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Optimizing bio-ethanol production from cabbage and onion peels waste using yeast (*Saccharomyces cerevisiae*) as fermenting agent

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Abstract

Production of bio-ethanol from lingocellulosic materials is providing a long-term sustainable for fuel supply. Cabbage and onion peels waste is one of cheap source of lignocellosic materials to serve as feedstock for bio-ethanol production. With the objective of evaluating its potential for bio-ethanol production, different concentrations (50g, and 100g) of Cabbage and onion peels waste treated with 1% diluted sulfuric acid and untreated were subjected to batch fermentation for 12 days with 0.5% and 1% yeast inoculums. Percent of bio-ethanol production, cell density and reducing sugars were measured at an interval of 4 days starting from the beginning. Results of these study showed that ethanol production was observed starting from the 4th day of fermentation, but its amount peaked 26.51 from 100g substrate with 1% inoculum on the 8th day of fermentation, and declined on 12th days (21.06%) from the same substrate concentration. Pretreated substrate showed significantly higher ethanol production than untreated. In agreement with ethanol production, cell density and reduction in reducing sugar were observed in the same pattern. Compared ethanol production between untreated substrates yield of 20.96%, and treated substrates yielded of 26.51%. Overall, this study showed that acid pre-treatment, inoculum concentration, fermentation period and substrate concentration affect the amount of bio-ethanol production. Finally, it can be concluded that the production of bioethanol from Cabbage and onion peels waste is economically and environmentally viable. Extensive use of these Wastes for bioethanol production may have twofold advantages, viz. reduction of its negative impact on environmental hygiene and generation of bio-ethanol.

Keywords: Acid Pre-treatment; Bio-ethanol; Cabbage; Onion peels waste; Distillation; Saccharomyces cerevisiae

1 Introduction

Fossil fuel is depleting day by day throughout the world. This limitation along with the problem of Green House Gas (GHG) emissions leads findings for alternative energy that are environmentally and commercially feasible. Bio-ethanol is one of the liquid fuels found to have an efficient calorific content capable of being used in automobiles and that have the ability to fulfill needed of world energy crisis by using as alternative energy that are environmentally and commercially feasible. It is derived from renewable feedstock sources such as sugary, starchy and lignocellulosic biomass (material). However, sugary and starchy biomasses are used mainly as food for humans and animal consumption that when used for energy production, the rapidly increasing world population will face food crises. As a result, alternative biomass sources such as agricultural and municipal wastes, and other lignocellulosic materials from non-food plants being sought as alternatives source.

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This project is prepared on title Optimization of Bio-ethanol production from Cabbage and onion peels waste using yeast (*Saccharomyces cerevisiae*) as a fermenting agent. The substrate used for this research; Cabbage and onion peels waste was collected from Wachemo university main campus student's cafeteria waste discharged. Cabbage and onion peels waste is one of the problems of environments waste in all most all university. Based on this truth the output of this research was:

- It may solve the problems of environmental hygiene in our campus and furthermore it used to solve the problems of environmental hygiene of all our countries university
- It may use as core steeps for the researcher who went to do research on the same area
- If is use as direction show based on the result of this outcome to produce bioethanol from Cabbage and onion peels waste to use bioethanol for energy use needed and if it scales up it may use to overcome world energy crisis because this waste is discharge from every home every day.

Bio-ethanol derived from renewable feedstock sources such as sugary, starchy and lignocellulosic biomass. Among the three major types of raw materials, production of bio-ethanol from sugary and starchy materials is easier as compared to lingo-cellulosic materials since it requires additional technical challenges such as pretreatment [10]. Moreover, nonfood biomass sources, mainly lignocellulosic materials, such as agricultural residues, waste materials or municipal solid wastes are gaining more attention than feedstock that are used as food for humans and other animals [1]. Using bio-ethanol as a fuel decreases fossil fuel consumption and increases energy supply security. It is considered biodegradable and sulphur free, during the combustion process it does not contribute to the increase of CO_2 in the atmosphere, reducing global warming [11, 20]. Bio-ethanol can be produced from cellulose and hemicelluloses that originate from many sources of biomass. In order to produce sugars from the biomass, the biomass is pre-treated with acids or enzymes in order to reduce the size of the feedstock and to open up the plant structure. The cellulose and the hemi cellulose portions are broken down (hydrolyzed) by enzymes or dilute acids into sucrose then fermented into bio-ethanol.

Bio-ethanol is a liquid obtained by distillation of fermented sugar by different yeast and bacterial species. The yeast *Saccharomyces cerevisiae* and facultative bacterium *Zymomonas mobilis* are better candidates for industrial alcohol production. The high specific rates of sugar uptake and ethanol fermentation by *Z. mobilis*, and its relatively small genome size, make it a promising candidate for metabolic engineering pathways for bioethanol synthesis from agricultural and forestry waste [9, 10, 21]. *Zymomonas mobilis* was recombinant bacterial species that possesses advantages over *S. cerevisiae* with respect to bio-ethanol productivity. However, bio-ethanol is produced commercially by yeast because it ferments glucose to ethanol as a virtually sole product and it is known for its high bio-ethanol tolerance, rapid fermentation rates and insensitivity to temperature and substrate concentration [10].

Many countries have been using bio-ethanol as alternative to petrochemicals like diesel and petrol by blending it with gasoline in fixed proportion. Maximum of 20 % bio-ethanol can be blended with gasoline to be used as alternative fuel source in same carbonator engine. This technique has been adopted in Brazil and USA [10]. In Ethiopia, the blending of Ethanol with Benzene was started in September 2008 with 5% Ethanol and 95% benzene [13]. Using ethanol blended fuel for automobiles can significantly reduce petroleum use and exhaust greenhouse gas emission. Ethanol is a simple alkyl alcohol that can be used as a transport fuel in spark ignition engines. Bio-ethanol has high octane levels and can be either blended into petrol or used in unmodified vehicles, or run as 100 percent ethanol in a converted engine [17, 21]

Fossil fuel is depleting day by day throughout the world. This limitation along with the problem of Green House Gas (GHG) emissions leads findings for alternative energy that are environmentally and commercially feasible. Main sources of biomass which are used for ethanol production are sugarcane, corn, wheat bran, cassava, sweet potato *etc*. However, this biomass is used mainly as food for human's consumption that when used for energy production, the rapidly increasing world population will face food crises. Many agricultural raw materials (wastes) rich in fermentable carbohydrates were tested worldwide for bioconversion from sugar to bioethanol, but the cost of carbohydrate raw materials has become a limiting factor for large scale production by the industries employing fermentation processes. Since the price of feedstock contributes more than 55% to the production cost, inexpensive feed stocks such as lingo-cellulosic biomass and agro-food wastes, are being considered to make bio-ethanol competitive in the open market [21]. The production of bio-ethanol from comparatively cheaper source of raw materials using efficient fermentative microorganisms is the only possible way to meet the great demand for bio-ethanol in the present situation of energy crisis. As a result, alternative biomass sources such as agricultural and municipal wastes, and other lignocellulosic materials from non-food plants being sought as alternatives source [15]

Agricultural waste is recognized as refuse and defined as the unwanted materials or byproducts generated from various agricultural activities, which account for over 30% of worldwide agricultural productivity. However, agricultural waste contains a large amount of carbohydrates and various bioactive compounds and can therefore be harnessed as a useful resource in the biorefining industry (chemicals, energy, foods, medicines, etc.) [23]. The recycling of waste materials using the bioconversion process has become a major component of environmental protection, which involves reducing the amount of carbon dioxide produced [3]

Moreover, Cabbage and onion peels waste is one of the problems of environments waste of tropical and subtropical Africa, Arabian Peninsula and a small part of India. In Ethiopia, especially in the town, it is the most pollution and environmental pollution factor due to the wastes is not used for other thing and wrong way discharge of the wastes. Extensive use of these harmful wastes for bioethanol production may have twofold advantages, viz. reduction of its negative impact on environmental hygiene and generation of bio-ethanol production. Therefore, the research was initiated to production of bio-ethanol from Cabbage and Onion peels wastes with following general and specific objectives.

General objective

- To determine the amount of bio-ethanol production from Cabbage and onion peels waste by a batch culture using *S. cerevisiae.*

Specific objectives

- To identify the optimum substrate concentration for maximum bio ethanol production from Cabbage and onion peels waste
- To determine the effect of inoculum (yeast) concentration on the rate of ethanol production from Cabbage and onion peels waste
- To determine the cell density and reducing sugar concentrations at different fermentation time
- To assess the effect of acid pre-treatment on the rate of ethanol production from Cabbage and onion peels waste

2 Material and methods

2.1 Description of the Study area

The experiment was conducted in Wachemo university biotechnology and Chemistry laboratories and Haramaya university central laboratory. Wachemo university located in Hadiya Zone Hosanna which occur in SNNP far from Addis Ababa 230km. Hosana tow lies on the geographical coordinates of at latitude of 7º 35’ 0” N, longitude of 37º 53’ 0”E and altitude of 2722m.a.s.1

2.2 Inoculums Preparation

Inoculums were prepared from dried baker's yeast, *Saccharomyces cerevisiae* that was purchased from market. Inoculums, 0.5% and 1% was prepared by dissolving 0.5g and 1g of yeast in 99.5 and 99ml of distilled water, respectively. For fermentation of each substrate 10ml of the solutions will be used as inoculums concentration for batch fermentation process [7, 22]

2.3 Preparation of Nutrient Solution

Nutrient supplements were prepared by adding 1g KH_2PO_4, 0.5 g $CaCl_2$, 0.05 g $MgSO_4$, 0.1 g Na_2SO_4 and 1 g $(NH_4)_2SO_4$ in 100ml distilled water and autoclaved at 121 °C for 15 minutes and 100ml of this solution was added to all sample [17].

2.4 Substrate preparation

Cabbage and Onion peels waste is a substrate that was used in this experiment. This substrate was collected from Wachemo university students’ cafeteria discharge waste and brought to the laboratory. The Cabbage and Onion peels waste was washed using tap water to remove adhering dirt and cut into pieces of 2-3 cm and dried in oven at 45^{o}C for 8hr and then ground into fine powder using high speed grinder and kept in a refrigerator until use [7, 23].

2.5 Pretreatment of substrates

The substrates were acid pre-treated using dilute sulphuric acid (1.5%). The purpose of acid hydrolysis is to remove lignin from the substrate. Cabbage and Onion peels wastes was completely steeped in dilute sulphuric acid (1.5%) for primary acid hydrolysis pretreatment. Ground fine powder of cabbage and onion peels waste sample was grouped in to 50 g and 100g and put in 250ml Erlenmeyer flasks and 10ml of 1.5% H_2SO_4 was add to each gram of substrate and autoclave the sample for 15 min at temperature of 121°C [16].

2.6 Fermentation process and Experimental design

Different amounts (50g and 100g) of acid pre-treated substrates were separately inoculated with 0.5 or 1% *S. cervisiae* and 100ml of the nutrient supplement was added and incubated in an incubator at 30 °C.

Table 1 Batch fermentation of Cabbage and Onion peels waste with different combinations of substrates and yeast inoculums

Batch No	Replications	Substrate Concentration	Nutrient Volume	Inoculums%	Inoculums Volume	Types of sample
Sample A	1	50grams	100ml	0.5%	10ml	Treated
	2	50grams	100ml	0.5%	10ml	
	3	50grams	100ml	0.5%	10ml	
Sample B	1	50grams	100ml	0.5%	10ml	Untreated
	2	50grams	100ml	0.5%	10ml	
	3	50grams	100ml	0.5%	10ml	
Sample C	1	500grams	100ml	1%	10ml	Treated
	2	500grams	100ml	1%	10ml	
	3	500grams	100ml	1%	10ml	
Sample D	1	50grams	100ml	1%	10ml	untreated
	2	500grams	100ml	1%	10ml	
	3	50grams	100ml	1%	10ml	
Sample E	1	100grams	100ml	0.5%	10ml	Treated
	2	100grams	100ml	0.5%	10ml	
	3	100grams	100ml	0.5%	10ml	
Sample F	1	100grams	100ml	0.5%	10ml	untreated
	2	100grams	100ml	0.5%	10ml	
	3	100grams	100ml	0.5%	10ml	
Sample G	1	100grams	100ml	1%	10ml	Treated
	2	100grams	100ml	1%	10ml	
	3	100grams	100ml	1%	10ml	
Sample H	1	100grams	100ml	1%	10ml	untreated
	2	100grams	100ml	1%	10ml	
	3	100grams	100ml	1%	10ml	

The pH of the solutions was adjusted to 4.5 with buffer solution (NaOH) and the flasks were sealed completely to induce an anaerobic condition for efficient fermentation. The fermentation process was allowed for 12 days at 30 degree

centigrade temperature so that the sugar was converted into Bioethanol by action of enzyme produced by *Saccharomyces cereviceae* [16]. 250ml E.flasks were used for batch fermentation [1]. Production of bio-ethanol and other parameters were estimated at the interval of 4 days starting from the beginning of fermentation. The experimental design was based on CRD [Completely Randomized factorial] Design factorial with three replications for each treatment and control. The control was non-pretreated substrates.

2.7 Analytical procedure

The samples were collected from each flask at interval of 4 days and allowed to centrifuges at 1500rpm for 30 minutes to remove the cells suspension. The supernatant fluid was filtered through Whitman filter paper No.1 and the 30 ml of filtrate was collected after filtration. 27ml of the filtrate was used to determine ethanol and reducing sugar concentration and the remaining 3ml will be used to determine cell density [2].

2.7.1 Quantitative analysis of reducing sugar

The amount of reducing sugar in the fermenting sample was estimated spectrophotometriclly following the method used by Nelson (1944) using D-glucose as standard. A fermenting sample (0.05ml) was mixed with 0.35ml citrate buffer (pH=6.5) and 0.6ml of Dinitrosalicylic acid (DNS) and then the mixture was boiled for 5 minutes immediately to stop the reaction. Then the absorbance was measured at 540nm using spectrophotometer. The amount of reducing sugar in the sample was calculated by relating absorbance of different concentrations of glucose on standard curve [16].

2.7.2 Qualitative determination of bioethanol

Presence of alcohol in the distillate was checked by functional group classification test [4]. For this, 1 drop of the unknown was added to 1 mL of reagent-grade acetone in a test tube. Then a drop of the sulfuric acid reagent was directly added into the solution and the mixture was shaken. A primary or secondary alcohol was reducing the orange-red sulfuric acid reagent to an opaque green or blue suspension of Cr (III) salts in 2–5 s.

2.8 Quantitative Estimation of Bio-ethanol

The fermented broth was assayed for quantitative estimation of ethanol using the acidified dichromate titration method. 25ml of the fermented sample was taken into 500ml Pyrex distillation flask containing 30ml of distilled water and distilled. The distillate was collected in 250ml flask containing 25ml of potassium dichromate solution (33.76g of $K_2Cr_2O_7$ dissolved in 400ml of distilled water with 325ml of 1% sulphuric acid) and volume raised to 1 litter [20]. About 20ml of distillate was added in each sample and the flasks were kept in a water bath maintained at 6o°C for 20 minutes. The flasks was cooled to room temperature and add distilled water until the volume raised to 50ml. Five ml of this solution was diluted with 5ml of distilled water for measuring the optical density at 600nm using spectrophotometer [5]. A standard curve was prepared under similar set of conditions by using standard solution of ethanol containing 0 to 20% (v/v) ethanol in distilled water and then ethanol content of each sample was estimated by relating absorbance of known ethanol concentration from the standard curve [12].

2.9 Determination of Cell Density (Biomass)

Cell density was measured using spectrophotometer (Humas Think HS 3300, Korea) at 600 nm absorbance (Summer *et al.*, 2004). Dry weight method of cell measurement will be used. The cell in the broth sample was separated by centrifugation and the wet weight of the culture was measured immediately and allowed to dry in oven at 100 degree cent grade for six hours. Then the sample was diluted and measured the absorbance of it with a spectrophotometer at 600nm.

2.10 Data Analysis

The data was summarized into tables and graphs by analyzing using two ways of variance (ANOVA) on Microsoft office excel spreadsheet and analyzed using Statistical Package for Social Studies (SPSS) version 17. LSD (least significant difference) test was used to identify significant differences among treatment means. P values < 0.05 was considered significant in all cases.

3 Results and discussion

3.1 Effects of acid pre-treatment, substrate and inoculums volume and Fermentation Period on Ethanol Production

Effects of acid pre-treatment, substrate and inoculums concentrations and fermentation period on ethanol production were observed and results were indicated in table 7. The results revealed that, acid pre-treated substrate produce more ethanol than untreated substrate in all the substrate and inoculums concentration. Acid-treated substrates significantly produce higher (26.51±0.02) amount of ethanol than untreated substrates (20.96%) at the concentration of 100g substrate inoculated with 1% yeast at 8th day of fermentations. This might be due to acid pre-treatment of lignocellulose breaks down lignin in to fermentable sugars and increase accessibility of enzymes and microbes to carbohydrates. An efficient method of combined steam pre-treatment and enzymatic hydrolysis was employed to obtain high conversion efficiency and ethanol production from lignocellulose [11]. In line with this [17] also reported that treatment of lignocellolosic substrates by acid prior to fermentation facilitates the availability of fermentable substances to enzymatic reaction. Young Jung *et al,* (2013) also reported that dilute sulfuric acid pretreatment can result in high reaction rates and significantly improve cellulose hydrolysis. In both acid treated and untreated substrates, ethanol production found to increase with increasing concentration of inoculums, suggesting more enzymes from yeast are facilitating conversion of more sugar into ethanol. This result agrees with [8] who reported that bio-ethanol in high quantity can be derived from cellulosic biomass through acid or enzymatic hydrolysis followed by fermentation.

The rate of ethanol production also depends on the substrate concentration, when the substrate concentration increased from 50 gram to 100-gram, rate of ethanol production also significantly increased. The highest amount of ethanol production was observed in 100 gram of acid pre-treated substrate with 1% inoculums concentration (26.51±0.02%). This argues with [16]1 kg rice straw will contain 390 g of cellulose. This amount is theoretically enough to produce 220 g or 283 ml of ethanol, however considering the practically achievable best yield as 74%. Enzymatic hydrolysis is sensitive to the substrate concentration. In addition to substrate concentration, pretreatment of cellulosic materials and hydrolyzing conditions such as temperature and pH are among factors influencing the effectiveness of enzymatic hydrolysis [15]. For every enzymatic reaction, there is optimal substrate level to yield maximum product and decreasing or increasing the amount of substrate beyond optimal level may be limited catalytic activities of enzyme produced in the system (Hoyer *et al.*, 2010).

Table 2 Ethanol production from Cabbage and Onion peels wastes using S. cerevisiae (mean ± SD, n=3)

Substrate (gm)	Treated untreated	Ethanol produced (%) at different fermentation period		
		4th day	8th day	12th day
A	Treated	6.54 ± 0.03^{dK}	11.16 ± 0.02^{Bk}	8.57 ± 0.01^{aK}
B	Untreated	4.09 ± 0.03^{dK}	9.87 ± 0.08^{cL}	6.23 ± 0.05^{aL}
C	Treated	7.13 ± 0.07^{cI}	15.14 ± 0.04^{bJ}	11.10 ± 0.03^{aJ}
D	Untreated	5.63 ± 0.02^{cJ}	12.02 ± 0.09^{aK}	8.03 ± 0.08^{aL}
E	Treated	9.89 ± 0.01^{dG}	18.44 ± 0.07^{cH}	14.46 ± 0.03^{aH}
F	Untreated	6.51 ± 0.06^{eI}	14.77 ± 0.04^{dI}	11.04 ± 0.07^{aI}
G	Treated	14.09 ± 0.02^{dD}	26.51 ± 0.02^{bE}	21.06 ± 0.06^{aE}
H	Untreated	11.04 ± 0.07^{eD}	20.96 ± 0.05^{cF}	17.66 ± 0.05^{aF}

Note: Means followed by different small letters in row are significant at 0.05 probability levels. Means followed by different capital letter in column are significantly different at 5% level of significance. A= 50g + 0.5% yeast (treated), B=50g + 0.5% yeast (untreated), C= 50g + 1% yeast(treated), D= 50g + 1% yeast(untreated), E= 100g + 0.5% yeast(treated), F= 100g + 0.5% yeast(untreated), G= 100g + 1% yeast(treated) and H= 100g + 1% yeast(untreated).

The amount of ethanol production was also depending on fermentation period, in this experiment's ethanol production was observed on the 4th day of fermentation in both acid pre-treated and untreated substrates of all the treatments. On the 4th day of fermentation, the amount of ethanol was found to be lesser quantity as compared to other days of fermentation. This is the period of adaptation for yeasts cells where they can synthesize enzymes and essential metabolites for their growth and development [7]. The maximum ethanol production was observed on the 8th day of fermentation (26.51±0.02). This indicated that yeast cells started to grow after log phase and dividing at maximal rate,

under which they are growing and producing ethanol at maximum quantity. Increasing incubation temperature with fermentation period results in increasing growth rate of microorganisms and productivity of microbial products [4]. On the 12th day of fermentation ethanol production (21.06±0.06%) was less than 8th day of fermentation at the concentration of 100g substrate inoculated with 1% yeast. This might be due to reduction of substrate concentration or due to decrease in the number of viable yeast cells [1].

The rate of ethanol production also depended on inoculum concentration. Increasing inoculum concentration from 0.5% to 1% also increase rate of ethanol production significantly. The maximum amount of bio-ethanol (26.51±0.02) were observed from 100g of pre-treated substrate inoculated with 1% yeast cells whereas minimum ethanol production (4.09±0.03%) was observed in untreated low substrate (50g) inoculated with 0.5% inoculums concentration. In all substrate concentration inoculum concentration (volume) were preform rapid bioconversion of sugars in to ethanol. In line with [6] used fed batch enzymatic saccharification strategy to achieve 110g/l sugar concentration and when these hydrolyzates was fermented with *S. cerevisiae*, of enough volume concentration almost 95.3g/l sugar was consumed to produce 45.7g/l ethanol with an ethanol yield of 94%. [14] have achieved an ethanol yield of 0.48g/g from the enzymatic hydrolyzates of pretreated *P. juliflora* and *L. camara* containing 36.5 and 37.5g/l sugars respectively by fermented with *S. cerevisiae*. The increasing ethanol production with increasing cell biomass indicated that the amount of yeast influenced ethanol production in agro wastes [1].

3.2 Qualitative Determination of Bio-ethanol

The presence of alcohol in the distillate was checked by the functional group classification test of chromic acid reagent grade. Experimental group and controlled group were prepared. After that 10ml of potassium dichromate and a 4ml of unknown sample was added in to experimental test tube that had 1ml of reagent grade acetone. Then a drop of the sulfuric acid reagent was directly added into the solution and the mixture was shaken. The primary or secondary alcohols were reduced the orange-red potassium dichromate reagent to blue green color suspension confirms positive result [11]. This indicated that the presence of alcohol in the distillate which was produced through fermentation process of Cabbage and Onion peels wastes.

3.2.1 Effects of acid pre-treatment, substrate concentration and inoculums volume and Fermentation Period on reducing sugar yield

Concentration of reducing sugar were measured on the 4th day of fermentation was found to be significantly higher in both acid pretreated and untreated substrates and found to be decline with increasing days of fermentation (Table 8). A maximum of 11.32±0.36mg/ml reducing sugar was recorded from the acid pretreated 100g of substrate inoculated with 1% yeast cell on 4th day of fermentation. However, with increasing fermentation period, the amount of reducing sugar was declined, and this may be due to some sugars contained in the substrate were consumed by yeasts and converted into ethanol. The comparison of reducing sugar concentration between acid pre-treated and untreated substrates showed that the amount of reducing sugar was significantly higher when substrates were pretreated with diluted sulphuric acid (Table 7). This may be the facilitation of hydrolysis of lignocellulosic material by the acid for conversion into sugar.

Acid pretreatment of lignocellulose is important to break down lignin and increase the availability of sugar for microbes to grow on and convert it to ethanol [9]. In all the substrates concentration; increasing yeast concentration from 0.5% to 1% resulted in significant increase in ethanol concentration and cell density; But decrease sugar concentration with increasing fermentation period. Rate of decrement of reducing sugar agrees with the amount of ethanol production, suggesting that the more is the reduction in reducing sugar means the more it is converted to ethanol by yeast. In fact, that, during fermentation process, the ethanol production was increased while the reducing sugar decreased as a result of the fact that during fermentation the yeast (*S. cerevisiae*) utilized the sugar as a source of carbon and energy, and ethanol is produced as a result [12].

Rate of reduction in reducing sugar also increase in pre-treated substrate than untreated substrate initial. Pretreatment of substrate open up plant structure and microbial easily accessible plant structure to degrade glucose. Therefore, an initial pretreatment is necessary for breakdown lignocellulose structure to make them more susceptible to an enzymatic attack and sugar easily utilized by microbes to change it in to energy and ethanol and carbon were produced as a result. The main advantage of pretreatment methods is the higher recoveries of sugars derived from hemicellulose and make sugar easily fermentable and produce energy and other microbial metabolites [3]. The dilute acid pretreatment has the advantage of not only solubilizing hemicelluloses but also converting solubilized hemicelluloses to fermentable sugars [18].

Table 3 Reducing sugar concentration (mg/ml) measured at 540nm from Cabbage and Onion peels wastes (values are Mean ±SD, n=3)

Substrate (gm)	Treated untreated	Reducing sugar concentration(mg/ml) at different fermentation period		
		4th day	8th day	12th day
A	Treated	7.93±0.035^{aG}	5.44±0.018^{bG}	3.0±0.062^{cG}
B	Untreated	7.25±0.077^{aH}	5.27±0.031^{bG}	3.20±0.049^{Cg}
C	Treated	9.30±0.045^{aE}	7.30±0.046^{bD}	5.0±0.042^{cD}
D	Untreated	9.19±0.036^{aE}	7.18±0.027^{bD}	5.92±0.062^{Cc}
E	Treated	9.96±0.013^{aF}	8.95±0.044^{bE}	6.0±0.085^{Cc}
F	Untreated	9.46±0.017^{aF}	8.31±0.045^{bF}	6.46±0.055^{cC}
G	Treated	11.32±0.36^{aC}	8.26±0.017^{bC}	7.06±0.072^{Cb}
H	Untreated	10.19±0.10^{aC}	8.62±0.026^{dD}	7.15±0.031^{Cb}

Note: Means followed by different small letters in row are significant at 0.05 probability levels. Means followed by different capital letter in column are significantly different at 5% level of significance. A= 50g + 0.5% yeast (treated), B=50g + 0.5% yeast (untreated), C= 50g + 1% yeast(treated), D= 50g + 1% yeast(untreated), E= 100g + 0.5% yeast(treated), F= 100g + 0.5% yeast(untreated), G= 100g + 1% yeast(treated) and H= 100g + 1% yeast(untreated).

The result of the study revealed that reducing sugars released from acid pre-treated plant sample were greater than that of untreated. Pretreated substrate yields more fermentable sugar than untreated substrates (Table 8). [2] pointed out that the main advantage of dilute acid pretreatment related to other pretreatment methods is the higher recovery of sugars derived from hemicellulose. The dilute acid pretreatment has the advantage of not only solubilizing hemicelluloses but also converting solubilized hemicelluloses to fermentable sugars [18].

3.2.2 Effects of acid pre-treatment, substrate and inoculums concentrations and Fermentation Period on Cell Density

The results showed that yeast biomass was increased in all the substrate concentration from 4thday to 8thday of fermentation period. However, after 8thday of fermentation the cell biomass concentration found to be decreased (Table 9).

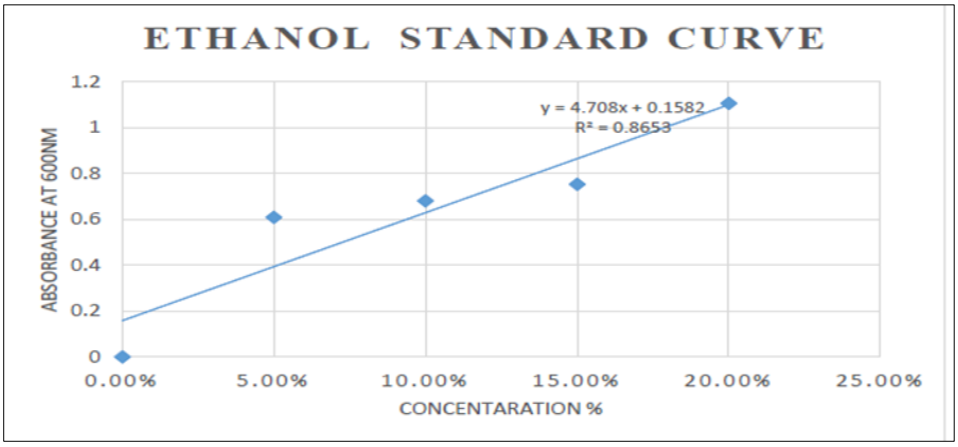

Figure 1 Standard curve prepared under similar set of conditions by using standard solution of ethanol containing 0 to 25% (v/v) ethanol in distilled water

This may be due to high alcohol content or cyto-toxicity of ethanol or decrease in fermentable sugar. In all the substrate concentrations, pre-treated substrate showed higher cell density as compared to untreated substrates. The maximum cell density (4.53±0.015mg/ml) was obtained from 100g of acid pretreated substrate with 1% of yeast inoculums on 8th day of fermentation. Moreover, at the same substrate concentration observed more ethanol production (26.51±0.02%).

In line with this [1] reported that increasing ethanol production with increasing cell biomass indicated that the amount of yeast influenced ethanol production. However; after 8th day of fermentation period cell biomass found to decline (3.06±0.023).

During fermentation period there were output of fermented broth to measure ethanol product, reducing sugar and cell density at different range of fermentation. This makes decrements of cell density at dead/decline phase of microbial cell growth. The 12th day of this fermentation period show dead phase of microbial cell growth. The out let of fermentation reaction contains; microbial metabolites, microbial cell, microbial enzyme, nutrient sources and all other material that incubated in the media [1].

Table 9 Effects of acid pre-treatment, substrate and inoculums concentrations and Fermentation Period on Cell Density (Values are Mean ±SD, n=3)

Substrate (gm)	Treated untreated	Cell density observed from fermented Cabbage and Onion peels wastes at 600nm (mg/ml) at different fermentation period		
		4th day	8th day	12th day
A	Treated	2.21 ± 0.040^{bF}	2.41 ± 0.018^{aC}	2.00 ± 0.017^{cF}
B	Untreated	2.02 ± 0.083^{bF}	1.9 ± 0.054^{aD}	1.64 ± 0.050^{cF}
C	Treated	2.81 ± 0.073^{bD}	3.44 ± 0.027^{aC}	3.01 ± 0.054^{bD}
D	Untreated	2.79 ± 0.013^{bD}	2.52 ± 0.032^{aC}	2.13 ± 0.015^{bD}
E	Treated	2.45 ± 0.027^{bE}	4.0 ± 0.018^{aC}	3.11 ± 0.044^{aC}
F	Untreated	2.35 ± 0.027^{bE}	3.12 ± 0.058^{aC}	2.12 ± 0.025^{aC}
G	Treated	3.15 ± 0.027^{bC}	4.53 ± 0.015^{aB}	3.06 ± 0.023^{cD}
H	Untreated	3.09 ± 0.018^{bC}	3.6 ± 0.013^{aB}	2.90 ± 0.067^{bD}

Note: Means followed by different small letters in row are significant at 0.05 probability levels. Means followed by different capital letter in column are significantly different at 5% level of significance. A= 50g + 0.5% yeast (treated), B=50g + 0.5% yeast (untreated), C= 50g + 1% yeast(treated), D= 50g + 1% yeast(untreated), E= 100g + 0.5% yeast(treated), F= 100g + 0.5% yeast(untreated), G= 100g + 1% yeast(treated) and H= 100g + 1% yeast(untreated).

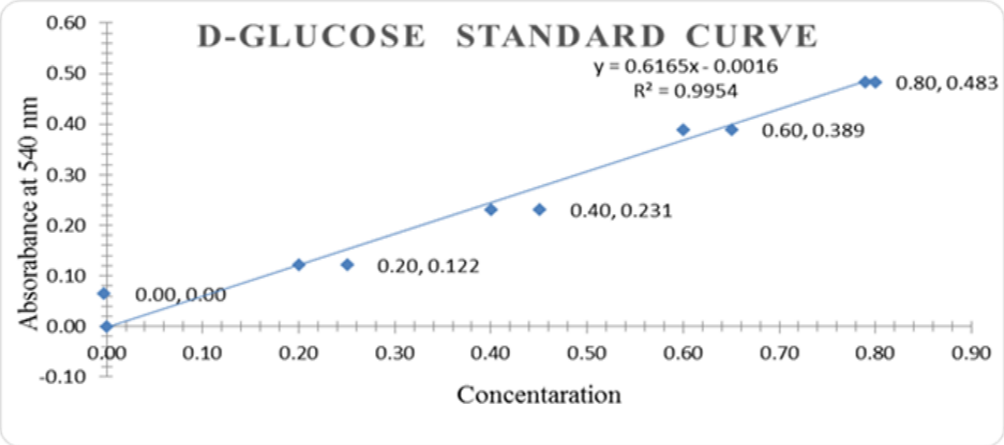

Figure 2 Standard curve for the determination of reducing sugar by using D- glucose concentration prepared by taking 1g/ml already prepared standard D- glucose

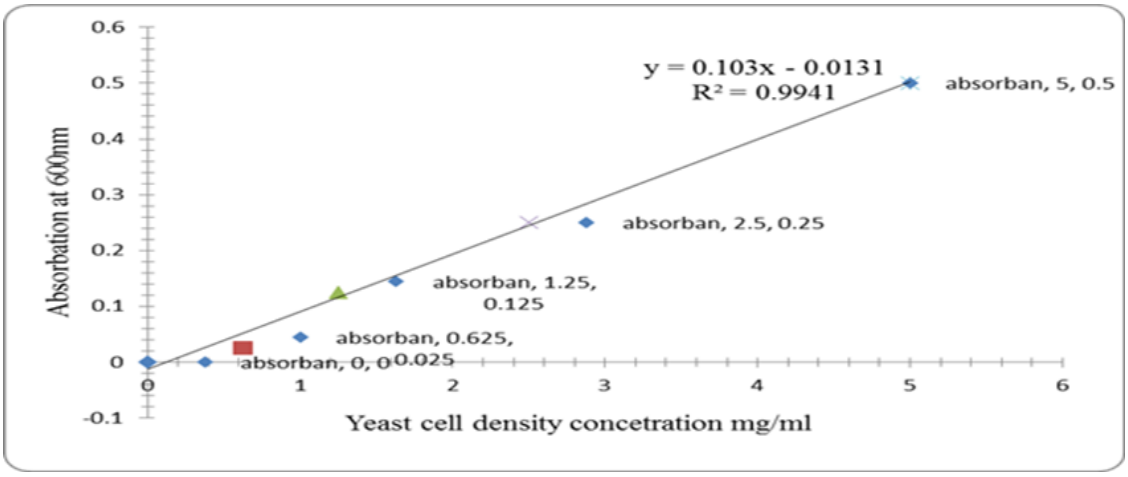

Figure 3 Standard curve for determination of cell density prepared by taking 10g baker yeast and dissolve by 10ml distilled water with appropriate serial dilution

4 Conclusion

Bio-ethanol has been identified as the mostly used bio-fuel worldwide since it significantly contributes to the reduction of crude oil consumption and environmental pollution. It can be produced from various types of feed-stocks such as sucrose, starch, lignocellulosic and algal biomass through fermentation process by microorganisms. Cabbage and onion peels waste is one of the problems of environments waste of tropical and subtropical Africa, Arabian Peninsula, and a small part of India. In Ethiopia, especially in the town, it is the most pollution and environmental pollution factor due to the wastes is not used for other thing and wrong way discharge of the wastes. Extensive use of these wastes for bioethanol production may have twofold advantages, viz. reduction of its negative impact on environmental hygiene and generation of bio-ethanol production. Therefore, the research was initiated to production of bio-ethanol from Cabbage and Onion peels wastes

The finding of present study revealed that Cabbage and Onion peels wastes exhibited significant result for the production of bio-ethanol. The amount of bio-ethanol production depended on substrates concentration and effect was statistically significant at $p<0.05$. Among the different substrate concentrations, 100 gram of treated substrate showed the highest (26.51±0.02%) percentage of ethanol production with high yeast cells (1%). Therefore, substrate concentration and inoculums concentration are directly proportional until it reached the optimum level for ethanol production. Comparatively the reducing sugar utilization was more in pretreated substrates than untreated ones. Bio-ethanol production increased slightly when initial substrate concentration increased. But, it decreased after 8th day of fermentation. This may be due to substrate limitation, decrease in cell biomass (yeast) and production of some toxic substance formation in the mixture.

Finally, it can be concluded that the production of bio-ethanol from Cabbage and Onion peels wastes is economically and environmentally viable. Beside that production of bio-ethanol from Cabbage and Onion peels wastes is important for environmental hygiene by reducing impacts of the wastes on environmental hygiene and the produced ethanol can be a good substitute of Petrol.

Recommendation

Based on the findings of the experiments, the following recommendations were suggested

- To check the bio-fuel quality of Cabbage and Onion peels wastes HPLC too.
- Further study is very important to describe how absolute bio-ethanol can be produced from Cabbage and Onion peels wastes by using Fed batch and continuous fermentations for inclusive use of substrate
- To produce high yield of bio-ethanol from Cabbage and Onion peels wastes estimation of the ethanol yield according to AOAC (1990) by calculation using the formula may followed.

Ethanol yield = 100Where sugar consumed is Equal to initial sugar concentration minus final sugar concentration (concentration of sugar left after fermentations)

Compliance with ethical standards

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Disclosure of conflict of interest

The authors declare that they have no competing interests.

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