

CONVERSION OF FOOD WASTE TO USEFUL CHEMICALS/PRODUCTS

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF
BACHELOR OF TECHNOLOGY (CHEMICAL ENGINEERING)

Submitted By
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Session: 2009-10



Under the guidance
of
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2010



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CERTIFICATE

This is to certify that the work for the thesis entitled “CONVERSION OF FOOD WASTE TO USEFUL CHEMICALS/PRODUCTS” submitted by Srishail Kumar in partial fulfillment of the requirements for the award of Bachelor of Technology Degree in Chemical Engineering (Session 2006-2010) at National Institute of Technology Rourkela (Deemed University) is an authentic work which was carried out by him under my supervision and guidance

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To the best of my knowledge, the matter embodied in the thesis has not been submitted to any other University/Institute for the award of any Degree or Diploma.

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ACKNOWLEDGEMENT

I would like to express sincere gratitude and appreciation to my project guide Dr.R.K.Singh, Professor, Department of Chemical Engineering, National Institute of Technology, Rourkela for his kind support, guidance, constructive criticism and timely advice during every stage of this project without which my project would not have completed. I am also thankful to Prof. S.K.Agarwal, Head of the Department, for providing the necessary opportunities for the completion of our project.

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ABSTRACT

In the following analysis, study was done based on the principles of fermentation. The raw materials involved were jack fruit and banana peels. Fermentation experiments were carried out on the aforementioned raw materials with the major chemical extracted being ethanol. To ensure that a measurable amount of chemical is derived the raw material was enhanced in starch content by adding plain sugar. However, excessive sugar added would cause the microorganisms to wither away due to a phenomenon called substrate inhibition. Therefore, to ensure no substrate inhibition, initial experiments were carried out to determine the upper limit to the amount of sugar being added. This was done using the growth kinetics phenomenon of the yeast added.

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CHAPTER – 1

INTRODUCTION

Food waste is "any food substance, raw or cooked, which is discarded, or intended or required to be discarded", according to the legal definition of waste by the EU Commission [1,2]. Proper management and recycling of huge volumes of food waste is one of the challenges faced by today's world [3]. Every tone of food waste means 4.5 ton of CO₂ emissions. The food wastes are generated largely by the fruit-and-vegetable/olive oil, fermentation, dairy, meat, and seafood industries [4].

Food wastes are a result of non uniform food trends al over the world and may also be attributed to the variations in food trends in different parts of the world. The eating habits in different places varies with the climatic conditions and the economy of the area[5].

Food waste is the discarding of food that is potentially usable. Both edible and inedible foods may be considered garbage and therefore wasted. Edible foods are considered inedible when their quality deteriorates until they become unhealthy or noxious. Deterioration of food occurs from microbial contamination or from rotting due to overproduction, storage problems, or improper preparation. Food is also wasted through food use that returns little nutritional value, like over processing and overconsumption [6].

Edible foods are also wasted when cultural or individual preferences say that food is undesirable. For example, some people dislike bread crusts, so they remove them and discard them. Societies with abundant food supplies often consider reusing leftover foods as inconvenient, while less food-rich societies regard food reuse as imperative. Specific parts of animals and plants considered edible in some cultures are considered inedible in others. Animal parts viewed as waste may include bones or shells, skins or scales, fat, blood, intestines, brains, eyes, and stomachs. Plant parts viewed as waste may include cores, seeds, stems, outer leaves, shells, rinds, husks, or peels [6].

1.1 Cultural Variations in Food Waste

Food systems in different cultures vary in the proportion of food waste that is discarded. Cultural variations exist in what is considered garbage, and understanding cultural food rules is crucial in examining food waste. For example, intestines and other internal organs are considered delicacies in China but are discarded as offal in many Western countries. Animal fats are consumed or used as fuel in societies like the Inuit, but in postindustrial nations fats are often trimmed and discarded to reduce caloric intake. Blood is an ingredient in dishes like black pudding in Britain but is discarded in many other societies.

Cultural differences in beliefs about what is edible versus inedible exist more often for animal foods than for plant foods. This may be because animals are similar to humans, so that edibility involves more symbolic meanings. Also, plant food wastes often constitute parts indigestible by humans that therefore have no nutritional value, such as vegetable rinds.

Moral values in most cultures admonish food waste. However, food protests and food riots may intentionally waste food to make ideological and ethical points. Many groups are proud of their efficient use of all parts of a slaughtered animal, such as Cajun claims to use "everything except the squeal" of hogs. Agricultural societies often feed plant food wastes to animals, while many industrial societies' process by-products of animal slaughter into livestock feed. Such practices recycle undesired by-products into edible foods and minimize actual food waste. Some societies accept the waste of less-desirable portions of animals and plants as a sign that they have attained a state of affluence and can afford to consume only high-quality items [6].

1.2 Food Systems and Food Waste

Postindustrial societies waste food across all stages of the food system. Food production wastes preharvest food through natural disasters, diseases, or pests; harvested food by inefficient collection of edible crops or livestock; and postharvest food in storage or contamination losses. Food processing wastes food in spillage, spoilage, discarding substandard edible materials, or removing edible food parts in inefficient processing. Food distribution wastes food by offering more food than consumers will purchase and then discarding unsold products. Food acquisition wastes food when consumers purchase more food than they use. Food preparation wastes food

by removing edible parts of foodstuffs, spilling or contaminating foods, and rendering foods inedible through improper handling and overcooking. Food consumption wastes food by taking larger portions than can be eaten or by spilling food. Digestion, transport, and metabolism of foods in the body waste nutrients through inefficient absorption, storage, or utilization, thereby failing to use all nutrients that were ingested.

Waste streams in the food system are the by-products of human production and consumption. Garbology, the study of human waste behaviors, identifies food waste as a significant portion of the total human waste stream. Food waste comprises about 10 percent of the total municipal solid waste streams in postindustrial nations and higher percentages in societies lacking mechanized refrigeration and durable packaging.

The four principal methods of disposing of food waste are dumping, burning, minimizing, and recycling. Dumping is the most common method of food waste disposal, but it may create sanitation and landfill problems. Burning food waste is convenient and minimizes the amount of solids needing to be disposed, but burning reduces air quality and is banned in many places. Minimizing food waste occurs through food trades, gifts, donations, and conservation during preparation and after consumption, such as reusing leftovers. Recycling often involves feeding food waste to livestock or composting food refuse. Compost can be used as fertilizer to grow more food, reducing the absolute food waste [6].

1.3 The Cost of Food Waste

Food waste significantly impacts environmental, economic, and community health. The accumulation of discarded food in landfills contributes to air and water pollution, and the burning of food refuse also affects air quality. Economic and nutritional losses are incurred from the calories lost in discarded food as well as from the energy and materials used to transport food waste to landfills. Wasted food means fewer nutrients are available for human consumption, which jeopardizes community food security.

There are also costs associated with the use of salvaged foodstuffs. For example, feeding animal slaughter by-products to livestock has caused outbreaks of bovine spongiform encephalopathy

(BSE) and hoof and mouth disease in several European nations. Consumption of leftover foods that were not prepared or stored properly is implicated in many cases of food borne illness [6].

1.4 Historical Changes in Food Waste

Historical transformations have changed the type and amount of food waste generated. Hunter-gatherer cultures often discarded bones as their primary food waste. The development of agriculture added more plant materials to the food waste stream. Industrialized agriculture increased organic waste by-products from large-scale food processing. Increased population growth and urbanization multiplied and concentrated the amount of food waste, which was increasingly dumped as the cities that generated waste became located farther from agricultural areas.

Historical shifts occurred in the conception of food waste. The term "garbage" originated in the French word for entrails and once referred exclusively to food waste. Later the word signified all refuse, since food waste embodies the most unacceptable characteristics of solid waste, putrefaction and attraction of vermin.

Material prosperity reduces the economic necessity for food conservation and reuse, and conspicuous consumption and disposal are demonstrations of social status. Food in postindustrial societies is inexpensive relative to total income, and wasting food is increasingly accepted. Technology that improves the durability of foods, such as plastic packaging, has reduced food waste from spoilage but has created a new waste problem as food packaging contributes more to the waste stream than food itself. Regardless of consumption and disposal practices, the growing world population has increased food waste [6].

CHAPTER – 2

LITERATURE REVIEW

2.1 Bioconversion of food wastes

Bioconversion of food processing wastes is receiving increased attention with the realization that waste components represent an available and utilizable resource for conversion to useful products. Liquid wastes are characterized as dilute streams containing sugars, starches, proteins, and fats. Solid wastes are generally cellulosic, but may contain other biopolymers. The greatest potential for economic bioconversion is represented by processes to convert cellulose to glucose, glucose to alcohol and protein, starch to invert sugar, and dilute waste streams to methane by anaerobic digestion. Microbial or enzymatic processes to accomplish these conversions are described [7].

Bioconversion of food waste for energy production is a process that can produce a significant amount of power. This is energy that does not come from foreign oil or other fossil fuel sources, and it is a much cleaner source of energy than many other energy sources. Food waste is discarded in large amounts all across the US, and the world, and bioconversion technology has the ability to turn this food waste into significant amounts of energy that can be used to power homes and vehicles. Food waste is biomass, and can be used in the biomass fermentation process to produce energy. Food waste can also be used in the biodiesel process. With all of the food waste that is thrown away, the bioconversion process would allow this waste to be reused instead, and become a source of energy for electricity, and fuel for your car [8].

The biodiesel process can use bioconversion technology to turn food waste into biodiesel fuel. Biodiesel fuel burns cleaner, has far fewer emissions, and does not pollute like some other fuels do. Biodiesel is also much safer, both for the earth and the people and animals on it. The biodiesel process offers an increase in local jobs, and more taxes going into the local governments. Because waste food does not need to be transported far away to undergo the bioconversion process, transportation costs are decreased, and all the pollution that usually accompanies transporting things long distances. Biomass fermentation can be done locally, using waste food and producing energy in the local community. This means less waste to fill the

landfill, and a renewable and sustainable alternative energy source which is part of the municipal waste stream [8].

Biomass fermentation can produce bioethanol as well. Bioethanol is a biofuel that can be used in place of gasoline. The fermentation process converts sugars in the biomass into biomass ethanol, which can be used in vehicles as a biomass fuel. Waste food that gets thrown away in the country, and around the world, is full of sugars and oils and fats which are perfect for creating biofuels. Many restaurants throw out thousands of gallons of used cooking oil and frying fat every single day, and this represents a large amount of biodiesel fuel that could be created using the waste food and bioconversion technology. In addition, households represent sixty percent of all the waste food thrown out, and this equates to tons of table scraps and discarded food which could be used to heat your home, power your light and appliances, and fuel your vehicle as well. Bioconversion technology makes it possible for food to leave your home as garbage and come back as electricity or another energy form [8].

Using alternative renewable energy sources which do not rely on fossil fuels means making the earth a better place. Bioconversion technology makes it possible to have the energy you need without putting a strain on the earth with high carbon emissions and pollution, or relying on foreign countries for fossil fuel supplies. The future of America and the world depend on using new energy sources, and waste food bioconversion is one that is renewable and sustainable. There will always be waste food and municipal waste, and using bioconversion technology keeps the landfills from becoming full, while getting rid of garbage and providing an eco-friendly energy source. There are many products that are possible using waste food and the available bioconversion technology. Biodiesel, bioethanol, biogas, electricity, heat, and more are possible from waste food [8].

2.2 Food Waste Treatment Methodologies

Since food industries produce a substantial amount of pollution, it is becoming more and more imperative to solve this problem. As the constraints related to environmental issues are becoming quite stringent, it is necessary to develop optimized systems for food waste treatment. Among the several biological and chemical processes in this chapter, the following are described: composting, anaerobic digestion, aerobic digestion, thermophilic anaerobic digestion, sequencing

batch reactor, electro dialysis, wet oxidation, pyrolysis, incineration, solid state fermentation and ozonation. Most of them have a high capacity to degrade concentrated and difficult substrates (plant residues, animal wastes, food industry wastewater and so forth) [9].

The process of degradation of organic bioreactive waste in landfills involves not only biological process but also inter-related physical and chemical processes. The organic components of the waste are degraded by micro-organisms in the landfill. The organic materials occurring in waste can be classified into main biological groups represented by proteins, carbohydrates and lipids or fats. Carbohydrates are by far the major component of biodegradable wastes and include cellulose, starch and sugars. Proteins are large complex organic materials composed of hundreds or thousands of amino acids groups. Lipids or fats are materials containing fatty acids. Five main stages of degradation of biodegradable wastes have been identified (Kjeldsen *et al.*, 2002). There are numerous factors influencing the degradation of the waste, and these have been reviewed by Westlake (1995) and Christensen *et al.* (1996). Among them, the most important ones are; site characteristics, waste characteristics, moisture content of the waste, temperature and acidity [10].

As an alternative to landfill, wastes containing combustible material may be incinerated or combusted. Incineration is the oxidation of the combustible material in the waste to produce heat, water vapor, nitrogen, carbon dioxide and oxygen. Depending on the composition of the waste, other emissions may be formed including, carbon monoxide, hydrogen chloride, hydrogen fluoride, nitrogen oxides, sulphur dioxide, volatile organic carbon, dioxins and furans, polychlorinated biphenyls, heavy metals, etc. (European Commission, 2004). Incineration is a treatment route which can be applied to a wide variety of wastes. Incineration of waste has a number of advantages over landfill[10];

- i) incineration can usually be carried out near the point of waste collection,
- ii) the waste is reduced into a biologically sterile ash product which for municipal solid waste is approximately 10% of its pre-burnt volume and 33% of its pre-burnt weight,
- iii) incineration produces no methane, unlike landfill,
- iv) Waste incineration can be used as a low cost source of energy,

- v) The bottom ash residues can be used for material recovery or secondary aggregates in construction and
- vi) Incineration is the best practicable environmental option for hazardous wastes

However, there are also disadvantages such as [10];

- i) generally there are much higher costs and longer pay-pack periods,
- ii) lack of flexibility,
- iii) removal of materials such as paper and plastics for recycling may reduce the overall caloric value of the waste and
- iv) Emitted levels may still have an adverse effect on health

2.3 Impact of food processing on the environment

Each step in the food industry system — food production, processing, transportation, storage, distribution and marketing — has some impact on the environment and there is much concern about environmental pollution. Due to the highly diversified nature of the food industry, various food processing, handling and packaging operations create wastes of different quality and quantity, which, if not treated, could lead to increasing disposal problems and severe pollution problems. Additionally, they represent a loss of valuable biomass and nutrients if not recovered by appropriate methods and technologies for upgrading, bioconversion or reutilization. Research should be intensified to improve efficiency in waste treatment, and to minimize waste in food processing and manufacturing operations through advanced manufacturing practices, and constructive utilization of what is unavoidable by bioconversion of by-products and waste into edible food, feed or industrial chemicals in order to decrease environmental loadings as a consequence of better integrated waste management. This review deals with the general characteristics and treatment operations of by-products, wastes and effluents from different categories of the food processing industry and their impact on the environment [11].

The environmental rules are extremely stringent and must ensure that the waste products are either disposed off or utilized to the full to ensure no harmful effects to the environment.

In general, major types of food processing industries associated with environmental objectives may be regarded as [11]

- (a) Agricultural industry
- (b) The meat and fish processing industry
- (c) Fruit and vegetable industry
- (d) Dairy industry
- (e) Packaging industry

Agricultural industry:

Public concern has forced authorities to consider environmental protection as a key strategy in agricultural development model aiming to balance protection of environment and development in the agricultural sector. Currently, one of the most important tasks in agriculture is seeking new progressive methods for solving problem of agricultural wastes due to critical status of the environment. Agricultural inputs such as fertilizers, pesticides, feed additives and irrigation water have been responsible for many of the recent gains in agricultural productivity, but unfortunately a number also have had, or threaten to have, adverse side-effects on the environment. The most significant environmental-associated problems which vary in character from region to region include concerns about the contamination of ground and surface waters by pesticide and fertilizer residues, the effects of ammonia emissions on surrounding vegetation and their contribution to acid rain, and the accumulation of heavy metals in soils . However, on the other hand it must also be realized that agriculture can make a significant positive contribution to the quality of the environment, e.g. utilization of atmospheric carbon dioxide and production of renewable resources via photosynthesis, provision of food and nutritional security. Nevertheless, with modern technology and increased support for agriculture, a conflict has arisen between agricultural and environmental objectives. The intensification of agricultural practices, stimulated by agricultural policies, has had an adverse effect on the environment. Approaches to the control of these effects of agricultural inputs, however, vary significantly among countries, and, in many cases, product quality standards for these inputs have already been introduced to reduce the risks of immediate and long-term cumulative negative impacts on the environment. But, in order to reduce agricultural pollution furthermore, effective agricultural policies should

be developed by introduction of appropriate management agreements, by setting and enforcement of regulatory standards, or by financial support for environmental-protecting strategies. In the future, emphasis should be laid on the use of alternatives to toxic and environment-polluting agricultural chemicals and on integrated pest management systems to cut down on the number of pesticide applications by determining the most appropriate time for application. The development of effective pesticides which do not pose long-term risks to consumer and environment, as well as genetic modifications of raw products so they better withstand typical harvesting, handling, storage and processing will play an important role in future agricultural objectives. This underlines the urgent research needs that will help the entire agricultural system identify, develop, and implement new systems for producing high-quality and wholesome foods with better utilization of raw materials and, above all, reduced adverse effects on the environment[11].

Meat processing industry:

The meat industry is composed of a large number of slaughterhouses and meat packaging plants of widely varying sizes. In general, the characteristics of the waste are much the same, regardless of the size of plants. Waste water from the meat industry, which has a considerable organic load, is strongly polluting the environment and can have adverse effects if discharged into rivers without adequate treatment.

Wastes from the meat industry may be divided into three classifications: (a) stockyard wastes, (b) slaughterhouse wastes, and (c) packaging house wastes. All these types of wastes are highly putrescent and malodorous. When discharged uncleaned to a water course they led to a rapid depletion of dissolved oxygen, damage to aquatic life, and production of odours, sludge deposits and unsightly floating scum. The main sources of polluting matter in the waste water are faeces and urine, blood, grease, washings from carcasses, floors and utensils, undigested food from the paunches of slaughtered animals, waste water from the cooking, curing and pickling of meat and condensate from rendering of offal and other by-product processing. The extent of processing differ greatly from plant to plant, and there is considerable variation in the volume and composition of the waste water generated. It is often possible to make significant reductions in polluting load by good housekeeping within the factory or by modification of the processes,

particular by separately recovering blood and paunches. However, these waste waters behave in a reasonably predictable manner when subjected to various methods of recovery operations and wastewater treatment[11].

Fish and seafood processing industry

The wastes from the seafood processing industry are either solid waste or waste water. Historically, there has been little concern for water pollution control, however, the recent world emphasis on these pollution problems has led to increased efforts to avoid pollution of the environment. Solid wastes consist of flesh, shell, bone, cartilage, and viscera. Waste water solutions contain dissolved materials and suspended solids. Most of the solids can be removed by filtration techniques. Even after filtration, however, the water can contain protein and oil in sufficient quantities to make recovery worthwhile and at the same time to avoid environmental pollution. Seafood waste management alternatives, including dry cleanup, water conservation, water recycling, and by-product recovery, are effective in reducing environmental loadings [11]

Fruit and vegetable processing industry

Large amounts of fruit and vegetable processing wastes are produced from packaging plants, canneries, freezing and drying operations, etc., which are generally derived from similar processes, namely washing, peeling, blanching, transport, instrument washing and sterilization. These wastes are characterized by chemical constituents such as carbohydrates, starches, pectin etc. They are not only as strong in biological oxygen demand (BOD) as domestic sewage but are also highly variable in strength.

Dairy industry:

Wastes in this industry include four major types of [11]

- (a) Milk from flushing and spills
- (b) Dairy products from machinery malfunctions and retail returns
- (c) Whey from cheese and casein production

(d) Ultra filtration permeate from production of cheese and whey

Whey has high organic strength and chemical oxygen demand and so poses high disposal problems. Methods for recovery and utilization of fat, protein and sugar content of waste water using evaporation, centrifugation, ultra filtration and reverse osmosis and also bioconversion processes are available for reduction of environmental loadings.

2.4 Application of hydrothermal reaction

The recovery of waste substances is not only important for prevention of environmental issues, but also for rational utilization of natural resources. Hydrothermal reaction is a prominent method for the treatment of organic wastes and has been attracting worldwide attention. During the process, various reactions such as oxidation, hydrolysis, dehydration and thermal decomposition can be carried out energetically so that the reaction can be successfully used for oxidizing organic wastes to CO₂ and other innocuous end products, as well as for conversion of organic wastes to fuels or useful materials, such as biocrude, hydrogen, glucose, lactic acid, acetic acid, amino acids, etc [12].

2.5 Citric acid production using banana peel as a substrate:

Citric acid is extensively used in dairy, food, beverage, pharmaceutical and biochemical industries. Factors such as moisture content, temperature, pH, inoculum level and incubation time affecting the citric acid production by *A. niger* were standardized by adopting the search technique by varying one factor at a time. To study the effect of moisture content on citric acid production, different sets of banana peels were steamed with water for different periods to get different moisture content ranging from 50–90%. The moisture content favoring maximum citric acid production was followed for subsequent experiments.

To find out the influence of temperature, the citric acid fermentation was carried out at 26, 28, 30, 32 and 34 °C. The temperature giving high amount of citric acid was taken as an optimum temperature. The optimum temperature for citric acid production derived from this experiment was applied for subsequent evaluation.

The effect of initial pH of the substrate was also evaluated by conducting experiments with initial pH of 2, 3, 4, 5, 6 and 7. The other parameters were kept at their optimum levels.

To define an appropriate level of inoculums for citric acid production, *A. niger* spores at various spore concentration levels such as 104, 106, 108, 1010 and 1012/ml were used. The inoculums level giving maximum citric acid production was taken as an optimum level for fermentation.

Experiments were conducted to find out the effect of incubation period by conducting fermentation for 1–5 days. Optimum levels of all the other derived parameters were used. The incubation time giving maximum citric acid production was determined as an optimum incubation time [13].

Table 2.1 [13]

Composition of banana peels (% dry matter, DM).

| <u>Parameters</u> | <u>% Dry matter</u> |
|-------------------|---------------------|
| Dry matter | 14.3 |
| Crude protein | 8.1 |
| Crude fat | 12.1 |
| Crude fiber | 8.2 |
| Carbohydrate | 60.2 |
| Moisture | 78.9 |

2.6 Fermentation process:

Fermentation technology is the oldest of all biotechnological processes. the term is derived from the Latin word “fevere”, meaning to boil, the appearance of food extract or malted grain acted upon by yeast, during the production of alcohol.

Fermentation is the process of chemical change, caused by organisms or their products, usually producing effervescence or heat.

Microbiologists consider fermentation as “an energy generating process in which organic compounds act both as electron donors and acceptors”, hence, fermentation is an anaerobic process where energy is produced without the participation of oxygen or other electron acceptors”.

Chemical equation: $C_6H_{12}O_6 + 2ATP + 2ADP + 4NADH \rightarrow 2C_2H_5OH + 2CO_2 + 4ATP + 2H_2O + 4NAD^+$

2.7 Microorganisms

Several species belonging to the following categories of micro organisms are used in fermentation processes:

Prokaryotic cells

Unicellular- bacteria, cyanobacteria

Multicellular- cyanobacteria

Eukaryotic cells

Unicellular- yeasts, algae

Multicellular- fungi, algae

Unicellular and micro fauna are rarely a part of fermentation processes while isolated cells of multi cellular animals are frequently cultured.



Fig 2.1: Microorganism Petridish

2.8 Microbial growth

(a) Requirements for artificial culture

The growth of organisms requires complex energy based processes. The rate of growth of microorganisms is dependant on several culture conditions, which should provide for the energy required for various chemical reactions. The production of a specific compound requires precise culture conditions at a specific growth rate. Many systems now operate under computer control.

The rate of growth of microorganisms and hence the synthesis of various chemical compounds under the artificial culture require organism specific chemical compounds as the growth medium(nutrient).

The kinds and relative composition of the medium, the pH, temperature, purity influence microbial growth and hence production of the biomass (the total mass of cells or organisms being cultured) and synthesis of various compounds.

Table 2.2 [13]

Nutrient sources for industrial fermentation

| <u>Nutrient</u> | <u>Raw material</u> |
|-------------------|-----------------------------------|
| (Carbon source) | |
| Glucose | corn sugar, starch, cellulose |
| Sucrose | sugarcane, sugar beet, molasses |
| Lactose | milky whey |
| Fats | vegetable oils |
| Hydrocarbons | petroleum fractions |
| (Nitrogen source) | |
| Protein | soya bean meal, corn steep liquor |
| Ammonia | pure ammonia or ammonium salts |
| Nitrogen | nitrate salts |
| Nitrate | air |
| Phosphorus source | phosphorus salts |

2.9 Phases of microbial growth

When a particular organism is introduced into a growth medium, the medium is inoculated with the organism. Growth of the organism doesn't occur immediately, but takes time, this is called the "lag phase".

Following the lag phase, the rate of growth of the organism increases steadily, for a certain period, this is known as "the growth or the exponential phase".

After a certain time, the rate of growth decreases gradually due to continuously falling concentrations of the nutrients or the continuously increasing concentrations of the toxic substances. This is where the rate of growth is checked and is known as the "deceleration phase".

After decoration, the culture ceases to grow and the concentration reaches a constant value, known as "stationary or steady phase". The biomass remains constant, except during chemo lysis (when accumulated chemicals in the culture lyse the cells).

Mutation of organisms in the cells can also be a source of contamination, called "internal contamination".

2.10 Substrate inhibition

Inhibition of an enzyme activity by a substrate of the reaction catalyzed by that enzyme; often, this type of inhibition occurs at elevated substrate levels in which the substrate is binding to a second, non-active site on the enzyme[14].

The large concentrations of substrate molecules lead to the micro organisms to wither away without functioning to their full capacity. This reduced functionality may be avoided by determining an upper level of the substrate concentration and using concentrations at values lower than this, thereby avoiding any chances of inhibition due to substrate.

CHAPTER-3

EXPERIMENTAL METHODS

3.1 Preparation of glucose solution

A 250 ml broth was initially prepared in a conical flask with all the nutrients and glucose added in desired quantities in 250 ml of distilled water.

The flask contents were then shaken to dissolve its contents completely.

The glucose concentration was initially varied from 5% onwards with an increase of 5% concentration.

The nutrients used are

- (a) Urea – 2 gm/l.
- (b) Magnesium sulphate – 0.5 gm/l.
- (c) Copper sulphate – 0.1 gm/l.
- (d) Zinc sulphate – 0.1 gm/l.
- (e) Glucose – desired concentration

The nutrients added and their functions may be enumerated as follows:

- (a) Magnesium ions insulate cells against stress factors temperature, alcohol and osmotic pressure.
- (b) Zinc ions help cells to produce fermentation enzymes. Their absence may cause slow or sluggish fermentation and poor yield.
- (c) Calcium ions help to stimulate cell growth and cell wall permeability.
- (d) Copper assists with cell internal enzyme production.
- (e) Potassium assists with storage of ATP inside the cells.

The nutrients are added in the desired quantities and weights are measured accurately using an electronic weight balance. Volumes and weights measurement accuracy should be maintained to

ensure minimum error during the process. Therefore, even smallest amounts of chemicals or the nutrients must be measured with absolute accuracy.

3.2 Cleaning of the different apparatus

The laminar flow was first cleaned with ethanol and then the ultraviolet light was switched on for 20 minutes. This was done to free the hood storage space off all bacterial contaminants.

The glass wares to be used were also washed with distilled water before being put to use. Initial warmth was provided in the oven to remove any water particle left sticking to the glass wares.

The process sterility is of utmost importance and must be ensured at all times.

The apparatus should be cleaned and maintained in a sterile environment during the experiment.

The equipment should look, smell and feel clean, even when it's not in use, which would cause fewer problems keeping everything sanitized.

Clean primary fermenters should be stored and carboys closed with a little chlorine solution inside (one capful unscented household bleach per litre cold water). Nothing will grow in them, so sanitizing will be a snap.

Lapses in sanitation are responsible for 90% of all home brewing failures. This can be avoided.

Word equation

Glucose + Inorganic phosphate + Adenosine Tri-phosphate + Adenosine di-phosphate + (Reduced) Nicotinamide adenine dinucleotide -----> Ethanol + Carbon dioxide + Adenosine tri-phosphate + water + Nicotinamide adenine dinucleotide

This equation is not comprehensive and the actual process of fermentation incorporates 12 individual enzyme controlled reactions.

Breakdown of sugars by bacteria and yeasts using a method of respiration without oxygen (anaerobic). The enzymes in yeast break down glucose to give two products: ethanol (alcohol) and carbon dioxide. Fermentation processes have long been utilized in baking bread, making beer and wine, and producing cheese, yogurt, soy sauce, and many other foodstuffs.

3.3 Preparation of pure culture

Vial pure culture of freeze dried *Saccharomyces cerevisiae* placed in a flask containing the broth with specified concentration of glucose. The yeast was added at a concentration of 5% solution in distilled water and then slowly added to the broth using a sterilized glass rod.

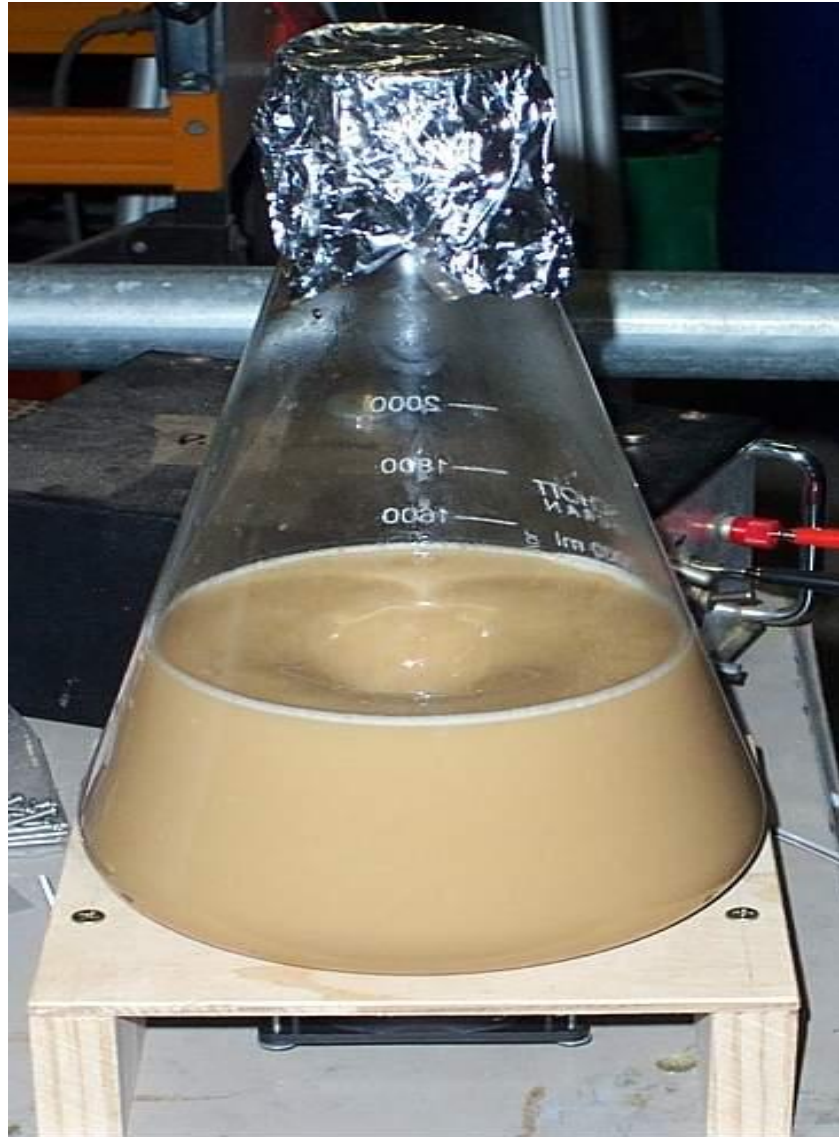


Fig.3.1 Fermentation Broth

3.4 Process conditions

The pH was maintained of the broth solution at about 5-5.5 using addition of sulphuric acid or sodium hydroxide. A pH meter was used to monitor the pH levels [15].

The contents of flask were then autoclaved at 121°C at 15 psig for 30 minutes. An extra amount of distilled water was also autoclaved for further usage [15].

The autoclaved broth was then allowed to cool to room temperature (30-35°C) .the distilled water autoclaved was also kept aside for cooling and future use.

3.5 Addition of yeast culture to broth

After the broth attained room temperature, the entire setup was then assembled in a laminar flow hood chamber in which a spirit lamp was lighted. The cleaning and sterility of the chamber was done a few minutes before the assembling of the setup .

Now the yeast which was stored at cool temperature was brought and a solution of 20 gms yeast was prepared in 250 ml of the autoclaved distilled water[15]. This was then slowly introduced into the broth solution, both of the solutions essentially being at room temperatures.

3.6 Incubation

The above (broth+culture) solution was then placed in a shaker incubator .the incubator was maintained at room temperature and rotated at an rpm of 100. There it was left for 24 hrs to ferment. The solution samples were taken out for observation at intervals of 8 hrs [15].

The incubator should be handled carefully and should be turned off at regular intervals to ensure no over heating due to prolonged usage.

The incubator should be maintained contamination free by keeping the hood closed at all times by keeping the hood closed and air tight. The process sterility can only be ensured when at all times the contamination due to other microbes is avoided by constant cleaning or keeping the apparatus away from contamination sources. The cleanliness is of utmost importance to ensure no interference from foreign and unwanted particles.

3.7 Sample estimation

After intervals of 8 hrs. , the sample was taken out of the incubator shaker for sample estimation. This was done by using a spectrometer. Absorbance% and transmittance% were measured by the spectrometer.

Graphs of absorbance % vs. time were plotted for each concentration of glucose. With increasing glucose concentration, the stationary phase period continuously increases for the cell growth kinetics. Plots were done for each concentration of glucose. At a definite glucose concentration, the aforementioned phase period begins to decrease, this is the breakaway point as at glucose concentrations beyond this level, the yeast cells begin to die away due cell wall rupture and there is no replication of the cells. Thus, for further experiments, this would be the maximum amount of glucose concentration to be added to the raw material to ensure proper functioning and growth of the cells.

A spectrophotometer consists of two instruments, namely a spectrometer for producing light of any selected color (wavelength), and a photometer for measuring the intensity of light. The instruments are arranged so that liquid in a cuvette can be placed between the spectrometer beam and the photometer. The amount of light passing through the tube is measured by the photometer. The photometer delivers a voltage signal to a display device, normally a galvanometer. The signal changes as the amount of light absorbed by the liquid changes.

A spectrophotometer (or spec as it is generally called), measures the absorbance of a sample at a particular wavelength set by the user. The user has to choose the wavelength maxima of the sample and input this value before taking any readings.

A blank solution has to be prepared - one that contains all the sample components except the analyte. The instrument measures the absorbance of this blank and assigns it a value of zero. Sample reading are taken with reference to this zero setting.

The absorbance values that are obtained from samples are also referred to as the optical density

3.8 Determination of %alcohol in fermented broth:

Now, after determining the maximum glucose concentration to be added to the raw material, the amount of alcohol derived by the fermentation process was determined by distilling the fermented broth.

The distillation setup consisted of a round bottom flask(500 ml), a condenser tube, a collection conical flask, a heating mantle ,a test tube stand, a water connection (for the cooling circuit), two bends at either ends of the condenser tube and pipes for the water connections.



Fig 3.2. Distillation setup

For the process, equal volumes of the fermented solution and distilled water were taken in the round bottomed flask. The mantle heating temperature was maintained at 78°C (the boiling point of ethanol). Mantle was switched on and it was ensured that the entire setup was leak proof. Distillation was allowed to carry on until the same amount of condensate was collected at the outlet of the condenser tube.

This amount of condensate is then placed in a measuring cylinder of suitable volume and a specific gravity spindle is then inserted into the cylinder. Then the reading on the spindle was noted down and also temperature of the broth.

Corresponding to the above spindle reading and temperature, an alcohol index (A) value was determined [16]. This value was then inserted in the following formula to determine the alcohol percentage

$$\% \text{ alcohol} = (100 - A) \times 0.5714$$

The spindle readings are divided as compared with the reading of 55. A spindle reading above 55 would indicate upper grade alcohol and that below 55 would be indicative of the lower grade alcohol.

Other products produced during the aforementioned processes would include methane, ethane and similar other hydrocarbon gases evolved during fermentation. Entrapment of the gases and their study is normally done using an HPLC setup, also known as high performance liquid chromatography.

It is a liquid chromatography which involves the separation of the compounds on the basis of their polarity. It is used to analyze, identify, purify & quantify the compounds.

It involves passing a mixture dissolved in a "mobile phase" through a stationary phase, which separates the analyte to be measured from other molecules in the mixture based on differential partitioning between the mobile and stationary phases

3.9 Tables and Graphs

Table 3.1: 5% glucose

| Time(hrs) | %absorbance |
|-----------|-------------|
| 0 | 0.3026 |
| 8 | 0.5152 |
| 16 | 0.6989 |
| 24 | 0.7059 |
| 32 | 0.6889 |

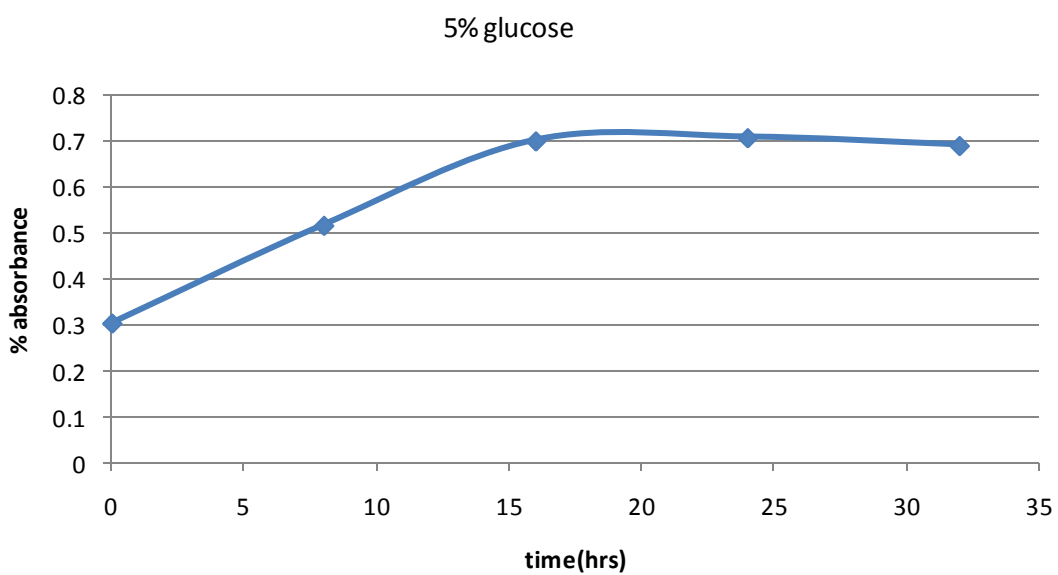


Fig. 3.3: graph of % absorbance vs. time

Table 3.2:10 % glucose

| Time(hrs) | % Absorbance |
|-----------|--------------|
| 0 | 0.7 |
| 8 | 0.8236 |
| 16 | 0.905 |
| 24 | 0.9046 |
| 32 | 0.9041 |
| 40 | 0.8862 |

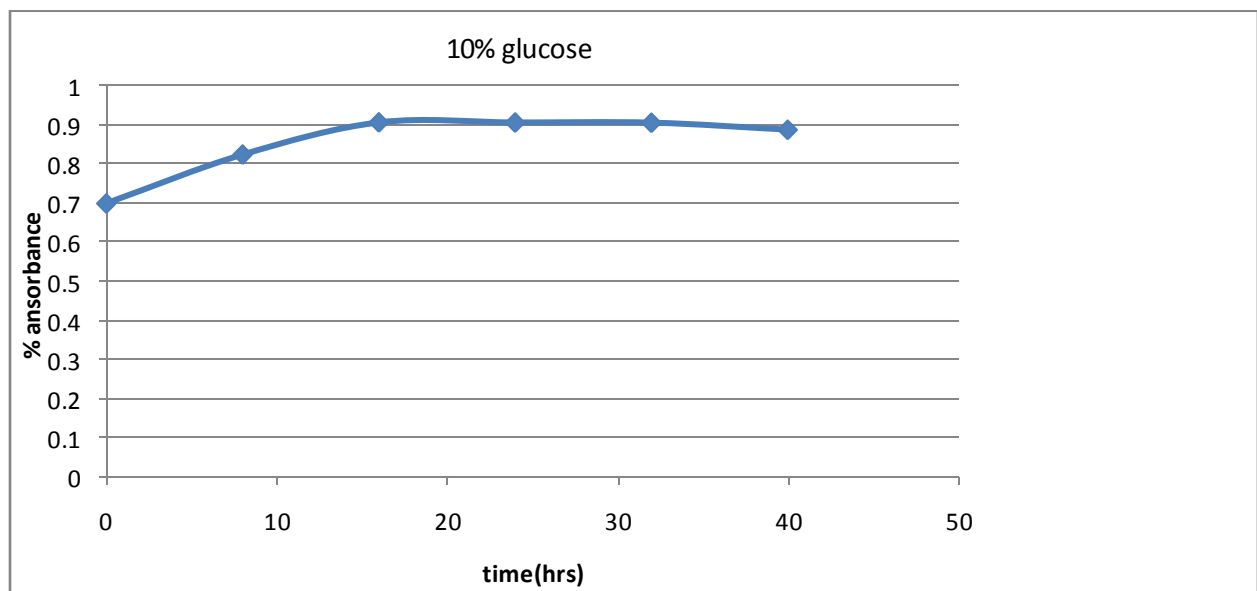


Fig. 3.4: graph of % absorbance vs. time

Table 3.3:15% glucose

| Time(hrs) | % absorbance |
|-----------|--------------|
| 0 | 1.02 |
| 8 | 1.2 |
| 16 | 1.25 |
| 24 | 1.365 |
| 32 | 1.44 |
| 40 | 1.445 |
| 48 | 1.452 |
| 56 | 1.47 |
| 64 | 1.39 |

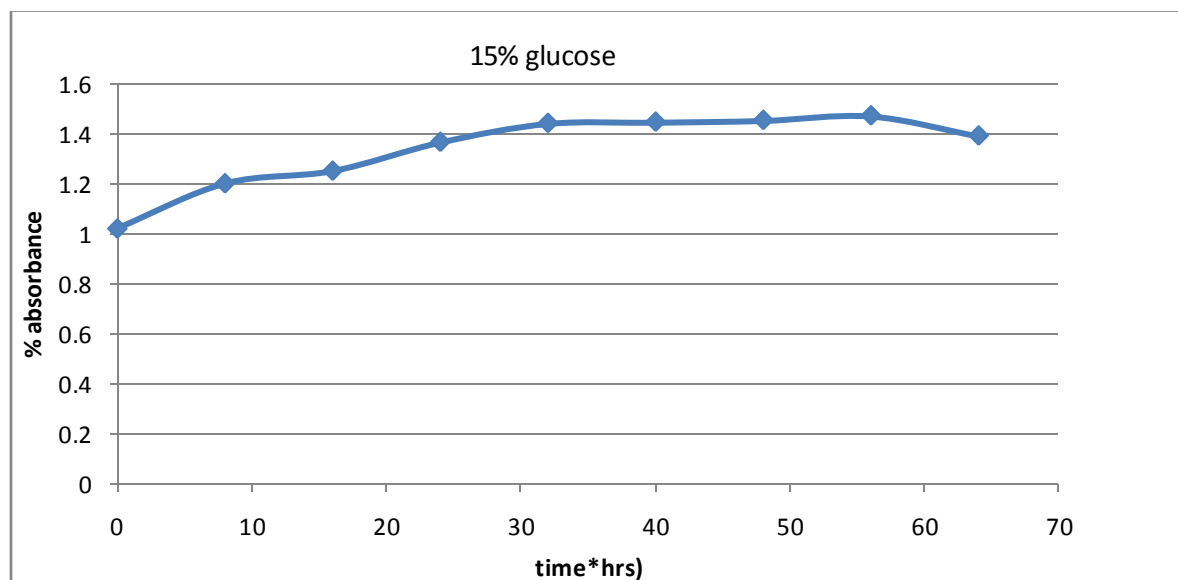


Fig. 3.5: graph of % absorbance vs. time

Table 3.4:20% glucose

| Time(hrs) | % absorbance |
|-----------|--------------|
| 0 | 1.58 |
| 8 | 1.6012 |
| 16 | 1.62 |
| 24 | 1.704 |
| 32 | 1.704 |
| 40 | 1.706 |
| 48 | 1.706 |
| 56 | 1.7078 |
| 64 | 1.6956 |

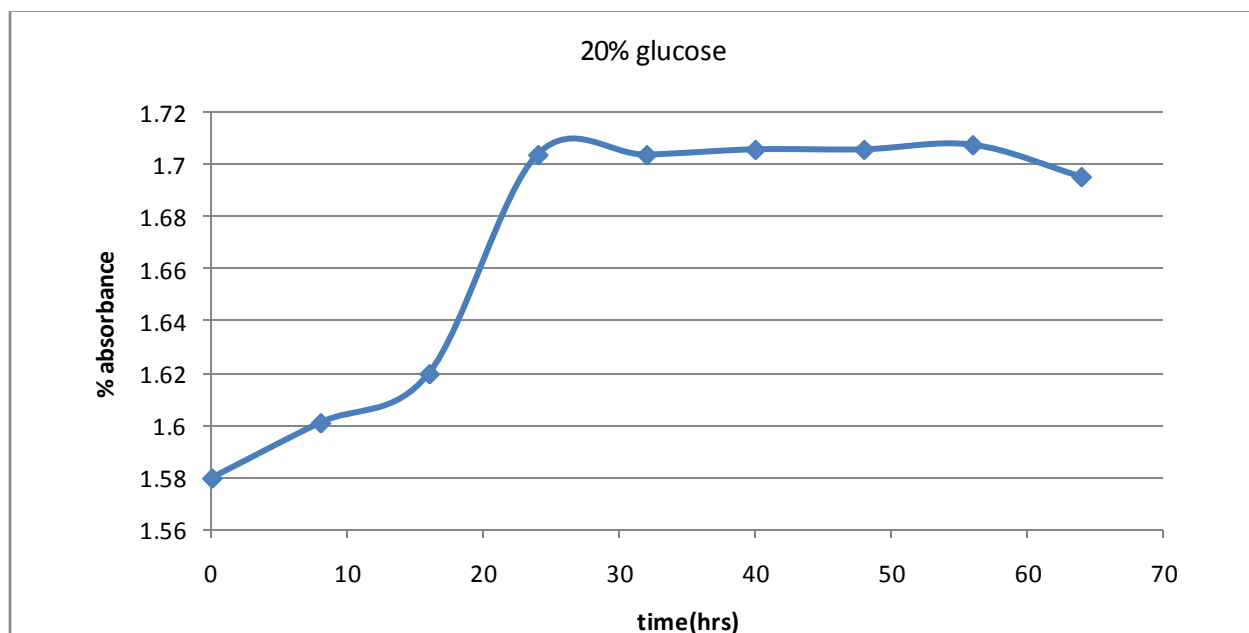


Fig. 3.6: graph of % absorbance vs. time

Table 3.5:25% glucose

| Time(hrs) | % absorbance |
|-----------|--------------|
| 0 | 1.519 |
| 8 | 1.659 |
| 16 | 1.66 |
| 24 | 1.667 |
| 32 | 1.6673 |
| 40 | 1.6598 |

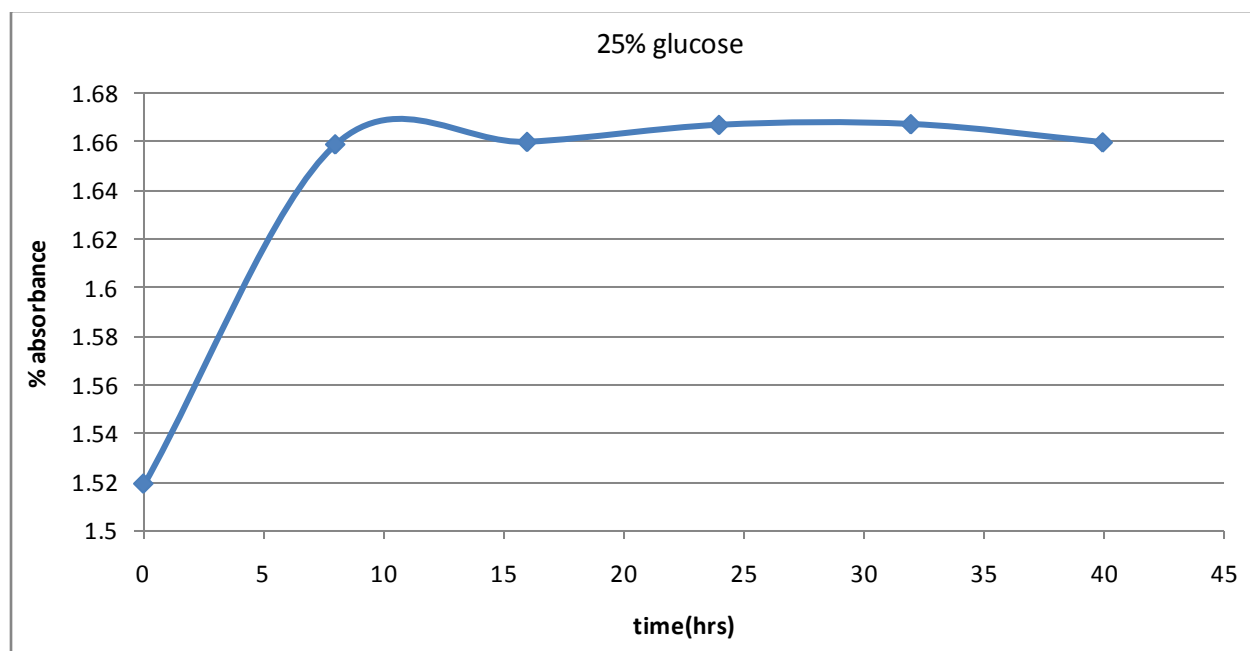


Fig. 3.7: graph of % absorbance vs. time

Table 3.6:21% glucose

| Time(hrs) | %absorbance |
|-----------|-------------|
| 0 | 1.43 |
| 8 | 1.534 |
| 16 | 1.635 |
| 24 | 1.723 |
| 32 | 1.724 |
| 40 | 1.7238 |
| 48 | 1.725 |
| 56 | 1.725 |
| 64 | 1.698 |

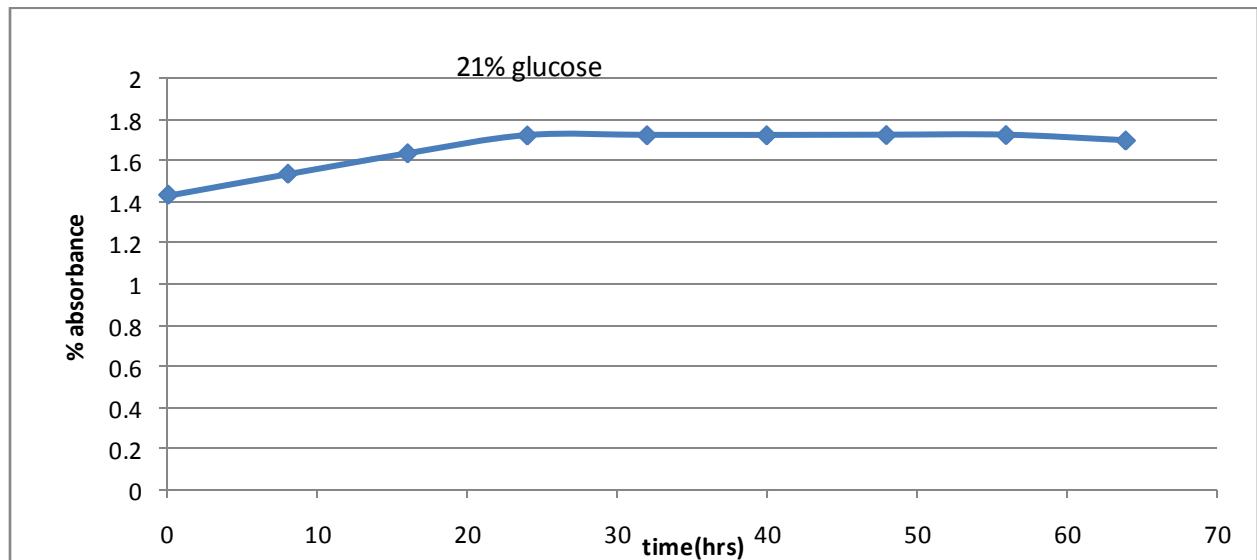


Fig. 3.8: graph of % absorbance vs. time

Table 3.7:22% glucose

| Time(hrs) | % absorbance |
|-----------|--------------|
| 0 | 1.469 |
| 8 | 1.554 |
| 16 | 1.641 |
| 24 | 1.7325 |
| 32 | 1.733 |
| 40 | 1.7346 |
| 48 | 1.7325 |
| 56 | 1.7355 |
| 64 | 1.7248 |

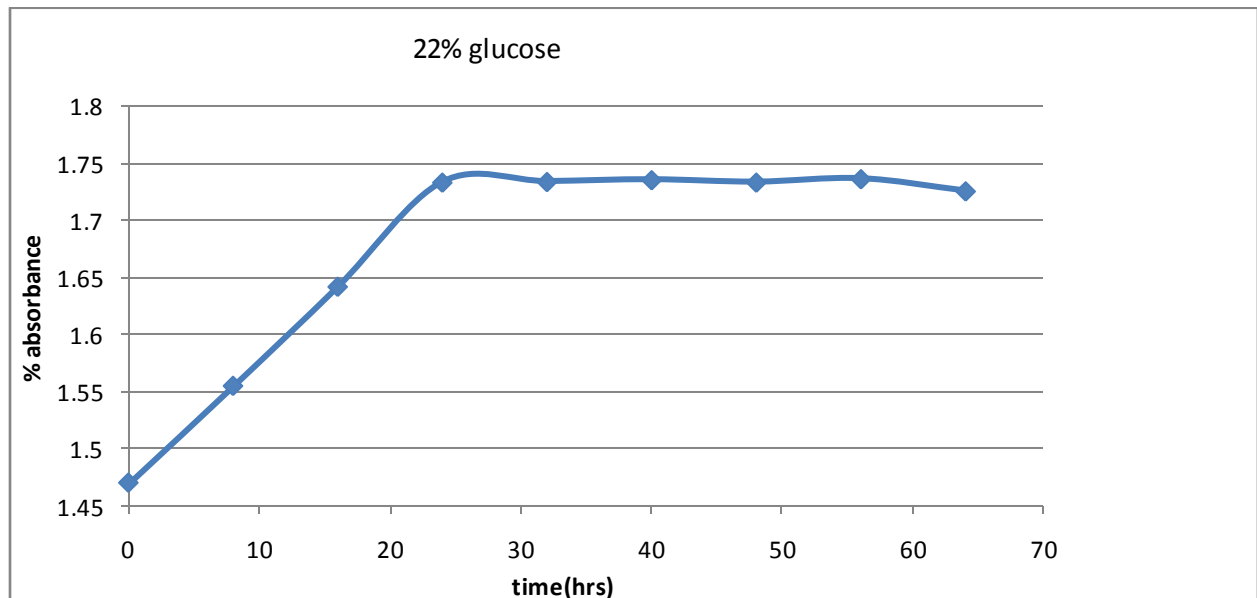


Fig. 3.9: graph of % absorbance vs. time

Table 3.8:23% glucose

| Time(hrs) | % absorbance |
|-----------|--------------|
| 0 | 1.4833 |
| 8 | 1.5916 |
| 16 | 1.688 |
| 24 | 1.777 |
| 32 | 1.778 |
| 40 | 1.7802 |
| 48 | 1.7658 |

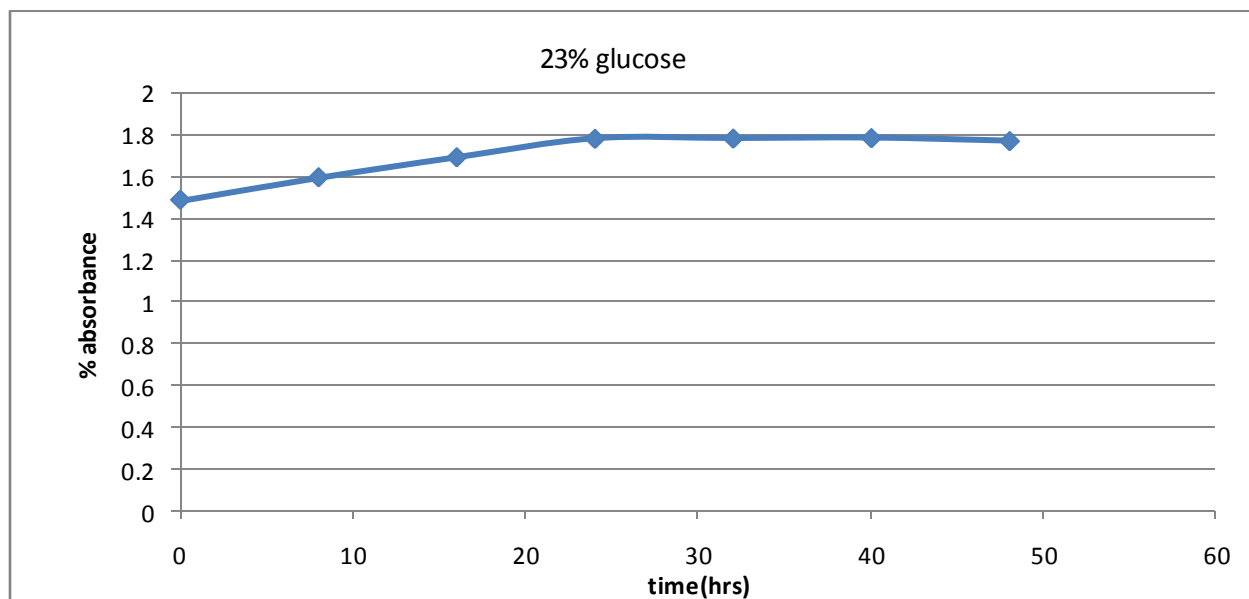


Fig. 3.10: graph of % absorbance vs. time

From the preceding graphs, it is evident that a maximum concentration of 23% glucose can be added along with the raw material to ensure no substrate inhibition.

3.10 Jack fruit seed fermentation

Seed pretreatment:

The jack fruit seeds were collected and dried in an oven at 60°C for 8 hrs. [23]. Then the dried were peeled off their skin and grinded to a fine powder. The powdered seeds were then sieved to and even finer size using sieves of mesh size 40[23].

Table 3.9: jack fruit seeds powder contents [23]

| | Jackfruit seed flour |
|-----------------|----------------------|
| Composition (%) | Dry milled |
| Moisture | 6.34 |
| Protein | 11.83 |
| Fat | 2.19 |
| Ash | 3.74 |
| Amylose | 36.67 |
| pH | 6.81 |

Slurry preparation:

To 125 gms of the above formed seed powder, 500 ml. of distilled water was added along with 0.520 gms. of alpfa amylase enzyme. [24]

Conditions: pH was maintained at 7 using sodium hydroxide or sulphuric acid.

Incubation was done for 10 min at 90 °C [24]

The fermentation and distillation processes were then carried out as described before.

3.11 Banana peels fermentation

Banana peels were from the fruit stall and from leftovers from bananas .Pre-processing of the banana peels was done by air drying for 72 hrs[19] and oven drying at 60 °C for 45 hrs[19] to constant the weight. The sample was then ground and weighed to amount to 62.5gms.

The powdered form of the banana peels was then mixed with 250 ml. of distilled water to a concentration of 25%. Plain sugar was also added to compensate for the low glucose content in

the banana peels to a concentration of 20 %(the reduced value from that determined above as 23% may be accounted to compensate for the alcohol produced from the peels).

The rest of the fermentation process was then carried out as described before.

The distillation and alcohol determination was done as earlier.

Table 3.10: Nutritional composition of Bananna Peel[25]

| Parameter | Concentration |
|-------------------------|---------------|
| Moisture (%) | 6.70 |
| Ash (%) | 8.50 |
| Organic matter(%) | 91.50 |
| Protein(%) | 0.090 |
| Crude lipid(%) | 1.70 |
| Carbohydrate(%) | 59.00 |
| Crude fibre(%) | 31.70 |
| Hydrogen cyanide(mg/gm) | 1.33 |
| Oxalate(mg/gm) | 0.51 |
| Phylate(mg/gm) | 0.28 |
| Saponins(mg/gm) | 24.00 |

CHAPTER – 4

RESULTS AND DISCUSSIONS

1. Glucose:

T=87.8°F

Spindle reading= 52

Sykes reading= 84.9

% Alcohol = $(100 - 84.9) \times 0.5714 = 8.62\%$

2. Jack Fruit:

T=87.8°F

Spindle reading= 55.7

Sykes reading=91.0

% Alcohol = $(100 - 91) \times 0.5714 = 5.14\%$

3. Banana Peel:

T= 87.2°F

Spindle reading=51.5

Sykes reading=84.1

% Alcohol = $(100 - 84.1) \times 0.5714 = 9.1\%$

The above values of alcohol percentages obtained are varying from the standard values due to the inconsistencies that have crept into the experiments. One of the major drawbacks may be described as the interference of other microorganisms which could not be eliminated completely by the precautions taken.

The studies conducted on the amount to pure glucose to be added to the raw material have indicated that beyond a certain value of glucose concentration, the microorganisms die away and the amount of enhancement should be confined to a value within this upper limit.

The other factors affecting the results would be slight variations in the temperatures of incubation, which could not be kept constant throughout the fermentation period.

Another factor affecting the results could be the performance of the spectrometer studies. The absorbance values could be having slight deviations from the actual values.

CHAPTER – 5

CONCLUSION

The studies conducted above indicate that a viable amount of alcohol could be produced from banana peels as the substrate, under the condition that some amount of plain sugar as a source of carbon is added.

The jack fruit seeds could be used as the substrate in the case of unavailability of a carbon source or when the use of a carbon source as an enhancer is prohibited.

It is seen that the alcohol produced from jack fruit seeds was lesser as compared to that from the banana peels in spite of its higher carbohydrate content. This may be attributed to the fact that the additional glucose added to banana peels increases its alcohol production.

The graphs and tables indicate that the amount of pure glucose to be added to the raw material should be maintained at levels determined by experiments to ensure no effect on the growth of the microorganisms.

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