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STUDIES ON PRODUCTION OF BIOETHANOL FROM WASTE POTATOES USING CO-CULTURE OF *SACCHAROMYCES CEREVISIAE* AND *ASPERGILLUS NIGER*

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Abstract

Bioethanol production from starchy materials such as potato, sweet potato, corn flour is based on bioconversion of starch into sugar and then ethanol by fermentation process. In the present scenario, there is a growing interest for ecologically and economically sustainable biofuel production. In the present scenario, bio-fuels have increased in popularity because of rising oil prices and the need for energy security. India imports >70% of it's required crude oil, which leads a major expenditure of foreign currency. India can cut it's crude oil import by using bioethanol for vehicles. In the past years, feasibility of lignocelluloses containing materials for ethyl alcohol production has been explored depending upon the availability in the region (Shindo and Tachibana, 2006). Waste potatoes, as byproduct, are easily available and account for 5-10% of the crop produced in India. The present study was carried out with the objectives of analyze the major chemical constituents of waste potatoes collected from different locations, optimize the fermentation variables for better yield of bioethanol using co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger* and quality evaluation of bioethanol produced. In this study for bioethanol production main fermentation variables were optimized in solid state fermentation (SSF) and simultaneous Saccharification and fermentation (SiSF) methods using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196. The results of various experiments revealed that with the SSF technique the highest yield of bioethanol (5.8%) using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196 was obtained at incubation temperature of 30°C after 96 hr of incubation period. In case of simultaneous Saccharification and fermentation (SiSF), the results of various experiments revealed that by employing co-culture of yeast and fungi the highest yield of bioethanol (5.3%) was obtained at a pH of 4.5 with incubation temperature of 25°C after 96 hr of incubation period. The results of various quality attributes of the bioethanol produced showed that there were no major differences in values of density, viscosity, of the bioethanol produced from both methods of fermentation.

Key words : Bioethanol, bio-fuel, ligno-celluloses, solid state fermentation (SSF), Simultaneous Saccharification and fermentation (SiSF), *Saccharomyces cerevisiae*, *Aspergillus niger*.

Introduction

The rapidly growing demand for energy, a dwindling and unstable supply of petroleum and the emergence of global warming from the use of fossil fuels have rekindled a strong interest in pursuing alternative and renewable energy sources. Bioethanol as an alternative to fossil fuels has been expanded in the last few decades in the whole world. Use of bioethanol as a renewable transportation fuel will minimize the amounts of fossil-derived carbon dioxide (CO_2) to the Earth's atmosphere. Now these days' bio fuels have increased in popularity because of rising oil prices and the need for energy security. Bioethanol is an alcohol made by fermentation, mostly from carbohydrates produced in sugar or starch crops such as corn , sugarcane and potatoes. Ethanol can be used as a fuel for vehicles in its pure form, but it is usually used as a gasoline additive to increase octane and improve vehicle emissions. Bioethanol is a clean burning, renewable resource that can be produced from fermentation of glucose rich substrates (Yu and Zhang, 2004). A new biotechnological approach for the production of ethanol by fermentation from the renewable carbohydrate materials for use as an alternative liquid fuel has been attracting worldwide interest (Ward and Singh, 2002). Nowadays, ethanol production from renewable resources has received great attention because of the increasing petroleum shortage (Nadir *et al.*, 2009). Biomass fuels

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such as ethanol are renewable and help reducing greenhouse gas emissions from fosssil fuels (Ibeto *et al*., 2011). Bioethanol can be blended with petrol or used as neat alcohol in dedicated engines taking advantage of it's higher octane number and higher heat of vaporization as it is an excellent fuel for hybrid vehicles (Kim and Dale, 2005). Keeping in view all the advantages, biomass based fuel development technology should rapidly gain momentum and barrier imposed earlier have to be removed for successfully attempting the production of bioethanol at commercial level. Presently, the fermentation of sugar to ethanol is best established process for conversion of biomass to energy (Classen *et al*., 1999). Fermentation of starchy materials leading to the production of biofuel is economical and should be practiced in developing countries like India (Sharma *et al*., 2006). In India, ethanol is produced mainly by the fermentation of substrates containing invert sugars by using various strains of *Saccharomyces cerevisiae* (Pranavya *et al*., 2015). Production of bioethanol from starch rich substrates such as potato tubers is based on the utilization of waste potatoes (Lim *et al*., 2013). Waste potatoes are easily available from 5-10% of crops as byproduct in potato cultivation (Liimatainen *et al*., 2004 and Ghosal *et al*., 2013). A large quantity of inexpensive waste potatoes from farm is available in surplus in the state of Madhya Pradesh for utilization as substrate in the production of bioethanol. These cheaply available waste potatoes contain a considerable amount of starchy material required for optimum growth of yeast used for production of bioethanol. Keeping in view the above fact following research work is planned with objectives as to analyse the proximate composition of waste potatoes collected from different locations, along with optimize the fermentation variables for maximum yield of bioethanol using co-culture of *Saccharomyces cerevisiae* and *Aspergillus niger* and evaluate the quality of bioethanol produced.

Materials and Methods

The present study was conducted in the Fermentation Technology Laboratory, Biotechnology Centre, Jawaharlal Nehru Krishi Vishwa Vidyalaya, Jabalpur (M.P.), India. Waste potato tubers were purchased from Adhartal vegetable market, Jabalpur (M.P.), India. The bioethanol producing micro organisms co-culture *viz*. *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196 were obtained from Institute of Microbial Technology (IMTECH), Chandigarh, Punjab (figs. 1 and 2). In this experiment, waste potatoes were taken as starch source (substrate). The culture of *Saccharomyces cerevisiae* and *Aspergillus niger* MTCC 2196 were grown and maintained on Yeast Extract Peptone Dextrose (YEPD) and Czapek Yeast Extract Agar (CYEA) media, respectively. The culture of *Saccharomyces cerevisiae* and *Aspergillus niger* were maintained by sub culturing them every 15 days on YEPD and CYEA agar plates, incubating for 24 hrs and 7 days respectively at 30°C and thereafter storing in a refrigerator at 4°C until further use. Inoculum of *Saccharomyces cerevisiae* and *Aspergillus niger* was prepared separately in YEPD and CYEA broth. A loopful of 24 and 7 days old culture of *Saccharomyces cerevisiae* and *Aspergillus niger* was inoculated and incubated at 30°C on a rotary shaker at 200 rpm for 24 and 48 hrs respectively. These inoculums were used to inoculate sterilized potatoes samples. *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196. Fermentation methods were used for production of bioethanol from waste potatoes by employing solid state fermentation (SSF) as described by Sharma *et al.* (2006) and simultaneous saccharification and fermentation (SiSF) as described by O'Leary (2000) was adopted for conducting the experiment. For solid state fermentation (SSF) and simultaneous saccharification and fermentation (SiSF) method, different variables *viz*. temperature, pH, and incubation period were studied for better recovery of bioethanol. In SSF, by maintaining the optimum condition of moisture content at 60% level, production of bioethanol was carried out at different incubation temperatures *viz*. 25, 30 and 35°C for different incubation periods *viz*. 3, 4, 5 and 6 days in order to attain for maximum recovery of bioethanol using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196. In SiSF, the process of fermentation was carried out at different temperatures *viz*. 25, 30 and 35°C for different incubation periods *viz*. 3, 4, 5 and 6 days with different ranges of pH *viz*. 4.5, 5.0 and 5.5 pH for maximum recovery of bioethanol using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196. The yield of bioethanol was determined by distillation and dehydration process adopted by O'Leary (2000). Distillation and dehydration was done using rotatory evaporator at 78±2°C under vacuum. Potato tubers were analysed for various chemical constituents like moisture, dry matter content, amylase and amylopectin contents according to AOAC (1980). Total starch content (Keer, 1950), total sugar (Miller, 1959) were also recorded. Quality of bioethanol produced was assessed using three different parameters like density determination using pycnometer (Caylak and Sukan 1998), viscosity by Ostwald viscosimeter (Bernnan and Tipper, 1967) and Boiling point determination as par O' Leary (2000).

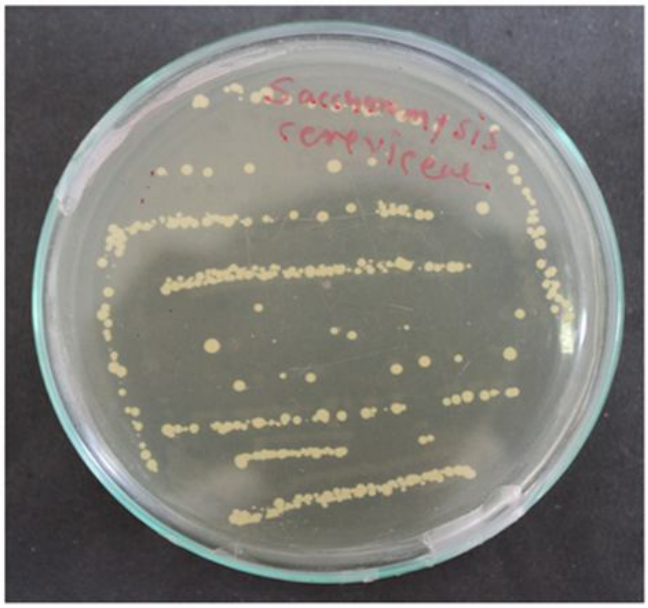

Fig. 1 : Strains of *Saccharomyces cerevisiae* MTCC 170.

Fig. 2 : Strains of *Aspergillus niger* MTTC 2196.

Results and Discussion

The effect of incubation temperatures and incubation periods on yield of bioethanol using co-culture of the *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196 applying the process of solid state fermentation (SSF) is summarized in table 1. This depicts that co-culture gave maximum yield (5.8%) of bioethanol at incubation period of 96 hr having maintained optimum incubation temperature of 30°C. The values of bioethanol yield were found to be lowest and recorded as 2.2% from the co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196 at incubation temperature of 25°C with incubation period of 144 hr. It was interesting to note that with the advancement in incubation period from 72 to 96 hrs, there was a relative increase in bioethanol yield and thereafter it got reduced. Various workers have also reported the similar findings using yeast and fungi (Liimatainen *et al*., 2004; Ming-Xong *et al*., 2009; Manikandan and Viruthagiri, 2010; Rath *et al*., 2014; Praveenkumar *et al*., 2014). Magdy *et al*. (2011) reported the maximum bioethanol concentration rates about 10 g/lit. through solid state fermentation process using yeast. Similarly, Ming-Xiong *et al*. (2009) reported 10.53 g/L of bioethanol yield from raw sweet potato starch using genetically engineered *Zymomonas mobilis* after 96 hr of incubation period. The findings in the present investigation indicated that the efficiency of bioconversion of starch into bioethanol was greater due to maximum enzymatic activity at a incubation period of 96 hr with a incubation temperature of 30°C for the co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196. Hence, it was concluded that the incubation temperature of 30°C for the co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196 with the incubation period of 96 hr for found to be optimum under SSF technique for achieving the maximum yield of bioethanol. The findings obtained in the present investigation showed that these are in agreement with the reported observations by earlier workers (Rai *et al*., 2013 b). Although, some variations observed in the values in present investigation might be due to the genetic variability of the strains used and culture conditions maintained.

The effect of pH, incubation temperature and incubation period on yield of bioethanol in Simultaneous Saccharification and Fermentation (SiSF) was also studied taking 50g substrate with 50ml distilled water (table 2). The maximum yield of bioethanol (5.3%) at a incubation temperature of 25°C with incubation period of 96 hr and having maintained the pH at 4.5 was achieved using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196. It was also observed that there was a relative increase in bioethanol yield at incubation temperature of 25°C with the relative increase in incubation period upto 96 hr. However, the bioethanol yield further got decreased at a incubation period of 120 and 144 hr. The decrease in bioethanol yield might be due to less enzymatic activity after an incubation period of 96 hr. Several workers have also reported the bioethanol yield almost in the similar range from bioconversion of starch rich substrates using co-culture of *Saccharomyces cerevisiae* MTCC 170 and

Table 1 : Effect of incubation temperature on bioethanol yield at different incubation period in SSF method.

Substrate taken - 50 g, Water added - 30 ml

S. No.	Incubation period (hr)	Yield of bioethanol (%)		
		Temperature (°C)		
		25	30	35
1.	72	4.3	5.3	2.6
2.	96	5.4	5.8	4.0
3.	120	4.1	5.2	3.8
4.	144	2.2	4.7	3.3

* Values are average of triplicates.

Table 2 : Effect of different pH on yield* of Bioethanol in Simultaneous Saccharification and Fermentation (SiSF) at different incubation temperatures and incubation periods using co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196.

Substrate taken - 50 g, Water added - 30 ml

S. No.	Incubation Period (hr)	pH	Yield of bioethanol (%)		
			Temperature (°C)		
			25	30	35
1	72	4.5	3.2	3.0	2.5
		5.0	2.5	2.8	2.0
		5.5	2.6	2.4	2.5
2	96	4.5	5.3	4.3	2.8
		5.0	3.2	3.5	3.0
		5.5	3.5	3.1	3.0
3	120	4.5	4.3	3.9	4.2
		5.0	4.0	3.4	4.2
		5.5	4.0	4.1	4.2
4	144	4.5	3.5	3.2	3.0
		5.0	3.5	2.8	3.0
		5.5	3.4	3.2	3.5

*Values are average of triplicates.

Table 3 : Effect of incubation temperature on residual sugar in solid state fermentation (SSF) at different incubation periods.

Substrate taken - 50 g, Water added - 30 ml

S. No.	Incubation Period (hr)	Residual sugar after Fermentation (mg/ml of fermented broth)		
		Temperature (°C)		
		25	30	35
1.	72	0.330	0.350	0.380
2.	96	0.221	0.280	0.290
3.	120	0.120	0.130	0.150
4.	144	0.080	0.075	0.090

* Values are average of triplicates.

Table 4 : Effect of diffrent pH on residual sugar in Simultaneous Saccharificaiton and Fermentation (SiSF) at different incubation temperatures and incubation periods.

Substrate taken - 50 g, Water added - 30 ml

S. No.	Incubation Period (hr)	pH	Residual sugar after Fermentation (mg/ml of fermented broth)		
			Temperature (°C)		
			25	30	35
1	72	4.5	0.315	0.390	0.320
		5.0	0.330	0.338	0.370
		5.5	0.320	0.370	0.340
2	96	4.5	0.140	0.230	0.150
		5.0	0.150	0.210	0.200
		5.5	0.120	0.210	0.205
3	120	4.5	0.090	0.120	0.105
		5.0	0.108	0.092	0.110
		5.5	0.090	0.115	0.100
4	144	4.5	0.050	0.065	0.080
		5.0	0.070	0.082	0.092
		5.5	0.065	0.075	0.045

* Values are average of triplicates.

Aspergillus niger MTCC 2196 (Abouzied *et al*., 1986; O'Leary, 2000; Rath *et al*., 2014 and Azad *et al*., 2014).

Initial sugar present in the fermentation medium got reduced relatively with the progressive increase in the incubation period upto 120 hr irrespective of the co-culture (*Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196) and incubation temperature (25, 30 and 35°C) used in the method of solid state fermentation (SSF) (table 3). It was also observed that there was a relative decrease in the level of residual sugar after a fermentation period of 120 hrs proceeding to 144 hr. In case of the co-culture of *Saccharomyces cerevisiae* MTCC 170 and *Aspergillus niger* MTCC 2196, the level of residual sugar after fermentation was found to be minimum (0.075 mg/ml of medium) at a incubation temperature of 30° C and incubation period of 144 hr. These observations indicated that the enzymatic hydrolysis of sugar must have taken

place at a higher rate under the above mentioned fermentation conditions resulting in maximum reduction in the level of residual sugar and in turn giving rise to maximum production of bioethanol. Various workers have also reported the level of residual sugars with respect to initial sugar level, incubation temperature and period of incubation (Hoskins and Lyons, 2009).

Different observations depicted in table 4 on different levels of pH (4.5, 5.0 and 5.5) at different incubation temperatures (25, 30 and 35°C) and incubation periods (72, 96 120 and 144 hrs) showed that the bioconversion of sugar into bioethanol was relatively low at a pH of 5.0 as compared to pH of 4.5 and 5.5 irrespective of the incubation temperature, incubation period used in the Simultaneous Saccharificaiton and Fermentation (SiSF) process of fermentation. It was also observed that pH of 4.5 was found to be optimum for better conversion of sugar using co-culture at a incubation temperature of 25° C and incubation period of 96 hr. The findings in the present investigation showed that the levels of residual sugar were found to be minimum under above mentioned fermentation conditions. The reason for higher efficiency of conversion of sugar might be the higher activity of enzymes involved in the hydrolysis of sugar into bioethanol. Several reports have also been published in the literature on the utilization of sugar in simultaneous saccharification and fermentation (SiSF) process under varied fermentation conditions (Ado *et al*., 2009; Rani *et al*., 2010; Rath *et al*., 2014; Azad *et al*., 2014). It was further reported that the concentration of sugar got reduced rapidly and consistently during 24 hr of fermentation and thereafter decrease was found to be gradual upto 96 hr of incubation period. These findings in the present investigations are in agreement with the result of earlier workers as reported above.

The quality of bioethanol, produced using two different methods (SSF) and (SiSF) of fermentation was assessed by various quality attributes such as density, viscosity and boiling point .The results analysed showed (table 5) that with the SSF technique, the density of bioethanol produced by co-culture was found to be 1.0208 g/ml, whereas the viscosity value for bioethanol produced by co-culture was found to be 0.96 centipoise. Likewise, the value of boiling point of bioethanol produced by co-culture was found to be 78.2°C. In case of SiSF method, the density of bioethanol produced from co-culture was found to be 1.0234 g/ml. Likewise, the viscosity of bioethanol produced by co-culture was found to be 0.98centipoise. Similarly, the value of boiling point of bioethanol produced from co-culture was found to be 78.1°C. Several workers have also reported the density, viscosity and boiling point of bioethanol under varied fermentation conditions (Caylak and Sukan, 1998; Ghobadian *et al*., 2008; O'Leary, 2000). Bioethanol production from waste potato has also been reported using bacteria (*Z. mobilis* MTCC 2427) and yeast (*S. cerevisiae*) separately by Rai *et al*. (2013 a) and Rai *et al*. (2013 b), respectively.

Table 5 : Quality attributes* of bioethanol produced from co-culture of two different strains using SSF and SiSF methods.

Quality attributes	SSF	SiSF
Density (g/ml)	1.0208	1.0234
Viscosity (centipoise)	0.96	0.98
Boiling point (° C)	78.2	78.1

*Values are average of triplicates.

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