferment waste. The priority is placed on the

organic solid waste produced during fermentation, as it makes up a large amount of the waste products and has a high nutrient content (Rahman, 2006). The spent fermentation feedstock is assumed to comprise a similar chemical composition to traditional brewery spent grain. In line with the sustainability design criterion, the goal is to repurpose the waste of the fermentation process, similarly to how the project repurposes bread waste. The intention is to explore the suitability of ferment waste for composting in order to close the loop in the food supply chain. While the main outlet of brewery wastes was previously animal feed, alternative uses, such as as a substrate in composting, have been explored in recent years (Rahman, 2006). Novel applications to recycle fermentation waste include using spent grains to cultivate oyster mushrooms (Blair, 2017) and raising black soldier flies on brewery waste to replace soy as a sustainable protein in animal feed (Entocycle, 2020). Although the development of sustainable applications for fermentation waste is encouraging, its application in soil additions and composting has been extensively reported as beneficial for plant growth, notably due to its valuable nutrient content and high water retention (Kanagachandran and Jayaratne, 2006; Krishnamoorthy et al., 2017; Liasu, 2008; Rahman, 2006; Stocks et al., 2001). A study by Liasu (2008) showed that spent grains can be used as an organic supplement for growing tomatoes, however only after a considerable period of composting. Therefore, the diversion of the spent feedstock waste to a composting facility can help reduce the project's waste stream while continuing the use of a valuable resource.

9. Conclusion

Value-added production using food destined for landfill can be an efficient way to reduce the alarming amount of food wasted in Canada. Many studies on biorefinery from food waste have been centered on the production of biofuels. The objective of this design project is to valorize food waste into a marketable and consumable product at a local level. To achieve this, the team suggests locally sourcing bread waste as a more sustainable alternative to traditional feedstocks for vodka production at Cirka Distilleries in Montreal. After comparing alternative fermentation pre-treatments, microwave irradiation followed by simultaneous saccharification and fermentation was selected for the design of the experimental procedure. The reduction of energy use and operating costs, while maximizing ethanol yield, ease of manipulation, and product quality are the determining criteria of the feasibility of this alternative for the client. An experimental optimization of two influential parameters was conducted, investigating the effects of particle size reduction methods and solid loading on the final ethanol yield. Solid loading was found to have a greater effect on ethanol produced than both the size reduction method and the interaction of both factors. The results of a statistical analysis, using a linear regression model, showed that solid loading had a significant positive effect on ethanol yield, for both size reduction methods. Overall, the wet blending method at the highest value of solid loading tested achieved the greatest ethanol production by weight. This preconditioning method is adapted to include drying the bread pieces in order to facilitate the availability and storage of the feedstock.

Despite not being able to test the effectiveness of the microwave SSF pre-treatment, the results allowed the team to establish the overall scaled-up process, from the bread waste feedstock to final vodka production. Further testing is necessary to optimize other parameters such as, temperature, duration of microwave irradiation, fermentation time, and filtration methods. A CLD, LCA, risk factor matrix, SWOT, market survey, cost benefit analysis, and economic risk analysis were performed to contextualize the project from an economic, environmental and social standpoint. The market survey displayed general consumer interest and the cost benefit analysis generated a positive NPV. However, the economic feasibility of the project is still impacted by a considerable risk of failure. Meanwhile, the production of vodka from bread waste has a significantly lower environmental impact than production from virgin wheat, and its waste stream can be used in composting. Overall this project shows potential for a craft-scale distillery application. Although the team's final experiments and completion to a distilled product were impacted by the pandemic, further additional research could optimize the production of vodka from bread waste.

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12. Appendices

Appendix A: Energy and Cost Calculations of Pre-treatment Processes

Water heating for alpha-amylase hydrolysis energy use and cost

The following calculations will be based on the volume used for the baseline method experiment. A total mass of 9.6kg of bread is processed. The estimations will be based on a bread volume of approximately 20 L in its cut and water-saturated state.

The equation for energy requirement in sensible heat change:

$$Q = m \times C_p \times \Delta T$$

Where

Q = Energy (kJ);

m = mass of water (kg);

Cp = Specific heat capacity of water = 4.1796 kJ/kg K;

ΔT = Temperature rise (K)

Atmospheric pressure is assumed for the calculation.

$$Q = 29.6\,kg \times 4.1796 \frac{\text{kJ}}{\text{kg.K}} \times (80 - 20)K = 7422.97\,kJ\ per\ experimental\ batch = 2.06\ \text{kWh}$$

This calculation doesn't account for the inefficiency of the heating device and the heat loss from the container to the environment. Newton's law of cooling will be helpful to estimate energy requirements to reach and keep water at temperature for an hour. Considering the energy use of the hot plate itself is more adapted from a business expense standpoint as it represents what the company ultimately pays for and accounts for the considerable heat losses of the heating equipment. A $1 000 heavy-duty Velp Scientifica ceramic hot plate of 800 W will be used for the following calculations (ITM, 2019). Based on the preliminary experiment, the hot plate was used at close to full power for 3 hours for the alpha-amylase hydrolysis. Hydro Quebec's industry tariffs are used to estimate the cost of operation (Hydro Quebec, 2019).

$$Energy\ use = Wattage \times Time\ of\ use$$

$$Energy\ use = 800\,W \times 3h = 2.4\,kWh$$

$$Energy\ cost = Energy\ use \times HydroQuebec\ tariff$$

$$Energy\ cost = 2.4\,kWh \times 0.0328 \frac{\$}{kWh} = 0.079\,\$$$

As expected, the actual energy expense is higher than the estimated one from the sensible heat formula.

Microwave energy use and cost

The assumptions are kept from the previous calculation but the 9.6kg of bread represents approximately 30 L in its cut, dry and slightly pressed state. Half the volume would fit in an industrial grade microwave and thus could be processed in two batches. The cost of such a microwave would carry around $1 500 (Global Industrial, 2019). The wattage and time of use are those recommended in a pre-treatment comparison study (Pietrzak, 2014) but could most likely be optimized through extensive testing.

$$Energy\ use = Wattage \times Time\ of\ use$$

$$Energy\ use = 400\ W \times 0.0333h \times 2 = 0.0266\ kWh$$

$$Energy\ cost = Energy\ use \times HydroQuebec\ tariff$$

$$Energy\ cost = 0.0266\ kWh \times 0.0328 \frac{\$}{kWh} = 0.0008\$$$

Sonifcation energy use and cost

The assumptions used are the same as for microwave irradiation. For a similar capacity, an ultrasonic bath is more expensive than a microwave at around $4 000 (ITM, 2019). Again, Hydro Quebec's industry tariffs are used.

$$Energy\ use = Wattage \times Time\ of\ use$$

$$Energy\ use = 250\ W \times 0.0833h \times 2 = 0.0416\ kWh$$

$$Energy\ cost = Energy\ use \times HydroQuebec\ tariff$$

$$Energy\ cost = 0.0416\ kWh \times 0.0328 \frac{\$}{kWh} = 0.0014\$$$

Appendix B: Mass Transfer Calculations Tables

Experiment Bloc A

Table B1. Nutritional content of the waste bread feedstock

Bread type	Quantity	Total Weight (g)	Energy (Calories)	Fat (g)	Sodium (g)	Carbohydrates (g)	Fibre (g)	Sugar (g)	Protein (g)
Dense baguette	5,00	1359,10	780,00	3,00	1,26	156,00	6,00	6,00	30,00
Whole wheat baguette	2,00	466,00	700,00	5,00	1,40	130,00	10,00	5,00	30,00
White baguette	4,00	940,40	700,00	5,00	1,45	130,00	5,00	5,00	25,00
Rustic baguette	1,00	320,60	832,00	0,00	1,73	172,80	6,40	0,00	25,60
TOTAL	-	2765,50	8932,00	45,00	16,63	1732,80	76,40	60,00	335,60
Per 100g mix	-	100,00	322,98	1,63	0,60	62,66	2,76	2,17	12,14

Table B2. Content in key macronutrients for each replicate and replicate information

Test	Weight pre-ferm (g)	Actual loading (%)	Solid weight (g)	Energy (Calorie)	Fat (g)	Carbohydrates (g)	Fibre (g)	Sugar (g)	Protein (g)
A-T1-S1	403,10	16,34	65,85	212,68	1,07	41,26	1,82	1,43	7,99
A-T1-S2	410,90	16,34	67,12	216,80	1,09	42,06	1,85	1,46	8,15
A-T1-S3	401,00	16,34	65,51	211,58	1,07	41,05	1,81	1,42	7,95
A-T2-S1	420,60	21,89	92,05	297,31	1,50	57,68	2,54	2,00	11,17
A-T2-S2	420,70	21,89	92,07	297,38	1,50	57,69	2,54	2,00	11,17
A-T2-S3	420,70	21,89	92,07	297,38	1,50	57,69	2,54	2,00	11,17
A-T3-S1	395,30	27,62	109,16	352,57	1,78	68,40	3,02	2,37	13,25
A-T3-S2	396,20	27,62	109,41	353,37	1,78	68,55	3,02	2,37	13,28
A-T3-S3	396,20	27,62	109,41	353,37	1,78	68,55	3,02	2,37	13,28

Table B3. Fermentation mass balance components and theoretical ethanol production

Test	Sugar content (g)	Water content (g)	Post hydrolysis water content (g)	Ethanol content (g)	CO2 produced (g)	Weight post-ferm (g)
A-T1-S1	43,66	337,25	333,03	22,33	21,33	379,20
A-T1-S2	44,51	343,78	339,47	22,76	21,75	364,30
A-T1-S3	43,44	335,49	331,29	22,21	21,22	378,80
A-T2-S1	61,04	328,55	322,64	31,22	29,82	357,70
A-T2-S2	61,05	328,63	322,72	31,22	29,83	386,20
A-T2-S3	61,05	328,63	322,72	31,22	29,83	386,20
A-T3-S1	72,38	286,14	279,14	37,02	35,37	317,60
A-T3-S2	72,55	286,79	279,77	37,10	35,45	300,90
A-T3-S3	72,55	286,79	279,77	37,10	35,45	293,80

Table B4. Brix results and calculated ethanol production and efficiency

Test	Initial Brix	Brix sugar content (g)	Theoretical max ethanol (g)	Final Brix	Brix differential	Fermented sugar (g)	Actual ethanol (g)	Fermentation efficiency %	Ethanol by weight (% w/w)
A-T1-S1	13,20	53,21	27,21	5,20	8,00	32,25	16,49	73,85	4,09
A-T1-S2	13,20	54,24	27,74	5,40	7,80	32,05	16,39	72,01	3,99
A-T1-S3	13,20	52,93	27,07	5,50	7,70	30,88	15,79	71,08	3,94
A-T2-S1	21,40	90,01	46,03	8,40	13,00	54,68	27,96	89,58	6,65
A-T2-S2	21,40	90,03	46,04	8,10	13,30	55,95	28,61	91,65	6,80
A-T2-S3	21,40	90,03	46,04	8,50	12,90	54,27	27,75	88,89	6,60
A-T3-S1	26,00	102,78	52,56	10,10	15,90	62,85	32,14	86,83	8,13
A-T3-S2	26,00	103,01	52,68	10,50	15,50	61,41	31,41	84,65	7,93
A-T3-S3	26,00	103,01	52,68	9,90	16,10	63,79	32,62	87,92	8,23

Table B5. Adaptation of results into ABV and comparison of actual and theoretical results

Test	Weight (g)	Volume (ml)	Density (g/ml)	ABV % (theory)	ABV % (actual)	Fermentation efficiency %
A-T1-S1	379,20	353,29	1,07	8,01	5,91	73,85
A-T1-S2	364,30	339,41	1,07	8,50	6,12	72,01
A-T1-S3	378,80	352,92	1,07	7,98	5,67	71,08
A-T2-S1	357,70	315,63	1,13	12,53	11,23	89,58
A-T2-S2	386,20	340,77	1,13	11,61	10,64	91,65
A-T2-S3	386,20	340,77	1,13	11,61	10,32	88,89
A-T3-S1	317,60	283,57	1,12	16,54	14,36	86,83
A-T3-S2	300,90	268,66	1,12	17,50	14,81	84,65
A-T3-S3	293,80	262,32	1,12	17,92	15,76	87,92

Experiment Bloc B

Table B6. Nutritional content of the waste bread feedstock

Bread type	Quantity	Total Weight (g)	Energy (Calories)	Fat (g)	Sodium (g)	Carbohydrates (g)	Fibre (g)	Sugar (g)	Protein (g)
1/2 Baguette Toledo	2,00	320,60	416,00	0,00	0,80	86,40	3,20	0,00	12,80
Dense baguette	1,00	282,00	780,00	3,00	1,30	156,00	6,00	6,00	30,00
Stick bread	1,00	120,00	264,00	0,96	0,58	52,80	2,40	2,40	9,60
Belgian bread	2,00	570,00	798,00	5,70	1,82	153,90	5,70	5,70	28,50
Whole wheat square	1,00	500,00	1200,00	10,00	2,20	220,00	20,00	10,00	50,00
Sliced miche	1,00	250,00	600,00	0,00	1,30	130,00	5,00	0,00	20,00
white sliced bun	1,00	396,00	1080,00	14,00	1,64	196,00	8,00	0,00	36,00
White square bread	1,00	500,00	1100,00	4,00	2,40	220,00	10,00	10,00	40,00
TOTAL	-	2938,60	7452,00	43,36	14,66	1455,40	69,20	39,80	268,20
Per 100g mix		100,00	253,59	1,48	0,50	49,53	2,35	1,35	9,13

Table B7. Content in key macronutrients for each replicate and replicate information

Test	Weight pre-ferm (g)	Actual loading (%)	Solid weight (g)	Energy (Calories)	Fat (g)	Carbohydrates (g)	Fibre (g)	Sugar (g)	Protein (g)
B-T1-S1	424,70	16,24	68,96	174,88	1,02	34,16	1,62	0,93	6,29
B-T1-S2	419,40	16,24	68,10	172,70	1,00	33,73	1,60	0,92	6,22
B-T1-S3	402,10	16,24	65,29	165,58	0,96	32,34	1,54	0,88	5,96
B-T2-S1	412,70	21,18	87,39	221,61	1,29	43,28	2,06	1,18	7,98
B-T2-S2	409,90	21,18	86,80	220,11	1,28	42,99	2,04	1,18	7,92
B-T2-S3	400,30	21,18	84,76	214,95	1,25	41,98	2,00	1,15	7,74
B-T3-S1	350,70	27,45	96,26	244,12	1,42	47,68	2,27	1,30	8,79
B-T3-S2	350,00	27,45	96,07	243,63	1,42	47,58	2,26	1,30	8,77
B-T3-S3	351,10	27,45	96,37	244,39	1,42	47,73	2,27	1,31	8,80

Table B8. Fermentation mass balance components and theoretical ethanol production

Test	Sugar content (g)	Water content (g)	Post hydrolysis water content (g)	Ethanol content (g)	CO2 produced (g)
B-T1-S1	36,04	355,74	352,23	18,43	17,61
B-T1-S2	35,59	351,30	347,83	18,20	17,39
B-T1-S3	34,12	336,81	333,48	17,45	16,67
B-T2-S1	45,67	325,31	320,86	23,36	22,32
B-T2-S2	45,36	323,10	318,68	23,20	22,16
B-T2-S3	44,30	315,54	311,22	22,65	21,64
B-T3-S1	50,31	254,44	249,54	25,73	24,58
B-T3-S2	50,21	253,93	249,04	25,68	24,53
B-T3-S3	50,37	254,73	249,82	25,76	24,61

Table B9. Brix results and calculated ethanol production and efficiency

Test	Initial Brix	Brix sugar content (g)	Theoretical max ethanol (g)	Final Brix	Brix differential	Fermented sugar (g)	Actual ethanol (g)	Fermentation efficiency %	Ethanol by weight (% w/w)
B-T1-S1	14,70	62,43	31,93	5,5	9,2	39,07	19,98	108,41	4,7
B-T1-S2	14,70	61,65	31,53	6	8,7	36,49	18,66	102,52	4,45
B-T1-S3	14,70	59,11	30,23	5,5	9,2	36,99	18,92	108,41	4,7
B-T2-S1	17,90	73,87	37,78	7,60	10,30	42,51	21,74	93,07	5,27
B-T2-S2	17,90	73,37	37,52	7,50	10,40	42,63	21,80	93,98	5,32
B-T2-S3	17,90	71,65	36,64	7,40	10,50	42,03	21,49	94,88	5,37
B-T3-S1	22,60	79,26	40,53	10,50	12,10	42,43	21,70	84,35	6,19
B-T3-S2	22,60	79,10	40,45	10,00	12,60	44,10	22,55	87,83	6,44
B-T3-S3	22,60	79,35	40,58	10,50	12,10	42,48	21,73	84,35	6,19

Table B10. Adaptation of results to ABV and comparison of actual and theoretical results

Test	Weight (g)	Volume (ml)	Density (g/ml)	ABV % (theory)	ABV % (actual)	Fermentation efficiency %
B-T1-S1	370,00	340,00	1,09	6,87	7,45	108,40
B-T1-S2	393,00	370,00	1,06	6,23	6,39	102,52
B-T1-S3	374,00	350,00	1,07	6,32	6,85	108,42
B-T2-S1	351,00	290,00	1,21	10,20	9,50	93,08
B-T2-S2	336,00	310,00	1,08	9,48	8,91	93,97
B-T2-S3	328,00	295,00	1,11	9,73	9,23	94,86
B-T3-S1	282,00	255,00	1,11	12,78	10,78	84,34
B-T3-S2	294,00	260,00	1,13	12,51	10,99	87,82
B-T3-S3	269,00	240,00	1,12	13,60	11,47	84,36

Appendix C: *R* Code for Statistical Analysis

```
#############################################################################
#
#Statistical Analysis of Fermentation Experiment Results
#Author: Meaghan Kilmartin
#Date: March 2020
#
#############################################################################
#1. Loading and inspecting the data
#############################################################################

#Set the working directory to the folder "Experiments".
#Open the first .csv data files and assign it the name them 'data1' and 'data2':

        data1 = read.csv("DesignExperiment1.csv")
        data2 = read.csv("DesignExperiment2.csv")

#### Data exploration ####
        names(data1)
        str(data1)
        head(data1)
        summary(data1)

        names(data2)
        str(data2)
        head(data2)
        summary(data2)

#############################################################################
#2. Linear model: Linear Regression
#############################################################################

# Regression allows to model a linear relationship between a response (Y) and a dependent variable (X)

# Our hypothesis is that ethanol_produced is a function of solid loading.
# First check that R is treating both variables as continuous
        data1$ethanol_produced = as.numeric(data1$ethanol_produced)
        str(data1$ethanol_produced)
        data1$solid_loading = as.numeric(data1$solid_loading)
        str(data1$solid_loading)

        data2$ethanol_produced = as.numeric(data2$ethanol_produced)
        str(data2$ethanol_produced)
        data2$solid_loading = as.numeric(data2$solid_loading)
        str(data2$solid_loading)
```

```
# Run the linear model: Regression of ethanol produced on solid loading
        lm1 <- lm(data1$ethanol_produced ~ data1$solid_loading) #Experiment A
        lm2 <- lm(data2$ethanol_produced ~ data2$solid_loading) #Experiment B

# Run through the diagnostic plots (to do before looking at p-values)
        opar <- par(mfrow=c(2,2))
        plot(lm1)
        plot(lm2)
        par(opar)

# Can also extract the residuals using
        hist(resid(lm1))
        hist(resid(lm2))

# Plot the data and the regression line
        plot(data1$ethanol_produced ~ data1$solid_loading, pch=18, col="coral", ylab="Ethanol Produced",
        xlab="Solid Loading")
        abline(lm1, lwd=2)

        plot(data2$ethanol_produced ~ data2$solid_loading, pch=19, col="gold2", ylab="Ethanol Produced",
        xlab="Solid Loading")
        abline(lm2, lwd=2)

# Is the data normally distributed?
        hist(data1$ethanol_produced,col="coral", main="Untransformed data", xlab="Ethanol Produced")
        hist(data1$solid_loading, col="coral", main="Untransformed data", xlab="Solid Loading")

        hist(data2$ethanol_produced,col="gold2", main="Untransformed data", xlab="Ethanol Produced")
        hist(data2$solid_loading, col="gold2", main="Untransformed data", xlab="Solid Loading")

#############################################################################
#3. Model Summary and Final Plotting
#############################################################################

# look at the model coefficients and p-values
        summary(lm1)
        summary(lm2)

# call up the coefficients of the model
        lm1$coef
        lm2$coef

# Standard error, t-value and R squared
        summary(lm1)$coefficients # Std. Error = Standard Error of the estimate
        summary(lm2)$coefficients

        summary(lm1)$r.squared # R2 (aka coefficient of determination; SSreg/ SStotal)
        summary(lm2)$r.squared
```

```
# plot the data and the regression line side by side
        opar <- par(mfrow=c(1,2))

        plot(data1$ethanol_produced ~ data1$solid_loading, pch=18,col="coral",xlab="Solid Loading", ylab =
        "EthanolProduced")
        abline(lm1, lwd=2)
        plot(data2$ethanol_produced ~ data2$solid_loading, pch=19,col="gold2",xlab="Solid Loading", ylab =
        "EthanolProduced")
        abline(lm2, lwd=2)

# plot on the same axes for comparison
        opar <- par(mfrow=c(1,1))

        plot(data1$ethanol_produced ~ data1$solid_loading, main="Linear Regression of Solid Loading on
        Ethanol Produced",  pch=18,col="darkorange1",xlab="Solid Loading, wt%", ylab = "EthanolProduced,
        wt%", xlim=c(15, 29), ylim=c(3.5, 9.5))
        abline(lm1, lwd=2, col="coral")
        par(new=TRUE)
        plot(data2$ethanol_produced ~ data2$solid_loading, main="Linear Regression of Solid Loading on
        Ethanol Produced", pch=18,col="gold2",xlab="Solid Loading, wt%", ylab = "EthanolProduced, wt%",
        xlim=c(15, 29), ylim=c(3.5, 9.5))
        abline(lm2, lwd=2, col="gold2")
```

Appendix D: Economic Analysis Table

Table D1. Accounting analysis of the project. Net benefits, PV net benefits, payback, NPV and IRR of the project.

Year	0	1	2	3	4	5	6	7	8	9
BENEFITS										
Annual income	$495 021,18	$502 446,49	$509 983,19	$517 632,94	$525 397,43	$533 278,39	$541 277,57	$549 396,73	$557 637,69	$566 002,25
Residuals										$1 308,80
TOTAL BENEFITS	$495 021,18	$502 446,49	$509 983,19	$517 632,94	$525 397,43	$533 278,39	$541 277,57	$549 396,73	$557 637,69	$567 311,05
COSTS										
NPV utilities	$13 088,00									
Operational costs	$298 567,30	$298 567,30	$298 567,30	$298 567,30	$298 567,30	$298 567,30	$298 567,30	$298 567,30	$298 567,30	$298 567,30
Bottles	$177 107,58	$180 561,17	$184 082,12	$187 671,72	$191 331,32	$195 062,28	$198 865,99	$202 743,88	$206 697,38	$210 727,98
TOTAL COSTS	$488 762,88	$479 128,47	$482 649,42	$486 239,02	$489 898,62	$493 629,58	$497 433,29	$501 311,18	$505 264,69	$509 295,28
Net benefits	$6 258,30	$23 318,02	$27 333,77	$31 393,92	$35 498,82	$39 648,82	$43 844,28	$48 085,55	$52 373,00	$58 015,77
PV NB	$6 258,30	$20 819,66	$21 790,32	$22 345,57	$22 560,14	$22 497,80	$22 212,88	$21 751,46	$21 152,58	$20 921,07
Payback	$6 258,30	$27 077,96	$48 868,28	$71 213,85	$93 773,99	$116 271,79	$138 484,67	$160 236,13	$181 388,71	$202 309,77
NPV	$202 309,77				**IRR**	#NUM!				

As seen in Table D1, the IRR of the project can't be determined. This is due to the fact that the project's payback starts at year 0. In other words, in this configuration, there will always be positive net benefits throughout the duration of the project. The NPV of the project in this case is $202 309.77.

Appendix E: Risk Factor Analysis Matrix

Table E1. Risk Factor Matrix for Rank Attribution

Severity \ Risk	1 Very unlikely	2 Unlikely	3 Notable chance	4 Very likely	5 Almost certain
1 Insignificant	1	2	3	4	5
2 Minor injuries	2	4	6	8	10
3 Notable injuries	3	6	9	12	15
4 Severe injuries	4	8	12	16	20
5 Death	5	10	15	20	25

Table E2. Colour-Coded Risk Ranking Categories

Description	Colour code	Recommended actions
Immediate Danger		Stop the process and implement controls.
High Risk		Investigate the process and implement controls immediately.

Medium Risk	Keep the process going. A control plan must be developed and implemented as soon as possible.
Low Risk	Keep the process going and monitor regularly. A control plan should be investigated.
Very Low Risk	Keep monitoring the process.

Table E3. Risk Analysis Table

Risk Contributors	Risk Rank	Risk Factors	Control Methods
Physical tasks	6	- Body sprains and strains	- Provide mechanical devices for lifting heavy objects. - Design workplace to prevent lifting heavy objects - Define specific areas by weight classes. Store heavy items accordingly. - Use safe lifting techniques.
Slippery surfaces	9	- Body sprains - Bone fractures - Concussion	- Control and monitor humidity and spillage. - Monitor pipe leakages and tank spills. - Clean floors and slippery surfaces regularly. - Inform the presence of slippery surf by putting signs on the floor. - Use mats and rugs on surfaces likely to be slippery. - Install texture flooring.

Height and ladders	8	- Body sprains - Bone fractures - Concussion - Head and spinal injuries	- Reduce tasks at high elevation - Prevent falls by installing protected work platforms and catwalks. -Use mechanical lifts when possible. - Train workers to work at heights. - Monitor the safety procedures and equipment. - Do not carry of lift heavy objects up to a platform. - One person at the time on a ladder. - Do not skips ladders when using one. - Store ladders in a designated area.
Cluttered areas	2	- Head and spinal injuries - Bone fractures	- Monitor and control the workspace regularly. - Keep housekeeping records to find trends and root causes for common issues. - Keep paths free of tools and equipment. - Label with bright colours apparent pipes. - Watch for hoses when moving one.

Confined space	15	- Asphyxia - Death	- Determine and monitor all confined spaces. - Train workers to enter confined spaces if needed. - Ventilate and purge the atmosphere to prevent concentration build-ups. - Monitor levels of CO_2 and other gases resulting from fermentation. - Prevent a worker from entering a confined space alone. Use a standby worker. - Develop an emergency plan to rescue asphyxiated workers. - Coordinate with local competent authorities.
Hot surfaces	12	- Minor burns - Severe burns - Death	- Monitor temperatures of pipes, tanks, and other heated equipment. - Ensure workers wear the appropriate equipment and clothing to withstand heat. This includes protection glasses, cotton clothes, temperature resistant gloves, face shields, etc. - Consider insulating hot surface. - Identify potential spill areas and monitor them. - Install boil-over protection systems in the brew kettles, if possible. - Keep a water hoses nearby hot surfaces and equipment.

Chemical manipulation	12	- Minor skin irritation - Serious burns - Asphyxia	- Get safety data sheets (SDS) for all chemical used on the workplace and update them. - Keep the SDSs readily available. - Store chemicals in confined areas. Post warnings signs. - Use protecting equipment when manipulating chemicals. - Provide an aerated environment for workers. - Ensure that only compatible chemicals are stored together. - Install eyewash and shower stations close to the working environment. - Acids and bases should be stored in different locations to prevent mixing in case of a spill.
Machinery and mobile equipment	8	- Serious cuts - Crushing injuries - Bone fractures - Amputations	- Make sure that all machines' guards are in place before using the equipment. Replace the older ones. - New equipment must be inspected to identify hazards and monitored. - Follow manufacturer's instructions. - Do not wear loose clothes near working machines. - Keep long hair and bears contained. - Do not bypass safety devices. - Test the load capacity by lifting the load by a few centimeters. - Use a spotter when driving forward using a forklift. - Do not leave a working machine unattended. - Use your seatbelt.

Flammable products and by-products	16	- Explosions - Fire	- Never leave a still unattended. - Keep the working environment aerated. - Charge the still boiler with alcohol with less than 40% ABV. - Dilute highly concentrated alcohol as quickly as possible to prevent flash ignition. - Place the alcohol receiver in non-flammable areas. - Keep the distillate levels as low as possible to prevent a spill. - Ensure that electric equipment follow the Electric Code requirements. - Keep the heaters at least 3m away from the distilling, pouring, and blending areas. - Ensure that the fire sprinkler system meets the fire jurisdiction's requirements regarding a distillery.
Broken glass	8	- Minor cuts - Loss of fingers or eyes	- Wear cut-resistant gloves. - Clean up broken glass immediately. - Wear safety equipment when manipulating glass. - Avoid bottle-to-bottle impacts.
Delivery operations	8	- Falls - Bone fractures - Crushing hazard - Death	- Use mechanical aids for loading/unloading products. - Wear high-visibility clothing when working on loading areas. - Secure loads before moving them. - Inspect and maintain your delivery vehicles. - Implementing a safe-driving program to prevent accidents. - Limit driving speed on site.

High-pressure	12	- Explosion - Fire hazard	- Store high-pressure cylinders in confined, well-aerated areas. - Prevent compressed cylinders and equipment from falling. - Monitor pressure levels in pipes. - Inspect kegs periodically. Discard or repair damaged kegs. - Monitor pressure when filling up the kegs. - Do not drop pressurized equipment. - Post a “No smoking” sign near the entrance of confined areas. - Keep compressed cylinders outside the building, if possible.
Noise	4	- Hearing loss	- Wear appropriate equipment when working in noisy environments. - Workers must not be exposed to more than 85 dB daily or 140 dB peak. - Train workers regarding hearing protection and noise damages. - Monitor noise levels in the most sensible areas. - Post warning signs accordingly. - Proceed to annual hearing tests as required by the Regulation.

Appendix F: Energy Expense Calculations (Holman, 2016)

The following calculations are for a single 625 L fermentation tank with a mash at a solid loading of 25%. The density of water is assumed to be 1000 g/L and the density of the bread waste in solution is based on bread crumb density which is assumed to be the densest form of cut bread at 473 g/L (Aqua-Calc, 2020).

From the density, the water fraction of the solution can be determined with the following calculations:

$$Volume = \frac{Fraction\ bread * total\ mass}{Density\ bread} + \frac{Fraction\ water * total\ mass}{Density\ water} \quad (1)$$

$$625\ L = \frac{0.25 * X\ kg}{0.473\ kg/L} + \frac{0.75 * X\ kg}{1.000\ kg/L}$$

$$X = 488.83\ kg$$

$$Water\ mass = Total\ mass * Fraction\ water$$

$$Water\ mass = 366.67\ kg$$

The energy needed to bring the volume of water of the solution up to a boil is calculated with the equation for sensible heat change

$$Q = m \times C_p \times \Delta T \quad (2)$$

Where

Q = Energy (kJ);

m = mass of water (kg);

Cp = Specific heat capacity of water = 4.1796 kJ/kg K;

ΔT = Temperature rise (K)

Atmospheric pressure is assumed for the calculation and the ambient temperature is set at 22 ˚C.

$$Q = 366.7\ kg \times 4.1796\ \text{kJ/kg K} \times (100 - 22)K$$

$$Q = 119\,550\ KJ = 119.55\ MJ = 33.21\ kWh$$

To calculate the surface area of the outside of the tank, a perfect cylinder is assumed with the dimensions of the actual tank: Width = 82 cm; Height = 166 cm (Home Brew Supplies, 2020).

$$Surface\ area\ of\ cylinder = (2\pi r * h) + 2\ (\pi r^2)$$

$$Surface\ area\ of\ cylinder = (2\pi(0.41\ m) * 1.66\ m) + 2\ (\pi(0.41\ m)^2) = 5.33\ m^2$$

The energy needed to keep to temperature ideal for the SSF process can be calculated from Newton's law of cooling:

$$\frac{dQ}{dt} = h * A * (T - T_a) \quad (3)$$

Where

Q = Energy (J);

t = time (s)

A = Object surface area (m^2);

h = Heat transfer coefficient = 16 $W.m^{-2}K^{-1}$ for type 304 stainless steel (food safe); (Engineering ToolBox, 2020)

T = Temperature of object surface (K)

T_a = Ambient fluid (air) temperature (K)

The ambient temperature is set to be at 22 ˚C and the temperature of the object at 30 ˚C. An important assumption is that the temperature of the steel matches the temperature of the mash inside the tank as it can be simplified as a thin wall.

$$\frac{dQ}{dt} = 16\ \mathrm{W.m^{-2}.K^{-1}} * 5.33\ m^2 * (303 - 295) = 682.24\ W$$

Since the fermentation takes place for 72 hours, the total energy lost by keeping the mash at temperature is calculated for the whole process:

$$682.24\ W = 682.24 \frac{J}{s} * 72\ h * 60 \frac{min}{h} * 60 \frac{s}{min} = 176\,836.61\ KJ = 49.12\ kWh$$

The total energy use of the fermentation process will thus be:

$$E = Initial\ energy\ input + Temperature\ maintenance \quad (4)$$

$$E = 33.21\ kWh + 49.12\ kWh = 82.33\ kWh$$