STUDIES ON THE APPLICATION OF ENZYME CONSORTIA ON SUGARCANE BAGASSE & PRODUCTION OF VALUE-ADDED PRODUCTS

A thesis submitted towards the partial fulfilment of the requirements for the degree of Master of Technology in Food Technology and Biochemical Engineering course affiliated to Faculty of Engineering and Technology, Jadavpur University

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DECLARATION OF ORIGINALITY AND COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as part of my Master of Technology in Food Technology and Biochemical Engineering.

All information in this document have been obtained and presented in accordance with academic rules and ethical conduct.

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ABSTRACT

Agricultural wastes such as rice straw, sugar beet, and sugarcane bagasse have become a critical environmental issue due to growing agriculture demand. This study aimed to investigate the valorization possibility of sugarcane bagasse waste. For every ton of sugarcane processed, approximately 250-280 kg of bagasse is produced. This study focuses on a biological method to obtain reducing sugar from the SCB by using mold. *Aspergillus niger was used* to produce enzyme like amylase, cellulase, xylanase and applied them to the SCB to obtain reducing sugar. Later on value-added products like bioethanol, silica powder and activated carbon was produced from the bagasse.

Bioethanol is a new and renewable energy source. The second-generation bioethanol production process from lignocellulosic materials has development opportunities. This is because the first generation of bioethanol raw materials is generally a food source. Diversification of raw materials for the bioethanol production process can be developed through the use of non-food or waste sources. Lignocellulosic biomass is considered as the future feedstock for ethanol production because of its low cost and its huge availability. For large-scale biological production of ethanol, it is desirable to use cheaper and more abundant substrates. When producing ethanol from maize or sugarcane the raw material constitutes about 40–70% of the production cost. By using waste products from forestry, agriculture and industry, the costs of the feedstocks be reduced. The residual SCB would be used to synthesis silica from it. Bagasse ash is rich in silica (SiO2), the amount of SiO_2 present in the raw sugarcane bagasse ash is 53.10% while the silica composition in acid treatment sample is 88.13%. It is thus an alternative source for silica extraction. In this study, a low-energy and low-chemical consumption method is proposed to obtain silica from bagasse ash using alkali extraction and acid precipitation. A silica yield of 74% were achieved. Activated Carbon (AC) is used for absorbing substances of crystalline form, having a large internal pore structures that make the carbon more suitable absorbent. In this study activated carbon was obtained from the residual SCB after alcohol production and residual ash after silica synthesis. The carbons were activated through chemical activation process using phosphoric acid (H3PO4) as activating agents at room temperature. Iodine value and surface area, Dye removal capacity of the activated carbons produced were investigated. Preparation of activated carbon from sugarcane bagasse is a promising approach to produce cheap and efficient adsorbent for gas pollutants removal. It may be also a solution for the agricultural waste problems in big cities. Methylene Blue adsorption tests suggest that the activated have high adsorption capacity.

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LIST OF ABBREVIATIONS

- O_2 – Oxygen
- % - Percentage
- w/v – Weight per volume
- µm – Micrometer
- US – United States
- ^{0}c – Degree centigrade
- M – Molarity
- N – Normality
- ml – Millilitre
- min – Minute
- hr – hour
- ml/hr – Millilitre/hour
- w/w – Weight per weight
- g/L – Gram per litre
- g – Gram
- L – Liter
- rpm – Revolutions per minute
- mm – Millimetre
- mg/ml – Milligram per millilitre
- cm – Centimetre
- nm – Nanometer

- UV – Ultraviolet
- Vis – Visible
- Conc. – Concentration
- eqn. – Equation
- Pa – Pascal
- SCBP – Sugarcane bagasse powder
- Temp – Temperature
- NaOH – Sodium hydroxide
- HCL – Hydrochloric acid
- DW – Distilled water
- Fig – Figure
- aq – Aqueous
- S – Solid
- l – Liquid
- AC – Activated carbon

Chapter 1:
Introduction and Literature review

1 Introduction:

Sugarcane (Saccharum officinarum) belongs to the genus Saccharum, and family Poaceae. It is believed to have originated in New Guinea and then cultivated throughout the tropical and subtropical regions of the world. Sugarcane cultivated in India belongs to two main groups: (a*) S. barberi* and *S. Sinense* and (b) *S. officinarum*. Sugarcane typically contains 63-73% water, 11-16% fiber, 12- 16% soluble sugars, and 2-3% non-sugar carbohydrates [2]. Temperature ranging from 19-210℃ to 27-380℃ is best suited for sugarcane cultivation. Its byproduct includes ethanol, bagasse, molasses, crude wax etc.

Bagasse is a fibrous material left behind after harvesting sugarcane. It has a multitude of uses, especially in the foodservice packaging industry as a sustainable alternative to conventional plastic. Bagasse, otherwise known as sugarcane pulp, is a by-product of the sugarcane industry. It's the fibrous substance that's left behind after the juice of the sugarcane plant is harvested. Sugarcane belongs to the family Poaceae of the genus Saccharum. Its taxonomy and nomenclature have been difficult to record over the years but essentially the genus *Saccharum* (S.) consisting of six species known as *cinarum*, *S. spontaneum*, *S. robustum*, *S. sinense*, *S. barberi*, and *S. edule*, and some of its agricultural characteristics. Among the sugarcane species, cinarum contains high sucrose, however it has poor resistance to disease *S. spontaneumis* highly adaptable and may grow in various altitudes in tropical and subtropical climates.[7]

Due to the susceptibility to some diseases found in the most common specie (cinarum), breeding methods of sugarcane have been developed. In the last three to four decades, one of the major contributions of breeding sugarcane has been to produce higher sugar yield. Hence cation in sugarcane chemical composition, for example the breeding varieties from 2011 had higher lignin and ash content than that of 2009, potentially leading to lower glucose yield [3].

Sugarcane is one of the most important cash crops in India. India being a major consumer of sugar occupies the second place in sugarcane production, next to Brazil. In recent times, sugarcane has become a preferred crop for renewable and eco-friendly energy production. Excepted rise in demand for energy has improved the scope of sugarcane production. Being a water-intensive crop, sugarcane accounts for about 86% of the sugar crops and it is mostly cultivated for its sucrose content. Of late, sugarcane has been recognized as an important energy source in terms of bio-ethanol production. The widely known fact is that the sugar industry produces the largest amount of waste known as bagasses. Sugarcane bagasse is a waste product from sugar refining industries, obtained after the extraction of juice for production of sugar. The sugar cane fibrous matter (lignocelluloses) has been crushed to extract the juice. India is second largest sugarcane producer in the world, therefore the waste management of these bagasses needs special attentions[5].

Without proper treatment and disposal of these agricultural wastes, they may cause a serious pollution problem in the environment. Historically, the bagasse waste has been burned in the fields, and thereby creating a large amount of pollution. However, in the present days the tends are changing and the environmental awareness driving the prudent use of the bagasse. Some of the area where these bagasse have being utilized are manufacturing biodegradable and compostable food service products, green building brickes made with clays and sugarcane bagasse ash, sustainable acoustic absorber, multiple utility of SCB with other additive or chemical composite materials, producing glass – ceramic materialsand cogeneration industry as active pozzolans for manufacture etc. Over the last two decades, intensive efforts have been dedicated to the development of biotechnological conversion of lignocellulose into cellulosic ethanol globally. [6,7]

However, although the purchase price of lignocellulose feedstock is generally competitive with petroleum on an energy basis, the cost of conversion using the present technology is high. The key factor responsible for the high cost of processing lignocellulosic biomass using current technology is the difficulty of its conversion to reactive intermediates (fermentable sugars). Such a recalcitrance barrier is manifested in costs associated with the

two unit operations aimed at rendering cellulosic biomass fermentable: thermochemical pretreatment and enzymatic hydrolysis. Therefore, the production of cellulosic ethanol as a single product is still not cost competitive on a large scale. Thus, the production of co-products alongside cellulosic ethanol, the so-called biorefinery concept, has been recognized as the most promising strategy that can benefit not only process economics but also environmental performance.[1]

1.1 Chemical Composition:

Vegetable fibers that are derived from stalks or stems are called bast fibers. Jute, Flax, Ramie, etc., are bast fibers, while these are also classified dicotyledons as these plants have net veined leaves. Sugarcane contains parallel-veined leaves. The fiber bundles are randomly arranged throughout the stem of the fiber, but in bast fiber, the fiber bundles are arranged in a certain ring pattern, and that's why it is not classified as bast fiber[14].

The sugar cane stalk can be divided into two portions, the outside rind and an inner pith. The outside rind portion contains longer and finer bundles of fibers, while the inner portion contains the short fibers. Bagasse actually contains both types of fibers. Cellulose covers about one-third of the plant tissues of sugarcane. Sugarcane bagasse contains about 40–50% cellulose and 25–35% hemicellulose. [10]

The rest contains lignin, wax, etc. Cellulose has a crystalline structure (about 50–90% crystalline depending on the source of cellulose), while hemicellulose is an amorphous structure containing xylose, glucose, etc. Cellulose is more like a natural linear polymer containing anhydroglucose units linked by β 1, 4 glycosidic C-2 and C-3 got secondary –OH groups while a primary –OH can be found at C-6 position. These hydroxyl groups help to produce strong intermolecular and intramolecular hydrogen bonds. These cellulose polymers are distributed in fibrils that are surrounded by hemicellulose and lignin. Lignin actually works as a glue between cellulose and hemicellulose and helps the material to gain rigidity. It is a three-dimensional polymer containing three different phenyl-propane precursor monomer namely, p-coumaryl, coniferyl, and sinapyl alcohol, which are joined together by alkyl-aryl, aryl-aryl, and alkyl-alkyl bonds. This composition stands for untreated raw sugarcane bagasse. But different pretreatments can help to reduce the contents, such as hemicellulose, lignin, etc., so that 55–89% cellulose can be yielded from these bagasse samples [3].

The chemical composition of untreated bagasse samples and of samples submitted to acid (sulfuric acid (H2SO4), 1%) and alkaline pretreatments (NaOH 0.25% to 4%) is presented in the table below. Percentages of cellulose, hemicellulose, lignin and ashes were calculated on a dry weight basis. Values for cellulose included glucose, cellobiose and hydroxymethylfurfural amounts quantified by HPLC.[3]

Hemicellulose comprised xylose, arabinose, furfural, glucuronic and acetic acids, while the total lignin amount was calculated by adding up the concentrations of soluble and insoluble lignins. Ash was the remaining inorganic fraction after the bagasse sample was carbonized in a muffle. Mass closure was obtained by adding cellulose, hemicellulose, lignin and ash percentages for each sample, and the total value obtained is shown. Biomass yield after each step is also given in the right column of Table. Untreated bagasse has 35% cellulose and similar amounts of hemicellulose (25%) and lignin (22%). The cellulose amount increased continuously after each acid or base pretreatment, ranging from an initial 35% content to circa 85% under pretreatments using NaOH 2% or higher. Most of the hemicellulose fraction was removed using acid, by its percentage decrease from circa 25% to 7.8%. Smaller hemicellulose fractions were removed in the subsequent base steps, reaching minimum values for NaOH 0.5% or higher. Finally, the lignin relative percentage in the sample increased slightly with acid pretreatment due to the removal of other components (mainly hemicelluloses) and then decreased progressively with pretreatments using NaOH concentrations between 0.5% and 2%. Ash percentage was quite high (approximately 20%) when compared to results found in the literature for other bagasse samples[3].

1.1.1 Cellulose:

Cellulose is a homopolysaccharide chain composed of anhydroglucose units linked byβ-(1,4)-glycosidic bonds which form a crystalline structure due to extensive intra- and intermolecular hydrogen bonds that facilitates fibrils. An average molecular weight of sugarcane bagasse cellulose ranges from 157 800- to 168 400g mol. The size of the fibers range from 1.0 to 1.5 mm. A cellulose unit, known as elementary fibrils, which can then be cross-linked by hemicellulose matrices brils, which creates resistance to chemical and enzymatic degradation. Degree of polymerization pertains to the number glucose unit in the molecular polymer. The average degree of polymerization (DPw) in sugarcane bagasse cellulose ranges from 974 to 1039. Diferent cellulose isolation methods and is dependent on the intrinsic viscosity (η). Intrinsic viscosity is the measure of volume occupied by the macromolecule and their ability. When sugarcane bagasse was subjected and delignification and potassium hydroxide isolation the DPw was 1406.5 while the combination of acetic and nitric acid lowered DPw to 822.5, indicating that the acetic and nitric acid mixture degrades the macromolecule of cellulose more than potassium hydroxide. The predominant polymorph of cellulose is known as cellulose-I andsolid state cross polarization magic-angle spinning carbon-13 nuclear magnetic resonance (CP/ MAS 13CNMR) spectroscopy. XRD is one of the most commonly used techniques for crystallinity index (CrI, %) analysis and it showed that the untreated bagasse contains an average CrI of 56.7%. The CrI of is index is frequently used to determine relative quantity of crystalline material present in sugarcane bagasse. CP/MAS 13CNMR has shown to be extremely resourceful in the morphological modifications analyzed in cellulose throughout corresponds to the C-6 crystalline cellulose signal[10].

1.1.2 Hemicellulose:

Hemicellulose is a heteropolysaccharide of low molecular weight. It averages 7380g m composition of hemicellulose in bagasse is primarily galactose (11.5–39.9%), xylose (15.5–28.9%) and glucose (17.5–50.5%) with smaller amounts of arabinose (5.35– 14.31%), mannose (0.0–14.0%), rhamnose (2.5–10.6%) and uronic acids (1.0–2.3%). Hemicellulose in sugarcane bagasse is composed oβf -(1,4)-xylo-pyranose backbone, having about 200β-xylopyranose residues linked by 1,4-glycosidic bonds, glucomannas and galactomannans, xyloglucans, β-glucans and small amounts of uronic acids. The degree of branching in hemicellulose is given by the arabinose/xylose ratio; hence, the lower the ratio the higher the degree of polymerization and likewise the higher the ratio the shorter the polymer chain. Values for the arabinose/xylose ratio are approximately 0.2 for bagasse. Hemicellulose is linked to lignin by covalent bonds, whereas the linkage between hemicellulose and cellulose is by the way of hydrogen bonds, which integrate easily with one another creating stability exibility. In sugar cane the hemicellulose content is found to be low, on average 19.90%, compared to that of sugarcane bagasse which is approximately between 30 and 35%. There are many methods of extraction of hemicellulose such as autohydrolysis, active oxygen species (oxygen and hydrogen peroxide) and solid alkali, and alkaline peroxide. In general, isolation comes from multiple alkaline extractions causing the cleavage of ester linkages and extracting the hemicellulose from the lignocellulosic matrix. Potassium hydroxide and sodium hydroxide with hydrogen peroxide pre-treatments for hemicellulose isolation have shown high values for xylose, 83.1–84.6 and 85.02%, respectively. In comparison, dewaxed and distilled water, and water with solid alkali precooking resulted in much lower xylose yields, 55.20% and 57.43%, respectively [10].

1.1.3 Lignin:

Lignin, the most abundant aromatic polymer, is an amorphous 3d phenolic biopolymer. Biosynthesis of lignin could be considered to arise from polymerization of three types of phenylpropane units as the monolignols: p- coumaryl, sinapyl and coniferyl alcohols. Monolignols can then give rise to the p-hydroxyphenyl (H), syringyl (S), and guaiacyl (G) lignins units. Lignin in bagasse has a molecular weight average range of 507–3973mol g−1. Lignin in sugarcane is extremely low, average of 2.37% and 4.16%, compared to ~25% found in sugarcane bagasse. Additionally, it has been reported that lignin content in sugarcane genotype IACSP04-627 to be 8.12% and IACSP04- 065 to be 4.32%. The lignin content in different sugarcane bagasse.[5]

Lignin affects most pre-treatment methods as well as enzymatic hydrolysis, thus multiple studies have been made for structural information of lignin, such as ionic liquid, ammonia, dilute acid, etc. Characterization methods for bagasse such as pyrolysis coupled to gas chromatographymass spectrometry (Py-GC-MS) showed that both coumaric and ferulic acid play an important role in the structure of bagasse. Ferulates however, showed acylation of arabinosyl residue from arabinoxylan chains revealing that ferulates are generally linked to carbohydrates in the cell-wall. Ionic liquid and hot water pre-treatment both hydrolyzed the b-O-4 inter-unit links. Different methods for lignin extraction processes showing the cleavage of b-O-4' linkages such as: dilute acids followed by steam explosion and ethanol washing, as well as alkali followed by steam explosions. [8]

Proton, carbon, phosphorus and two-dimensional heteronuclear single quantum coherence nuclear magnetic resonance (1 H, 13 31 P and 2D HSQC NMR) are primarily used to identify the functional groups in the isolated lignin structure.An assessment of various hydroxyl groups in lignin, with results S-OH: 0.58 mmol g−1 , G-OH:0.47, H-OH:0.53 among others, can be obtained using P NMR spectra. Isolated lignin from sugarcane bagasse was reported to be composed of 83%bo-O- f 4' links, alkyl-aryl ether bonds, and very few quantities, 6% of b-5, phenylcoumarans and molar ratio of H:G:S concluding bagasse is S-rich. The lignin of bagasse is derived from mature stem and therefore is rich in syringyl (S) lignin, which is present in the mature tissue[10].

1.1.4 Ash and extractives

Sugarcane bagasse ash is black in color and contains 2.39 g c− m3 of particle density with irregular shaped particles. It contains about 87.8% of sand with particles sizes greater than 63 µm, 11.50% of slit with particles sizes ranging from 2 to 63µm, 0.7% of clay with less than 2µm and 10.32% of organic matter. In Brazil, sugarcane bagasse ash generation was estimated to be approximately 2.5 million tons per year and is discarded primarily as soil fertilizer.

Economical and technological development for the application of ash have grown in the past few years. Ash from bagasse is rich in silica and hence may be used for recycling purposes such as ceramic raw material, additive to cement, concrete and fine aggregate in mortars. X-Ray raction (XRD) has been used to determine the inorganic oxide content in bagasse ash. Extractives in sugarcane bagasse range from 2.3 to 10.5% of total chemical composition. Extractives are hydrophobic and can be fatty acids, waxes, and proteins, among others. Research has reported that about 0.9% of acetone extractives contained about 140 mg k −1 of n-fatty acids, 700 mg k −1 g of n-aldehydes and 330 mg k−1 of n-fatty alcohols in bagasse. For chemical analysis purposes, extractives are usually removed from sugarcane bagasse [10].

1.2 PRODUCTION SCENARIO OF SUGARCANE

India is the largest producer and consumer of sugar in the World. About 45 million sugarcane farmers, their dependents and a large agricultural force, constituting 7.5 percent of the rural population, are involved in sugarcane cultivation, harvesting and ancillary activities. This enabled India to become the largest producer of sugarcane and sugar in the world leaving the other major producers Brazil and Cuba. The major sugarcane crop growing states in India are Uttar Pradesh, Bihar, Assam, Haryana, Gujarat, Maharashtra, Karnataka and Tamil Nadu. The sugarcane cultivation and sugar industry in India plays a vital role towards socio-economic development in the rural areas by mobilizing rural resources and generating higher income and employment opportunities.

The major problem of sugarcane in India is based on monsoon and water supply. The cyclical nature in sugar production has caused distortions in the export of sugar in India. This study analyzes the state-wise production and reasons for the changes in production of sugarcane in the time period of 2000-2010. The production of sugar is spread across the country. In the above states, they are classified into three groups according to its production capacity. They are, the high sugar producing states are Maharashtra and Uttar Pradesh. The second category was the medium sugar producing states. They are Gujarat, Andhra Pradesh, Tamil Nadu, Karnataka, and Haryana. The remaining States are low producing States. They were Bihar and Assam[2].

In India, sugarcane occupy about 4.0 million hectare area and is produced in most of the states having the highest area of 47.05% in Uttar Pradesh followed by Maharashtra (17.52%), Karnataka (7.76%), Tamil Nadu (7.47), Gujarat (4.57%) and Andhra Pradesh (3.76%) contributing about 88% of the total area. The rest of the 12% area is shared by Bihar, Uttarakhand, Haryana, Madhya Pradesh, Chhattisgarh, Punjab etc. However, the yield of sugarcane per hectare is highest in Tamil Nadu, followed by West Bengal, Karnataka and Maharashtra. The average cane yield in India is about 70.0 tonnes per hectare while the sugar recovery is around 10.0 percent Sugarcane is a major cash crop in India responsible for the overall socio-economic development of the farming community. Molasses, sugar and khandsari etc, are produced from the juice of sugarcane. Production of the crop is mainly located in the states of Uttar Pradesh, Maharashtra, Tamil Nadu, Karnataka and Gujarat. Sugarcane cultivation needs temperature of 15 degree to 40 degree and rainfall of 100 to 150 centimeters and fertile loamy soil or hard soil.

Sugarcane is a long duration crop which produces huge amounts of biomass, requiring large quantities of water, which typically are supplied through 25-30 irrigation cycles per crop season sugarcane is cultivated from Kanyakumari (southern Part) to Punjab (north – west) but it is more cultivated in Uttar Pradesh, except these States, sugarcane is an important crop in Maharashtra, Tamil Nadu, Andhra Pradesh, Karnataka, Punjab, Haryana, and Bihar etc. In India, the sugar industry is the second largest agriculture based industry after textile fibers. It arises over INR 225 billion in taxes for the common wealth and state governments[5].

1.3 ECONOMICAL ASPECTS:

The sugar industry of India is the second largest agro-based industry after textiles and it has successfully contributed towards providing employment and economic development of country. The sugar industry as a whole has supported 6 million farmers and their families. Sugarcane is considered as the crop for the future because of its contribution to production of sugar, jaggery, khandsari and many by products like molasses, bagasses and press mud and also certain renewable sources of green energy in the form of bioethanol and many bio-based products.

In India, the agro-climatic regions of sugarcane cultivation can be divided into two: tropical and sub-tropical. The sub-tropical region constitutes the northern states of Uttar Pradesh, Bihar, Uttarakhand, Punjab, Haryana comprises of 55% of total area under sugarcane and contributes 47% of country's sugarcane production. The tropical region constitutes mainly the southern states of Maharashtra, Karnataka, Tamil Nadu and Andhra Pradesh. Despite having lesser area i.e. 42% of the total area under sugarcane, the tropical region contributes

higher i.e. 51% of country's sugarcane production as the longer duration crop and favorable climatic condition causes higher productivity and better sugar recovery. In this era of globalization and when almost everything is decontrolled, sugar industry continues to be tightly regulated. The different regulations in the form of cane reservation area, regulated release mechanism for the produced sugar, levy sugar obligation and dual pricing of sugarcane and state determined state advised price (SAP)) has adversely affected the competitiveness and growth performance of mills and therefore led to delayed payment to sugarcane farmers.

However after the deregulation of 2013, sugar sales and prices are freed but sugarcane quantity and prices are still controlled. Sugarcane crop requires much higher amount of inputs because of its longer duration nature and the cost of sugarcane production has shown an increasing trend over the years. Out of various factors of production of sugarcane, labour and land accounted for 32 % each in the total C2 cost of production in TE 2013-14. The prices of farm inputs including farm wages have shown an increasing trend over the years.The productivity and sugar recovery from sugarcane has remained stagnant over the years which are major challenges for Indian sugar sector[7].

1.4 Waste generation due to Sugarcane:

The sugarcane industry in India generates significant amounts of waste due to the extensive processing involved. Here are the primary types of waste generated:

1.4.1 Types of Waste

A. **Bagasse:** The fibrous residue left after extracting juice from sugarcane.
Volume: For every ton of sugarcane processed, approximately 250-280 kg of bagasse is produced.
Uses: Primarily used as a biofuel for energy production, in paper manufacturing, and for producing biodegradable products.

B. **Molasses**: A by-product of the sugar extraction process.
Volume: Roughly 4-5% of the sugarcane processed ends up as molasses.
Uses: Used in the production of ethanol, as an animal feed additive, and in the production of various chemicals and food products.

C. **Press Mud (Filter Cake):** The solid residue from the juice filtration process.
Volume: Around 3-4% of the processed sugarcane mass.
Uses: Utilized as a fertilizer, soil conditioner, and in biogas production.

D. **Vinasse:** A liquid by-product from the distillation of molasses to produce ethanol.
Volume: For every liter of ethanol produced, around 10-15 liters of vinasse is generated.
Uses: Can be used as a fertilizer after treatment, or in the production of biogas, though its high organic content poses environmental challenges.

E. **Trash:** Leaves and tops of the sugarcane plant, often left in the fields after harvest.
Volume: Accounts for about 20-25% of the total biomass of the sugarcane plant.
Uses: Can be used as mulch, for composting, or in bioenergy production, though often it is simply burned, contributing to air pollution.

1.4.2 Environmental Impact:

- **Air Pollution:** Burning of sugarcane trash and bagasse in open fields or inefficient boilers leads to significant air pollution.

- **Water Pollution**: Improper disposal of press mud and vinasse can lead to contamination of water bodies, affecting aquatic life and water quality.

- **Soil Degradation:** Continuous use of chemical fertilizers over organic waste like press mud can lead to soil health deterioration.

1.4.3 Value added products from sugarcane waste:

Utilizing sugarcane waste to create value-added products can enhance sustainability and provide economic benefits. Here are some notable value-added products derived from sugarcane waste:

From Bagasse:

1. Paper and Pulp Products- Bagasse can be processed into pulp to manufacture paper products like writing paper, cardboard, and packaging material. It provides a sustainable alternative to wood pulp, reducing deforestation.
2. Bio-composites- Bagasse fibers can be combined with resins to create biodegradable and eco-friendly composites used in manufacturing furniture, building materials, and automotive components. It offers a lightweight and sustainable alternative to traditional materials like plastic and wood.
3. Biofuel and Energy- Bagasse is a rich source of biomass and can be used to generate electricity and steam in cogeneration plants. It provides renewable energy, reducing reliance on fossil fuels.
4. Biodegradable Tableware- Bagasse can be molded into plates, bowls, and other disposable tableware. It will reduce plastic waste and provide an eco-friendly alternative for single-use items.

From Molasses:

1. Ethanol- Fermentation of molasses produces ethanol, which can be used as a biofuel and as a raw material in the chemical industry. It reduces greenhouse gas emissions when used as fuel and diversifies the energy portfolio.
2. Animal Feed- Molasses is rich in nutrients and can be used as a feed additive for livestock. Enhances the nutritional value of animal feed, promoting better health and productivity in livestock.
3. Fermentation Products- Molasses can be used to produce yeast, citric acid, and other fermentation-based products. It supports the food and pharmaceutical industries with essential ingredients.

From Press Mud (Filter Cake):

1. Biofertilizers- Press mud is rich in organic matter and nutrients, making it an excellent soil conditioner and fertilizer. Enhances soil health and fertility, promoting sustainable agriculture.
2. Biogas Production- Press mud can be anaerobically digested to produce biogas, which can be used for cooking, heating, and electricity generation. Provides a renewable source of energy and reduces greenhouse gas emissions.

From Vinasse:

1. Fertilizer- Treated vinasse can be used as a liquid fertilizer due to its high potassium content. Offers a sustainable fertilization option, reducing the need for chemical fertilizers.
2. Biogas Production- Vinasse can be processed in biogas plants to produce methane-rich biogas. Converts waste into energy, reducing environmental pollution.

From Trash (Leaves and Tops):

1. Mulch and Compost- Sugarcane trash can be used as mulch to retain soil moisture and suppress weeds, or composted to create nutrient-rich organic matter. Improves soil health and reduces the need for chemical inputs.
2. Biochar- Pyrolyzing sugarcane trash produces biochar, a carbon-rich material used to enhance soil fertility and sequester carbon. Contributes to carbon sequestration and improves soil quality.

Innovative Uses:

1. Nanocellulose- Bagasse can be processed to extract nanocellulose, used in high-strength materials, biomedical applications, and electronics. Opens new avenues in advanced material science with sustainable raw materials.
2. Platform Chemicals- Advanced biorefineries can convert sugarcane waste into platform chemicals like furfural and levulinic acid, used in various industrial applications. Supports the development of a bio-based chemical industry, reducing dependence on petrochemicals.

By converting sugarcane waste into these value-added products, the industry not only addresses waste management challenges but also creates new revenue streams and promotes environmental sustainability.

1.5 Bioethanol from SCB:

Lignocellulosic biomass such as sugarcane bagasse (SCB) is a renewable and abundant source for ethanol production. Sugarcane bagasse is composed of cellulose, hemicellulose, lignin, extractives, and several inorganic materials. Pretreatment methods of SCB are necessary for the successful conversion of SCB to ethanol. Each pretreatment process has a specific effect on the cellulose, hemicellulose, and lignin fraction. The conversion of SCB to ethanol typically consists of four main steps: pretreatment, enzymatic hydrolysis, fermentation, and distillation. There are many types of pretreatments such as physical, chemical, physico-chemical, and biological pretreatments. [17]

If 76% of bagasse is pre-treated and enzymatically hydrolyzed for sugar, this sugar could be fermented to yield up to 149.3 liters of ethanol per ton of bagasse. Other studies suggest that in a two step pre-treatment using a dilute acid pretreatment condition at 175°C for 40 min and 1% H_2SO_4 concentration with a 1:1 solid liquid ratio, followed by an organosolv treatment, it is possible to achieve 192 liters of ethanol per ton of bagasse. Whereas using similar conditions, 120°C for 40 min with 1% H_2SO_4 concentration with a 1:4 solid liquid ratio followed by organosolv, ethanol generation could produce up to 180 liters of per ton of sugarcane bagasse. Different pre-treatments and pathways for hydrolysis and fermentationhave varying effects on glucose concentration available for fermentation contributing to different ethanol production. [17]

Cogeneration systems are very common in sugar mills. When using cogeneration system for first and second generation production simultaneously in different boiler pressures (2.2–9.0 MPa), it was shown that the system using 2.2 MPa achieved a maximum yield of anhydrous

ethanol production of 113.7 liters per ton of sugarcane. The distillation process of sugarcane bagasse is compared to conventional process, the distillation columns operates under vacuum pressures ranging from 19 to 25 kPa and rectification columns pressure ranges from 101 to 135 kPa with an extractive distillation process using monoethyleneglycol (MEG) for ethanol dehydration, providing higher yields of anhydrous ethanol from bagasse than conventional. Hemicellulose pentoses, mainly cult to transform for the production of second-generation bioethanol, hence metabolic microorganisms are necessary to succeed on pentose. Thus pre-treatment of bagasse is necessary in order to modify chemical composition, size and structure in such manner in which hydrolysis can be carried out promptly and with increased yields[19].

1.6 Silica from SCB:

Sugarcane bagasse is a signifcant renewable energy source for the sugar and bioethanol industries. Bagasse ash is the waste from the combustion process and is mostly disposed of as landfill. Only a small quantity of bagasse ash is utilized as pozzolan in concrete, and a considerable quantity is left unused due to its high carbon and crystallite content. Generally, bagasse ash is rich in silica (SiO2), and it is thus an alternative source for silica extraction.

The chemical composition of bagasse ash depends on the burning condition, i.e., burning temperature, burning duration and air intake. Owing to the incomplete burning of bagasse, carbon is also found in bagasse ash.[22]

Generally, bagasse ash has a high silica (SiO2) content. Silica content is influenced by the availability of silicon in soil, sugarcane roots play a significant role in the absorption of silicic acid from the soil and transporting it toward the shoots, where it is deposited as amorphous silica. The transpiration process in the plants enhances silica deposition in all parts by water transmission. However, the amount of silica in bagasse varies depending on the species and maturity of the sugarcane, geological and soil conditions, fertilizer used, and cultivation practice.[23]

Silica forms in bagasse ash consist of amorphous phases and crystalline phases (quartz and cristobalite). It has been reported that quartz could also come from sand stuck to the sugarcane during harvest. Cristobalite is a crystal morphology of silica resulting from the high combustion temperature of bagasse. Researchers have reported that bagasse ash has the potential for use as pozzolan in concretes since it contains amorphous phases that can react with chemical compounds in a cement mixture. Bagasse ash needs to be ground to increase its reactivity through increased fineness and surface area. However, the incorporation of a high volume of bagasse ash adversely affects the binding and physical properties of pozzolanic cement, and so small quantities of bagasse ash are used. Bagasse ash contains high levels of SiO2, similar to rice husk ash, and it is used as a source material for the extraction of SiO2.[22]

Table 1: Composition of Sugarcane bagasse Ash

Compounds	Concentration(%wt)
SiO_2	66.3
Al_2O_3	4.6
CaO	0.8
Fe_2O_3	10.4
SO_3	0.2
MgO	1.7
K_2O	1.2
Na_2O	0.2

1.4 Activated carbon from SCB:

Activated carbon (AC) is a carbon material like graphite with an irregular and imperfect arrangement structure of microcrystalline carbon. The activated carbon has a porous structure which increases the surface area and decreases density. AC is one of the best adsorbents for removing trace contaminants from air, soil, and water due to its strong physical adsorption. This results from the advantages of the AC such as porous properties, high chemical/thermal stability, unique surface area, surface functional groups, and physicochemical nature of AC. Activated carbons are prepared through physical or chemical activation methods. The physical activation has been reported as more beneficial due to its larger surface area, higher yields, and highly developed porous structure.[25]

Activated carbon is used in gas purification, gold purification, metal extraction, water purification, medicine, sewage treatment, air filters in gas masks and respirators, filters in compressed air and many other applications. Activated carbon can be produced from carbonaceous materials such as coconut shell, saw dust, agricultural residues. Sugarcane bagasse is composed largely of cellulose and lignin. Hence, it is a potential resource of cheaper raw material for activated carbon (AC) production if pyrolyzed under controlled conditions or with some chemical treatment. This could offer the combined benefits of decreasing the volume of agricultural wastes and simultaneously producing a valuable adsorbent with lower cost than commercial activated carbons. Activated carbon adsorption was repeatedly reported to be an effective technology for environmental remediation, for industrial processing, and to remove trace contaminants from both air and water.[28]

Chapter 2:
Materials & Methods

2.1 PREPARATION OF SUGARCANE BAGASSE POWDER(SCBP):

The bagasse was collected from a local sugarcane juice shop and dried under the sunlight. Then it was cut 1cm long with a help of a scissors. After that the pieces were grounded into a coarse powder.

2.2 PROXIMATE TESTS OF SCBP:

2.2.1 MIOSTURE CONTENT:

Apparatus required- Hot air oven, Petri dish, Weighing balance, Spatula.

Procedure- About 5gm of sample was taken in a petridish. Then the sample was kept in a hot air oven at 105℃ temperature. After 2hrs. the sample was taken out from the oven and it was cooled in a desiccator until reaches to room temperature. Then the weight of the sample was taken.

Wet basis Moisture content(%)= $[(W_1-W_2)/(W_1-W)]*100$

Dry basis Moisture content(%)= $[(W_1-W_2)/(W_2-W)]*100$

Where,

W = Weight of empty petri dish.

W_1 = Weight of petri dish + sample before drying.

W_2 = Weight of petri dish + dried sample.

2.2.2 ASH CONTENT:

Apparatus required- Muffle furnace, Porcelain crucible, Weighing balance, Spatula.

Procedure- Ash content of the sample was determined by using muffle furnace. 5gm of sample was taken in a porcelain crucible and kept in a muffle furnace at 550℃ temperature for 4hrs. After completion of ashing, the weight of the sample was taken. The process of heating was repeated for 30 minutes, cooling in a desiccator and weighing until the difference between two successive weighing's is less than 1 mg.

Total ash (% on dry weight) = $[(W_2 – W) \times 100 \times 100] / [(W_1 – W) \times (100 – M)]$

Where,

W = Weight of empty porcelain crucible.

W_1 = Weight of porcelain crucible + sample

W_2 = Weight of porcelain crucible + ash

M = Moisture % of the sample.

2.2.3 FAT CONTENT:

Apparatus required- Thimbles, Soxhlet extraction apparatus, Heating mantles, Filter paper, Glass beads, Weighing balance.

Reagents required- Petroleum ether (60°-80℃)

Procedure- 5gm of dried sample was taken and placed in a thimble. The thimble was placed in the Soxhlet extractor. 90 ml of petroleum ether was taken in a round bottom flask and

placed the whole setting on a heating mantle and allowed the petroleum ether to boil. This extraction process was continued for 6 hrs. The extraction unit was removed from the heat source and the extractor and condenser were detached. The flask was replaced on the heat source and the solvent was evaporated. The flask was placed in an oven at 102ºC and the contents were dried until a constant weight is reached (1-2 hours). Cool The flask was cooled to room temperature in a desiccator and weigh of the flask with contents was taken.

Crude fat (%) = $[(W_2 – W_1)/S]*100$

Where,

W_1= Weight of empty flask

W_2= Weight of flask and extracted fat

S= Weight of sample

2.2.4 CRUDE FIBER CONTENT:

Reagents required- Sulfuric acid, Sodium hydroxide, Acetone

Procedure- 3gm sample was accurately weighed into the fiber flask and 100ml of 0.25(N) Sulfuric acid was added. The mixture was heated under reflux for 1hour with the heating mantle. The hot mixture was filtered through a fiber sieve cloth. The filtrate obtained was thrown off and the residue was returned to the fiber flask to which 100ml of 0.31(N) Sodium hydroxide was added and heated under reflux for another 1hour. The mixture was filtered through a fiber sieve cloth and 10ml of acetone was added to dissolve any organic constituent. The residue was washed with about 50ml hot water twice on the sieve cloth before it was finally transferred into the crucible. The residue was oven dried at 105℃ to drive off moisture. The oven dried crucible containing the residue was cooled in a desiccator and later weighed. The crucible was transferred to the muffle furnace for ashing at 550℃ for 4hours. The crucible containing white or grey ash (free of carbonaceous material) was cooled in the desiccator and weighed.

Crude fiber (%)= $[(W_2 – W_3) / W_1]*100$

Where,

W_1 = Sample weight

W_2 = Crucible weight with fiber and ashes, after drying in an oven at 105°C

W_3 = Crucible weight with ashes, after muffle at 550 °C for 4 hours

2.2.5 PROTEIN CONTENT (Kjeldahl Method):

Apparatus required- Digestion bench placed in digestion chamber, Kjeldahl Distillation Unit, Kjeldhal flask, Burette, Conical flask, Volumetric flask, Measuring cylinder, Weighing Balance,Pipettes

Reagents required- Potassium sulphate, Copper sulphate, Sodium hydroxide (NaOH), Commercial sulphuric acid, Methyl red Indicator

Procedure- 3gm of the sample weighed and place in digestion tube. Then 7 gm of catalyst, 3 to 5 anti-bumping granules and 20 ml of cone H_2SO_4 were added to it. Also a tube containing the above chemicals was prepared as blank. The tube was covered with exhaust manifold and placed in the preheated digestor and digested at about 110-130℃ for 15 mins.

Turn the digestor to digestion temperature normally around 420°C and the sample was digested until the solution is light green and then a further 15 mins. The tube was removed and leave to stand until sample is cooled. 60 ml distilled water was added. Switch on distillation apparatus and pre-washed for 10 mins. 25 ml 4% boric acid was dispensed into a 250 ml conical flask and placed the flask under the condenser, ensuring that the condenser tip is immersed in the boric acid solution. The digestion tube was connected containing the sample digest to the distillation apparatus. 60 ml 40% NaOH was dispensed carefully into digested sample. Immediately turned on the steam supply valve to initiate the distillation. Heated for 4 mins until all ammonia has passed over into the boric acid. Then lowered the conical flask ensuring the condenser tip is not immersed in solution and continue heating for further 1 min. Approximately 120 ml distillate was collected. Conical flask containing ammonia distillate was placed on magnetic stirrer. 1 ml indicator added and titrate the sample with standard 0.1N sulfuric acid until the solution change from green to pinkish.

Protein (%) = ((B-A) ×0.1 ×14)/W ×100 × (6.25)/1000

W = Weight of the sample

A = volume (ml) of 0.1N H_2SO_4 used in blank titration

B = volume (ml) of 0.1N H_2SO_4 used in sample titration

14.00 = atomic weight of nitrogen

1000 = the conversion of mgN/100 g to gN/100 g sample

6.25 the protein-nitrogen conversion factor

2.2.6 CARBOHYDRATE CONTENT (Phenol-Sulfuric Acid Method):

Apparatus required- Boiling tube, Centrifuge tube, Spectrophotometer
Reagents required- Phenol, Sulphuric Acid 96% reagent grade, Glucose (standard solution), Sodium carbonate, Hydrochloric acid

Procedure- 100mg of sample weighed into a boiling tube then hydrolysed by keeping it in boiling water bath for 3hrs with 5ml of 2.5N HCL and cooled it to room temperature. After that neutralized with sodium carbonate until the effervescence stops and the volume made up to 100ml and centrifuged. 0.2, 0.4, 0.6, 0.8 and 1ml of the working standard pipetted out in a series of test tube. 0.1 and 0.2ml of the sample solution was taken out in two separate test tube and made the volume up in each test tube till 1ml with distilled water. The blank was set with 1ml of distilled water. 1ml of phenol solution and 5ml of 96% H_2SO_4 were added to each test tubes and shook the contents well after every 10 mins.

UV-Vis Spectrophotometer was used to read the absorbance at 490nm and calculation for the total amount of carbohydrate present in the sample solution is done using the standard graph of glucose.

2.3 EXTRACTION OF REDUCING SUGAR FROM SCBP:

2.3.1 TRIAL 1:

Method 1: 10gm of the sugarcane bagasse powder (SCBP) was soaked with 200ml of distilled water and kept for 24hrs. Then the mixer was filtered and the reducing sugar content of the filtrate was measured using fehling's method.

2.3.1.1 Fehling's method of sugar estimation:

Apparatus required: Burette, Pipette, Weighing machine, Mantle heater

Reagents required: Fehling's A, Feling's B, Dextrose, Methylene blue indication

Reagent preparation:

1. Fehling's A Solution: 34.639 gm of crystallized copper sulphate (CuSO4.5H2O) of highest purity was dissolved in water and,the volume made up to 500 ml.

2. Fehling's B Solution: 173 gm of Rochelle salt (Na-K-tartrate) and 50 gm of sodium hydroxide were dissolved in water to make up to 500 ml.

3. 1% solution of pure dextrose was prepared for standardizing the Fehling's reagent.

Procedure:

1.Standardization of the Fehling's reagent:

i) Preliminary titration: To a mixture of 5ml each of Fehling's A and Fehling's B solution and 30ml of water were taken in a porcelain basin. Little by little from the burette the solution of the known dextrose solution was added and heated for 2 mins after each addition. This process continued until the solution over the red precipitate is practically colorless.

ii) Final titration using 1% aqueous solution of methylene blue as indication: On the basis of the preliminary titration, from the burette the sugar solution was added within 1ml of the required amount. Then gently boiled for 2mins then while still boiling, 3-5 drops of indicator was added and the titration was completed. Calculated the mg of sugar equivalent to one ml of Fehling's solution.

The sample solution was placed in the burette and the same procedure was repeated for finding the strength of the sample solution.

2.3.2 TRIAL 2:

Method 2: 10gm of SCBP was mixed with 120ml of 10% Sodium Hydroxide solution and 80ml of distilled water. It was autoclaved for 15min at 15 psi. then kept for 24hrs. After this 25ml of petroleum benzine was added and agitated vigorously. When the two layers formed, with the help of a separating funnel the bottom layer was separated and the reducing sugar content of the extract was measured by Fehling's method.

2.3.3 TRIAL 3:

Inoculum Preparation:

Czapek Dox media is a semisynthetic medium containing nitrate s the sole source of nitrogen. It is used for cultivation of fungi. This media has good buffering action due to the presence of different salts. This media supports abundant growth of almost all saprophytic Aspergilli.

Components:

Item	Weight(gm)
Sucrose	4.5
Sodium Nitrate	0.3
Dipotassium Phosphate	0.15
Magnesium Sulfate	0.075
Potassium Chloride	0.075

Ferrous Sulfate	0.0015

All the reagents were suspended in 150ml distilled water and the pH was adjusted to 4 with 0.1(N) HCL and sterilized by autoclaving at 15psi (121℃) for 15 minutes. Then the media was inoculated with a loop full of *Aspergillus niger* from a slant culture in aseptic condition and kept for 24hrs and 48hrs at 35℃ in shaking condition.

The growth media was filtered and the assay of enzymes (Amylase, Cellulase & Xylanase) were done.

2.3.3.2 ASSAY OF ENZYMES (AMYLASE, CELLULASE, XYLANASE) IN CELL FREE EXTRACT:

2.3.3.2.1 Construction of Dextrose standard curve by DNS method:

3,5 – dinitrosalicylic acid (DNS) oxidizes the free aldehyde (aldose) or ketone (ketose) functional group present in reducing sugars and gets reduced to 3 – amino – 5 – nitrosalicylic acid under alkaline conditions. The final product is an aromatic reddish-brown complex that has a maximum absorbance at 540 nm. The intensity of the color is proportional to the amount of reducing sugar present in an unknown sample.

Apparatus Required: Test/Boiling tubes, Pipettes, Water bath, UV-Vis Spectrophotometer
Reagent Required: 3,5-dinitrosalicylic acid [DNS], Dextrose working solution, Sodium potassium tartarate, Sodium hydroxide

Preparation of Reagents:

1. 3,5-dinitrosalicylic acid [DNS]: About 1g of DNS is dissolved in 50ml of distilled water. To this solution about 30g of sodium potassium tartarate tetrahydrate was added in small lots, the solution turns milky yellow in color. Then 20ml of 2(N) NaOH was added, which turns the solution to transparent orange yellow color.
   The final volume is made to 100 ml with the distilled water. This solution was stored in an amber colored bottle.
2. Dextrose working solution: 180mg of dextrose is weighed and made up to 100ml with distilled water.

Procedure: Standard dextrose solution in the range of 0.2, 0.4, 0.6, 0.8 and 1 ml were taken out into 5 separate test tubes. A test tube containing a blank solution is also prepared. Using distilled water, the volume brought up to 2ml in each test tube, including the test tube containing the blank solution. 1 ml of DNS reagent added to each tube and covered the test tubes with aluminium foil. The contents were heated in the test tubes in a boiling water bath for 5 minutes. Then removed from water bath and cooled to room temperature. 9ml distilled water added to each test tube and mixed well.

Samples from each test tube were taken into different cuvettes and placed each cuvette in a UV-Vis spectrophotometer and the intensity of dark orange red colour at 540 nm as the 'absorbance' or OD was recorded.
A graph plotted with the amount of dextrose on X axis Vs OD at 540nm (A540nm) on Y axis.

2.3.3.2.2 Estimation of Enzyme Activity:

Apparatus Required: Test/Boiling tubes, Pipettes, Water bath, UV-Vis Spectrophotometer
Reagent Required: 3,5-dinitrosalicylic acid [DNS], KH_2PO_4, K_2HPO_4, Starch

Preparation of Reagents:

1. **Phosphate buffer 0.1(M) pH 7.8:**

a. (M) KH2PO4: 1.360 g of KH_2PO_4 dissolved in 100 ml of distilled water.
b. 0.1 (M) K2HPO4: 1.7418 g of K_2HPO_4 dissolved in 100 ml of distilled water. The solutions were mixed at 1:1 ratio and the pH was adjusted to 7.8

2. **Sample**:
Sample(I)- 100µl of 24 hours old cell free growth media was mixed with 1900 µl phosphate buffer.

Sample(II)- 100µl of 48 hours old cell free growth media was mixed with 1900 µl phosphate buffer

3. **Substrate:**

a) 1% starch solution: 1gm soluble starch dissolved in 90 ml of distilled water and boil to get clear solution. The volume made up to 100 ml with distilled water.
b) 1% Cellulose solution: 1gm cellulose dissolved in 90 ml of distilled water and boil to get clear solution. The volume made up to 100 ml with distilled water.
c) 1% Xylane solution: 1gm xylane dissolved in 90 ml of distilled water and boil to get clear solution. The volume made up to 100 ml with distilled water.

Procedure:

1. Blank: 2 ml of phosphate buffer, 0.5 ml of substrate and 0.5 ml of distilled water were taken in a clean dry test tube.
2. Test: 2 ml of phosphate buffer, 0.5 ml of substrate and 0.5 ml of sample were taken in a clean dry test tube.
3. The contents of the tubes were mixed by vortexing / shaking the tubes and incubated for 30 min at 37°C.
4. 0.5 ml of DNS added to all the test tubes, mixed the contents of the tubes by shaking the tubes and incubated for 10 min in a boiling water bath and cooled to room temperature.
5. Then to the cooled test tubes 0.5 ml of distilled water added, and the absorbance recorded at 540 nm against blank.

   This process was performed for both the samples [Sample(I) & Sample(II)] with 3 different substrates (Starch, Cellulose, Xylane).

Enzyme activity (EA)

= [Standard Curve Value * 60 * Dilution Factor (20)] / [Sample taken (ml) * Time of incubation (min)

(µg of glucose released/ml of sample/hour at 37°C)

= (EA * 1000)/ (Molecular weight of glucose*60)

IU/min

Method 3: 5gm of SCBP was submerged with 20ml inoculum (with cell mass) and 80ml distilled water and incubated for 3 hours at room temperature. Then it was filtered and the sugar content was measured by Fehling's method.

2.4 PROCESS OPTIMIZATION:

1. Effect of Inoculum:

Inoculum with cell mass and without cell mass both were taken to study the effect of it on extraction of reducing sugar from SCBP. 20ml of each inoculum were taken with 80ml of D.W and 5gm of SCBP was submerged in it and incubated for 3 hours at room temperature. Then it was filtered and the sugar content was measured by Fehling's method.

2. Effect of SCBP amount:

2.5gm and 5gm of SCBP were taken to study the effect of it on extraction of reducing sugar from SCBP. 2.5gm and 5gm of SCBP were submerged in 20ml 24hours old cell free extract and 80ml distilled water and incubated for 3 hours at room temperature and the filtered. In order to study the effect on sugar extraction, sugar content of the filtrate was measured by Fehling's method. The amount showing the highest sugar content was used for further study.

3. Effect of pH:

Different pH such as 3.5, 5, 6.5, 8, 9.5, 11 were taken to study the effect of different pH on extraction of reducing sugar from SCBP. 0.1 (N) HCl and 0.1 (N) NaOH were used for pH adjustment. 2.5gm of SCBP were submerged in 20ml 24hours old cell free extract and 80ml distilled water and incubated for 3 hours at room temperature and then filtered. In order to study the effect of pH on sugar extraction, sugar content of the filtrate was measured by Fehling's method. The amount showing the highest sugar content was used for further study.

4. Effect of Incubation period:

Different incubation periods such as 3hours, 4 hours, 5 hours were taken to study the effect of different incubation time on extraction of reducing sugar from SCBP. 2.5gm of SCBP were submerged in 20ml 24hours old cell free extract and 80ml distilled water and incubated for 3 hours, 4 hours and 5 hours at room temperature and then filtered. In order to study the effect of incubation period on sugar extraction, sugar content of the filtrate was measured by Fehling's method. The amount showing the highest sugar content was used for further study.

5. Effect of Temperature:

Different temperatures like 35℃, 40℃, 45℃ were taken to study the effect of different temperature on extraction of reducing sugar from SCBP. 2.5gm of SCBP were submerged in 20ml 24hours old cell free extract and 80ml distilled water and incubated for 3 hours at 35℃, 40℃ & 45℃ and then filtered. In order to study the effect of temperature on sugar extraction, sugar content of the filtrate was measured by Fehling's method. The amount showing the highest sugar content was used for further study.

2.5 PRODUCTION OF ETHANOL FROM SCBP BY USING *SACCHAROMYCES CEREVISIAE*:

Apparatus required: Weighing balance, Autoclave, Laminar air flow, Rotary BOD incubator shaker, pH meter, vacuum filter, conical, burette, measuring cylinder, cotton, spatula, glass rod, filter paper.

Reagents required:

Growth Media:

Composition	Concentration(w/v)
Dextrose	2%
Yeast extract	1%
Peptone	2%
KH_2PO_4	0.5%
$(NH_4)SO_4$	1.5%
$MgSO_47H_2O$	0.25%
Antifoam	0.01%

pH- 5.5

Temperature- 30℃

Fermentation Media:

Composition	Concentration(w/v)
Sugar from SCBP	5%
Yeast extract	0.5%
Peptone	1%
KH_2PO_4	0.5%
$(NH_4)SO_4$	1.5%
$MgSO_47H_2O$	0.25%
Antifoam	0.01%

pH- 5.5

Temperature- 30℃

PROCEDURE:

1. At first the reducing sugar solution collected from 50gm of SCBP was reduced to 250ml for the fermentation media. Then the growth media and fermentation media was prepared with all the reagents and strelized along with 100ml D.W and required glass apparatus.
2. The inoculum is prepared by taking one loop full of fresh culture of *Saccharomyces cerevisiae* in 10ml of sterile water.
3. 2ml of inoculum is added to 100ml of growth media in a sterile conical flask and set to incubation in a rotary incubator shaker at 30℃ for 8-20 hrs and the micro-organisms are allowed to grow in an aerated condition.
4. Before the start of fermentation, the percentage of sugar in the freshly prepared fermentation media is estimated by Lane Eynon method.
5. 15ml of inoculated growth media is pitched into 250ml of fermentation media.
6. The media is incubated at 30℃ in an anaerobic condition.
7. After 48-72 hours the broth is taken out, strained and analysed for alcohol.
8. The filtrate was distilled and alcohol was collect in a round bottom flask. Alcohol percentage was estimated with alcohol meter.

2.6 SYNTHESIS OF SILICA POWDER FROM THE RESIDUAL SCBP AFTER ALCOHOL PRODUCTION

Apparatus required: Magnetic stirrer, Centrifuge, Hot air oven, magnetic beads, beaker, filter paper, mantle heater

Reagents required: 1(M) Hydrochloric acid, 1(M) Sodium hydroxide solution, 2.5(M) HCL

Procedure:

I. Bagasse ash: The residual SCBP after the extraction of reducing sugar was dried in the hot air oven at 105℃ for 5hrs. Then it was ignited to form into ash in the presence of atmospheric air.

II. Bagasse ash pre-treatment: To remove the oxide compounds of minor elements from bagasse ash, an acid treatment was performed. 100 g of as-received bagasse ash was suspended in 600 ml 1(M) HCl and stirred continuously by magnetic stirrer in a controlled 25 °C room for 2 hours. The ash was then filtered and washed with hot water until the pH of the filtrate was 7. Subsequently, the pre-treated ash was dried in an oven at 100±5 °C for 5hours.

III. Silica extraction: The acid-treated bagasse ash was dispersed in 1M NaOH in a beaker with ash-to-base ratios of 1:8 w/v. The mixture was heated at 90±5 °C for 1hour and stirred by a magnetic stirrer. The beaker was covered with watch glass during the heating process, after which, the mixture was left to cool to room temperature. The ash residue was filtered of, and the filter cake was washed with hot water (double the volume of NaOH used). The filtrate, i.e. sodium silicate and washing water, was adjusted to pH of 7 with 2.5 M HCl and left for silica aging at room temperature for 24hours. The silica was then filtrated and washed with hot water and dried at 105℃ for 5hours. The percentage yield of silica was calculated based on the silica content of the bagasse used.

2.7 PRODUCTION OF ACTIVATED CARBON FROM THE RESIDUAL SCBP AFTER ALCOHOL PRODUCTION

Apparatus required: Muffle furnace

Reagent required: 30% aq. Solution of Phosphoric acid

Procedure:

The residual SCBP after the extraction of reducing sugar was dried in the hot air oven at 105℃ for 5hrs. Then it was placed in a brass container compactly and the lid was sealed to maintain an anaerobic condition. The container was placed in a muffle furnace at 500℃ for 2hours.

Produced carbon was then treated with 30% aqueous solution of phosphoric acid (H_3PO_4) in a weight ratio 1:1 for 24 hrs and then washed with double-distilled water several times and dried in hot air oven.

2.8 PRODUCTION OF ACTIVATED CARBON FROM THE RESIDUAL SCBP ASH AFTER SILICA SYNTHESIS

Apparatus required: Magnetic stirrer, Centrifuge, Hot air oven, magnetic beads, beaker, filter paper, mantle heater

Reagent required: 30% aq. Solution of Phosphoric acid, Hydrochloric acid

Procedure:

After the sodium silicate was collected for silica synthesis, the residual carbon was wash with double distilled water until neutral pH was achieved and then filtered. Produced carbon was then treated with 30% aqueous solution of phosphoric acid (H_3PO_4) in a weight ratio 1:1 for 24 hrs and then washed with double-distilled water several times and dried in hot air oven.

2.8.1 CHARACTERIZATION OF ACTIVATED CARBON

2.8.1.1 Moisture Content

Apparatus required: Hot air oven, Petri dish, Weighing balance, Spatula.

Procedure- About 3gm of activated carbon was taken in a petridish. Then the sample was kept in a hot air oven at 105℃ temperature. After 1hrs. the sample was taken out from the oven and it was cooled in a desiccator until reaches to room temperature. Then the weight of the sample was taken.

Moisture content (%)= [(A-B)/A]*100

Where,

A= weight of the sample before drying

B= weight of the sample after drying,

2.8.1.2 Iodine solution test

Apparatus required: Magnetic stirrer, Burette, pipette, filter paper

Reagent required: 0.1 (N) Iodine solution, Sodium Thiosulphate (NaS2CO3.5H2O), Starch

Procedure:

Step 1: 10ml of 0.1 N Iodine solution was put into a conical flask and drops of starch solution was added to it. The pale yellow color of iodine solution turns blue. Titration of the formed solution was done with 0.05 N Sodium Thiosulphate (NaS2CO3.5H2O) till it becomes colorless. Burette reading corresponds to blank reading (B) was taken.

Step 2: 0.1 gm of Activated carbon was weighed accurately. It was poured into a dry flask. 10ml of 0.1 N Iodine solution was added into the flask containing the activate carbon. The flask was shaken properly for 4 minutes and then filtered. The filtrate was the titrated against standard Sodium Thiosulphate (NaS2CO3.5H2O) using starch as indicator. Burette reading corresponds to A was taken.

The Iodine valve (ID) was calculated using this equation,

ID = C × Conversion factor (mg/gm)

Conversion factor =[Molar weight of Iodine (127) * Normality of Iodine] / [Weight of sample *Blank reading]

C= B-A

Where,

B=Blank reading

A=Burette reading of sample

2.8.1.3 Surface Area:

Apparatus required: Magnetic stirrer, Burette, pipette, pH meter
Reagent required: Hydrochloric acid, Sodium chloride, Sodium hydroxide

Procedure: The specific surface area of activated carbon was estimated using sear method by agitating 1.5 g of activated carbon samples in 100 ml of dilute hydrochloric acid at pH= 3. Then a 30 g of sodium chloride was added while stirring the suspension. The volume was made up to 150 ml with distilled water. The solution was titrated with 0.1N NaOH to rise the pH from 4 to 9 and volume V recorded.

The surface area according to this method was calculated as:

S =32V - 25

Where,

S = surface area of activated carbon

V = volume of sodium hydroxide required to raise the pH sample from 4 to 9.

The value 32 in equation is a dimensional constant measured/cm.

2.8.1.4 Methylene blue adsorption test:

Apparatus required: Magnetic stirrer, Burette, pipette, BOD Incubator, UV-Vis Spectrophotometer
Reagent required: Hydrochloric acid, Sodium chloride, Sodium hydroxide

Procedure:

Standard curve of methylene blue solution: Various concentration of methylene blue solution was prepared (C_0= 0.3mg/L to 1.5mg/L) and the absorbency was measured by UV-Vis spectrophotometer at 625nm to produce a standard curve.

Adsorption test: The adsorption performance of the sugarcane bagasse based activated

carbon was done by methylene blue solution. Dye solution of various initial concentrations (0.1mg/L to 1mg/L) were agitated with 0.05 g of the prepared activated carbon using a water-bath shaker at 25ºC for 48 hours to attain an equilibrium concentration. After equilibrium, the samples were filtered and the dye concentrations in the solutions (Ce) were measured using an UV-Vis spectrophotometer at 625 nm.

The dye removal percentage was calculated by using the equation,

Dye removal (%) = $[(C_0-Ce)/C_0]*100$

Chapter 3:
Results & Discussions

3.1 PROXIMATE TESTS:

Table 2: Proximate test results

Moisture(Wet basis)	**7.5%**
Moisture(Dry basis)	**8.1%**
Ash content	**1%**
Crude Fat content	**2.5%**
Crude fiber content	**32.4%**
Protein content	**1.56%**
Total carbohydrate	**54.6%**

The proximate properties gave indications on how SB could benefit when used as an ingredient. Low moisture content suggested the ability to prolong storage life by preventing microbial growth and spoilage. low crude fat meant SB could be incorporated into low fat food with reduced calories value. Relatively high crude fiber and carbohydrate content indicates its use as a substrate in fermentation.

3.2 TRIAL 1: In this method only 4.3% dextrose equivalent sugar can be extracted. This is due to the fact that cellulose, hemicellulose, lignin could noy be broken by this method.

3.3TRIAL 2: Bluish green color was detected during the titration which indicates the presence of lignin in the filtrate.

3.4TRIAL 3:

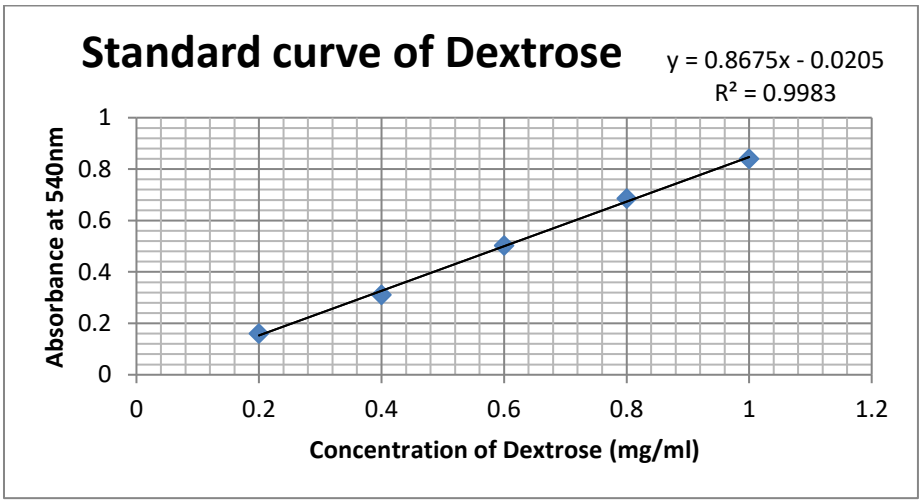
3.4.1 ASSAY OF ENZYMES (AMYLASE, CELLULASE, XYLANASE) IN CELL FREE EXTRACT:

3.4.1.1 Standard Curve for dextrose: A standard curve for dextrose was constructed using UV – Vis spectrophotometer at 540 nm. The lactose conversion percentage was calculated using the standard curve given in Fig 1. The absorbance values for different amounts of glucose is given in Table 3.

Table 3. – Standard curve for Glucose

Concentration (mg/ml)	**Absorbance at 540nm**
0.2	0.160
0.4	0.311
0.6	0.503
0.8	0.686
1	0.840

Fig.1 Standard curve of dextrose



3.4.1.2 Estimation of Enzyme Activity:

Table 4: Enzyme activity of 24hrs old inoculum (IU/min)

Amylase	Cellulase (CMC)	Cellulase (Filter paper)	Xylanase
9.87	6.704	6.356	7.370

Table 5: Enzyme activity of 48hrs old inoculum (IU/min)

Amylase	Cellulase (CMC)	Cellulase (Filter paper)	Xylanase
4.154	3.411	4.355	2.856

Aspergillus niger is a fungi that are present in nearly all soils and other diverse habitats. These are highly efficient producers of many extracellular enzymes. They are used commercially for production of cellulases, pectinase, amylases, and other enzymes that degrade complex polysaccharides.
Lag phase of *Aspergillus* varies from 6-13hrs. In the early log phase around 24hr, *Aspergillus* produces highest amount of enzymes. For this reason the inoculum from 24hrs old culture was used for this study.

3.4.2 OPTIMIZATION PARAMETERS:

Effect of Inoculum: Inoculum with cell mass and without cell mass both were taken to study the effect of it on extraction of reducing sugar from SCBP. As *Aspergillus* highly produces extracellular enzymes so the sugar content of both the filtrate was the same. Cell free extract was further used.

Effect of SCBP amount: 2.5gm and 5gm of SCBP were taken to study the effect of it on extraction of reducing sugar from SCBP. As the enzyme to substrate ratio is high for 2.5gm SCBP so the sugar content was higher for this. 2.5gm sample was used for further studies.

Effect of pH: Different pH such as 3.5, 5, 6.5, 8, 9.5, 11 were taken to study the effect of different pH on extraction of reducing sugar from SCBP. At pH 9.5 the sugar content was the higher among this. This may be due to the fact that more NaOH is present in this solution. NaOH solution breakdown the hydrogen bonding between the lignocellulosic component which cause the lignin, hemicellulose, and lower molecular fractions to dissolve in alkali solutions. So, both the NaOH and enzyme works upon the degradation of cellulosic components. But when the pH rises more, the effectiveness of the enzyme decreases and due to that the sugar content also decreases.

Effect of Incubation period: Different incubation periods such as 3hours, 4hours, 5hours were taken to study the effect of different incubation time on extraction of reducing sugar from SCBP. The sugar content measured for each of the samples was closed to each other. So, for feasibility 3hrs incubation period was chosen.

Effect of Temperature: Different temperatures like 35℃, 40℃, 45℃ were taken to study the effect of different temperature on extraction of reducing sugar from SCBP. The incubated in 35℃ showed the best result among these.
This is due to the fact, that *Aspergillus niger* grows rapidly at 30℃-35℃ temperature range. Above that temperature the protein and nucleic acids denatures.

Table 6 : Effect of different parameters on extraction of reducing sugar:

Sample	Inoculum	Amount of SCBP (gm)	pH	Incubation Period(Hrs)	Temperature	Extractable sugar in 100gm SCBP (%) Dextrose equivalent
1	With cell mass	5	–	3	35	19.8
2	Without cell mass	5	–	3	35	20
3	Without cell mass	5	–	3	35	20.7
4	Without cell mass	2.5	–	3	35	24.34
5	Without cell mass	2.5	3.5	3	35	24.16
6	Without cell mass	2.5	5	3	35	24.61
7	Without cell mass	2.5	6.5	3	35	25.84
8	Without cell mass	2.5	8	3	35	29.4
9	Without cell mass	2.5	9.5	3	35	32.1
10	Without cell mass	2.5	11	3	35	26
11	Without cell mass	2.5	9.5	3	35	32.08
12	Without cell mass	2.5	9.5	4	35	32.61
13	Without cell mass	2.5	9.5	5	35	32.34
14	Without cell mass	2.5	9.5	3	35	32.08
15	Without cell mass	2.5	9.5	3	40	26.14
16	Without cell mass	2.5	9.5	3	45	20.93

3.5 PRODUCTION OF ETHANOL FROM SCBP BY USING SACCHAROMYCES CEREVISIAE:

Alcoholic fermentation is a complex biochemical process during which yeasts convert sugar to ethanol, carbon dioxide and other metabolic byproducts that contribute to the chemical composition and sensorial properties of different fermented food products. Current industrial ethanol fermentation is mainly carried out with the yeast because of its low pH and high ethanol tolerance. The conversion of sugars to ethanol by yeast under anaerobic conditions is the process used to make the renewable transportation fuel, bioethanol.

Growth (Aerobic):

$C_6H_{12}O_6(+ O_2) \rightarrow$ Biomass

$C_{12}H_{22}O_{11}(+ O_2) \rightarrow$ Biomass

Secondary metabolites (Anaerobic):

$C_6H_{12}O_6 \rightarrow 4$ Ethanol $+ 2CO_2$

$C_{12}H_{22}O_{11} \rightarrow 4$ Ethanol $+ 4CO_2$

The raw material for ethanol production can be any material containing appreciable amounts of sugar or substances that can be converted to sugar. Conventioal production uses sugar (from sugarcane and sugar beet), starch (from corn, wheat, or potatoes) or other polysaccharides. The production process of second-generation ethanol also called cellulosic alcohol, uses cellulosic feedstock (e.g from agricultural residue) which require further pretreatment. In industrial fermentation, carbohydrates are not the only important group of compounds in the medium. It has been suggested that an appropriate amount and diversity of nitrogenous compounds are important for the successful completion of industrial fermentation processes and product quality.

After distillation of the fermentation media, the alcohol percentage was measured by a alcohol meter.

Alcohol percentage after distillation = 7%

Which means around 17.5 ml of alcohol can be obtained from 250ml fermentation media by using SCBP.

3.6 SYNTHESIS OF SILICA POWDER FROM THE RESIDUAL SCBP AFTER ALCOHOL PRODUCTION

When the acid-treated bagasse ash was extracted with NaOH solution at 90±5 °C for silica, sodium silicate solution (Na2SiO3) was obtained. The precipitation reaction of silica from Na2SiO3 with HCl acid is

$$SiO_2(s) + 2NaOH(aq) \rightarrow Na_2SiO_3(aq) + H_2O(l)$$

$$Na_2SiO_3(aq) + 2HCl(aq) \rightarrow SiO_2(s) + 2NaCl(aq) + H_2O(l)$$

It has been reported that a high concentration of NaOH (> 1M) does not significantly improve the silica yield, and so 1(M) NaOH is recommended for the extraction of silica. Silica was extracted from black bagasse ash and dissolved into NaOH solution forming a liquid phase of sodium silicate solution.
The silica started to precipitate at a pH lower than 10 from the obtained sodium silicate solution using 2.5M HCl. In the process, a pH of 7 was maintained and the gel was formed during this silica aging period.
It should be noted that an increase in the HCl concentration (>2.5M) had an adverse effect on silica yield owing to the re-dissolution of silica in a high acid environment. After filtration, washing the silica with hot water helped lower the Na and K contents of the final products, and whiten the silica resulting in purer silica. Silica from this synthesis method was xerogel type with liquid phase in pores and was removed by evaporation.
In this experiment 4.9gm silica powder was obtained from 10gm of SCBP ash (Yield- 74%).

3.7 CHARACTERIZATION OF ACTIVATED CARBON:

3.7.1 Moisture Content: The activated carbon obtained from residual sugarcane bagasse after alcohol production has the moisture content of 5%. And the activated carbon obtained from residual ash after silica synthesis has the moisture content of 3%.

3.7.2 Iodine solution test: This is the most fundamental parameter used to characterize activated carbon performance. It is a measure of activity level (Higher degree indicates higher activation), often reported in mg/g (with typical range of 500 – 1200 mg/g). It is a measure of the microspore content of the activated carbon by adsorption of iodine from solution. It is equivalent to surface area of activated carbon between 900 m^2 /g and 1100 m^2 /g.
The activated carbon obtained from residual sugarcane bagasse after alcohol production has an Iodine value (ID) of 628.31mg/g. And the activated carbon obtained from residual ash after silica synthesis has an Iodine value (ID) of 687.31mg/g.

3.7.3 Surface Area: The smaller the surface area of a material, the higher the adsorption capacity within the material. The activated carbon obtained from residual sugarcane bagasse after alcohol production has a surface area of 451.8 m^2/g. And the activated carbon obtained from residual ash after silica synthesis has a surface are of 447.4 m^2/g.

3.7.4 Methylene blue adsorption test:

Fig 2: Methylene blue standard curve

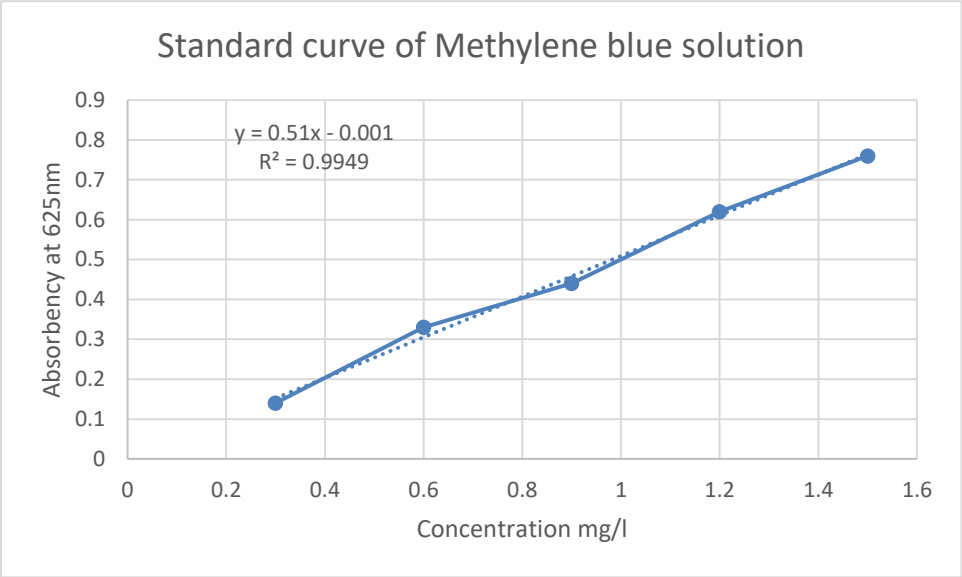


Table 7: Dye removal (%):

A= Activated carbon obtained from residual sugarcane bagasse after alcohol production.

B= Activated carbon obtained from residual ash after silica synthesis.

Sample	Initial dye concentration (C_0) mg/l	Final dye concentration (Ce) mg/l	Dye removal (%) [(C0-Ce)/C0]*100
A	1	0.21	79%
A	0.1	0.026	74%
B	1	0.05	95%
B	0.1	0.003	97%

The result shows that sample B has higher level of Dye removing capacity then sample A. The results indicated that the maximum dye removal percentage was around 97%, thus, the activated carbon is more efficient in methylene blue adsorption from aqueous solution. Hence it can be confirmed that the prepared activated carbon is mesoporous, since the methylene blue is used to characterize mesoporous activated carbons.

Chapter 5:

Conclusion

Conclusion:

The following conclusions were drawn from the work carried out. The SCBP produces maximum 32% of dextrose equivalent sugar in the enzyme consortia after the process parameters (pH- 9.5, Incubation period- 3hrs, Temperature- 35°C) were optimized. *Aspergillus niger* produces maximum amount of extracellular enzymes like Amylase, Cellulase and Xylanase in the initial log phage (Amylase- 9.87IU/min, Cellulase- 6.704IU/min, Xylanase- 7.307IU/min). This is a biological process to extract the sugar from SCB with using any harsh chemicals. 7% Alcohol was produced with the extracted sugar solution. And the residual bagasse was used to produce silica powder and activated carbon. Bagasse ash has the potential to be a source for amorphous silica extraction using a process with a low concentration of alkaline. This method provides low chemical and energy consumption compared to the traditional method of silica production from molten quartz sand. Acid pre-treatment is recommended to remove impurities in the ash. The silica obtained was light gray in color owing to a high carbon content from incomplete burning. Residual bagasse produces silica powder with a yield of 74%. With the residual bagasse after alcohol production and the residual bagasse after silica synthesis both were chemical activated with phosphoric acid to get activated carbon. This research work proves the effectiveness of activated carbon produced from sugarcane bagasse waste. The prepared activated carbon by using chemical process has Iodine value of-

The activated carbon obtained from residual sugarcane bagasse after alcohol production has an Iodine value (ID) of 628.31mg/g. And the activated carbon obtained from residual ash after silica synthesis has an Iodine value (ID) of 687.31mg/g. The surface area was measured by sear method. The activated carbon obtained from residual sugarcane bagasse after alcohol production has a surface area of 451.8 m^2/g. And the activated carbon obtained from residual ash after silica synthesis has a surface are of 447.4 m^2/g. The dye removal capacity of the carbons was measured by methylene blue. From the methylene blue adsorption from the aqueous solution experiment it has been shown that the maximum dye removal was around 97% by the activated carbon obtained from residual ash after silica synthesis.

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