

CHAPTER ONE

1. Introduction

Acetic acid (CH_3COOH) is a weak carboxylic acid with a pungent odour that exists as a liquid in room temperature. The word acetic acid comes from the word acetum which in Latin means sour and relates to the fact that acetic acid is responsible for the bitter tastes of fermented juices. Acetic acid is produced naturally and synthetically in large quantities for industrial purposes. It is formed when ubiquitous bacteria of the genera acetobacter and clostridium convert alcohols and sugars to acetic acid [1]. Vinegar is a dilute aqueous solution for acetic acid. The literal meaning of vinegar is sour wine. Vinegars are produced from ciders, grapes (wines), sucrose, glucose, or malt by successive alcoholic and acetous fermentations. In the first step, sugars in the plant material are converted into alcohol and carbon dioxide through the action of the enzymes produced by yeasts. The next step involves the conversion of alcohol into vinegar by acetobacter. This bacterium provides the enzymes to convert the alcohol first to acetaldehyde and then to acetic acid. The three distinct approaches used in vinegar fermentation are the open vat method, the trickle method and the bubble method. In all the three methods the air supplies the oxygen needed for the conversion of alcohol to vinegar. Vinegar is used as an acidifier, flavor enhancer, flavoring agent, pH control agent, pickling agent, solvent, and used very often for its anti microbial properties. Vinegars are extensively used in food as it is of low cost and also has an antimicrobial action on foods.[2]

The acetic acid fermentation is a highly aerobic process essentially a biotransformation by acetic acid bacteria, which involves the incomplete oxidation of ethanol to acetic acid. The ethanol may be derived from many different sources including wine, cider, beer or fermented fruit juice, or it may be made synthetically from natural gas and petroleum derivatives. Industrial acetic acid bacteria which are members of Acetobacter can be obtained by bacterial species such as *A. aceti*, *A. rancens*, *A. xylinum* and *A. europaeus* since the conversion of acetic acid is an oxidation process, there need to be a sufficient amount of oxygen to facilitate the oxidation.

1.1 BACKGROUND

Acetic acid was used as a medicinal agent and was probably the first known antibiotic. For most of human history, acetic acid was produced by fermentation of sugar to ethyl alcohol and its subsequent oxidation to acetic acid by microorganisms. This process was supplemented in the nineteenth century by wood distillation. In 1916, the first dedicated plant for the production of acetic acid by chemical rather than biological means became commercial. This method was based on acetylene-derived acetaldehyde, and it marked the advent of inexpensive, industrial grade acetic acid and the birth of a viable industry based on its use. The advantages of chemical synthetic routes include high Fermentation production routes have traditionally been aimed at the food market. Vinegar production usually requires lower capital investment, has shorter startup Times, and can generate different types and flavors of vinegar when different carbohydrate sources are used. Furthermore, the raw material (e.g., corn, sugarcane, and sugar beet) is a Renewable resource.

1.2 HISTORY

Vinegar was known early in civilization as the natural result of exposure of beer and wine to air, because acetic acid-producing bacteria are present globally. The use of acetic acid in alchemy extends into the 3rd century BC, when the Greek philosopher Theophrastus described how vinegar acted on metals to produce pigments useful in art, including white lead (lead carbonate) and verdigris, a green mixture of copper salts including copper(II) acetate. Ancient Romans boiled soured wine to produce highly sweet syrup called sapa. Sapa that was produced in lead pots was rich in lead acetate, a sweet substance also called sugar of lead or sugar of Saturn, which contributed to lead poisoning among the Roman aristocracy.

In the 16th-century German alchemist Andreas Libavius described the production of acetone from the dry distillation of lead acetate, ketonic decarboxylation. The presence of water in vinegar has such a profound effect on acetic acid's properties that for centuries chemists believed that glacial acetic acid and the acid found in vinegar were two different substances. French chemist Pierre Adet proved them identical.[8][9]

In 1845 German chemist Hermann Kolbe synthesised acetic acid from inorganic compounds for the first time. This reaction sequence consisted of chlorination of carbon disulfide to carbon tetrachloride, followed by pyrolysis to tetrachloroethylene and aqueous chlorination to trichloroacetic acid, and concluded with electrolytic reduction to acetic acid.[10]

By 1910, most glacial acetic acid was obtained from the pyroligneous liquor, a product of the distillation of wood. The acetic acid was isolated by treatment with milk of lime, and the resulting calcium acetate was then acidified with sulfuric acid to recover acetic acid. At that time, Germany was producing 10,000 tons of glacial acetic acid, around 30% of which was used for the manufacture of indigo dye. [11]

Because both methanol and carbon monoxide are commodity raw materials, methanol carbonylation long appeared to be attractive precursors to acetic acid. Henri Dreyfus at British Celanese developed a methanol carbonylation pilot plant as early as 1925. However, a lack of practical materials that could contain the corrosive reaction mixture at the high pressures needed (200 atm or more) discouraged commercialization of these routes. The first commercial methanol carbonylation process, which used a cobalt catalyst, was developed by German chemical company BASF in 1963. In 1968, a rhodium-based catalyst ($\text{cis-}[\text{Rh}(\text{CO})_2\text{I}_2]^-$) was discovered that could operate efficiently at lower pressure with almost no by-products. US chemical company Monsanto Company built the first plant using this catalyst in 1970, and rhodium-catalyzed methanol carbonylation became the dominant method of acetic acid production. In the late 1990s, the chemicals company BP Chemicals commercialised the Cativa catalyst ($[\text{Ir}(\text{CO})_2\text{I}_2]^-$), which is promoted by iridium for greater efficiency. This iridium-catalyzed Cativa process is greener and more efficient and has largely supplanted the Monsanto process, often in the same production plants. [12]

Acetic acid is produced both synthetically and by bacterial fermentation. Today, the biological route accounts for only about 10 percent of world production, but it remains important for vinegar production, as many of the world food purity laws stipulate that vinegar used in foods must be of biological origin. About 75 percent of acetic acid made for use in the chemical industry is made by methanol carbonylation, explained below. Alternative methods account for the rest.

Total worldwide production of virgin acetic acid is estimated at 5 Mt/a (million metric tons per year), approximately half of which is produced in the United States. European production stands at approximately 1 Mt/a and is declining, and 0.7 Mt/a is produced in Japan. Another 1.5 Mt are recycled each year, bringing the total world market to 6.5 Mt/a. The two biggest producers of virgin acetic acid are Celanese and BP Chemicals. Other major producers include Millennium Chemicals, Sterling Chemicals, Samsung, Eastman, and Svensk Etanolkemi. [13][14]

1.3 STATEMENT OF THE PROBLEM

We are imported acetic acid due to lack of production factory in our county. To import this product we expend so many birr. Due to this problem we are initiated to produce this product. Acetic acid is the most important organic acid mainly used for the production of cellulose acetate for photographic film as well as synthetic fibers and fabrics due to these multiple application. We can use molasses as raw material to produce maximum amount of acetic acid.

1.4 Objectives

1.4.1 General objective

- The production of acetic acid from molasses

1.4.2 Specific objective

- Preparation of culture for acetobacter.
- Effect of alcohol content on fermentation of acetic acid.
- Characterization of the product by acid content, PH and alcohol content.
- To calculate material and energy balance of acetic acid production process
- To analysis economic issue
- To design equipment sizing

1.5 SCOPE OF THE STUDY AND LIMITATION

Scope:

This study work ranges from raw material preparation, production of acetic acid, characterization the product. Beside this it also includes plant design that contains material and energy balance, economic evaluation and feasibility .the plant layout is also included. The acetic acid that we are going to produced have a great advantageous for the people of Ethiopia since it distribute the whole regions of the country.

Limitation:

- Shortage of necessary equipment (distillation, autoclave, incubator, ph meter).

CHAPTER TWO

2 LITERATURE REVIEW

2.1 Introduction

Acetic acid also called ethanoic acid is organic compound. Acetic acid produced via fermentation. Its pathway is conversion of glucose to ethanol and ethanol to acetic acid. In first step, *Saccaromyces cerevesiae* (yeast) converts fermentable sugar of molasses into ethanol and carbon dioxide. In second step, *acetobacter aceti* (acetic acid bacteria) converts ethanol into acetic acid and water. After completing process, the separation of product is carried out via centrifugation. Mixture of acetic acid and water is separated by distillation.[3]

2.2 MOLASSES TYPES AND ITS UTILIZATION

The name molasses is derived from Latin word Mel, meaning honey and can be defined as the final effluent obtained in the manufacture of sugar in the repeated crystallization from various raw materials. It is a residual substance from which no crystalline sucrose can be obtained by simple means. Cane molasses is a major byproduct of the sugar industry. There are various types of molasses which depends on the source from which they are; beet molasses, cane molasses, black strap molasses, refinery molasses and high test molasses are among the common ones. Molasses can be converted into many value-added products by application of modern technologies. Many products can be made theoretically and Practically, but in actual practice, the production of only a few products is commercially viable and hence, commercial scale plants are working in different countries to produce; ethyl alcohol, baker's yeast, torula yeast protein molasses, L-lysine, acetic acid, acetone-butanol, citric acid, lactic acid, glutamic acid and mono sodium glutamate.

The industrial use of molasses arises from its sugar constituents. When compared to others, there was less molasses utilization in Ethiopia at present mainly due to low technological Development and low market availability. The uses of power alcohol from molasses source for vehicles increase the demand of molasses in most other countries and there is a promising move towards production and use of power alcohol in Ethiopia also. The common process for ethanol production from any biomass source consists of two steps: Converting polysaccharides into monosaccharide through acid hydrolysis or enzymatic process and then converting the monosaccharide into ethanol by fermentation.

Ethanol is produced by the fermentation of sugar, a well-established process used in the production of beer and alcohol. Carbohydrates such as starch from cereal and tuber crops– which is enzymatic converted into simple sugars and natural sugars from sugar beet, sugar cane and sweet sorghum crops are fermented using yeast to produce a mash containing ethanol, water and unfermented solids. Distillation columns separate ethanol from the fermented mash, with additional purification taking place in rectification columns to produce a mixture. Dehydration is the step of removing the last of the water, taking the mixture beyond its azeotropic equilibrium to produce anhydrous ethanol, or bio-ethanol.

Ethanol, also known as ethyl alcohol or grain alcohol is a high-octane, water-free alcohol produced from the fermentation of sugar or converted starch. It is a colorless clear liquid with mild characteristic odor that boils at **78°C** and it has no basic or acidic properties and can therefore be used as liquid fuel in internal combustion engines either on its own or blended with petroleum products. The largest single use of ethanol is as a motor fuel and fuel additive.

2.3 Current status of ethanol and sugar production in Ethiopia

The worldwide recent awareness for the use of ethanol to replace petroleum and generation of Power along with sugar mill plants should have led to setting up of number of ethanol plants and Co-generations. There is only one sugar mill producing ethanol & few distilleries participating in Downstream chemicals from alcohol in the country at present due to poor economy of scales. Among molasses derived products ethanol takes the largest part, but its utilization must attract the attention of the government policy makers in order to utilize as a bio-ethanol. Bio-ethanol or Bio-fuel is ethanol-based products that can process into liquid fuels for either transport or heating purposes. With the coming into being of the sugar sector expansion and modernization in the country, implementation of the different domestic measures for bio-ethanol fuels utilization has to take place. At present there was about 8000 cubic meter annual production of ethanol.

Ethiopia started modern sugar industry in 1951 at Wonji sugar factory as a share company founded by Ethiopian government and foreign investors followed by Shao and Metehara sugar factories in 1962 and 1969 respectively. Five years later, in 1974, all sugar factories became in charge of the government ownership following the change of government and started operating under the Ethiopian Sugar Corporation which has embarked upon alcohol production program from molasses for use as additive to gasoline in 1979.

Table 2.1 Ethanol from Molasses Distillation in Li

Ethanol production	2006/7	2007/8	2008/9	2009/10	2010/11	2011/12
Finchaa	8,000	8,000	17,000	18,600	18,600	18,600
Wonji/shoa		12,245	17,809	20,836	25,153	25,153
Metehara				17,676	21,301	24,480
Tendaho			23,296	47,508	64,051	60,616
Total	8,000	20,245	58,105	104,620	129,106	128,849

2.4 Raw material source identification

Acetic acid also called ethanoic acid is organic compound. Acetic acid produced via fermentation. Its pathway is conversion of glucose to ethanol and ethanol to acetic acid. In first step, *Saccaromyces cerevesiae* (yeast) converts fermentable sugar of molasses into ethanol and carbon dioxide. In second step, *acetobacter aceti* (acetic acid bacteria) converts ethanol into acetic acid and water.

Ethyl alcohol may be produced from any fermentable sugar by yeast under suitable conditions. Since starches and certain other carbohydrates may be hydrolyzed to fermentable sugars by biological or chemical means there are available many possible sources of sugar.

➤ The raw material may be classified into three principle types.

✓ Saccharine Materials

These types of materials are sugar-cane, sugar beets, molasses and fruity juices.

✓ Starchy Material

The starchy materials include cereals, corn, potatoes, malt, oats, rye, barley, rice, wheat, grain etc.

✓ Cellulosic Material

This type includes wood and waste sulfite liquor. The starch in the corn is fermented into sugar, which is then fermented into alcohol. Other crops such as, barley, wheat, rice, sorghum, sunflower, potatoes, sugar cane and sugar beets can also be used to produce ethanol.

Sugar cane and sugar beets are the most common ingredients for ethanol in other parts of the world. Since alcohol is created by fermenting sugar, sugar crops are the easiest ingredients to convert into alcohol. Brazil, the country with the world's largest ethanol production, makes most of its ethanol this way. Today, many cars in Brazil operate on ethanol made from sugar cane.

A. Molasses

Molasses is defined as final residue obtained in the preparation of sugar. It is the residual syrup incapable of crystallizing after concentration or cooling is called Blackstrap Molasses in a raw sugar house and refinery black strap or barrel syrup in a refinery. It is a dark viscous product with a bitter taste. The approximate weight of one gallon is 12.3lb.

One liter=1.474kg

The US Customs Laboratory defines blackstrap as molasses in which the non-sugar are more than 6% of the total soluble solids excluding any foreign substance that may have been added or developed in the product. It is roughly estimated that 4 to 5 tons of molasses are required preparing one ton of alcohol. It has been found that on average 4 tons of molasses (88brix) sp. gravity 1.47, is produced from every 100 tons of cane crushed.

Composition of blackstrap molasses:

Final molasses is composed of organic and inorganic matter and water. About 52% of blackstrap molasses is total sugar about 105 or more in inorganic salts or ash. 10-20% water and the balance is organic matter. Potassium and sodium salts are considered as molasses forming.

The analysis of a 50% sugar containing molasses has the sugar.

Inverted sugar (glucose and fructose) =12-18%

Non fermentable sugars (lactulose) =6-7 %

So in the 50% sugars fermentable sugars are about 44% and about 6% are non-fermentable sugars are present

The analysis of blackstrap and particularly of sugars in it may vary considerably depending on the variety of sugarcane soil, climate, period of the crop, efficiency of factory operation, system of sugar boiling, type and capacity of crystallizers, etc.

Table 2.2Analysis of molasses

%age (usual rang)	Component
17-25	Water
35-40	Sucrose
4-9	Dextrose
5-14	Laevulose
1-5	Other reducing substance
2-5	Other carbohydrate
7-15	Ash
2-6	Nitrogenous compound
2-8	Nitrogenous acid
0.1-1	Wax, sterols, pigment

B. Water

The water is that used to distillation or to make pure acetic acid is soft water. Soft water is water which has relatively low concentration of calcium carbonate and other ions. It describes types of water that contain few or no minerals like calcium or magnesium ions. The term is usually relative to hard water, which does contain significant amounts of such ions. Hard water may be better for the heart than soft water. Its calcium and magnesium ions may be a dietary supplement for some. The main disadvantage of hared water is it deposits calcium carbonate on pipes, especially hot water pipes. This is called “scale formation”. It may cause blockage of pipes and reduce boiler efficiency. In all fermentation process water plays high role to dilute high molasses brick (78-85%) to low brick molasses (10-16) use water. Water is also used as a general cleaning.

C. Yeast

Certain types of yeast are desirable, namely, those which are able to produce and tolerate high concentrations of alcohol and which possess uniform and stable characteristics. Strains of *saccharomyces cerevisiae* are commonly used, but other yeast's such as *S.anamensis* and *schizosaccharomyces pombe*, may be employed under certain conditions. Having selected the yeast for fermentation and having isolated it in pure culture, a starter is then prepared. A starter of large volume is required to "pitch" (inoculate) the main mash, which frequently may have a magnitude of several thousand gallons.

D. Di ammonium phosphate (DAP)

Di ammonium phosphate (DAP) is the world's most widely used phosphorus (p) fertilizer. It is made from two common constituents in the fertilizer industry and it is because of its relatively high nutrient content and its excellent physical properties. Di ammonium phosphate is used during fermentation it is a main food for yeast.

E. Sulfuric acid (H_2SO_4)

The concentrated H_2SO_4 of 96% concentrated is added at the unit of low bricks mash preparation. During the production of yeast the low bricks molasses syrup is prepared. The H_2SO_4 is added to the prepared low bricks tank, reaction takes place & transferred to yeast production tank. These is because of

- ◆ To restrict the production of bacteria & the movement of unwanted yeast & ready it for fermentation.
- ◆ To make the good & comfortable yeast PH & quality
- ◆ To reduced unnecessary yeast that share the fermentation yeast used to ferment ale.
- ◆ Creates good environment for yeast & give good quality alcoholic ale.

F. Acetic acid bacteria (AAB)

Acetic acid bacteria (AAB) are important micro-organisms in the food and biotechnological industries because of their ability to oxidize many types of sugar and alcohols. The production of acetic acid is one of the most important industrial processes in which these bacteria are involved.

2.5 Raw Materials for acetic acid Production process

Acetic acid is produced industrially both synthetically and by bacterial fermentation. About 75% of acetic acid made for use in the chemical industry is made by the carbonylation of methanol, explained below. The biological route accounts for only about 10% of world production, but it remains important for the production of vinegar because many food purity laws require vinegar used in foods to be of biological origin. As of 2003–2005, total worldwide production of virgin acetic acid was estimated at 5 Mt/a (million tonnes per year), approximately half of which was produced in the United States. European production was approximately 1 Mt/a and declining, while Japanese production was 0.7 Mt/a.[15][14] Another 1.5 Mt were recycled each year,

bringing the total world market to 6.5 Mt/a. Since then the global production has increased to 10.7 Mt/a (in 2010), and further; however, a slowing in this increase in production is predicted.[17] The two biggest producers of virgin acetic acid are Celanese and BP Chemicals.

2.5.1 Methanol carbonylation

Most acetic acid is produced by methanol carbonylation. In this process, methanol and carbon monoxide react to produce acetic acid according to the equation:



The process involves iodomethane as an intermediate, and occurs in three steps. A catalyst, metal carbonyl, is needed for the carbonylation (step 2).

1. $\text{CH}_3\text{OH} + \text{HI} \rightarrow \text{CH}_3\text{I} + \text{H}_2\text{O}$
2. $\text{CH}_3\text{I} + \text{CO} \rightarrow \text{CH}_3\text{COI}$
3. $\text{CH}_3\text{COI} + \text{H}_2\text{O} \rightarrow \text{CH}_3\text{COOH} + \text{HI}$

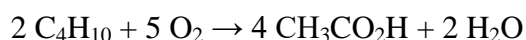
Two related processes for the carbonylation of methanol: the rhodium-catalyzed Monsanto process, and the iridium-catalyzed Cativa process. The latter process is greener and more efficient [18] and has largely supplanted the former process, often in the same production plants. Catalytic amounts of water are used in both processes, but the Cativa process requires less, so the water-gas shift reaction is suppressed, and fewer by-products are formed.

By altering the process conditions, acetic anhydride may also be produced on the same plant using the rhodium catalysts. [19]

2.5.2 Acetaldehyde oxidation

Prior to the commercialization of the Monsanto process, most acetic acid was produced by oxidation of acetaldehyde. This remains the second-most-important manufacturing method, although it is usually not competitive with the carbonylation of methanol. The acetaldehyde can be produced by hydration of acetylene. This was the dominant technology in the early 1900s. [20]

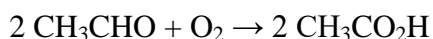
Light naphtha components are readily oxidized by oxygen or even air to give peroxides, which decompose to produce acetic acid according to the chemical equation, illustrated with butane:



Such oxidations require metal catalyst, such as the naphthenate salts of manganese, cobalt, and chromium.

The typical reaction is conducted at temperatures and pressures designed to be as hot as possible while still keeping the butane a liquid. Typical reaction conditions are 150 °C (302 °F) and 55 atm. Side-products may also form, including butanone, ethyl acetate, formic acid, and propionic acid. These side-products are also commercially valuable, and the reaction conditions may be altered to produce more of them where needed. However, the separation of acetic acid from these by-products adds to the cost of the process.

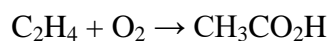
Under similar conditions and using similar catalysts as are used for butane oxidation, the oxygen in air to produce acetic acid can oxidize acetaldehyde. [21]



Using modern catalysts, this reaction can have an acetic acid yield greater than 95%. The major side-products are ethyl acetate, formic acid, and formaldehyde, all of which have lower boiling points than acetic acid and are readily separated by distillation.

2.5.3 ETHYLENE OXIDATION

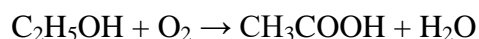
Acetaldehyde may be prepared from ethylene via the Wacker process, and then oxidised as above. In more recent times, chemical company Showa Denko, which opened an ethylene oxidation plant in Ōita, Japan, in 1997, commercialised a cheaper single-stage conversion of ethylene to acetic acid. [22] The process is catalyzed by a palladium metal catalyst supported on a heteropoly acid such as silicotungstic acid. Similar process uses the same metal catalyst on silicotungstic acid and silica: [23]



It is thought to be competitive with methanol carbonylation for smaller plants (100–250 kt/a), depending on the local price of ethylene. The approach will be based on utilizing a novel selective photocatalytic oxidation technology for the selective oxidation of ethylene and ethane to acetic acid. Unlike traditional oxidation catalysts, the selective oxidation process will use UV light to produce acetic acid at ambient temperatures and pressure.

2.5.4 Oxidative fermentation

For most of human history, acetic acid bacteria of the genus *Acetobacter* have made acetic acid, in the form of vinegar. Given sufficient oxygen, these bacteria can produce vinegar from a variety of alcoholic foodstuffs. Commonly used feeds include apple cider, wine, and fermented grain, malt, rice or potato mashes. The overall chemical reaction facilitated by these bacteria is:



A dilute alcohol solution inoculated with *Acetobacter* and kept in a warm, airy place will become vinegar over the course of a few days. Industrial vinegar-making methods accelerate this process by improving the supply of oxygen to the bacteria. [24]

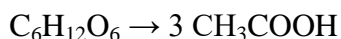
The first batches of vinegar produced by fermentation probably followed errors in the winemaking process. If must is fermented at too high a temperature, *acetobacter* will overwhelm the yeast naturally occurring on the grapes. As the demand for vinegar for culinary, medical, and sanitary purposes increased, vintners quickly learned to use other organic materials to produce vinegar in the hot summer days before the grapes were ripe and ready for processing into wine. This method was slow, however, and not always successful, as the vintners did not understand the process. [35]

One of the first modern commercial processes was the "fast method" or "German method", first practised in Germany in 1823. In this process, fermentation takes place in a tower packed with wood shavings or charcoal. The alcohol-containing feed is trickled into the top of the tower, and fresh air supplied from the bottom by either natural or forced convection. The improved air supply in this process cut the time to prepare vinegar from months to weeks.

Nowadays, most vinegar is made in submerged tank culture, first described in 1949 by Otto Hromatka and Heinrich Ebner. In this method, alcohol is fermented to vinegar in a continuously stirred tank, and oxygen is supplied by bubbling air through the solution. Using modern applications of this method, vinegar of 15% acetic acid can be prepared in only 24 hours in batch process, even 20% in 60-hour fed-batch process. [26]

2.5.5 Anaerobic fermentation

Species of anaerobic bacteria, including members of the genus *Clostridium* or *Acetobacterium* can convert sugars to acetic acid directly without creating ethanol as an intermediate. The overall chemical reaction conducted by these bacteria may be represented as:



These acetogenic bacteria produce acetic acid from one-carbon compounds, including methanol, carbon monoxide, or a mixture of carbon dioxide and hydrogen:



This ability of *Clostridium* to metabolize sugars directly, or to produce acetic acid from less costly inputs, suggests that these bacteria could produce acetic acid more efficiently than ethanol-oxidizers like *Acetobacter*. However, *Clostridium* bacteria are less acid-tolerant

than *Acetobacter*. Even the most acid-tolerant *Clostridium* strains can produce vinegar in concentrations of only a few per cent, compared to *Acetobacter* strains that can produce vinegar in concentrations up to 20%. At present, it remains more cost-effective to produce vinegar using *Acetobacter*, rather than using *Clostridium* and concentrating it. As a result, although acetogenic bacteria have been known since 1940, their industrial use is confined to a few niche applications. [28]

2.6 CHEMICAL AND PHYSICAL PROPERTIES

2.6.1 Molasses Properties

Computation of molasses

- Total Solids: 75 to 88 % Wt.
 - Total reducing sugars: 44 to 60 % Wt. (52%)
 - Unfermentable Sugars: 4 to 5 % Wt. (5%)
 - Fermentable Sugars: 40 to 55 % Wt. (48%)
- Total In organics: 8 to 12 % Wt.
 - Settable dry sludge : < 3.5% Wt.
 - Specific Gravity : 1.38 to 1.52 (1.4)
 - Titrable volatile acidity : 3000-20,000 ppm
 - pH at 40 deg. Dilution : 4.5 to 5.6
 - Caramel(OD) : 0.2 to 0.6

TABLE 2.3 COMPOSITION OF MOLASSES

Water	20%
Sucrose	35%
Glucose	7%
Fructose	9%
Ashes	12%
Wax,colorants,carbohydrates	17%

2.6.2 Ethanol properties

Chemical and physical properties of ethanol

Ethyl Alcohol (otherwise known as ethanol) is one of the most widely used chemicals in the world, and provides a plethora of benefits. It is used as a fuel for combustion engines, for disinfection, as a drink, and more.

Molecular formula of ethanol

The molecular formula of ethyl alcohol/ethanol is $\text{CH}_3\text{CH}_2\text{OH}$. It can be abbreviated to $\text{C}_2\text{H}_6\text{O}$. The 'C' stands for carbon, and the 'H' stands for hydrogen. Each ethanol molecule contains two carbon atoms, 6 hydrogen atoms, and one oxygen atom.

Heat capacity of ethanol

The heat capacity of ethanol is 111.46 J/(mol K).

Appearance, Odor and Taste

At room temperature, ethanol is a clear, colorless, volatile liquid with a characteristic odor.

When diluted, it is somewhat sweet, but concentrated alcohol has a strong, burning taste.

Solubility

Ethanol is highly soluble in water and organic solvents, but poorly soluble in [fats](#) and oils.

Density

Density of ethanol at 68 °F (20 °C) is 0.789 g/mL

pH

Pure ethanol is neutral (pH ~7). Most alcoholic beverages are more or less acidic: table wine pH = 3.3-3.7, beer pH ~ 4.

Boiling Point

Boiling point of ethanol is 173.3 °F (78.5 °C)

Melting Point

Melting point of ethanol is -173.4 °F (-114.1 °C)

Freezing Point

- Wine (10% alcohol by volume): 25 °F (-4 °C)
- Vodka (40% alcohol by volume): -10 °F (-23 °C)
- Pure alcohol (100% alcohol by volume): -175 °F (-115 °C) ^[5]

Flash Point

Flash point is the temperature at which ethanol is flammable – can catch fire:

- Beer (5% alcohol by weight) can catch fire, if ignited, at 144 °F (62 °C),
- Wine (10% alcohol by weight) at 120 °F (49 °C),
- Vodka (40% alcohol by weight) at 79 °F (26 °C) ^[5] and
- Concentrated alcohol (96% alcohol by weight) can catch fire at 63 °F (17 °C) ^[5].

Table2. 4 Ethanol physical properties.

Properties	Ethanol
Flash point	55°F
Ignition temeprature	793°F
Specific gravity	0.79
Vapour density	1.49
Vapour presure	44mmHg
Boiling point	173°F or 78.5 ⁰ c
Flammable range	3.3% 19%
Conductivity	Yes
Smoke character	Slightly to none
Toxicity	Lower
Soulibity	Highly

2.6.3 Physical and Chemical Properties of acetic acid

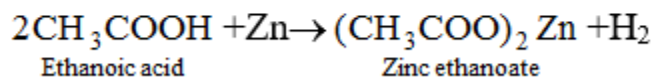
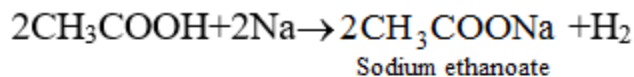
Physical properties:

- (i) Ethanoic acid is vinegar smelling liquid. The lower carboxylic acids are liquids whereas higher ones are solids.
- (ii) Ethanoic acid is sour in taste. Other lower carboxylic acids are also sour in taste.
- (iii) Ethanoic acid has boiling point 391 K. Carboxylic acids have higher boiling points than corresponding alcohols, aldehydes and ketones.
- (iv) Acetic acid is soluble in water, i.e., it is miscible with water in all proportions. The lower carboxylic acids are soluble in water but solubility in water decreases with increase in molecular weight.
- (v) Acetic acid freezes at 290 K. Thus, in cold weather crystallization of acetic acid may take place that is why pure acetic acid is called glacial acetic acid.

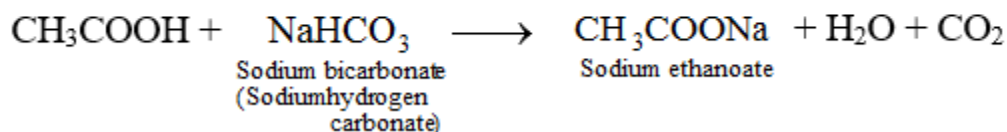
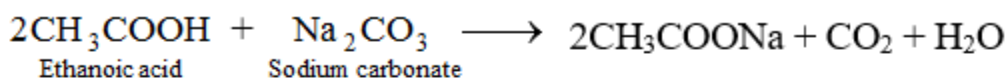
Chemical Properties:

- (i) Ethanoic acid is weak acid but it turns blue litmus red.

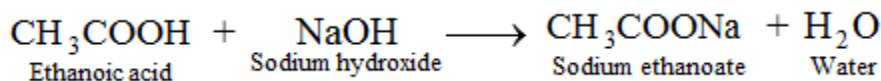
(ii) Reaction with Metale. Ethanoic acid reacts with metals like Na, K, Zn etc. to form metal ethanoates and hydrogen gas.



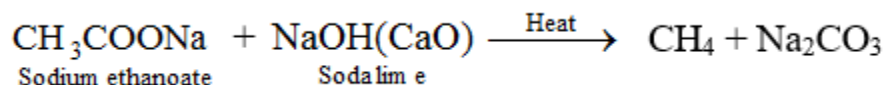
(iii) Reaction with Carbonates. Ethanoic acid reacts with bicarbonates and carbonates and produces brisk effervescence due to formation of carbon dioxide, CO₂.



(iv) Reaction with Base. Ethanoic acid reacts with sodium hydroxide to form sodium ethanoate and water.

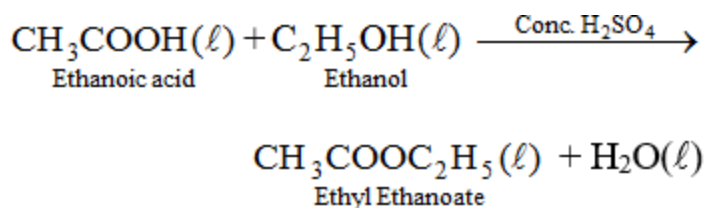


(v) Decarboxylation (Removal of CO₂) When sodium salt of ethanoic acid, i.e., sodium ethanoate is heated with soda lime (3 parts of NaOH and 1 part of CaO), methane gas is formed.



This reaction is known as decarboxylation because a molecule of CO₂ is removed from a molecule of acid.

(vi) Reaction with alcohols. Ethanoic acid reacts with ethanol in presence of concentrated sulphuric acid to form esters which are pleasant fruity smelling compounds.



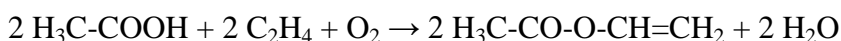
2.7 Important Uses & Applications of Acetic Acid

1. Production of chemical compounds

Acetic acid is used as a chemical reagent for the production of a number of chemical compounds. It is mainly used in the production of vinyl acetate monomer, acetic anhydride and ester production.

➤ Vinyl acetate monomer

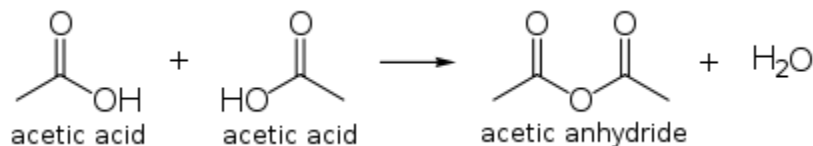
The major use of acetic acid is for the production of vinyl acetate monomer (VAM). This application consumes approximately 40 to 45 percent of the world's production of acetic acid. The reaction is of ethylene and acetic acid with oxygen over a palladium catalyst.



Vinyl acetate can be polymerised to polyvinyl acetate or to other polymers, which are applied in paints and adhesives.

➤ Acetic anhydride

The condensation product of two molecules of acetic acid is acetic anhydride. The worldwide production of acetic anhydride is a major application, and uses approximately 25 to 30 percent of the global production of acetic acid. Acetic anhydride may be produced directly by methanol carbonylation bypassing the acid, and Cativa plants can be adapted for anhydride production.



Acetic anhydride is a strong acetylation agent. As such, its major application is for cellulose acetate, a synthetic textile also used for photographic film. Acetic anhydride is also a reagent for the production of aspirin, heroin, and other compounds.

2. Purification of organic compounds

To purify organic compounds, acetic acid is used as a solvent for re crystallization.

Glacial acetic acid is an excellent polar protic solvent, as noted above. It is frequently used as a solvent for recrystallisation to purify organic compounds. Pure molten acetic acid is used as a solvent in the production of terephthalic acid (TPA), the raw material for polyethylene terephthalate (PET). Although currently accounting for 5–10 percent of acetic acid use worldwide, this specific application is expected to grow significantly in the next decade, as PET production increases.

Acetic acid is often used as a solvent for reactions involving carbocations, such as Friedel-Crafts alkylation. For example, one stage in the commercial manufacture of synthetic camphor involves a Wagner-Meerwein rearrangement of camphene to isobornyl acetate; here acetic acid acts both as a solvent and as a nucleophile to trap the rearranged carbocation. Acetic acid is the solvent of choice when reducing an aryl nitro-group to an aniline using palladium-on-carbon.

Glacial acetic acid is used in analytical chemistry for the estimation of weakly alkaline substances such as organic amides. Glacial acetic acid is a much weaker base than water, so the amide behaves as a strong base in this medium. It then can be titrated using a solution in glacial acetic acid of a very strong acid, such as perchloric acid.

3. Medical Use

Acetic acid can be used as an antiseptic against pseudomonas, staphylococci, enterococci, streptococci and others. It is also used in cervical cancer screening. World Health Organization has listed acetic acid in its list of essential medicines. It is also used for the treatment of outer ear infections like the growth of fungus and bacteria.

4. Food additive

Acetic acid is used in vinegar, which is used as a condiment & in the pickling of raw vegetables and other foods.

5. Manufacture of inks and dyes

Acetic acid is used for the manufacture of inks and dyes.

6. Perfume industry

Acetic acid is also used in making perfumes.

2. 8PROCESS DESCRIPTION OF ACETIC ACID FROM MOLASSES

2.8.1 Ethanol production process

Ethanol producing plants practice a combination of physical and biological processes in the production. Fermentation of sugar with yeast followed by concentration to ethanol grade by distillation is employed to produce the ethanol. There are three sub-units called molasses treatment, fermentation, and distillation which are implemented in the process of bio-ethanol production. These are described below in brief as explained and understood during the literature review and the site visit.

I. Molasses treatment:

The first sub-unit in the production of ethanol is molasses treatment. This stage mainly focuses on a reduction in the level of impurities. This assures better performance regarding distillation where the reduction of impurities is significant and allows improved yields and lower steam consumption. Thus, molasses that comes from the sugar factory with a concentration of 86⁰ Brix goes through heating to a temperature of 95 ⁰c to 100 ⁰c and dilution using process water and steam condensate to 50⁰ Brix in order to reduce its viscosity. While heating, acidification is carried out using sulphuric acid to a pH of 4.7 to 4.9 before it is sent to decanters in order to take out solid materials through sedimentation. The diluted juice in decanters is then cooled to a temperature of 55⁰c to 60⁰c and diluted more to a final concentration of 20 Brix to which is now named Mash. This Mash is now free of huge fraction of the impurities and is appropriate to obtain good quality fermentation.

II. Fermentation stage

The second sub-unit in the production of ethanol from molasses is fermentation which has two stags.

- The first stage, yeast propagation, is a pre-fermentation process aiming to achieve optimal yeast cell concentration needed for fermentation.
- Fermentation

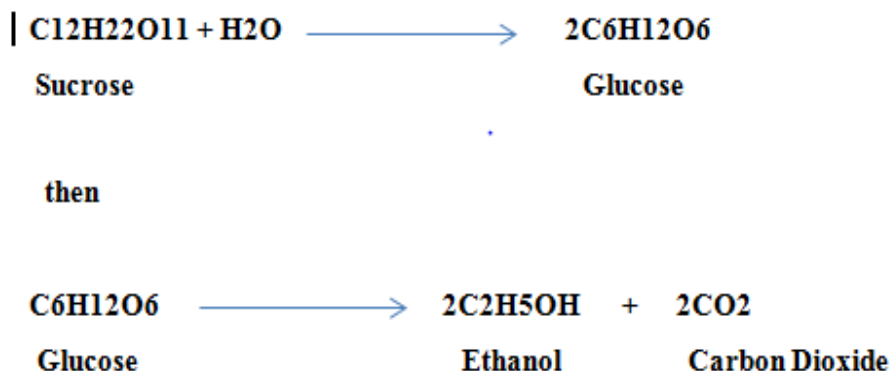
III. Yeast propagation:

The process is referred as aerobic fermentation as it is supplied with air and nutrients. The nutrients to be supplemented are nitrogen and phosphorous, because of the poor content of these nutrients in the raw material (molasses) for the yeast to propagate and be active. Nitrogen, added

in the form of ammonia sulphate, is significant for both cell multiplication and fermentation phases mainly as a result of protein and nucleic acid synthesis. Thus, cell growth, fermentation speed, and the productivity of fermentation decrease when Nitrogen deficiency occurs.

IV. Fermentation

The second stage, final fermentation, is a process wherein alcohol and carbon dioxide are produced. The complete fermentation process needs around 24 to 30 hours, with resulting beer (Fermented mash) containing 7% to 9% ethanol by volume. The fermentation process operates in batch mode and converts the fermentable sugars in the feed stock into alcohol using yeast. It can be defined as a process in which chemical changes are brought about in the organic substrate through the action of biochemical catalysts, called enzymes, elaborated by specific types of living microorganism. Yeast is added to the mash to ferment the sugars to ethanol and carbon dioxide. Using a continuous process, the fermenting mash flows through several fermenters until the mash is fully fermented and leaves the tank. In a batch fermentation process, the mash stays in one fermenter.



During the fermentation process, care should be taken to prevent oxygen from getting into contact with the ethanol which would otherwise oxidize to acetic acid.

V. Distillation:

The third sub-unit is distillation, in which the fermented mash is distilled to pull off ethanol. By conventional distillation processes about 96% ethanol by volume can be concentrated, which is called hydrous or technical ethanol and is utilized by beverage industries, pharmaceuticals, and others. The anhydrous bio-ethanol is ethanol that can be used as a fuel blended with gasoline and it should be concentrated further but as the composition forms an azeotrope or a constant boiling, further distillation cannot enhance this percentage. Thus, the remaining water can be removed

through a step that follows conventional distillation called dehydration. Hence, aromatic benzene is added to commercial grade bio-ethanol in order to obtain anhydrous bio-ethanol in the dehydration step. Benzene is chosen because of its lowest price as compared to other solvents and is consumed at about 1 to 2 liters per 1000 liters of bio-ethanol produced

.Production of bio-ethanol is coupled with by-products. The by-products from the plants are carbon dioxide, fuel oil, and spent wash (stillage). Carbon dioxide evolves at the stage of fermentation and is released to the environment. The amount of CO₂ released is as great as the alcohol production. On the other hand fuel oil can be separated at the stage of distillation. It was used to be separated and stored in a specified storage tank. However, it is not the case now as no application has been found and no market has been identified so far and thus it is left to be in the final by-product. The other by-product yet important to consider is the spent wash produced during fermentation that will affect the water body if it is released directly due to its high BOD content and acidity. The spent wash from the bio-ethanol plants has been protected by a pre-treatment unit comprised of neutralization and clarification followed by an effluent treatment unit when the plants are in operation.

VI. Dehydration:

Ethanol is dried using the pressure swing regeneration as opposed to a heating or thermal swing. The wet ethanol containing about 94.68- 95% (v/v) alcohol is fed to the molecular sieve dehydration unit which will remove the water content of the wet alcohol to produce the-anhydrous ethanol of strength of not less than 99.9%. The dehydration unit consists of two fixed adsorption beds which operate in alternative, that is, while one bed is dehydrating, the other one is regenerating. Molecular sieves are synthetic zeolites (crystalline aluminum silicates) that have strong affinities for water. The zeolites adsorb water at low temperature conditions and they desorb the water molecules when heated. The complex crystalline structure of the zeolites give the material the ability to absorb or reject materials based on their molecular sizes. The ethanol-water mixture passes over the zeolite beads and the pressure forces the water into the sieve pores where it adsorbs to the walls of the internal cavities. The ethanol molecules, being too large to enter the pores, pass over the beads and through the bed leaving the water behind. The purified ethanol vapor is passed through a condenser where it is condensed with the help of cooling water. Condensed ethanol is collected in the product receiver.

2.8.2 Acetic acid production process

1 Fermentation

Dilute solution of ethanol was charged in to fermenter. Oxygen in the form of air was supplied through the solution of alcohol in fermenter. An aerobic bacterium like acetobacter was added into the fermenter for aerobic fermentation. Oxidation of ethanol was carried out at 26 – 36⁰C temperature.

The fermentation process operates in batch mode and converts the ethanol in the feed stock into acetic acid using acetic acid bacteria.

2 Distillations

Distillation is a physical process where compounds are separated by virtue of their different boiling points and vapor pressures. The separation in distillation occurs when a mixture of compounds in the still is brought to boil. As a simplification, assume that the still contains only acetic acid and water. Water has a boiling point of 100°C and acetic acid has a boiling point of 118°C.

Block diagram and process flow diagram

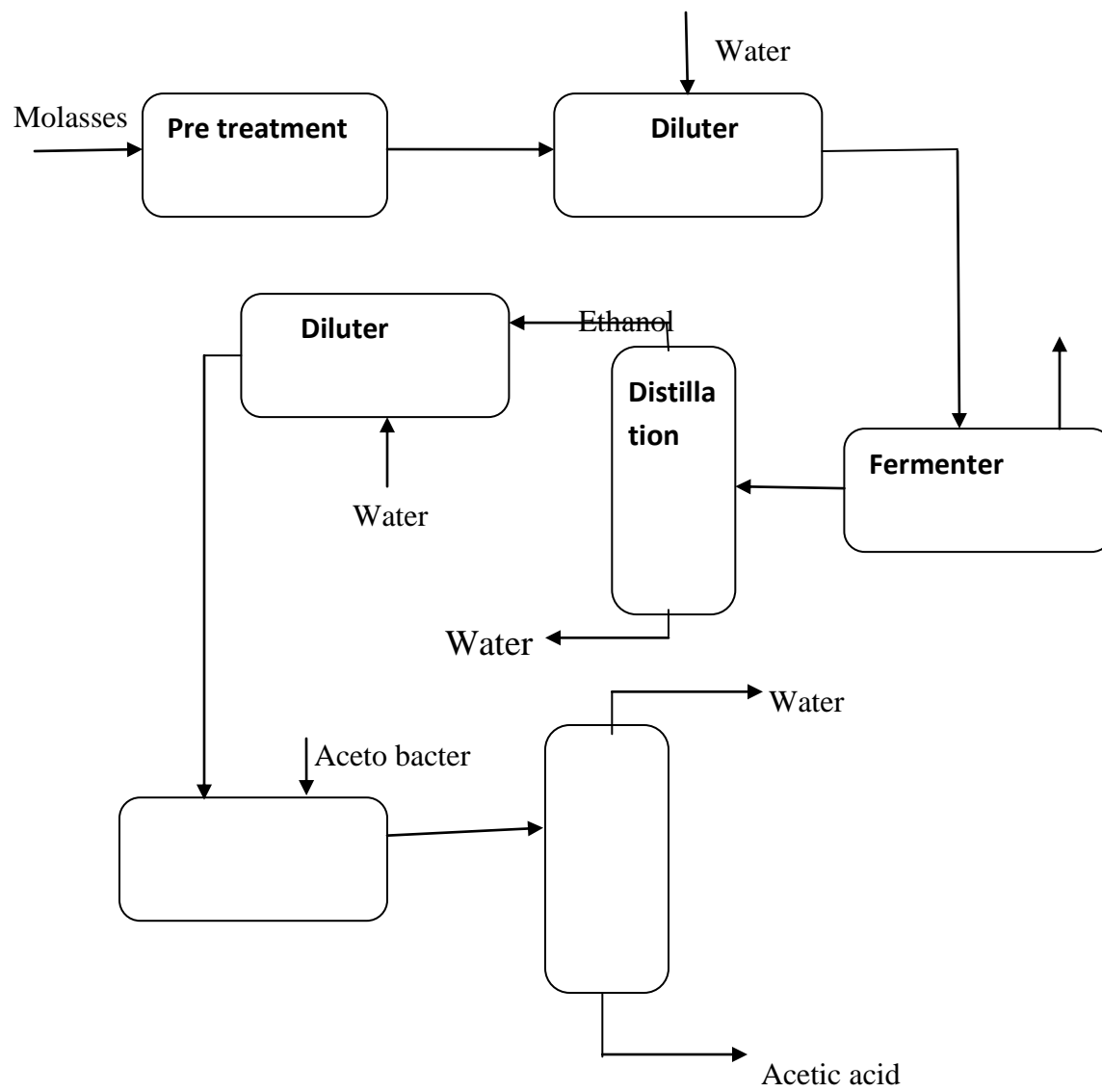


Figure2.1 production of acetic acid

CHAPTER THREE

3.METHODOLOGY

3.1 MATERIALS AND METHOD

3.1.1 Materials

1. Molasses

Molasses is defined as final residue obtained in the preparation of sugar .It is the residual syrup incapable of crystallizing after concentration or cooling is caller Blackstrap Molasses in a raw sugar house and refinery black strap or barrel syrup in a refinery. It is dark viscous product with a bitter taste.

Molasses were obtained from a sugar factory and were used as a fermentation medium.

Table 3.1 Composition of the sugar cane molasses used for experiment

Molasses type	Water content(%)	Soluble solid	Total sugar	Total N ₂	Mineral substance	PH
Sugar cane molasses	18.2	81.8	54.6	0.5	6.2	7.6

Sugar cane molasses were diluted with water to a resultant sugar content of 180-200 g/l. pH value of culture media was adjusted with 10 % v/v sulphuric acid to the value of 4.50.

2. Yeast

yeast is the most important ingredient in ethanol manufacturing and a single-celled organism .yeast is living creature,metabolizig,reproducing, and living of ingredient in ethanol. it is responsible for converting sugar into alcohol and carbondioxide(CO₂) in fermetation stage.

3. Acetic acid bacteria

Acetic acid bacteria is a large group of Gram-negative bacteria and strict aerobe,which has a marked feature that when ethanol is exposed to air and under the function of vinegar bacteria, it can be converted into acetic acid and water. Acetobacteraceae belongs to Proteobacteria, Alphaproteobacteria, Rhodospirillales, which include Acetobacter and Gluconobacterasia. The

oxidation of glucose is stronger for Acetobacteraceae. Therefore, most acetic acid is produced by Acetobacter. Acetic acid bacteria are the main strains which can determine the yield and quality of acetic acid.

Equipment

- .pre-heater: to heat the solution for killing micro organism
- Digital pH meter: - to measure the pH of the hydrolyzes before fermentation.
- Autoclave: - for sterilization and hydrolysis.
- Fermenter: to ferment the mixed solution
- Distillation: to produce ethanol and acetic acid
- Bicker
- Incubator
- Measuring cylinder
- Titrants
- Alcohol measuring instrument
- Titrand
- petri dishes

Chemicals

- ✓ Sulfuric Acid (H_2SO_4):- used as a pretreatment and hydrolysis sawdust.
- ✓ Sodium Hydroxide (NaOH):- used to adjust the pH of molasses before fermentation.
- ✓ Distilled water
- ✓ Iodine solution: used for identifying whether presence of sugar solution
- ✓ NaOH :- Acidity by using titration means by using known concentration of NaO determining unknown concentration of acetic acid
- ✓ Phenophitaline
- ✓ Nutrient agar medium
 - Peptone
 - sodium chloride
 - beef extract
 - yeast extract
 - agar and
 - Distilled water

3.1.2METHOD

3.1.2.1 Procedure of experiment ethanol

The followings were basic steps for the production of ethanol alcohol, these processes were:-

- Sample collection.
- A pre- treatment phase of molasses to hydrolysis.
- Hydrolysis to break down the molecules of fructose into simple sugars.
- Distillation to produce alcohol

1 Sample preparation

The sample that was acquired had to be prepared and conditioned for pretreatment, hydrolyze, fermentation and Molasses is defined as final residue obtained in the preparation of sugar .It is the residual syrup incapable of crystallizing after concentration or cooling is caller Blackstrap Molasses in a raw sugar house and refinery black strap or barrel syrup in a refinery and its brix around 88degree brix.

2 Pretreatment

The purpose of the pretreatment was to reduce molasses concentration by diluting with distilled water the ratio 1:4, 1:2, 1:1 because raw material molasses have 88 brix from sugar factor. Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of byproduct inhibitors and must be cost effective.



Figure 3.1 diluted molasses

3 Steam pre-heater

Steam pretreatment uses steam at 121°C temperatures for 15 min the purpose of pre heater was to kill microorganism, first prepared sample was at standard temperature then put into pre-heater which is called autoclave for 121°C, when the temperature was 60°C we can open pressure release valve to live atmospheric pressure for the purpose of sample. At temperature around 90°C pressure release valve should close and wait till temperature reach 120°C keep for 15 min. Finally the samples was kept in autoclave for the given pretreatment time and temperature and allowed to cool.



Figure 3.2 autoclave equipment

Procedures in Steam Pretreatments

- Add 100ml molasses to 500 ml conical flasks
- Add 400ml, 300, 100 of distilled water.
- The conical flasks capped with the help of rubber plugs.
- Autoclave at a temperature of 121°C for 15 minutes.
- After finishing the given pretreatment time and temperature the sample in autoclave and allowed to cool and separate soluble from the non-soluble portion.

4 Acid Hydrolysis

Before it is fermented for alcohol production. Even though there are many types of hydrolysis types, dilute acid hydrolysis is an easy and productive process and the amount of alcohol produced in case of acid hydrolysis is more than that of alkaline hydrolysis. 100ml molasses were used for each experiment and the factors for hydrolysis were time (5 to 30 minutes), hydrolysis temperature (100 to 132°C), and acid concentration (3 to 8%).

Procedures for Acid Hydrolysis

- ❖ Add 10ml of 5% of diluted sulfuric acid to 250ml of molasses. Pretreatment steps in the order of experimental design for all experiment and soak for 24hr.
- ❖ The molasses was then hydrolyzing in the reactor between 100 and 132°C for 5 to 30min.
- ❖ After hydrolysis, neutralize with 10 M NaOH until the pH became around between (5-6) ranges.
- ❖ To obtain the original sugar concentration in the hydrolyzed boil the solution till homogenize the solution then adjust it PH.



Figure 3.3 Diluted by 5% sulfuric acid

PH Adjustment

Before addition of any micro-organism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 5.0 -6.0 is maintained

Procedures in pH adjustment

- > Mix pretreated and hydrolyzed solution, filtered, shaken substrate primarily checked for pH using a digital pH meter. The pH then adjusted to 5.0-6.0.
- > Mix samples (pretreated and hydrolyzed) were acid hydrolyzed, so it needs highly basic solution to bring the pH in the range of 5.0-6.0.
- > Sodium hydroxide solution was added drop wise to the other flask with constant stirring until the pH reaches to a range of 5.0-6.0.

If suppose the pH goes beyond 5.0-6.0, concentrated sulfuric or hydrochloric acid was added drop wise to maintain the pH in the range.

5 Fermentation

The aim of the experiment was to measure the ethanol production by the fungus (*Saccharomyces cerevisiae*) using molasses hydrolyzed as energy and carbon source. The clear solutions then go to fermentation. The fermentation was carried out under anaerobic condition at a temperature of 30-35°C medium, pH 5.5 for 3 days. Mix the following nutrients in there proportion. 4 gm. of yeast, *Saccharomyces cerevisiae* (instant premium) was added in a 250 ml conical flask. The conical flasks were properly covered with aluminum foil.

The procedure of fermentation

The sample was conditioned to temperature of 30°C before fermentation step was started. This was the temperature at which all fermentation experiments were carried out. The parameters of fermentation i.e. fermentation time, yeast concentration and fermentation temperature were set to be at 72 hour and 30°C temperature. And after 72 hours of fermentation, the samples were taken out and distilled

6 Distillation

Distillation was the last step in the production of ethanol. It is the purifications steps. Distillation is the method used to separate two liquid based on their different boiling points. However, to achieve high purification, several distillations are required. In this experiment separation were used by rotary evaporator at a temperature of 85°C for 3hrs. All distillation experiments were carried out at a temperature of 85°C and a distillation time of 3 hours.

3.1.2.2 Procedures of experiment acetic acid

The first activity in order to effective product produced preparation of culture with accurate dilution of percent(%) to produce aceto bacteria at optimum temperature then the produced aceto bacteria go to fermenter with ethanol and also optimum temperature and concentration for 5days

3.1.2.2 .2 Procedures of Acetobacter aceti

Nutrient agar medium. It is general-purpose medium and was used for identification of Acetobacter aceti. Medium composition:

- Peptone
- sodium chloride
- beef extract
- yeast extract
- agar; and
- Distilled water

➤ 7 gram Nutrient agar medium mix 250 ml Distilled water

After dissolving all ingredients completely in distilled water by slight heating, the medium was autoclaved at 121⁰C for 15 minutes at 15 lb pressure. The medium was then cooled to 50-55⁰C and pH of the medium was maintained up to 7.2. Incubation was made at 30⁰C for 48 hours and growth was observed.



Figure 3.4 Nutrient agar stirred by stirrer



figure 3.5 balance nutrient agar



figure 3.6 uniformly



Figure 3.7 Slight heating figure 3.8 Autoclaved at 121°C for 15 minutes figure 3.9 add Petri dishes



Figure 3.10 Incubator figure 3.11 after 2 day growth bacteria

The production of acetic acid

The way production of acetic acid from ethanol through fermentation process by adding acetobacter and ethanol through fermenter there is spontaneously formed acetic acid from ethanol.

Dilute solution of alcohol was first sterilize in autoclave and then charged in to fermenter. Oxygen in the form of air was supplied by bubbling through the solution of alcohol in fermenter. An aerobic bacterium like acetobacter was added into the fermenter for aerobic fermentation. Oxidation of ethanol was carried out at $26 - 36^{\circ}\text{C}$ temperature. Liquid mixture coming out from fermenter containing acetic acid and water was separated in acetic acid by distillation.



Figure 3.12 Fermentation



figure 3.13 distillation



Figure 3.14 Final acetic acid

CHAPTER FOUR

4 RESULT AND DISCUSSION

4.1 Analysis of the experimental results

The process consists of many parts: pretreatment to remove other particle from raw material molasses, reduce its brix of sugar molasses, sterilize the step of homogenize pre-heated sample, dilute acid hydrolysis, fermentation to produce ethanol, distillation to separate ethanol, dilute ethanol production of acetic acid, fermentation to produce acetic acid and distillation to separate acetic acid from water. The experimental outcomes of those particular results are measured. During the experiment many things would be affect our result not to obtain high efficiency product.

Molasses weight (g)	Water added for dilution (ml)	pH	Sugar concentration % (w/v)	Yield% (w/v)
100	400	4.8	10	5.5 ± 0.20
100	200	4.8	15	7.8 ± 0.10
100	100	4.8	20	11 ± 0.40

Table 4.1

Effect of sugarcane molasses concentration

Sugar molasses concentrations were varied from 100ml, 200ml, 400ml sample was taken in experiment but there is varies product have been produced. as the concentration of molasses increase the content of sugar increase in the ethanol production to produce high quality .

Effect of Temperature on Ethanol Product

Fermentation condition is an important parameter of fermentation process. Controlled condition can ensure maximum product from fermentation. In our experimental study Ethanol production is maximized by controlling fermentation temperature by simulation for suitable condition

Samples were maintained in the optimum between pH 5.0 and pH 4.6 with the temperature range from 28, 30, 32, 34, 36 and 38°C as shown in Figures above, The samples were fermented for 48 h; after analyzing the samples it found that the optimum yield was achieved in 34°C and range of 5.0 pH with the fermentation efficiency of 88%.

Effect of fermentation time

For optimum yield, the fermentation time was important when the sample was over fermented it lose its quality as well as when the sample was late also it have not sufficient product was produce therefore to be gain high quality wait the sample until it enough fermentation.

The effect insufficient equipment

There is the effect on production with the shortage of equipment, treatment of ethanol can affectively high quality can produce with distillation process in the case our experimental duration we have not distillation column we in forced to produce our product by use of stock equipment in the place of distillation, such equipment not produce high quality product, therefore our product a little bit affected

Result for acetic acid

ethanol weight (g)	Concentration %	pH	Fermentation Temperature	Yield% (w/v)
100	8		30	
100	10		30	
100	13		30	

Table 4.2

We done by apply three flask in order to test better result by using different concentration of ethanol those concentration are 8%, 10% and 13% ethanol we seen from those concentration was better results can be obtained alcohol is this flask we used 10% ethanol.

Characterization of product

The above product from diagram acetic acid making characterization in order to determined it is acetic acid or not by using parameter

- Acidity
- Alcohol content

➤ PH

Physical property like Oder, color and test

The following table is the result of measuring parameter for characterization

Parameter	Standard value	Measured value
PH	6.78	6.4
Alcohol	12%	16%
Titrateable Acidity	10.47%	5.9

Table 4.3 characterization of acetic acid

How to determine the above parameter

- PH direct measuring by using PH meter
- Alcohol content:-by using picno meter
- Acidity by using titration means by using known concentration of NaOH determining unknown concentration of acetic acid

Method for titration

Material use

- ✓ Phenolphthalein
- ✓ Bicker
- ✓ NaOH
- ✓ Titrant
- ✓ Titrated, Measuring cylinder

Titrateable acidity (TA) was determined as ml of 1N NaOH used to obtain a pink color endpoint with phenolphthalein. Dry phenolphthalein (4-5 droop) was added into each 10ml sample acetic acid solution. The formula to calculate %TA as acetic is as below:

$$\%TA = \frac{(\text{ml of NaOH}) \times (\text{N of NaOH}) \times (60.05)}{10 \times \text{Sample Weight}}$$

3.3. EFFECT OF TEMPERATURE ON ACETIC ACID FORMATION

The temperature the main considering parameter in order to obtain required result from fermentation because fermentation operates under mild condition

The main cause for mild condition of fermentation the involving microorganism (acetobacteria) dies is occur. So without acetobacteria the fermentation or conversion ethanol to acetic acid cannot be successes.

Totally no result without extremely high temperature means there is no aceto bacteria

Then we use the optimum temperature used from fermentation at 30°C is suitable for the cultivation of aceto bacter.

3.4. EFFECT OF ALCOHOL CONTENT ON FERMENTATION OF ACETIC ACID

The alcohol content increase the conversion of acetic acid decrease approximately there was no result occurred because the existence of acetobacteria affected by it these aceto bacteria has the major role on the formation of acetic acid. so we used the alcoholic content 10% was suitable for the propagation of aceto bacter for the production of acetic acid.

CHAPTER FIVE

MATERIAL AND ENERGY BALANCE

Material quantities, as they pass through processing operations, can be described by material balances. Such balances are statements on the conservation of mass. Similarly, energy quantities can be described by energy balances, which are statements on the conservation of energy. If there is no accumulation, what goes into a process must come out. This is true for batch operation. It is equally true for continuous operation over any chosen time interval. Material and energy balances are very important in an industry. Material balances are fundamental to the control of processing, particularly in the control of yields of the products. The first material balances are determined in the exploratory stages of a new process, improved during pilot plant experiments when the process is being planned and tested, checked out when the plant is commissioned and then refined and maintained as a control instrument as production continues. When any changes occur in the process the material balances need to be determined again. The increasing cost of energy has caused the industries to examine means of reducing energy consumption in processing. Energy balances are used in the examination of the various stages of a process, over the whole process and even extending over the total production system from the raw material to the finished product. The energy balance determinations are also made to determine the energy requirements of the process, the heating, cooling and power required.

4.1 Material balance

Basic Assumptions:

- Steady state for all calculations i.e. Input=output
- Production capacity of the plant is = 5000 ton/year
- Raw material capacity of the plant is =12000 ton/year
- Plant working time is=320day/year
- Basis: one operation day/24hr

Material Balance around fermentor(ethanol)

Basics:-amount on final sugar cane molasses supplied from sugar factory is 12000 ton/hr.

Molasses supplied = 12000 ton/year.

According to research article of high level ethanol from sugar cane molasses, the initial concentration of molasses 88 degree brix

After wards feeding of diluted molasses to give final concentration 20% (w/v) sugars was fed to the fermenters to enable yeast cells to utilize sugars in the molasses for conversion into ethanol.

➤ Molasses + process water = 12000 ton/year.*0.88 /0.2 = 52800ton/year.

➤ Process water =52800ton/year –12000 ton/year. = 39800 ton/year.

➤ used 4gm/L yeast extract,

✓ 0.8031kg molasses = 1 litter molasses

✓ 39800000 kg molasses = ?

❖ 495579623 litter molasses

1 litter molasses = 4 gram yeast

495579623 litter molasses = ?

❖ 198231852gram yeast

❖ 198.2 ton/year

➤ Carbon Dioxide

1ton of molasses = 150 kg of CO₂

52800ton/year = ?

❖ 7920 ton/year carbon dioxide

➤ Mass of media 15% of dilute ethanol

Experimental/laboratory material balance

The general material balance equation for a system is represented by

Input = output

- ❖ Input: enters through system boundaries
- ❖ Output: exits through system boundaries

Assumption:

- ❖ Batch reactor
- ❖ Steady state
- ❖ Law of conservation mass

Material balance on diluter (molasses)



$$M1=100 \text{ ml}$$

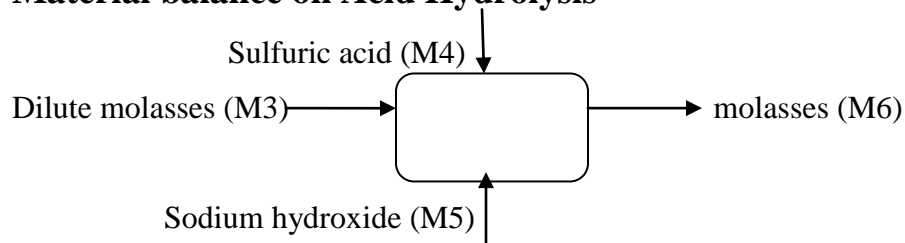
$$\text{Density of molasses} = 1412.42 \text{ kg/m}^3 = 1.412 \text{ gm/ml}$$

$$M1=141.2 \text{ gm}$$

$$M2=100 \text{ gm}$$

$$M3=M1+M2=141.2 \text{ gm}+100 \text{ gm}=241.2 \text{ gm}$$

Material balance on Acid Hydrolysis



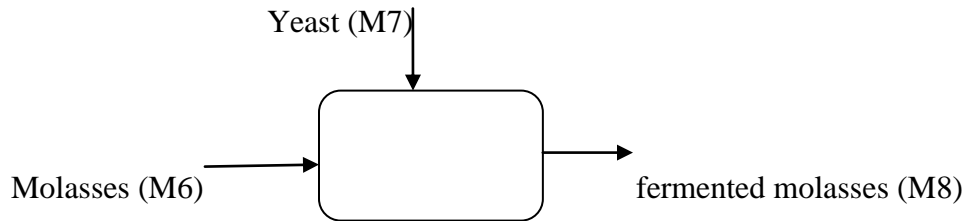
$$M3=241.2 \text{ gm}$$

$$M4=9 \text{ gm}$$

$$M5=3 \text{ gm}$$

$$M6=M3+M2+M5=253.2\text{gm}$$

Material balance on fermentation

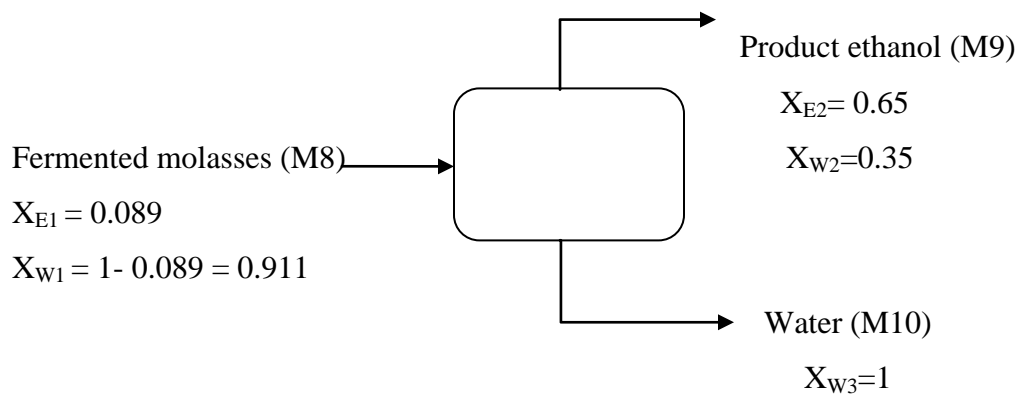


$$M6=253.2\text{gm}$$

$$M7=2\text{gm}$$

$$M8=M6+M7=253.2\text{gm}+2\text{gm}=255.2\text{gm}$$

Material balance on distillation

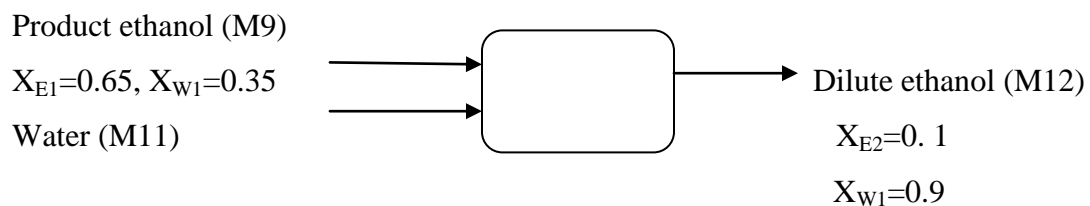


$$M8=200\text{gm}$$

$$M9=200\text{gm} \times 0.089 / 0.65 = 27\text{gm}$$

$$M10=M8-M9=200\text{gm}-27\text{gm}=173\text{gm}$$

Material balance on dilute (ethanol)

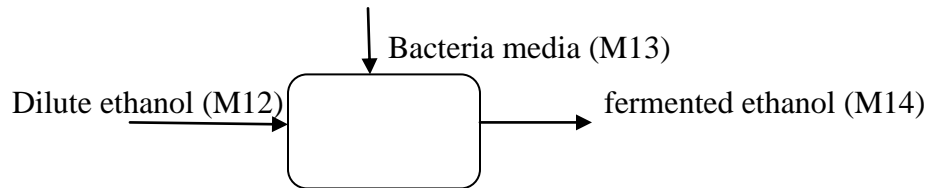


$$M9=20\text{gm}$$

$$M_{11}=110\text{gm}$$

$$M_{12}=M_9+M_{11}=130\text{gm}$$

Material balance on fermentation

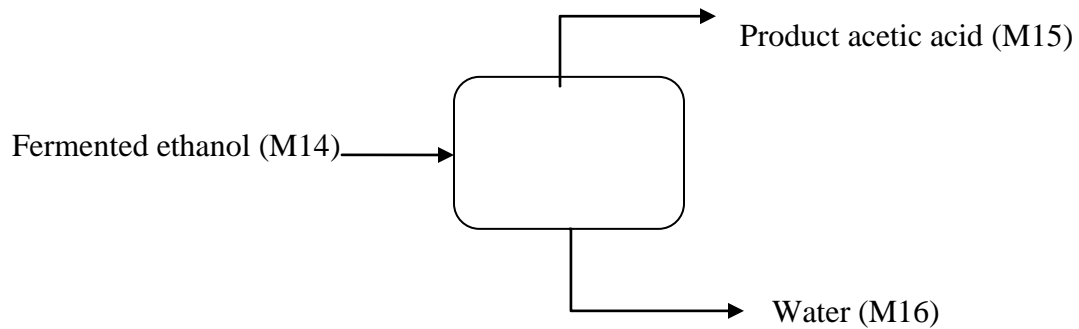


$$M_{12}=130\text{gm}$$

$$M_{13}=15\% \text{ of } M_{12}=0.15 \times 130\text{gm}=19.5\text{gm}$$

$$M_{14}=M_{12}+M_{13}=130\text{gm}-19.5\text{gm}=280.5\text{gm}$$

Material balance on distillation



$$M_{14}=280.5\text{gm}$$

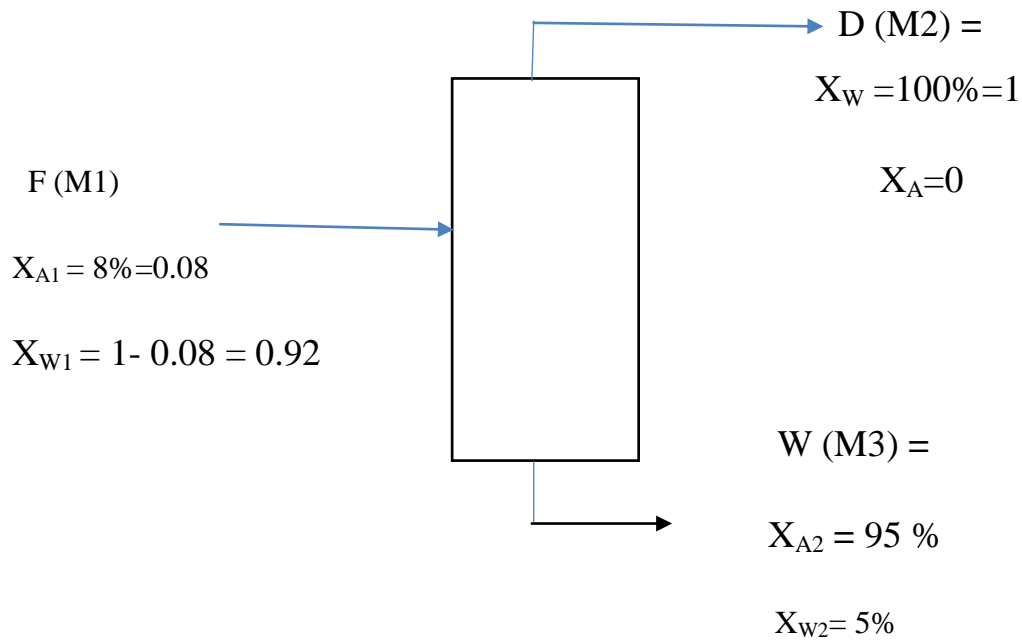
$$M_{15}=$$

$$M_{16}=M_{14}-M_{15}$$

INDUSTRIAL SCALE UP

4.1.1 Material balance on distillation column

It is the equipment used for the purification of acetic acid from water-acetic acid mixture. The mixture from fermentation enter in to the distillation with the mixture of 9.58% acetic acid and the rest water, then with two series distillation column the purification of acetic acid come to 95%



Where, X_{A1} = fraction of acetic acid

X_{w1} = fraction of water

$F (M1)$ - Fermentation broth or hydrous acetic acid

$D (M2)$ - mass of distil (water)

$W (M3)$ - mass of bottom(acetic acid) = 5000ton/year

From acetic acid component balance

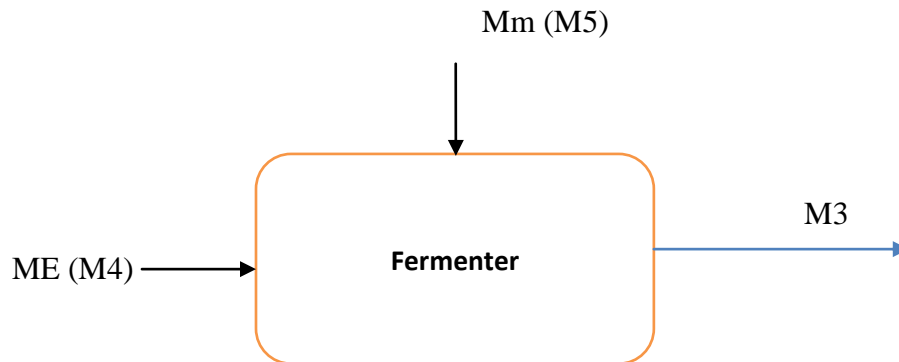
$$F \cdot X_{A1} = W \cdot X_{A2} + D \cdot X_A$$

$$F \cdot 0.08 = 5000 \text{ton/year} \cdot 0.95 + W \cdot 0$$

$$F=59375 \text{ ton/year}$$

$$D=F-W=59375 \text{ ton/year}-5000 \text{ ton/year}=54375 \text{ ton/year}$$

4.1.2 Mass balance for fermenter



➤ From the reaction of fermenter

Ethanol to acetic acid

Where, $M_m (M5)$ = mass of media = 15% of ethanol = 15% $M4$

$M_F (M3)$ = mass of fermented = 59375 ton/year

$M_E (M4)$ = mass of dilute ethanol

$$M4 = M3 - M5$$

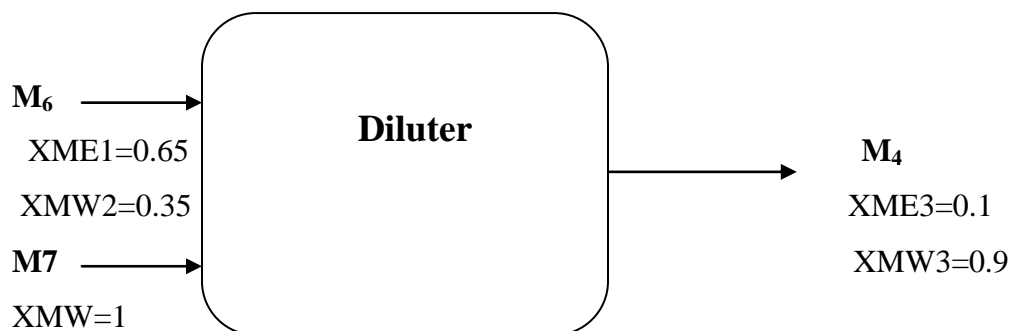
$$= 59375 \text{ ton/year} - 15\% M4$$

$$= 39583 \text{ ton/year}$$

$$M5 = 0.15 M4$$

$$= 59375.75 \text{ ton/year}$$

4.1.3 Material balance on diluter



M_6 = mass of ethanol

M_7 = Process Water

M_4 = ethanol from Diluter= 39583 ton/year

X_{ME} = fraction of acetic acid

X_{MW} = fraction of water

From ethanol component balance

$$M_6 * X_{ME1} = M_4 * X_{MA3}$$

$$M_6 * 0.65 = 39583 \text{ ton/year} * 0.1$$

$$M_6 = 6090 \text{ ton/year}$$

From total mass balance

$$M_6 + M_7 = M_4$$

$$M_7 = M_4 - M_6$$

$$= 39583 \text{ ton/year} - 6090 \text{ ton/year}$$

$$= 33493 \text{ ton/year}$$

4.1.4 Material balance on distillation column (ethanol)

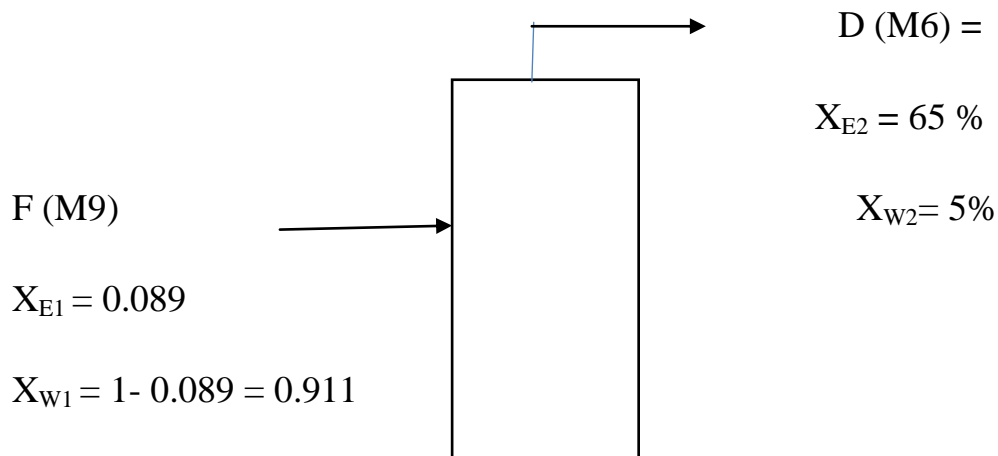
% of ethanol which exits from fermenter and enters into distillation column is=

ethanol=8.89%

$$\text{So, \% water} = 100\% - 8.89\% = 91.11\%$$

65% of ethanol is achieved at the top of distillation column.

100% of water out bottom



$$\begin{array}{l} \longrightarrow \\ W (M8) = \\ X_w = 100\% = 1 \end{array}$$

Where, X_{E1} = fraction of ethanol

X_{w1} = fraction of water

F (M9) - Fermentation broth

D (M6)-mass of distil mass of ethanol=7669.4 ton/year

W (M8)- mass of bottom or water

From ethanol component balance

$$M9 * X_{E1} = M8 * X_{E2} + M6 * X_{E3}$$

$$M9 * 0.089 = M8 * 0 + 6090 \text{ ton/year} * 0.65$$

$$M9 = 44478 \text{ ton/year}$$

From total mass balance

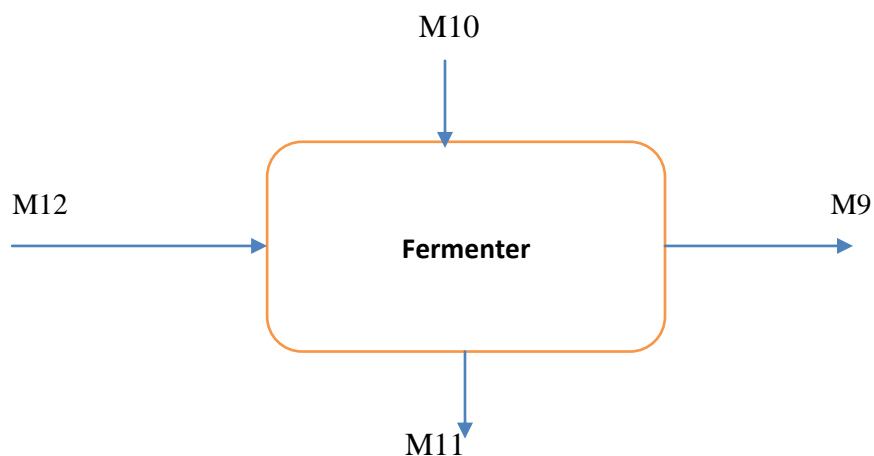
$$M9 = M6 + M8$$

$$M8 = M9 - M6$$

$$= 44478 \text{ ton/year} - 6090 \text{ ton/year}$$

$$= 38388 \text{ ton/year}$$

4.1.5 Material balance on Fermenter



M12- diluted molasses = 52800ton/year.

M10- yeast culture = 198.2 ton/year

M11- carbon dioxide

M9- Fermentation broth

$$\begin{aligned}
 M_{11} &= (M_{12} + M_{10}) - M_9 \\
 &= (52800 \text{ ton/year} + 198.2 \text{ ton/year}) - 44478 \text{ ton/year} \\
 &= 14611 \text{ ton/year}
 \end{aligned}$$

4.1.6 Material balance on diluter



M1- molasses= 12000ton/year

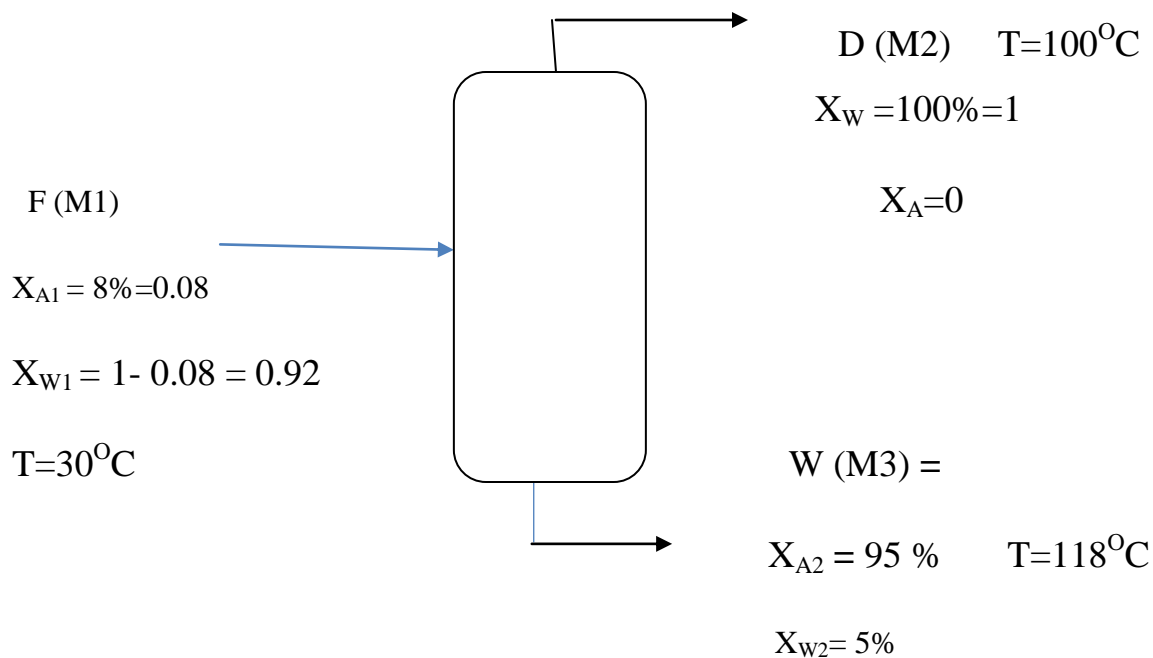
M2- water = 39800

M3- dilute molasses = 52800ton/year

4.2 Energy balance

Energy is an important and costly input in the production process of ethanol. After feedstock costs, it is the most expensive variable cost. This energy is utilized in two forms, thermal (steam) and electrical.

4.2.1 Energy balance for distillation (acetic acid)



Where, X_{A1} = fraction of acetic acid

X_{w1} = fraction of water

F (M1) - Fermentation broth or hydrous acetic acid

D (M2) - mass of distil (water)

W (M3) - mass of bottom (acetic acid) = 5000ton/year

Heat capacity on feed

$C_p, \text{ feed} = 0.08 \cdot 2.72 + 0.92 \cdot 4.18 = 4.0632 \text{ KJ/kgk}$

Heat capacity on top

$$C_p, \text{ distillate} = 0.95 \cdot 2.72 + 0.05 \cdot 4.18 = 2.793 \text{ KJ/kgK}$$

Heat capacity on bottom

$$C_p \text{ water} = 4.18 \text{ kJ/kgK}$$

4.3. SIZING OF THE EQUIPMENT'S

Table 5.1 density of substances

Substances	Density(kg/m ³)
Acetic acid	1050
Ethanol	789
Water	1000
Molasses	1424.42

Take the safety factor 10% of the capacity of the equipment.

Sizing on distillation (acetic acid)

Volume of acetic acid

➤ W (M3) - mass of bottom(acetic acid) = 5000 ton/year

$$15625 \text{ kg/day} \cdot 1 \text{ day/4 batch} = 3906.25 \text{ kg/batch}$$

Volumetric of acetic acid = mass of acetic acid / density of acetic acid

$$= 3906.25 \text{ kg/batch} / 1050 \text{ (kg/m}^3\text{)}$$

$$= 3.7 \text{ M}^3$$

Volume of water

➤ D (M2) - mass of distil (water) = 54375 ton/year

$$169922 \text{ kg/day} \cdot 1 \text{ day/4 batch} = 8345 \text{ kg/day}$$

Volumetric of water = mass of water / density of water

$$= 8345 \text{ kg/day} / 1000 \text{ kg/m}^3$$

$$= 8.3 \text{ M}^3$$

$$\text{Total volume} = 3.7 \text{ M}^3 + 8.3 \text{ M}^3 = 12 \text{ m}^3$$

10% allowance will be:

$$\begin{aligned}
 V &= V + 0.1v \\
 &= 12\text{m}^3 + 0.1 * 12\text{m}^3 \\
 &= 13.2 \text{ m}^3
 \end{aligned}$$

Sizing fermenter(acetic acid)

Volume of ethanol

$$\begin{aligned}
 \text{➤ Mass of ethanol} &= 39583 \text{ ton/year} \\
 &= 123697\text{kg/day} * 1\text{day}/4\text{batch} = 30924\text{kg/batch}
 \end{aligned}$$

$$\begin{aligned}
 \text{Volumetric of ethanol} &= \text{mass of ethanol}/\text{density of ethanol} \\
 &= 30924\text{kg/batch}/789\text{kg/m}^3 \\
 &= 39.2\text{M}^3
 \end{aligned}$$

$$\begin{aligned}
 \text{Volume of media} &= 15\% \text{ of dilute ethanol} \\
 &= 0.15 * 39.2\text{M}^3 \\
 &= 5.88\text{M}^3
 \end{aligned}$$

$$\begin{aligned}
 \text{Total volume} &= 39.2\text{M}^3 + 5.88\text{M}^3 \\
 &= 45.08\text{M}^3
 \end{aligned}$$

$$\text{With 10\% safety factor, } 45.08\text{M}^3 + 0.1 * 45.08\text{M}^3 = 49.5\text{M}^3$$

Sizing diluter (acetic acid)

Volume of ethanol

$$\begin{aligned}
 \text{➤ Mass of ethanol} &= M6 = 6090 \text{ ton/year} \\
 &= 19031.25\text{kg/day} * 1\text{day}/4\text{batch} = 4758\text{kg/batch}
 \end{aligned}$$

$$\begin{aligned}
 \text{Volume of ethanol} &= \text{mass of ethanol}/\text{density of ethanol} \\
 &= 4758\text{kg/batch}/789\text{kg/m}^3 \\
 &= 6\text{M}^3
 \end{aligned}$$

Volume of water

$$\begin{aligned}
 \text{➤ Mass of water} &= M7 = 33493 \text{ ton/year} \\
 &= 104665.625\text{kg/day} * 1\text{day}/4\text{batch} = 24166\text{kg/batch}
 \end{aligned}$$

$$\text{Volume of water} = 24.2\text{M}^3$$

$$\text{Total volume} = 6\text{M}^3 + 24.2\text{M}^3 = 30.2 \text{ M}^3$$

Sizing on distillation (ethanol)

Volume of ethanol

- Mass of ethanol= D (M6)-mass of distil mass of ethanol=7669.4 ton/year
23967kg/day*1day/4batch=5991.7kg/batch

Volume of ethanol =mass of ethanol/density of ethanol
 $=5991.7\text{kg/batch}/789\text{kg/m}^3=7.6\text{M}^3$

Volume of water

- Mass of water= W (M8)- mass of bottom or water=38388 ton/year
119962kg/day*1day/4batch=29991kg/batch

Volume of water =mass of water/density of water
 $=29991\text{kg/batch}/1000\text{kg/m}^3$
 $=29.99\text{M}^3$

Total volume= $7.6\text{M}^3+29.99\text{M}^3$
 $=37.6\text{M}^3$

With 10% safety factor, $=37.6\text{M}^3+0.1*37.6\text{M}^3$
 $=41.3\text{M}^3$

Sizing on fermenter (ethanol)

Volume of molasses

- Mass of molasses M12- diluted molasses = 52800ton/year.
165000kg/day*1day/4batch=41250kg/batch

Volume of molasses =mass of molasses/density of molasses
 $=41250\text{kg}/1424.42\text{kg/m}^3$
 $=29\text{M}^3$

Volume of yeast

- 1 litter molasses=0.004 litter yeast
29000 litter molasses=?
=116 litter yeast
 $=0.116\text{M}^3$

Total volume= $29\text{M}^3+0.116\text{M}^3$
 $=29.116\text{M}^3$

With 10% safety factor, $=29.116\text{M}^3+0.1*29.116\text{M}^3$
 $=32\text{M}^3$

Sizing on diluter (ethanol)

- Mass of molasses M12- diluted molasses = 52800ton/year.
 $165000\text{kg/day} \times 1\text{day}/4\text{batch} = 41250\text{kg/batch}$

Volume of molasses = mass of molasses/density of molasses

$$= 41250\text{kg} / 1424.42\text{kg/m}^3$$

$$= 29\text{M}^3$$

With 10% safety factor, $= 29\text{M}^3 + 0.1 \times 29\text{M}^3$

$$= 31.9 \text{ M}^3$$

4.3 EQUIPMENT DESIGN

Fermenter:

Commercial fermentations are usually performed especially in vessels held under positive pressure of sterile air to resist entry of contamination micro-organism. However, the production of pathogenic organisms for medical purposes or for biological, welfare operates below atmospheric pressure because the safety of the plant operators is more important than the integrity of the product. Fermentation formerly distinguished processes from which oxygen was absent, but the term has now been extended to aerobic processes. The function desired in fermentation are gas liquid contacting, on-line sensing of concentrations, mixing, heat transfer, foam control, and feed of nutrients, or reagents such as those of pH control. The workhorse of the fermentation industry is the conventional batch fermenter. There is extensive process piping, and copper or brass fittings are avoided because of the highly deleterious effects of copper on many biological systems.

Design Calculations for fermenter:

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