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Bioethanol Production from Potato Peels Using *Saccharomyces cerevisiae* Treated with ZnO and ZnO/g-C_3N_4 Nanomaterials

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Abstract

Bioethanol is a promising biofuel produced from agricultural wastes. The problem is that the bioconversion of cellulose to bioethanol takes a long time for excellent results. Predominantly, efficient enzymes and active microorganisms (yeast) can enhance the enzymatic saccharification and fermentation bioprocesses, respectively. The addition of nutrients and electron acceptors in form of nanomaterials was found to modify the bioenvironment and to biostimulate the microorganisms to accomplish the target bioprocesses efficiently. The objective of this investigation was to increase bioethanol production from agricultural wastes using nanomaterials. In this study, the bioethanol production from potato peels (as an example of agricultural wastes) was increased using ZnO nanomaterials and ZnO/g-C_3N_4 nanomaterials with the concentration of 5, 10, 15, 50, 100, and 150 mg/L each as well as the control (without the addition of nanomaterials). It was hypothesized that yeast treatment with nanomaterials (nutrients) leads to biostimulate yeast cells and increases cell activity. Consequently, it is hypothesized that these procedures increase bioethanol production from potato peels over a shorter Hydraulic Retention Time (HRT), i.e., residence time. It was found that the biostimulation of the fungi (yeast) *Saccharomyces cerevisiae* using 150 mg/L of ZnO/g-C_3N_4 nanomaterials generated the highest bioethanol concentration of 33.2% compared to all other treatments.

Keywords: Bioethanol; Fungi; Agricultural wastes; Nanotechnology; Saccharomyces cerevisiae. .

1. Introduction

The US National Nanotechnology Initiative defined "Nanotechnology" as the study, manipulation, and use of materials between 1 and 100 nanometers in particle size called "Nanoparticles", where 1 nanometer (nm) is equal to 10^{-9} meter. Nanotechnology as defined by size is very broad, including fields of science as diverse as surface science, biochemistry, microbiology, bioengineering, biomedical engineering, optics, electronics, semiconductor physics, microfabrication, etc. The main features of implementing nanotechnology are that the physical, chemical, electrical, thermal, and further engineering properties of the synthesized nanoparticles differ intrinsically from the original material properties (Saini et al., 2010; Petcharoen and Sirivat, 2012; Kumar et al., 2013; Attia and Mohamed, 2022).

Agricultural wastes (e.g., rice straw, wheat straw, corn stover, sugarcane bagasse), agro-industrial wastes (e.g., potato peel waste, citrus pulp) and water hyacinth (*Eichhornia* sp.) are causing severe environmental problems. The processes of pretreatment, saccharification and fermentation are the major obstacles in bioethanol production from wastes and must be overcome by efficient novel technologies. With reference to bioconversion technology the hindrances are biomass processing, precisely enzymatic saccharification and fermentation bioprocesses. Regarding the enzymatic saccharification bioprocess, the challenge is to accomplish a competent bioprocess for depolymerization of cellulose and hemicelluloses to produce fermentable monomers with high concentration. Afterwards, metabolizing the monomers by yeast to generate bioethanol and energy

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for themselves which is subjected to the yeast cell activity and efficiency (Gupta et al., 2015; Gupta and Verma, 2015; Nasidi et al., 2015). These bioprocesses have several challenges and limitations such as biomass handling and efficient pretreatment process for removing the lignin from the lignocellulosic agro-residues. Predominantly, efficient enzymes and active microorganisms (yeast) will enhance the enzymatic saccharification and fermentation bioprocesses, respectively. Bioconversion of cellulose to bioethanol requires some new pretreatment, enzymatic saccharification and fermentation technologies, to make the whole process effective (Gupta et al., 2015). Due to the complex crystallinity of cellulose structure, cellulose enzyme is required to convert cellulose chain into glucose; however, it takes long time for good results (Gupta and Verma, 2015).

Nanomaterials are hypothesized to biostimulate the yeast cell and to increase cell activity. Consequently, nanomaterials are hypothesized to enhance the bioconversion process of agricultural biowastes to bioethanol and to accelerate the saccharification and fermentation processes, which increase the bioconversion efficiency and, therefore, the bioethanol production. Additionally, it is hypothesized that the addition of trace metals (yeast nutrients) in form of nanomaterialswill reduce not only the Hydraulic Retention Time (HRT), i.e., residence time, but also the time to achieve the highest bioethanol percentage and yield. This is since the cell uptake rate of nutrients is inversely proportional with the nutrient particle size. In other words, the reduced size of nutrient particle to nanoparticle is easily up taken by the cell with the highest uptake rate.

The main objective of this study is to increase bioethanol production from agricultural biowastes using nanotechnology.

This general objective can be further elaborated in terms of the following specific objective: Biostimulating the yeast cells using nanomaterialsfor improving the bioconversion process of agricultural biowastes to bioethanol and, therefore, increasing bioethanol production.

Based on our previous paper (Saeed et al., 2022), different concentrations of ZnO and ZnO/g-C_3N_4 nanomaterials (10, 15, and 50 mg/l) were tested to optimize the bioethanol production of the *Saccharomyces cerevisiae* and consequently the bioconversion process of potato peels, as an example of agricultural biowastes, using the minimum amount of nanomaterials.

2. Material and methods

All chemicals were obtained from Sigma-Aldrich and used without further purification. Images from scanning electron microscopy (SEM) were taken using a Philips CM20 microscope with an accelerating voltage of 200 kV, using ZEISS FE-SEM ULTRA Plus (equipped with an EDX analyser). Measurements of X-ray diffraction (XRD) with Philips PW1710 X-ray diffractometer were carried out with Cu Ka radiation (λ = 1.5418 Å). XRD patterns were recorded at 0.020° 2H and collection 10 s/step from 20° to 80° 2H. On the PerkinElmer FT-IR spectrometer, the spectra were recorded as a thin film.

2.1. Experimental Setup

The experimental setup that was implemented in this study can be elaborated as follows: designing a bioreactors system, specifying the suitable enzymes and yeast strain, identifying the appropriate nanomaterials, and selecting the agricultural biowastes.

2.1.1. Design of Bioreactors

The proposed bioreactor design (Fig. 1) was based on the bioconversion processes of biomass to bioethanol, the design parameters and lab-scale models of bioreactors described in the literature (Badwal et al., 2015; Cotana et al., 2014; Hasunuma and Kondo, 2012; Kang et al., 2015; Saha et al., 2015; Saha et al., 2013; Singhania et al., 2014; Tormin et al., 2015; Wang et al., 2014). The bioreactors were manufactured and implemented in this study.

The design parameters can be specified as follows:

1. Bioreactor volume: 5.54 L
2. Substrate volume: 5 L
3. Stirrer speed: 150 rpm
4. Enzymatic saccharification temperature: 40-50ºC
5. Fermentation temperature: 38ºC
6. Hydraulic Retention Time (HRT), i.e., residence time: 5 days
7. Bioreactor’s mode of operation: batch flow

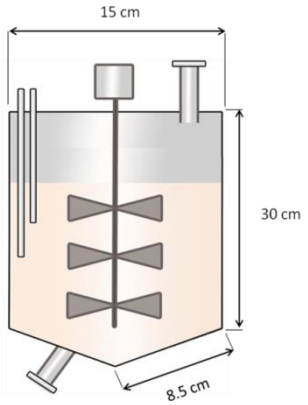

Figure. 1: The bioreactor design.

2.1.2. Yeast and Enzymes

In this study, the yeast strain *Saccharomyces cerevisiae* (Thermosacc® Dry, Lallemand, WI, USA) was implemented in a dry and active form as described by Ali (2015) and Vancov et al. (2015). The yeast inoculum was prepared as described by Ali (2015), where it was grown aseptically in 500 mL Erlenmeyer flasks containing 250 mL of cultivation medium which was used for bioreactor runs and contain (g/L):2g $(NH_4)_2SO_4$; 5g KH_2PO_4; 1g $MgSO_4.7H_2O$ and 1g yeast extract; 0.5 g bacto-peptone; and 0.05 M sodium citrate buffer at a pH of 4.8±0.2. A loopful of 24- and 7-days old culture of Saccharomyces cerevisiae was inoculated and incubated at 30°C on a rotary shaker at 200 rpm for 24 and 48 hr respectively. These inoculums were used to inoculate sterilized potatoes samples. This medium with a working volume of 100 mL was transferred into a 250 mL Erlenmeyer flask and sterilized by autoclaving at 121°C, 15 psi for 30 min. The flask was autoclaved at 121°C at 15 psi for 20 min and cooled at 30 °C. After this, Saccharomyces cerevisiae inoculums 5% (V/W) of were added. The process of fermentation was carried out on shaker incubator at temperatures 30°C for incubation period 48 h. After the fermentation period, the fermented mass was centrifuged at 12000 rpm for 15 min. and then supernatant was collected by filtration which was further used for distillation process. On the other hand, the following enzymes were used (Ali, 2015): Celluclast 1.5 L (cellulase enzyme) and Novozyme 188 (β-glucosidase enzyme), both manufactured by Novozymes, A/S, Bagsværd, Denmark. A commercial enzyme mixture was used for enzymatic saccharification throughout this study following the methodology described by Ali (2015).

2.1.3. Potato Peels

The potato peel wastes (PPW) were collected from Banha chips factories. They were dried at 50 ºC for 48 h, ground and sieved to get particles with particle size between 400 and 800 μm. It was stored at room temperature (25 ± 5°C) until use (Saeed et al., 2022). The scientific name of the used potato peel is (*Solanum tuberosum* L.). The Potato peel contains numerous polyphenols and phenolic acids such as gallic acid, protocatechuic acid, vanillic acid, caffeic acid, chlorogenic acid, p-hydroxybenzoic acid and p-coumaric acid. Potato peel contains starch (25%), non-starch polysaccharide (30%), protein (18%), acid-soluble and acid-insoluble ligenin (20%), lipids (1%) and ash (6%) on dry basis. The lipid fraction includes long-chain fatty acids, alcohols, triglycerides and sterol esters. In addition, lignin units have been found in the cell wall of potatoes. Potato peel is rich in starch (52% dry weight). Elemental analysis of potato peel shows that it contains (in % dry basis): C (43.78 ± 0.15), H (5.96 ± 0.12), N (4.06 ± 0.01) and O (46.21 ± 0.28). The C/N ratio of potato peel is 10.7 and its pH 6.5. The calorific value of potato peel is 17.37 ± 0.38 (MJ/kg).

2.2. Nanomaterial's preparation

Graphitic carbon nitride nanosheets (g-C_3N_4) were prepared and characterized using thermal polymerization (Saeed et al., 2022); Zhu et al. 2017; Ma et al. 2017; Liu et al. 2011).

ZnO nanoparticles were prepared via co-precipitation method (Mohamed and Attia, 2020; Attia and Abdel-Hafez, 2022) by adding 50 mL NaOH solution (4 M) into 50 mL of $ZnSO_4$ solution (0.2 M) at an approximate rate of 5 mL/min under vigorous stirring and the stirring was continued for 12 h. Then, the precipitate obtained was filtered and washed thoroughly with deionized water. The precipitate was dried in an oven at 100 ºC and ground to fine powder using agate mortar. The obtained powder was calcined at 500 ºC for 2 h.

ZnO/g-C_3N_4 nanocomposites were prepared by adding 0.5 g of g-C_3N_4 and 0.4 g of ZnO NPs in 40mL ethanol under sonication for 30 minutes. Then the solution stirred at 80 °C for 4 hours to get the fine powder.

2.3. *Determination of C_2H_5OH concentration*

This spectrophotometric technique was implemented for determination of C_2H_5OH concentration after nanomaterial treatment to the

PPW, where C_2H_5OH concentration was quantified by solvent extraction and dichromate oxidation reaction using modified protocol (Xue et al., 2013). This approach included solvent extraction of C_2H_5OH from broth accompanied by determination of the C_2H_5OHconcentration by the dichromate oxidation technique. Dibutyl phthalate (DBP) (1 mL) and aqueous sample (1 mL) were blended in a microtube and subsequently blended robustly for 1 minute. The blend was centrifuged at 3,420 xg for 5 min towards breaking up to 2 phases. Lower phase (DBP layer) was translucent, while upper phase was turbid. Subsequently, 500 µL of upper phase was moved to a microtube and blended with 500 µL of dichromate reagent (containing 2% w/v of $K_2Cr_2O_7$ in 1 N of H_2SO_4), and blended robustly for 1 min. The blend was left for 10 min at room temperature to permit oxidation resultant in lower phase to manifest its tint as blue green. An amount of 100 µLof the oxidation resultants were watered down using 900 µL of deionized H_2O. The density at 570 nm (A570) of analyzed specimen was quantified using UV-visible spectrophotometer. The C_2H_5OH concentration in the specimen was quantified using the C_2H_5OH benchmark curve showing the correlation between A570 and the C_2H_5OH concentrations to compute the C_2H_5OH percent of the hydrolysate. Our calculations were based on the calibration curve (Figure 2). Calibration curve for estimation the ethanol percent was obtained by plotting the absorbance (at 570 nm) vs. concentrations of standard ethanol. in the range of 10-90%. The concentrations of ethanol were daily prepared by dilution of the stock solution. The calibration equation of ethanol standard was determined to be y = 0.0077x+0.2659, (R2 = 0.9515) where y is the peak area of ethanol and x is the concentration of ethanol.

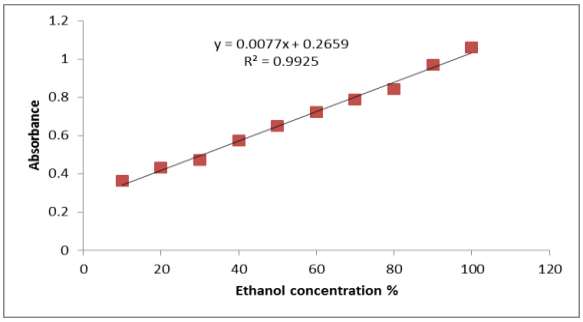

Figure 2: Calibration curve of standard Ethanol from 0% to 100%.

Results

ZnO nanoparticles were successfully synthesized by the co-precipitation method using zinc sulfate and sodium hydroxide as precursors. The TEM image of ZnO NPs is depicted in Figure 3a. It is observed that for ZnO NPs have a spherical structure with a mean diameter ranging from 35 to 44 nm. The chemical composition of ZnO NPs was analyzed by an energy dispersive spectrometer (EDS) spectrum (Fig. 3b). The result shows the peaks of O, and Zn with (28.42 wt%, and 72.58 wt%), respectively confirming that the sample is of high purity. The UV–Vis spectrum of the prepared ZnO NPs is shown in figure (3c). It's clear that ZnO NPs a semi-conductor with a wide bandgap (3.3eV) that makes it a suitable absorber of UV radiation and exhibit a sharp band at 375 nm. In figure 3d, it was shown that the XRD pattern of the as-prepared ZnO NPs. The diffraction peaks are quite similar to those of bulk ZnO, which can be indexed as the hexagonal wurtzite structure ZnO and diffraction data were in agreement with the JCPDS card for ZnO (JCPDS 36-1451). The FTIR spectrum of ZnO NPs was recorded in the range 390–4000 cm^{-1}, and it is given in Fig. 3e. A significant vibration band ranging from 400 cm^{-1} to 500 cm^{-1} is assigned to the characteristic stretching mode of the Zn-O bond. The broad peak 3434cm^{-1} (stretching) indicates the presence of hydroxyl groups of absorbed H_2O molecules.

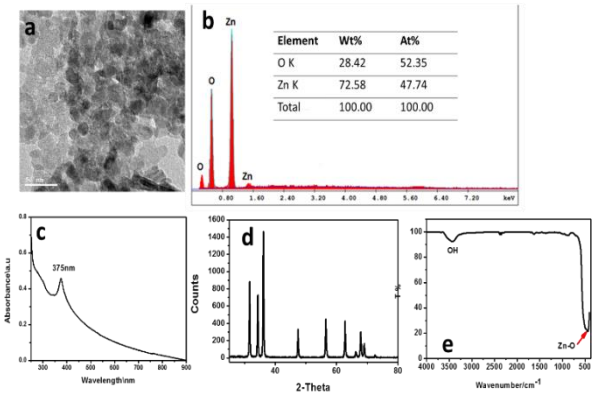

Figure 3: **T**EM image of the prepared ZnO NPs (a), EDS spectrum (b), UV-Vis absorption spectrum (c), XRD patterns (d), and FTIR spectrum (e), respectively.

3.1. Effect of ZnO nanomaterials on C_2H_5OH production

All concentrations of the ZnO nanomaterials have a clear stimulating effect on fermentation processes compared with the control. When the potato waste peels (PPW) were treated with 0 (control), 5, 10, 15, 50, 100 and 150 mg, respectively of ZnO nanomaterials added to 1 liter of the PPW autoclaved hydrolysate obtained from 15 g of dry powdered plant materials that was used as source of glucose for C_2H_5OH production. Under ambient conditions, it was found the ethanol percent increased from 3.443% to 17.73% with increasing concentrations of the ZnO nanomaterials.The obtained results indicated that the best concentrations of ZnO nanomaterials from this group was 150 mg/L which minimized the lag phase and the time to attain the peak bioethanol generation contrasted with the other concentrations added to the PPW substrate. When 150 mg/L of ZnO nanomaterials were added, it was found that the yield of bioethanol reached to 17.73% in comparison with the control (***Table 1 and Figure 4***).

Table 1. The effect of different concentrations of ZnO NPs on the Ethanol Production.

Concentration of ZnO (mg/L)	Ethanol (%)
0 (control)	3.44
5	4.73
10	6.01
15	8.3
50	9.16
100	13.01
150	17.73

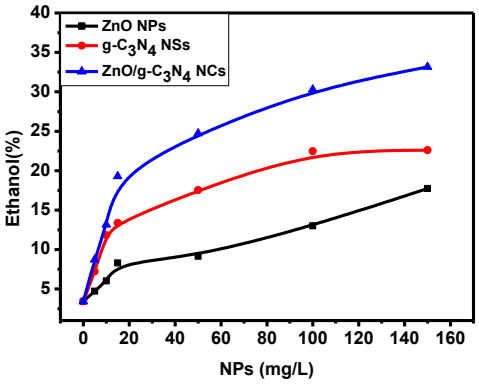

Figure 4: The effect of different concentrations of ZnO nanoparticles, g- C_3N_4 nanosheets, ZnO/g-C_3N_4nanocomposites on the Ethanol percent.

3.2. Effect of ZnO/g-C_3N_4 nanocomposites on C_2H_5OH production

All concentrations of the ZnO/g-C_3N_4 nanocomposites have a clear stimulating effect on fermentation processes compared with the control. When the potato waste peels (PPW) were treated with 0 (control), 5, 10, 15, 50, 100 and 150 mg, respectively of ZnO/g-C_3N_4 nanocomposites added to 1 liter of the PPW autoclaved hydrolysate obtained from 15 g of dry powdered plant materials that was used as source of glucose for C_2H_5OH production. Under ambient conditions, it was found the ethanol percent increased from 3.443% to 33.2% with increasing concentrations of the ZnO/g-C_3N_4 nanocomposites. The obtained results indicated that the best concentrations of ZnO/g-C_3N_4 nanocompositesfrom this group was 150 mg/L which minimized the lag phase and the time to attain the peak bioethanol generation contrasted with the other concentrations added to the PPW substrate. When 150 mg/L of ZnO/g-C_3N_4 nanocomposites were added, it was found that the yield of bioethanol reached to 33.2% in comparison with the control (***Table 2 and Figure 4***).

Table2. The effect of different concentrations of g-C_3N_4 nanosheets and ZnO/g-C_3N_4nanocomposites on the Ethanol Production.

Concentration of NPs (mg/L)	Ethanol (%) g-C3N4	ZnO/g-C3N4
0 (control)	**3.44**	**3.44**
5	**7.23**	**8.73**
10	**11.83**	**13.16**
15	**13.38**	**19.3**
50	**17.54**	**24.73**
100	**22.48**	**30.3**
150	**22.61**	**33.16**

Discussion

The biostimulation of yeast cells using nanomaterials increases the bioethanol production from biomass. Specifically, it was found that the nanomaterials have biostimulating effects on the cells activity during the startup of the fermentation process of the substrate and through the hydraulic retention time (HRT) to the end of the experiments, which agrees with the statements of Abdelsalam et al. (2019a,b) and Attia et al. (2018, 2021). The addition of nanomaterials with large specific surface area and large band gap enables the absorption of a large amount of the visible light (380-760 nm) which creates a rich light media around the yeast cells responsible of bioethanol production from organic matter, which agrees with the statements of Abdelsalam et al. (2019c).

In the present paper, we are investigating nanomaterials with the concentrations of 10, 15, and 50 mg/l. However, in Saeed et al. (2022), the

nanomaterials were investigated with the concentrations of 0, 5, 100, and 150 mg/l. Therefore, the present paper completes the missing ranges in the study of Saeed et al. (2022).

Future research will focus on the irradiation with laser to accelerate the growth of several cell cultures. Laser radiation has been found to photobiostimulate a large variety of bioresponses (Abdelsalam et al., 2018). Mitochondria are susceptible to irradiation with monochromatic visible light. The illumination of mitochondria raises the adenosine triphosphate (ATP) synthesis and the intake of oxygen. It was found that irradiation of cells with laser radiation boosts the ATP synthesis and accelerates the proliferation of cells (Abdelsalam et al., 2019a,b). At the same time, elevated activity of mitochondrial respiratory chain enzymes and ATP synthesis was revealed in irradiated cells using laser radiation (Abdelsalam et al., 2021a,b; Samer et al., 2021a,b).

Further future research will focus on the photoactivation of nanomaterials using laser radiation to increase the activity of nanomaterials, which creates a rich light media around the cells which photobiostimulates the cells and increases bioethanol production from the substrate (Abdelsalam et al., 2021a,b; Samer et al., 2021a,b; Hijazi et al., 2020a,b).

Conclusions

According to the outcomes of this research, it was concluded that the addition of ZnO/g-C_3N_4nanomaterials improved the bioethanol yield compared to the control which is without nanomaterials addition. This shows the role of nanomaterials in the fermentation of PPW samples in which glucose was converted into C_2H_5OH. The addition of 150 mg of ZnO/g-C_3N_4nanomaterials delivered the highest bioethanol yield compared to all other treatments and, therefore, using 150 mg of ZnO/g-C_3N_4nanomaterials is recommended.

Conflicts of interest

There are no conflicts to declare.

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