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# Effect of Different Parameters on the Growth of Cellulose Decomposing Bacteria

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**Cellulose is the major constituent found in the Lignocellulosic biomass should be degraded by microorganism isolated from various sources such as farm yard manure and rhizosphere of the sugarcane. Total 7 bacteria was isolated among these 3 bacteria from farm yard manure designated as Fym1,Fym2,Fym3 and 4 bacteria from sugarcane rhizosphere sr1,sr2,sr3,sr4 having higher potential for cellulose decomposition examined on the Cellulose Congored plate showed the clear zone. Effect of different parameter such as pH, Substrate concentration, temperature on bacterial growth. All the 7 strain of the bacteria could grow on high pH 8 to10, so it may be placed in the group of alkalophilic. Concentration of substrate 1 to 2 % showed the higher growth of the bacteria. While at bacteria also survived at higher temperature, but maximum enzyme activity should be achieved at 37ºC.**

**Key words:** Lignocellulosic biomass, Rhizosphere, Cellulose Congored plate, Alkalophilic, Enzyme activity.

Lignocellulosic biomass is the most abundant material on earth, mainly found in raw materials like, agricultural residues (e.g. corn stover and wheat straw), forestry residues (e.g. sawdust and mill wastes), portions of municipal solid waste (e.g. waste paper) etc. Lignocellulose is the collective term used for the three main components of plant material, namely cellulose, hemicellulose and lignin. In plants, linear cellulose chain provides tensile strength, lignin gives chemical resistance and hemicellulose is responsible for bonding between cellulose and lignin. Cellulose is a major component of lignocelluloses, composed of linear β-1, 4-linked D-glucopyranose chains. While β-1, 4-linked glucose is the chemical repeating unit, the structural repeat is β-cellobiose (Varrot *et al*., 2003). Cellulose molecule contains tightly bound glucose units by virtue of van der Waals forces and hydrogen bonds. The interactions between both populations lead to complete degradation of cellulose, releasing carbon dioxide and water under aerobic conditions, and carbon dioxide, methane and water under anaerobic conditions. Varieties of microorganisms are known for produce series of enzymes that could degrade cellulose to soluble sugars, primarily cellobiose and glucose. Enzymes responsible for this reaction are known as cellulases. Cellulases is a family of at least 3 groups of enzymes endo-(1,4)-β-D-glucanase exo-(1,4)-β-D-glucanase and β-glucosidases (Percival *et al*., 2006). Such enzymes are usually secreted as part of multienzyme complexes that may include dockerins and carbohydrate-binding modules (Bras *et al*., 2008). In recent year, the application and

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interest in cellulases has particularly with the utilization of the enzymes in the production of bioethanol from lignocelluloses (Sun and Cheng, 2002). Bioethanol can be blended at low concentrations with petrol. Cellulases are very important in agricultural field for composting and plant waste management (Lu *et al*., 2004; Maswaka and Magan, 1998). Insects like termites (Isopteran) and bollworm (Lepidoptera) contains syntrophic symbiotic microflora in their guts responsible for cellulosic feed digestion (Dillon and Dillon, 2004; Saxena *et al.,* 1993). Cellulolytic bacterial species include *Trichonympha, Clostridium, Actinomycetes, Bacteroides succinogenes, Butyrivibrio fibrisolvens, Ruminococcus albus, and Methanobrevibacter ruminantium* (Schwarz, 2001; Milala *et al.,* 2005). Cellulases have their own biotechnological potential in various industrial processes like in food, animal feed, fermentation processes, brewing industries, paper-pulp preparation, laundry detergent preparation, agricultural fields etc. Application of cellulases in pulping process resulted in energy saving and increases shelf life of paper (Bhat, 2000). Cellulases are used in textile industries for improving hand and appearance of textiles (Hebeish and Ibrahim, 2007). Enzymatic treatment has certain advantages like eco-friendliness and cheaper (Ghosh ans singh, 1993; Chander Kuhad *et al*., 2010). Glucanases are used in fermentation to hydrolyze glucan that reduces the viscosity of wort and improve the filterability (Canales *et al*., 1988; Bamforth, 2009). Cellulases are applied with various hydrolytic enzymes to produce protoplast of plant cell used to produce hybrid strains. Cellulase producing microorganisms like *Aspergillus, Chaetomium, Trichoderma* and actinomycetes can be used to produce high quality of compost (Fontaine *et al*., 2004)

## MATERIAL AND METHODS

### Sample collection

For the isolation of cellulose decomposing organisms, various samples like FYM, Rhizosphere soil from sugarcane crop were collected from farm of Navsari Agricultural University, Navsari, Gujarat, India. Samples were characterized in terms of physical parameters.

### Enrichment of organisms

A pinch of sample was added in 50 ml sterile cellulose broth containing (g/l): $KH_2Po_4$: 0.5; $MgSo_4$: 0.25; Cellulose: 2.0; Gelatin: 2.0; Congo red: 0.2; pH- 7.2. After enrichment, a loopful of suspension was streaked on cellulose agar media containing (g/l): $KH_2Po_4$: 0.5; $MgSo_4$: 0.25; Cellulose: 2.0; Gelatin: 2.0; Congo red: 0.2; Agar-agar- 30.0; pH- 7.2 by four sector method. Well isolated and pure culture was obtained by subsequent streaking on the agar plate. Total 19 cellulase producing isolates were obtained designated as; fym-1, fym-2, fym-3, sr-1, sr-2, sr-3, sr-4.

### Maintenance of the Culture

The isolates were transferred on the N-agar slant and stored at 4º C. The organisms were sub cultured monthly.

### Inoculum preparation

For inoculum preparation, a loopful of culture from the slants was added in 25 ml sterile cellulose broth containing (g/l): $KH_2PO_4$: 0.5; $MgSO_4$: 0.25; Cellulose: 2.0; Gelatin: 2.0; Congo red: 0.2; pH- 7.2 and incubated on shaker for 24 hrs at 37o C. Then from the activated culture 5 ml was inoculated into 100 ml of sterile broth and incubated for 24 h on shaker at 37ºC.

### Screening for extracellular cellulase production

For primary screening, cellulose agar plates were prepared. Actively growing cultures of different isolates were inoculated and plates were incubated for 24 h at 37oC. After 24 hrs, efficiency of isolates was judged on the basis of zone of clearance.

### Effect of pH, Substrate and temperature on Growth and enzyme Secretion

### Effect of pH

To monitor the effect of pH on growth and enzyme secretion, the pH of medium (cellulose agar plate) was set by adding different amount of HCl or NaOH (pH 4, 5, 6, 7, 8, 9, 10). The enzyme secretion was monitored after 24 hrs.

### Effect of Substrate

The effect of substrate on growth and enzyme secretion of isolates was studied on cellulose agar plates at varying substrate concentration (1-5% w/v). After incubation for 24 hrs at 37º C, enzyme secretion was detected on the basis of zone ratio.

**Effect of temperature**

To investigate the effect of temperature on growth and enzyme secretion, cellulose agar plates were incubated at different temperature i.e. 25°C, 37°C, 50 °C and room temperature. The enzyme secretion was monitored after 24 hrs on the basis of zone ratio.

## RESULTS

**Sample collection**

The samples (farmyard manures, rhizosphere soil from sugarcane) were collected from the field or Navsari Agricultural University, Navsari, Gujarat, India. Samples were selected from various habitats in order to get diversified microbial population. All the samples were characterized in terms of physical properties (Table 1).

**Enrichment and screening of organisms**

After addition of pinch of sample in cellulose broth, flasks were incubated under shaking conditions for 4 days at 37ºC. Total 19 isolates were obtained after streaking of enriched liquid medium on cellulose agar plate containing Congo-red dye

**Effect of pH on growth and cellulase production**

pH is the factor which affects growth and metabolism of organisms. To investigate the effect of pH on growth and enzyme production, all the isolates were grown on solid media containing cellulose and different pH i.e. 4, 5, 6, 7, 8, 9, and 10. Three isolates from FYM significantly affected by pH of the medium. Fym-1 and Fym-3 isolates produce maximum enzyme at pH 10 and were showing ability for growth and enzyme production on entire pH range tested. Fym-2 was found to be alkalophilic, produces enzyme at pH 8 to 10 with 10 being optimum (Graph-1). Four isolates from sugarcane rhizosphere produces maximum enzyme in the pH range of 6-8. However, all have ability to secrete extracellular cellulases in entire pH spectrum tested, except sr-4. (Graph-2)

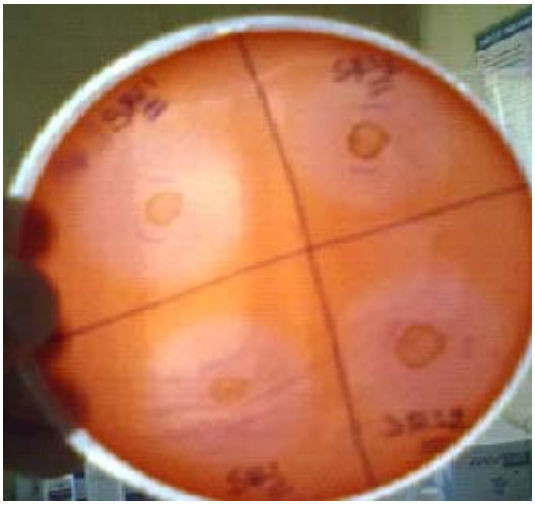
**Effect of substrate concentration on cellulase production**

Fym-1 and Fym-3 isolates showed highest cellulase production at 1% substrate concentration while Fym-2 showed highest cellulase production at 2% concentration. 1% substrate concentration was optimum for sr-1, sr-2 and sr-4 isolates whereas 2% substrate was found to be optimum for sr-3.

**Table 1.** Physical characterization of the sample

| Sample | Characteristics | Particle density (Mg/gm) | Bulk density (Mg/gm) | % Porosity (Mg/gm) | Weight of soil (t/ha) | Maximum water holding capacity (%) | Isolated organisms |
|---|---|---|---|---|---|---|---|
| FYM | Grayish black in colour, smell like fermenting dung | - | - | - | - | - | Fym-1, Fym-2, Fym-3 |
| Rhizosph ere soil-Sugarcane | Black in colour with residue of sugarcane rhizosphere, odour less | 1.67 | 1.15 | 31.14% | 1725 | 51.87 | sr1,sr2,sr3, sr4 |

Sugarcane Rhizosphere sr1, sr2, sr3, sr4



Farm Yard Manure Fym1 , Fym2, Fym3

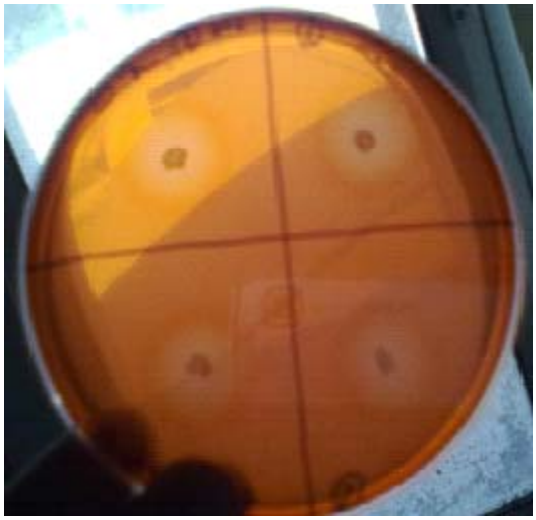
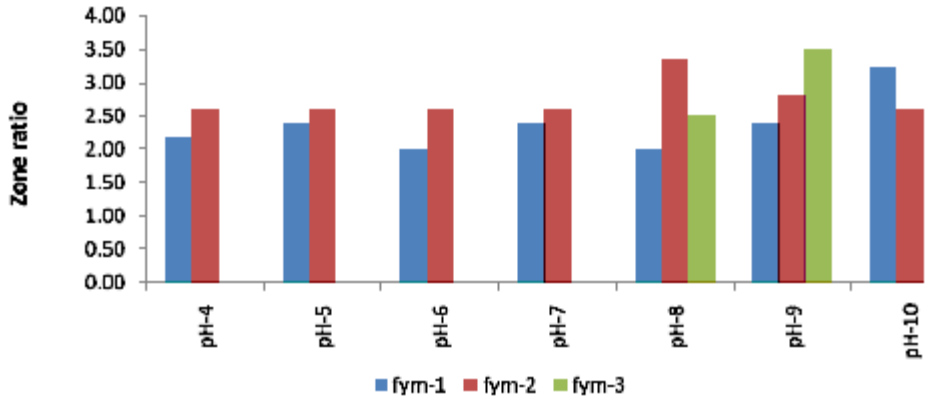
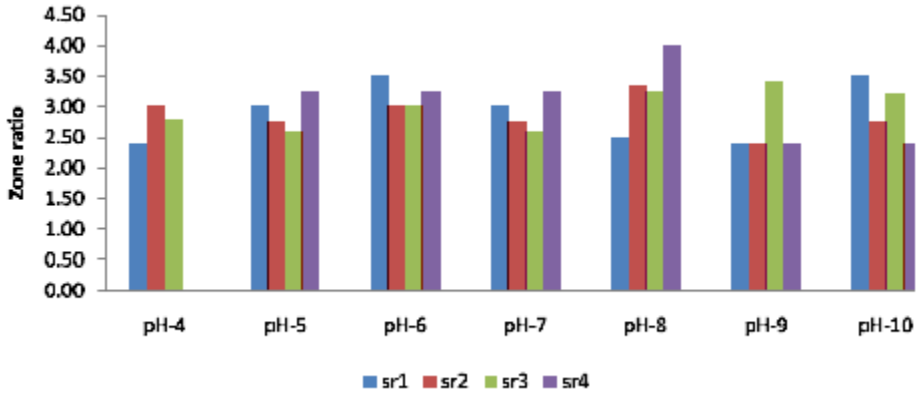


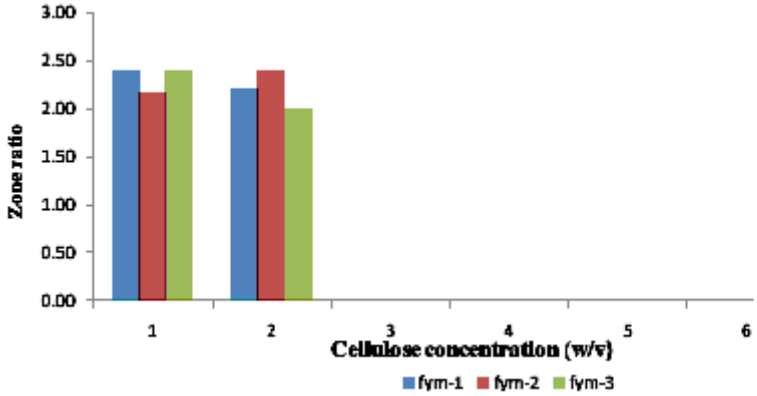
**Fig. 1.** Zone of Clearance of CMC Congored Plate



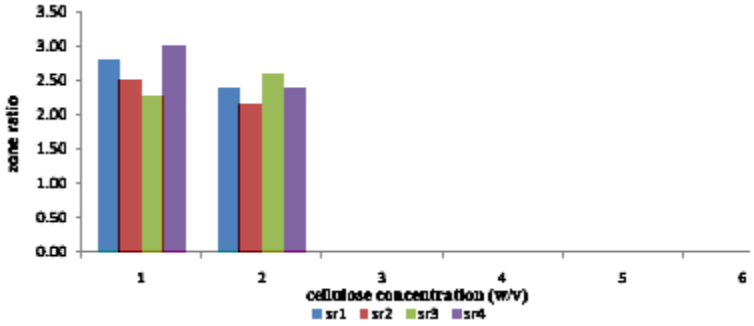

**Graph 1.** Effect of pH on cellulase production from Fym isolates



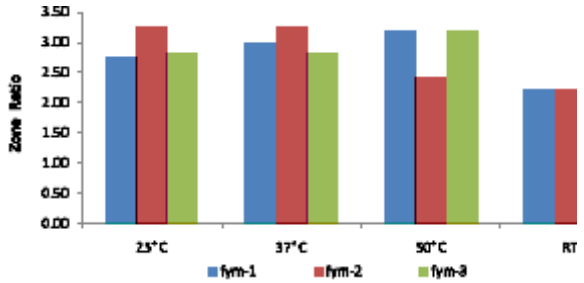

**Graph 2.** Effect of pH on cellulase production from Sugarcane rhizosphere isolates



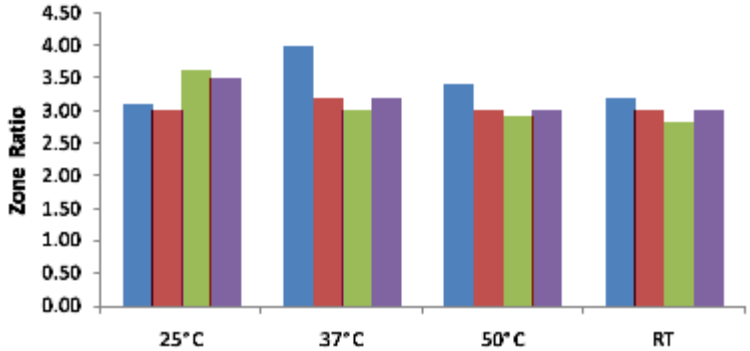

**Graph 3.** Effect of substrate concentration on Cellulase production from FYM isolates




**Graph 4.** Effect of pH on cellulase production from Sugarcane rhizosphere isolates




**Graph 5.** Effect of temperature on Cellulase production from FYM isolates




**Graph 6.** Effect of temperature on cellulase production from Sugarcane rhizosphere isolates

None of the isolates out of these showed cellulase production at 3-6% substrate concentration after 24 hours of incubation.

**Effect of temperature on cellulase production**

All the Fym isolates showed different pattern of growth and enzyme production with different temperature tested. However, room temperature was not found suitable for enzyme production. This might due to continuous fluctuation in temperature. Isolate rr showed optimum growth and enzyme production at 50° C followed by 37° C. This indicates moderate thermophilic nature of the isolate.

## CONCLUSION

Among 7 cellulase producing bacterial isolates were obtaining from various Farm yard manure and Sugarcane rhizosphere. Most of the isolate produced maximum cellulase at 1% concentration of carboxy methyl cellulose , 5-8 pH and 37°C temperature Fym-1 and Fym-3 isolates produce maximum enzyme at pH 10 and were showing ability for growth and enzyme production, Fym-2 was found to be alkalophilic, produces enzyme at pH 8 to 10.

## REFERENCES


1. Bamforth, C. W. Current perspectives on the role of enzymes in brewing. *J Cereal Sci*., 2009; **50**: 353–357.
2. Bhat, M. K. Cellulases and related enzymes in biotechnology. *Biotech Adv.*,2000; **18**: 355–383.
3. Bras, N., Cerqueira, S.A., Fernandes, P.A., Ramos, M.J. Carbohydrate Binding Modules from family 11: Understanding the binding mode of polysaccharides. *Int. J. Quant. Chem.,* 2008; **108**: 2030-2040.
4. Canales, A. M., Garza, R., Sierra, J. A., Arnold, R. The application of cellulose. *FEMS Microbiol Rev.*, 1988; **13**: 25-58.
5. Chander, K. R., Mehta, G., Gupta, R., Sharma, K. K. Fed batch content in the hydrolysates and eventually the ethanol fermentation by *Saccharomyces cerevisiae. Biomass Bioenerg*., 2010; **34**: 1189–1194.
6. Dillon, R. J., Dillon, V. M. The gut bacteria of insects nonpathogenic degrading bacteria from termite gut and mould soils of India. *Indian J Microbiol*., 2004; **33**: 55–60.
7. Fontaine, S., Bardoux, G., Benest, D., Verdier, B., Mariotti, A., Ghosh, P., Singh, A. Physicochemical and biological treatments for enzymatic saccharification of newspaper cellulosics improves the sugar content in the hydrolysates and eventually the ethanol fermentation by *Saccharomyces cerevisiae*. *Biomass Bioenerg*.,1993; **34**: 1189-1194.
8. Hebeish, A., Ibrahim, N. A. The impact of fronteir sciences on textile industry," *Colourage.*, 2007; **54**: 41-55.
9. Lu, W.J.H.T., Wang, H.T., Nie, Y. F. Effect of inoculating flower stalks and vegetable waste with ligno-cellulolytic microorganisms on the composting process. *J. Environ. Sci. Health., Part B.* 2004; **39**: 871-887.
10. Milala, M. A., Shugaba, A., Gidado, A., Ene, A.C., Wafar, J.A. Studies on the use of agricultural wastes for cellulase enzyme production by *A. niger*. *J. Agric. & Biol. Sci*.., 2005; **1**: 325-328.
11. Mswaka, A. Y., Magan, N. Wood degradation and cellulase and ligninase production, by Trametes and other wood-inhabiting basidiomycetes from indigenous forests of Zimbabwe. *Mycol Res*.,1998; **102**: 1399-1404.
12. Percival Zhang,Y.H., Himmel, M. E., Mielenz, J. R. Outlook for cellulase improvement: screening and selection strategies. *Biotechnol Appl*., 2006; **24**: 452–481.
13. Schwarz, W. H., The cellulosome and cellulose degradation by anaerobic bacteria. *Appl. Microbiol. Biotechnol*.,2001; **56**: 634–649.
14. Sun ,Y., Cheng, J. Hydrolysis of lignocellulosic material for ethanol production: a review. *Bioresourse Technol*., 2002; **83**: 1-11.
15. Varrot, A., Frandsen, T.P., Vonossowski, I., Boyer, V., Cottaz, S., Driguez, H., Sculein, M., Davies, G.J., Structural basis for ligand binding and processivity in cellobiohydrolase Cel6A from *Humicola insolens. Structure.*, 2003; **11**: 855-864.