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Full Length Research Paper

Sustainable Biogas Enhancement Using Marine *Streptomyces clavuligerus*-Derived Enzymes: A Novel Eco-Friendly Strategy

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The marine actinomycete strain MAC 9 was used for the production of cellulase and xylanase from wheat bran with inducers oats and soy meal as substrate. Out of 30 actinomycetes screened from sediments of Tiruchendhur coastal areas of Tamil Nadu, India only 8 strains showed both cellulase and xylanase activity. The marine actinomycete exhibited highest enzyme activity at alkaline pH 8-9 with temperature ranging from 40-55°C. The enzymes were used in pretreatment of rice and wheat straw waste for biogas production. More biogas production was observed in the agricultural waste with the pretreatment of cellulase, xylanase and the combination of enzymes.

Key words: Biogas, cellulase, energy, marine actinomycete, solid state fermentation, thermophilic, wheat bran, xylanase.

INTRODUCTION

Energy from biomass holds a promising scope under Indian conditions because this sector encourage the effective management of agricultural waste in an eco-friendly way. The most serious environmental problem associated with the burning of fossil fuels is the release of large quantities of carbon dioxide which has the potential to raise the atmospheric temperature and consequent melting of ice caps and sea level rise. Biogas plants seem to be the ultimate answer to energy crisis at present with simple and perennially available raw material involving simple technology and less cost with efficiency. Codigestion of solid waste increased the rate of biogas production. Biogas production was observed from codigestion of cow dung with rice husk, coconut pith, rice chaff (Elijah Iyagba et al., 2009; Radhika et al., 1983; Vivekanandan and Kamaraj, 2011).

The microbial enzymes are relatively more stable and active with extraordinary properties (Bull et al., 2000). Solid state fermentation (SSF) has more advantages than submerged fermentation (SMF) due to low capital investment, simplification of the fermentation media, absence of complex machinery, reduced energy requirement and improved product recovery. The metabolites are more thermostable in nature (Ghildyal et al., 1985). Mixed solid substrate fermentation (Benjamin et al., 1998) was a novel process. Most actinomycete strains secreting high activity xylanases like Streptomyces sp. (Keskar et al., 1989) and *Streptomyces roseiscleroticus* (Grabski and Jeffries, 1991) are thermotolerant in nature. Actino-mycetes are the important group of microorganisms used for the efficient biodegradation of lignocellulosic waste. Nowadays the interest in cellulases and xylanases has increased due to many potential applications like bioenergy, biofuel production (Zaldivar et al., 2001). Xylanases are glycosidases (O-glycoside hydrolases, EC 3.2.1.x) which catalyze the endohydrolysis of 1,4-β- D-xylosidic linkages in xylan. Cellulase and xylanase are used for the biodegradation of lignocellulosic residues and enhanced

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the rate of biogas yield.

MATERIALS AND METHODS

Source of materials

The actinomycetes were isolated from marine sediment sample of Tiruchendur coastal area. The wheat and rice straw were collected from the local market, Coimbatore, Tamil Nadu, India.

Isolation of actinomycetes from marine sediment sample

The actinomycetes were isolated from the marine sediment samples collected from two different points of Tiruchendur (Latitude: 8°29.80 N Longitude: 78°07.73 E) , Tuticorin District , Tamil Nadu, East Coast of India. The seacoast is about 85 miles in length extends along the Gulf of Mannar. Rolling dunes of White Sea sand are thrown up at many points along this coast by the continued action of the surf and the prevailing southwest wind. The collected samples were immediately transported to the laboratory. The air dried samples were incubated at 55°C for 10 min and serially diluted. It was spread on actinomycete isolation agar media g/L (sodium caseinate 2, L - asparagine 0.10, sodium propionate 4, di-potassium phosphate 0.5, magnesium sulphate 0.1, ferrous sulphate 0.001, agar 15) at pH 9 and incubated for 7-14 days. 20 mg/l nystatin and cycloheximide100 mg/L was added to control bacterial and fungal contaminations. The pure actinomycetes cultures were maintained on nutrient agar media.

Screening of cellulase and xylanase producing actinomycetes

The actinomycete isolates were tested to produce cellulase and xylanase by growing them on carboxyl methyl cellulose (CMC) agar medium (nutrient agar media with 1.5% CMC) and xylan-agar medium (nutrient agar medium with 2.5 g/L xylan) for 2 days. The plates were then stained with Congo red solution composed of 0.5% Congo Red and 5% ethanol for 15 min and destained with 1 M NaCl. The cellulase and xylanase producing isolates were screened by observing yellow zones around the colonies against the red background (Teater et al., 1982). The actinomycete showed the maximum zone of clearance was selected for further analysis.

Inoculum production

A loopful of cells from a freshly grown slant culture was inoculated into 100 ml of sterile modified mineral salt solution. The composition of media (g/ 100 ml) was as follows: Magnesium sulphate 0.05, Dipottasium hydrogen phosphate 0.1, Sodium chloride 4, Ferrous sulphate 0.001, manganous chloride 0.001, zinc sulphate 0.001.It was incubated at 40°C in an incubator shaker at 180 rpm for 72 h.

Solid state fermentation

The agricultural waste with additives soymeal and oats were taken for enzyme production [wheat bran (3.3 g) + soymeal (3.4 g) + oats (3.3 g)]. The waste substrates were dried at room temperature to reduce the moisture content and ground to the desired size. It was added with 80 ml of modified mineral salt solution and autoclaved at 15 lbs pressure, 120°C for 20 min. After cooling, 10 ml of inoculum was added to modified mineral salt media (pH 9) with solid waste as substrate and incubated at 45°C in an incubator shaker at 180 rpm for 7 days.

Optimization of different parameters

The different parameters like pH of the medium ranging from (6 - 11), Temperature (35 ¯ 60°C) and Sodium chloride concentration (1-5%) were optimized for enzyme production. All the experiments were carried out in 250 ml Erlenmeyer flask containing 100 ml of medium. It was incubated for 7 days in an incubator shaker at 180 rpm.

Enzyme extraction

The crude enzyme from the fermented substrate was extracted by using 0.05 m sodium phosphate buffer (pH 9.0). The fermented substrate was mixed with 100 ml of buffer and was kept in the rotary shaker (180 rpm) at 40°C for 1 h. The raw extract was obtained by pressing the mixture and subsequent centrifugation at 10000 rpm for 15 min at 4°C. The clear supernatant obtained from centrifugation was used to determine enzyme activity.

Enzyme assay

Cellulase and Xylanase activity was assayed by incubating 1.0 ml of enzyme extract with each 1 ml of 1% CMC and oat spelt xylan, 4 ml of sodium phosphate buffer (pH 9.0) at 50°C for 30 min. A control without enzyme was used in this assay. Released reducing sugar was measured by dinitrosalicylic acid (DNS) method (Miller, 1959). To this mixture 3 ml of DNS reagent was added and heated in a boiling water bath for 10 min. At the time of cooling 1 ml of freshly prepared 40% sodium potassium tartarate solution was added and the samples were read at 510 nm in U.V. Spectrophotometer. The enzyme activity was expressed as IU/ml.

Partial purification of enzyme

Acetone in chilled condition (60% (v/v) was added to the crude enzyme extract and stored overnight at -4°C for precipitation and subjected to centrifugation at 10000 rpm for 10 min. The dried precipitate was suspended in sodium phosphate buffer and incubated overnight at 4°C. The enzyme was dialyzed against same sodium phosphate buffer. It was loaded on column (2.5 × 17 cm) of DEAE Sephadex A-50 already equilibriated with sodium phosphate buffer. The partially purified enzyme was collected and stored at 4°C for further use.

Utilization of cellulase and xylanase in biogas production

Four sets of batch digesters were taken. In 4 digesters 1:1 ratio of wheat and rice straw (100 g) was chopped into pieces and mixed with 100 ml of water and incubated overnight at 90°C. To this 200 g of cow dung, 400 ml of sterile water and 100 ml of one unit digested slurry were added. Set 1 was used as control. In set 2, 3 and 4, 10 ml of cellulase, xylanase, cellulose + xylanase enzymatic solution was added and incubated for one day. This mixture was placed in the digester and allowed to biomethanation at 27 ± 1°C with pH 7 as a batch process over a period of 30 days hydraulic retention time (HRT). Strictly anaerobic condition was given to this set up. Biogas production was measured daily on volume basis by water displacement method (Baba et al., 2012).

RESULTS AND DISCUSSION

Screening of potential isolate for cellulase and xylanase activity

On the basis of larger clear zone formation on CMC and

Table 1. Influence of different pH, temperature and NaCl concentration on xylanase activity by *S. clavuligerus*.

pH	Enzyme activity (IU/ml)	Temperature (°C)	Enzyme activity (IU/ml)	NaCl concentration (%)	Enzyme activity (IU/ml)
6	109.54±0.44	35	243.45±0.27	1	201.27±0.31
7	204.83±0.15	40	325.26±0.30	2	302.22±0.30
8	325.53±0.30	45	371.46±0.35	3	371.46±0.35
9	371.46±0.35	50	341.25±0.23	4	273.32±0.42
10	334.75±0.23	55	315.21±0.22	5	237.40±0.30
11	315.44±0.35	60	302.48±0.30		

Table 2. Influence of different pH, temperature and NaCl concentration on cellulase activity by *S. clavuligerus*.

pH	Enzyme activity (IU/ml)	Temperature (°C)	Enzyme activity (IU/ml)	NaCl concentration (%)	Enzyme activity (IU/ml)
6	176.50±0.31	35	194.51±0.22	1	151.61±0.36
7	201.70±0.26	40	335.34±0.36	2	335.34±0.36
8	335.34±0.36	45	302.52±0.26	3	290.60±0.29
9	314.24±0.25	50	290.37±0.30	4	248.57±0.30
10	280.58±0.33	55	285.24±0.27	5	230.42±0.37
11	234.29±0.18	60	255.59±0.35		

*Values are based on Mean±SD of 3 individual observations.

xylan agar medium the marine actinomycete MAC 9 was selected as a potential isolate among 8 actinomycetes screened for both cellulase and xylanase activity. It was identified as *Streptomyces clavuligerus* by 16S rRNA gene sequencing method. (GENBANK accession number for nucleotide sequence: JQ801297).

Effect of different pH, temperature and sodium chloride concentration on cellulase and xylanase activity

Tables 1 and 2 show the xylanase and cellulase activity for different pH, temperature and sodium chloride concentration. The organism exhibited maximum xylanase activity at 45°C (371.46±0.35) with pH 9 and 3% sodium chloride concentration. From Table 2 it was observed that cellulase activity was maximum at 40°C with pH 8 (335.34±0.36). The maximum enzyme activity was observed at high alkaline conditions ranging from pH 8-10, temperature from 40-60°C and sodium chloride concentration 2-4%. It confirmed the nature of marine actinomycete which needed high sodium chloride concentration level and high alkaline conditions for it's growth. It too preferred high temperature from 40-60°C. This indicated the highly alkaline and thermophilic nature of the marine species and the respective enzymes. According to Nascimento et al. (2003) *Streptomyces malaysiensisi* produced highest xylanase activity from 162 actinomycetes isolated from Brazilian soil. Aboul-Enein et al. (2010) reported about the highest cellulase activity at pH 8 (212 IU/ml). The cellulase and xylanase enzymes production by *Streptomyces clavuligerus* was very high than the above mentioned reports.

Biogas production from enzyme treated agricultural waste

Figure 1 show the results of biogas production in control and enzymatic pretreated samples. The biogas production was observed from 2nd day onwards up to 30th day by water displacement method. In first interval (1-10 days) the gas production was gradually increased in both control and enzyme treated samples. The second interval (10-20 days) showed the peak rate in gas production in all the samples and it was highest in the combination of enzymes on 20th day. The digester contents were mixed properly at regular intervals of time. Water is very essential to methane formation as the nutrients for the microbes must dissolve in water before they can be assimilated. The gas production was decreased in the third interval (20-30 days) in all the samples. pH was maintained nearly 7 for biogas production. In the initial stage the biogas production was very less due to the production of volatile fatty acids by acid forming bacteria that inhibited the methanogenesis and reduced the pH (Cuzin et al., 1992).

The methanogenic bacteria are mesophilic in nature. So the digesters were maintained at 27°C with strict anaerobic condition (Elijah Iyagba et al., 2009). Higher concentrations of solids due to high viscosity of the

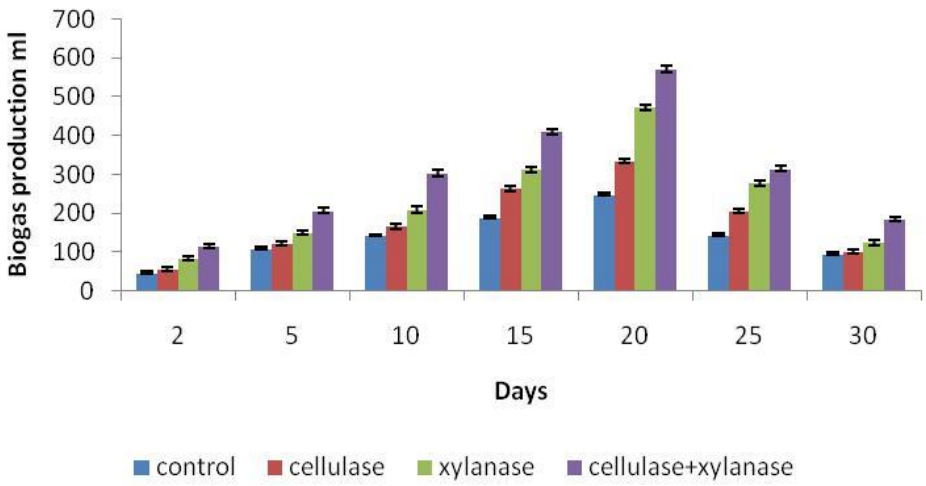

Figure 1. Comparative study of biogas production in control, cellulase, xylanase, cellulase+ xylanase treated samples.

medium delay the initiation of gas production in the first interval. The viscosity level was very high in control. So it produced the least gas production. But the enzymatic pretreated samples showed more biogas production due to the easier digestion of the solid substrates and reduce the viscosity levels. The rate of gas production increased gradually, reaching maximum and decreased gradually in both control and enzyme treated samples (Sathya priya, 2000). The highest biogas production was observed (Figure 1) in xylanase and cellulase treated sample (573.53±7.12 ml). The xylanase treated sample showed higher biogas production (473.36±6.45 ml) than cellulase treated sample (334.83±5.25 ml) and control (246.66±4.5 ml) on 20th day.

The biogas production using combination of cow dung and rice chaff gave 161.5 ml (Vivekanandan and Kamaraj, 2011). Codigestion of cow dung and rice husk showed a cumulative biogas production of 161.5 ml at the end of the 38th day of the experiment (Elijah Iyagba et al., 2009). The results indicated that cellulase and xylanase enzyme pretreatment increased the digestivity of lignocellulosic agricultural waste and enhanced the yield of biogas production than nonenzymatic sample. It showed the utility of cellulase and xylanase enzyme as an enhancer in biogas production and increased the biogas yield.

Conclusion

The waste was utilized as substrate in solid state fermentation. It showed the efficient management of waste for enzyme production. The waste with inducers (wheat bran+ soy meal + oats) showed highest cellulase and xylanase production. The organism (*S. clavuligerus*) preferred highly alkaline pH and increased temperature for maximum cellulase and xylanase production. The enzymes cellulase, xylanase, and both enzymes easily degrade the feedstock used for biogas production. It showed a very high yield in biogas production when the feed stock was treated with enzymes. It is one of the best ecofriendly method for the management of solid waste using utilization of enzymes for pretreatment of the feed stock used in biogas production. Biogas is one of the alternative, ecofriendly energy which should not cause any air pollution effects.

REFERENCES

Aboul Enein A, Abou elalla F, Serour E, Hussien T (2010). Purification and characterization of a novel thermoactive cellulase from thermophilic actinomycetes isolated from soil sample of Egypt. Int. J. Acad. Res., 2(1): 81-86.

Baba Shehu Umar , Ibn Abubakar , Nasir Ismail (2012). Anaerobic digestion of cow dung for biogas production. ARPN J. Eng. Appl. Sci., 7: 169-172.

Benjamin S, Pandey A (1998). Mixed solid substrate fermentation: A novel process for enhanced lipase production by *Candida rugosa*. J. Acta Biotechnol., 18: 315-324.

Bull AT, Ward AC, Goodfellow M (2000). Search and discovery strategies for biotechnology, The paradigm shift. Microbiol. Mol. Biol. Rev., 64: 573-606.

Cuzin N, Farinet JL, Segretain C, Labat M (1992).Methanogenic fermentation of cassava peel using a pilot plug flow digester. Bioresour. Technol., 41: 259-264.

Elijah Iyagba T, Ibifuro Mangibo A, Yahaya Sayyadi M (2009). The study of cow dung as co-substrate with rice husk in biogas production. Sci. Res. Essay, 4(9): 861-866.

Ghildyal NP, Lonsane BK, Sreekantiah KR, Sreenivasa Murthy V (1985). Economics of submerged and solid state fermentations for the production of amyloglucosidase, J. Food Sci. Technol., 22: 171-176.

Grabski AC, Jeffries TW (1991). Production, purification characterisation of B- (1-4) endoxylanase of *Streptomyces roseiscleroticus.* Appl. Environ. Microbiol., 57: 987-992.

Keskar SS, Srinivasan MC, Deshpande VV (1989), Chemical modification of xylanase from a thermotolerant *Streptomyces*. J. Biol. Chem., 261: 49-55.

Miller GL (1959). Use of dinitrosalicylic acid reagent for determination of

reducing sugar. Anal. Chem., 31: 426-428.
Nascimento RP, Marques S, Alves L, Girio F, Amaralcollaco MT, Sacramento DR, Silva Bon EP, Coelho RR(2003).A novel strain of *Streptomyces malaysiensisi* from Brazilian soil produces high endo-1,4-xylanase titres. World J. Microbiol. Biotechnol., 19: 879-881.
Radhika LG, Seshadri SK, Mohandass PM (1983). A study of biogas generation from coconut pith. J. Chem. Technol. Biotechnol., 3: 189-194.
Sathya Priya B (2000). Indigenous technique for the conversion of terrestrial biomass-A new source of energy (Biofuel/Biogas). Unpublished Master's thesis, Bharathiar University, Coimbatore, Tamil Nadu, India.
Teater RM, Wood PJ (1982). Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen, Appl. Environ. Microbiol., 43: 777-780.
Vivekanandan S, Kamaraj G (2011). The study of biogas production from rice chaff (karukka) as co-substrate with cow dung. Indian J. Sci. Technol., 4(6): 657-659.
Zaldivar J, Nielsen J, Olsson L (2001). Fuel ethanol production from lignocellulose: A challenge for metabolic engineering and process integration. Appl. Microbiol. BIotechnol., 56: 17-34.