Applied Biological Research 15 (2): 154-158; (2013) **Short Communication**

BIOETHANOL PRODUCTION FROM WASTE POTATOES USING BACTERIUM *Zymomonas mobilis* MTCC 2427

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ABSTRACT

The present study was carried out to assess bioethanol production from waste potatoes and optimize some fermentation variables for *Zymomonas mobilis* MTCC 2427. The waste potatoes contained 76% starch. Under solid state fermentation (SSF) maximum bioethanol of 14.17 g 100 mL^{-1} medium was achieved at 35°C when incubated for 120 hr. Under simultaneous saccharification and fermentation (SiSF), highest ethanol yield of 14.36 g 100 mL^{-1} medium was attained at 35°C, pH 5.5 and 120 hr. The ethanol produced was volatile, flammable, colourless and miscible in water and organic solvents having pleasant smell. SiSF fermentation gave more ethanol recovery than SSF method.

Key words: Bioethanol, biofuels, SiSF, fermentation, potato, *Zymomonas mobilis*

INTRODUCTION

Bioethanol is an alternative fuel because the source crops can be grown renewably under almost all climates. Bioethanol is generally CO_2 neutral as the CO_2 is absorbed by plants which, in turn, release O_2 in the same amount. In comparison, fossil fuels emit CO_2 and other poisonous gases. Initially Brazil and USA started mass production of bioethanol from sugarcane and corn and later small scale production was started in Spain, France and Sweden from wheat and sugar beet (EUBIA, 2006). Modern biotechnological approaches for ethanol production by fermentation of plant materials have attracted worldwide attention (Ward and Singh, 2002). Besides being renewable, biomass fuels minimize greenhouse gas emissions (Ibeto *et al.,* 2011) and lessen dependence on oil imports (Jegannathan *et al.,* 2011). Biofuels produced from lingocellulosic/starchy materials can be blended with petrol or used as neat alcohol in dedicated engines taking advantage of its higher octane number and higher heat of vaporization as it is an excellent fuel for hybrid vehicles (Kim and Dale, 2005). The efforts are on to overcome the impediments in bioethanol production at commercial level. Fermentation of starchy materials to biofuel is economical especially for developing countries like India (Sharma *et al.,* 2006). Swain *et al.* (2013) reported *Trichoderma* sp. and *Saccharomyces cerevisiae* as effective bioethanol producers which use sweet potato as substrate. Murugan and Rajendran (2013) reported the use of agave leaves for bioethanol production by *S. cerevisiae* and *Zymomonas mobilis.* Pradeep *et al.* (2012) optimized variables for ethanol production from finger millet. Waste potatoes, as byproduct, are easily available and account for 5-10% of the crop produced in India (Ghosal *et al.,* 2013). A large quantity of waste potatoes from farm is available in surplus in Indian states such as Madhya Pradesh, Maharashtra, etc. These waste potatoes contain a good amount of starch sufficient for optimum yeast growth. Keeping in view the cost and availability of waste potatoes, the present work was undertaken to chemically analyze waste potatoes for suitability in bioethanol production and to optimize some fermentation variables for optimum bioethanol recovery.

MATERIALS AND METHODS

The present study was conducted in the Fermentation Technology Laboratory, Biotechnology Centre, JNKVV, Jabalpur, Madhya Pradesh (India). Waste potatoes, unfit for human consumption, were purchased from Adhartal Vegetable Market, Jabalpur. The bioethanol producing bacterium *Zymomonas mobilis* strain MTCC 2427 was obtained from the Institute of Microbial Technology (IMTECH), Chandigarh (Punjab). The strain was selected on the basis of its high starch conversion ability without forming unwanted end products. *Z. mobilis* MTCC 2427 culture was grown on yeast extract peptone dextrose (YEPD) for 24 hr at 30°C before use. The culture was maintained on YEPD medium by first growing it for 48 hr and then storing in a refrigerator at 4°C until use. Two methods of fermentation viz., solid state fermentation (SSF) [Sharma *et al.*, 2006] and simultaneous saccharification and fermentation (SiSF) [O'Leary, 2000] were followed. Further, different variables viz., temperature, pH and incubation period were assessed for better bioethanol recovery. In order to attain maximum bioethanol recovery by using *Z. mobilis* MTCC 2417, bioethanol production was assayed under optimum moisture (60%) conditions in SSF using 3 incubation temperatures (25, 30 and 35°C) and 4 incubation periods (72, 96, 120 and 144 hr). In SiSF the fermentation was conducted at 3 pH levels (4.5, 5.0 and 5.5) to optimize pH for maximum bioethanol recovery. The yield of bioethanol was determined by distillation and dehydration process (O'Leary, 2000). Distillation and dehydration was done by using rotary evaporator at 78±2°C under vacuum. Potato tubers were analyzed moisture, dry matter content, amylose and amylopectin contents as per AOAC (1980). Total starch (Keer, 1950) and total sugar contents were assayed. The quality of bioethanol was assessed using 3 para-meters viz., density determination using pycnometer (Caylak and Sukan, 1998), viscosity by Ostwald viscosimeter (Brennan and Tipper, 1967) and boiling point determination as per O'Leary (2000).

RESULTS AND DISCUSSION

The moisture, dry matter and starch contents in waste potato were 80.5, 19.5 and 76%, respectively. The amylose and amylopectin were 60.1 and 15.9%. Under solid state fermentation (SSF) *Zymomonas mobilis* MTCC 2427 yielded maximum ethanol (14.17 g 100 mL^{-1} medium) at 35°C when incubated for 120 hr and lowest yield (11.85 g 100 mL^{-1}) at 25°C when incubated for 72 hr (Table 1). Liimatainen *et al*. (2004) observed bioethanol yield of 7.6 g 100 g^{-1} from different potato cultivars while Lim *et al.* (2013) reported ethanol yield of 14.92% after 60 hr in SSF under optimal conditions. Similarly, Izmirlioglu and Demirci (2010) observed higher ethanol content (22.75 g L^{-1}) and production rate (2.22 g L^{-1} hr^{-1}) through liquefaction and saccharification process (SiSF) using yeasts. Ming-Xiong *et al*. (2009) reported ethanol yield of 10.53 g L^{-1} after 96 hr incubation from raw sweet potato using genetically engineered *Z. mobilis*. Higher conversion efficiency of starch into bioethanol seems due to higher enzymatic activity at 120 hr and 35°C.

Under SiSF, highest ethanol yield (14.36 g 100 mL^{-1}) was seen at 35°C, pH 5.5 and 120 hr (Table 2). Less ethanol yield of 12.21 g 100 mL^{-1} was recorded at 25°C, pH 4.5 and 72 hr. A remarkable increase in ethanol yield was observed with increase in incubation period upto 120 hr; however the yield declined later. Izmirlioglu and Demirci (2010) reported bioethanol yield in almost similar range from bioconversion of starch-rich substrates using bacteria. The use of *Z. mobilis* gave better bioethanol yield using starch-rich substrates (Bandaru *et al*., 2006; Cazetta *et al*., 2007).

Table 1: Optimization of temperature for bioethanol yield at various incubation periods in solid state fermentation (SSF) using *Z. mobilis*

Incubation period (hr)	Bioethanol yield (g 100 mL^{-1} medium)		
	Temperature (°C)		
	25	30	35
72	11.85	12.75	12.73
96	12.51	12.92	13.30
120	12.73	13.87	14.17
144	12.35	13.15	13.70

Substrate used 50 g; water added 50 mL

Under SSF at 25°C the residual sugar showed 9.41% decrease with the advancement of incubation from 0 hr to 120 hr (Table 3). Above 120 hr the amount of residual sugar was slightly more (10.25%)

due to less sugar fermentation. The residual sugar was 0.0355 mg mL^{-1} fermented broth showing 9.57% reduction at 96 hr. At 30°C, the initial sugar of 0.371 mg mL^{-1} medium was reduced with increase in incubation period. The lowest amount of residual sugar (0.0211 mg mL^{-1}) was seen after 120 hr incubation. At 35°C, the initial sugar (0.37 mg mL^{-1}) was reduced to 0.0157 mg mL^{-1} after 120 hr incubation. The residual sugars were 0.031 and 0.0264 mg mL^{-1} fermented broth after 72 and 96 hr, respectively. The enzymatic sugar hydrolysis seems to have occurred at higher rate under above conditions leading to the maximum reduction in residual sugar, thereby yielding higher bioethanol.

The amount of residual sugar levels in SiSF revealed that at 25 and 30°C bacterial fermentation the amount of residual sugars were reduced in decreasing order with rise in incubation period upto 120 hr but increased thereafter (Table 4). At 35°C and initial pH of 4.5 the residual sugars were 0.0356, 0.0304, 0.0271 and 0.0347 mg at 72, 96, 120 and 144 hr, respectively. Ado *et al*. (2009) reported that by using co-culture of *Aspergillus niger* and *S. cerevisiae* under optimized culture conditions the sugar concentration in cassava starch was reduced in SiSF method. Behera *et al*. (2010) reported that ethanol production concomitantly increased with increase in sugars. The high conversion of sugar to ethanol may be attributed to the higher hydrolytic enzyme activity.

Table 2: Effect of pH on bioethanol yield in simultaneous saccharification and fermentation (SiSF) at different incubation temperatures and incubation periods using *Z. mobilis*

Incubation period (hr)	pH	Yield of bioethanol (g 100 mL^{-1} medium)		
		25°C	30°C	35°C
72	4.5	12.21	12.58	12.54
	5.0	12.54	12.77	13.08
	5.5	12.73	13.12	13.29
96	4.5	12.57	12.84	12.77
	5.0	12.68	13.15	13.39
	5.5	13.17	13.59	13.47
120	4.5	13.16	13.28	13.20
	5.0	13.21	13.64	14.11
	5.5	13.57	13.93	14.36
144	4.5	12.52	12.85	12.65
	5.0	12.93	13.21	13.62
	5.5	13.11	13.37	13.82

Substrate taken 50 g; water added 50 ml

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

Incubation period (hr)	Residual sugar after fermentation at					
	25°C		30°C		35°C	
	mg mL^{-1}	%	mg mL^{-1}	%	mg mL^{-1}	%
72	0.0473	12.75	0.0349	9.41	0.0311	8.39
96	0.0355	9.57	0.0306	8.25	0.0264	7.12
120	0.0349	9.41	0.0211	5.69	0.0157	4.24
144	0.0380	10.25	0.0312	8.41	0.0207	5.58

Substrate 50 g & water 50 ml; Initial sugar: 0.371 mg mL^{-1}

Table 4: Effect of pH on residual sugar in simultaneous saccharification and fermentation (SiSF) at different incubation temperature and incubation periods

Incubation period (hr)		Residual sugar after fermentation at					
		25°C		30°C		35°C	
		mg mL^{-1}	%	mg mL^{-1}	%	mg mL^{-1}	%
pH 4.5	72	0.0376	12.97	0.0357	12.32	0.0356	12.28
	96	0.0357	12.32	0.0313	10.80	0.0304	10.49
	120	0.0268	9.25	0.0243	8.38	0.0271	9.35
	144	0.0355	12.25	0.0308	10.63	0.0347	11.97
pH 5.0	72	0.0356	11.72	0.0304	10.00	0.0267	8.79
	96	0.0347	11.42	0.0269	8.85	0.0234	7.71
	120	0.0262	8.62	0.0221	7.27	0.0155	5.11
	144	0.0315	10.37	0.0262	8.62	0.0207	6.81
pH 5.5	72	0.0349	11.23	0.0267	8.59	0.0252	8.11
	96	0.0269	8.65	0.0237	7.63	0.0235	7.56
	120	0.0233	7.50	0.0188	6.05	0.0154	4.96
	144	0.0285	9.17	0.0215	6.92	0.0187	6.02

Substrate taken 50 g; water added 50 ml; Initial sugar (0 day): 0.290, 0.304 and 0.311 mg mL^{-1}medium, respectively

At 25°C the residual sugars decreased upto 120 hr incubation and thereafter it increased. The residual sugar at 30 and 35°C got reduced with increase in incubation period upto 120 hr. At initial pH of 5.5, the residual sugars at 25 and 30°C got reduced on 72 to 120 hr incubation (Table 4). At 35°C the minimum residual sugar was seen at 120 hr and maximum at 72 hr incubation. The bioconversion of sugars into bioethanol was

relatively low at a pH 4.5 than at pH 5.0 and 5.5, irrespective of the temperature and incubation period. The pH 5.0 was optimum for better conversion of sugar using *Z. mobilis* at 30°C for 120 hr with less residual sugars.

Table 5: Quality attributes of bioethanol produced by *Z. mobilis*

Quality attributes	SSF	SiSF
Density (g mL^{-1})	0.9764	0.9769
Viscosity (Centipoise)	1.02	1.07
Boiling point (^oC)	78.0	78.0

The quality of bioethanol produced by two methods of fermentation i.e. SSF and SiSF revealed that bioethanol produced was a volatile, flammable, colourless liquid with a pleasant smell (Table 5). It was found to be completely miscible with water and organic solvents. In SSF method density was recorded as 0.9764 g mL^{-1}, viscosity 1.02 centipoise and boiling point 78^{o}C, whereas in SiSF method, these values were 0.9769 g mL^{-1}, 1.07 centipoise and 78^{o}C, respectively. The results are in conformity with other workers (Ghobadian *et al*., 2008, Oyeleke and Jibrin, 2009, Qadri *et al*., 2009).

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