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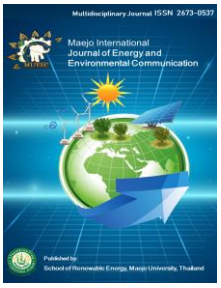

ARTICLE

Influence of nanoparticles inclusion on the production of bioethanol from corn stalks and leaves

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ABSTRACT

Bioethanol is a viable alternative to petroleum-derived fossil fuels. It is renewable, low-cost, and the preferred fuel for most developing countries worldwide. Although it is possible to make bioethanol from corn stalks and leaves wastes, second-generation biofuels made from agricultural waste feedstocks represent a significant step forward. In the present research, nickel oxide (NiO) nanoparticles (NPs) were used as a biocatalyst to achieve maximum ethanol output. The pretreatment of 2% NaOH with NiO NPs, 15-min autoclave condition, showed the highest total and reducing sugar yield was 162.69 g/L and 43.75 g/L. After hydrolysis, the suitable total and reducing sugar yield of 185.43 g/L and 125.42 g/L was chosen for further fermentation with the expansion of *Saccharomyces cerevisiae* cells. Separate hydrolysis and fermentation (SHF) using corn stalk and leaf waste was significantly assisted by incorporating a nanocatalyst; ethanol concentration was increased to 15.8 g/L at 24 hours incubation period. The study revealed critical information regarding ways NiO NPs could be employed to improve the efficiency of the ethanol production bioprocess.

1. Introduction

Due to the impending depletion of fossil fuels, numerous scientists are attempting to generate energy from lignocellulosic biomass, the most abundant renewable source of biofuels on Earth (Wannapokin et al., 2018). Biomass is any biological material that comes from living or recently living organisms (Pimpimol et al., 2020). This includes virgin wood, energy crops, agricultural residues, food waste, and industrial and co-products. Biomass energy from wood and/or energy crops is controversial because it takes up land and freshwater that could be used for food crops (Junluthin et al., 2021; Kumaran et al., 2022). In addition, because of the fertilizers and pesticides used to grow these crops on a large scale, could also add to pollution problems. As an alternative, you could use crop residues that can be used to make both food and energy (Wannapokin et al., 2017; Chutturi et al., 2022).

Several technologies can be used to turn crop waste into liquid or gaseous biofuels or electricity that can be used (Sangkharak et al., 2020). Thermochemical processes like direct combustion, pyrolysis, and gasification are used to make heat, steam, electricity, and biofuels. Fermentation and anaerobic digestion are two biological processes that produce alcohols, methane, hydrogen, and even biodiesel (Van Tran et al., 2020). Some of these technologies are far enough along that they can be used right away. Biofuels like ethanol, hydrogen, methane, and diesel are needed to replace fossil fuels and reduce their negative effects on the environment. Bioethanol is a valuable renewable fuel that has many benefits. It is used extensively in the automotive industry due to its environmental and financial benefits (Menon et al., 2010). Bioethanol has a broad range of flammability, high evaporation energy, and high-octane numbers, all considered favourable characteristics.

Bioethanol can be used with crude oil-derived hydrocarbon fuels because it has these characteristics (Khammee et al., 2021). Today, most of the world's bioethanol comes from food crops like sugarcane and rice. The fermentation process results in the production of ethanol by combining feedstocks with the inoculum (yeast, bacteria) during the fermentation. This is why ethanol made

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from food crops is known as first-generation bioethanol. However, consuming edible crops can pose a threat to food availability (Mussatto et al., 2012). Scientists were encouraged to look for alternative feedstocks because of this dilemma. Second-generation bioethanol, which is made from non-edible materials such as molasses and sawdust, grasses or corn straw, rice straw, and other biomasses (molasses), can be used. Third-generation bioethanol (algal biomass), was used to accelerate the production of bioethanol from inedible materials. This was done to accelerate the process of making bioethanol from inedible material (Ramaraj & Unpaprom, 2019).

Because they are inexpensive and easily available, lignocellulosic material is a good substrate for large quantities of bioethanol production. An estimated 200 billion tons of lignocellulosic biomass will be produced each year. The four main phases of creating bioethanol from biomass include biomass pretreatment, Catalyst recovery, enzymatic hydrolysis, fermentation, and ethanol synthesizer (Nguyen et al., 2020). Due to several constraints, it is currently not possible to use lignocellulosic bioethanol feedstocks on a commercial scale. First, lignocellulosic bioethanol has a complex structure. It consists of lignin, hemicellulose, cellulose, and other insignificant components. To extract hemicellulose or cellulose from feedstocks, it is necessary first to deconstruct the structural components of the feedstock molecules (Manmai et al., 2021). During the pre-treatment, *Saccharomyces cerevisiae* creates several inhibitors. These include compounds of phenolic, furans, carboxylic, and furan acids. Plant-based materials may also contain contaminants that can cause *Saccharomyces cerevisiae* to stop its metabolism and reduce bioethanol production.

Nanoparticles are gaining increasing interest among researchers due to their exquisite properties, which enable them to be applied in lignocellulosic pre-treatment (Abdelsalam et al., 2016). They are also being explored in biofuels in order to improve the performance of these bioprocesses. Furthermore, it elucidates the different types of nanomaterials (metallic, nanofibers, and nanotubes) that have been used in these bioprocesses. It also evaluates the effects of nanoparticles on bioethanol and the ability of nanoparticles to suppress inhibitory compounds under certain conditions effectively. As a result, in this work, nanoparticles are introduced to the production of bioethanol fermentation from maize stalks and leaves, and the parameters that influence nanoparticle performance on bioethanol production processes are explored.

2. Material and Methods

2.1 Feedstock preparation

Corn samples were collected from the Mae Tange farming area in Chiang Mai, Thailand. Samples were transported within two hours to the Energy Research Center at Maejo University in Sansai, Chiang Mai, Thailand, 50290, to be identified and investigated. The sample was also chosen depending on the research plan. The plant samples were also dried and stored to be disassembled and processed further. The samples are dried using the portable solar drier. The monomeric sugars were estimated using the National Renewable Energy Laboratory (NREL) methods (Ruiz & Date, 1996). Overall experimental processes were highlighted by the schematic diagram (Figure 1).

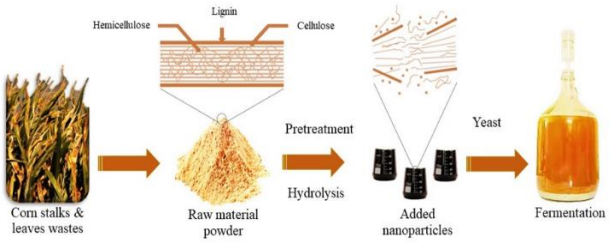

Figure 1 Overall experimental processes bioethanol production

2.2 NiO nanoparticle synthesis

Abdelsalam et al. (2016) NiO nanoparticle synthesis procedure was adopted for this study. NiO nanoparticles were created by dissolving 4.75 g of nickel chloride hexahydrate ($NiCl_2.6H_2O$) in distilled water (20 mL), then adding ammonia dropwise to achieve a pH of 10. To finish the reaction, the mixture was microwaved at 700 W for 3 minutes. The resulting NiO NPs precipitate was carefully washed and oven dried for 6 hours at 100°C.

2.3 Yeast culture preparation

The yeast strain used in this study (*Saccharomyces cerevisiae*) was obtained from the Program in Biotechnology, Faculty of Science, Maejo University, Chiang Mai. The most common rich medium, called yeast extract peptone dextrose (YEPD) was utilized. To prepare the inoculum, the yeast was inoculated into a 250 mL Erlenmeyer conical flask containing 100 mL of YEPD medium. A rotary shaker was used to incubate the yeast at 30°C and 120 revolutions per minute for a period of 12 hours, which was long enough to achieve the exponential growth phase. After that, the culture was utilized as the inoculum for the experiments on the production of bioethanol.

2.4 Pre-treatment and hydrolysis process

During the process of this work, a nanoparticle-assisted autoclave pre-treatment was created. The sample was processed in an autoclave designed explicitly for use in laboratories. First, the pulverized corn stalks and leaves waste substrate was added alkaline solution and 100 mL of nanoparticle solution at a solid loading, and then the mixture was autoclaved at a specific temperature and for a certain amount of time. Next, different sodium hydroxide concentrations (0, 2, 4% NaOH) with NiO NPs and autoclave times (0, 15, 30 min) were applied. After that, sample aliquots of two mL were taken out for the decreasing sugar analysis.

The effect of pre-treatment on enzyme hydrolysis was investigated; the pre-treated pulverized corn stalks and leaves waste substrate was subjected to enzyme hydrolysis using commercial cellulase enzyme with a 2 %(v/v) enzyme loading. In a hydrolysis flask, 3 g of hydrotrope pre-treated substrate was suspended in 1 M sodium citrate buffer (pH 4.5). The samples were incubated in a shaking water bath at room temperature for 24 hours. Total and reducing sugar analyses were performed on the hydrolysate (Dubois et al., 1956; Miller, 1972; Vu et al., 2017).

2.5 Fermentation process and ethanol estimation

Fermentation was carried out in a hermetically sealed 1L flash containing feedstock solution and yeast. The fermentation set-up is presented in Figure 2. All cultures were purged with nitrogen gas to eliminate the oxygen. Later, cultures were shaken at 120 rpm in an orbital shaker. All cultures were evaluated in dark fermentation at 24, 48, 72, and 96 hours. The ethanol determination procedure was adopted by Vu et al. (2018).

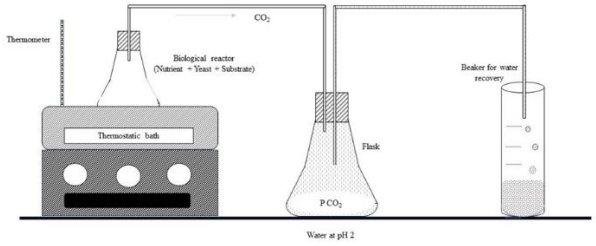

Figure 2 Schematic diagram of fermentation process on bioethanol from corn stalks and leaves

3. Results and discussion

3.1 Feedstock characteristics

First, agricultural wastes are composed of four components that differ in their average composition: cellulose, extractives, hemicellulose and lignin (Mejica et al., 2022). It is important to accurately characterize the beginning biomass to determine the products' yields and characteristics (Ramaraj et al., 2021). Corn is the most widely grown crop in the world (*Zea mays*).

It was the most popular for the last decade. The most common agricultural waste in Thailand is corn stalks and leaves. Corn stover, a morphologically diverse biomaterial, includes cobs, stalks, husks, leaves and stalks. Technological solutions enable the separation of corn stover fractions based on harvest term, tissular, and chemical composition. It is important to analyse elements, as lignin (and cellulose) significantly impact biomaterial composition.

An in-depth characterization of the lignocellulose material and elemental content analyses (including microelements and macroelements) helps predict maize stover fractions. The chemical composition, proximate and ultimate analyses of the corn stalks and leaves utilized in bioethanol production are detailed in the reports presented in Table 1. In addition, the levels of carbon, hydrogen, nitrogen, sulfur, ash, and oxygen that were discovered on air-dried stalks after they had been dried with a portable solar dryer are also reported in the same table. This also identifies the best time to harvest the crop for bioethanol fermentation.

3.2 Pretreatment and hydrolysis

The need for pretreatment and fractionation of biomass feedstocks to obtain adequately pure fermentable carbohydrates is one of the primary challenges in lignocellulosic ethanol production (Manmai et al., 2019). When compared to dried biomass, the primary by-product of corn biomass ethanol, the value of the by-products (hemicellulose and lignin fraction) is low (Manmai et al., 2020). This research looked into the feasibility of fermentative bioethanol production from lignocellulosic biomass treated with a combination of additional nanoparticles, alkali, and heat (autoclave). Corn leaves and stalk powder were pre-treated in NaOH solutions of 0, 2, and 4 % for periods of 0, 15, and 30 min before fermentation. The maximum total sugar yield (162.69 g/L) and decreasing sugar yield (43.75% g/L) were achieved with pre-treatment of 2% NaOH with NiO NPs under the 15-minute autoclave condition (Figure 3).

The fibre structure of any biomass is opened up and partially dissolved during the pre-treatment process (Whangchai et al. 2021). After this step, in most instances, a liquid reject is removed, and the residual solids are transported to the hydrolysis step. It should be noted that there is a significant amount of variation within each technological configuration. This finding leads one to hypothesize that the majority of the glucan and xylan can be preserved in the solid fraction and then transferred to the hydrolysis step if one pays adequate attention (Gupta et al., 2009). After the glucan and xylan have been converted into dissolved glucose and xylose, respectively, by the hydrolysis process, the liquid fraction is passed to the fermentation step. In the earlier, enzymes were frequently employed if the hydrolysis process was one that required assistance (Bukhari et al., 2017). In all of the experiments, cellulase was utilized either as a standalone enzyme or as part of an enzyme cocktail, and it was sometimes combined with glucosidase or xylanase as a supplement. In this study, 2% of cellulase was utilized. Both total and reducing sugar yields of 185.43 g/L and 125.42 g/L, respectively, are satisfactory following hydrolysis (Figure 3).

Table 1 Chemical composition, proximate and ultimate analyses of corn stalks and leaves

Parameters	Dried in a solar dryer
Chemical composition g.(g dry substrate)$^{-1}$	
Glucan	0.349
Galactan	0.031
Arabinan	0.134
Mannan	0.072
Limin	0.960
Xylan	0.185
Proximate analysis (%)	
Moisture	2.11
Ash	4.37
Volatile	75.78
Fixed carbon	19.85
Total	100
Ultimate analysis (%)	
Carbon	51.13
Hydrogen	5.16
Nitrogen	0.89
Sulfur	0.11
Oxygen	42.71

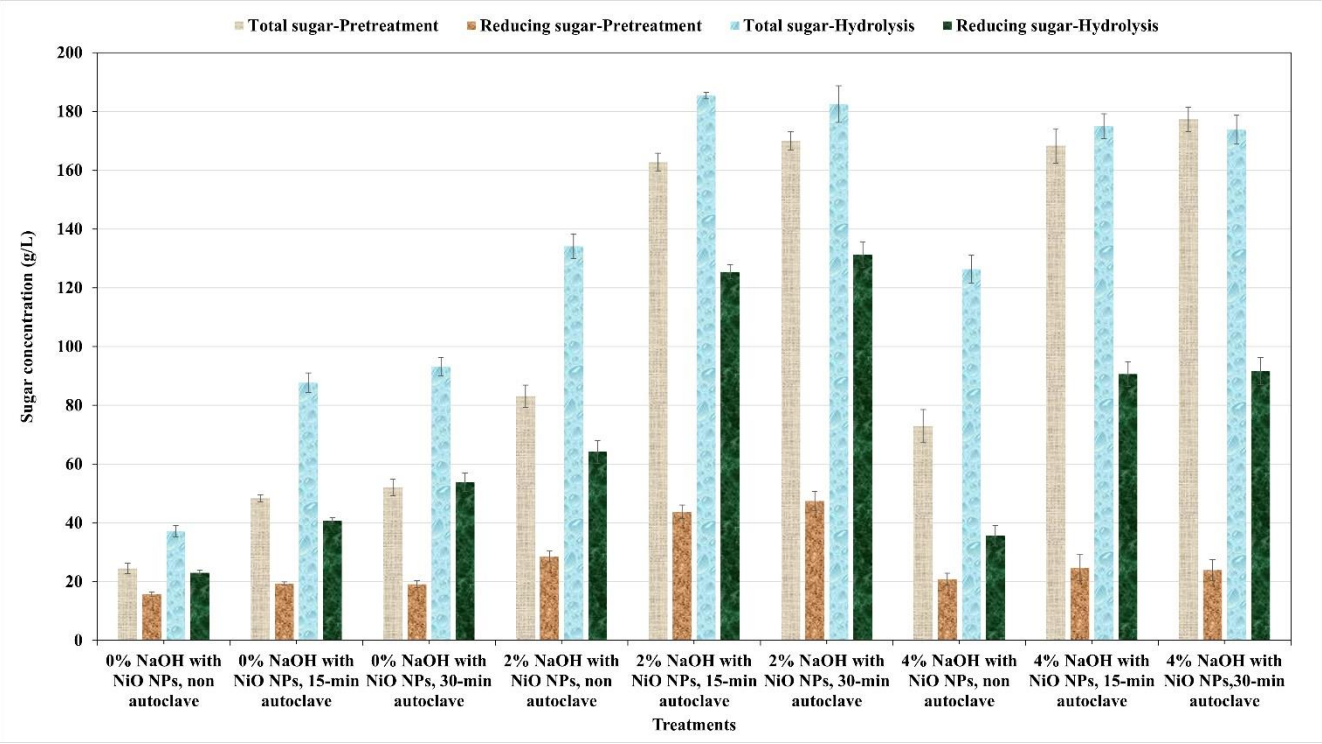

Figure 3 Pre-treatment and hydrolysis of corn stalks and leaves

By measuring bioethanol yield in fermented broth every 24 hours for 72 hours, the optimal pretreatment condition for efficient corn stalks and leaves substrate was identified. The presence of solid material in the slurry used in the fermentation that came before it probably made it difficult for the organisms to move about, reduced the amount of dissolved oxygen, and prevented them from accessing nutrients. This experiment was able to record substantially higher levels of bioethanol production as a result of the filtrate media's improved rheological parameters.

The experiment's findings, which were depicted, indicate that an increase in ethanol yield of 15.8 g/L in a fermentation period of 24 hours had the effect illustrated in the Figure 4. Choosing the best pretreatment and hydrolysis procedures for producing bioethanol could reduce production costs and the amount of time needed for fermentation. Additionally, it has been linked to an increase in yeast's capacity for consuming sugar as well as a higher level of product production (Asachi & Karimi, 2013). Compared to some earlier research on the production of bioethanol utilizing a variety of lignocellulosic materials, the largest amount of bioethanol achieved in our work under optimal conditions performed quite favorably (Table 2).

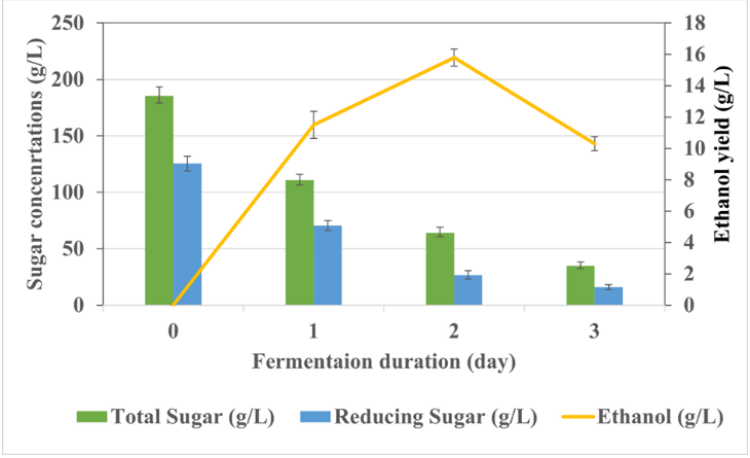

Figure 4 Bioethanol production from corn stalks and leaves

Table 2 Chemical composition, proximate and ultimate analyses of corn stalks and leaves

Lignocellulosic	Pretreatment	Organism	Ethanol (g/L)	Reference
Sugarcane bagasse	NaOH	*S. cerevisiae*	14.00	Bhadana & Chauhan, 2016
Oil palm bunch hydrolysate	NaOH + H_2SO_4	*S. cerevisiae*	8.49	Duangwang & Sangwichien, 2015
Cassava pulp	H_2SO_4 + Ca $(OH)_2$	*S. cerevisiae*	11.90	Akaracharanya et al. 2011
Sago pith residue hydrolysate	Autoclave	*S. cerevisiae*	14.3	Vincent et al. 2015
Corn stalks and leaves	NaOH with NiO NPs, Autoclave	*S. cerevisiae*	15.8	This study

4. Conclusion

This research found that utilizing NiO NPs as a biocatalyst led to an increase in both biomass concentration and ethanol output. As part of a report on the nanocatalyst process condition, the substrate was used to detail *S. cerevisiae* growth and ethanol generation. In addition, S. cerevisiae consumed more fermentable sugar when cultured in the presence of nanocatalysts. Findings from this research have significant implications for using NiO NPs to mitigate inhibitors throughout the fermentation process and maximize product yield. In addition, using NiO NPs increased bioethanol production from corn stalks and leaves debris, demonstrating the potential of employing nanoparticles to promote biofuel production from starch-based agricultural leftovers. This study looks at agricultural waste and the processes used to remove the lignin from them. It also looks at the enzymatic hydrolysis and fermentation processes used to turn simple sugar into bioethanol. Also, it has been confirmed that agro-waste biomasses could be used to make more bioethanol.

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