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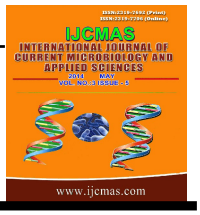

Original Research Article

Bio-Ethanol Production from Banana peel by Simultaneous Saccharification and Fermentation Process using cocultures *Aspergillus niger* and *Saccharomyces cerevisiae*

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A B S T R A C T

Keywords

Aspergillus niger, *Saccharomyces cerevisiae*, Simultaneous Saccharification and Fermentation (SSF)

Simultaneous Saccharification and Fermentation (SSF) of banana peels to ethanol by cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae* was investigated at different temperatures (20°C to 50°C) and at different pH (4 to 7). Fermentation was done for 7 days for banana peels and the ethanol content was measured every 24 hours. The optimum pH and temperature for the fermentation of banana peels was found to be 6 and 30°C. With the optimized pH and temperature, fermentation was then carried out at different yeast concentration 3% to 12%. With the change in the concentration of yeast, the time required for the completion of fermentation decreased dramatically. Using a 12%, 9%, 6%, 3% yeast inoculum, maximum ethanol production was completely achieved in 2, 3, 5, 7 days respectively.

Introduction

Bioethanol as an alternative source of energy has received special attention world wide due to depletion of fossil fuels. In India, sugar cane molasses is the main raw material for ethanol production. But the short supply and increased cost is the main hindrance for its use. The cellulosic materials are cheaper and available in plenty but their conversion to ethanol involves many steps and is therefore expensive. Under such circumstances a novel approach is essential to use renewable substrates such as fruit waste.

Banana is one of major constitute the principal food resources in the world and occupy the fourth world rank of the most significant foodstuffs after rice, corn and milk (INIBAP, 2002). Most of the fruit peels/residues are dried, ground, pelletized, and sold to the feed manufacturers at a low price which is not considered a highly viable proposition (Mamma *et al.,* 2008). As per the FAO statistics, India is the largest producer of banana in the world and accounts for nearly 30% of the total world production

of banana. Though banana peel is a fruit residue, it accounts for 30–40% of the total fruit weight (Emaga *et al.*, 2008) and contains carbohydrates, proteins, and fiber in significant amounts. Banana peels are readily available agricultural waste that is under utilized as potential growth medium for yeast strain, despite their rich carbohydrate content and other basic nutrients that can support yeast growth (Brooks, 2008; Essien *et al*., 2005; Hueth and Melkonyan, 2004). Since banana peels contain lignin in low quantities (Hammond *et al.*, 1996), it could serve as a good substrate for production of value-added products like ethanol.

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 (Favela-Torres *et al*., 1986; Pandey *et al*., 2000; Akin-Osanaiye *et al*., 2008). In this respect, inexpensive raw materials such as agricultural wastes, cellulosic wastes, fruit wastes, vegetable wastes, municipal and industrial wastes can be used to produce ethanol cheaply (Park and Baratti, 1991; Schugerl, 1994; Joshi *et al*., 2001; Akin-Osanaiye *et al*., 2008). Increased yield of ethanol production by microbial fermentation depends on the use of ideal microbial strain, appropriate fermentation substrate and suitable process technology.

An ideal microorganism used for ethanol production must have rapid fermentative potential, improved flocculating ability, appropriate osmotolerant, enhanced ethanol tolerance and good thermo tolerance (Benitez *et al*., 1983; Divanya *et al*., 1992). In most of these studies the preferred candidate for industrial production of ethanol has been *S. cerevisiae*. Yeast also has the ability to produce ethanol which is not contaminated by other products from the substrate (Jones *et al*., 1981).

The production of industrial and fuel ethanol from starchy biomass commonly involves a three-step process (Laluce and Mattoon, 1984) : (i) liquefaction of starch by an endoamylase such as α-amylase; (ii) enzymatic saccharification of the low-molecular-weight liquefaction products (dextrins) to produce glucose; and (iii) fermentation of glucose to ethanol. Commercial amylases (frequently those produced by *Aspergillus* species) are used for liquefaction and saccharification of starch and represent a significant expense in the production of fuel alcohol from starchy materials.

Fruits are highly perishable products, currently most of the perishable fruits are lost during their journey through the agrifood chain, due to spillage, physiological decay, water loss, mechanical damage during harvesting, packaging and etc so recent years effort have been directed towards the utilization of cheap and renewable agricultural sources such as banana peels waste as an alternative substrate for production of alternative biofuel like ethanol.

The purpose of this study is the elimination of the enzymatic liquefaction and saccharification step by using symbiotic co- cultures of amylolytic and sugar-fermenting organisms and to evaluate a single-step system for the enhanced fermentation of banana peels to ethanol by using symbiotic cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae*.

Materials and Methods

Isolation of microorganisms and its maintenance

Soil samples were collected randomly from the top 2 cm of the soil profile at three different places. Approximately 50 g of soils were collected from each site and put into plastic bags and brought to the laboratory. Soil samples were air-dried at room temperature (27±1°C) for 24 to 48 h. The dried soil samples were processed to remove stones and plant residues. 100 mg of each soil samples were transferred to labeled test tubes containing five milliliters of sterile saline (0.9% NaCl) (Knudsen *et al*., 1995). In order to suppress bacterial growth, 30 mg/l of streptomycin was added. The test tubes were vortex mixed until. 100 µl of each of the suspension was evenly spreaded on PDA plates with a spreader and incubated at 27±1°C. Mixed colonies on the plates were observed after 5–7 days. Pure culture of *Aspergillus niger* was obtained by streak plate method. It was then maintained on PDA slants at 4^{o}C. Yeast strain *Saccharomyces cerevisiae* (Bakers yeast) (Kwality, India) was obtained from the local market.It was maintained on PDA slants at 4^{o}C.

Starch hydrolysis test of isolated strains of *Aspergillus niger*

An inoculum from a pure culture was streaked on a sterile plate of starch agar. The inoculated plate was incubated at 27°C for 5 to 7 days. Iodine reagent was then added to flood the growth. Presence of clear zone surrounding colonies confirmed the positive result and accounts for their ability to digest the starch and thus indicates presence of alpha-amylase.

Pretreatment of Banana peel substrates

Banana peel wastes were procured from local market in Allahabad, Uttar Pradesh, India. Before processing ripe waste banana peels, it was cleaned, chopped (3-5 cm) and disinfected with 70% ethanol. It was sun dried for 7 days and ground to fine powder.

Simultaneous Saccharification and Fermentation (SSF) of Banana peels

Ethanol fermentation was carried out in 200 ml flasks containing 5g powdered banana peels in 96 ml distilled water. The flasks were sterilized by autoclaving at 121°C for 30 min and a 4% (v/v) inoculum of *Aspergillus niger* and 3% (w/v) inoculum of *Saccharomyces cerevisiae* was added. Fermentation was done for 7 days and the ethanol content was measured every 24 hours.

Effect of temperature, pH and yeast inoculum on ethanol production

Fermentation of banana peels was carried out at different temperatures (20°C to 50°C) at pH 6 and at different pH (4 to 7) at 30°C. The optimum temperature and pH obtained during the course of investigation was used for fermentation at different yeast concentration 3% to 12%.

Estimation of ethanol content by gas chromatograph

A gas chromatograph (Chemito, 2000) equipped with a flame ionization detector (FID) and data acquisition system with computer software (IRIS 32) was used to analyze the ethanol concentration. The installed column was a Capillary column (30 m). Temperature programming was implemented for the liquid sample

analysis. During the analysis, the oven temperature was maintained at 80°C. The injector and detector temperatures were 120 and 160°C, respectively. The flow rate for carrier gas (Nitrogen) was set at 30 ml/min. The injection sample volume was 0.2 µl. The volume of standard ethanol used was 0.2 µl. The area of standard ethanol was found to be 1500. In each set of experiments, the data points were reported.

The formula used for the calculation of percentage of ethanol is given below.

$$Conc.\ of\ Ethanol = \frac{Volume\ of\ Standard\ ethanol \times Area\ of\ unknown\ sample}{Area\ of\ standard\ Ethanol}$$

$$\% \text{ of Ethanol} = 100 - \left(\frac{Volume\ of\ Standard\ ethanol \times Conc.\ of\ Ethanol}{Volume\ of\ Standard\ ethanol} \times 100 \right)$$

Results and Discussion

The result of the investigation showed that the fermented banana peels produced a significant amount of ethanol. The volumetric production of ethanol was varied according to the variations in temperature, pH and at different yeast concentrations. It was also varied according to fermentation time and fungal strains.

Effect of pH on ethanol production

The ethanol production of inoculated samples was studied for 7 days regularly and the observations were noted down. The percentage of ethanol production from banana peels at 24 hours interval for seven days at different pH by *Aspergillus niger* strain A, strain B and strain C is indicated in table 1 - 3 respectively. The variation in ethanol yield from banana peels for different *Aspergillus niger* strains at pH 6 is indicated in table 4. The variation in ethanol yield from banana peels with the change in pH (4 to 7) for seven days by *Aspergillus niger* strain B is indicated in figure 1. The variation in ethanol yield from banana peels for different *Aspergillus niger* strains at pH 6 is indicated in figure 2. *Aspergillus niger* strain B was found to be efficient strain yielding a higher value of ethanol production as compared to other *Aspergillus niger* strains A and C. The highest bioethanol production was shown by *Aspergillus niger* strain B at pH 6 (6.287%) followed by pH 5 (5.638%), pH 7 (2.877%) and pH 4 (2.364%). Mohamed and Reddy (1986) has reported that the ethanol production from potatoes by cocultures of *Aspergillus niger* and *Saccharomyces cerevisiae* was optimal in the pH range 5 to 6. Neelakandan and Usharani (2009) has reported that the maximum ethanol yield from cashew apple juice using immobilized yeast cells by *Saccharomyces cerevisiae* was obtained at pH 6. Shafaghat *et al.* (2010) has reported that the maximum ethanol production from molasses was achieved at pH 5.6 by *Saccharomyces cerevisiae*. Togarepi *et al*. (2010) reported that the rate of ethanol production was maximum at pH 6. Mark *et al.* (2007) reported that fermentations at initial pH 6.0 produced the most ethanol. Jannani *et al*.(2013) also reported maximum ethanol production at pH 5.4 from grape fruit waste by using *Saccharomyces cerevisiae.* Ado *et al*. (2009) found maximum ethanol production at pH 5 corn cobs using cocultures of *Saccharomyces cerevisiae* and *Aspergillus niger*. Shilpa *et al*. (2013) reported maximum ethanol production from banana peels at pH 5.5. Thippareddy

and Agrawal (2010) also produced maximum ethanol at pH 5.5 followed by pH 6 by using *Aspergillus niger* for hydrolysis and *Saccharomyces cerevisiae* for fermentation of agriculture waste.

Effect of temperature on ethanol production

The ethanol production of samples was studied for inoculated sample for 7 days regularly and the changes were noted down. Percentage of ethanol production from banana peels at 24 hours interval for seven days at different temperatures by *Aspergillus niger* strain A, strain B and strain C is indicated in table 5-7 respectively. The variation in ethanol yield from banana peels by different *Aspergillus niger* strains at 30ºC is indicated in table 8. The variation in ethanol yield from banana peels with the change in temperature (20ºC to 50ºC) for seven days by *Aspergillus niger* strain B is indicated in figure 3. The variation in ethanol yield from banana peels by different *Aspergillus niger* strains at 30ºC is indicated in figure 4. *Aspergillus niger* strain B was the most efficient strain yielding a higher value of ethanol as compared to other *Aspergillus niger* strains. It was observed that the maximum ethanol production was at temperature 30ºC with 6.434%, followed by 40ºC, 20ºC and 50ºC in which bioethanol was decreased to 5.691%, 2.637% and 1.957% respectively. Hadeel *et al*. (2011) reported that the maximum ethanol production from rambutan fruit biomass using yeast *Saccharomyces cerevisiae* was at temperature 30ºC. Neelakandan and Usharani (2009) reported that the maximum ethanol yield from cashew apple juice using immobilized yeast cells by *Saccharomyces cerevisiae* was obtained at 32ºC. Manikandan and Viruthagiri (2010) reported that in the ethanol production from corn flour *Aspergillus niger* and non starch-digesting and sugar-fermenting *Saccharomyces cerevisiae,* the optimum value of the temperature was found to be 30ºC. Togarepi *et al.* (2010) reported that a maximum rate of ethanol production was achieved at a temperature of 30 ºC. Thippareddy and Agrawal (2010) also observed maximum ethanol at temperature 30°C by using *Aspergillus niger* and *Saccharomyces cerevisiae* from agriculture waste. Magdy *et al*. (2011) reported that temperature in the range of 25-30°C is commonly found optimum for thermophilic *S. cerevisiae* strain for production of ethanol in SSF of various substrates, i.e. apple pomace (Hang *et al*., 1986), carob pod (Roukas, 1994), sweet sorghum (Kargi and Curme, 2004). Manikandan *et al*. (2008) reported maximum ethanol production at temperature 33°C followed by 30°C. Jannani *et al*. (2013) also reported maximum ethanol production at Temperature 30°C from banana waste by using *Saccharomyces cerevisiae*

Variation of ethanol production due to yeast concentration

The ethanol production of samples was studied for inoculated sample for 7 days regularly and the changes were noted down. With the increase in the concentration of *Saccharomyces cerevisiae,* the time required for the completion of fermentation decreased dramatically. Using a 12%, 9%, 6% and 3% yeast inoculum, maximum ethanol production was completely achieved in 2, 3, 5, 7 days respectively. The percentage of ethanol production from banana peels at 24 hours interval for seven days at different yeast concentrations by *Aspergillus niger* strain A, strain B and strain C is indicated in table 9-11

Table.1 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different pH by *Aspergillus niger* strain A at 30ºC

	Production of Ethanol (in %)						
pH / Days	1	2	3	4	5	6	7
4	0.344	0.497	1.025	1.213	1.745	2.142	2.263
5	0.289	0.512	0.767	0.874	0.996	1.141	1.161
6	0.638	0.891	2.377	3.010	4.800	5.733	6.005
7	0.511	0.615	2.137	3.032	3.973	5.006	5.365

Table.2 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different pH by *Aspergillus niger* strain B at 30ºC

	Production of Ethanol (in %)						
pH / Days	1	2	3	4	5	6	7
4	0.386	0.478	1.144	1.367	1.898	2.212	2.364
5	0.557	0.850	2.368	3.148	4.204	5.421	5.638
6	0.645	1.001	2.888	3.457	4.997	5.923	6.287
7	0.525	0.753	1.355	1.657	2.341	2.772	2.877

Table.3 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different pH by *Aspergillus niger* strain C at 30ºC

	Production of Ethanol (in %)						
pH / Days	1	2	3	4	5	6	7
4	0.348	0.435	0.944	1.227	1.835	1.951	2.006
5	0.365	0.544	1.900	2.730	3.543	4.072	4.440
6	0.506	0.933	2.239	2.948	4.289	5.295	5.466
7	0.421	0.633	1.013	1.453	1.676	2.125	2.336

Table.4 Percentage of ethanol production from banana peels at 24 hours interval for seven days by different *Aspergillus niger* strains at pH 6 and at 30ºC

	Production of Ethanol (in %)						
Strain / Days	1	2	3	4	5	6	7
A	0.638	0.891	2.377	3.010	4.800	5.733	6.005
B	0.645	1.001	2.888	3.457	4.997	5.923	6.287
C	0.506	0.933	2.239	2.948	4.289	5.295	5.466

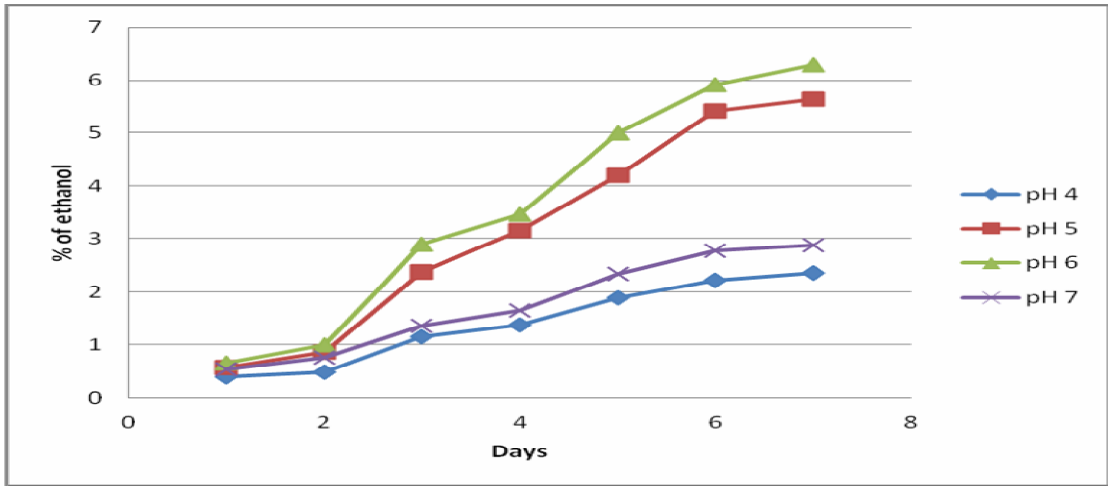


Figure.1 Variation in ethanol yield from banana peels with the change in pH (4 to 7) for seven days by *Aspergillus niger* strain B at 30ºC

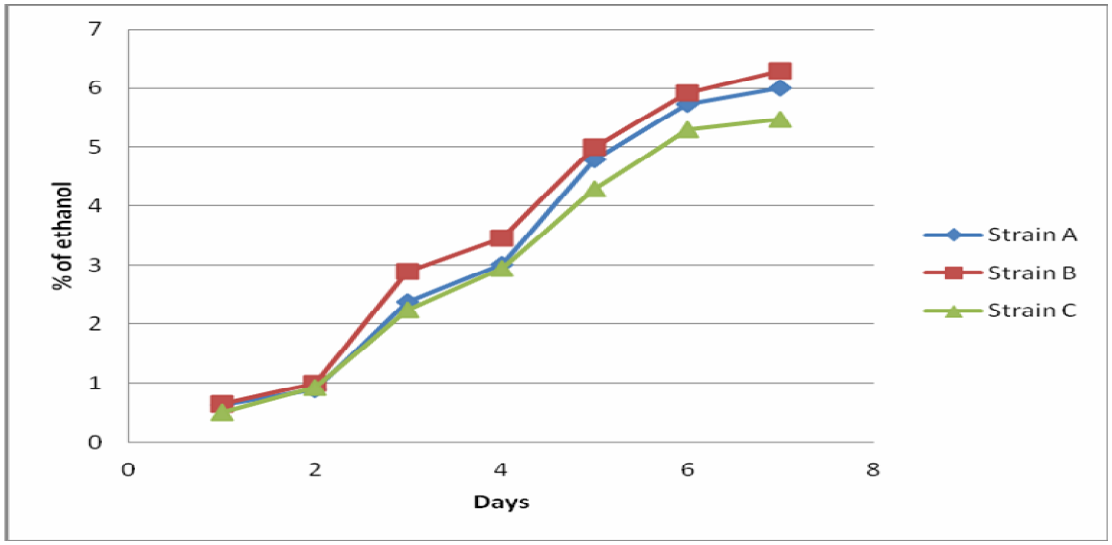


Figure.2 Variation in ethanol yield from banana peels by different *Aspergillus niger* strains at pH 6 and at 30ºC

Table.5 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different temperatures by *Aspergillus niger* strain A at pH 6

	Production of Ethanol (in %)						
Temp / Days	1	2	3	4	5	6	7
20ºC	0.455	0.573	0.958	1.250	1.848	2.239	2.509
30ºC	0.527	0.979	2.492	3.328	4.678	5.514	6.146
40ºC	0.477	0.738	2.289	2.824	3.445	5.071	5.507
50ºC	0.342	0.584	1.031	1.310	1.707	1.733	1.868

Table.6 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different temperatures by *Aspergillus niger* strain B at pH 6

	Production of Ethanol (in %)						
Temp / Days	1	2	3	4	5	6	7
20ºC	0.42	0.541	1.214	1.486	1.882	2.417	2.637
30ºC	0.605	1.131	2.888	3.474	4.820	5.797	6.434
40ºC	0.566	0.852	2.352	3.025	4.046	5.408	5.691
50ºC	0.386	0.445	1.078	1.284	1.676	1.854	1.957

Table.7 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different temperatures by *Aspergillus niger* strain C at pH 6

	Production of Ethanol (in %)						
Temp / Days	1	2	3	4	5	6	7
20ºC	0.335	0.497	0.804	1.053	1.362	1.565	1.684
30ºC	0.413	0.728	2.423	3.186	4.380	5.010	5.501
40ºC	0.597	0.710	1.759	2.499	2.967	3.805	4.441
50ºC	0.380	0.500	0.735	0.981	1.100	1.267	1.333

Table.8 Percentage of ethanol production from banana peels at 24 hours interval for seven days by different *Aspergillus niger* strains at pH 6 and at 30ºC

	Production of Ethanol (in %)						
Strain / Days	1	2	3	4	5	6	7
A	0.527	0.979	2.492	3.328	4.678	5.514	6.146
B	0.605	1.131	2.888	3.474	4.820	5.797	6.434
C	0.413	0.728	2.423	3.186	4.380	5.010	5.501

Figure.3 Variation in ethanol yield from banana peels with the change in temperature (20ºC to 50ºC) for seven days by *Aspergillus niger* strain B at pH 6

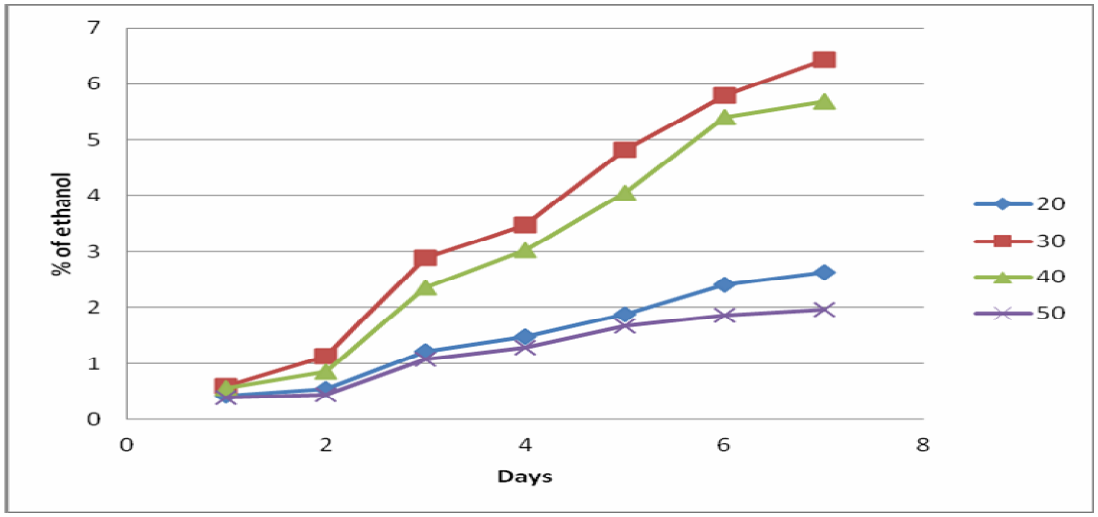


Figure.4 Variation in ethanol yield from banana peels by different *Aspergillus niger* strains at pH 6 and at 30ºC

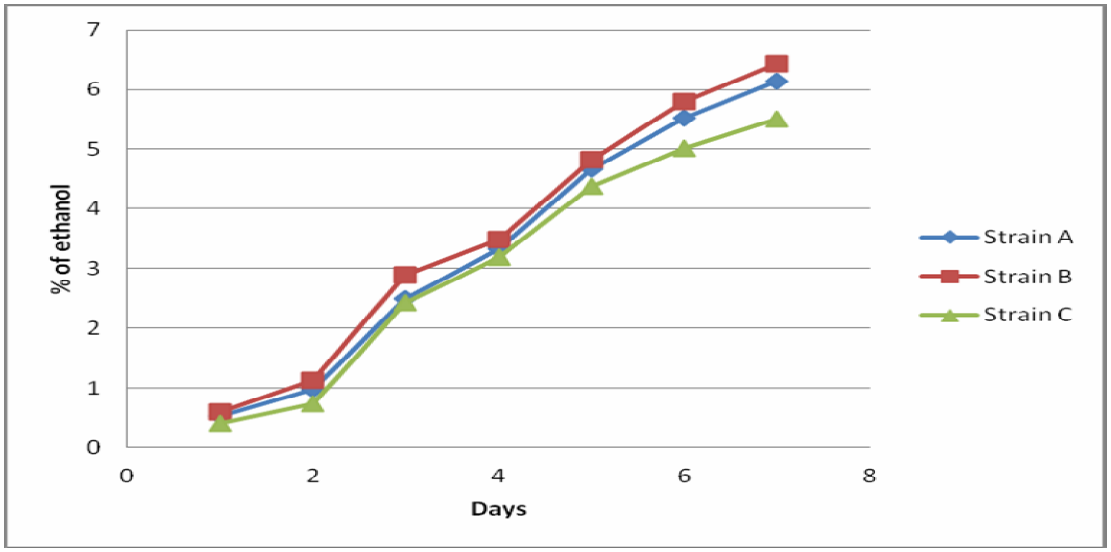


Table.9 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different yeast concentrations by *Aspergillus niger* strain A at the optimum temperature (30ºC) and at the optimum pH (pH 6)

	Production of Ethanol (in %)						
Yeast Conc. / Days	1	2	3	4	5	6	7
3%	0.533	0.981	2.69	3.407	4.809	5.894	6.244
6%	0.984	2.159	3.615	5.228	6.183	6.191	6.192
9%	1.651	3.627	6.239	6.243	6.246	6.258	6.266
12%	2.953	6.385	6.860	6.392	6.396	6.405	6.414

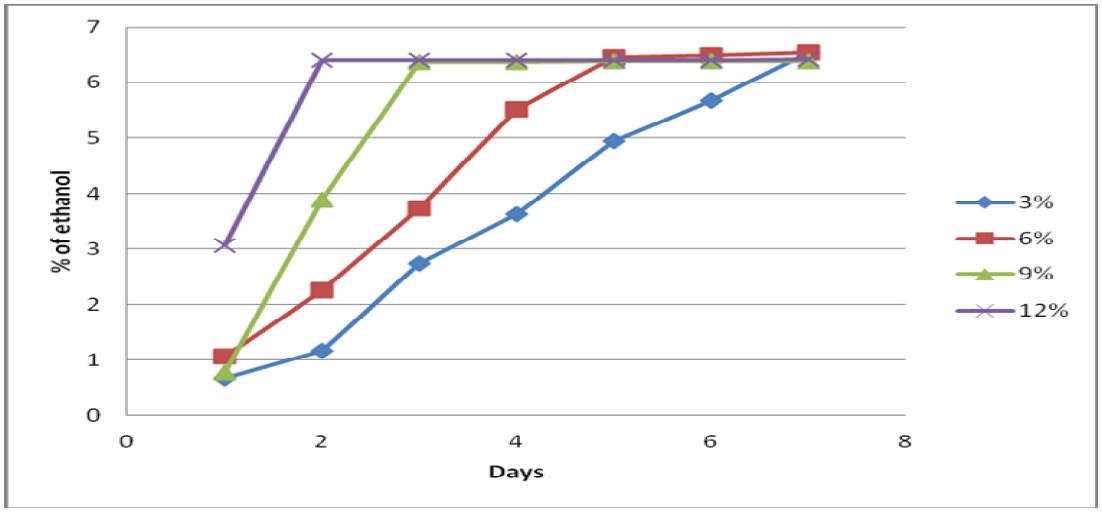
Table.10 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different yeast concentrations by *Aspergillus niger* strain B at the optimum temperature (30ºC) and at the optimum pH (pH 6)

	Production of Ethanol (in %)						
Yeast Conc. / Days	1	2	3	4	5	6	7
3%	0.668	1.168	2.746	3.641	4.947	5.679	6.533
6%	1.058	2.257	3.734	5.518	6.450	6.477	6.540
9%	0.778	3.890	6.371	6.372	6.378	6.383	6.385
12%	3.061	6.396	6.396	6.398	6.400	6.405	6.407

Table.11 Percentage of ethanol production from banana peels at 24 hours interval for seven days at different yeast concentrations by *Aspergillus niger* strain C at the optimum temperature (30ºC) and at the optimum pH (pH 6)

	Production of Ethanol (in %)						
Yeast Conc. / Days	1	2	3	4	5	6	7
3%	0.596	1.089	2.259	3.195	4.437	5.168	5.585
6%	0.822	1.943	3.268	4.932	5.622	5.645	5.651
9%	1.526	3.266	5.548	5.548	5.570	5.638	5.647
12%	2.515	5.616	5.622	5.622	5.674	5.681	5.687

Figure.5 Variation in ethanol yield from banana peels with the change in yeast concentration (3% to 12%) for seven days by *Aspergillus niger* strain B at the optimum temperature (30ºC) and at the optimum pH (pH 6)



respectively. The variation in ethanol yield from banana peels with the change in yeast concentration (3% to 12%) for seven days by *Aspergillus niger* strain B at the optimum temperature (30ºC) and at the optimum pH (pH 6) is indicated in figure 5. Mohamed and Reddy (1986) has reported that the increasing *Saccharomyces cerevisiae* inoculum in the cocultures *Aspergillus niger* and *Saccharomyces cerevisiae* from 4% to 12% gave a dramatic increase in the rate of ethanol production from potato starch. 15% yeast suspension. Togarepi *et al*. Ocloo and Ayernor (2010) has reported that the yeast concentration significantly affected the time taken for the fermentation to be completed, that is, to achieve maximum alcohol yield. The results obtained supported the fact that the speed of fermentation depends on the yeast concentration, the higher the concentration, the shorter the fermentation period required to achieve maximum alcohol yield (Kordylas, 1990). Ueda *et al*. (1981) reported of 5 days fermentation period for raw cassava root starch using (2010) reported that for the yeast

concentration the rates increased rapidly with the increase in the amount of yeast added, up to the yeast concentration of 8 g/20 g fruit pulp (Fig. 3). Beyond that point the rates no longer significantly increased. At this point the substrate becomes limiting and increasing the yeast amount does not increase the rate of reaction.

The maximum ethanol yield from banana peels was 6.540 %. Fungal Strain B gave a higher value of ethanol. The amount of ethanol content increased with the increase in fermentation time. Simultaneous fermentation of starch to ethanol can be conducted efficiently by using cocultures of the amylolytic fungus *Aspergillus Niger* and a non-amylolytic sugar fermenter, *Saccharomyces cerevisiae.* Therefore the findings of this work suggest that banana peels could be a good substrate for ethanol production.

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