ADDIS ABABA UNIVERSITY

ADDIS ABABA INSTITUTE OF TECHNOLOGY

SCHOOL OF CHEMICAL AND BIOENGINEERING

DEPARTMENT OF CHEMICAL ENGINEERING

# *Investigation of Biogas Production by Co-Digestion of Brewery Wastewater and Brewery Spent Yeast*

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**A thesis submitted to School of Chemical and Bio Engineering, Addis Ababa Institute of Technology in partial fulfillment of the requirements for the Degree of Master of Science in Chemical Engineering (Environmental Engineering)**

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This is to certify that the thesis prepared by Getachew Abrha entitled, “***Investigation of Biogas Production by Co-digestion of Brewery Wastewater and Brewery Yeast***” and submitted in partial fulfillment of the requirements for the degree of Master of Science in Chemical Engineering (Environmental Engineering) complies with the regulations of the university and meets the accepted standards with respect to originality and quality.

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# Abstract

*Production of biogas through anaerobic digestion of organic waste materials provides an alternative environmentally friendly renewable energy. In this study, biogas production from co-digestion of brewery wastewater, spent yeast and brewery sludge in five mix ratios was evaluated under mesophilic condition (35 ℃, 37 ℃, 39 ℃) using a batch digester for 21 days. For all substrates, total solids (TS), biological oxygen demand (BOD), chemical oxygen demand (COD), temperature (T), total nitrogen (TN), total phosphorus (TP), and pH were measured before and after digestion. For the experimental design, different mix ratios of yeast and wastewater by volume (0 %, 1 %, 6 %, 11 %, 100 %) were adopted for all the anaerobic digestion employed. All measured physico-chemical parameters of the sample substrate significantly varied before and after anaerobic digestion AD. Gas production was measured for all the samples periodically starting from fourth day of digestion experiment and went to zero at about 21 days in all substrates. Assessment of cumulative biogas production revealed that substrate in a mix ratio of 1 %, temperature 37 ℃, pH 6.5 (3417 mL/800 mL sample) spent and 6 %, 37 ℃, 6.5 (3325.5 mL/800 mL sample) showed the highest biogas production and the lowest was in 11 % volume ratio, 35 ℃ temperature and pH of 5 (2173 mL/800 mL sample) and 11 %, 39 ℃, 5 pH suggesting the first mix ratio of the two substrates is an optimal mix to yield better biogas. Overall the results indicated that biogas yield and TS, BOD, COD reduction can be significantly enhanced when brewery spent yeast and brewery wastewater are co-digested.*

*Key words: Biogas, Co-digestion, anaerobic digestion, BOD, COD, TS, TN, TP, spent yeast,*

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# 1. Introduction

## 1.1 Background

Abundant availability of energy for domestic, agricultural and industrial purposes is the most captivating features of any civilized communities (Sayibu, 2015). Energy is the source of economic growth and its consumption reflects the state of development of a Nation. Renewable energy utilizes natural resources with technologies ranging from solar power, wind power, hydroelectricity, micro-hydro, to biomass and biofuels. These sources are a feasible alternative to the problems relating to imminent fossil fuel shortage, the complicity of setting up hydroelectric and thermoelectric powers, thus, they are gaining significant attention. Increase in energy demand due to growth in the worlds' economies has resulted to change in energy consumption patterns, which in turn, vary depending on the source and availability of the energy source, conversion loss and end-use efficiency (Martins das Neves, 2009). Most developed and developing countries have shown interest in the production of biogas from renewable resources. Biogas plays an important role in the domestic and agricultural life of the rural dwellers for its application in cooking, crop drying and soil fertilizing (Samuel, 2013).

Anaerobic degradation of organic matter is a complex process carried out by multiple microbial populations interacting in a food web; referred as bio-methanization, it is a natural process that takes place in absence of air (oxygen). It involves the biochemical decomposition of complex organic material by various biochemical processes with the release of energy-rich biogas and production of nitrous effluents. Biogas is produced when bacteria degrade biological materials in the absence of oxygen, during anaerobic digestion (Weedermann, 2015). Anaerobic treatment involves the breakdown of organic matter in the absence of oxygen and the stabilization of these materials by converting them to methane and carbon dioxide gases (Rabah, 2010) . Biogas can be converted to heat and/or electricity, and its purified derivative, biomethane, which are suitable for every function for which fossil natural gas is used. Varieties of diverse microbes, including members of the Eubacteria and Archaea degrade the complex molecules to a mixture of $CH_4$ and $CO_2$. The composition of this microbial consortium depends on various environmental and internal factors such as substrate ingredients, temperature, pH, mixing, or the geometry of the anaerobic digester (Bayer, 2004) (Cime, 2007). The coexistence of different microbial populations as a result of the change in the reactor operating conditions provides unprecedented control over their overall contribution to the degradation of the organic matter (Jalowicki, 2016) Anaerobic digestion of waste from food and beverage industries can contribute positively to the environmental management since it combines both waste removal and stabilization with net fuel (Biogas) production. Effluent from food and beverage industries contain a high level of organic matter that could be converted into energy as a supplement for fossils (Martins das Neves, 2009). In the food industry, the brewing sector holds a strategic economic position: beer is the fifth most consumed beverage in the world, with an average consumption of 23 L/person/year (Fillaudeau, L., Blanpain-Avet, P., Daufin, G., 2006) .

The biogas sector in Ethiopia started with the launch of the National Domestic Biogas (NBPE) programme in 2008, which has led to the dissemination of over 8000 biodigesters so far, about 60% of what was initially intended. The use of domestic biogas has been triggered by the energy crisis in Ethiopia and the suitability of the technology with the physical geography. However, the dissemination has been affected by factors such as economic instability, poverty and illiteracy. Also, many Ethiopian farmers are trapped in a lock-in, where due to their limited purchasing power they cannot afford the niche technology; at least in the way it is being disseminated. Within the emerging biogas sector, the NBPE designated a diverse set of actors to contribute to the implementation of the niche technology. However, their alignment is poor and the private sector is not involved. Expectations have had to be lowered because targets were not met. Also, learning processes are not optimal (Linda Manon Kamp, 2016).

Other studies show, around 1100 biogas plants were constructed in different regions of the country. Currently, around 40 % of these plants are not functional due to lack of effective management and follow up, evacuation of ownership, less priority given by the government, technical problems, reduced animal holding and water problems. Other reasons for the limited success of the technology in Ethiopia includes the adoption of a project-based standalone approach without follow-up structure in place, variations in design, and the absence of a standardized biogas technology (National Biogas Program (NBP), 2007). On the other hand, Getachew et al, (Eshete, 2006), indicated that at least over one million households in Tigray, Amhara, Oromia and Southern Nations, Nationalities and Peoples regional states qualify for the installation of a domestic biogas plant. The domestic biogas technology attracted interest mainly due to consideration of the animal dung, the raw material that is plenty in many rural households in the country. After the establishment of the National Biogas Program Ethiopia in 2009, close to 859 biogas plants have been constructed and are in regular use. Out of the 859 functional biogas plants, 206 are found in Tigray Region, 143 are in Amhara Region, 330 in Oromia Region and 180 are found in SNNP regional states (Claudia, 2011).

Co-digestion is the simultaneous digestion of a homogenous mixture of two or more substrates. The most common situation is when a major amount of a main basic substrate (e.g. manure or sewage sludge) is mixed and digested together with minor amounts of a single, or a variety of additional substrates. The expression co-digestion is applied independently to the ratio of the respective substrates used simultaneously. Until quite recently anaerobic digestion (AD) was a single substrate, single-purpose treatment. For example, manure was digested to produce energy; sewage sludge was anaerobically stabilized and industrial wastewater was pretreated before final treatment in a wastewater treatment plant. Today, the limits and the possibilities of AD are better known and co-digestion has, therefore, become a standard technology.

Generally, co-digestion is applied in wet single-step processes such as continuously stirred tank reactors (CSTR). The substrate is normally diluted with dry solid contents of around 8 to 15 %. Wet systems are particularly useful when the digestate can be directly applied on fields and green lands without solid’s separation. Since agriculture requires high-quality fertilizers without pollutants, co-digestion lends itself towards blends of well-controlled industrial wastes, grass clippings from parks, food industry wastes, dairy wastes, etc. together with dilute substrates such as animal manures or sewage sludge.

There are two major drivers which helped to promote co-digestion: the first one is Digesters in wastewater treatment plants are usually oversized. Addition of co-substrates helps to produce more gas and consequently more electricity at only marginal additional cost. The extra electricity produced allows covering the energy needs of wastewater treatment at a reasonable cost. The second driver is; Agricultural biogas production from manure alone (which has a relatively low gas yield) is economically not viable at current oil prices. Addition of co-substrates with a high methane potential not only increases gas yields but above all increases the income through tipping fees.

Co-digestion offers several ecological, technological and economic advantages:

Improved nutrient balance: The digestion of a variety of substrates instead of a single waste type improves the nutrient ratio of TOC(total organic carbon):N:P which optimally should be around

300:5:1. It also maintains a reasonable mix of minerals (Na, K, Mg, Mn, etc.) as well as a balanced composition of trace metals. Co-digestion, therefore, helps to maintain a stable and reliable digestion performance and a good fertilizer quality of the digestate. Optimization of rheological qualities Wastes with poor fluid dynamics, aggregating wastes, particulate or bulking materials, and floating wastes can be much easier digested after homogenization with a dilute substrate such as sewage sludge or liquid manure. The mixing of different substrates allows some flexibility to be able to compensate for seasonal mass fluctuations of wastes. Underloading and overloading of digesters can be avoided and the digestion process can be maintained at a constant rate. Gate fees and biogas recovery in agricultural digesters the application of co-substrates can considerably improve the overall economics (payback time) of the plant.

In general the merits of co-digestion are the following ; Improved nutrient balance for an optimal digestion and a good fertilizer quality, homogenisation of particulate, floating, or settling wastes through mixing with animal manures or sewage sludge, Increased, steady biogas production throughout the seasons, higher income thanks to gate fees for waste treatment, additional fertilizer (soil conditioner), renewable biomass production for digestion ("Energy crop") as a potential new income of agriculture (Braun Rudolf, 2009). There is abundant literature about the utilization of co-digestion, such as co-digestion of organic fraction of municipal solid waste (OFMSW) and agricultural residues, organic solid wastes and sewage sludge or more specific wastes (Neczaj, Bien, Grosser, Worwag, & Kacprzak, 2012).

Ethiopia has a large population of dairy and beef cattle, generating large amounts of surplus manure that can be used in biogas plants to produce renewable energy. However, the high water content, together with the high content in fibers, are the major reasons for the low methane yields when cattle manure is an-aerobically digested, typically ranging between 10 and 20 $m^3$ $CH_4$ per ton of manure treated (Tamrat, Mebeaselassie, & G., 2013). There are many practices of producing biogas by co-digestion in Ethiopia. Mohammed gedefaw have tried to produce biogas by co-digestion of Cow dung and food waste. At an average of 56 ℃ temperature the biogas production rate was 0.035 $m^3/kg$ of food waste (Mohammed gedefaw, 2015). Similar study done by Alemayehu Gashaw and Abile teshita show high production of biogas (Alemayehu Gashaw & Teshita., 2014).

The brewing industry produces large quantities of wastewater with a high concentration of degradable organic pollutants, which are ideal for the production of biogas. There are many conventional ways reported in recent literature about how to successfully treat brewery wastewater, ranging from fluidized-bed bioreactors to anaerobic sequencing batch reactors (ASBR) (Alvarado-Lassman, 2008). Breweries produce approximately 2-6 hecto-liter(hl) of wastewater per hecto-liter(hl) of beer produced. The wastewater typically has COD in the range 2000-6000mg/l with a BOD/COD ratio around 0.5- 0.7. The COD consists mainly of easily biodegradable organic compounds such as sugars, ethanol, and soluble starch. Because of its high biodegradability, biological treatment (anaerobic and aerobic) is the most widely used treatment process for brewery wastewater. During aerobic treatment, organic compounds are oxidized to carbon dioxide and water by aerobic bacteria, in the presence of air. The energy requirement in anaerobic system is typically 0.7-1kWh/kg COD and the applied volumetric loading rate 0.5-2.0 kg COD/m$^3$/d.

During anaerobic treatment, organic compounds are reduced to methane gas by anaerobic bacteria in an environment free of oxygen. The energy requirement in anaerobic treatment is typically 0.07-0.1kWh/kg COD – an order of magnitude less than that needed for aerobic treatment. Volumetric reactor loading rates can be as high as 25kg COD/m$^3$/d, resulting in relatively small reactor volumes. Sludge production is low, and the excess biomass can often be sold as a seed source for quick start-up of new anaerobic plants so, by comparison with aerobic plants, sludge disposal costs are negligible. Anaerobic and aerobic processes are often used in combination to treat brewery wastewater. In the anaerobic stage, around 70-85 % of the COD is converted into biogas. This is followed by an aerobic post-treatment stage which increases the overall COD reduction to about 98 %. Using the resulting biogas as a fuel in the brewery boilers or in a combined heat and power (CHP) plant can generate a positive energy balance. Whether it is better to burn the biogas directly in the boiler or to set up a separate CHP plant has to be evaluated economically for each project, and will depend on local conditions, the scale of operation and so on. Oil or gas fuel can partly be replaced by biogas, resulting in operational cost saving and the resulting reduction in fossil fuel use makes the brewery more sustainable. CHP plants produce both electrical and thermal energy, which attract subsidies that make them economically feasible in spite of the initial investment cost. Therefore, the objective of the research is to investigate the potential production of biogas from brewery wastewater and spent yeast in a lab-scale anaerobic digester.

## 1.2 Statement of the problem

The brewery industry is the largest manufacturing industry in Ethiopia. There are more than ten major brewery factories which use 3-9 hectoliter water/hectoliter beer and corresponding yeast, grain and hop; producing much wastewater, spent grain, spent yeast and other wastes (ABIWSI, 2004). Environmental pollution derived from domestic and industrial activities is the main threat to the surface and groundwater qualities in Ethiopia. It is reported that the majority of industries in the country discharge their waste into nearby water bodies and open land without any form of treatment (Mohammed Ibrahim, 2015). Most of the effluent discharged by industries including breweries in Ethiopia does not meet the national discharge standards as many of them release their effluent with little or no prior treatment (US/ETH/99/068/Ethiopia, 2011). For this reason, environmental issues are a critical factor in today's industrial competitiveness. The brewery industry discharges many offensive pollutants. These discharges include wastewater, spent grains, waste yeast, and dripped beer from fillers packaging and chemicals used for clean In Place (CIP) units (e.g. caustic soda, phosphoric acid, and nitric acid). Of those, wastewater and spent yeast are usually characterized by wide variation both in the discharge volume and the strength of pollutants such as BOD, COD, solids, and pH. The key environmental issues associated with the brewing include high water and energy consumption, emissions to air (dust and volatile organic compounds (VOCs) and large volumes of wastewater and untreated spent yeast with a high organic load which contributes to BOD, COD, suspended solids, and nutrients such as N and P. Some Ethiopian brewery factories like Meta Abo brewery factory have constructed wastewater treatment plant which is a series of aerobic and anaerobic reactors; but because of poor construction and operation those treatment plants are not effective (Dejene Beyene and Dr. P.V.V. rasada Rao, 2017). Some of the Ethiopian brewery industries treat spent yeast in a highly energy-intensive process. But most of the brewery industries discharge to nearby rivers, because of its high energy requirement, as a result it is causing many environmental problems around where it is dumped off. Some information shows many animals have exploded because of eating this untreated dumped yeast around Sebeta (Fikreslasie, 2011). Spent yeast is highly pollutant waste because of its high BOD, COD, TS, TN, and TP contents, causing eutrophication, bad smelled environment, poisoning animals and other living organisms (Anca Corina Fărcaş, 2017) (Thiago Rocha, 2014). This study is focused to convert those potential environmental pollutants to valuable bioenergy in the form of methane (Biogas production) using lab-scale anaerobic digester. Also, Biogas production from those mixtures (wastewater + Spent yeast) didn't get attention and not studied well; which have dual benefits i.e. environmental protection and overcoming energy shortage.

## 1.3 Objective of the research

### 1.3.1 General objective

The main objective of this thesis is to investigate Biogas production from co-digestion of brewery wastewater and spent yeast

### 1.3.2 Specific objectives

The specific objectives of this research work are to:

- ✓ characterize the raw material for TS, BOD, COD, pH, T, TN, TP
- ✓ determine and characterize the product (amount of gas produced and it's constituents like $CO_2$, $CH_4$, $O_2$, $H_2S$),
- ✓ investigate the effect of volume ratio, temperature, and pH on methane production
- ✓ characterize the digestate for TS, BOD, COD, pH, T, TN, TP, TK

## 1.4 Significance of the study

Converting environmental pollutants to valuable products does not only increase profitability but also, it ensures the safety of workers, society around and the environment.

Generally, the significance of this study can be pointed out as follows:

- ✓ It encourages companies to see their waste as wealth, not as difficulty
- ✓ It will ensure the safety of the people, animals, plant, and the environment in general
- ✓ It will play a positive role in energy management
- ✓ It will open further research study in waste management areas

## 1.5 Scope of the study

This work generally covers from the production of Biogas up to characterization of both the main product and sludge left (fertilizer). This study will also be focused on the major factors that have a significant effect on the production of biogas such as volume ratio, temperature, and pH.

# 2. Literature review

## 2.1 Brewing industry

Beer is one of the most consumed beverages in the world, with an average consumption of 23 L per person per year. Thus, the brewing industry is one of the leading economic contributors to most countries in the world (Hay JXW, 2017). However, huge volumes of by-products and wastewater with a high content of degradable organic pollutants are produced. Two important by-products are: (1) spent grain (SG), a mixture of barley grain husk, pericarp and fragments of endosperm, resulting from the mashing process (Pires EJ, 2012), and (2) trub (Tr), also known as dead yeast or surplus yeast, from the fermentation step (Ferreira IMPLVO, 2010). SG and Tr may reach 30 and 3 % of the volume of beer produced, respectively (Fillaudeau L, 2006). Currently, waste (water) management and disposal represent a significant cost for the brewery industry and an important aspect of concern for a sustainable brewery operation (Unterstein, 2000). The main use of brewery by-products regards animal nutrition but alternative options are worth to be investigated for valuing (Fillaudeau L, 2006).

The brewery has a significant economic value in the agro-food sector as one of the traditional industries. Beer is produced through the fermentation of sugars derived from the saccharification of starch from malted grains (such as barley, rice, and wheat). It can be flavored using hops, herbs or fruits. As one of the oldest beverages produced by humans, a wide variety of beer has been cultivated and established and can vary in alcohol content, bitterness, pH, turbidity, color, and most importantly, flavor (Goldammer, 2008). Beer is the most consumed alcoholic beverage in the world and the third most popular beverage after water and tea. Globally, a beer culture has been established and beer festivals, such as the widely known Oktoberfest in Munich, Germany, are held in a number of countries. Generally, Kenya leads in beer production in the East African Community (EAC) region with a production capacity of 2.8 million hectoliters for the year 2003, followed by Tanzania 2.1 million hectoliters and Uganda 1.3 million hectoliters (Export processing zones, 2005). Processing of beer involves both chemical and biochemical reactions which include mashing, lautering, hops boiling, fermenting and maturation. In the mashing process, malts (germinated and dried grains) are mixed with adjunct flavorings and liquor (pure water) and heated to allow enzymes to break down starch into sugars. This process yields a mixture of malt and wort (sugar water) called mash for the lauter tun. In the lauter tun, the mash is separated into clear liquid wort and residual malt. Lautering consists of three steps: mashout, recirculation, and sparging. During mash out, the temperature is raised to stop the enzymatic conversion of starches to a fermentable fluid. Recirculation consists of drawing off the wort from the bottom of the mash and adding it to the top. After recirculation, water is trickled through the grain to extract the sugars in the sparging process. Care has to be taken during the sparging process, as wrong temperature or pH during sparging can extract tannins from the grain husks, which results in an unpleasant and extremely bitter taste. Once the mash is sparged, the resultant wort is sent to a hops boiler where hops are added tor flavor and boiled according to a recipe hops schedule based on the individual company. Eventually, the wort is sent to a fermenter where the sugars undergo fermentation, via the glycolysis which has the overall chemical reaction as illustrated in the equation below

$$C_6H_{12}O_6 \longrightarrow 2CH_3OH + 2CO_2 \qquad \text{Equation-A}$$

The duration for fermentation depends on the desired final alcohol content of the beer After fermentation the beer is drained and moved into bright tanks where it is allowed to condition, and For every 1,000 tons of beer produced, 137 to 173 tons of solid waste may be created in the form of spent grain, trub from wort production and waste yeast (Caliskan, 2014).

Effluent characteristics play an important role in the selection of treatment process of the wastewater (Rana, 2014); (Udayakumar, 2015) and. Biological oxygen demand (BOD;), Chemical oxygen demand (COD), total suspended solids (TSS), total dissolved solids (TDS), total nitrogen and phosphorous are some of the physicochemical parameters used to characterize waste water. BOD; measures the amount of oxygen required by bacteria for breaking down to simpler substances, the decomposable organic matter present in any wastewater or treated effluent. It is a measure of the concentration of organic matter present in any water. The greater the decomposable matter present, the greater the oxygen demand and the greater the BOD; values (Singh, 2012). COD is a measure of the oxygen required to oxidize all organic material into carbon dioxide and water, and the values are always greater than BODs. The BOD5 to COD ratio is commonly used as an indicator for biodegradability of the waste and is dependent on the characteristics of the waste (Samudro, 2010) (Hammam, 2014). However, C/N/P is also an important parameter for the successful anaerobic degradation of organic wastes.

### 2.1.1 Brewery Process Description

Beer is a fermented beverage with low alcohol content made from barley malt, adjuncts, hops, water, and yeast. Production methods will differ from brewery to brewery as well as according to beer types, brewery equipment, and national legislation. The production steps include wort production process, fermentation processes, and pasteurization and packing processes.

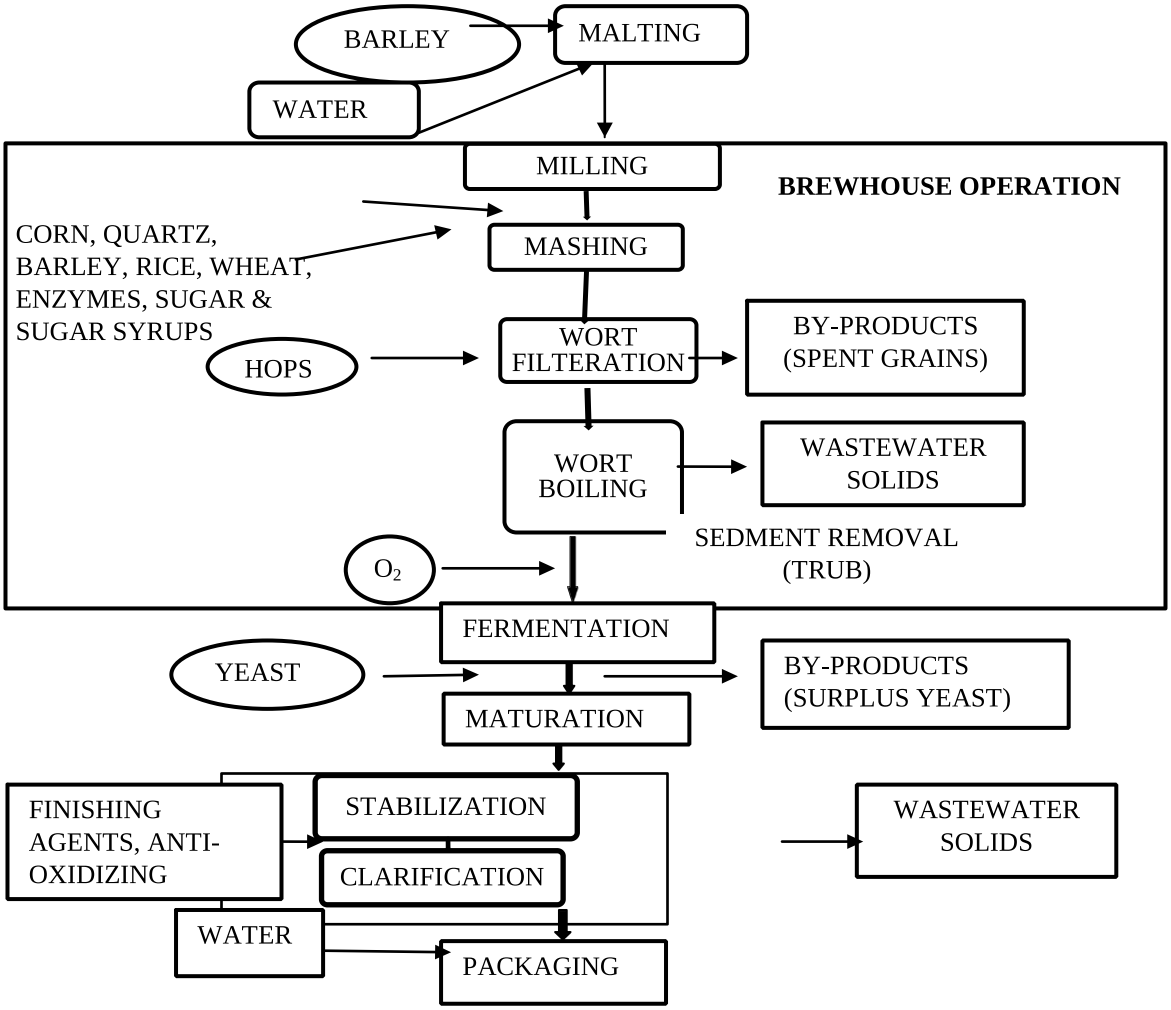

*Figure 2-1 Technological Process in Breweries and Main Waste Generated*

(EEPA, 2003)

#### 2.1.1.1 Wort Production Process

Malted barley is ground (either dry or wet) in a malt grinder so that the husk is left intact while the rest becomes very coarse powder, rich in starch and enzymes. The enzymes quickly degrade the starch to sugar on contact with water. The product, called sweet wort, is a mixture of partially degraded starch, sugars, enzymes, proteins and water (Binnie, 2010). When the mashing is completed the insoluble solids called the brewer's grains are separated from the wort by straining. The wort is boiled with hops and then finished wort is cooled to 9 °C depending on the yeast and the fermentation process chosen. The cooled wort is hereafter transferred to the fermentation area. The major wastewater sources from this section of a brewery include cleaning of the cereal for grinding, filtration of the mash, removal of trub, before and after brewing rinse of the mash tun, lauter tun and wort kettle as well as the water used for cooling purposes (IFC, 2007). The COD, BOD and nutrients contribution of these sources is high as it contains hop residues and some protein material from the malt (EPA, 2008).

#### 2.1.1.2 Fermentation Processes

Fermentation can be preceded by separation of the cold break consisting mainly of protein formed during cooling of the wort. This separation is performed by wort sedimentation in kettles, flotation, centrifugation or filtration. Yeast is added to the cold wort and aerated to support the development of the yeast to induce fermentation of sugar wort which is converted to $CO_2$, alcohol, heat and new yeast cells. When the fermentation process has reached completion, the yeast is drawn off and used for a new batch of wort with the excess being disposed of as a by-product. The surplus yeast can be resold as animal feed (Binnie, 2010). Following the primary fermentation, the produced beer (green beer) is transferred to storage or maturation vessels for a certain period of time before filtration.

#### 2.1.1.3 Filtration and Stabilizer of Beer

Following maturation, beer is filtered to remove remaining yeast to obtain bright beer which has the specified level of clarity and prolonged shelf life. Finings (fish collagens) are added after maturation to promote flocculation of any remaining yeast or proteins and the mixture is filtered through a filtration unit coated with filter slurry of Kieselguhr or filter aid. The result is a clear or bright beer and a spent filter slurry which is highly polluting and a particular problem for municipalities because it settles very easily and tends to block sewers and pipes. If high gravity brewing is practiced, it is usual to blend with sterile de-aerated water to normal gravity after fermentation. Other additions at this stage include stabilizers to promote longer shelf life and foam improvers to retain stable, white foam when the beer is poured (Binnie, 2010). Prior to filtration, the beer may be centrifuged, cooled to -1 °C to $-1.5^0$C to precipitate any suspended solids. The beer is then filtered in a Kieselguhr (diatomaceous earth) filter followed by a filter cloth. The $CO_2$

concentration in the beer is then adjusted and the beer is transferred to the bright beer tank for packaging. It is important that all process equipment and pipes are kept clean and disinfected. Cleanness is done by means of CIP plants where cleaning agents are circulated through the equipment or spread over the internal surface of the tanks (EPA, 2008). The spent filter aids are washed out of the filter with water during backwashing and discharged to the drains contributing to the suspended solids, nitrogen and phosphorous of the wastewater.

#### 2.1.1.4 Pasteurization and Packing Processes

From the bright beer tanks, the beer is pumped to the packaging area where it is bottled or canned. Bottling is usually preceded with bottle washing to remove any residual beer mold, labels, and dust particles. In addition to the packaging lines and reuse, bottles require systematic cleaning. Since the bottle washer consumes large quantities of energy, water and caustic, large quantities of wastewater are discharged. The use of nonreturnable packaging material reduces the consumption of energy, water and caustic, therefore reducing wastewater generation (EEPA, 2003).

## 2.2 Spent Yeast

During the fermentation process, yeast cells can multiply numerous times, which results in a markedly greater yeast mass than what is added at the commencement of fermentation. The fermentation conditions of each brewery influence the yeast growth rate. Huige, claims that the typical volume of spent yeast collected from a lager fermentation is approximately 0.6–0.8 lbm/bbl (pounds per barrel) or 1.71 kg/m$^3$ – 2.28 kg/m$^3$ of the final volume of beer produced (Huige, 2006).

Similar to spent grain, some breweries sell their spent yeast as animal feed as a source of protein and water-soluble vitamins (Djuragic, Levic, & Serdanovic, 2010). Various trials have been published which examine the use of brewers’ spent yeast as a feed supplement in diets for ruminants, horses, poultry, swine, and fish (Djuragic, Levic, & Serdanovic, 2010) (Mussatto, 2014) (Ferreira, Pinho, Vieira, & Tavarela, 2010). Spent yeast also takes up a significant position in human nutrition due to its high nutritional benefits. Well-known brands of yeast spreads are produced with spent brewers’ yeast in numerous countries, although these products use extracts of yeast as it is uncommon to use whole brewers’ spent yeast in human food applications. Yeast extracts are also used in food manufacturing to provide flavor (Munch, Hofmann, & Schieberle, 2005) (Sombutyanuchit, Suphantharika, & Verduyn, 2001). Furthermore, the use of spent yeast for their potential as a source of minerals and B-complex vitamins, and high quantities of essential amino acids might provide additional opportunities to the brewing industry in terms of sustainability (Vieira, et al., 2016).

The results showed that proteins (49.63 %), carbohydrates (31.55 %), minerals (7.98 %), ribonucleic acid (RNA) (8.12 %) and total lipids (4.64 %) predominate in the biomass composition. It was also observed that the yeast was an excellent source of some microelements, such as selenium, chromium, nickel, and lithium; that it is also a good source of dietary fiber, particularly soluble fibers; and that the content of lipids was low, with a predominance of saturated and mono-unsaturated fatty acids with 10, 16 and 18 carbon atoms (Sideney Becker Onofre, 2017).

*Table 1Average elemental compositions of yeast biomass and COD contribution of the constituents*

(Villadsen, 2011).

| Constituent | Elemental composition | Weight contribution % | Yield g-COD/g-yeast cell | COD contribution |
|---|---|---|---|---|
| Protein | $CH_{1.58}O_{0.31}N_{0.27}S_{0.004}$ | 57 | 0.763 | 55.8 % |
| RNA | $CH_{1.25}O_{0.75}N_{0.38}P_{0.11}$ | 16 | 0.167 | 12.2 % |
| DNA | $CH_{1.15}O_{0.62}N_{0.39}P_{0.10}$ | 3 | 0.032 | 2.4 % |
| Carbohydrates | $CH_{1.67}O_{0.83}$ | 10 | 0.143 | 10.5 % |
| Phospholipids | $CH_{1.91}O_{0.23}N_{0.02}P_{0.02}$ | 10.8 | 0.200 | 14.6 % |
| Neutral fat | $CH_{1.84}O_{0.12}$ | 2.5 | 0.052 | 3.8 % |
| Cellular metabolites | $CH_{1.8}O_{0.8}N_{0.2}S_{0.01}$ | 0.7 | 0.011 | 0.8 % |
| Yeast biomass | $CH_{1.596}O_{0.396}N_{0.216}S_{0.0024}P_{0.017}$ | 100 | 1.367 | 100 % |

Elemental composition and weight contribution data sourced from Villadsen et al (Villadsen, 2011). COD is calculated according to the theoretical oxygen requirement of a combustion reaction.

## 2.3 Brewery wastewater

Brewery wastewater, as with many foods and drink related wastewaters, can be treated very efficiently using high rate anaerobic biological processes such as Veolia Biobed® technology. The anaerobic process can typically remove up to 85 % of the total Chemical Oxygen Demand (COD) load from the wastewater and convert it into biogas, which can be used as a renewable energy source to replace fossil fuel in the brewery boilers, or as a fuel source to generate electricity in a CHP (Combined Heat and Power) plant. Brewery wastewater is a significant waste or negative product in a brewery process. Although, substantial technological improvements have been made by breweries in South Africa to reduce wastewater generation, yet an estimated 5 to 6 L of waste effluent is generated per liter of beer produced. Wastewater is mostly water by weight with other waste materials making up a small portion. At other times, large quantities of these other materials may be present that require some form of pre-treatment before discharging the wastewater into the sewage system. The brewing process usually generates large amounts of wastewater that need to be disposed of or treated in the least costly way to meet discharge regulations (Breggie V .P. and Michael B. F., 2012). Brewery wastewater has a high temperature in the range of 25 to 38°C. The high pH range of between 2 and 12 are influenced by the amount and type of chemicals used in the cleaning and sanitation processes, such as caustic soda, phosphoric acid, and nitric acid. Brewery solid waste includes spent grains, spent yeast, diatomaceous earth (DE) slurry and packaging materials (Driessen W, 2003).

Table 2-2 typical characteristics of brewery wastewater

(Driessen W, 2003)

| Parameter | Unit | Brewery wastewater | Typical brewery Benchmark |
|---|---|---|---|
| Flow | - | - | 2-8 hl effluent/hl beer |
| COD | Mg/l | 2000-6000 | 0.5-3 kg COD/hl beer |
| BOD | Mg/l | 1200-3600 | 0.2-2 kg BOD/hl beer |
| TSS | Mg/l | 200-1000 | 0.1-0.5 kg TSS/hl beer |
| T | $^0$C | 18-40 | |
| PH | - | 4.5-12 | |
| Nitrogen | Mg/l | 25-80 | |
| Phosphorous | Mg/l | 10-50 | |

The composition of brewing effluents can fluctuate significantly as it depends on various processes that take place within the brewery, but the amount of wastewater produced depends on water consumption during the process. In general, water consumption per volume of produced beer attain 4.7 m3 /m3 but it should be pointed that the wastewater to beer ratio is often 1.2 m3 /m3 to 2 m3

/ m3 less because part of the water is disposed of with by-products and lost by evaporation Organic components in brewery effluent are generally easily biodegradable and mainly consist of sugars, soluble starch, ethanol, volatile fatty acids, etc., leading to a Biological Oxygen Demand (BOD)/COD a ratio of 0.6 to 0.7. The effluent solids consist of spent grains, kieselguhr, waste yeast, and “hot” trub. The pH levels are determined by the amount and the type of chemicals used at the CIP (clean in place) units (e.g. caustic soda, phosphoric acid, nitric acid). Nitrogen b and phosphorous levels are mainly depending on the handling of raw material and the amount of spent yeast present in the effluent. High phosphorous levels can also result from the chemicals used in the CIP unit. Table 1 summarizes some of the most important environmental parameters (Brito A.G., 2007).

### 2.3.1 Environmental Impact of Brewery Wastewater

The primary environmental impacts that can be attributed to the production of beer are the result of noise, emissions to air, wastewater discharges and inefficient waste handling system (Programme, 2003).The discharge of nutrient rich wastewaters of domestic and industrial origins can have deleterious consequences on the ecological balance and functioning of the receiving environment as well as the public health of downstream end-users of the polluted water sources. Such devastating consequences manifest as: toxicity to fish and other aquatic organisms; depletion of the dissolved oxygen in receiving water bodies as ammonia or ammonium ions are oxygen consuming; eutrophication when nitrogen and phosphorus are made available to aquatic plants as nutrients; and potential public health risk (methemoglobinemia) in drinking water, especially when consumed by infants (Bitton, 2005). Nitrate and nitrite nitrogen constitute a public health concern, primarily related to methemoglobinemia and carcinogenesis. Ammonia nitrogen may deplete dissolved oxygen in natural waters by way of microbial nitrification reactions. Consequently, there is a direct need to control the pollution of surface and ground water since the public health and well-being of the people have a direct link with the availability of adequate quantity of good quality water (Binnie, 2010).

### 2.3.2 Brewery Wastewater Treatment Techniques

The principal objective of any wastewater treatment is generally to allow industrial effluents to be disposed of without danger to human health or unacceptable damage to the natural environment. From environmental, economic, political and social perspectives, wastewater treatment represents one of the most pressing issues facing industries today. Wastewater treatment technologies range from simple clarification in a settling pond to a complex system in advanced treatment requiring ample equipment and highly skilled operators (Huei, 2005).

Basically, wastewater treatment methods can be divided into three categories which are physical, chemical and biological wastewater treatment processes (Olajumoke, 2010).For almost all combinations of requirements in terms of effluent quality, land availability, construction and running costs, mechanization level and operational simplicity there will be one or more suitable treatment processes. The selections of specific unit processes depend not only on the nature of wastewater, including degradability and treatability by selected processes, but also on discharge requirements. Other important factors are environmental impact, land availability, projected life of plant design and cost (Eddy & Revised by Tchobanoglous, G. and Burton, F.L. Wa, 2004) (LemosChernicharo, 2006).

#### 2.3.2.1 Physical Wastewater Treatment Process

Physical treatment is for removing coarse solids and other large materials, rather than dissolved pollutants. It may be a passive process, such as sedimentation to allow suspended pollutants to settle out or float to the top naturally. The physical units most commonly used in wastewater treatment include screening, grit removal, mixing and flocculation, sedimentation, clarification, aeration, and volatilization and stripping of volatile organic compounds (VOCs). These processes are mostly used at Pre-treatment stage (Arcadio, 2002) (Olajire, 2012).

#### 2.3.2.2 Chemical Wastewater Treatment Process

Chemical treatment processes are the processes used for the treatment of wastewater in which change is brought about by means chemical reactions. The principal chemical processes used for wastewater treatment include chemical coagulation, precipitation, disinfection and oxidation, ion exchange and others. This type of treatment mainly relies on addition of chemicals and is applied when the wastewater cannot be treated biologically (Gillberg, 2003) (Angassa, 2011). A significant disadvantage of this treatment process is its additive. As a result, there is a net increase in the dissolved constituent and secondary pollutants in the wastewater (Huei, 2005). Besides that, another disadvantage of chemical treatment process is that the cost of most chemicals is related to the cost of energy (Gillberg, 2003).

#### 2.3.2.3 Biological Wastewater Treatment Process

Due to its simplicity and flexibility biological wastewater treatment is the most utilized and the best economical solution for treatment of biodegradable wastewater. Organic components in brewery effluent are generally easily biodegradable as these mainly consist of sugars, soluble starch, ethanol, volatile fatty acids (Driessen W., 2003). The effluent has a low content of non-biodegradable components. Brewery wastewater normally has a COD: BOD ratio of 1.5 to 1.7 indicating that the wastewater is easily degradable. The principal process used for the biological treatment of wastewater can be classified with respect to their metabolic function as aerobic and anaerobic (Huei, 2005).

Aerobic Waste Water Treatment Process**:** Organic material is converted into $CO_2$ and sludge (biomass) through aerobic treatment. The conversion is done with $O_2$ supplied to the reactor tank either mechanically or by diffusion from the atmosphere. For aerobic processes molecular oxygen ($O_2$) is the electron acceptor. Thus, it’s needed to foresee sufficient aeration in the bioreactor to obtain a controlled aerobic degradation of substrate. In an anoxic environment molecular oxygen is absent. However, oxygen is available in bound state, mostly as nitrate ($NO_3^-$). Certain genera (e.g. *Pseudomonas*, *Thiobacillus, Paracoccus,)* are able to use this nitrate as final electron acceptor. Nevertheless, they only do this when there is a lack of $O_2$. Only when there is absolutely no $O_2$ available they will adapt their respiration chain and will consume nitrate. The overall basic reactions during aerobic treatment can be summarized as: -

Organic matter + $O_2 \rightarrow CO_2 + H_2O$ + Aerobic Biomass Equation-A

Since it is an oxidative biological reaction, large amounts of biomass are produced which settle as sludge which requires further disposal. The aerobic treatment of brewery effluent requires a comparatively large energy input compared to anaerobic treatment (Bitton, 2005). In contrast to the anaerobic process, this process is quite fast which means that bacteria are able to make better use of the substrate and can create biomass more readily (Driessen W, 2003).

Anaerobic Wastewater Treatment Process**:** Anaerobic treatment of wastewater is a process that takes place in the absence of electron acceptor. The overall basic reactions during anaerobic system can be summarized as: -

Organic matter → $CH_4$ + $CO_2$ + Anaerobic Biomass Equation-B

This process is characterized by biological conversion of organic compound into biogas (methane 70-85 % and carbon dioxide 15-30 % with traces of hydrogen sulphide) (Huei, 2005). The interest in the use of anaerobic treatment processes can be addressed by considering the advantages and disadvantages of these processes. The principal advantages of the anaerobic treatment are the fact that the process is net energy producer instead of an energy user, its low biomass production, the low nutrient requirements and the high volumetric loadings possible.

The disadvantages of the anaerobic compared with aerobic processes are mainly operational considerations, such as long startup time, more sensitive to toxic substances than aerobic, the possible need to neutralize the acidity by adding alkaline; and that further treatment may be required, odor generation from $H_2S$ and other sulfur compounds, and it only reduces the organic pollution by 85-90 %, which means that a second step, usually anaerobic stage, is needed to guarantee high effluent quality (Tchobanoglous G., 2003).

Post Treatment**:** After the completion of the anaerobic digestion, the remaining biodegradable organic material, odour compounds mainly $H_2S$ from anaerobic effluent, is subjected further to post-treatment processes (Tchobanoglous G., 2003). This includes dewatering, aeration and leachate treatment. The importance of aeration process in post- treatment is to remove the left-over biodegradable organics by aerobically reducing to valuable material which is used as soil conditioner.

## 2.4 Anaerobic Digestion (AD)

Anaerobic digestion is a complex physico-chemical and biological process involving different factors and stages of change. In the context of energy recovery, anaerobic wastewater treatment systems are generally preferred because of their low energy consumption and low nutrient requirements, besides energy recovery in the form of methane gas ($CH_4$) has an added attraction. High strength wastewaters can be treated easily and profitably by anaerobic biological systems. Biogas is produced during anaerobic degradation of wastewaters. The average composition of biogas is 50–70 % $CH_4$, 30–40 % $CO_2$, 5–10 % $H_2$, 1–2 % $N_2$ and trace amounts of $H_2O$ and $H_2S$. Organic compounds are degraded by different classes of microorganisms such as (i) acidogens, which degrade organic compounds to a mixture of fatty acids and (ii) methanogens, which produce $CH_4$ from some of the products of acidogenesis such as acetic acid, hydrogen and carbon dioxide. However, in nature, it is very common to find the group of bacteria known as sulfate-reducing bacteria (SRB) along with the methanogens. These SRB reduce the oxidized forms of sulphur present in the waste and produce $H_2S$, which appears as a component of the biogas. This is undesirable for the efficient utilization of $CH_4$ because, during combustion, $H_2S$ is oxidized to $SO_2$ and $SO_3$. The gases $H_2S$, $SO_2$, and $SO_3$ create air pollution and $SO_3$ causes corrosion of chimney stacks and other equipment in treatment plants in different industries (Ahammad Skz, 2008).

Anaerobic digestion (AD) processes appear as a good alternative to add value to these by-products, recovering, in this way, energy in the form of biogas. Raw spent grain biomethane potential can range from 271 L kg−1 of volatile solids (VS) of substrate (Čater M, 2015) to 387 L kg−1 (VS of substrate) (Bochmann G, 2015), reaching 89 % of chemical oxygen demand (COD) removal efficiency (Gonçalves IC, 2015). Still, improvements can be achieved if some pre-treatments were applied, such as enzymatic hydrolysis (Wang H, 2015). Regarding trub, its treatment by AD processes used to involve dilution with brewery wastewater (Yu H, 2000); (Xiangwen S, 2008); (Gregor Drago Zupančič, 2017)or application as a co-substrate with brewery spend grain (Vitanza R, 2016).

### 2.4.1 Factors Affecting Anaerobic Process

Due to its low sludge production, anaerobic wastewater treatment has a lower capacity to adapt itself to changing circumstances and recover from toxic shocks compared to aerobic wastewater treatment processes. As anaerobic digestion is a biological process, it is strongly influenced by environmental factors such as temperature, pH, retention time, the chemical composition of wastewater, the competition of methanogens with sulfate-reducing bacteria, and the presence of toxicants (Bitton, 2005).

#### 2.4.1.1 Temperature

The anaerobic degradation process is strongly influenced by temperature and the microorganisms can be divided into the following classifications: psychrophilic (0-20 °C), mesophilic (20-42 °C), and thermophilic (42-75 °C) (Nyns, 2010). Generally, the rate of all reactions varies with temperature (Diaz, 2001). In biological systems, temperature increase is not as great as for chemical reactions. Methane production has been documented under a wide range of temperatures, but bacteria are most productive in either mesophilic conditions 25 - 40 ℃, or in the thermophilic conditions, at 50 – 65 ℃ (Ostrem, 2004) (Biey E. M., 2003). The hydrolysis and acidogenesis processes are not significantly affected by temperature, as among the mixed population there are always some bacteria that have their optimum within the temperature range in which the reactor is being operated. The acetogenesis and methanogenesis stages are carried out by fewer specialized species of microorganisms and are thus more likely to be more sensitive to temperature (Nyns, 2010).

#### 2.4.1.2 pH and Alkalinity

The first steps of anaerobic digestion can occur at a wide range of pH values, while methanogenesis only proceeds when the pH is neutral (Alemu, 2008). For pH values outside the range 6.5 - 7.5, the rate of methane production is lower (Mosey, 2002). A sufficient amount of hydrogen carbonate frequently denoted as bicarbonate alkalinity in the solution is important to maintain the optimal pH range required for methanogenesis. The pH determination is really useful and is important to relate its value to other process parameters: alkalinity, VFA concentration, and biogas production and composition (Lin, 2002). The amount of carbon dioxide and volatile fatty acids produced during the anaerobic process affects the pH of the digester contents.

#### 2.4.1.3 Volatile Fatty Acids

The concentration of VFAs is one of the most important parameters in the monitoring of the anaerobic digestion process. It is commonly agreed that VFA build-up is the result of unbalanced digestion conditions. The decrease in pH accompanying accumulation of VFAs is the main cause of toxicity and reactor failure in the anaerobic digestion process. This is because the toxicity of VFAs is pH dependent since only the non-ionized forms are toxic to microorganisms. VFAs are toxic at pH values where they exist in protonated forms, as they then can penetrate the cell membrane. When they are inside the cell, where the pH is around 7, they are ionized and the hydrogen ion released will cause a decrease in the intracellular pH (Björnsson, 2000).

#### 2.4.1.4 Toxic Substances

Under certain circumstances removal efficiencies in an activated sludge system can suddenly decrease sharply or stop completely. Toxic compounds in the influent could be responsible for this phenomenon. Methanogens are considered the most sensitive to toxicity in anaerobic digestion.

Examples of inhibition are: toxicity by heavy metals, poisoning by particular organic compounds such as phenols, cationic detergents, antibiotics and presence of strong oxidizers. In the case of oxygen, the facultative bacteria present in the granules can use this element before it can affect the methanogens (Diaz, 2001).

However, during a hydraulic shock load, the amount of oxygen can exceed the capacity of the facultative bacteria present in the granule or floc, causing inhibition of methanogenesis (Renato Carrha´ Leit, 2005). Products in the chain of simultaneous biochemical reactions, such as $NH_3$, $H_2S$, and VFA are pH dependent since only the non-ionized forms exhibit Microbial toxicity (Wilson, 2004). Toxicity of heavy metals is determined by their concentration, oxidation state, the environmental pH and redox potential, type of microbial system that is poisoned and the adaptation potential of the bacteria (Werner Kossmann, 2000).

### 2.4.1.5 Nutrients

Organic and inorganic substances are necessary for anaerobic digestion process. Very low metal concentrations may make the anaerobic degradation process inefficient (Osuna, 2003); but, above a certain threshold level, it can be inhibitory. As a rule of thumb, a COD/**N** ratio of 400/5 is taken an amount of nitrogen that is necessary for a healthy and stable anaerobic digestion (Mata-Alvarez J. , 2003). For wastewaters containing a lot of N, it is necessary to control the pH very well in the reactor to prevent $NH_3$-inhibition. A COD/P ratio of 400/1 is considered a minimum for the phosphorus need of anaerobic treatment.  At high concentrations, it is possible that undesired precipitations like $Ca_3(PO_4)_2$ or $NH_4MgPO_4$ occur. When the amounts of precipitation are larger than the production of anaerobic sludge, it has problem with the sludge composition. Heavy metals have also been reported to inhibit the degradation of VFAs to methane in anaerobic digestion (Björnsson, 2000).

### 2.4.1.6 Retention Time

The amount of time that wastewater stays in the digester is known as retention time or residence time; the longer a substrate is kept under proper reaction conditions, the more complete its degradation will be. The rate of the reaction, however, will decrease with increasing residence time, indicating that there is an optimal time that will achieve the benefits of digestion in a cost-effective way. The appropriate time depends on the feedstock, environmental conditions and intended use of the digestion (Mahony, 2002). Recent research has shown that volatile suspended solids in a digester could be reduced by 64- 85 % after only 10 hours, but retention times of 10 days were typical for complete digestion (Lin, 2002).

#### 2.4.1.7 Organic Loading Rate (OLR)

Every wastewater treatment plant is developed to be able to treat a certain amount of organic material or design load. This amount is most of the time expressed as kg BOD or COD per day. If there is an excess of biodegradable matter fed to the digester, this can lead to the overproduction of VFAs then a drop in pH and a reduction in methane production will occur. Underfeeding the reactor could also lead to a reduction in the digester performance due to insufficient nutrients for microbial growth (Etawah, 2006).

A higher OLR will demand more of the bacteria, which may cause the system to crash if it is not prepared. One danger of increasing the OLR would be that the acidogenic bacteria, which act early in the digestion process and reproduce quickly given enough substrate, would multiply and produce acids rapidly (Etawah, 2006). The methanogenic bacteria, which take longer to increase their populations, would not be able to consume the acids at the same pace. The pH of the system would then fall, killing most of the methanogenic bacteria and leading to a positive feedback loop, eventually halting digestion. An early indication of this is lowered biogas production and eventually a lower pH (Cloonan, 2004).

#### 2.4.1.8 Bacterial Consortia

Anaerobic digestion is carried out by a group or consortia of bacteria, working together to convert organic matter to gas and inorganic constituents. A wide variety of physical, chemical, and biological reactions take place. The bacterial consortia catalyze these reactions. Consequently, the most important factor in converting waste to gas is bacterial consortia. The bacterial consortia are essentially the bio-enzymes that accomplish the desired treatment. A poorly developed or stressed bacterial consortium will not provide the desired conversion of waste to gas and other benefits (Dennis A, 2001).

## 2.5 Brewery Waste management

In the brewery industry, brewing and packaging are the operations that give rise to waste. Water and wastewater management constitutes a practical problem for the food and beverage industry including the brewing industry. Wastewater management and waste disposal have become a significant cost factor and an important aspect in the running of brewery operation. Brewery managers need to view wastewater and waste disposal problems as proof of inefficiencies in their production process rather than as inevitable by-products of production. As brewery managers strive to improve on their environmental performance due to pressures from society, traditional pollution prevention techniques seem no longer to be cost-effective. It appears that process waste-reduction is a better cost-effective solution to traditional end-of-pipe strategies. Brewery managers and accountants should endeavor to bridge communication problems in order to have access to environmental information especially on the quantities and values of wasted products at all times (Breggie V .P. and Michael B. F., 2012).

## 2.6 Brewery Sludge

Sludge is a major by-product of almost all industrial effluents. Yale defined sludge as solids, semi-solid, or liquid waste derived from municipal, commercial, or industrial treatment facilities, wastewater treatment plants, and air pollution control. The US Environmental Protection Agency (EPA) defines sludge as the semi-liquid residue or slurry remaining from the treatment of industrial water and wastewater. Spinosa also defined sludge as an accumulation of solids removed from sewage during waste treatment. Industrial wastewater solids are also referred to as sludge, whether generated from biological or physical-chemical processes (Metcalf & Eddy, 1981). The sludge that has been treated could be used or disposed of in an environmentally acceptable manner. Direct discharge into water bodies, landfill, land reclamation, composting, soil conditioning, irrigation (effluent recovered from sludge), brick making, etc. are possible disposal methods and reuse options. Land application and use of sludge as a soil conditioner appear to be the most acceptable alternatives. Sludge, such as organic manure, should be regarded as a valuable commodity. For sludge to serve its useful purposes it must be treated and the treatment options used are dependent to a large extent on the results obtained from its characterization and the end-use of the sludge.

Brewery sludge has COD of 2072 mg/l, the turbidity of 1034 NTU and BOD of 640mg/l. The Total Solids (TS) and Suspended Solids (SS) of brewery sludge are 7307.50 mg/l and 2067.50 mg/l, respectively.

*Table 2-3 Characteristics of Sludge*

(Osaro K. Ize-Iyamu, Osayanmo Eguavoen, & Micheal Osuide, 2011)

| Parameters | Mean ± S.D(standard deviation) (From Triplicate Determinations) |
|---|---|
| pH | 6.7 ± 0.10 |
| Turbidity NTU | 1034.00 ± 4.10 |
| Suspended Solids (SS) mg/l | 2067.50 ± 5.20 |
| Volatile Solids (VS) mg/l | 5242.00 ± 5.45 |
| Total Solids (TS) mg/l | 7307.50 ± 7.60 |
| DO mg/l | 2.65 ± 0.04 |
| $BOD_5$ mg/l | 640.00 ± 6.00 |
| COD mg/l | 2072.19 ± 6.55 |
| $HCO_3$ mg/l | 47.00 ± 2.00 |
| Ca mg/l | 26.85 ± 1.25 |
| Mg mg/l | 27.80 ± 1.20 |
| K mg/l | 12.80 ± 0.55 |
| $PO_4$ mg/g | 14.05 ± 0.48 |
| $NH_3$-N mg/l | 56.00 ± 2.78 |
| $NO_2$-N mg/l | 12.60 ± 0.60 |
| $NO_3$-N mg/l | 15.50 ± 0.42 |
| Fe mg/l | 0.91 ± 0.02 |
| Zn mg/l | 3.20 ± 1.00 |
| Cr mg/l | ND |
| Pb mg/l | 0.29 ± 0.01 |
| Elect. Conductivity µs/cm | 154.00 ± 3.20 |
| Temperature ℃ | 30.5 ± 0.20 |
| Total Coliform Count TCC | $3.90 \times 10^8 \pm 60.00$ |
| Sludge Volume Index SVI mL/g | 69.50 ± 2.40 |

## 2.7 Environmental, Economic and Social Benefits of Biogas

### 2.7.1 Energy and climate concerns

Biogas is a renewable source of energy. The carbon dioxide that is released when biogas is combusted and mixed with the oxygen in the air does not contribute to the greenhouse effect. The carbon in the methane molecule produced by the biogas process originates from carbon dioxide in the air that growing plants have previously taken up by photosynthesis. The use of biogas is thus an important step in climate change mitigation. The development of biogas represents a strategically important step away from oil dependency that will contribute to a sustainable energy supply in the long term. Renewable means that there will be no “peak biogas”; rather biogas will be continuously available and thus offers improved energy security. Biogas is also produced locally meaning that it is not dependent on trade relationships. This also contributes to improved energy security (Lars, 2012).

### 2.7.2 Increase in agricultural productivity

Anaerobic digestion increases nitrogen fixation. The digestate is used as fertilizer. Anaerobic digestion kills certain bacteria, parasites and weed seeds that otherwise might have had negative effects on crop production. Organic bio-gas production helps to ensure food security (Florian, 2013). In Ethiopia, it leads to a reduction in agricultural productivity as a result of using dung and crop residue as fuel instead of using these as soil nutrients. Due to the use of dung as a source of domestic energy it is estimated that 10 % of the annual grain production is lost for the Tigray region. Through the bio-gas Program the utilization of slurry is promoted, thus contributing to increased crop production (National Biogas Programme Ethiopia, 2008).

### 2.7.3 Sustainable development

Production of bio-gas offers many benefits to society and is an important contribution to a sustainable development. One of the most important tasks we face today is to reduce our exploitation of the earth's finite resources and to develop systems for re-cycling of nutrients and energy that are sustainable in the long term. In the bio-gas process, waste is converted into energy and nutrients and hence, the exploitation of finite resources is reduced. The biogas process has many advantages from the point of view of the environment, especially since it results in two environmentally friendly final products: biogas and bio fertilizer (Lars, 2012).

### 2.7.4 Carbon revenues

A biogas installation results in greenhouse gas (GHG) abatement. This abatement is denoted as "carbon offsets" and has a value under the clean development mechanism (CDM) or the voluntary carbon market. These offsets can be sold as carbon credits and utilized for policies to stimulate biodigester adoption by for instance, providing subsidies or soft loans. Consequently, these carbon revenues can cover a part of the required capital investments to tackle the impact of the low ambient temperature on biogas production (Buysman, 2009). All the CDM certified and biogas projects under validation are studied to determine the claimed carbon reduction per digester to estimate carbon income.

### 2.7.5 Biogas and recycling of nutrients

When biogas is produced from organic waste, manure or food waste, the residue, digestate, contains all the nutrients in the original substrate. These nutrients are retained in soluble and plant-available forms in the residue, and cannot be lost by leaching, since the digestion takes place in closed containers. Using the digestion residue as a bio fertilizer reduces the need for mineral fertilizers. The return of the bio fertilizer to arable land constitutes an excellent case of recycling of a natural resource.

### 2.7.6 Biogas as a bio-fuel

Biogas is a high quality bio-fuel. As any fuel it can be used to produce electricity and heat, or both in CHP equipment. As it consists of methane it is easily adaptable to existing processes where natural gas, also methane, is used. Methane is a fuel in demand by industry, partly because it is a gas, which gives a high-quality combustion that can be precisely controlled. Methane burns with a clean and pure flame, which means that boilers and other equipment are not clogged by soot and cinders. This leads to a cleaner workplace environment and less wear and tear on the plant. Biogas is the most environmentally friendly vehicle fuel on the market today (Lars, 2012). Biogas gives the smallest emissions of carbon dioxide and particulate matter of all vehicle fuels on the market. Emissions of carbon monoxide, hydrocarbons, Sulphur compounds and nitrogen oxides are less than when petrol or diesel is used as fuel. A gas engine is quieter and vibrates less than a diesel engine, which means a better working environment for professional drivers. Biogas is lighter than air. If a leakage occurs, methane rises through the surrounding air. Biogas has a higher temperature of ignition than petrol and diesel, which reduces the risk of fires and explosions at accidents (Lars, 2012).

### 2.7.7 Decrease eutrophication

If manure is just unloaded in the environment, it will leak and be carried by water to the nearest water course. Leaking manure is a main cause of Eutrophication of surface waters in the region. Besides from this, anaerobic digestion also to a great extent reduces the pathogenic contents of the manure. Also, the process greatly reduces the smell of the manure (Lars, 2012).

### 2.7.8 Used as waste management option

Biogas is also produced with organic waste as substrate. This is a great advantage in waste management; the waste does not need to be land filled or just incinerated for recovery of its heat content. When fermenting organic waste, the two important resources are recovered, the biogas and the nutrients in the residue (Lars, 2012).

### 2.7.9 Woman empowerment and health

Biogas is widely accepted in Ethiopia as a cooking fuel and will mainly benefit women and children (National Biogas Programme Ethiopia, 2008). Cooking on biogas has also a significant health advantage over traditional cooking with an open fire. The major point is the fact that cooking is smokeless and that will diminish the number of eye infections and respiratory problems among in particular women usually in charge of cooking and small children being near their mothers. Moreover, in rural area collecting fire wood takes time. Always this activity is done by women. It is expected that biogas will reduce the overall workload of women by providing the daily energy demand and increase women empowerment (National Biogas Programme Ethiopia, 2008). Also, the danger that children burn themselves while cooking is less when using a biogas stove (Jan, 2010).

### 2.7.10 Reduce deforestation

The energy saving aspect and thus saving on cost for firewood is from the point of view of the farmer household an important aspect. Moreover, it is one of the major considerations of a government to promote this technology because it reduces the burden on the environment. It saves trees and helps thereby to combat erosion and to store carbon (Jan, 2010). In Ethiopia, more than 90 % of the energy demand of the country provided by biomass, a dire energy situation exists due to a high rate of depletion of the country’s forest cover (National Biogas Programme Ethiopia, 2008).

### 2.7.11 Reduce greenhouse gas (GHG)

The conversion of animal wastes and manure to methane/biogas can yield significant health and environmental benefits. Methane is a GHG that has 21 times more global warming potential than carbon dioxide in trapping heat in the atmosphere. By trapping and utilizing the methane, GHG impacts are avoided (Serjio, 2010).

# 3. Methodology

## 3.1 Materials

One-liter (1 L) plastic polyethylene (PET) bottles were used for anaerobic digestion process which was placed inside a temperature-controlled water bath. The bottles were filled with 400 mL of sludge from anaerobic brewery wastewater treatment plant and 400 mL mixture of wastewater and spent yeast which was mixed at different percentages. 1000 mL syringe was used to measure the volume of gas accumulated at the gas storage attached at the end of plastic bottles which is 1.420L urine bag. The percentage of methane, carbon dioxide, oxygen, and hydrogen sulfide were analyzed using GeoTech BIOGAS 5000 gas analyzer.

The raw input materials were analyzed for TS, TN, TK, TP, chemical oxygen demand (COD), biological oxygen demand (BOD) and turbidity using equipment and apparatus such as rubber hose, incubator, drying oven, furnace, Spectrophotometer, test tube, measuring cylinder and weighing scale throughout the experiment. The most important equipment and apparatus used in the analysis procedure are described in the annex.

## 3.2 Collection of Wastewater, anaerobic sludge, and spent yeast

The wastewater used in the experiments was taken directly from the brewery sludge effluent storage of St. Georg brewery (BGI). The typical mass concentration of total solids and volatile solids, chemical oxygen demand, biochemical oxygen demand was determined according to international standards (American Public Health Association (APHA), 2005). The anaerobic degradability of the collected wastewater was investigated in all experiments and the result was recorded from the Gas Analyzer.

The excess yeast was collected directly from the yeast collection tank of the lager beer production line of St. Georg brewery. The typical, mass concentrations of TS, VS, and COD in yeast was measured. The yeast and wastewater were mixed in a prepared cylindrical tank prior to the filling to the reactor.

Anaerobic sludge was collected directly from anaerobic wastewater treatment plant of St. George brewery. The sludge was taken from bottom openings of the reactor. It was better to test from all positions of the reactor but the reactor has only one opening. The typical mass concentration of total solids and volatile solids, chemical oxygen demand, biochemical oxygen demand was determined according to international standards. Also, the total solids, chemical oxygen demand, the biological oxygen demand of the above three wastes were determined.

## 3.3 Experimental design

The laboratory experiment was used based on the factorial design of three treatment factors (i.e. volume ratio, temperature, and pH) having three levels each were analyzed for the different combinations of their test levels. Using General Factorial method, the optimum combination of the operational factors was determined.

Randomization of the experimental runs as well as appropriate analysis technique was ensured through proper software (Design expert 11). The factors and levels are shown in table 3-1 below.

*Table 3-1: Factors and Levels*

| Numerical factors | | |
|---|---|---|
| Volume ratio (%) | Temperature ($^0$C) | pH |
| 1, 6, 11 | 35, 37, 39 | 5, 6.5, 8 |

The above design factors were selected in a rational basis, that is the volume ratios 1, 6, 11 were selected based on the brewery effluent composition ratio of wastewater to spent yeast and to use the already designed anaerobic wastewater treatment plant. The ratio of wastewater to spent yeast at the brewery effluent ranges from 99% to 75% in volume. The temperature and pH were selected based the characteristics of the consortia of bacteria present on the digestion process. As obtained from different literatures the pH and temperature range at which those bacteria are more fertile (Nyns, 2010) (Ostrem, 2004) (Diaz, 2001) (Alemu, 2008) (Mosey, 2002) (Lin, 2002) (Biey E. M., Start-up of the multi stage system for biogas production and solid waste treatment in low-tech countries, vol. 48, 2003). The anaerobic bacteria are active at those range of temperatures and pH, as their metabolic and reproduction activities are highly pronounced. This long range of temperature and pH allows the survival of diverse types of anaerobic bacteria.

## 3.4 Operation

A series of 27 experiments were performed at different combination of temperature, yeast to wastewater ratio, and pH and results were recorded at different period of time. The experiments were carried out using the constant volume of brewery sludge and various yeast and wastewater mixture ratios. The used volume are as follows, sludge 400 mL which is the same for all the experiments, and 400 mL of a mixture of wastewater and yeast. In the experiment, a mixture of 1% (v/v) of yeast in the wastewater was used which is 400 mL×0.01=4 mL of yeast, and 400-4 mL= 396 mL of wastewater. The same calculation was performed to 0, 6, 11, 100 % (percentage of yeast in a mixture of wastewater and yeast by volume) in all experiments. After four days of operation, the produced gas volumes were measured and analyzed for the percentage of methane production. Again after 14 days and 21 days, the same measurement was held. Both the volume and percentage of the produced gas were recorded. In all experiments, the raw inputs were characterized in component-wise and mixture-wise (i.e. sludge inoculum, spent yeast, wastewater and the mixture of them).

## 3.5 Analytical methods

The mass concentrations of total solids (TS), volatile solids (VS), total nitrogen (TN), COD, and BOD were analyzed according to American Public Health Association Standard methods (American Public Health Association (APHA), 2005). The mass concentrations of COD and BOD were monitored and analyzed during all cycles conducted. The VFA content was determined in all cycles, and mass concentrations of TKN and $NH_4$-N were measured at the end of each experiment. The COD was determined in accordance with the procedure of ISO 6060:1989 (Standardization & Switzerland:, 1989).

### 3.5.1 Laboratory analysis

#### 3.5.1.1 Total solids, Total dissolved solids (TDS), Total suspended solids

Total solids were determined by gravimetric method and then suspended solids were calculated by using the equation below: (American Public Health Association (APHA), 2005)

$$TS = TSS + TDS \qquad \text{Equation -C}$$

#### 3.5.1.2 Biochemical Oxygen Demand (BOD)

BOD was estimated by preparing the required volume of dilution water with the addition of nutrients namely phosphate buffer, magnesium sulfate, calcium chloride, and ferric chloride. The diluted samples were transferred to BOD bottles. After determining initial DO, final DO was estimated of the bottles kept for the incubation period of five days. The bottles were kept for DO determination and the blank was fixed by adding 2 mL manganese sulfate ($MnSO_4$), 2 mL of alkali iodide azide ($NaOH + KI + NaN_3$) (American Public Health Association (APHA), 2005).

#### 3.5.1.3 Chemical Oxygen Demand (COD)

COD determination was carried out with dichromatic reflux method with the addition of 10 mL of 0.25 N potassium dichromate ($K_2Cr_2O_7$) and 30 mL $H_2SO_4 + Ag_2SO_4$ reagent in 20 mL diluted sample. The mixture was refluxed for 2 hours and was cooled to room temperature. Then the solution was diluted to 150 mL by using distilled water and excess $K_2Cr_2O_7$ remained was titrated with ferrous ammonium sulfate (FAS) using ferroin indicator. The COD values were determined using: (American Public Health Association (APHA), 2005)

$$\mathrm{COD} = ((A - B) \times N \times 8 \times 1000\ \mathrm{mL/L})/V \qquad \text{Equation-D}$$

Where A is the mL of FAS used for blank; B is the mL of FAS used for the sample, V is volume of the sample N is the normality of FAS and 8 is milliequivalent weight of oxygen.

#### 3.5.1.4 Total Nitrogen

The Nitrogen levels were determined using the Kjeldahl method, developed by Johan Kjeldahl,

where the sample was weighed, digested, neutralized and the nitrogen estimated by titration (American Public Health Association (APHA), 2005).

#### 3.5.1.5 Phosphorous

The phosphorus levels were determined using UV spectrophotometer. Prior to measurement, the samples were digested in 1:1 HCl on a sand bath. After digestion, distilled water was used to take to mark and a complexing reagent (molybdate) added and color change observed. The sample was then read on the instrument (American Public Health Association (APHA), 2005).

## 3.6 Production Process

In this specific study the biogas production process followed the following steps:

Raw material (sludge, wastewater, spent yeast) were collected from St. George beer factory and brought to Addis Ababa university, Environmental engineering laboratory. The physico-chemical characteristics of the raw materials were determined after that the desired proportion of the collected wastes were prepared for anaerobic digestion. The anaerobic digestion was held in one litter polyethylene plastic bottles which were put inside a temperature-controlled water bath. The pH of the samples was adjusted once before anaerobic digestion and pH of the effluent was measured. After incubation for 4 days, the amount of biogas volume produced was measured by 1000 mL syringe. The percentage of carbon dioxide, methane, hydrogen sulfide and oxygen were measured using gas analyzer GeoTech 5000. This measurement was continued until the volume of obtained gas approached zero milliliter. The time of measurement was at 4th, 14th, and twenty first day of the digestion process. At the end of the digestion process the effluent was characterized for it's potential as fertilizer, and other parameters were measured.

# 4. Results and Discussion

## 4.1 Characterization of the raw materials

### 4.1.1 Characterization of spent yeast

The physical and chemical characteristics of brewery yeast waste used in the study were determined and the observed results are displayed in Table 4-1. The total solid of the brewery yeast waste was 124,445 ± 2,061.8 mg/L.

*Table 4-1 Characteristics of spent yeast*

| Parameters | Unit | Yeast waste (St. George beer) |
|---|---|---|
| pH | | 5.65 ± 0.2 |
| TS | mg/L | 124445 ± 2061.8 |
| COD | mg/L | 182000 ± 707 |
| $BOD_5$ | mg/L | 120500 ± 1118 |
| TN | mg/L | 41.85 ± 7.85 |
| TP | mg/L | 65.15 ± 9.05 |

The brewer's spent yeast is a concentrated suspension of yeast cells in beer. The cell or solids concentration of the suspension differs according to the beer recovery process after fermentation. In this study, the total COD of spent yeast used ranged between 181293 and 182707 mg/L with an average (of 27 samples) of 182000 ± 707 mL/L. However, Neira & Jeison measured average TCOD concentration of 201,180 ± 842 mg/L with soluble COD concentration of 90 g-COD/L. The disparities in the concentrations presented above are mainly a result of varying solids (cell) concentrations in the spent yeast suspensions, which is influenced by the beer recovery strategy of the specific brewery.

Protein is the most abundant constituent of yeast cells (Villadsen, 2011). As such, the degradation of protein is important in achieving high degradation efficiencies. Carbohydrates and phospholipids make up the majority of the COD not contributed by protein. Together, protein, carbohydrates, and phospholipids make up about 80 % of the cell mass. The value recorded in table 4-1 above shows that the spent yeast has high percentage of organic content to be used a source of food for anaerobic bacteria hence production of biogas.

Zupančič et al. (2012) reported spent yeast from Brewery Laško having an average total solids concentration of 188 gTS/L of which 95 % were volatile solids. The average measured total COD concentration of the spent yeast was 200000 ± 642 mg/L (Zupančič et al. 2012). The spent yeast liquor is essentially unrecovered beer, which is made up of a variety of readily digestible volatile compounds. In addition to various forms of carbohydrates and alcohol, low amounts of other compounds such as lactic and pyruvic acids, adenosine, uridine, tyrosine have been found in beer (Almeida, 2006).

### 4.1.2 Characterization of Brewery sludge

The raw sludge from anaerobic wastewater treatment was investigated for chemical oxygen demand COD, biological oxygen demand BOD, total solids TS, total nitrogen TN, total phosphorus TP, PH, and the following results were recorded as shown in table 4-3 before digestion. For the sake of comparison, literature results are tabulated alongside the results of this study. Skimming all along the table, the results fairly agree with results from literature. The most important part here is the content of the total solids (TS) concentrations, the total phosphorus (TP), the total nitrogen (TN), biological oxygen demand (BOD) and the chemical oxygen demand (COD) from which Biogas specifically Methane is going to be generated. One can, therefore, conclude that those findings are good enough to make conclusion about the nature of the brewery sludge.

Table 4-2 Characteristics of brewery sludge

| Parameters | Obtained parametric value (mg/l) | Findings from literature | | | |
|---|---|---|---|---|---|
| | | (Enitan, 2015) | (Helmers, 2014) | (Driessen W., 2003) | (Mataa, 2012) |
| Total organic content (mg/l) | 1157.62 ± 150 | 1137.33 ± 1074.34 | n.d | n.d | n.d |
| Chemical oxygen demand (mg/l) | 3190.97 ± 200 | 3340.97 ± 2265 | n.d | 200-6000 | 635±412 |

| | | | | | |
|---|---|---|---|---|---|
| Biological oxygen demand(mg/l) | 240.15 ± 130 | 215.27 ± 870.92 | 217.0(ppm) | 200-3600 | n.d |
| Total nitrogen(mg/l) | 68.44 ± 12 | n.d | 29(ppm) | 25-80 | 54±7 |
| Total phosphorus(mg/l) | 42.89 ± 7 | n.d | 7(ppm) | 10-50 | n.d |

As it is shown from the above table 4-2 the result obtained from the measurement of the characteristics of anaerobic sludge is comparable and is within the range of different reported research studies. Those compositions are sourced basically from the raw inputs of the brewing process, like grain, hops, yeast, and other organic and inorganic additives. The sludge contributes in the digestion a lot in term of COD, BOD, TS, TP, so this contribution should be considered in the calculation of overall composition of the co-digestion process. The value recorded in table 4-2 above shows that the brewery sludge has considerable percentage of organic content to be used a source of food for anaerobic bacteria hence production of biogas.

### 4.1.3 Characterization of Brewery Wastewater

The raw brewery wastewater was investigated for COD, BOD, TS, TN, TP, PH, and the following results were recorded.

*Table 4-3 characteristics of brewery wastewater*

| Parameter | Unit | Brewery effluent composition |
|---|---|---|
| Chemical oxygen demand (COD) | mg/L | 2480±395 |
| Biochemical oxygen demand (BOD) | mg/L | 1413 ± 218 |
| Total suspended solids (TSS) | mg/L | 40 ± 6.5 |
| Temperature | $^0$C | 35.62 ± 2 |
| pH | | 8.7 ± 1.5 |

| | | |
|---|---|---|
| Total nitrogen | mg/L | 37.5 ± 8 |
| Total phosphorus | mg/L | 55.1 ± 22 |

Brewery effluent wastewater had COD value ranging from 2085 mg/L to 2875 mg/L with the average value of 2480±395 mg/L. This COD value was within the range reported by (Driessen W., 2003) (2000 mg/l to 6000 mg/L). The obtained $BOD_5$ value ranged from 1195 mg/L to 1631 mg/L with the average value 1413±218 mg/L) and it was found that 1200 mg/L to 3,600 mg/L was reported by the same authors. The high value of standard deviation showed that the variability of brewery wastewater composition from batch to batch production. Because the brewery production activities are varied by season, by day of the week and by the time of day, wastewaters are extremely variable (EPA, 2008). According to the environmental protection agency (EPA, 2008), the high organic loads in the wastewater arise from dissolved carbohydrates, the alcohol from beer wastes, and a high content of suspended solids, such as spent grain, malt, and yeast. It is also explained in many reports that the raw materials like malt and adjuncts, and the discharge of trub, weak wort, surplus yeast, emptying and rinsing of process tanks, pre and after- runs of Kieselguhr filtration and drip beer could possibly be the sources of high COD and $BOD_5$ in the wastewater.

The composition of brewing effluents can fluctuate significantly as it depends on various processes that take place in the brewing. Organic components in brewery effluent which have $BOD_5$/COD ratio of 0.6 to 0.7 are generally easily biodegradable (EPA, 2008). The results indicated that the $BOD_5$ /COD ratio was 0.6, which indicates easily biodegradability of organic matter.

The brewery wastewater was also characterized for its nutrients (N and P) content. In this study, the results show that the brewery effluent had TN and TP value ranged from 29.5 mg/L to 45.5 mg/L, 33.1 mg/L to 77.1 mg/L, with average values of 37.5 ± 8 mg/L and 55.1± 22 mg/L (Table 4-3). The high P levels obtained in the study could be attributed to the use of phosphorous-containing chemicals mainly composed of phosphoric acid used in the CIP (Cleaning In Place) units and the variation in the concentration resulted from the variation in the amount of cleaning agents (EPA, 2008) (Driessen W., 2003).

The raw materials malt and adjuncts, nitric acid used for cleaning and the amount of spent yeast present in the effluent could be the sources of nitrogen in the wastewater (Driessen W., 2003) (EPA, 2008). The sources of the nitrogen forms could be malt processing followed by protein hydrolysis for $NH_4$-N and $NO_3$-N. Also, it might have come from the dissociation of the nitric acid used in the CIP units as well as ammonification and nitrification of the ammonium nitrogen in the wastewater during the periods of sample collection for analysis.

Brewing activities like malt processing, filtration and packaging could be the cause for the high TSS values, which indicated that brewery solids mainly of spent grains, Kieselguhr, surplus yeast, cold break and possible label pulp from the bottle washer (Driessen W., 2003) (EPA, 2008). The TSS analysis of the wastewater gave values ranging from 284 mg/L to 510 mg/L (Table 4-3). This TSS value was found within the range reported by (Driessen W., 2003).

The wastewater analysis also showed pH values ranging from 7.2 to 10.2 pH units which were within the wide range (2-12) pH values given by different reports like (Driessen W., 2003) for the discharges from different sections of a brewery. The variation in the wastewater's pH could be attributed to the batch processing nature of the brewery and the amount and type of chemicals (e.g. caustic soda, phosphoric acid, nitric acid, etc.) used at the CIP units.

### 4.1.4 Characterization of the Mixture before digestion

*Table 4-4 Characterization of the Mixture before digestion*

| parameter | Volume Ratio, i.e percentage of yeast volume in the mixture(yeast+wastewater) | | | | |
|---|---|---|---|---|---|
| | 0 % | 1 % | 6 % | 11 % | 100 % |
| BOD | 476.3 ± 17 | 514 ± 21 | 1502 ± 75 | 2489 ± 45 | 30085.5 ± 67 |
| COD | 1400 ± 23 | 1232.7 ± 60 | 2729 ± 102 | 4226 ± 35 | 50430 ± 105 |
| TS | 526.75 ± 61 | 558.5 ± 54 | 1595.2 ± 112 | 2631.8 ± 97.2 | 31626.75 ± 122.4 |
| pH | 7.7 ± 1.2 | 7.65 ± 1.3 | 7.4 ± 1.25 | 7.25 ± 1.05 | 6.2 ± 1.31 |
| Temperature | 32.75 ± 2 | 31 ± 1.5 | 30.5 ± 1.5 | 30.36 ± 1.4 | 27.75 ± 1.7 |

### 4.1.5 Characterization of the Mixture after digestion

*Table 4-5 Characterization of the Mixture after digestion*

| Parameter | Volume Ratio, i.e percentage of yeast volume in the mixture(yeast+wastewater) | | | | |
|---|---|---|---|---|---|
| | 0 % | 1 % | 6 % | 11 % | 100 % |
| BOD | 37.8 ± 11 | 55.7 + 6.2 | 87.38 ± 13 | 173.8 ± 17 | 902.6 ± 135 |

| | | | | | |
|---|---|---|---|---|---|
| COD | 227 ± 9.5 | 236 ± 13 | 275 ± 6.2 | 297 ± 8.5 | 1020 ± 26 |
| TS | 395 ± 55 | 413 ± 50 | 1236 ± 96.3 | 2350 ± 87 | 22732 ± 120 |

As it is shown from figure 4-4 and 4-5 there is high reduction of BOD, COD, and TS because of the anaerobic digestion process. The BOD has reduced by 92, 89, 94.18, 93, 96.7 % at 0, 1, 6, 11, 100 % volume ratio respectively. The COD of the mixture has reduced by 83.7, 80.9, 89.9, 92.9, and 97.9 % at 0, 1, 6, 11, and 100 % volume ratio respectively. Also, the total solid of the mixture has shown a decrement after the digestion; that is, 25, 26, 22.5, 10.7, and 28 % at 0, 1, 6, 11, and 100 % volume ratio. This result is comparable to other research reports. Example kebena Bula (2014) reported that the percentage of removal of different parameters after digestion of from anaerobic wastewater plant were 94.70 % (BOD5), 92.20 % (COD), 85.30 % (S2-), 87.30 % (TSS), 48.90 % (TDS), 44.98 % (NO3-N), 42.70 % (EC), 15.15 % (NH4-N), 10.30 % (TN); Gizachew (2011) also reported that; the removal efficiency after digestion was 82% of COD, and 66.1 % of total solids. Except during the addition of yeast (1%), the TN, COD, BOD, and TP increases in all the mixtures as concentration or contribution of yeast increases. The result was expected and this is due to the high TN, COD, BOD, and TP nature of spent yeast. As it is shown from this study and referenced studies the percentage of organic matter reduction is good enough showing that there is biodegradation of organic substances which in return indicating high production of biogas.

## 4.2 The yield of the Experiments

Table 4-6 summarizes the yield of methane obtained from brewery waste at each of the 27 experimental runs. Evidently, a maximum extraction yield of methane (0.198 L/D/L) was obtained at run number (14).

*Table 4-6 Selected experimental runs showing the methane production rate per used volume of sample (Liter of methane produced per day per liter of the sample(L/D/L))*

| | | Factor 1 | Factor 2 | Factor 3 | Response 1 |
|---|---|---|---|---|---|
| Std | Run | A: Temperature | B: pH | C: Volume Ratio | Methane Yield |
| | | ℃ | | % | L/D/L |
| 1 | 15 | 35.00 | 5.00 | 1.00 | 0.142 |
| 2 | 8 | 37.00 | 5.00 | 1.00 | 0.153 |
| 3 | 1 | 39.00 | 5.00 | 1.00 | 0.148 |
| 4 | 20 | 35.00 | 6.50 | 1.00 | 0.159 |
| 5 | 9 | 37.00 | 6.50 | 1.00 | 0.203 |
| 6 | 10 | 39.00 | 6.50 | 1.00 | 0.19 |
| 7 | 24 | 35.00 | 8.00 | 1.00 | 0.156 |

|  |  |  |  |  |  |
|---|---|---|---|---|---|
| 8 | 4 | 37.00 | 8.00 | 1.00 | 0.172 |
| 9 | 21 | 39.00 | 8.00 | 1.00 | 0.163 |
| 10 | 6 | 35.00 | 5.00 | 6.00 | 0.136 |
| 11 | 26 | 37.00 | 5.00 | 6.00 | 0.147 |
| 12 | 17 | 39.00 | 5.00 | 6.00 | 0.14 |
| 13 | 11 | 35.00 | 6.50 | 6.00 | 0.149 |
| 14 | 27 | 37.00 | 6.50 | 6.00 | 0.198 |
| 15 | 12 | 39.00 | 6.50 | 6.00 | 0.183 |
| 16 | 23 | 35.00 | 8.00 | 6.00 | 0.147 |
| 17 | 25 | 37.00 | 8.00 | 6.00 | 0.164 |
| 18 | 3 | 39.00 | 8.00 | 6.00 | 0.154 |
| 19 | 2 | 35.00 | 5.00 | 11.00 | 0.129 |
| 20 | 16 | 37.00 | 5.00 | 11.00 | 0.137 |
| 21 | 5 | 39.00 | 5.00 | 11.00 | 0.133 |
| 22 | 22 | 35.00 | 6.50 | 11.00 | 0.148 |
| 23 | 7 | 37.00 | 6.50 | 11.00 | 0.19 |
| 24 | 14 | 39.00 | 6.50 | 11.00 | 0.173 |
| 25 | 13 | 35.00 | 8.00 | 11.00 | 0.138 |
| 26 | 18 | 37.00 | 8.00 | 11.00 | 0.153 |
| 27 | 19 | 39.00 | 8.00 | 11.00 | 0.143 |

*Table 4-7 Selected experimental runs showing the percentage of methane produced*

|  |  | Factor 1 | Factor 2 | Factor 3 | Response 1 |
|---|---|---|---|---|---|
| Std | Run | A: Temperature | B: pH | C: Volume Ratio | Methane Yield |
|  |  | 0C |  | % | % |
| 1 | 15 | 35.00 | 5.00 | 1.00 | 42 |
| 2 | 8 | 37.00 | 5.00 | 1.00 | 45.3 |
| 3 | 1 | 39.00 | 5.00 | 1.00 | 43.7 |
| 4 | 20 | 35.00 | 6.50 | 1.00 | 47.1 |
| 5 | 9 | 37.00 | 6.50 | 1.00 | 60 |
| 6 | 10 | 39.00 | 6.50 | 1.00 | 56.4 |
| 7 | 24 | 35.00 | 8.00 | 1.00 | 46.3 |
| 8 | 4 | 37.00 | 8.00 | 1.00 | 51 |
| 9 | 21 | 39.00 | 8.00 | 1.00 | 48.26 |
| 10 | 6 | 35.00 | 5.00 | 6.00 | 40.13 |
| 11 | 26 | 37.00 | 5.00 | 6.00 | 43.6 |
| 12 | 17 | 39.00 | 5.00 | 6.00 | 41.5 |
| 13 | 11 | 35.00 | 6.50 | 6.00 | 44.23 |
| 14 | 27 | 37.00 | 6.50 | 6.00 | 58.6 |
| 15 | 12 | 39.00 | 6.50 | 6.00 | 54.2 |
| 16 | 23 | 35.00 | 8.00 | 6.00 | 43.4 |
| 17 | 25 | 37.00 | 8.00 | 6.00 | 48.6 |

| | | | | | |
|---|---|---|---|---|---|
| 18 | 3 | 39.00 | 8.00 | 6.00 | 45.7 |
| 19 | 2 | 35.00 | 5.00 | 11.00 | 38.34 |
| 20 | 16 | 37.00 | 5.00 | 11.00 | 40.62 |
| 21 | 5 | 39.00 | 5.00 | 11.00 | 39.5 |
| 22 | 22 | 35.00 | 6.50 | 11.00 | 44 |
| 23 | 7 | 37.00 | 6.50 | 11.00 | 56.3 |
| 24 | 14 | 39.00 | 6.50 | 11.00 | 51.3 |
| 25 | 13 | 35.00 | 8.00 | 11.00 | 40.8 |
| 26 | 18 | 37.00 | 8.00 | 11.00 | 45.3 |
| 27 | 19 | 39.00 | 8.00 | 11.00 | 42.5 |

Noticeably, percentage yield varied within the range of 38.4 % to 60 %. The minimum percentage yield was at 35 °C temperature, 5 pH and 11 % spent yeast volume ratio at the run number (19) which was observed at the lowest pH and temperature and average spent yeast volume ratio. On the other hand, the maximum percentage yield was at 36.5 °C temperature, 6.5 pH and 1 % spent yeast volume ratio, which are the average values of all the factors considered for this study. This result is not only comparable but better than other co-digestion studies. For example Gizachew Assefa reported that From the digestion of 1:2 mix of Municipal solid waste (MSW): cow manure (CM): 0.47 m3/d/m3 biogas with 51.5% methane content was produced; 0.45 m3/d/m3, 0.35 m3/d/m3, 0.26 m3/d/m3, and 0.23 m3/d/m3, biogas with 31.4%, 42.9%, 20.3% and 33.7 % methane composition is produced from 1:1 mix of OFMSW: CM, CM alone, FMSW alone and 2:1 1 mix of OFMSW: CM respectively (Gizachew, 2011). Similar study was reported by Alemayehu Gashaw & Abile Teshita (2014).

*Table 4-8 Model summary*

| Source | Sequential p-value | Adjusted $R^2$ | Predicted $R^2$ | |
|---|---|---|---|---|
| Linear | 0.0716 | 0.1614 | 0.0456 | |
| 2FI | 0.9960 | 0.0385 | -0.2460 | |
| Quadratic | < 0.0001 | 0.8350 | 0.7437 | Suggested |
| Cubic | 0.2173 | 0.8716 | 0.6980 | Aliased |
| Std. Dev. | 0.0083 | $R^2$ | 0.8921 | |
| Mean | 0.1573 | Adjusted $R^2$ | 0.8350 | |
| C.V. % | 5.30 | Predicted $R^2$ | 0.7437 | |
| | | Adeq Precision | 14.4924 | |

The response of the analysis was methane yield. The response yield was arranged from 0.129 L/D/L – 0.203 L/D/L. The aim of the model fit summary was to maximize the adjusted R-Squared and predicted R-Squared values. Model significance was checked for the model and model factors. Linear model factors such as temperature (A), pH (B), and volume ratio and, quadratic model factors namely pure quadratic terms ($A^2$, $B^2$, $C^2$) and interaction quadratic terms (AB, AC, BC) based on the F and p-values were checked. The predicted R² of 0.7437 is in reasonable agreement with the adjusted R² of 0.8350; i.e. the difference is less than 0.2. The "Pred R-Squared is the measure of the extent this developed model can be used to predict ranges of data this study has not considered, which, therefore, means that this model has 74.37 % precision in fitting to overall ranges of data. The R-squared, on the other hand, shows how much of the difference in the outcome is explained by the model. This model is, therefore, almost is fairly good in accomplishing this task as demonstrated through its high value (89.21 %). "Adeq Precision" measures the signal to noise ratio. A ratio greater than 4 is desirable. In this case, the ratio of 14.492 indicates an adequate signal. Hence, it can be concluded that this model can be used to navigate the design space.

### 4.2.1 Analysis of variance (ANOVA) for Quadratic model

*Table 4-9 ANOVA for Quadratic model*

| Source | Sum of Squares | df | Mean Square | F-value | p-value | |
|---|---|---|---|---|---|---|
| Model | 0.0098 | 9 | 0.0011 | 15.62 | < 0.0001 | Significant |
| A-Temperature | 0.0008 | 1 | 0.0008 | 12.08 | 0.0029 | |
| B-pH | 0.0009 | 1 | 0.0009 | 12.48 | 0.0026 | |
| C-Volume Ratio | 0.0011 | 1 | 0.0011 | 16.11 | 0.0009 | |
| AB | 2.083E-06 | 1 | 2.083E-06 | 0.0300 | 0.8646 | |
| AC | 8.333E-06 | 1 | 8.333E-06 | 0.1198 | 0.7335 | |
| BC | 0.0000 | 1 | 0.0000 | 0.2025 | 0.6584 | |
| $A^2$ | 0.0017 | 1 | 0.0017 | 24.44 | 0.0001 | |
| $B^2$ | 0.0052 | 1 | 0.0052 | 75.07 | < 0.0001 | |
| $C^2$ | 6.667E-07 | 1 | 6.667E-07 | 0.0096 | 0.9232 | |
| Residual | 0.0012 | 17 | 0.0001 | | | |
| Cor Total | 0.0110 | 26 | | | | |

The Model F-value of 15.62 implies the model is significant. There is only a 0.01 % chance that an F-value this large could occur due to noise.

P-values less than 0.0500 indicate model terms are significant. In this case temperature, pH, volume ratio, temperature², pH² are significant model terms. Values greater than 0.1000 indicate the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model.

*Table 4-10 Coefficients in Terms of Coded Factors*

| Factor | Coefficient Estimate | df | Standard Error | 95 % CI Low | 95 % CI High | VIF |
|---|---|---|---|---|---|---|
| Intercept | 0.1884 | 1 | 0.0042 | 0.1795 | 0.1974 | |
| A-Temperature | 0.0068 | 1 | 0.0020 | 0.0027 | 0.0110 | 1.0000 |
| B-pH | 0.0069 | 1 | 0.0020 | 0.0028 | 0.0111 | 1.0000 |
| C-Volume Ratio | -0.0079 | 1 | 0.0020 | -0.0120 | -0.0037 | 1.0000 |
| AB | 0.0004 | 1 | 0.0024 | -0.0047 | 0.0055 | 1.0000 |
| AC | -0.0008 | 1 | 0.0024 | -0.0059 | 0.0042 | 1.0000 |
| BC | -0.0011 | 1 | 0.0024 | -0.0062 | 0.0040 | 1.0000 |
| $A^2$ | -0.0168 | 1 | 0.0034 | -0.0240 | -0.0097 | 1.0000 |
| $B^2$ | -0.0295 | 1 | 0.0034 | -0.0367 | -0.0223 | 1.0000 |
| $C^2$ | -0.0003 | 1 | 0.0034 | -0.0075 | 0.0068 | 1.0000 |

The coefficient estimate represents the expected change in response per unit change in factor value when all remaining factors are held constant. The intercept in an orthogonal design is the overall average response of all the runs. The coefficients are adjustments around that average based on the factor settings. When the factors are orthogonal the variance inflation factors (VIFs) are 1; VIFs greater than 1 indicate multi-collinearity, the higher the VIF the more severe the correlation of factors. As a rough rule, VIFs less than 10 are tolerable. In this case, one can simple observe that a good multi-collinearity exists for all the significant factors. Final Equation in Terms of Coded Factors is as follows:

$$\text{Methane Yield} = 0.1884 + 0.0068 \times A + 0.0069 \times B - 0.0079 \times C + 0.0004 \times A \times B - 0.0008 \times A \times C - 0.0011 \times B \times C - 0.0168 \times A^2 - 0.0295 \times B^2 - 0.0003 \times C^2 \qquad \text{Equation-E}$$

The equation in terms of coded factors can be used to make predictions about the response for given levels of each factor. By default, the high levels of the factors are coded as +1 and the low levels are coded as -1. The coded equation is useful for identifying the relative impact of the factors by comparing the factor coefficients.

*Table 4-11 Final Equation in Terms of Actual Factors*

| Methane Yield | = |
|---:|---|
| -6.26496 | |
| +0.314431 | Temperature (A) |
| +0.170802 | pH (B) |
| +0.002604 | Volume Ratio (c) |
| +0.000139 | Temperature * pH |
| -0.000083 | Temperature * Volume Ratio |
| -0.000144 | pH * Volume Ratio |
| -0.004208 | $\text{Temperature}^2$ |
| -0.013111 | $\text{pH}^2$ |
| -0.000013 | $\text{Volume Ratio}^2$ |

The equation in terms of actual factors can be used to make predictions about the response for given levels of each factor. Here, the levels should be specified in the original units for each factor. This equation should not be used to determine the relative impact of each factor because the coefficients are scaled to accommodate the units of each factor and the intercept is not at the center of the design space.

### 4.2.2 Model Adequacy Checking

The normal probability plot of residuals is shown in Figures 4-2. If data from experiments form a normal distribution, the residuals fall on a straight line, which implies that the errors are spread in a normal distribution. Here a residual means difference in the observed value (obtained from the experiment) and the predicted value or fitted value (value that can be predicted by the developed model equation). Hence, observing this normal plot, it can be noticed that all the points line up well and the deviation of points for extract yield from normality is insignificant, which means that the methane yield is precisely modeled.

This can also be confirmed by the variations between the residuals and model predicted values analyzed through residual graphs as presented in Figure 4-3. The plot is a random scatter (constant range of residuals across the graph). Thus, the model does not need any form of transformation as it is well fitted.

On the other hand, Figure 4-1 shows the plot of residuals versus the experimental run order. It checks for lurking variables that may have influenced the response during the experiment. The plot shows a random scatter and thus no more blocking or any other solutions were needed.

The plot of predicted versus actual (Figure 4-4), which is the graph of the predicted response values versus the actual response values, is also another important diagnostics plot that should be taken into consideration. The purpose is to detect a value, or group of values, that are not easily predicted by the model. Looking into the graph, all values fall along the straight line, which shows the high power of prediction of the model developed.

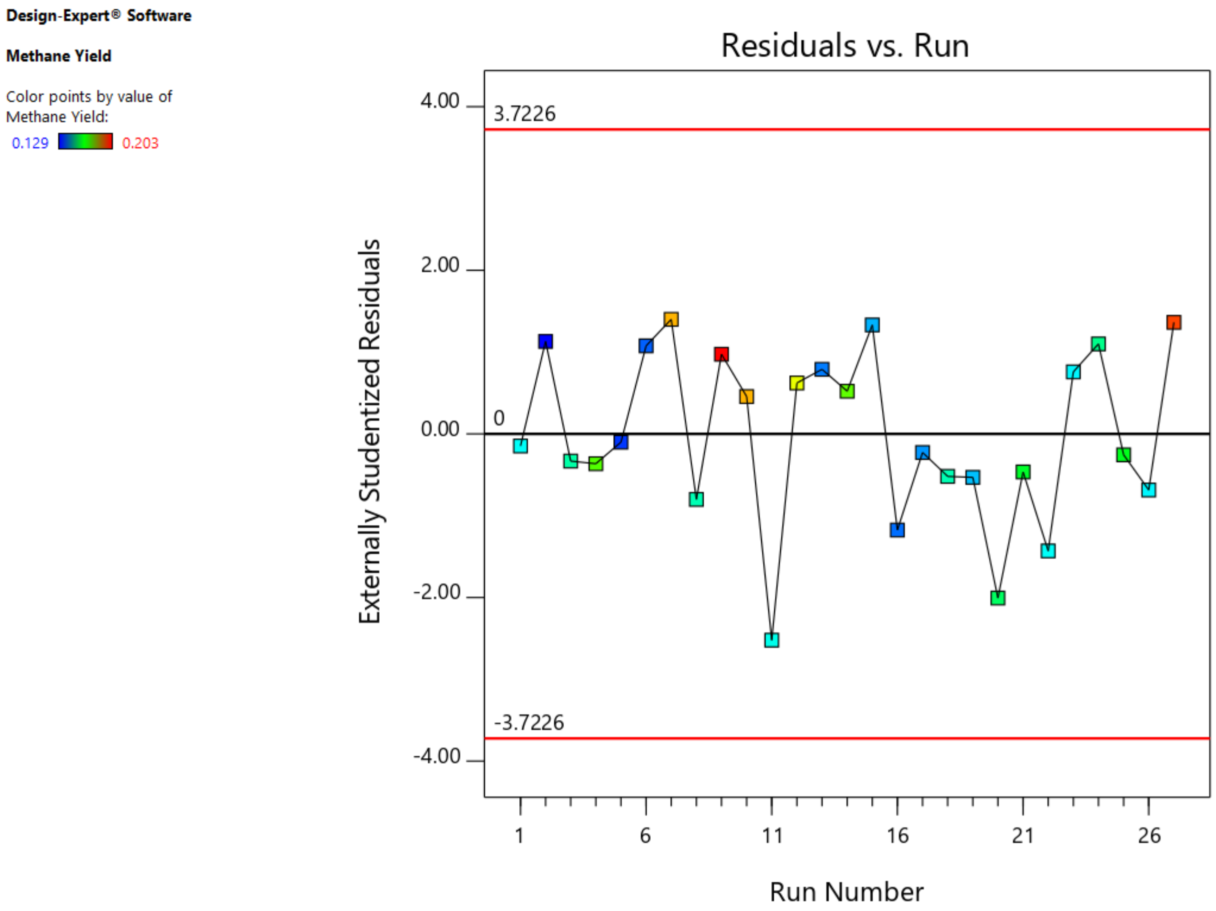

*Figure 4-1Residuals vs. Run*

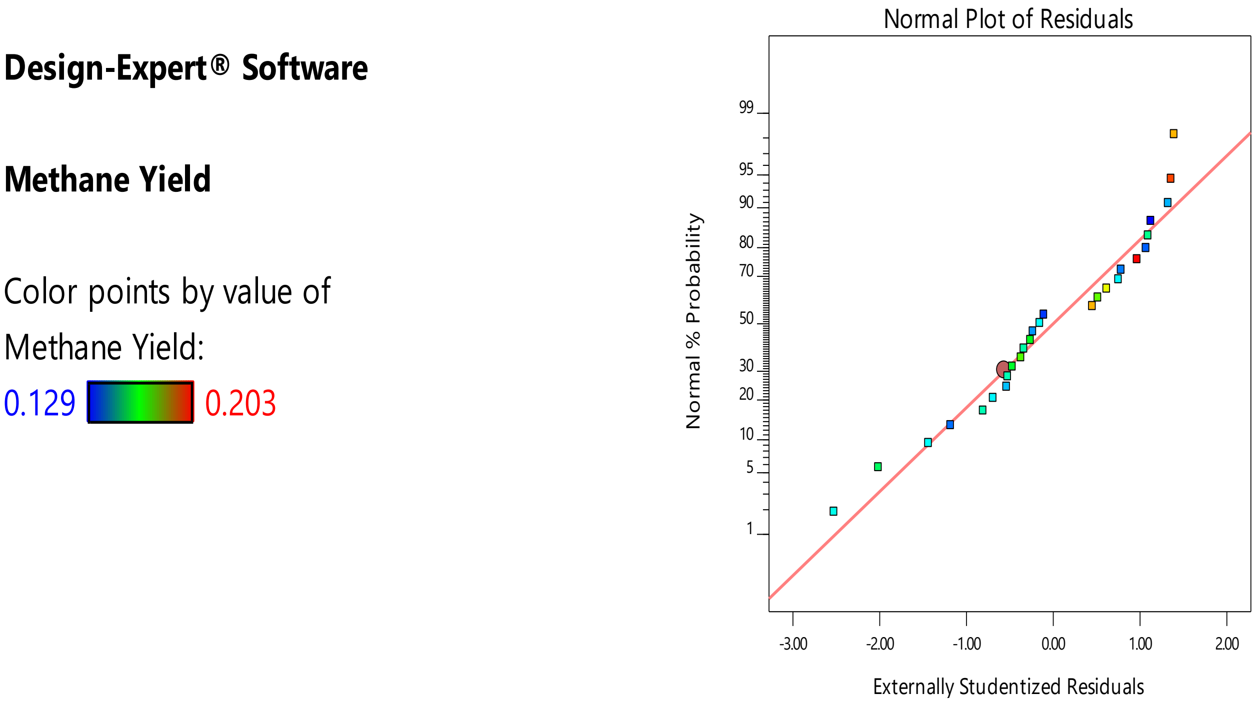

*Figure 4-2 Probability of Normal methane yield vs Residuals*

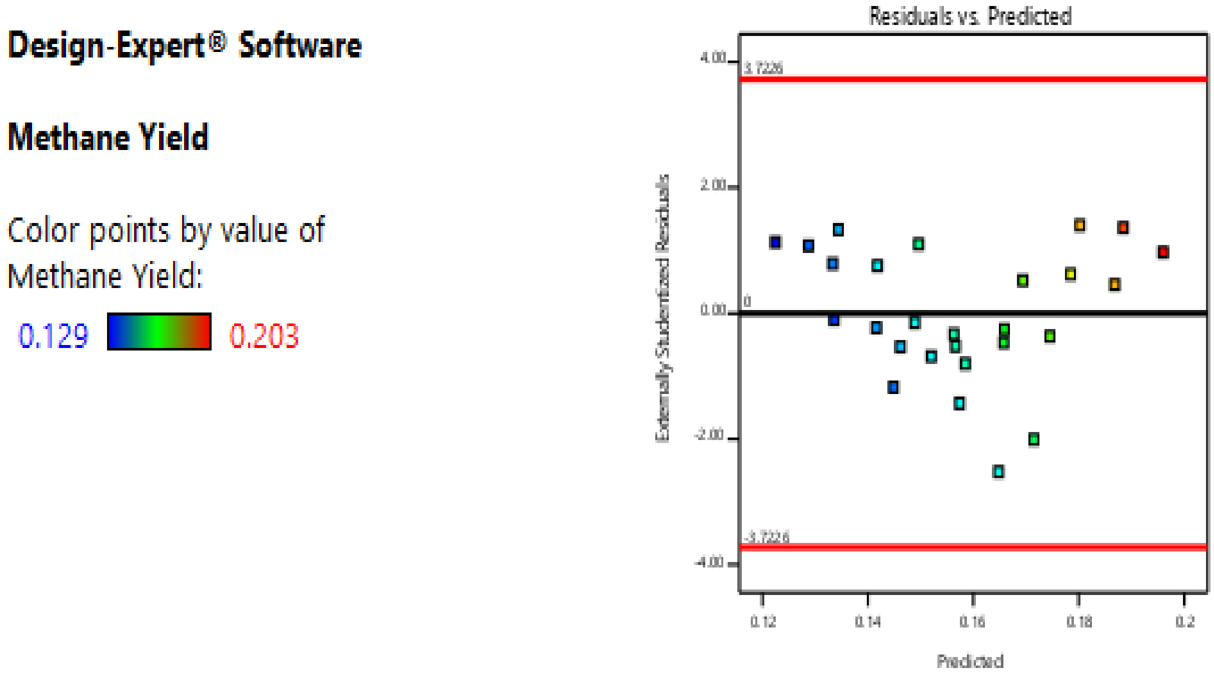

*Figure 4-3 Residuals vs Predicted*

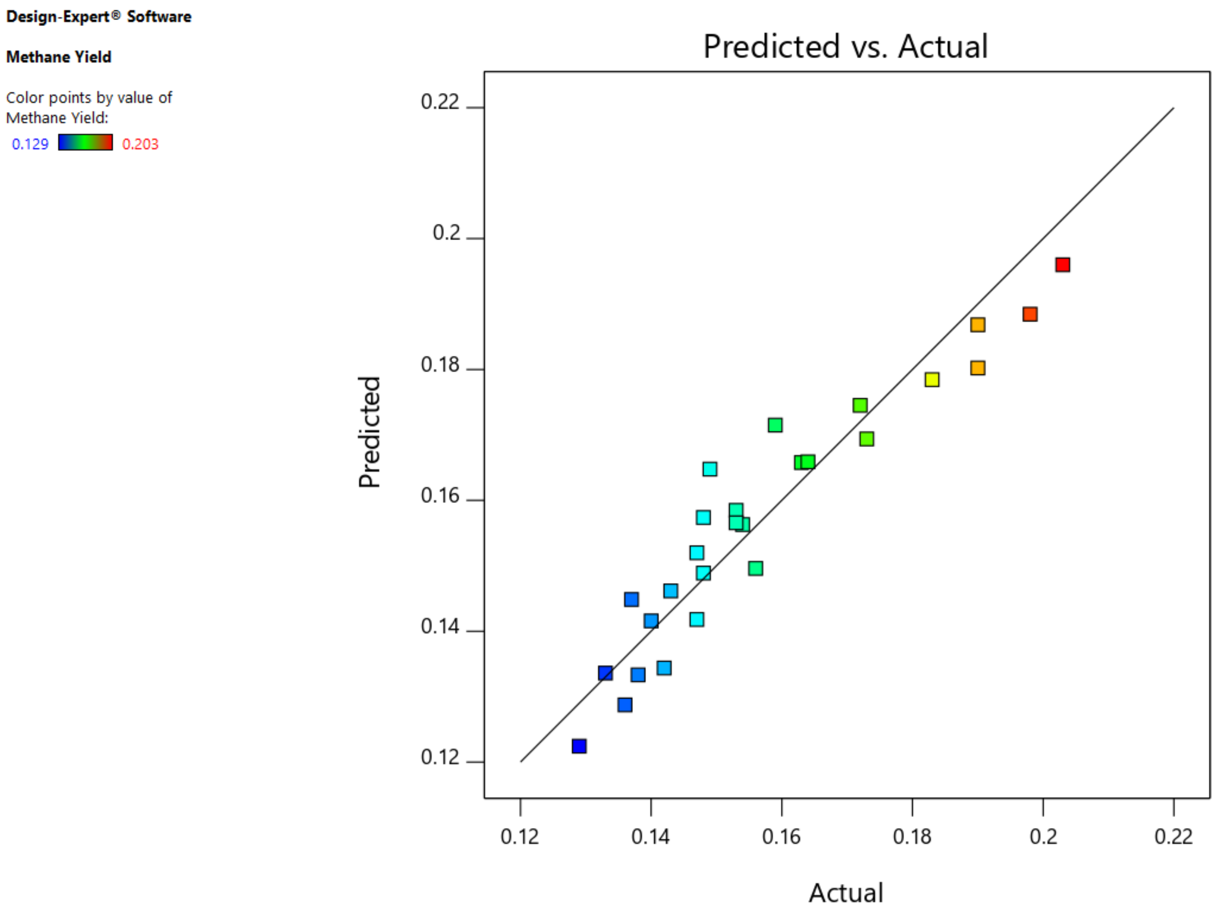

*Figure 4-4 Predicted vs Actual*

### 4.2.3 Effect of Temperature on the methane yield

As shown in figure 4-5 below the temperature has direct proportional relationship with methane production rate until the temperature of 38 ℃. as temperature of the digestion process increases from 35 to 38 ℃ the methane yield increased from 0.129 L/D/L to 0.203 L/D/L. but above this temperature the yield begun to decrease due to creation of uncomfortable zone for the anaerobic bacteria involved. Those bacteria are highly sensitive to small change in temperature and pH. The obtained result is comparable to other studies held before.

Generally, the rate of all reactions varies with temperature. In biological systems, temperature increase is not as great as for chemical reactions. Methane production has been documented under a wide range of temperatures, but bacteria are most productive in either mesophilic conditions 25 - 40 ℃, or in the thermophilic conditions, at 50 - 65 ℃ (Ostrem, 2004) (Biey E. M., 2003). Cecchi and Pavan et al. (1993) observed that temperature is a factor that affects the kinetics and the composition of the mixed microbial population (Cacchi, 1993).

In order to achieve reasonable methane production, the temperature should be above 20 °C, and this fact makes anaerobic treatment more attractive in tropical countries (Björnsson, Evaluation of parameters for monitoring an anaerobic co-digestion process, Applied Microbiology and Biotechnology annual thesis presentation, 2000) (Pena-Varo, 2002). Other authors have claimed success with the anaerobic digestion of waste at ambient temperature, which is known to be cost effective (Biey E. M., 2003). Temperature has a positive effect on digestion rate, resulting in higher volumetric methane production (Mahony, 2002).

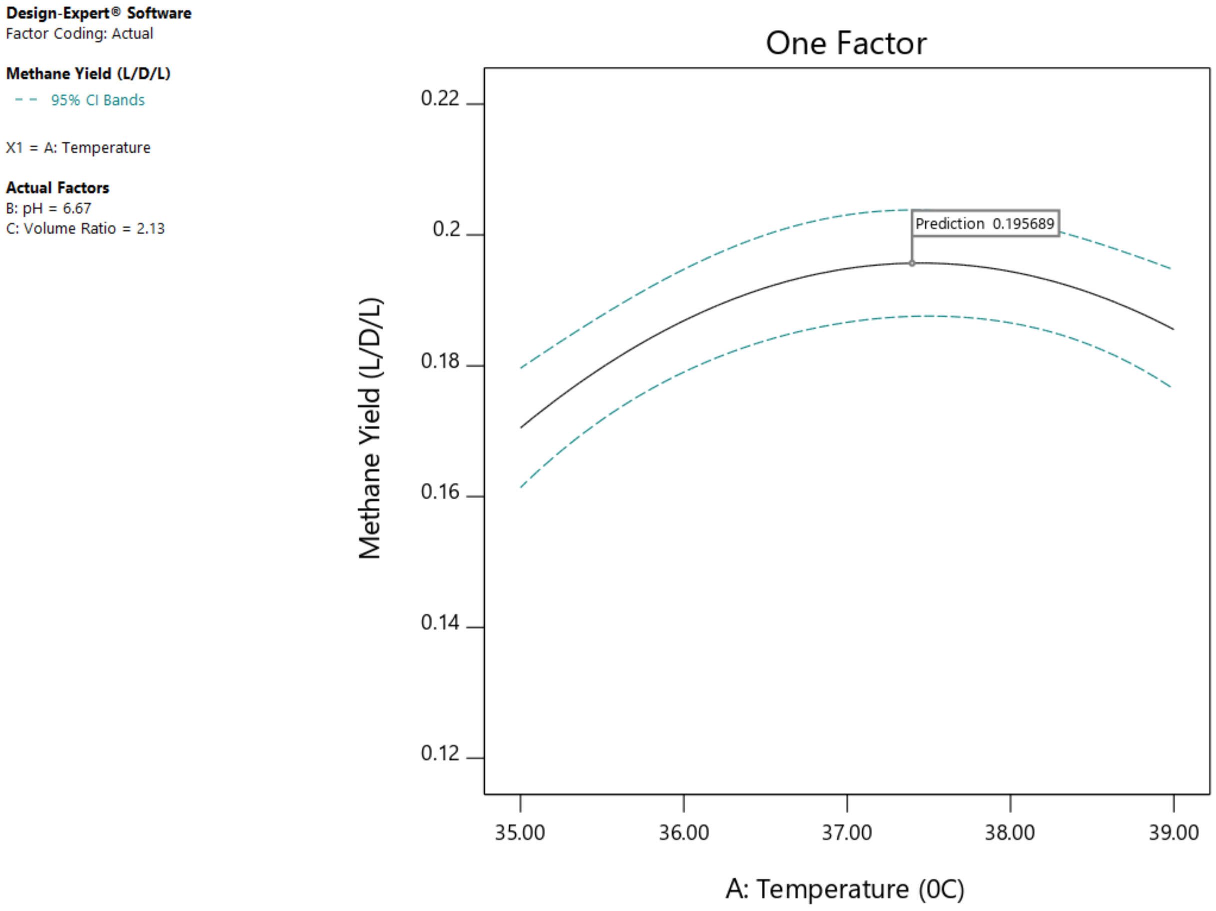

*Figure 4-5 Effect of Temperature on the methane yield*

### 4.2.4 Effect of pH on the methane yield

As shown in figure 4-6 below the pH has direct proportional relationship with methane production rate until the pH of 7. As pH of the digestion process increases from 5 to 7 the methane yield increased from 0.129 L/D/L to 0.203 L/D/L. but above this pH the yield begun to decrease due to creation of uncomfortable zone for the anaerobic bacteria involved. Methanogenic bacteria are highly sensitive to change in pH, especially when it is above 7.5. The obtained result is comparable to other studies held before.

Each of the microbial groups in anaerobic degradation has specific pH optimum and can grow in a specific pH range (Biey E. M., 2003). A pH outside 6.0 to 8.5 pH interval can lead to imbalance. The methanogens and acetogens have pH optimum at approximately 7, while acidogens have lower pH optimum around 6. Methanogens grow very slowly at pH lower than 6.6 (Mata-Alvarez J. , 2003) (Björnsson, Evaluation of parameters for monitoring an anaerobic co-digestion process, Applied Microbiology and Biotechnology annual thesis presentation, 2000). The pH determination is really useful and is important to relate its value to other process parameters: alkalinity, VFA concentration, and biogas production and composition (Mata-Alvarez J. , 2003). The amount of carbon dioxide and volatile fatty acids produced during the anaerobic process affects the pH of the digester contents. Jain and Mattiasson (1998) found that above pH 5.0, the efficiency of CH4 production was more than 75 % (Mattiasson, 1998)

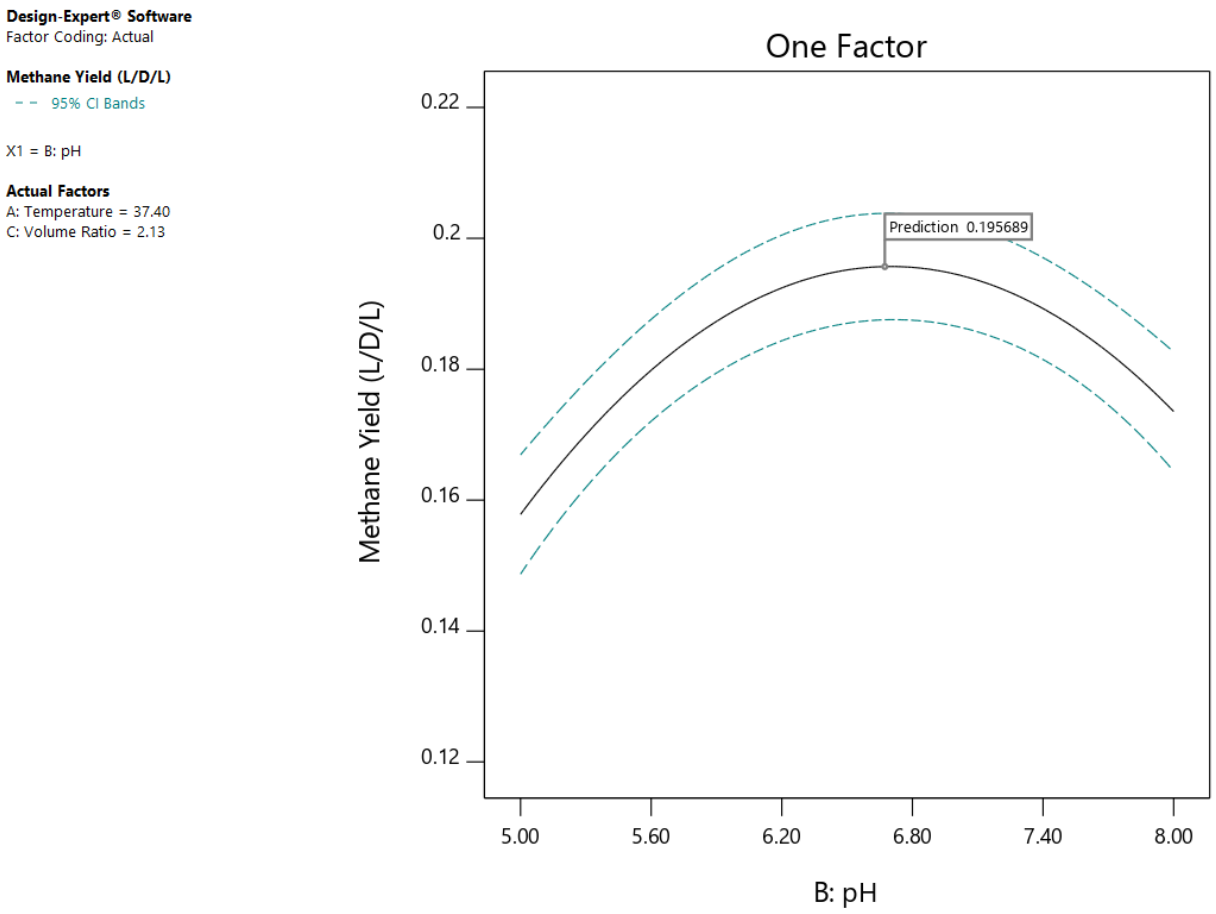

*Figure 4-6 Effect of pH on the methane yield*

### 4.2.5 Effect of volume ratio on the methane yield

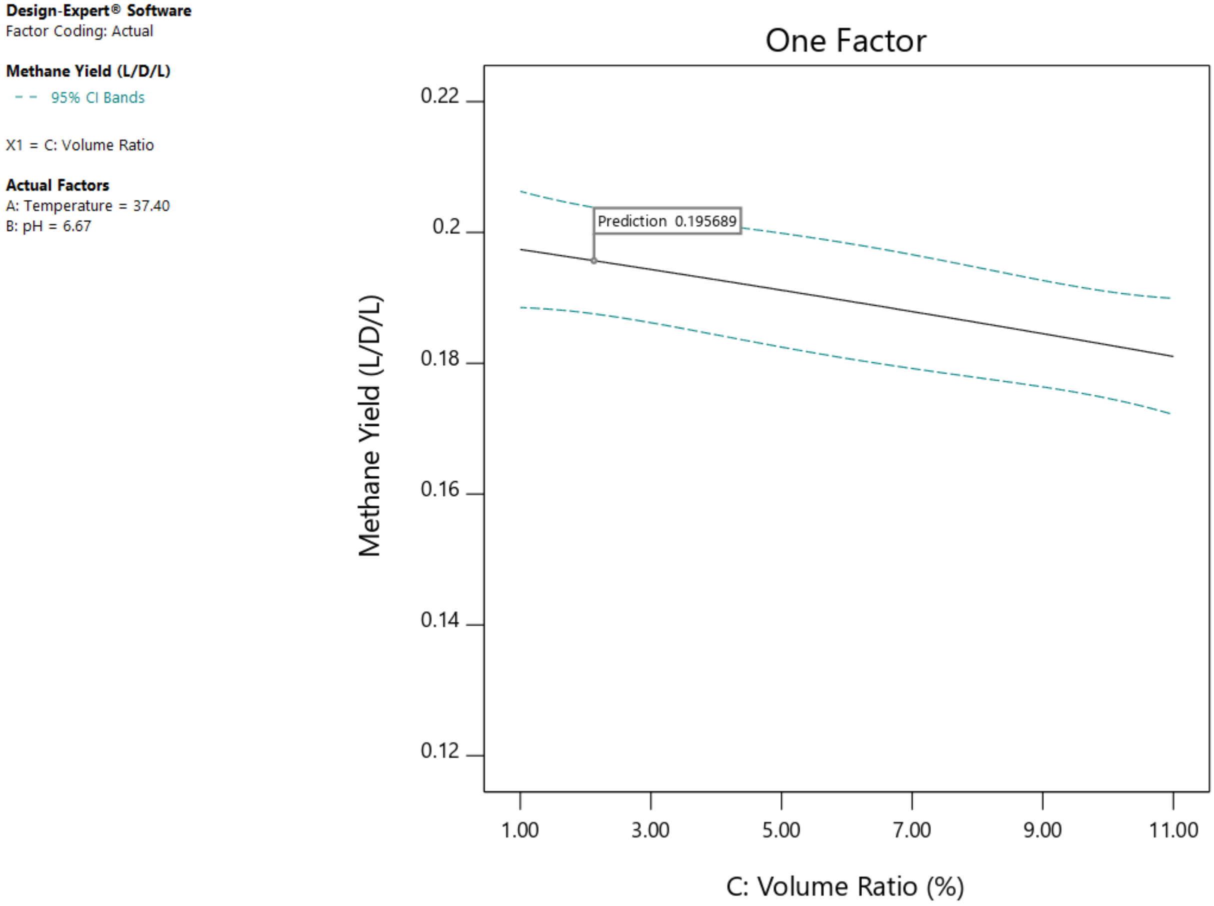

*Figure 4-7 Effect of volume ratio on the methane yield*

As shown in the in figure 4-7 the highest percentage of methane is obtained at 1 % volume ratio and the lowest percentage of methane is recorded at volume ratio of 11 %. This dramatic difference is due to accumulation of yeast cells. When the volume ratio of spent yeast is small then all the added yeast will be utilized by the anerobic bacteria, hence there is no accumulation of extra material on the digester; in the contrary when the volume ratio of yeast is high, there exist accumulation of those yeast cells. This accumulation of organic material hinders the action anaerobic bacteria causing low rate of biogas production.

### 4.2.6 Comparison of the Factor Effect at a Point

Figure 4-9 below is called a perturbation plot, which helps to compare the effects of all the factors at a point in the design space. The response is plotted by changing only one factor over its range while holding all the other factors constant. A steep slope or curvature in a factor shows that the response is sensitive to that factor. A relatively flat line shows insensitivity to change in that factor. If there are more than two factors, the perturbation plot could be used to find those factors that most affect the response. In this case, as it can be seen from the plot, the increase in three of the factors has an increasing effect on the yield of methane. This is especially true until the reference point it reached. Past the reference point, however, the effect of each factors on the extraction yield is reverted.

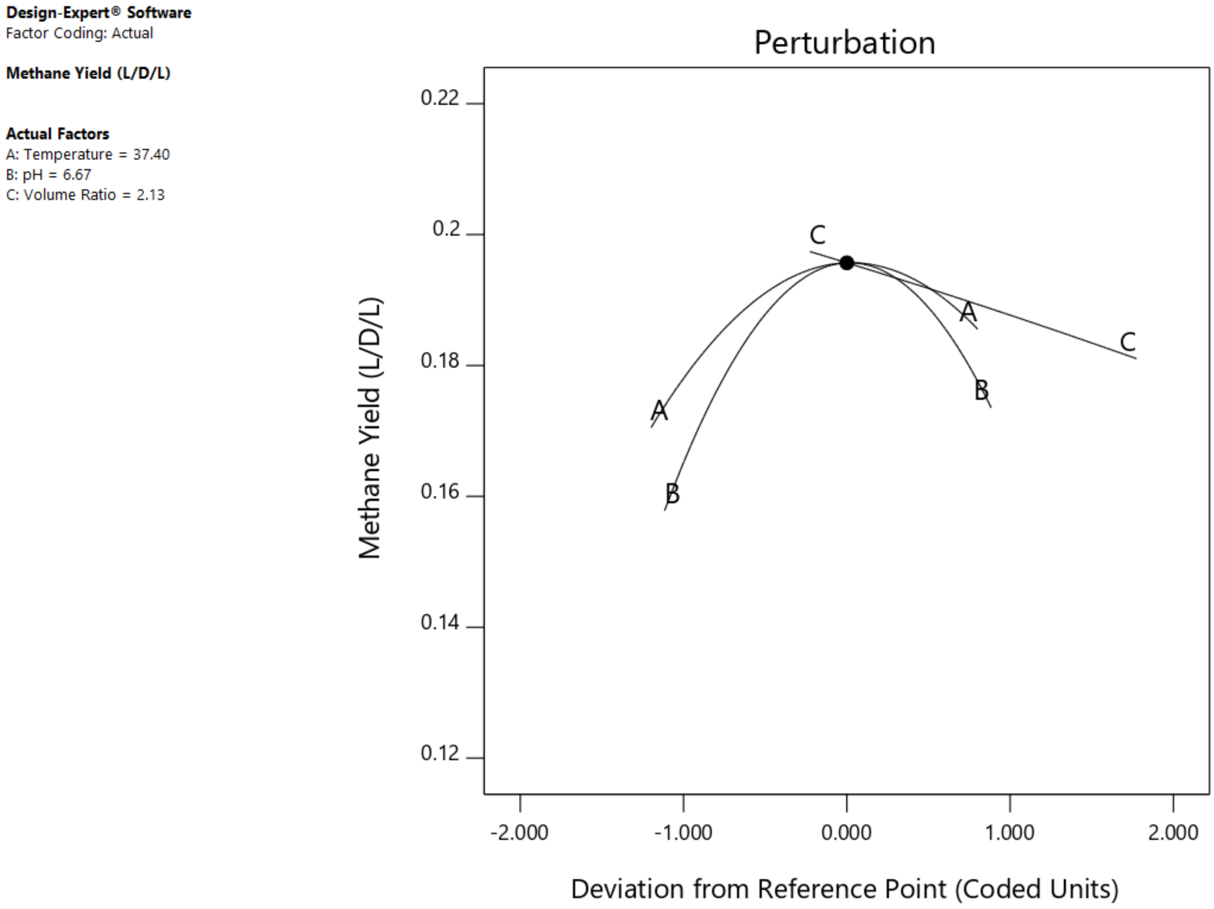

*Figure 4-8 Perturbation plots comparing the effect of all factors at a point in the design space*

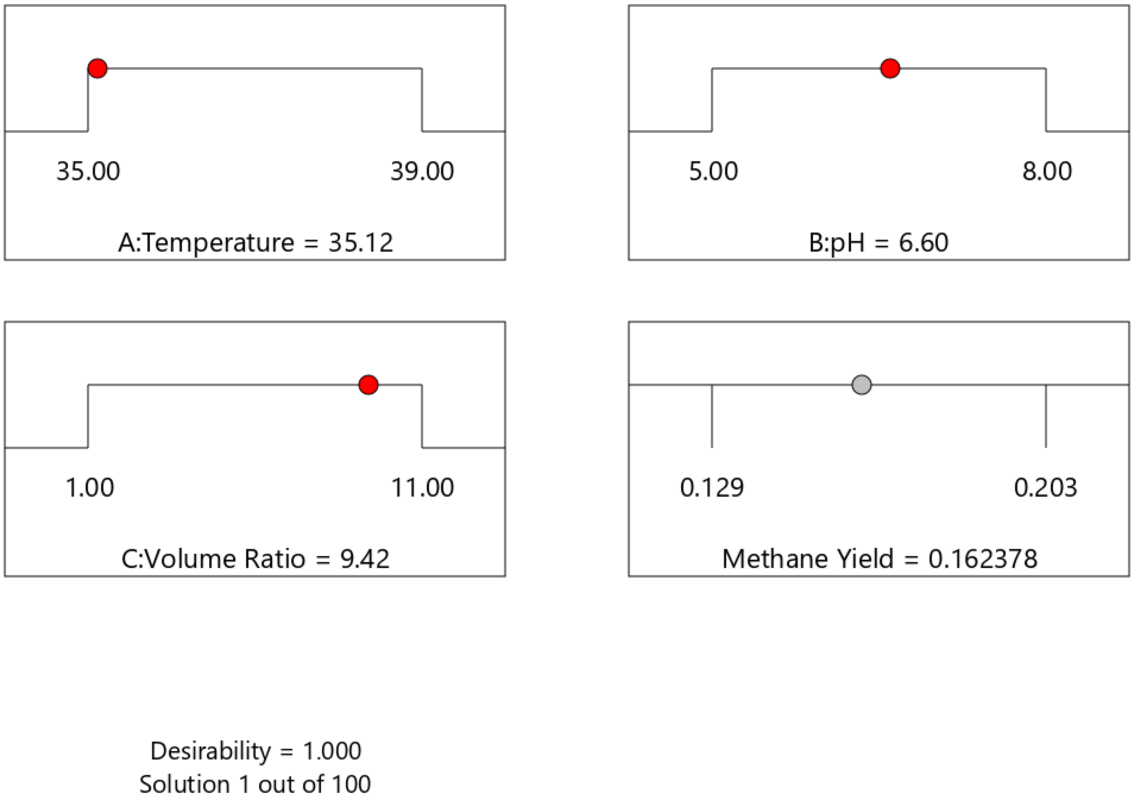

*Figure 4-9 Ramp Plot of Optimization Solution for the Response*

Depending on the parameters by compromising yield, economy and energy carrying, the best solution was selected among the alternatives. Undertaking the consideration of these constraints 35.12 ℃ temperature, 6.6 pH and 9.4 % volume ratio were selected which gave 0.162378 L/D/L methane yield as summarized by Figure 4-10.

## 4.3 Determination of potential of sludge after digestion as an organic fertilizer

Digestate is the by-product of methane and heat production in a biogas plant, coming from organic wastes. Depending on the biogas technology, the digestate could be a solid or a liquid material. Digestate contains a high proportion of mineral nitrogen (N) especially in the form of ammonium which is available for plants. Moreover, it contains other macro- and microelements necessary for plant growth. Therefore, the digestate can be a useful source of plant nutrients, it seems to be an effective fertilizer for crop plants. On the other hand, the organic fractions of digestate can contribute to soil organic matter (SOM) turnover, influencing the biological, chemical and physical soil characteristics as a soil amendment. Besides these favorable effects of digestate, there are new researches to use it as solid fuel or in the process of methane production

For the determination of fertilizing potential, the nitrogen, phosphorus and potassium content of the sludge after digestion was measured and the mean value & deviation from this mean are displayed in Table 4.13 below.

*Table 4-12 fertilizing potential of digestate*

| Parameter | Unit | 0 % spent yeast | 1 % spent yeast | 6 % spent yeast | 11 % spent yeast | 100 % spent yeast |
|---|---|---|---|---|---|---|
| Nitrogen | mg/L | 33.9 ± 8.500 | 46.2 ± 0.040 | 57.9 ± 5.7 | 63.24 ± 3.11 | 102 ± 21.1 |
| Phosphorus | mg/L | 56.35 ± 5.750 | 68.1 ± 0.015 | 72.9 ± 6.4 | 74 ± 4.18 | 120 ± 11.41 |
| Potassium | mg/L | 113.8 ± 53.375 | 120.5 ± 13 | 129.8 ± 14 | 135 ± 24 | 170 ± 9.45 |

The above table-12 shows that the digestate has a good capacity to be used as fertilizer. This result is comparable to other digestate potential from different biogas system. Not only is comparable but this digestate has all the contents at the best percentage composition. As reported by Torsten Rehl and Joachim Müller the following composition was seen from different biogas plant digestate product.

*Table 13 Digestate Composition of Different raw inputs*

(Torsten 2014)

| | Digestate code number 1 | Digestate code number 2 | Digestate Number 3 |
|---|---|---|---|
| Input substrates of fermentation process | 35% liquid manure 50% maize silage 15% crop residues | 30% liquid manure 70% maize silage | 30% liquid manure 20% maize silage 50% bio waste |
| Dry matter, % | 64.00 | 41.80 | 89.00 |
| Organic dry matter, % | 54.40 | 35.53 | 75.65 |
| Mineral dry matter | 9.60 | 6.27 | 13.35 |
| C, g/kg | 21.76 | 12.54 | 33.82 |
| $N_2$, g/kg | 5.05 | 2.63 | 7.57 |
| $NH_4^+$, g/kg | 3.31 | 1.27 | 4.43 |
| $P_2O_5$, g/kg | 1.12 | 0.43 | 2.82 |
| $K_2O$, g/kg | 3.60 | 2.66 | 4.71 |

P. Vilanova Plana & B. Noche also reported almost similar studies. The following table shows the nature and components of digestate.

*Table 14 Digestate characteristics*

(Noche, 2016).

| | **ABSOLUTE VALUES** | **CHANGE** |
|---|---|---|
| Organic DM (%DM) | 63.8–75.0 | -5 to -15 |
| Total N (%DM) | 3.1–14.0% | ? |
| Total N (kg Mg -1 FM) | 1.2–9.10 | ≈0 |
| Total $NH_4^+$ (kg Mg -1 FM) | 1.5–6.8 | ? |
| $NH_4^+$ share on total N (%) | 44–81% | +10 to +33 |
| Total C content (%DM) | 36.0–45.0 | -2 to -3 |
| C:N ratio | 3.0–8.5 | -3 to -5 |
| Total P content (%DM) | 0.6–1.7 | ? |
| Total P (kg Mg -1 FM) | 0.4–2.6 | ≈0 |
| Water soluble P (% of total P) | 25–45 | -20 to -47 |
| Total K (%DM) | 1.9–4.3 | ? |

| | | |
|---|---|---|
| Total K (kg Mg-1 FM) | 1.2–11.5 | ≈0 |
| Total Mg (kg Mg-1 FM) | 0.3–0.7 | ≈0 |
| Total Ca (kg Mg-1 FM) | 1.0–2.3 | ≈0 |
| Total S (kg Mg-1 FM) | 0.2–0.4 | ? |
| pH | 7.3–9.0 | +0.5 to + 2 units |

DM = Dry matter. FM = Fresh matter, ? = No data found/no data available.

*Table 15 Composition of different Digestates*

| Digestate source | Type of digestion process | Total-N ($N_t$) | $NH_4$-N | Total-P | Total-K | Source of data |
|---|---|---|---|---|---|---|
| Swine manure | mesophilic | 2.93 (g /L) | 2.23 (g /L) | 0.93 (g /L) | 1.37 (g/ L) | *Loria et al., 2007* |
| Liquid cattle slurry | mesophilic | 4.27 (% DM) | 52.9 (‰ $N_t$) | 0.66 (% DM) | 4.71 (% DM) | *Möller et al., 2008* |
| Energy crops, cow manure slurry and agro-industrial waste | thermophilic | 105 (g $kg^{-1}$ TS) | 2.499 (g $L^{-1}$) | 10.92 (g $kg^{-1}$ TS) | - | *Pognani et al., 2009* |
| Energy crops, cow manure slurry, agro industrial waste and OFMSW | thermophilic | 110 (g $kg^{-1}$ TS) | 2.427 (g $L^{-1}$) | 11.79 (g $kg^{-1}$ TS) | - | *Pognani et al., 2009* |
| Cow manure, plant residues and offal | mesophilic and thermophilic | 0.2013 (%m/m, fresh matter) | 0.157 (%m/m, fresh matter) | 274.5 mg $kg^{-1}$ (fresh matter) | 736.45 mg $kg^{-1}$ (fresh matter) | *Makádi et al.,2008b* |
| Clover/grass or pea straw or cereal straw or silage maize and clover/grass silage (mean) | mesophilic | 0.253 (%m/m, fresh matter) | 0.176 (%m/m, fresh matter) | 0.62 (% DM) | 18.5 (% DM) | *Stinner et al., 2008* |

Those related studies show that the digestate produced from this study has sufficient basic elements of fertilizer. The total nitrogen, total phosphorus, total potassium, and other important elements are sourced from the brewery input and spent yeast.

# 5. Conclusions and Recommendation

## 5.1 Conclusion

During beer production large quantities of surplus yeasts are generated. Surplus yeast is a residue containing high amounts of biodegradable organic matter, with a considerable potential for energy production through conversion to biogas. The digestion of surplus yeast in existing anaerobic digesters for wastewater treatment would enable taking advantage of its energy potential with little investment.

Co-digestion of yeast and brewery wastewater is feasible since no negative effects of the joint digestion were observed during batch tests compared to the result of anaerobic digestion of brewery wastewater. Furthermore, thermally pre-treated spent yeast tested during this research showed no significant improvements in biogas yield or COD removal efficiency, indicating that this pre-treatment, within the studied conditions, is not useful to enhance anaerobic digestion of surplus yeast. During 84 days of operation, no negative effects were observed when digesting wastewater and yeast in a lab scale one litter polyethylene bottle reactor.

## 5.2 Recommendation

Based on the findings of this study, the following recommendations are forwarded:

- Pilot plant operation similar to the one undertaken in this research should be undertaken over a much longer period to establish steady state conditions that will enable correct assessments of long term treatment efficiencies, optimal loading rates, optimal hydraulic retention times, suitable dilution ratio, and gas production potentials among others. Such long term experiments should provide the required information for developing design and operation/maintenance guidelines when using anaerobic digester for the treatment of brewery wastes.
- The type of microbes involved in anaerobic digestion should be isolated and identified for further investigation.
- Parameters such as design of digester should be taken into account to improve the biogas production from the co-digestion of brewery wastewater and spent yeast.
- This study with brewery spent grain as co-substrate should be researched and tested to increase the feasibility of the waste treatment and energy production.

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# Appendices

## Appendix A: procedure of COD measurement

**COD measurement:** dichromatic reflux method was used and its procedure is as follows:

1. Wash culture tubes and caps with 20% $H_2SO_4$ before using to prevent contamination.
2. Place sample (2.5 mL) in culture tube and Add $K_2Cr_2O_7$ digestion solution (1.5 mL).
3. Carefully run sulphuric acid reagent (3.5 mL) down inside of vessel so an acid layer is formed under the sample-digestion solution layer and tightly cap tubes or seal ampules, and invert each several times to mix completely.
4. Place tubes in block digester preheated to 150°C and reflux for 2 h behind a protective shield.
5. Cool to room temperature and place vessels in test tube rack. Some mercuric sulfate may precipitate out but this will not affect the analysis.
6. Add 1 to 2 drops of Ferroin indicator and stir rapidly on magnetic stirrer while titrating with standardized 0.10 M FAS.
7. The end point is a sharp color change from blue-green to reddish brown, although the blue green may reappear within minutes.
8. In the same manner reflux and titrate a blank containing the reagents and a volume of distilled water equal to that of the sample.
9. COD is given by

COD (mg $O_2$ /L) = $[(A-B) \times M \times 8000) / (V_{sample})$

Where: A = volume of FAS used for blank (mL)

B = volume of FAS used for sample (mL)

M = molarity of FAS

8000 = milli equivalent weight of oxygen (8) ×1000 mL/L.

# Appendix-B Design expert-11 results

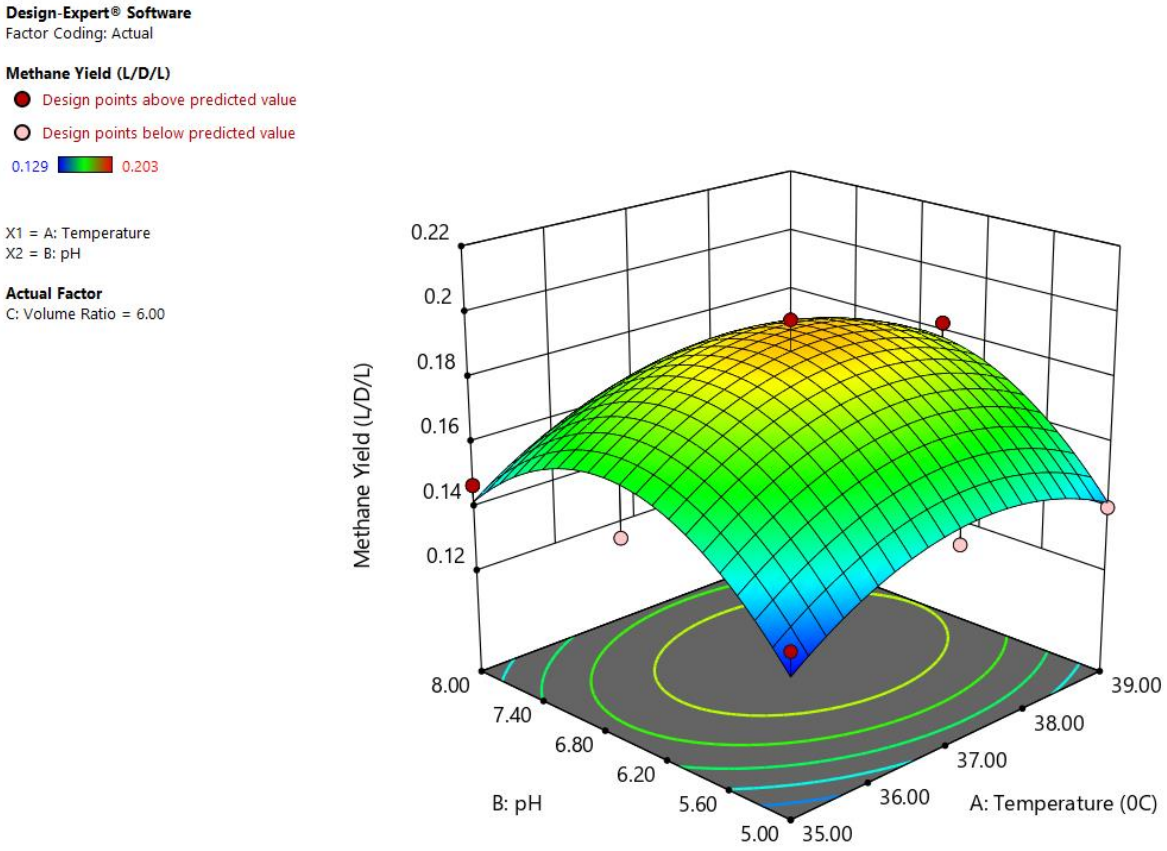

*Figure 10 Interaction effect of temperature and pH on methane yield at constant volume ratio of 6*

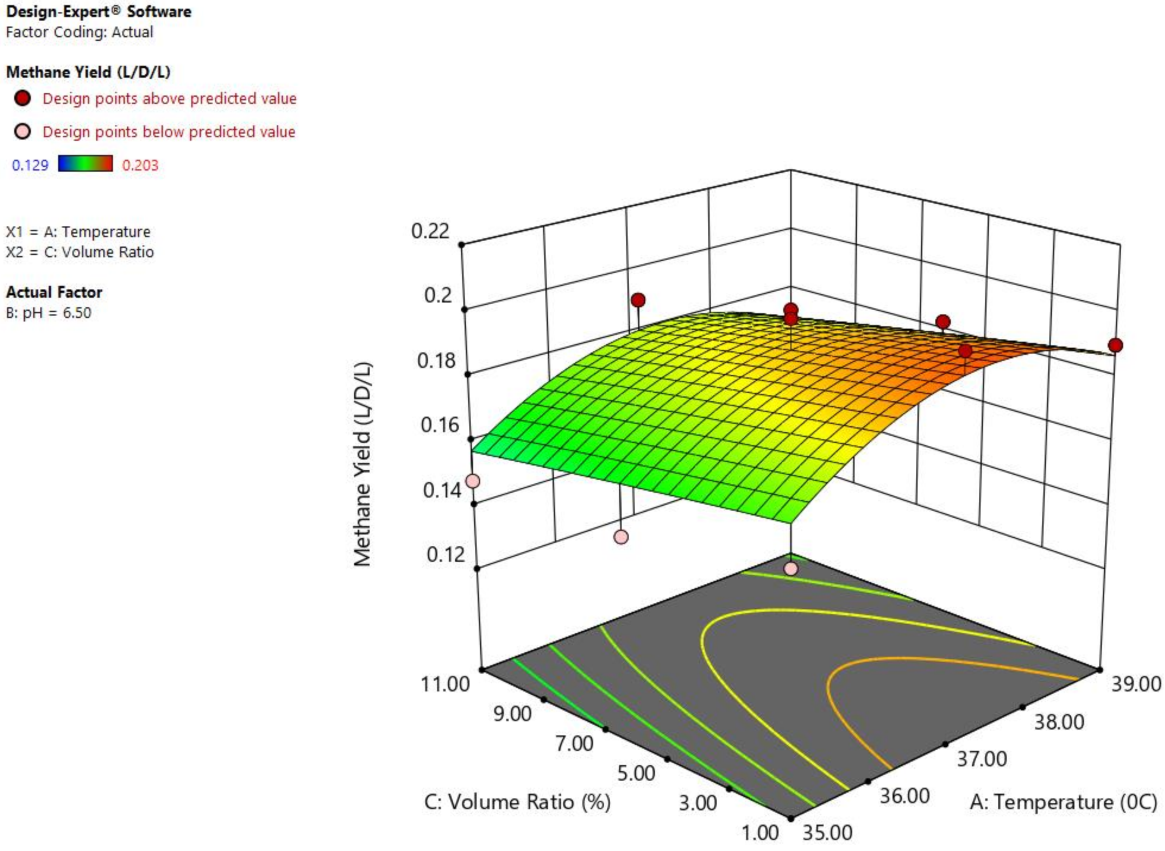

*Figure 11 Interaction effect of temperature and volume ratio on methane yield at constant pH of 6.5*

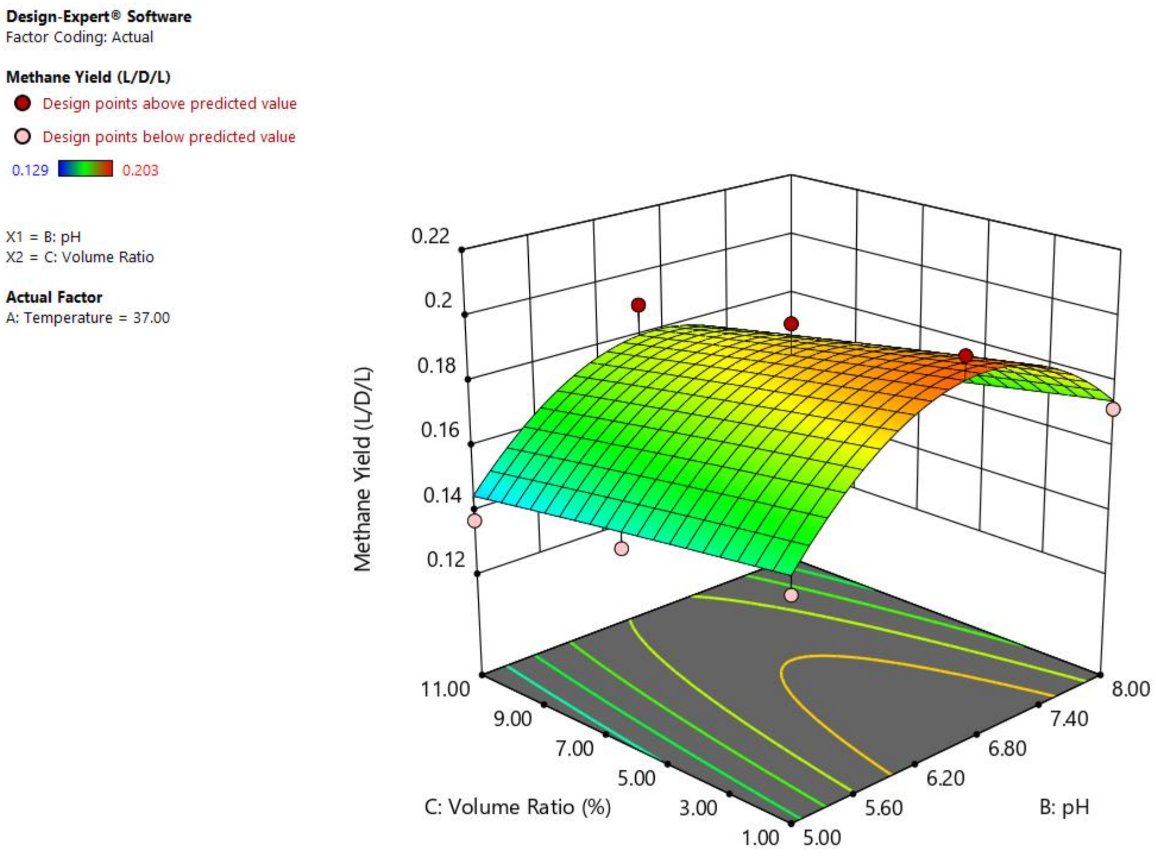

*Figure 12 Interaction effect of pH and volume ratio on methane yield at constant temperature of 37*