Global Science Research Journals

ISSN: 2465-7182 Vol. 3 (3), pp. 132-140, October, 2015

http://www.globalscienceresearchjournals.org/

Global Journal of Biochemistry and Biotechnology

Full Length Research Paper

Production of bio-ethanol from waste potato peel collected from University of Gondar, student's cafeteria

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Accepted 14 October, 2015

Ethanol is one of the bio-energy sources with high efficiency and low environmental impact. Various raw materials such as agricultural crops, industrial and domestic wastes can be used as carbon source for ethanol production. In the present study, potato peel waste discarded from University of Gondar, student Cafeteria was chosen as a sole carbon source for ethanol production using microbial liquefaction, saccharification and fermentation with sequential and co-culture techniques. *Aspergillus niger* and *Bacillus species* were used for hydrolysis while *Saccaharomyces cerevisae* of bakery and brewery yeast were used for fermentation of potato peel flour with 87% moisture content. The maximum crude supernatant (380mL) was obtained from sequential culture of *bacillus sp.* with bakery yeast whilst the minimum (74mL) was obtained from *A. niger* and brewery yeast. After distillation of 150mL of broth, the maximum ethanol distillate was obtained from sequential culture of *A. niger* and bakery yeast without autoclaving while the minimum was from *bacillus* and Brewery yeast. The ethanol concentration is maximum for autoclaved and non autoclaved sequential culture of *A. niger* and bakery yeast (42.5% and 41.3 % v/v respectively). The minimum concentration of ethanol (25.5) was obtained from co-culture of *A. niger* and brewery yeast followed by *bacillus* and brewery yeast (27% v/v). The standardized pure ethanol expected in mL was maximum for non autoclaved sequential culture of *A. niger* and bakery yeast (12mL) followed by autoclaved one (11.9mL). From this study, the use of sequential culture of *A. niger* and bakery yeast is far better than the other combination used interms of quantity of bio-ethanol.

Key words: *Aspergillus niger*, bakery and brewery yeast, bio-ethanol, potato peel, *Saccharomyces cerevisiae*

INTRODUCTION

Due to the potential exhausting nature of fossil fuels and the increasing price of petroleum together with environmental concerns, the search for alternative renewable fuels has attracted great attention in worldwide (Sharma *et al*., 2008). Attention has been given to the conversion of biomass into fuel ethanol, which is the cleanest liquid fuel alternative to non-renewable fossil fuels (Lin *et al*., 2006). The demand of energy for transportation, heating and industrial processing is increasing from day to day. As demand for the limited global supply of nonrenewable energy resources increases, the price of oil and natural gas keep increasing. Hence, a new biotechnological approach for the production of ethanol by fermentation from the

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renewable carbohydrate materials for use as an alternative liquid fuel has been attracting worldwide interest (Ward and Singh, 2002).

In recent years bioenergy sources have become more important as available and economical alternative to diminishing and much expensive fossil fuels (Duhan *et al*., 2013). Bio-ethanol is the only liquid transportation fuels that do not contribute to the green house gas effect (Anuj, *et al*., 2007). The increasing demand for bio-ethanol for various industrial purposes such as alternative source of energy, industrial solvents, cleansing agents and preservatives has necessitated its increased production. Ethanol production is usually accomplished by chemical synthesis of petrochemical substrates and microbial conversion of carbohydrates present in agricultural products. Owing to depleting reserves and competing industrial needs of petrochemical feedstocks, there is global emphasis in ethanol production by microbial fermentation process. Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology (Brooks, 2008). Yeasts such as *Saccharomyces cerevisiae* is one of the most important microorganisms in the production of bio-ethanol due to its high ethanol yield, high tolerance to ethanol concentration, high selectivity, low accumulation of by-products, high fermentation rate, good tolerance to substrate concentrations and lower pH value (Muhamud *et al*., 2009). Several authors (Abouzied & Reddy, 1986, Oyeleke, *et al*., 2012., Duhan *et al*., 2013) combined *saccharomyces cerevisiae* with other group of saccharifying fungi such as *Aspergillus species, Zymomonas mobilis, Kluyveromyces spp., Trichoderma spp., Gloeophyllum sepiarium* and *Pleurotus ostreatus* to enhance production of bio-ethanol.

The study of Abouzied & Reddy (1986) showed that co-culturing of an efficient sugar fermenter such as *Saccharomyces cerevisiae* with an *Aspergillus* species in a starch medium would prevent accumulation of inhibitory concentrations of reducing sugar and hence enhance the amylolytic activity, the amount of starch metabolizable, and the total ethanol. In such a case *Aspergillus* species hydrolyze starch to glucose, and *S. cerevisiae*, which is nonamylolytic ferments glucose to ethanol under anaerobic condition. Yeast is a facultative anaerobe. In an aerobic environment, it converts sugars into carbon dioxide and water. In an anaerobic environment, it converts sugars into carbon dioxide and ethanol. Thus, for an ethanol industry, it is important to exclude significant oxygen from its system.

The commonest ways of producing ethanol is fermentation of feedstocks which are rich in sugar or starch such as sugarcane, sugar beet, sweet sorghum, corn and cassava (Balat *et al*., 2008). However, the major disadvantage of this process is that most of these crops are food crops and tend to increase the cost of production. In order to make the fermentation method cost effective and to meet the great demand for ethanol, research studies are now being directed in two areas namely, the production of ethanol from cheaper raw materials and the study of new microorganisms or yeast strains efficient in ethanol production (Akin-Osanaiye *et al*., 2008). Inexpensive raw materials such as agricultural wastes, municipal and industrial wastes can be used to produce ethanol (Akin-Osanaiye *et al*., 2008).

Potatoes are one of the alternative feedstock utilized for ethanol production (Duhan *et al*., 2013). Potatoes are starchy high value crops which do not require complex pretreatment. About 5-20% of waste potato byproducts from potato cultivation could be utilized for bio-ethanol production (Limatainen *et al*., 2004, Adarsha *et al.,* 2010). Besides its vast industrial application, potato peel is discarded from various kitchens, Cafeterias, and industries as zero value waste (Israilides *et al*., 2008). However, the potato peel contains sufficient amount of starch that can be directly converted to bio-ethanol. Starch substrates can be pretreated by physical, physicochemical, chemical and biological systems. However, biological pretreatment is cost effective, eco-friendly and mostly preferred. Biological pretreatment is carried out under mild reaction condition with few side reactions. The system has very low chemical consumption, less energy demand and less susceptibility to pressure and corrosion as compared to other pretreatments (Lee, 1997; Samsuri *et al*., 2008). So, this study was aimed to produce bio-ethanol from waste potato peel discarded from University of Gondar student's cafeteria using two steps process: saccharification and fermentation using sequential and co-culture methods.

MATERIALS AND METHODS

Processing of waste potato peel

Waste potato peel was collected from University of Gondar student cafeteria and washed thoroughly to remove dust and debris. The sample was then dried in oven for 36hr at 70ºC. The dried peel of potato was grind to fine powder using grinder. The grinded sample was then sieved to obtain fine flour and packed in plastic container until use.

Liquefaction, Saccharification and fermenting organisms

Bio-ethanol production commonly involve three steps: Liquefaction of starch by α- amylase or intact organisms, enzymatic (microbial) saccharification of liquefied product to produce glucose, fermentation of glucose to ethanol (Sree *et al*., 2004). For this study two fungal isolates namely: *Aspergillus niger* and *Saccharomyces cerevisiae* and one bacterial isolate known to posses starch degrading activity was selected. Pure culture of *Aspergillus niger* was obtained from Ethiopian Institute of Health (EIH) whilst *Saccharomyces cerevisiae* was obtained

Table 1: Fungal and bacterial culture used for study

Treatments	Combination of organism used	Culture type
1	*Bacillus sp*. and bakery yeast	Sequential
2	*Bacillus sp.* and brewery yeast	sequential
3	*Aspergillus niger* and brewery yeast	Co-culture
4	*Aspergillus niger* and bakery yeast	Co-culture
5	*Aspergillus niger* and bakery yeast	sequential

from Dashen brewery industry, Gondar, Ethiopia. Commercially available bakery yeast was also purchased from local market for comparative study with brewery yeast. Pure isolate of starch utilizing *bacillus species* was obtained from one of MSc student of Biotechnology. Starter culture of both *Aspergillus niger* and *Saccharomyces cerevisiae* were maintained on slant culture of Potato Dextrose Agar (PDA) supplemented with 40mg/mL chloramphenicol to inhibit the growth of unwanted bacteria and incubated at 30ºC for three days until the mycelium sporulates give black conidia for *A. niger* (Ado, *et al*. 2009 and Manas, *et al*. 2013). The bacterial isolate was maintained on nutrient agar incubated at 37 ^{o}C for 24 hours.

Fermentation condition

Different protocols were compared for maximum ethanol yield and concentration. Amongst them, the optimized protocol of Swain *et al.* (2013) was selected with very little modification. Fermentation was conducted using ethanol production parameters of (pH, 5.0; temperature, 30° C; initial moisture content 87%; incubation period of six days; $(NH4)_2SO_4$, 0.2%; inoculums size, 10% for yeast , 4% for *A. niger* and 2% for bacteria.

Preparation of inoculums

Inoculums of *A. niger* was prepared in 200mL of appropriate medium containing 2g of soluble starch, 2g of glucose, 2g of peptone, 0.4g of malt extract, 0.2g of magnesium chloride, 0.4g of calcium carbonate, 0.4g of ammonium phosphate and 0.002g of ferrous sulfate. Prior to inoculation, the pH was adjusted to 4.5 and the medium was properly autoclaved for 25 minutes at 121ºC. After medium was cooled at room temperature, the *A. niger* culture maintained on two potato dextrose agar slants was washed by few drops of autoclaved distilled water in sterile condition and transferred to 200mL of medium. Finally the inoculums culture was incubated for 48hrs at 30ºC with 120rpm shaking**.**

Saccharomyces cerevisiae inoculums were prepared in 250mL Yeast-mold Medium (YMM) containing 0.75g of yeast extract, 1.75g of peptone, 0.75g of malt extract and 2.5g of glucose. The medium pH was adjusted to 5 and properly autoclaved, cooled at room temperature and inoculated using two slant cultures maintained on PDA. Finally it was incubated for 24hr at 30ºc with 120rpm.

The bacterial inoculum was prepared in 50mL of nutrient broth (0.65g) containing 2g of soluble starch. The solution was stirred and the pH was adjusted to 6.5, autoclaved and cooled at room temperature. Then the medium was inoculated with 100µl of *bacillus* isolates and incubated for 24hr at 37ºc.

Fermentation medium

Sixty gram (60g) of potato peel powder was measured for all treatments and dissolved in 490 mL of distilled water (w/v) to bring the final moisture content 89% in 800mL screw capped culture bottle. 0.2% of $(NH_4)_2SO_4$ was added in each bottle to supplement nitrogen for the microbes (Swain, *et al.,* 2013). All duplicate media prepared for *A. niger* and yeast were adjusted to a pH of 4.75 whilst the media for bacillus as starter culture were adjusted to pH of 6.5. All media were autoclaved at 121ºc for 30 minutes. The media were then cooled and inoculated with appropriate amount of inoculums.

Culture techniques and treatments

Two types of culture techniques; namely sequential and co-culture methods has been employed for this study. Sequential culture involves prior inoculation of medium with selected starch degrading organisms to generate convertible sugars followed by inoculation of ethanol producing organisms. The sequential culture was conducted for *A. niger* and *S. cerevisiae* of bakery yeast, *bacillus species* and brewery yeast and *bacillus* and bakery yeast. The co-culture method was conducted for *A. niger* and brewery yeast and *A. niger* and Bakery yeast giving a total of five treatments each with duplicate (Table 1).

In case of sequential culture of *A. niger* with both brewery and bakery yeast, the fermentation medium was first inoculated with 4% *A. niger* starter culture and incubated at 30 ^{o}C for three days. 10% of *S. cerevisiae* inoculum of bakery and brewery yeast was then added in sterile condition in separate fermentation bottle and incubated at 30 ^{o}C for extra three days in anaerobic condition. For sequential culture of bacteria and *S. cerevisiae,* fermentation medium was first inoculated with 2% of bacterial inoculums and incubated at 37^{o}C for two days. After two days, the broth medium was autoclaved, cooled and inoculated with 10% bakery and brewery yeast separately added and finally incubated for three extra days in anaerobic condition.

For co-culture techniques; the inoculums of *A. niger* and brewery yeast with a ratio of 1:2.5% has been used to inoculate the fermentation medium at the same time.

Table 2: Mean fermented broth obtained on top of each fermentation bottle

Treatments	Culture	Culture type	Mean crude supernatant (mL)
1	Bacillus sp. and bakery yeast	Sequential	380
2	Bacillus sp. and brewery yeast	Sequential	340
3	*A. niger* and brewery yeast	Co-culture	74
4	*A. niger* and bakery yeast	Co-culture	120
	A. niger and bakery yeast	Sequential non autoclaved	240
5		Sequential autoclaved	105

Co-culture methods of *A. niger* and bakery yeast was prepared similar to brewery yeast. The inoculated media were then incubated at 30 ^{o}C for six days. All treatments were mixed to promote uniform utilization of substrate with 24 hour interval.

Distillation Process

After fermentation, distillation has been carried out using fractional distillation apparatus. The fermented 150mL of top fermented broth was transferred into round bottom flask and placed on a heating mantle fixed to a distillation column enclosed in running tap water. Another flask was fixed to the other end of distillation column to collect the distillate at 78^{0}C (standard temperature for ethanol production).

Analytical methods for bio-ethanol production

Different analytical methods were used for further analysis of bio-ethanol after distillation.

I. **Identification of bio-ethanol:** About 5mL distillate sample has been taken and pinch a of potassium dichromate and a few drop of H_2SO_4 was added following methods of Caputi, *et al.* (1959).

II. **Density of bio-ethanol:** Bio-ethanol was transferred to measuring cylinder set to zero reading on electronic balance. The weight and volume of ethanol was recorded, and the density calculated according to Ademiluy, *et al.* (2013) using the formula: Density (g/mL) = mass ethanol/Volume of ethanol.

III. **Ethanol concentration:** Ethanol concentration was determined following Perry's Chemical Engineers' Handbook, seventh edition (Perry *et al.*, 1997) available online at http://www.handymath.com/cgi-bin/ethanolwater3.cgi?submit=Entry. This method uses density of bio-ethanol after distillation and calculates corresponding ethanol percentage in each of distillates. The amount of ethanol in mL was then calculated from obtained density data.

IV. **pH Test:** pH meter was first calibrated and then placed into the ethanol produced. The readings was then taken (Ademiluy, *et al.,* 2013)

RESULT

Crude supernatant obtained after fermentation

According to Kimbrough (2000), there should be a clear visible liquid layer on top of the yeast layer in fermentation bottle. This layer contains most of the alcohol produced after proper fermentation. After six days of incubation, the top yellowish layer supposed to contain bio-ethanol was measured for all treatment and given as mean crude supernatant for all treatments (Table 2, Figure 1 and 2). The minimum crude supernatant (74mL) was obtained from co-culture of *A. niger* and brewery yeast whilst the maximum top crude supernatant (380mL) was recorded from the sequential culture of bacteria (bacillus) and bakery yeast. Treatments with bacillus species with both yeast strains using sequential inoculation produced comparatively higher top supernatant. For the culture of *A. niger* with both yeast strains, sequential culturing method was relatively better than the use of co-culture methods.

The sequential fermentation media of *A. niger* was separated into two and one set of broth was autoclaved after proper hydrolysis and saccharification while one set was remained without autoclaving. The bakery yeast inoculum was then added into each treatment. Interestingly, there was big difference in both autoclaved and non autoclaved medium. The fermented crude supernatant was reduced from 240mL to 105mL for autoclaved medium.

Distillation, concentration and quantity of bio-ethanol produced

About 150mL clear yellowish supernatant was measured and directly distilled using fractional distillation apparatus. The maximum crude ethanol (31mL) was obtained from sequential culture of *Aspergillus niger* and bakery yeast without autoclaving after hydrolysis followed by the coculture *of A. niger* and bakery yeast (30mL). The minimum crude ethanol (18mL) was recovered from the sequential culture of bacillus species with bakery yeast.

The crude concentration of distillate bio-ethanol was estimated from density of ethanol using Perry's Chemical Engineers' Handbook and standard curve of commercial ethanol diluted to different concentrations. The maximum concentration (42.5%) of ethanol was obtained from the culture of *A. niger* and bakery yeast following sequential method of hydrolysis and fermentation. However, the minimum concentration (25.5 %) was recovered from co-culture of *A. niger* and brewery yeast (Table 2). Though, the crude ethanol produced was minimum for bacillus and bakery yeast combination, however this combination produced 40% of crude ethanol preceded only by sequential culture of *A. niger* and bakery yeast. Upon

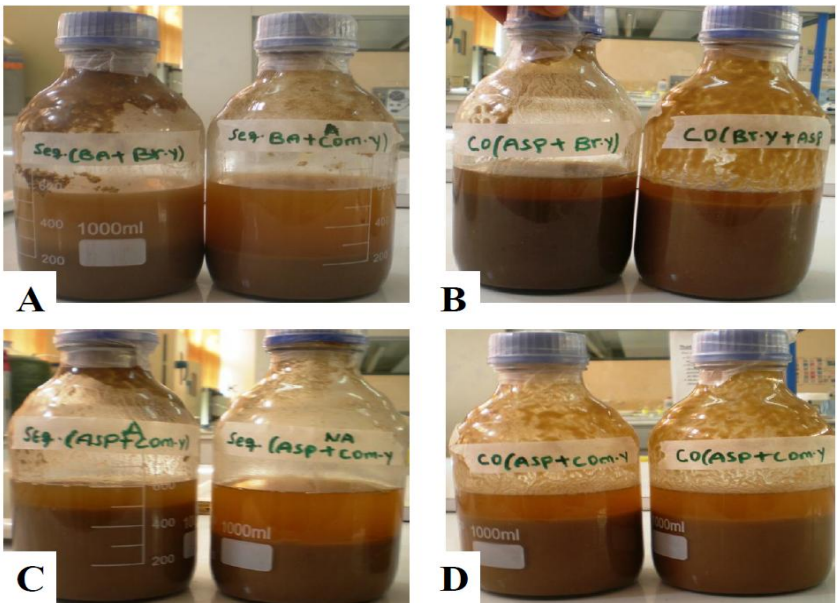

Figure 1. Fermentation of potato peel with sequential and co-culture of study organism (figure A left: sequential culture of *bacillus and brewery yeast*, right: sequential culture of *bacillus and bakery yeast*; **Figure B**: co-culture of *A. niger and brewery yeast* in duplicate; **Figure C**: left sequential culture of autoclaved *A. niger and bakery yeast* while the right contains non-autoclaved *A. niger*; **Figure D** indicates duplicate of co-culture of *A. niger and bakery yeast*).

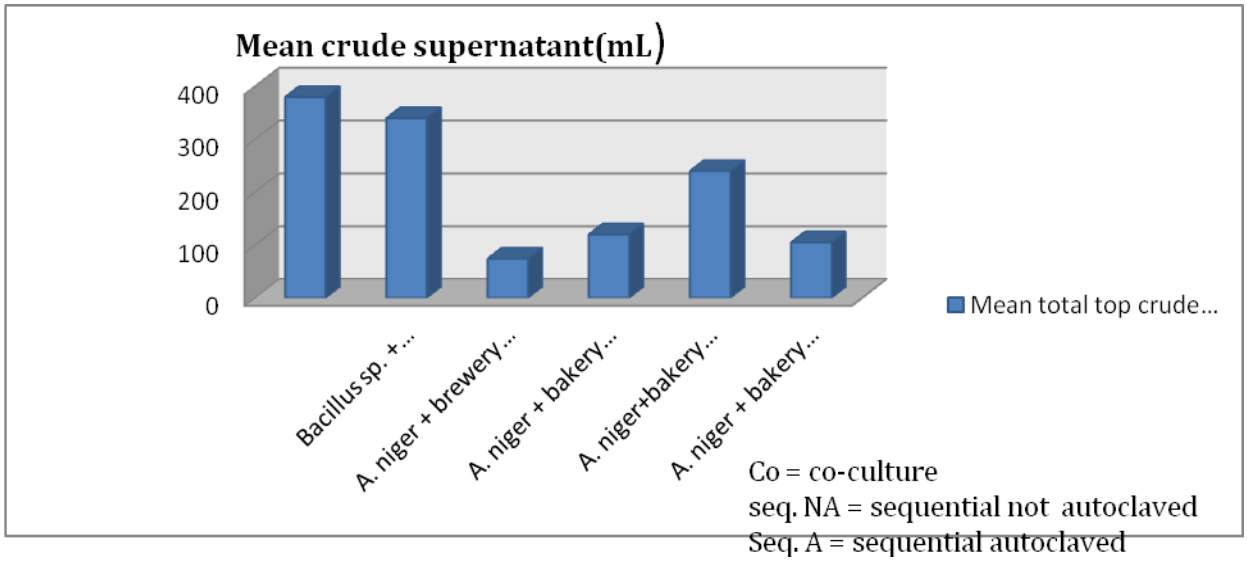

Figure 2: Top yellowish crude supernatant obtained at the end of fermentation

standardizing the distillate ethanol to absolute range, the maximum quantity of ethanol (12.8mL) was obtained from the sequential culture of *A. niger* (without autoclave) and bakery yeast followed closely by the same culture (11.9mL), containing autoclaved *A. niger* initially inoculated for hydrolysis of starch. Likewise, the minimum ethanol content (6.75mL and 7.2mL) was calculated for sequential culture of yeast strains with *bacillus species*. The pH value of crude ethanol ranged from 5.4 to 6.3.

Identification and final confirmatory test for bio-ethanol

Production of bio-ethanol was further confirmed using potassium dichromate test with the help of few drops of H_2SO_4 as indicated in Caputi, *et al*. (1959). The color of the crude distillate was changed from pink (dichromate color) to green. The formation of green color is strong

Table 3: crude ethanol obtained concentration, pH, and standardized pure ethanol expected.

No.	Culture	Culture type	Crude ethanol from 150mL	pH	Concentration of ethanol (%)	absolute ethanol concentration expected (mL)
1	Bacillus sp. and bakery yeast	Sequential	18	5.86	40	7.2
2	Bacillus sp. and brewery yeast	Sequential	25	5.9	27	6.75
3	*A. niger* and brewery yeast	Co-culture	29.5	6.0	25.5	7.39
4	*A. niger* and bakery yeast	Co-culture	30	5.5	34.8	10
5	*A. niger* and bakery yeast	Sequential non autoclaved	31	5.4	41.3	12.8
		Sequential autoclaved	28	6.3	42.5	11.9

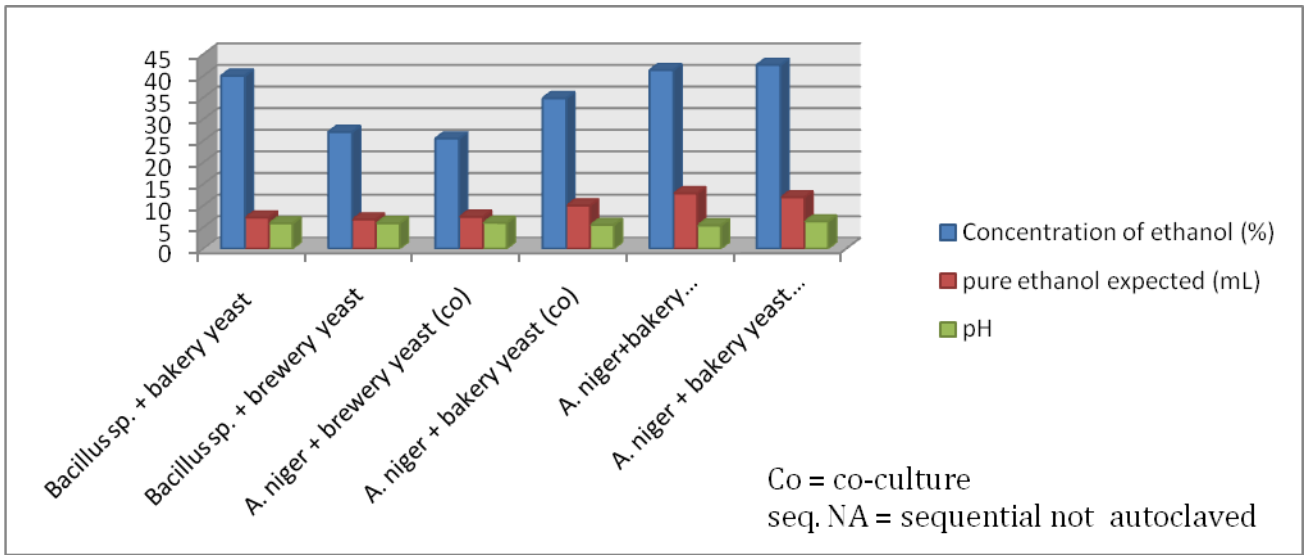

Figure 3: concentration of crude ethanol (in %), pH and quantity of pure ethanol expected when converted to absolute (dehydration)

Table 4: Density and concentration of ethanol in water

No.	Culture	Culture type	Density (g/mL)	Concentration of ethanol (v/v)	Concentration of ethanol in water (%)
1	Bacillus sp. and bakery yeast	Sequential	0.935	0.4	40
2	Bacillus sp. and brewery yeast	sequential	0.959	0.27	27
3	*A. niger* and brewery yeast	Co-culture	0.961	0.255	25.5
4	*A. niger* and bakery yeast	Co-culture	0.953	0.348	34.8
5	*B. niger* and bakery yeast	Sequential non autoclaved	0.933	0.413	41.3
		Sequential autoclaved	0.930	0.425	42.5

evidence for existence of ethanol in crude primary distillate.

DISSCUSION

In the present work bio-ethanol production was studied using *Aspergillus niger*, *bacillus* spp. and Yeast *Saccharomyces cerevisiae* in co-culture and sequential culture condition. The *A. niger and bacillus* were selected for saccharification as they have great potential to hydrolyze starchy substrates to simple sugars. Though this step can be carried out by commercially available alpha- amylase the process is very expensive for large scale production (Ali *et al.*, 2011). In this study, an attempt was made to design an economical process by

Figure 4: Overall flow chart of bio-ethanol processing from potato peel

the use of intact fungal and bacterial organism as a source of amylases instead of commercially available enzyme. For fermentative production of bio-ethanol yeast *Saccharomyces cerevisiae* of bakery yeast and brewery yeast were employed. Several studies recommend the use of *Apergillus niger* and *saccharomyces cerevisiae* (Abouzied, *et al.,* 1986; Ado, *et al.,* 2009) for co-culture production of bio-ethanol.

In co-culture condition, 60g of potato peel flour was dissolved in distilled water to bring 87% moisture content with little increment from 80% of optimal condition of Swain *et al.* (2013). 4% of *A. niger*, and 10% of *S. cerevisiae* was then added at the same time and incubated for six days. In sequential culture, the same amount of medium was first inoculated with 4% for *A. niger* and 2% for *bacillus* since bacteria are fast grower than fungi. 10% of *S. cerevisiae* inoculum was then applied after 3 days and incubated for extra three days for fermentation.

Kimbrough (2000), stated that a clear visible liquid supernatant on top of the yeast layer in fermentation bottle contains most of the alcohol produced after proper fermentation, Our result indicated that the maximum crude supernatant (380mL) was obtained from the sequential culture of bacillus bacteria with bakery yeast followed by sequential culture of bacillus with brewery yeast (340mL). This variation is may be due to substrate specificity of brewery yeast as compared to bakery yeast which became superior in this result. For fungal sequential culture (*A. niger* with bakery yeast), the maximum crude supernatant (240mL) was obtained when *A. niger* was not autoclaved after saccharification and co-used for anaerobic fermentation with bakery yeast. However, the supernatant liquid was decreased to 150mL when *A. niger* was autoclaved prior to inoculation with bakery yeast. The possible reduction of supernatant might be due to incomplete hydrolysis of starch at a given saccharification time that can also be converted even after addition of bakery yeast. The least crude extract 74mL was recorded from co culture of *A. niger* and brewery yeast. Our result clearly indicated that, the use of sequential culture is 68% more efficient than the co-culture depending on the value of supernatant obtained.

The maximum ethanol quantity interms of concentration (42.5%) was obtained from sequential autoclaved culture of *A. niger* and *bakery yeast* followed by sequential non autoclaved culture of the same combination (41.3%) when the same amount of (150mL) of supernatant was distilled irrespective of the amount of supernatant obtained. The concentration of bio-ethanol from the autoclaved one was 42.5% (v/v) and the non autoclaved was 41.3% (v/v) when the same amount of supernatant was distilled. Similarly, Hongzhi *et al.* (2009) obtained highest concentration of (50g/L) from autoclaved one and lower for non autoclaved (4g/L). Our result is higher than maximum quantity of ethanol produced (26%) by Oyeleke *et al.* (2012) from cassava peel using *G. sepiarium* and *P. ostreatus* for hydrolysis and *Z. mobilis* and *S. cerevisiae* for fermentation. However, Jubril, (2009) reported 63.8% for *A. niger* and *Z. mobilis* when used simultaneously for millet. Duhan *et al*. (2013) obtained maximum ethanol concentration of 7.89% (v/v)

from potato using 10% inoculum size of *Saccharomyces cerevisiae* alone from potato at pH 6 after 48 h. This large variation is due to only one organism he selected, short fermentation time and improper pH (6) used. Agunlejika, *et al*. (2005), also reported average ethanol concentration of 16% from spoilt fruits in contrast to the higher value in present result.

The result of co-culture and sequential culture of *A. niger* and bakery yeast indicated that the maximum supernatant, and concentration of ethanol (v/v) was produced from sequential culture.

Though the total crude supernatant of bacillus and bakery yeast was very high, the sample gave minimum quantity (18mL) of ethanol after distillation. The sequential culture of bacillus probably might contain other denser side product that decreased quantity of distillate interms of mL. The maximum crude ethanol distillate was obtained for sequential culture of *A. niger* and *S. cerevisae* (31mL) as indicated in table 3. The concentration of crude ethanol in water was standardized to absolute range and presented interms of absolute ethanol expected after proper removal of water. The maximum standardized pure bio-ethanol (12.8mL) was obtained for non autoclaved sequential culture of *A. niger* and Bakery yeast followed by autoclaved sequential culture of the same organisms (11.9mL).

CONCLUSION

Potato Peel is waste materials that have ability to pollute environment. Potato peel waste is available in plenty in every food industries and cafeterias. However, its commercial potential for fuel ethanol has not been reported in Ethiopia. Being a cheap source of fermentable carbohydrate bio-resource, it could be employed for the production of fuel ethanol. In present study two culture techniques, namely: sequential and co-culture has been utilized with three organism having potential of hydrolysis and fermentation. Based on the result, the best result interms of distillate quantity, concentration and standardized pure ethanol was obtained for sequential culture of *A. niger* and bakery yeast (medium was incubated with *A. ni*ger for saccharification for three days and addition of bakery yeast and fermentation for extra four days). Nutshell, this study showed that potato peel has sufficient quantity of starch that can be used for bio-ethanol production and has to be emphasized.

ACKNOWLEDGEMENTS

The authors acknowledge University of Gondar, department of biotechnology for research facilities and financial support; Dashen Brewery factory for provision of yeast strains; Ethiopian Institute of Health for provision of pure isolates of *Asperigillus niger;* Mr. Gebrekidan Weldegerema for provision of isolates of *bacillus* bacteria.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interest.

REFERENCES

Abouzied, MM and Reddy CA (1986). Direct fermentation of potato starch to ethanol by cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae*.. *Applied and Environmental Microbiology*, 52(5): 1055-1059.

Adarsha R, Asha DL, Balaji RR (2010). Production of bio-ethanol from *Pectobacterium carotovorum* induced soft rotten potatoes. *Afr. J. Microbiol. Res*. 4(12): 1340-1342

Ademiluy FT and Mepba HD (2013). Yield and Properties of Ethanol Biofuel Produced from Different Whole Cassava Flours. *Hindawi Publishing Corporation, ISRN Biotechnology*

Ado SA, Olukotun GB, Ameh JB & Yabaya A (2009). Bioconversion of cassava starch to ethanol in a simultaneous *saccharification* and fermentation process by co-cultures of *Aspergillus niger* and *Saccharomyces cerevisiae*. *Science World Journal, 4(1)* : *19-22*

Akin-Osanaiye BC, Nzelibe HC and Agbaji AS (2008). Ethanol production from *Carica papaya* (Pawpaw) fruit waste*. Asian J. Biochem.* 3(3):188-193.

Ali MN, Mohd MK and Mohiuddin M (2011). Ethanol fuel production through microbial extracellular enzymatic hydrolysis and fermentation from renewable agro based cellulosic wastes*. International Journal of Pharma and Bio-Sciences,* 2(2)

Anuj KC, Ravinder R, Lakshmi MN, Rao V and Ravindra P (2007). Economic and environmental impact of bio-ethanol production technology. *Biotechnology and Molecular Biology Review.*, 2(1): 14-32.

Balat M, Balat H and Oz C (2008). Progress in bio-ethanol processing. *Progress in Energy and Combustion Sci.* 34: 551-573.

Brooks AA (2008). Ethanol production potential of local yeast strains isolated from ripe banana peels. *Afri. J. Biotechnol.,* 7 (20) 3749-3752

Caputi A, Veda M and Brown T (1959). Spectrophotometric determination of ethanol in wine. American. *J. Enol. Viticul,* 19: 160-165

Duhan JS, Kumar A, and Tanwar SK (2013). Bio-ethanol production from starchy part of tuberous plant (potato) using *Saccharomyces cerevisiae* MTCC-170. *Afr. J. Microbiol. Res*. 7(46) : 5253-5260

Hongzhi, M., Qunhui, W., Dayi, Q., Lijuan, G.,Wenyu, Z. (2009). The utilization of acid-tolerant bacteria on ethanol production from kitchen garbage. Renewable Energy, 34(6): 1466-1470.

Israilides C, Arapoglou D, Varzakas T and Marchant R (2008). Integrated management of potato starch wastes , Institute of technology of Agricultural products ,Greece

Kimbrough D (2000). Fermentation and Distillation

Lee J (1997). Biological conversion of lignocellulosic biomass to ethanol. J. Biotechnol. 56:1-24.

Limatainen H, Kuokkanen T, Kaariainen J (2004). Development of bio-ethanol production from waste potatoes. In: Pongracz E (ed.) Proceedings of the Waste Minimization and Resources Use Optimization Conference. University of Oulu, Finland. Oulu University Press: Oulu. pp 123-129.

Lin Y & Tanaka S (2006). Ethanol fermentation from biomass resources: current state and prospects. *Appl. Microbiol. Biotechnol.*, 69, 627-42.

Manas R, Jyoti M and Hrudayanath T (2013). Bio-ethanol Production from Sweet Potato (Ipomoea batatas L.) Flour using Co-Culture of Trichoderma sp. and Saccharomyces cerevisiae in Solid-State Fermentation. *An International Journal*. Vol.56, n.2: pp. 171-179

Muhamud F and Bin I (2009). *Production of Bio-ethanol from Tapioca Starch using Saccharomyces cerevisiae.* University of Malaysia Pahang, Malaysia.

Oyeleke SB, Dauda BEN, Oyewole OA, Okoliegbe IN and Ojebode T (2012). Production of Bio-ethanol from cassava and sweet potato peels. *Advances in Environmental Biology*, 6(1): 241-245

Perry RH, Green D, Maloney JO (1997). *Perry's Chemical Engineers' Handbook., 7th edition*. McGraw-Hill Companies, USA.

Samsuri M, Gozan M, Hermansyah H, Prasetya B, Nasikin M and Watanabe T (2008). Ethanol production from bagasse with combination of cellulase- cellubiase in simultaneous saccharification and fermentation using white rot fungi pretreatment. J. Chem. Nat. Resource Eng. 3:20-32.

Sharma YC, Singh B & Upadhyay SN (2008). Biofuels: Global Impact on Renewable Energy, Production Agriculture, *Fuel*, 87,2355-2373.

Sree NK, Sridhar M, Suresh K, Bannat IM, Rao LV (2004). High alcohol production by repeated batch fermentation using an immobilized osmotolerant Saccharomyces cerevisiae. *J. Ind. Microbiol.,* 24: 222-226

Swain MR, Mishra J and Thatoi H (2013). Bio-ethanol Production from Sweet Potato (*Ipomoea batatas L.*) Flour using Co-Culture of Trichoderma sp. And Saccharomyces cerevisiae in Solid-State Fermentation. Braz. Arch. Biol. Technol. v.56 n.2: pp. 171-179

Ward OP, Singh A (2002). Bio-ethanol technology: developments and perspectives. *Adv. Appl. Microbiol.* 2002; 51: 53–80.