

**PRODUCTION AND CHARACTERIZATION OF CITRIC ACID  
FROM CARBOHYDRATE SOURCES**

**BY**

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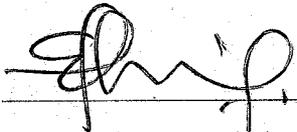
**A PROJECT SUBMITTED TO THE DEPARTMENT OF  
CHEMICAL ENGINEERING SCHOOL OF ENGINEERING AND  
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THE AWARD OF BACHELOR OF ENGINEERING (B.ENG)  
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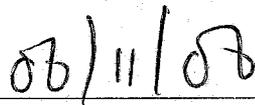
**OCTOBER 2006**

## CERTIFICATION

This is certify that this project tittles "Production and Characterization of Citric Acid from Carbohydrate Sources" was done by Abdullahi Yusuf Paiko under the supervision of Engr. Mrs. E.J Eterigho and submitted to the chemical Engineering Department, Federal University of Technology Minna, In partial fulfillment of the requirement for the award of Bachelor of Engr. (b.Eng.(B. Eng.) Degree in Chemical Engineering.



Engr. Mrs. Eterigho



Date & Signature

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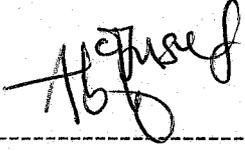
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## DECLARATION

I hereby declare that this research project topic "Production and Characterization of Citric Acid from Carbohydrate Sources" was wholly undertaken by me under the guidance and supervision of Engr. Mrs. E.J Eterigho. It has never been submitted to chemical Engineering for award of degree.



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Abdullahi Yusuf Paiko

## DEDICATION

This project report is dedicated to the Glorious one: ALLAH, who saw me through the darkest hours when I was in search of relevant materials to carry out this project and also for keeping me alive to be part of this programme today.

A special dedication also goes to my father Alhaji Yusuf Paiko for his financials and moral assistance through my B. Eng. Academic pursuit

Also this work is dedicated to all members of my family and my friends which include, Umar Ndanusa, Adamu Usman, Akeem Olatunji, and Suleiman Moh'd Idris.

Lastly, real dedication is for you my best and closest friend Miss Amina, you are such a qualify person that every body desires (I Love you.)

## ACKNOWLEDGEMENT

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## ABSTRACT

The aim of this project is to produce and characterize citric acid from carbohydrate sources which includes starch hydrolysate from cassava, cane juice from sugar cane and cane molasses under certain physical conditions such as: oxygen supply through air, Temperature, pH, time and concentration of enzymes.

All the carbohydrate source under go fermentation for about 6-8 days after the citric acid is recovered by precipitation and adsorption.

Finally, sugar cane juice yield 72%, due to its high concentration sugar content while molasses and starch hydrolysate yield 68% and 47% respectively.

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## CHAPTER ONE

### Introduction

Citric acid is one of the most commonly used acids in food and pharmaceutical industries on account of its high solubility and toxicity.

There is a great worldwide demand for citric acid consumption due to its low toxicity when compared with other acidulant used mainly in the pharmaceutical and food industries. Other applications of citric acid can be found in detergents and cleaning products, cosmetics and toiletries, and other. Global production has now reached 1.4 million tones and there is annual growth of 3.5–4.0% in demand/consumption of citric acid [1].

As a result of the adverse market conditions, only big producers have survived. Any increase in citric acid productivity would be of potential interest and hence there is an obvious need to consider all possible ways in which this might be achieved. The production by submerged fermentation is still dominating. However, solid-state processes can create new possibilities for producers. Many by-products and residues of the agro-industry can be used in the production of citric acid. A cost reduction in citric acid production can be achieved by using less expensive substrates. The use of agro-industrial residues as support in solid-state fermentation is economically important and minimizes environmental problems. Other perspectives for citric acid production sector are the improvement of citric acid producing strains, which have been carried out by mutagenesis and selection[6].

## **1.1 Scope**

To produce and compare the percentage composition of citric acid present in carbohydrate sources; cane Juice. Cane molasses and starch hydrolysates, and to characterize the physical properties of citric acid.

## **1.2 Aim and Objective**

The aim and objective of this project work is to produce and characterize citric acid form carbohydrate sources, under optimum conditions of fermentation.

## **1.3 Relevance of Work**

This project work is highly essential when trying to look at the importance of production of citric acid to Nigeria economy by comparing the percentage volume obtain from; cane juice, cane molasses and starch hydrolysates. If this is achieved it gives birth to processing industries thereby:

- Reducing unemployment
- Increasing per capital income of the economy.
- Making it readily available

## CHAPTER TWO

### 2.0 Literature Review

Citric acid is a colourless translucent crystalline acid,  $C_6H_8O_7$ , principally derived by fermentation of carbohydrate or from lemon, lime and pineapple juices and use in preparing citrates and in flavouring and metals polish.

Citric acid (2 – hydroxyl tricarboxylic acid; 2 – hydroxyl propane -1,2,3-tricarboxylic acid) is in greater demand than any other acid of immediate biological origin. Its poly functionality makes it suitable for a wide range of applications. The presence of one hydroxyl and three carboxylic radicals permits the formation of complex molecules which may be both soluble and capable of modifying the solubility of constituent material

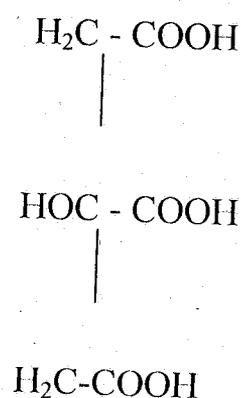


Fig. 2.1 2-hydroxyl propane 1, 2, 3-tricarboxylic acid

Citric acid is the most widely used crystalline organic acid in the food industry where its high solubility, mildly sour taste and palatability are of special value. Citric, lactic and relatively small quantities of Formic, and acetic acids are almost the only organic acid in foods. Citric acid is of special interest also to biochemist, because of its role in the terminal respiratory metabolism of almost all

living cells and its accumulation in some leaf, stem, and fruit tissue to a remarkably high concentration. The already commutability of citric acid has made it the preferred buffer in the pharmaceutical industry, citric acid occurs in all animal tissue.

## 2.1 History of Citric Acid

Citric acid fermentation was first observed as a fungal product by Wehmer in 1893 by a culture of *Penicillium glaucum* on sugar medium. After a few years, he isolated two new fungal strains with the ability to accumulate citric acid, which were designated *Citromyces* (*Penicillium*). However, industrial trials did not succeed due to contamination problems and long duration of fermentation. It was the work of Currie which opened up the way for successful industrial production of citric acid. In 1916, he found that numerous strains of *Aspergillus niger* produced significant amounts of citric acid.

The most important finding was that *A. niger* grew well at pH values around 2.5 –3.5 and high concentrations of sugars favour citric acid production. The first citric acid fermentations were carried out in surface cultures. In the 1930s, some units were implanted in England, in Soviet Union, and in Germany for the commercial production. In general, citric acid is commercially produced by submerged microbial fermentation of molasses; the fermentation process using *Aspergillus niger* is still the main source of citric acid worldwide. Although methods were well developed to synthesize citric acid using chemical means, better successes were achieved using microbial fermentations, and over the period of time, this technique has become the method of ultimate choice for its commercial production over chemical synthesis [2] [3].

Despite that, the introduction of submerged fermentation presented several problems, including the choice of productive strains with low sensitivity to trace elements. It was necessary to consider raw material much more carefully. Several works were dedicated to the optimization of the conditions for the utilization of cheap material like sugar cane molasses, beet molasses, starch and hydrolysate starch. Various processes for treating and purifying molasses were developed, especially for the removal of trace metals. Moreover, it was found that a small excess of copper ions was beneficial to achieve high yields of citric acid.

## 2.2 Microbial Production of Citric Acid.

### 2.2.1 Microorganisms

A large number of microorganisms including fungi and bacteria such as *Arthrobacterparaffinens*, *Bacilluslicheniformis* and *Corynebacterium*ssp., *Aspergillus niger*, *A. aculeatus*, *A. carbonarius*, *A. awamori*, *A. foetidus*, *A. fonsecaeus*, *A. phoenicis* and *Penicillium janthinellum*; and yeasts such as *Candida tropicalis*, *C. oleophila*, *C. guilliermondii*, *C. citroformans*, *Hansenula anomala* and *Yarrowia lipolytica* have been employed for citric acid production. Most of them, however, are not able to produce commercially acceptable yields due to the fact that citric acid is a metabolite of energy metabolism and its accumulation rises in appreciable amounts only under conditions of drastic imbalances. Among the mentioned strains, the fungus *A. niger* has remained the organism of choice for commercial production because it produces more citric acid per time unit. The problem in the production of citric acid for yeasts is the simultaneous formation of isocitric acid. The main advantages of using *A. niger* are its ease of handling, its ability to ferment a variety of cheap raw materials, and high yields. Industrial strains which produce commercial citric acid are not freely available and only a few can be obtained from international culture collections.

The improvement of citric acid producing strains has been carried out by mutagenesis and selection. The most employed technique has been by inducing mutations in parental strains using mutagens. Mutants of *Aspergillus niger* are used for commercial production. Among mutagens, radiation, UV radiation and chemical mutagens are often used. To obtain hyper-producer strains, UV treatment can frequently be combined with some chemical mutagens. The single spore technique and the passage method are the principal methods of selecting strains. The first one has the disadvantage that mineral acid and organic acids (gluconic and oxalic acids) simulate the presence of citric acid.

Different methods of fermentation can lead to different yields of citric acid production by the same strain. Thus, a strain which produces good yields in the solid fermentation or liquid surface is not necessarily good producer in the submerged fermentation. In that way, each method and raw material of industrial interest should be tested with known producer strains.

In any technique used in citric acid production the inoculation of microorganism is done by means of spores which are added into the fermentation medium. Spores can be inoculated either mixing them with the air, which is introduced in substrate, or in form of a spore suspension. Spores are produced in glass bottles on solid substrates at optimum temperature. The type of sporulation medium and time of incubation affect spore viability and citric acid production by mycelia grown from *A. niger*. It was mentioned that potato dextrose agar gives high citric acid yields. Viability increases with time of incubation, but higher production of citric acid was achieved in less than 7 days of spore incubation. The capacity of germination of the spores tends to reduce with the time but in some cases, short periods of up to 7-8 days do not present significant differences in relation to spores collected after 3 days.

### 2.2.2 Substrates

Several raw materials such as hydrocarbons, starchy and molasses, have been employed as substrates for commercial submerged citric acid production (Table 2.1), although citric acid is mostly produced from starch or sucrose based medium using merged fermentation. Generally, citric acid is produced by fermentation using inexpensive raw material, including crude natural products, such as hydrolysates starch, sugar cane broth and by-products like sugar cane and beet molasses.

Molasses is preferably used as the source of sugar for microbial production of citric acid due to its relatively low cost and high sugar content (40-55 %). Since it is a by-product of sugar refining, the quality of molasses varies considerably, and not all types are able for citric acid production. The molasses composition depends on various factors like the variety of beet and cane, methods of cultivation, conditions of storage and handling (transport, temperature variations), *etc.* Both beet and cane molasses are suitable for citric acid production, however, beet molasses is preferred to sugar cane due to its lower content of trace metals, supplying better production yields than cane molasses, but there are considerable yield variations within each type. In the case of cane molasses, generally it contains some metals (iron, calcium, magnesium, manganese, zinc) which retard citric acid synthesis and it requires some pretreatment for the reduction of them [4] [5] [9] [10].

Table 2.1. Raw materials employed in submerged and semi-solid production of citric acid

Raw material	Strain	(citric acid)kg /m <sup>3</sup>	(Yield )%
Beet molasses	A. niger ATTC 9142	109	-
Black strap molasses	A. niger GCM 7	86	-
Cane molasses	A. niger T55	-	65
	A. niger GCMC-7	113.6	100
Hydrolysate starch	A. niger UE-1	74	49

### 2.3 Citric Acid Production Techniques

Citric acid production synthesis by fermentation is the most economical and widely used way of obtaining this product. More than 90 %of the citric acid produced in the world is obtained by fermentation, which has its own advantages: operations are simple and stable, the plant is generally less complicated and needs less sophisticated control systems, technical skills required are lower, energy consumption is lower and frequent power failures do not critically affect the functioning of the plant.

Citric acid production by fermentation can be divided in three phases, which include preparation and inoculation of the raw material, fermentation, and recovery of the product. The industrial citric acid production can be carried in three different ways: by submerged fermentation, surface fermentation and solid-state fermentation. All of these methods require raw material and inoculum preparation. In industrial citric fermentation, the large-scale spore production is made by using appropriate means and conditions such as direct inoculation in the production

fermentor. Sometimes it is necessary to remove the remainder mineral of the raw material and add other nutrients such as phosphorous, magnesium and nitrogen for development of the mycelium and a good production of the citric acid.

### **2.3.1 Submerged fermentation**

The submerged technique is widely used for citric acid production. It is estimated that about 80% of world production is obtained by submerged fermentation. This fermentation process employed in large scale requires more sophisticated installations and rigorous control. On the other hand, it presents several advantages such as higher productivity and yields lower labour costs, flower contamination risk and labour consumption. Submerged fermentation can be carried out in batch, fed batch or continuous systems, although the batch mode is more frequently used.

Normally, citric fermentation is concluded in 5 to 12 days, depending on the process conditions.

### **2.3.2 Surface fermentation**

Liquid surface culture is the classic citric production process and was the first industrial manufacture; submerged fermentation was developed only after that. Surface fermentation is still used in industries of small and medium scale because it requires less effort in operation, installation and energy cost.

### **2.3.3 Solid-state fermentation**

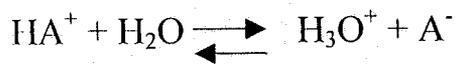
Solid-state fermentation was first developed in Japan where abundant raw materials such as fruit wastes and mainly rice bran are available. It is the simplest method for citric acid production and it has been an alternative method for using

agro-industrial residues. Solid-state culture is characterized by the development of microorganisms in a low-water activity environment on an insoluble material that acts both as physical support and source of nutrients. Some similarities are observed with the surface process since the fungus also develops on material surface. The substrate is solid and it is moistened to about 70% moisture, depending on the substrate absorption capacity. The initial pH of the material is normally adjusted to 4.5 –6.0 and the temperature of incubation is about 28–30°C, depending on the microorganism used. The solid culture process is completed within 96 hours under optimal conditions.

The most common organism used in solid-state fermentation is *A.niger*. However, there have also been reports with yeasts. The strains with large requirements of nitrogen and phosphorus are not ideal microorganisms for solid culture due to lower diffusion rate of nutrients and metabolites occurring at lower water activity in solid state process. The presence of trace elements may not affect citric acid production so harmfully as it does in submerged fermentation thus, substrate pretreatment is not required. This is one of the important advantages of the solid culture.

#### 2.3.4 Buffers

The most important type of buffer solution is made by dissolving a weak acid and a salt of the same weak acid in water or by dissolving a weak base and a salt of the weak base in water. For example, considering the first case where ionization will occur according to the reaction.



If a salt of the weak,  $\text{HA}^+$  is added, the  $\text{H}_3\text{O}^+$  concentration will decrease (pH increases) as predicted by Le-Chatelliers principle that is equilibrium is shifted to the left.

If strong acid or some other sources of hydroxonium ion is introduced into the solution, it dissociates with the anions  $\text{A}^-$ , reverse of the reaction above take place, since there is a large reservoir of  $\text{A}^-$ , little change in pH is observed.

Similarly, the introduction of strong base into the solution produces no appreciable change in pH because, the  $\text{H}_3\text{O}^+$  ion will interact with the base and since there is large reservoir of  $\text{H}_3\text{O}^+$  ion no change in pH is observed.

## 2.4 Properties Of Citric Acid

Citric acid is a colorless and odorless crystal, which is very soluble in water and ethanol and sparingly soluble in ether.

Citric acid is a strong organic acid as indicated by the first dissociation constants  $\text{p}k_1 = 3.13$  at  $25^\circ\text{C}$ /second and third dissociation constant are  $\text{P}k_2 = 4.76$ ,  $\text{P}k_3 = 6.40$  respectively.

Citric acid heated to  $175^\circ\text{C}$  losses water to form aconitic acid, which losses  $\text{CO}_2$  to form itaconic anhydride. Itaconic anhydride may be added to water to form itaconic acid or rearrange to form citraconic anhydride. Addition of water to citraconic anhydride gives citraconic acid. On heating in air or with an oxidizing agent, it yields acetone dicarboxylic acid which may further break up to carbon dioxide and acetone.

Citric acid is tribasic and manifests the used properties of poly basic acid. It forms three series of well defined salts and two different mono and di alkali salts. The various salts of the alkali metals are readily soluble, but the normal salts of the alkaline acid readily forms stable complex salts with many metallic ions.

## **2.5 Factors Affecting Citric Acid Production**

### **2.5.1 Chemical Factors**

Citric acid accumulation is strongly influenced by the type and concentration of carbon source. The presence of carbohydrates which are rapidly taken up by microorganisms has been found essential for a good production of citric acid. Among the easily metabolized carbohydrates, sucrose is the most favourable carbon source followed by glucose, fructose and galactose. As presented previously, several raw materials can be employed successfully for citric acid production. However, there are some critical factors that should be taken into account such as costs or need of pretreatment for choosing the type of substrate. Molasses has trace elements which must be precipitated by potassium Ferrocyanide. Table 2.2 shows the main factors that affect citric acid production.

Table 2.2 Chemical factors affecting citric acid Production

Factor	Positive effect	Level	Negative effect
Carbon source	Sucrose	14 –22 %	Starch
	Glucose		xylose
	Fructose		Arabinose
	Galactose		Sorbitol
			Pyruvic acid
Phosphorus source	potassium	low	
	Dehydrogen	(0.5-5.0g/L)	
	phosphate		
Nitrogen source	Ammonium nitrate	under 25%	High Conc.
	Ammonium sulphate	0.1 to 0.4gN/L	
	Peptone		
	Malt extract		
	Urea		
Trace elements	Zinc	Low levels	Manganese
	Copper		(1 ppm)
	Magnesium sulphate	(0.02-0.025%)	

## 2.5.2 Physical Factors

### (i) pH

The pH of a culture may change in response to microbial metabolic activities. The most obvious reason is the secretion of organic acids, such as citric acid, which will cause the pH decrease. Changes in pH kinetics also depend highly

on the microorganism. With *Aspergillus* sp., *Penicillium* sp. and *Rhizopus* sp., pH can drop very quickly to less than 3.0. For other groups of fungi such as *Trichoderma*, *Sporotrichum*, *Pleurotus* sp., pH is more stable between 4.0 and 5.0. The nature of the substrate and production technique also influence pH kinetics. In this way initial pH must be very well defined and optimized depending on the microorganism, substrate and production technique.

## (ii) Aeration

Since citric acid production is an aerobic process, oxygen supply has a determinant effect on its production. Increased aeration rates lead to enhanced yields and reduced process time. An interruption of aeration during batch fermentation is quite harmful. Dissolved oxygen concentration influences the citric acid formation directly. It is important to maintain the oxygen concentration above 25% saturation. Critical dissolved oxygen tension is 9–12 % of air saturation for growth phase and 12–13 % of air saturation for the production phase. The high demand of oxygen is reached by constructing appropriate aeration devices, which is also dependent on the viscosity of the fermentation broth. This is an additional reason why small compact pellets are the preferred mycelial forms of *A.niger* during the production. When the organism turns into development of filaments, the dissolved oxygen tension rapidly falls to less than 50 % of its previous value, even if the dry mass has not increased by more than 5%. Aeration should be performed through the medium during the whole process with the same intensity, even though, due to economic reasons, it is usually preferred to start with low aeration rates. The incorporation of the oxygen together with air in submerged process results in increment of citric acid production, but it is economically unviable. However, it is possible to circulate the oxygen in the fermentor since the carbonic

gas is removed from the process. High aeration rates lead to high amounts of foam, especially during the growth phase, so the addition of antifoaming agents and the construction of mechanical »defoamers «are required to tackle this problem.

## 2.6 Citric Acid Recovery

The recovery of citric acid from fermented broth is generally performed through three procedures: precipitation, extraction and adsorption (mainly using ion exchange resins). The first method is the most frequently used and it is applicable in all types of processes. The second one requires a fermented broth with little impurities. In both of the methods there is the need to remove the fermented broth, mycelles of the fungus, and materials in suspension by filtration.

## 2.7 Occurrence and Culture Of *Aspergillus Niger*

*Aspergillus* is a widely distributed fungi occurring in soil and almost all natural products. A few pathogenic may produce substances which inhibit the growth of other macro organisms, we have different species of *Aspergillus*, but the one of interest is "*Aspergillus niger*".

*Aspergillus niger* is a very common black mould occurring in soil and on food products of importance in spoilage of sugar, fat and chemical solutions. Its formation of citric fomic, gluconic or oxalic acids depends on the strain and substrate [8].

In order to develop a complex medium for industrial fermentations it is necessary to determine the following criteria;

- (a) Specific nutrient requirement of selected micro organism and procedures.

- (b) Exact composition of industrial nutrients, possible modification during storage, and influence of technological procedure in their fabrication.
- (c) Properties of nutrient in terms of storage and handling, as well as their behavior as component of complex media during media preparation (sterilization) fermentation, and product isolation
- (d) Cost of nutrient.

## 2.8 Extraction of Raw Materials

### (i) Sugar cane

When economic conditions have been suitable, prime quality sugar cane is being used for citric acid production, but ordinarily only low quality fruits are used.

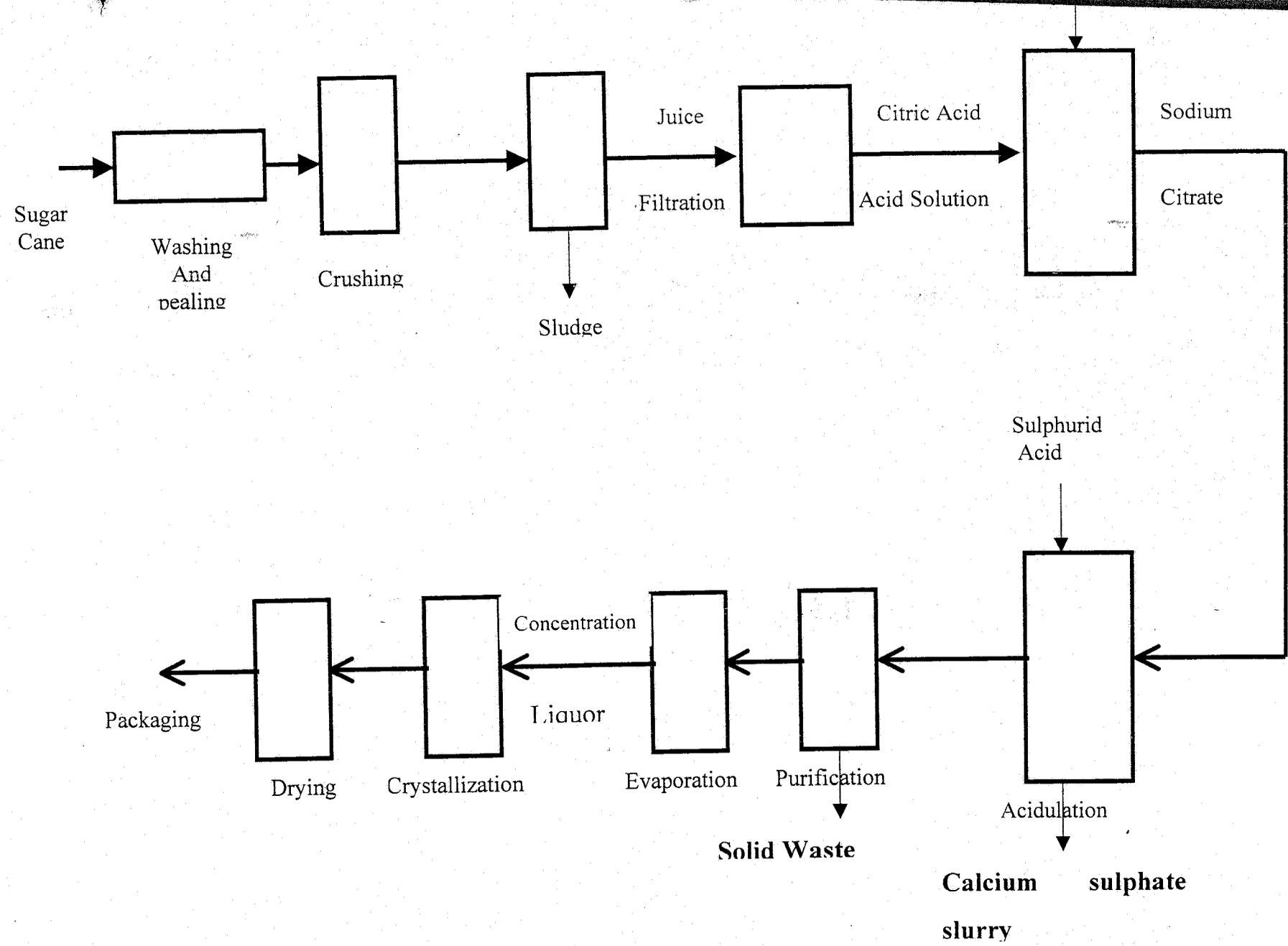
Sugar cane is washed and peeled. The pulp is shredded and crushed between rollers which press out the Juice. The pressed pulp may be extracted twice with water and the Juice. The brownish portion of the inside is fermented with the yeast for four to ten days.

As soon as the destruction is completed. The Juice is filtered with a diatomaceous earth used as a filter acid. Either a filter press or rotary re-coat filter may be used. The dilute clarified Juice, which contains 3 – 4% Juice acid is lined and calcium citrate is filtered off and washed as shown in figure 2.2. The product may be marketed as crude calcium or may be converted to citric acid.

### (ii) Cassava

Fresh cassava tubers obtained locally were washed and peeled. The process of starch extraction from these tubers consisted of wet milling the washed cassava roots followed by washing the starch from the fibrous mass. Starch was sedimented in settling containers and air – dried.

FIGURE 2.2 FLOW DIAGRAM FOR THE PRODUCTION OF CITRIC ACID FROM SUGAR CANE



## 2.9 Uses Of Citric Acid

The food and pharmaceutical industries utilize citric acid extensively because of its high solubility, pleasant sour taste, very low toxicity ready salability citric acid also finds application in some cosmetic preparations and in metal and chemical cleaning, electro pickling and copper plating, secondary oil recovery and other industrial uses.

### (a) Food

#### (i) Food Candy

Citric acid is added to enhance the flavours of citric, berries and other ingredients used in the manufacture of candy citric acid when added to the candy batch during the cooking process (a procedure known as "doctoring" the batch), causes conversion to simple sugar which would not crystallize readily.

#### (ii) Soft Drinks and Syrup

Since citric occurs naturally in fruit, it is the preferred acidulant for carbonated and still beverages. Citric acid adds refreshing properties to the drink, often duplicating natural fruit products. It acts as a preservative in syrups and the finished beverage and aids in obtaining the desired bouquet by modifying the sweet flavours. It sequesters harmful metals which cause haze and accelerates deterioration of colour and flavour.

The amount of citric added to soft drinks depends upon the flavours and particular end uses intended. Some syrup flavours, such as grape or orange, contain as little as 0.5 per gallon, sufficient citric acid be as high as 4 of citric acid per gallon, sufficient citric acid should be added to give a final PH of 2.5 to 4.5.

### **(iii) Soft Drink Tablets**

Tablets containing citric acid, and alkaline salt, a sweetening agent and various flavours have been placed on the market, which effervesce when dissolved in water. Carbonation of the water solution is caused by the release of carbon dioxide when citric acid reacts with a metal bicarbonate.

### **(iv) Fruit and Vegetable Juices**

In general, lower PH value extract a protective effect on fruit juice pigment. Straw berry fountain syrup in particular is colour stabilized by citric acid. The natural flavours of grape and other fruit juices are greatly enhanced by the tartness which citric acid gives. The lower PH increase the resistance to spoilage.

### **(v) Frozen Fruits**

Citric acid is used for two purposes in the processing of fruits for frozen packs. First since lye peeling operation are common, it is important after a thorough water washing to neutralize the residual lye by dipping fruits or vegetables in 1 to 2% citric acid solutions. Since residual alkali destroy natural ascorbic acid, it is extremely important that the last traces be neutralized. The citric further stabilizes Ascorbic acid by lowering the pH to inactivate the oxidative enzymes present. In addition, the citric acid retards destruction of Ascorbic acid by binding harmful metal contaminants.

### **(vi) Jellies, Jams and Preserves**

Citric acid serves to adjust the PH of jellies, jams or preserve mixtures to the optimum range, where pectin can act most effectively, certain foods would not naturally contain sufficient acids to give the proper PH. The use of citric acid also

gives the desired degree of flavour, which are important to jellies After the jelly juice – peptic sugar mixture is cooked and concentrated. Citric acid is added.

## **(b) Pharmaceuticals**

The citrate ion is rapidly and almost completely oxidized, less than 1% being excreted unchanged in the urine. Intravenous injection shortens the coagulation time of the blood, but in vitro the citrate ion acts as an anti coagulant.

The free acid is employed in pharmaceuticals preparations as an acidulant and to enhance the flavour of syrups, solutions and elixirs. With sodium bicarbonate, citric acid is used in many effervescent powders and tablets to liberate carbon.

Citric acid salt such as sodium citrate and potassium citrates are also used in the mixture. The free acid may also be employed as an acidulant in mild astringent preparations. Many of the salts are important pharmaceuticals.

## **(c) Cosmetics**

### **(i) Hair Rinses and Settling Fluids**

Citric acid rinses have enjoyed wide popularity for many years. Hair rinsed with a small quantity of the citric acid in water becomes lustrous. In addition, citric acid added to a solution of dextrin and glycerol with ammonium chloride, hydrogen sulphide and potassium hydroxide holding opportunities.

### **(ii) Lotions**

Citric acid has been suggested for use in astringent lotions to treat oily skin having large pores and in bleaching lotions where a mild acid is preferred to adjust pH and to act as a sequesterant.

## **(d) Other Industrial Application**

### **(i) Metal Cleaning**

Before the layers of rust, scale and tarnish can be removed efficiently from metals, the grease, dirt and oil must be eliminated by pre cleaning. Most oil and grease removal is accomplished with organic solvents, emulsions, or alkali system. The citric acid dip follows to remove tarnish and rust and produces a shiny surface.

Certain aluminum brightener formulations also contain citric acid, as do general metal cleaners and polishes for home use. Citric acid is the product of choice for home use because it is non toxic and completely safe. Citric acid, in combination as a paste or powder to copper surfaces, produces a brilliant shine.

### **(ii) Rust and Scale Removal**

Citric acid and Ammonium citrate has been used for rust and scale removal for many years. Ammoniac citric acid solutions in addition to their efficient cleaning actions, offer the desired advantage of being non toxic, personnel are not exposed to the hazards, customarily experienced with other compounds.

### **(iii) Copper Plating**

The addition of citric acid to copper cyanide plating solutions increased both cathode and anode efficiency and yields smooth, lustrous, light and streak free surfaces. The desirable effect on citrate ion in copper plating baths include, reduced effect of impurities and greater efficiency in plating on stainless steel magnesium, Zinc, lead and aluminum.

#### **(iv) Tanning**

Citric acid as a component of the tanning liquors does not cause undesirable smelling of the hide. The use of citric acid to adjust the PH of tanning liquors has been recommended as it is the acid, whose properties must closely approach those of the mixture of acids naturally present in tanning materials.

#### **(v) Secondary Oil Recovery**

Citric acid a powerful sequestering agent, keeps iron in solution and prevents injections rates from a previously plugged conditions, when a previously plugged well is cleaned, citric acid prevents re-plugging.

#### **(vi) Printing**

Citric acid acts as a stabilizer in diazole-sensitised materials used to make diazole paper. The most important function of this stabilizer is to prevent the "pre-coupling" of diazole and coupler while the product is still on the shelf.

#### **(vii) Miscellaneous**

Citric acid is the starting point in the manufacture of various esters and of the citrates of sodium ammonium, bismuth, calcium, ferric iron, lithium, magnesium, manganese, potassium and strontium.

Citric acid is also used in floor cement, linoleum, inks, silvering compounds and algacide formulations as well as in the dyeing of fabrics, in the processes, for removal of contamination of radio active isotopes.

## CHAPTER THREE

### 3.0 Experimental Materials and Equipments

#### 3.1 Materials

1. De-ionised water
2. Cane Juice (extracted from sugar cane)
3. Cane molasses (got from Bacita sugar industry)
4. Starch hydrolysate (extracted from cassava)

#### 3.2 Equipments

S/N	Equipments	Functions
1.	Water bath	For supplying the required experimental temperature.
2.	Thermometer	For measuring the temperature of the water in water bath.
3.	Fabricated Glass Tube	For supplying air to the Juice in the vessel.
4.	Fabricated Copper Tube	For supplying air used in all experiments.
5.	Ritameter	For measuring the air flow rate.
6.	Rubber hose	For supplying air to air bubbler from the rotameter.
7.	Resinous filter aid	For filtering air from compressors.
8.	200ml beaker	Used as vessel for the juice.
9.	Perforated lid	For covering the vessel.

#### 3.3 Experiments

##### 3.3.1 Pretreatment of Molasses

Cane molasses obtained from Basita sugar industry was used. Cane molasses contains.

- i. 20% Water
- ii. 62% Sugar Content
- iii. 10% Non sugar Content
- iv. 8% Inorganic Salts (Ash Content)

Making a blackish homogenous liquid with high viscosity. Ash contents include ions such as Mg, Mn, Al, Fe, and Zn in variable ratio.

Sugar content was diluted to about 25% sugar level. The molasses solution, after adding 35m of  $\text{I NH}_2\text{SO}_4$  per liter was boiled for half an hour, cooled, neutralized with lime water (CaO) and was left to stand over night for clarification. The clear supernatant liquid was diluted to 15% sugar level.

### 3.3.2 Preparation of Resin:

Spherical particles of cation exchange resin, Amberlite IR 120 was used. Prior to used the resin was pre-conditioned. The resin (100g) was washed thoroughly two times with HCL. After two washers with distilled water. The resin was then washed with NaoH until the filterate was alkaline. The resulting material Sodium salt of the resin was suspended in three (3) times its volume of NaoH and heated over a steam bath for 2 hours with occasional mix up. The supernatant fluid was decanted after 30 minutes of setting and replaced with fresh hot NaOH. The procedure was respected two times, the resin was filtered and washed with litres of distilled water to make it free of alkali.

### 3.3.3 Preparation of Aspergillus Niger of pg/ml Solution:

A quantity of 20mg of Aspergillus was scrapped with spatula from its medium, this quantity was charged into 250ml test tube filled with distilled water to the 250ml level. The materials was mixed thoroughly with the aid of centrifuge to form a complete solution. The solution given Aspergillus concentration of.

$$\frac{20 \times 10^3 \text{ pg}}{250\text{ml}} = 50\text{pg/ml}$$

### 3.3.4 Fermentation

(a) **Starch hydrolysate:** The starch was charged into a beaker and then was heated to 85°C at pH of 6.5. The cooled starch was then filtered and diluted with de-ionised water to give a starch concentration of 100g/c.

(b) **Cane Juice:** the fresh cane was crushed, filtered together with little volume of distilled water for easy passage through the screen into the beaker. The juice obtained was heated to 85°C at pH 6.5. The cooled Juice was then filtered and sucrose concentration of 100gl.

The following salts were added perlitre of the diluted Juice.  $\text{NH}_4\text{NO}_3$  1g,  $\text{KH}_2\text{PO}_4$  0.6g, Mg 504.  $7\text{H}_2\text{O}$  0.16g,  $\text{K}_4\text{Fe}(\text{CN})_6$  180mg. The initial PH was adjusted to 5.0

(C) **Cane Molasses;** The cane molasses was diluted with de-ionised water to give a sucrose concentration of 100g/l. the solution was heated to 85°C after adjusting the pH to 6.5. The cooled medium was filtered and then 1.8kg of  $\text{K}_4\text{Fe}(\text{CN})_6$  was added to the final medium.

After sterilization of the three media,  $\text{K}_4\text{Fe}(\text{CN})_6$  was added at a room temperature of 90oC, spore were inoculated to each medium at a concentration of  $1 \times 10^8$  litre. Fermentations were carried out in a sealed beaker. The fermentation of the *Aspergilles niger* were carried out at 30°C. The PH was kept at pH 3.0 by the addition of  $\text{Na}_2\text{CO}_3$  substrate and products concentrations were determined.

The dry weight of the mycelium was measured by filtering 20ml of the fermented broth through a balance filter paper and dried at 95°C till constant weight.

Citric acid production usually start at the second day of fermentation. After 6 to 7 days, when the citric acid production rate had slowed down, all fermentations were stooped. During fermentations no other by-product was defected except citric acid. Connection for single fermentation is shown in figure 3.1

### 3.3.5 Recovery of Citric acid from fermented liquor:

The quantity of calcium hydroxide to neutralize the calculated quantity of citric acid was determined from stoichiometry. The fermented Juice and hydolysate were filtered in a separate were heated to 60°C and Ca (OH<sub>2</sub>) was added to each with thorough mixing, calcium salt was precipitated.

The quantity of concentrated sulphuric acid required to acidulate calcium citrate was calculated from stoichiometry. The calcium sulphate were filtered off and dilute citric acid were obtained as filtrate.

A small quantity of activated carbon was heated with citric acid solution at 90°C which turns the solution colourless. The carbon was filtered off after cooling.

The colourless solution was evaporated in heating mantle at 60 – 70°C to a viscous liquid and this liquid was cooled rapidly with mixing to a temperature not less than the transition temperature not less than the transition temperature of 36.6°C. the acid crystals were obtained at the bottom with liquid at the top.

The crystals were separated from the liquor by decantation and mild washing of the crystals was carried out in an attempt to make the appearance better. The washed crystals were later dried in vacuum oven at 60°C.



### 3.3.6 Analysis of citric acid;

Citric acid can be detected and identified by the use of spot tests. Fusion of the acid with urea at 150°C yields citrazinic acid (2,6-dihydroxy -4- pyridine carboxylic acid), the ammonium salt of this product either as a solid or in solution shows blue fluorescence under ultraviolet light.

Various acids have been separated by thin-layered chromatography on silica-gel using 25:3:4 ethanol – water ammonium hydroxide as the developing solvent. Under these conditions, acid shows or R<sub>F</sub> value of 0.05, citric acid can be separated from mixture of acids by gas chromatography of methyl or ethyl esters.

### 3.4 Characterization of Citric And Anhydrate and Monohydrate.

Physical properties of citric acid such as; density solubility, boiling point elevation and freezing point depression were determined.

At 15°C various densities were obtained of citric acid monohydrate (% by weight) when dissolve in H<sub>2</sub>O and are given in table 3.1 Citric acid solubility in water were determine of various temperature ranges and are given in table 3.2 similarly, solubility of monohydrate and anhydrous citric acid in some organic solvent and ethyl alcohol at 25°C determined and are given in table 3.3 and 3.4 respectively. The freezing point depression and boiling point elevation of citric acid were also determined when the citric acid was dissolved in water, the various values obtained are given in table 3.5.

Table 3.1 Densities of Citric acid solution at 15°C.

Citric acid Monohydrate (% by weight).	Density at 15°C	Concentration of solution g/litre.
6	1.0227	61.36
10	1.0392	103.9
16	1.0632	170.1
20	1.0805	216.1
26	1.1.60	287.6
30	1.1244	337.3
36	1.1515	414.5
40	1.1709	468.4

Table 3.2 Solubility of citric acid in water.

Temperature °C	Citric acid (% by Weight)
10	54.0
20	59.2
30	46.3
36.6	67.3
40	68.6
50	70.9
60	73.5

Table 3.3 Solubility of hydrated and anhydrous citric acid in some organic solvent at 25°C.

Solvent	Density saturate solution 25°C		Solubility g/100g	
	Monohydrous	Anhydrous	Monohydrous	Anhydrous
Amyl acetate	0.8917	0.8861	5.980	4.22
Ether	0.7228	0.7160	2.174	1.05
Amyl alcohol	0.8774		5.430	
Ethyl	0.9175		5.276	

Table 3.4 Solubility of hydrated and anhydrous Citric acid in ethyl alcohol at 25°C

Monohydrate			Anhydrous		
Ethyl alcohol % wt	Density 25°C	Solubility g/100g	Ethyl alcohol % wt	Density 25°C	Solubility g/100g
20	1.286	66.0	20	1.297	32.3
40	1.257	64.3	40	1.246	59.0
60	1.216	62.0	60	1.190	54.8
80	1.163	58.1	80	1.120	48.5
100	1.068	49.8	100	1.010	38.3

Table 3.5 freezing point Depression and Boiling point Elevation of aqueous citric acid.

Concentration mole/1000g H <sub>2</sub> O	Freezing point depression °C	Boiling point elevation °C
0.010	0.023	-
0.505	0.042	-
0.100	0.203	-
0.500	0.965	0.284
1.00	1.940	0.577
2.00	1.00	1.214
5.00	-	3.512
10.00	-	8.390

## CHAPTER FOUR

### 4.0 Results and Discussions of Result

The following presentation are the result obtained from the experiments carried out to determine the conditions for optimum yield and recovery of citric acid from cane juice, cane mousses and starch hydrolysate, yield as used in this work is the citric formed during fermentation.

$$C_y = c_t - c_i$$

Where  $c_y$  = citric acid yield

$C_t$  = citric acid at time during fermentation

$C_i$  = citric acid present before fermentation

**Table 4.1 Result**

Materials	% Citric acid yield	Selectivity
Sugar cane juice	72	0.72
Cane molasses	68	0.60
Starch hydrolysate	47	0.41

### 4.2 Discussion of Result

#### 4.2.1 Time of fermentation

Separate experiments were carried out in order to determine the time for fermentation and the result of the experiment are shown in table below.

Table 4.2

Time (hrs)	Weight of citric acid in juice	Weight of citric Acid in molasses	Weight of citric Acid in hydroysate
0	1.50	1.45	1.345
7	1.80	1.68	1.49
14	1.54	1.38	1.31
21	1.47	1.26	1.22
28	1.40	1.18	1.16
35	1.34	1.15	1.11
42	1.28	1.12	1.10
49	1.28	1.12	1.10

From the above table, it can be seen that as the time increases the weight of citric acid in the different materials decreases but at 7 hrs of fermentation, the weights increases this is because, from time 0 to 7, the acid starts to be formed but, as the time continuous the weight decreases.

Before the conversion of sugar and starch to acid takes place, the oxygen supplied would have followed the steps below:-

- i Oxygen must cross the gas film to reach the gas liquid interface.
- ii Oxygen must cross the liquid film to reach the main body of liquid.
- iii Oxygen must cross the liquid film surrounding the growing cells to reach surface.
- iv Oxygen is used for the respiration of the growing cell.

**4.2.2 pH effect on citric acid production**

The PH of the fermenting liquor varied from 3.5-5.0 at 0.5 intervals. These range were chosen from the value obtained from previous works carried out on the citric acid production from sugar and starch, and these were done at a temperature

and air flow rate of 30°C and 11.892m<sup>3</sup>/hr. The result obtained are given in table below.

Table 4.3

Time of Fermentation (hrs)	% Weight of citric acid in fermenting liquor			
	pH 3.5	pH 4.0	pH 4.5	pH 5.0
0	1.601	1.601	1.345	1.372
1	1.617	1.633	1.345	1.345
2	1.665	1.665	1.345	1.313
3	1.729	1.729	1.393	1.281
4	1.729	1.745	1.393	1.249
5	1.825	1.809	1.441	1.217
6	1.873	1.841	1.521	1.175
7	1.921	1.841	1.521	1.14
8	1.910	1.760	1.511	1.14

## CHAPTER FIVE

### 5.0 Conclusion and Recommendation

#### 5.1 Conclusion

From table 4.1. For cane juice, the final yield obtained to 72% with high selectivity of 0.72 however for molasses and hydrolysate, the fermentation yielded 68% and 47% respectively, with lower selectivity of 0.6 and 0.41 respectively when compare to the cane juice. Since no acids other than citric acid were produced, more carbon source must have been used for growth and maintenance in the case of molasses and hydrolysate media. This is further supported by growth of *Aspergillus Niger*, cell growth was lowest in the cane juice medium which was rather unexpected because of its similarity to molasses medium with respect to yield and selectivity.

One explanation for this behaviour is that, the micro-organism in the cane juice medium requires less carbon source for its energy generation as compare to the requirement within the molasses and hydrolysate media. As a consequence, the specific acid production rate is highest in the conclusion that, by and large molasses and hydrolysate, with their low citric acid yield high growth which is unfavourable for submerged production of citric acid, despite their low cost. Based on my results, cane juice can be considered as an alternative favourable raw material, which gives good yield with tolerable higher growth.

Moreover, no problem arises due to fluctuating cane juice composition. The results with this source were reproducible, where the varied from 30% to 50% in the case of using molasses and starch hydrolysate as sources.

## 5.2 Recommendations

1. Base on my result obtained, cane juice can be considered as an alternative favourable raw material, which gives good yield with to favourable higher growth.
2. No problem arises due to fluctuating cane juice composition
3. Despite the low cost of both cane molasses and starch hydrolysates yield low citric acid and high growth of organism which is unfavourable for submerged production of citric acid.
4. The stirrer speed has direct influences on other scale-up parameters, such as power input or mass transfer of fermentation at different stirrers speed.

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## APPENDIX

### Recovery of citric acid from fermentation

1. Volume of juice recovered after pressing = 250ml
2. Volume of hydrolysate recovers = 200ml
3. Volume of water added = 750ml
4. Weight of juice recovered = 268g
5. Weight of hydrolysate recovered = 211g
6. Total weight of juice if 1000ml solution = 1072g
7. Total weight of hydrolysate if 1000 ml solution = 1055g
8. Litre volume from titration = 5.8ml

$$\% \text{ citric acid} = \frac{6.404 \times N \times V}{W}$$

Where

v = Titre volume

N = Normality of sodium hydroxide = 0.1

W = Weight of sample = 1g

% by the weight of citric acid before fermentation

$$\text{Total weight of citric acid of juice in 1072g solution} = 40.0 \text{ grams}$$

$$= \frac{3.37 \times 1072}{100}$$

$$\text{Total weight if citric acid of hydrolysate in 1055g} = \frac{3.73 \times 1055}{100}$$

Titre value after 8 hrs of fermentation

$$\% \text{ citric acid after fermentation} = \frac{6.404 \times 8.6}{1}$$

$$= 5.5\%$$

Total weight of juice in solution = 1072g

Weight of citric acid =  $\frac{5.5 \times 1072}{100} = 58.96\text{g}$

100

Total weight of hydrolysate in solution = 1055g

$\frac{5.5 \times 1055}{100} = 58.03\text{g}$

100

To calculate the quantity of calcium hydroxide required to neutralize 56g of citric acid in fermented liquor.

The equation of reaction is  $2 \text{C}_6 \text{H}_8 \text{O}_7 + 3 \text{Ca}(\text{OH})_2 \Rightarrow \text{Ca}_3(\text{C}_6 \text{H}_8 \text{O}_7)_2 + 6\text{H}_2\text{O}$

by stoichiometry, 384g of citric acid react with 222g of  $\text{Ca}(\text{OH})_2$

56 grams will react  $\Rightarrow \frac{222 \times 56}{384} = 32.37$  grams  
of  $\text{Ca}(\text{OH})_2$

$\text{Ca}(\text{OH})_2$  required = 32.37 grams

The quantity citrate produced is calculated as

$\frac{498 \times 56}{384} = 72.612$  grams

The quantity sulphuric acid required to separate citric acid from citrate is calculated on weight basis from the equation  $\text{Ca}_3(\text{C}_6 \text{H}_5 \text{O}_7)_2 + 3\text{H}_2\text{SO}_4 \Rightarrow 3 \text{CaSO}_4 + 2 \text{C}_6 \text{H}_8 \text{O}_7$

By stoichiometry, 498 of citrate reacts with 294 grams of  $\text{H}_2\text{SO}_4$

72.612g react  $\Rightarrow \frac{294 \times 72.612}{498} = 42.87$  grams

Sulphuric acid required = 42.87 grams

citric acid formed  $\frac{72.612 \times 384}{498} = 56$  grams

$\frac{408 \times 72.612}{498} = 59.49$  grams

Weight of citric acid recovered from fermentation after eight hours is 56-38  
= 18 grams

The concentrated citric acid solution gives a white colour because, the solution was dematerialized before evaporation. This is as a result of the ion exchange resin which absorbs the minerals present in the solution. Loss of acid were recorded due to lack of crystalliser and water formed during neutralization, and claimed part of the acid.

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