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Conversion of biomass wastes into biofuels: a sustainable technology

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Abstract

Bioethanol is a sustainable biofuel generated from lignocellulosic biomass wastes. It has been discovered that adding nutrients and electron acceptors in the form of nanomaterials might alter the bioenvironment and bio-stimulate microorganisms to carry out the intended bioprocesses effectively. In this study, XRD and UV-Vis spectroscopic techniques were used to confirm the purity, structure, and quality of green synthesized ZnO nanoparticles made using crude extract from banana peels. ZnO nanomaterials were used in combination with the control (i.e., no nanomaterials added) to improve the production of bioethanol from sugar cane bagasse. It was proposed that treating yeast with nutrients or nanomaterials would bio-stimulate the cells and boost their activity. It was found that the maximum concentration of bioethanol (19.75 g/L) was produced by bio-stimulating *Saccharomyces cerevisiae* with 200 mg/L of ZnO nanoparticles, as compared to the control (no nanomaterials added).

Key Words: lignocellulosic biomass wastes, sugarcane bagasse, *Saccharomyces cerevisiae*, ZnO nanoparticles, Bioethanol

1. Introduction

Due to the exponential expansion in population, industrial development and urbanization, the world's energy needs have grown over time. It is anticipated that energy usage will rise by 50% during the next ten years. With respective energy consumption rates of 23%, 17%, and 9%, China, India, and the US lead the world in energy consumption (Rebros et al.,2005,41; Zhang et al.,2016,140). The International Energy Outlook-2018 (IEO-2018) states that Africa, India, and China are the world's most densely inhabited regions. Their economies make use of nearly one-third of the of global energy. Gas, oil, and coal are the key fossil fuels that are now the primary sources of energy demands. Although they meet around 80% of the world's energy demands, conventional fossil fuels have a number of disadvantages (Hou et al.,2017,165). Due to their non-renewable nature, fossil fuels eventually run out, creating a major energy crisis. Cost increases are a major economic problem brought on by the growing use of energy sources, especially in countries developing. The use of these conventional fuels causes irreversible environmental damage, which is the most pressing worry. Acid rain, the greenhouse effect, and global warming are caused by the release of harmful substances such as methane (CH_4), carbon dioxide (CO_2), nitrogen oxide (NO_2), sulphur dioxide (SO_2), and others when fossil fuels are burned (Yong et al.,2018,266). Global CO_2 emissions from industry and fossil fuels have risen significantly during the past 20 years. There is increased awareness of CO_2 as a greenhouse gas as a result of these emissions detrimental effects on the planet due to global warming and climate change (Yao et al.,2011,46). If unchecked emissions are not reduced, the current CO_2 level of 394.5 parts per million volume (ppmv) is expected to rise to 500 ppmv by 2050 (Le Quere et al.,2018,1; Palareti et al.,2016,38). As a result of these problems, researchers are now focusing on ecologically friendly energy and efficient technology for deep sustainability through renewable energy resources (Mahmoodi et al.,2018,166). According to the research, because biofuels like biohydrogen, biodiesel, and bioethanol are produced from substances inexpensive, abundant, and environmentally benign, they are of particular interest to researchers (Adegboye et al., 2021). Biofuel acts as its ideal solution for the world's current crisis.

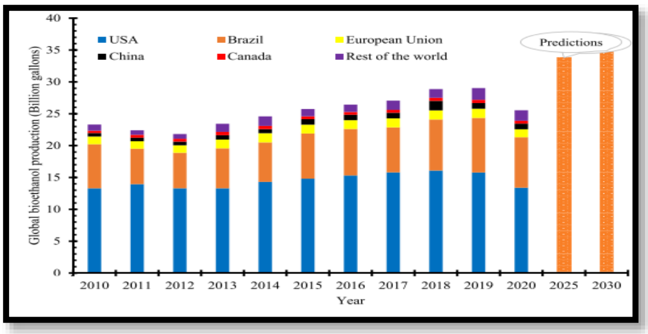


Figure (1): Global bioethanol production by country, billion gallons from 2010-2030.

Biofuels, particularly bioethanol, are seen as a possible alternative for traditional petroleum fuels due to its many and significant socioeconomic and environmental advantages (Devarapalli and Atiyeh, 2015 ,268). The primary benefits of using ethanol are its cleanliness, purity, and renewable nature as compared to traditional energy fuels (Wang et al., 2011,612). Bioethanol is the most popular liquid biofuel and is blended with petrol for use as vehicle fuel in the market and more than one million barrels of oil equivalent per day (mboe/d) of bioethanol were produced in 2020 in the United States and Brazil from starch and sugars, respectively (IEA, 2021).

The United States and Brazil led the ethanol producer chart (Figure 1), with global ethanol output rising from 23.31 billion gallons in 2010 to 29.03 billion gallons in 2019 (Renewable Fuels Association, 2023). Based on the present pace of production, it is estimated that the world will produce 33.8 billion gallons of ethanol in 2025 and an additional 34.87 billion gallons in 2030 (FAO. Biofuel, 2013). In petrol blends, bioethanol boosts the octane level (Dodo et al., 2017,28). The global market values expected increase demand for bioethanol due to the use of it in various applications such as bioplastics, sustainable aviation fuel, and green platform chemicals (Mandegari et al., 2018,76).

Ethanol made from sugars and starches such sugarcane juice, molasses, wheat, or corn is referred to as first-generation (1 G) bioethanol (Lennartsson et al., 2014,165). Nowadays, first-generation ethanol production accounts for over 99% of global industrial production, and the processes involved are well-established. However, there is a growing preference for waste-based biofuels over crop-based biofuels, with waste lignocelluloses being the preferred source of these biofuels ("RED II – Europex", 2021). On the other hand, lignocelluloses are the feedstock for second-generation (2 G) ethanol technologies, which are mostly being developed in the USA and Brazil. 2 G ethanol production is more difficult and expensive compared to 1 G ethanol production, but it has more environmental benefits and doesn't raise the question of fuel vs food. The usual four steps in the production of fuel bioethanol, that is Pretreatment, enzymatic or acidic hydrolysis, fermentation, and distillation (Figure 2).

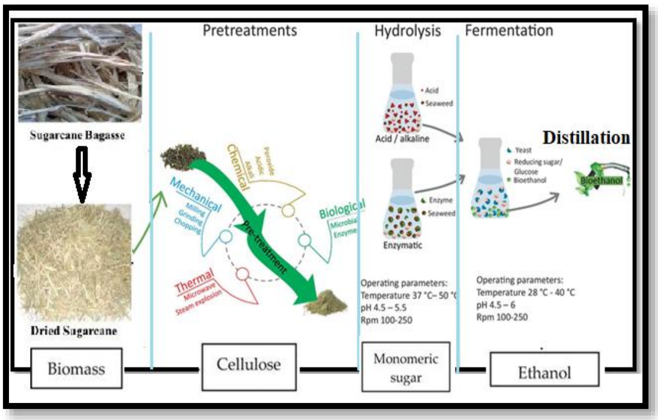


Figure (2): A four-step process for ethanol production from biomass.

2. The Theoretical Framework

In general, lignocellulosic biomass (LCB) refers to biomass that can be utilized as sustainable alternative feedstocks to produce bioethanol, such as woody materials, grasses, and agricultural residues like wheat straw, sugarcane bagasse, rice straw, etc. Furthermore, it is reported that lignocellulosic materials have the potential to yield 442 billion Liters of bioethanol Year (Haq et al., 2016, 1413). A secondary agricultural residue known as sugarcane bagasse is thought to be a viable feedstock to produce bioethanol (Anwar et al., 2014,163), which aids in the replacement of fossil fuels and the mitigation of greenhouse gas emissions (Thakur et al., 2015,1172). According to a 2010 International Energy Agency (IEA) assessment, 25% of residues that can be turned into biofuels can produce 13.0–23.3 EJ of energy, which means that by the end of 2030, they may cover 10.3–14.8% of the world's transport fuel demand (Niphadkar et al., 2018,229). After the juice from sugarcane is extracted, a solid residue known as sugarcane bagasse (SCB) is left behind. It contains polysaccharides that can be turned into bioethanol. Bagasse's polysaccharide (Figure 3) is made up of ∼ 45–50 % cellulose, 25–30 % hemicellulose, lignin covers ∼ 25 % and whereas along with 2.4–9.0% ash (Karp et al., 2013,679). Hemicellulose is an amorphous polymer that is typically made up of various C6 and C5 sugars, including L-arabinose, L-glucuronic acid, D-glucose, D-xylose, D-mannose, and D-galactose. Amorphous (Rodrigues et al., 2011, 1242), hemicellulose is a big, intricate carbohydrate molecule that aids in the cross-linking of cellulose fibres in plant cell walls (Williams et al., 2015 ,5). Hemicelluloses are also known as "crosslinking glucans" because they can create hydrogen bonds with lignin and cellulose (Pasangulapati et al., 2012, 663). Cellulose is a polymer made up of hydro glucose fractions joined by b-glycosidic bonds to carbon atoms four and one. This is supported by the formation of several van der Waals and hydrogen bonds within solid molecules as well as the presence of three reactive hydroxyl groups: OH number one to C-6, OH secondary to C-2, and OH secondary to C-3 (Kadla and Gilbert, 2000 ,197). It is arranged into fibrils, which are encased in a lignin and hemicellulose matrix.

Lignin is made up of phenylpropane polymeric units, which are insoluble in water and exhibit remarkable resistance to both chemical and physical forces. Lignin's primary job is to keep fibers together. As seen in Figure 2, p-hydroxyphenyl, guaiacyl, and syringyl are the three main constituents of lignin which are formed physical and chemical linkages with cell wall polysaccharides. The presence of lignin in the vegetal cell wall is known to hinder the accessibility of enzymatically or acid hydrolysis to conversion SCB into glucose (Mooney et al. ,1998,113). Some treatments efficiently remove the lignin fraction, while keeping the cellulose/hemicellulose proportion nearly unchanged (Chen and Dixon ,2007 ,25). Therefore, pretreatment process is essential for breaking the recalcitrant structures of SCB and making it more accessible for acid or enzyme catalysis.

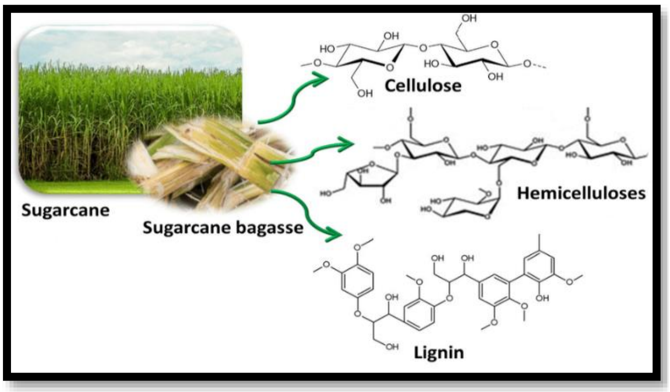


Figure (3): Sugarcane plant, sugarcane bagasse and its main constituents (cellulose, hemicellulose, and lignin).

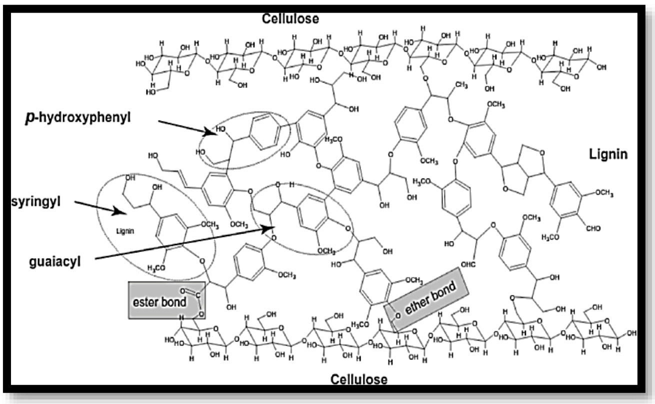


Figure (4): Chemical linkages between lignin and cellulose.

Pretreatment of SCB biomass is usually a prerequisite step to improve cellulose digestibility by removing hemicelluloses and or/ lignin or rearranging them in the cell wall, thus, increasing the cellulose accessibility (Antunes et al. 2018,189). to date, there are many developed forms of Pretreatment techniques, including chemical (alkaline, acid, organosolv, oxidative, deep eutectic solvents, ionic liquids), physiochemical (hydrothermal, steam explosion), biological, physical methods (milling, grinding, mechanical chipping) (Xu and Huang, 2014,43) or it can be combination of these methods. Among the existing different pretreatment techniques, sodium chlorite (SC) pretreatment has emerged as one of the most popular pretreatment methods in industries now in use due to its effectiveness, low corrosion, low sugar degradation, low inhibitor concentration, cost-effectiveness, and yield of digestible substrate (Siqueria et al., 2013 ,339). The creation of pretreatments with little to no inhibitor generation remains a difficulty in the synthesis of bioethanol, despite the availability of many pretreatments (Rabelo et al., 2011,2600). Pretreatments should be improved to provide a pretreatment that is both maximally efficient and cost-effective, while also not producing inhibitors. Considering all these factors, one therapy that may be carried out affordably, in a gentle manner, and without the need of acids is the combination of sodium chlorite with NaOH delignification pretreatment.

The SCB must be hydrolysed following the pre-treatment for bioprocesses like fermentation to convert it into useful materials for the synthesis of biofuels and biochemical products. This is because the SCB's polymeric sugars need to be disassembled into smaller components for microbiological action to occur. The word "hydrolysis" refers to the process of dissolving complex materials in the presence of water and a catalyst; in the case of SCB, the catalyst may be an enzyme or an inorganic chemical substance (Dionísio et al. 2020,121290). The biological process of converting a complex sugar into simple sugars by enzymatic action prior to fermentation is known as enzyme-catalysed hydrolysis. This is required to allow the complex sugar's glucose to become microbially vulnerable. Cellulase is the enzyme that breaks down cellulose; it is typically found in commercial cellulases like Novozym, Spezym, and viscozyme.

On the other hand, culture enzymes can also be used. The goal of hydrolysis in general and enzymatic hydrolysis in particular is to convert cellulose and hemicellulose into sugars known as hexose and pentose (Dekker and Wallis 1983, 3027), which are then used as suitable feedstocks for fermentation (Pramanik et al. 2021,100013).

Another technique for producing glucose syrup from SCB is acid hydrolysis, which involves dissolving the complex sugar in biomass into simple sugars using a diluted solution of an inorganic acid. Acid hydrolysis of SCB has been accomplished by using inorganic acids including phosphoric acid (Gámez et al. 2006, 78), nitric acid (Rodrĭguez Chong et al. 2004,143) and sulfuric acid. To hydrolyse SCB, Canilha et al. (2010) used a 2% w/v H_2SO_4 solution. According to De Moraes Rocha et al. (2011), a 90% optimal conversion of the hemicellulosic hydrolysate by the 1%, w/v H_2SO_4+1%, w/v CH_3COOH solution was obtained during the dilute acid hydrolysis of SCB by a mixed sulphuric and acetic acid solution. Velmurugan and Muthusumar (2011) assessed the pre-treatment of acid hydrolysis, utilising a new sono-assisted method. Therefore, the efficient conversion of SCB to reducing sugars is essential for the utilisation of biomass to produce biofuels and biochemical products. Acid hydrolysis has been shown to be preferred over enzymatic processes due to its faster and more efficient processing time.

In the last ten years, nanobiotechnology has found widespread application in a variety of scientific and technological fields, including drug delivery, biomedicine, wastewater treatment, bioremediation, fabrics, cosmetics, and coating (Al-Dhabi et al., 2019, 111529; Agayeva et al., 2020, 3258; Baskar et al., 2016, 23). Researchers from all around the world have shown a great deal of interest in the use of nanomaterials in the bioenergy sectors for the hydrolysis of lignocellulosic biomass and the subsequent manufacture of bioethanol (Aarti et al., 2021,164). According to Cherian et al. (2015), nanoparticles aid in increasing biomass pre-treatment efficiency, enzymatic hydrolysis, and reaction rate in addition to increasing productivity. nanoparticles impact the biochemical conversion process involved in the fermentation-based synthesis of liquid fuels. using a variety of nanoparticles, including silicon, nickel, iron, cobalt, silver, and magnetic nanoparticles (Singhvi and Kim, 2020, 5300). Because of their higher surface area, nanoparticles can boost enzyme loading and hence induce catalytic efficiency. For instance, Fe_3O_4 was used by Vijayalakshmi et al. (2021, 901) to fabricate bioethanol on an industrial scale using a variety of feedstocks. The study's conclusion demonstrated the nanoparticle's potential to significantly increase bioethanol yield when a variety of feedstocks are fermented. MnO_2 nanoparticles were used by Cherian et al. (Cherian et al., 2015 ,1223) to immobilise cellulase and increase the amount of bioethanol produced during the fermentation of sugarcane leaves. In a different study, Kim and Lee (Kim and Lee ,2016 ,139) used methyl-functionalized cobalt ferrite–silica nanoparticles ($CoFe_2O_4@SiO_2$-CH_3) for syngas fermentation to produce bioethanol, while Kim et al. (Kim et al.,2014 ,446) used methyl-functionalized silica (SiO_2–CH_3). According to the authors, the bioethanol yield increased by 166% when SiO_2-CH_3 nanoparticles were used, and by 213 % when $CoFe_2O_4@SiO_2$-CH_3 nanoparticle catalysts and five cycles of reusability were used. Sanusi et al. (Sanusi et al., 2020 ,386) provided evidence of the use of NiO nanoparticles to improve the production of bioethanol from potato peel waste. The study's findings demonstrated that artificial nanoscale NiO generated a bioethanol yield of 31.58 g/L, or roughly 65% more than the macroscale catalyst control experiment.

Scientists used to be fascinated with creating metal nanoparticles, but these days they are more interested in creating oxide nanoparticles in an environmentally friendly manner (Thema et al., 2015 ,1043; Thovhogi et al., 2015, 392). Zinc oxide is the most preferred oxide nanoparticle due to its adaptable properties and wide range of uses, including in electronic devices and cosmetics (Singh et al., 2019, 1061). In addition, ZnO nanoparticles are crucial for biomedical uses such medication administration and bioimaging (Mirzaei et al., 2017, 907). Zinc oxide (ZnO) is widely used in many applications since it is the second most prevalent metal and can be found in all six classes of enzymes. It is also considered biosafe due to its diverse morphological shapes and sizes (Das et al., 2013, 556). Zinc oxide nanoparticles can be produced using a variety of techniques, such as the hydrothermal (Stankovic et al., 2013, 21), solvothermal (Talebian et al., 2013, 66), sol-gel (Lallo et al., 2019, 440) and thermal breakdown method (Das et al., 2013, 556). Nevertheless, several of them need the use of difficult to access substrates and are time and money consuming. Via the green synthesis route, nanoparticles can also be obtained. This type of synthesis uses natural ingredients, such as plant peels, that are frequently recovered from agricultural waste. These substances serve as the reducing or stabilizing agents for nanoparticles. Every day, fruit markets, the food industry, and households worldwide generate tonnes of garbage from banana peels. Numerous studies have attempted to make use of this enormous biomass of banana peels, producing mycological medium (Essien et al.,2005, 1451), bioenergy (Clarke et al., 2008, 527), plant composition (Doran et al., 2005, 7), and pectin (Happi et al., 2008, 463).

The aim of this study was that sugar cane bagasse (SCB) was initially treated with sodium chlorite and dilute sodium hydroxide to achieve effective delignification and hemicellulose solubilization. Furthermore, dilute acid hydrolysis process was carried out on pretreated SCB to produce fermentable sugars and the fermentation process was carried out using *Saccharomyces cerevisiae* to efficiently utilize the fermentable for bioethanol production. Moreover, the objective of this investigation was to increase bioethanol production from SCB using nanomaterials. So, we used banana peels crude extract that is rich of carbohydrates and other phytochemicals to synthesize ZnO NPs using green chemistry that were characterized X-ray diffraction (XRD) and UV-Vis spectroscopic techniques. Then, the use of ZnO NPs as biocatalysts to enhance the bioprocess of ethanol production from fermentation of sugarcane bagasse.

3.Methods of Research and the tools used

3.1. Chemicals and materials

The bagasse from sugarcane was collected and all chemicals were obtained from Sigma-Aldrich and used without further purification.

3.2. Preparation of banana peel extract

The banana peel was washed with ethanol, cut into small pieces, and dried in oven at 70 °C for 24 h. 5 g of crushed banana peel was combined with 60 mL of distilled water, and the mixture was then placed in the steel autoclave. After maintaining the autoclave at 180 °C for the required 6 h, the autoclave was allowed to cool to room temperature. The filtering process was used to separate the extract from the medium (Şahin et al., 2024 ,448).

3.3. Green synthesis of ZnO catalyst

For the green synthesis of ZnO catalyst, 25 mL of banana peel extract was dropped into a beaker, and 30 mL of a 5 mM $Zn(NO_3)_2 \cdot 6H_2O$ solution was added and stirred. The pH of the solution was adjusted to 12 by adding 3 M NaOH. After stirring for 4 h at room temperature, the resulting mixture was filtered, and the resulting solid was dried at a temperature of 80 °C for 24 h and then calcined at 350 °C for 2 h (Imade et al., 2022).

3.4. Delignification of sugarcane bagasse

The bagasse from sugarcane collected and then dried and cut by scissors to small sizes. 90 g of bagasse was weighted and placed at 5 L beaker. The bagasse was soaked into 1.5 L of sodium chlorite solution (7 %) and adjusted the PH of solution to 13 by addition NaOH solution (3M). The treatment solution was to soak for 24 h. After the soaking time, the residue was removed from the solution by filtration and then washed with distilled water several times until PH of filtrate equal 7. The residue was dried in an oven at 70 °C for 48 h. Finally, the residue was reweighted. The weight difference was equated to the amount of lignin removed.

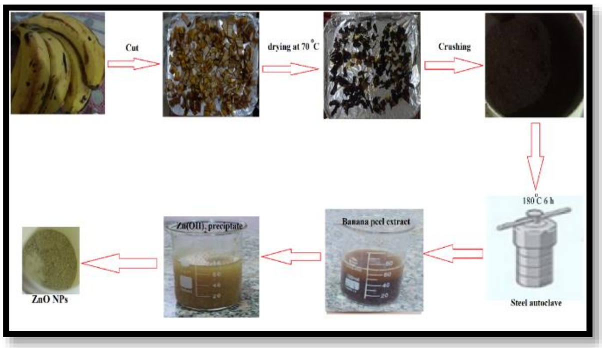


Figure (5): Synthesis of ZnO NPs from banana peel extract.

3.5. Acid hydrolysis treatment of bagasse

The aim of acid hydrolysis was to make cellulose available for yeast fermentation. We prepare two flasks; each flask contains 25 g of bagasse residue and 450 mL of H_2SO_4 (0.5 %). The two flasks were autoclaved for 15 min at 121° C under pressure 1.5 atm. The mixtures were centrifuge and filtered. The bagasse extract was neutralized with NaOH (1 M) and heated at 50 °C for 10 min.

3.6. Fermentation method

The fermentation experiments were carried out in sterile 100 mL Erlenmeyer flasks with a working volume of 50 mL The fermentation medium contained; glucose (20 g/L), ZnO nanoparticles (200 mg/L), nutrients (yeast extract (*Saccharomyces cerevisiae*): 5 g/L, $(NH_4)_2SO_4$: 2.5 g/L, KH_2PO_4: 1 g/L, $CaCl_2$: 0.1 g/L and $MgSO_4$:1 g/L). Then pH values (pH= 6), were achieved by adjusting the pH of the medium with 0.05 M sodium citrate buffer. Yeast inoculations were carried out at 5 % (v/v). After inoculation, the experiments were incubated under static conditions at 30 °C for 5 days of fermentation period. Then, we applied the same steps in glucose control on fermentation of sugarcane bagasse extract with and without ZnO.

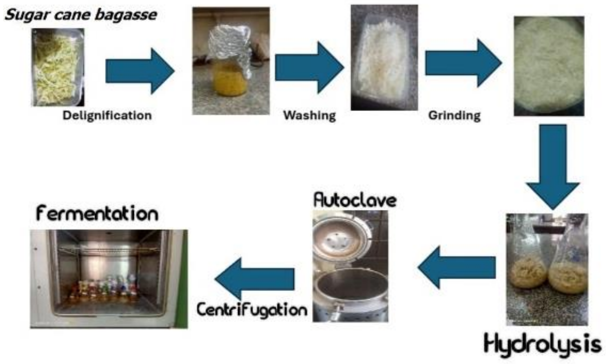


Figure (6): Pretreatment methods of sugarcane bagasse biomass.

3.7. Analysis of reduced sugars by DNS method

Miller's technique, which makes use of the dinitro salicylic acid method (DNS), was employed to estimate reduced sugars (Choong et al., 2016, 28) We used calibration curve of dilutions knowledge of the reducing sugar concentration to determine the reducing sugar concentration of samples (Figure 7).

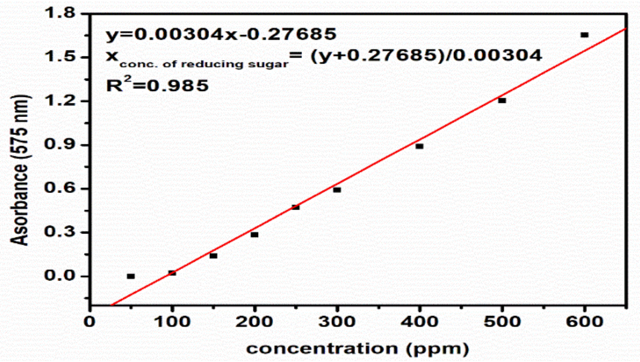


Figure (7): Standard calibration curve for the DNS method.

3.8. Characterization and Analytical assay

The structural and chemical identification of prepared ZnO NPs was obtained by X-ray powder diffraction analysis (XRD, Bruker D8 Advance X-ray diffractometer, Cu Kα radiation (λ= 1.5406 Å) at 10 kV and 10 mA). UV–Visible spectroscopy measurement of ZnO NPs was performed on UV–Vis spectrophotometer with wavelength ranging from 200 to 800 nm. The amount of ethanol produced was calculated using GC analysis of the bioethanol after the fermentation time ended. This was done using the Perkin Elmer Clarus-680 model with capillary column Elite-5MS, 30 m×0.25 mm ID, 250 µm df, in accordance with the method of Dizhbite et al. (2011). The 260°C injector temperature, the 260°C ion source with an EI of 70 eV, the 50–60 Da MS scan range, the 1 mL/min flow rate of helium as the carrier gas, and the 10:1 split ratio. While the relative area of each compound's peak was determined using the Turbo mass ver. 5.4.2 programme based on GC/MS data, the identification of each individual compound was carried out using the GC/MS chromatogram and Library NIST-2008 (Dizhbite et al.,2011).

4. Results of Research

4.1. Nanoparticles characterization

ZnO nanoparticles were successfully synthesized by green method using Banana peel extract acts as reducing agent. Figure 8 displays the XRD pattern of synthesized ZnO catalyst. The UV–Vis spectrum and band gap of the prepared ZnO NPs is presented in Figure 9. It is evident that

ZnO NPs have a broad absorption peak from 200 to 400 nm due to the intrinsic band gap of Zn–O absorption.

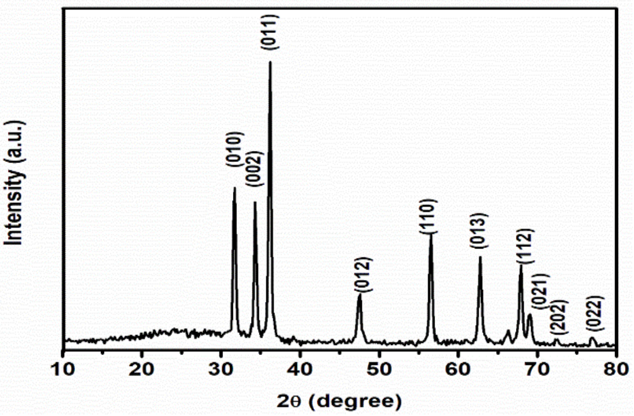


Figure (8): XRD diffractograms of zinc oxide nanoparticles.

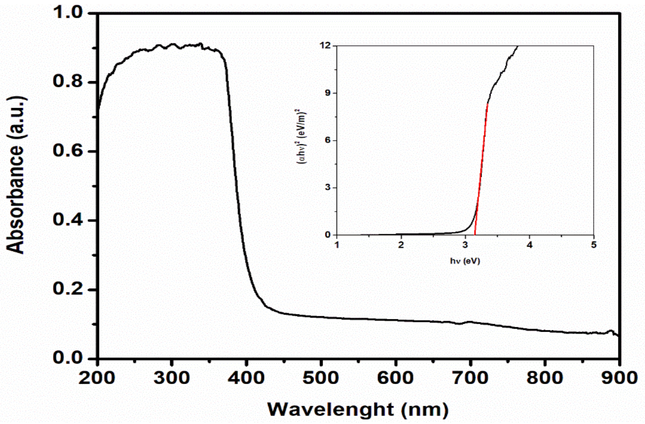


Figure (9): UV–Vis absorption spectra of ZnO nanoparticles (band gap of ZnO inset in Figure).

4.2. Bioethanol production from sugar cane bagasse

The addition ZnO NPs has effective effect on concentration of total reducing sugar and fermentation processes compared with the control (without ZnO NPs). The concentrations of consumed total reducing sugars are calculated from Standard calibration curve for the DNS method. Figure 10 demonstrates that the total reducing sugar was reduced after 5 days. Then bioethanol concentrations were measured after 5 days by GC analysis (Figure 11).

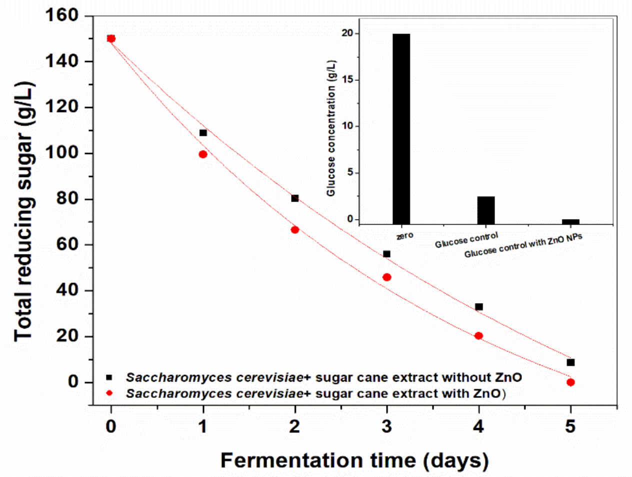
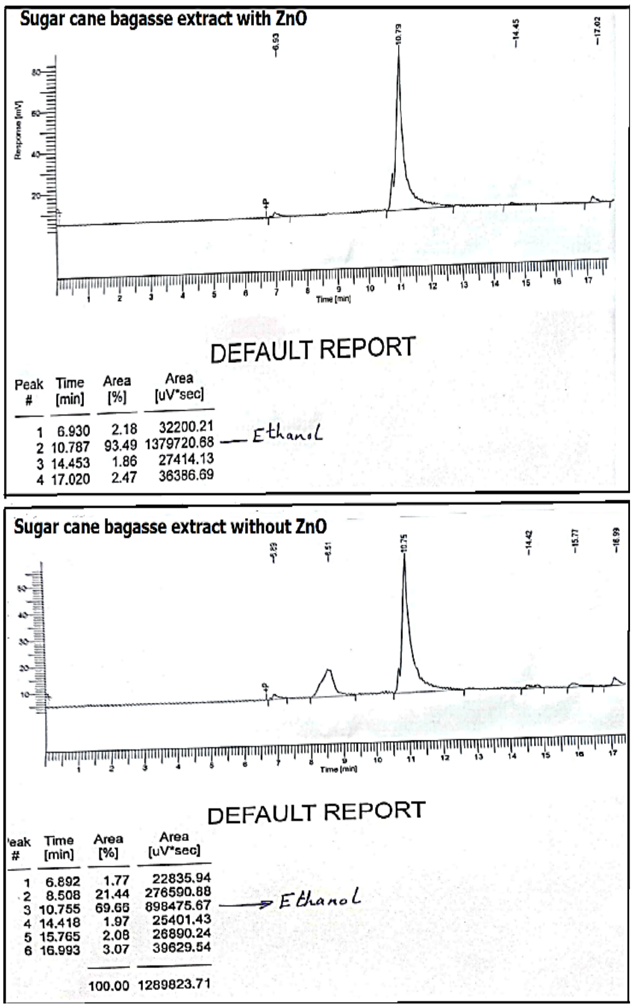


Figure (10): Diagram of consumed total reducing sugars after 5 days.


Sugar cane bagasse extract with ZnO

DEFAULT REPORT

Peak #	Time [min]	Area [%]	Area [uV*sec]	
1	6.930	2.18	32200.21	
2	10.787	93.49	1379720.68	— Ethanol
3	14.453	1.86	27414.13	
4	17.020	2.47	36386.69	

Sugar cane bagasse extract without ZnO

DEFAULT REPORT

Peak #	Time [min]	Area [%]	Area [uV*sec]	
1	6.892	1.77	22835.94	
2	8.508	21.44	276590.88	
3	10.755	69.66	898475.67	→ Ethanol
4	14.418	1.97	25401.43	
5	15.765	2.08	26890.24	
6	16.993	3.07	39629.54	
		100.00	1289823.71	

Figure (11): Chromatogram curve of sugar cane bagasse extract with and without ZnO.

5. Interpretation of Results

In Figure 8, XRD analysis showed the hexagonal crystal system (ICSD Card No.: 89-0511) is represented by the corresponding diffraction peaks at 31.77° (0 10), 34.42° (0 0 2), 36.26° (0 1 1), 47.6° (0 1 2), 56.6° (1 1 0), 62.85° (0 1 3), 67.9° (020), 68.1° (1 1 2), 69.15° (0 2 1) and 76.97° (0 2 2) that are attributed to the indexing planes of ZnO. The crystallite size was calculated 26.64 nm using highest intensity peaks for the synthesized ZnO by Debye-Scherrer equation [Crystallite size (D) = Kλ/(β cos θ)]. The UV–Vis spectrum (Figure 9) shows that ZnO NPs have a broad absorption peak from 200 to 400 nm due to the intrinsic band gap of Zn–O absorption. It is observed that ZnO NPs is a semi-conductor with a wide bandgap (3.15 eV) that makes it an effective UV radiation absorber.

After 5 days of the fermentation, the glucose concentration decreased from 20 g/L to 2.44 g/L and 20 g/L to 0 g/L for the control and the nano-administered processes, respectively as an indicator for activity of Saccharomyces cerevisiae and Saccharomyces cerevisiae with ZnO (inset in Figure 10). Furthermore, the fermented sugar cane extract with ZnO NPS decreased total reducing sugar concentration from 150 g/L to 0 g/L compared to 8.71 g/L for the sugar cane extract without ZnO (Figure 10). It was observed that ZnO NPs increases the concentration of bioethanol production up to 19.75 g/L as compared with 14.77 g/L control (without Nps), as shown in Fig. 11. The generation of bioethanol from biomass is increased with the use of nanomaterials in the bio-stimulation of yeast cells. Furthermore, it has been shown that zinc oxide nanoparticles have an electropositive glucose nanoparticle connection (Choong et al.,2016, 369), which is beneficial for substrate to cell contact. Moreover, because of their small atomic size and redox potential, nanoparticles have a greater attraction for electrons (Zhong et al., 2007,17). Under anaerobic conditions, a strong affinity was found within a few nanometers between bacteria and nanoparticles (Lower et al., 2001,1360). Additionally, it has been shown that both cell adsorption to the surfaces of NPs and the likelihood of nanoparticles being adsorbed to the cell surface occur (Han et al., 2011, 7903; Sanusi et al.,2020, 386). Therefore, for the nano-fermentation processes, better Saccharomyces cerevisiae substrate contact, cellular metabolism, and process performance were realized (Sanusi et al.,2020, 386; Verma et al., 2010, 2; Faramarzi et al.,2019, 622).

6. Conclusion

This research demonstrates the green synthesis of ZnO nanoparticles using crude extract from banana peels as a reducing and stabilizing agent. Then, the nanoparticles are used as a biocatalyst to produce ethanol from sugar cane bagasse. The ZnO that was biosynthesized was examined using XRD analysis and UV–Vis spectroscopy. In comparison to the control, which did not include the addition of nanoparticles, it was determined that the addition of ZnO nanomaterials increased the concentration of bioethanol. The zinc oxide nanoparticles enhance the activity of microbes involved in the fermentation of sugars from sugar cane bagasse into bioethanol. Thus, researchers are interested in the applications of nanomaterials in the generation of biofuel from sustainable and renewable feedstocks.

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References and Sources

Aarti, C., Khusro, A., Agastian, P.(2021). Lignocellulosic biomass as potent feedstock resource for bioethanol production: Recent updates. World News Nat. Sci., 37,164-181.

Adegboye, M. F., Ojuederie, O. B., Talia, P. M., Babalola, O. O. (2021). Bioprospecting of microbial strains for biofuel production: metabolic engineering, applications, and challenges. Biotechnology for Biofuels, 14, 1-21.

Agayeva, N.l., Rzayev, F.H., Gasimov, E.K., Mamedov, C.A., Anmadov, S., Sadigova, N.A., Khusro, A., At-Dhabi, N.A., Arasu, M.V. (2020). Exposure of rainbow trout (Oncorhynchus mykiss) to magnetite ($Fe_3 0_4$) nanoparticles in simplified food chain: Study on ultrastructural characterization. Saudi. Biol. Sci., 27, 3258-3266.

Al-Dhabi, N.A., Ghilan, A.K.M., Smyth, G. A., Arasu, M.V., Duraipandiyan, V., Ponmurugan, K. (2019). Environmentally friendly synthesis of silver nanomaterials from the promising Streptomyces parvus strain Al-Dhabi-91 recovered from the Saudi Arabian marine regions for antimicrobial and antioxidant properties. Journal of Photochemistry and Photobiology B: Biology, 197, 111529.

Antunes, F., Chandel A.K., Brumano L.P., Terán Hilares R., Peres G.F.D., Ayabe L.E.S., Sorato V.S., Santos J.R., Santos J.C., Da Silva S.S. (2018). A novel process intensification strategy for second-generation ethanol production from sugarcane bagasse in a fluidized bed reactor. Renewable Energy, 124, 189-196.

Anwar, Z., Gulfraz, M., Irshad, M. (2014). Agro-industrial lignocellulosic biomass a key to unlock the future bio-energy: a brief review. Journal of Radiation Research and Applied Sciences, 7, 163–173.

Baskar, G., Kumar, R.N., Metvin, X.H., Aiswarya, R., Soumya, S. (2016). Sesbania aculeate biomass hydrolysis using magnetic nano bio composite of cellulase for bioethanol production. Renew. Energy, 98, 23-28.

Canilha, L., Carvalho, W., de Almeida Felipe, M. d. (2010). Ethanol production from sugarcane bagasse hydrolysate using Pichia stipitis. Applied Biochemistry and Biotechnology, 161, 84-92**.**

Chen, F., Dixon, R. A. (2007). Lignin modification improves fermentable sugar yields for biofuel production. Nature Biotechnology, 25, 759-761.

Cherian, E., Dharmendirakumar, M., Baskar, G. (2015). Immobilization of cellulase onto Mn02 nanoparticles for bioethanol production by enhanced hydrolysis of agricultural waste. Chinese I. Catal., 36,1223-1229.

Choong, Y. Y., Norli, I., Abdullah, A. Z., Yhaya, M. F. (2016). Impacts of trace element supplementation on the performance of anaerobic digestion process: a critical review. Bioresource Technology, 209, 369-379.

Clarke, W. P., Radnidge, P., Lai, T. E., Jensen, P. D., Hardin, M. T. (2008). Digestion of waste bananas to generate energy in Australia. Waste Management, 28, 527–533.

Das, D., Nath, B. C., Phukon, P., Kalita, A., Dolui, S. K. (2013). Synthesis of ZnO nanoparticles and evaluation of antioxidant and cytotoxic activity. Colloids and Surfaces B: Biointerfaces, 111, 556–560.

Dekker, R., Wallis, A. (1983). Enzymic saccharification of sugarcane bagasse pretreated by autohydrolysis-steam explosion. Biotechnology and Bioengineering, 25, 3027-3048**.**

Devarapalli, M., Atiyeh, H. K. (2015). A review of conversion processes for bioethanol production with a focus on syngas fermentation. Biofuel Research Journal, 2, 268–280.

Diallo, A., Ngom, B. D., Park, E., Maaza, M. (2015). Green synthesis of ZnO nanoparticles by Aspalathus linearis: structural & optical properties. Journal of Alloys and Compounds, 646, 425–430.

Dionísio, S. R., Santoro, D. C. J., Bonan, C. I. D. G., Soares, L. B., Biazi, L. E., Rabelo, S. C., Lenczak, J. L. (2021). Second-generation ethanol process for integral use of hemicellulosic and cellulosic hydrolysates from diluted sulfuric acid pretreatment of sugarcane bagasse. Fuel, 304, 121290**.**

Dizhbite, T., Telysheva, G., Dobele, G., Arshanitsa, A., Kampars, V. (2011). Py-GC/MS for characterization of non-hydrolyzed residues from bioethanol production from softwood. Journal of Analytical and Applied Pyrolysis, 90, 126-132.

Dodo, C. M., Mamphweli, S., Okoh, O. (2017). Bioethanol production from lignocellulosic sugarcane leaves and tops. Journal of Energy in Southern Africa, 28 (3), 1–11.

Doran, I., Sen, B., Kaya, Z. (2005). The effects of compost prepared from waste material of banana on the growth, yield and quality properties of banana plants. Journal of Environmental Biology, 26, 7–12.

Essien, J. P., Akpan, E. J., Essien, E. P. (2005). Studies on mould growth and biomass production using waste banana peel. Bioresource Technology, 96, 1451–1456.

Faramarzi, S., Anzabi, Y., Fararizadeh-Malmiri, H. (2019). Selenium supplementation during fermentation with sugar beet molasses and saccharomyces cerevisiae to increase bioethanol production. Green Processing and Synthesis, 8, 622-628.

Food and Agriculture Organization. (n.d.). *Biofuels*.

Gámez, S., González-Cabriales, J. J., Ramírez, J. A., Garrote, G., Vázquez, M. (2006). Study of the hydrolysis of sugar cane bagasse using phosphoric acid. Journal of Food Engineering, 74, 78-88**.**

Gupta, K., Chundawat, T. S. (2019). Role of enzymes in synthesis of biologically important organic scaffolds. Asian Journal of Chemistry, 31, 2698–2706.

Han, H., Cui, M., Wei, L., Yang, H., Shen, J. (2011). Enhancement effect of hematite nanoparticles on fermentative hydrogen production. Bioresource Technology, 102, 7903-7909.

Happi Emaga, T., Ronkart, S. N., Robert, C., Wathelet, B., Paquot, M. (2008). Characterisation of pectins extracted from banana peels (Musa AAA) under different conditions using an experimental design. Food Chemistry, 108, 463–471.

Haq, F., Ali, H., Shuaib, M., Badshah, M., Hassan, S. W., Munis, M. F. H., Chaudhary, H. J. (2016). Recent progress in bioethanol production from lignocellulosic materials: a review. International Journal of Green Energy, 13, 1413–1441.

Hou, J., Ding, C., Qiu, Z., Zhang, Q., Xiang, W. N. (2017). Inhibition efficiency evaluation of lignocellulose-derived compounds for bioethanol production. Journal of Clean Production, 165, 1107-1114.

Huang, xu. z. (2014). Pretreatment methods for bioethanol production. Applied Biochemistry and Biotechnology, 174, 43-62**.**

Imade, E. E., Ajiboye, T. O., Fadiji, A. E., Onwudiwe, D. C., Babalola, O. O. (2022). Green synthesis of zinc oxide nanoparticles using plantain peel extracts and the evaluation of their antibacterial activity. Scientific African, 16, e01152.

International Energy Agency. (2021). *World Energy Outlook 2021*. Paris.

Kadla, J. F., Gilbert, R. D. (2000). Cellulose structure: a review. Cellulose Chemistry and Technology, 34, 197-216.

Karp, S.G., Woiciechowski, A.L., Soccol, V.T., Soccol, R. (2013). Pretreatment strategies for delignification of sugarcane bagasse: A review. 56, 679–689.

Kim, Y. K., Park, S. E., Lee, H., Yun, J. Y. (2014). Enhancement of bioethanol production in syngas fermentation with Clostridium ljungdahlii using nanoparticles. Bioresource Technology, 159, 446-450.

Kim, Y.K., Lee, H. (2016). Use of magnetic nanoparticles to enhance bioethanol production syngas fermentation. Bioresour Technol, 204, 139-144.

Lallo da Silva, B., Caetano, B. L., Chiari-Andréo, B. G., Pietro, R. C. L. R., Chiavacci, L. A. (2019). Increased antibacterial activity of ZnO nanoparticles: influence of size and surface modification. Colloids and Surfaces B: Biointerfaces, 177, 440–447.

Le Quéré, C., Andrew, R. M., Friedlingstein, P., Sitch, S., Pongratz, J., Manning, A. C., et al. (2018). Global Carbon Budget 2018 (pre-print). Earth System Science Data, 10, 2141-2194.

Lennartsson, P. R., Erlandsson, P., Taherzadeh, M. J. (2014). Integration of the first and second generation bioethanol processes and the importance of by-products. Bioresource Technology, 165, 3–8.

Lower, S. K., Hochella Jr, M. F., Beveridge, T. J. (2001). Bacterial recognition of mineral surfaces: nanoscale interactions between Shewanella and a-FeOOH. Science, 292, 1360-1363.

Mahmoodi, P., Karimi, K., Taherzadeh, M. J. (2018). Efficient conversion of municipal solid waste to biofuel by simultaneous dilute-acid hydrolysis of starch and pretreatment of lignocelluloses. Energy Conversion and Management, 166, 569-578.

Mandegari, M., Farzad, S., G¨orgens, J. F. (2018). A new insight into sugarcane biorefineries with fossil fuel co-combustion: techno-economic analysis and life cycle assessment.

Energy Conversion and Management, 165, 76–91.

Miller, G. L. (1959). Use of dinitrosalicylic acid reagent for determination of reducing sugar. Analytical Chemistry, 31, 426-428.

Mirzaei, H., Darroudi, M. (2017). Zinc oxide nanoparticles: biological synthesis and biomedical applications. Ceramics International, 43, 907–914.

Mooney, C. A., Mansfield, S. D., Touhy, M. G., Saddler, J. N. (1998). The effect of initial pore volume and lignin content on the enzymatic hydrolysis of softwoods. Bioresource Technology, 64, 113-119.

Niphadkar, S., Bagade, P., Ahmed, S. (2018). Bioethanol production: insight into past, present and future perspectives. Biofuels, 9, 229–238.

Palareti, G., Legnani, C., Cosmi, B., Antonucci, E., Erba, N., Poli, D., Pengo, V. (2016). Comparison between different D-Dimer cutoff values to assess the individual risk of recurrent venous thromboembolism: Analysis of results obtained in the DULCIS study. International Journal of Laboratory Hematology, 38, 42-49.

Pasangulapati, V., Ramachandriya, K. D., Kumar, A., Wilkins, M. R., Jones, C. L., Huhnke, R. L. (2012). Effects of cellulose, hemicellulose and lignin on thermochemical conversion characteristics of the selected biomass. Bioresource Technology, 114, 663–669.

Pramanik, S. K., Mahmud, S., Paul, G. K., Jabin, T., Naher, K., Uddin, M. S., Zaman, S., Abu Saleh, M. (2021). Fermentation optimization of cellulase production from sugarcane bagasse by Bacillus pseudomycoides and molecular modeling study of cellulase. Current Research in Microbial Sciences, 2, 100013**.**

Rabelo, S. C., Amezquita Fonseca, N. A., Andrade, R. R., Maciel Filho, R., Costa, A. C. (2011). Ethanol production from enzymatic hydrolysis of sugarcane bagasse pretreated with lime and alkaline hydrogen peroxide. Biomass and Bioenergy, 35, 2600-2607**.**

Rebros, M., Rosenberg, M., Stloukal, R., & Kristofíková, L. (2005). High efficiency ethanol fermentation by entrapment of Zymomonas mobilis into LentiKats. Letters in applied microbiology, 41, 412–416.

Renewable Fuels Association. (n.d.). Global ethanol production by country or region.

Rodrigues, J. A. R. (2011). From the mill to a biorefinery: the sugar factory as an industrial enterprise for the generation of biochemicals and biofuels. Química Nova, 34, 1242–1254.

Rodríguez-Chong, A., Ramírez, J. A., Garrote, G., Vázquez, M. I. (2004). Hydrolysis of sugar cane bagasse using nitric acid: a kinetic assessment. Journal of Food Engineering, 61, 143-152**.**

Şahin, Ö., Baytar, O., Kutluay, S., Ekinci, A. (2024). Potential of nickel oxide catalyst from banana peel extract via green synthesis method in both photocatalytic reduction of methylene blue and generation of hydrogen from sodium borohydride hydrolysis. Journal of Photochemistry and Photobiology A: Chemistry, 448, 115301.

Sanusi, A. I., Suinyuy, T. N., Lateef, A., Gueguim Kana, E. A. (2020). Effect of nickel oxide nanoparticles on bioethanol production: process optimization, kinetic and metabolic studies. Process Biochemistry, 92, 386-400.

Sanusi, I.A., Suinyuy, T.N., Jateef, A., Kana, G.E.B. (2020). Effect of nickel oxide nanoparticles on bioethanol prodction: Process optimization, kinetic and metabolic studies. process Biochem, 92, 386-400.

Singh, A., Sharma, P., Saran, A. K., Singh, N., Bishnoi, N. R. (2013). Comparative study on ethanol production from pretreated sugarcane bagasse using immobilized Saccharomyces cerevisiae on various matrices. Renewable Energy, 50, 488-493.

Singh, J., Kumar, S., Alok, A., Upadhyay, S.K., Rawat, M., Tsang, D.C.W., Bolan, N., Kim, K.H. (2019). The potential of green synthesized zinc oxide nanoparticles as nutrient source for plant growth. Journal of Cleaner Production, 214, 1061-1070.

Singhvi, M., Kim, B.S. (2020). Current developments in lignocellulosic biomass conversion into biofuels using nanobiotechnology approach. Energies, 13, 5300.

Siqueira, G., Várnai, A., Ferraz, A., Milagres, A. M. F. (2013). Enhancement of cellulose hydrolysis in sugarcane bagasse by the selective removal of lignin with sodium chlorite. Applied Energy, 102, 339-402**.**

Stanković, A., Dimitrijević, S., Uskoković, D. (2013). Influence of size scale and morphology on antibacterial properties of ZnO powders hydrothermally synthesized using different surface stabilizing agents. Colloids and Surfaces B: Biointerfaces, 102, 21–28.

Talebian, N., Amininezhad, S. M., Doudi, M. (2013). Controllable synthesis of ZnO nanoparticles and their morphology-dependent antibacterial and optical properties. Journal of Photochemistry and Photobiology B: Biology, 120, 66–73.

Thakur, I.S., Ray, A.K., Singhal, A. (2015). Ethanol from sugar cane bagasse of pulp and paper mill effluent by Cryptococcus albidus and Saccharomyces cerevisiae. Energy Sources, Part A Recovery, Util. Environ. Eff., 37, 1172–1179.

Thema, F.T., Beukes, P., Gurib-Fakim, A., Maaza, M. (2015). Green synthesis of Monteponite CdO nanoparticles by Agathosma betulina natural extract. I. Alloys Compd, 646, 1043-1048.

Thovhogi, N., Hands, A., Gurib-Fakim, A., Maaza, M. (2015). Nanoparticles green synthesis by Hibiscus Sabdariffa flower extract: main physical properties. I. Alloys Compd, 647, 392-396.

Velmurugan, R., Muthukumar, K. (2011). Utilization of sugarcane bagasse for bioethanol production: sono-assisted acid hydrolysis approach. Bioresource Technology, 102, 7119-7123**.**

Verma, A., Stellacci, F. (2010). Effect of surface properties on nanoparticle-cell interactions. Small, 6, 2-21.

Vijayalakshmi, G., Govindarajan, M., Al-Mulahim, N., Ahmed, Z., Mahboob, S. (2021). Cellulase immobilized magnetic nanoparticles for green energy production from *Allamanda schottii* L: Sustainability research in waste recycling. Saudi Journal of Biological Sciences, 28, 901-910.

Wang, M., Wang, J., Tan, J. X. (2011). Lignocellulosic bioethanol: status and prospects. Energy Sources, Part A: Recovery, Utilization, and Environmental Effects, 33, 612–619.

Williams, C. L., Dahiya, A., Porter, P. (2020). Chapter 1 - Introduction to bioenergy and waste to energy. Bioenergy (Second Edition). 5-44.

Yang, G., Wang, J. (2018). Improving mechanisms of biohydrogen production from grass using zero-valent iron nanoparticles. Bioresource Technology, 266, 413-420.

Yao, W., Wu, X., Zhu, J., Sun, B., Zhang, Y. Y., Miller, C. (2011). Bacterial cellulose membrane A new support carrier for yeast immobilization for ethanol fermentation. Process Biochemistry, 46, 2054-2058.

Zhang, Q., Zheng, Z., Liu, C., Liu, C., Tan, T. (2016). Biodiesel production using lipase immobilized on epoxychloropropane-modified Fe_3O_4 sub-microspheres. Colloids and Surfaces B: Biointerfaces, 140, 446-451.

Zhang, Y., Shen, J. (2007). Enhancement effect of gold nanoparticles on biohydrogen production from artificial wastewater. International Journal of Hydrogen Energy, 32, 17-23.