Article-32

Asian Jr. of Microbiol. Biotech. Env. Sc. Vol. 18, No. (3) : 2016 : 225-229

ISSN-0972-3005

A STUDY OF BIODEGRADATION OF PAPER WASTES BY USING BACTERIA ISOLATED FROM THE SOIL

DHUHA MAHDI JABIR AND MUSTAFA MAHDI JABIR

Faculity of Science, University of Alqadisya

***Keyword**: Biodegradtion, Paper Wastes,* Pseudomonas florecence.

Abstract–Ten soil samples have been collected randomly from local agriculture soils in Diwaniya city then the dilution 10^{-5}celtured in nutrient agar s after mixing with 1gm of paper wastes after 90 days incubation at 35° C the resulted bacteria hydrolysis the paper and gave a zone of clearance about 13 -3 mm then the growing bacteria have been cultured alone on carboxymethylcellulose (CMC)agar .after incubation period and biochemical test there were three genus of bacteria recognized *Pseudomonas florescence, E.coli and Bacillus subtillus*. The study also observed that *Pseudomonas florescence* gave the highest diameter of zone of clearance 15 mm while *E. coli* gave the lowest zone of clearance 5mm.

INTRODUCTION

Biodegradation is defined as the biologically catalyzed reduction in complexity of chemical compounds (Alexander M*et al*.,1994) Indeed, biodegradation is the process by which organic substances are broken down into smaller compounds by living microbial organisms (Marinescu *et al*., 2009). When biodegradation is complete, the process is called "mineralization". However, in most cases the term biodegradation is generally used to describe almost any biologically mediated change in a substrate (Bennet *et al*., 2002).For thousands of years, people managed solid waste by gathering it up, carting it out and dumping or burying it in isolated places. Crude as it was, this system worked because most of the waste consisted of biodegradable organic compounds that decomposed easily. In addition, the volume of rubbish was much lower than now because there were fewer people and less packaging materials (Kujawa, 2002).

Cellulose is the most abundant organic compound on earth (Tomme *et al*., 1995). Every year, plants make more than 10 metric ton of cellulose. A cellulose polymer is a linear chain of thousands of glucose molecules linked by β (1:4) –glucosidic bounds and the basic repeating unit is cellubiose. The cellulose polymer chain is a flat, ribbon like structure stabilized by internal hydrogen bounds between adjacent chain causes them to strongly with one another in parallel arranges of many chains that all have the same polarity (Bahkali, 1996)

Microorganisms are capable of degrading various wastes and therefore have been developed to be of use in recycling domestic solid waste as well as toxic substances, the paramount role of microorganisms in the global recycling of carbon and other elements has long been recognized (Magnelli and Forchiassin, 1999)

For example Microorganisms are important in conversion of lignocellulose wastes into valuable products like biofuels produced by fermentation (Shin, 2000).

Bacteria which have high growth rate as compared to fungi have good potential to be used in cellulase production. However, the application of bacteria in producing cellulase is not widely used. The cellulolytic property of some bacterial genera such as *Cellulomonas, Cellvibrio, Pseudomonas sp* (Nakamura and Kppamura, 1982)

Bacillus and *Micrococcus* (Immanuel, 2006). Cellulose is produced by a large number of microorganisms. They are either cell bound or extra cellular. Although a large number of microorganisms can degrade cellulose, only a few of them produce significant quantities of free enzymes capable of completely hydrolyzing crystalline cellulose (Koomnok, 2005). Cellulases are used in the textile industry for cotton softening and denim finishing; in laundry detergents for colour care, cleaning; in the food industry for mashing; in the pulp and paper industries for drainage improvement and fibremodification, and they are

***Corresponding author's email**: dhuhajabir@yahoo.com; Mustafahdlife47@gmail.com

even used for pharmaceutical applications (Cherry and Fidants, 2003).

In nutshell, the cellulose enzymes will be commonly used in many industrial applications and the demands for more stable, highly active and specific enzymeswill also grow rapidly. So, cellulose enzyme will be the most stirring technology of future. And continuous research for advances in speckled aspects for cellulose production (such as cost, substrate specificity, and specific activity) is desired to achieve improved techno economic feasibility. The present work was carried out to optimize the nutritional and environmental parameters for improving cellulose (Sonia Sethi *et al*., 2013) production by bacterial strains.

The aim of this research to investigate bacterial isolates capable to produce cellulose enzyme and use it in biodegradation of paper waste.

MATERIALS AND METHODS

Samples collections

Soil samples of 200 g were collected from 5 different sites in Diwniya city. The samples were collected with small sterile shovels into. The samples were collected with small sterile shovels into sterile plastic containers. The soil samples were sent to the laboratory within 30 min for analysis. The pH and temperature of soil samples were determined using digital pH meter and thermometer respectively. Humidity was determined by hygrometer.

Bacteria culture

A total one gram of paper was added to 50 mL of different soil suspensions in 100 mL rubber stopper flasks contain nutrient agar and incubate at 35ºC with shaking for approximately 90 days.

Screening of cellulolytic bacteria

Pure cultures of bacterial isolates were individually transferred in CMC agar plates. After incubation for 48 hours, CMC agar plates were flooded with 1 % congo red and allowed to stand for 15 min at room temperature. One molar NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis (Andro *et al*., 1984). The bacterial colonies having the largest clear zone were selected for identification and cellulase productionin submerged system.

Identification of cellulolytic bacteria

Identification of cellulolytic bacteria was carried out by methode as described by Cowen and Steel (Cowan, 1993) and Cullimore (Cullimore, 2000), which was based on morphological and biochemical tests.

RESULTS AND DISCUSSION

Cellulose is the main building block of plants and have major fraction of organic carbon in soil. Micro organisms, which live in soil, are accountable for recycling of this organic carbon to the environment (Wang *et al*., 2008). Degradation of cellulosic materials is a complex process and requires participation of microbial cellulolytic enzymes. Habitats where these substrates are present are the best sources for isolation of cellulolytic microorganisms (Das *et al*., 2010). About one fifth of fresh water and soil samples yield cellulose degrading bacteria after enrichment but some samples did not bear such kind of bacteria. This is due to existence of microenvironments where 2 degrading microorganism still continues (Ivanen *et al*., 2009).

The soil in the field of the present study was slightly basic, the humidity and temperature was 36% and 33°C respectively. The physiochemical properties of soil play an important role in the growth of microorganism. Table 1 physiochemical properties of soil.

Table 1. Physiochemical properties of soil samples.

Physiochemical property of soil	Readings
pH	8.1- 6.4
Temperature	34.5
Humidity	36%

In the present study, three cellulolytic bacteria were isolated, from soil samples. These three bacteria were screened for cellulase production in submerged fermentation process using suitable medium. Cellulase -producing bacteria were isolated from soil. Based on the morphological and biochemical characteristics, the isolates were identified as *Pseudomonas fluorescens*, *Bacillus subtilus* and *E. Coli* the characters and the shape of isolated bacteria are shown in Table 2 Fig. 1, 2 and 3 respectively.

The present study 1gm of papers have been

Table 2. The chareacterstics of of bacteria isiolated from the soil

Charecter	*E. coli*	*Pseudomonas fluorescens*	*Bacillus subtilus*
Gram stain	-	-	+
Motility	+	+	+
Spore forming	-	-	-
Catalase test	+	+	+
Oxidase teat	-	+	+
Oxidase demand	An aerobic	Aerobic	Obligate aerobic

cultivated with the bacteria mention above and the result were the zone of clearance was lower than the zone of clearance when the bacteria have been cultivated in CMC medium only. The diameter of zone of clearances showed in Table 3 below.

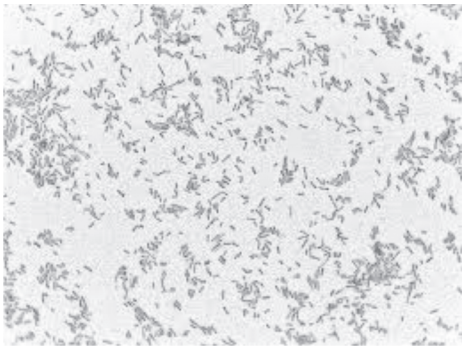

Fig. 1. *Pseudomonas fluorescens* after gram staining

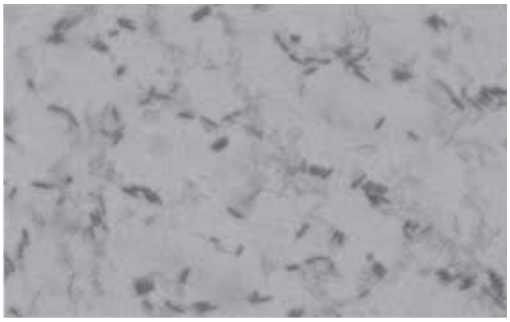

Fig. 2. *E. Coli* After gram staining

Enzymatic activity was determined through congo red test where zone of clearance was measured in mm and compared with standard cellulase. This indicated that these cellulolytic bacterial strains utilized the available sources of cellulose present in waste and carried out their degradation. Quantitative test of this isolate showed highest enzymatic activity in *Pseudomonas fluorescens*

Table 3. The diameter of zone of clearance in different isolates after cultured with papers

Pseudomonas fluorescens	10-13 mm
Bacillus subtilus	4-8 mm
E. coli	3 mm

which was 10- 15 mm and *Bacillus subtilus* which was 5-10mm the lowest clearance zone has been showed in *E. Coli* which was about 5mm. Table 4 showed these results.

Inspite of presence of more of cellulytic bacteria in the soil as many studies refer (Cullimore, 2000) but the present study only three types of bacteria have been found and that may belongs to the nature of the soil or to the cultivating conditions .

The diffrence in the zone of clearance among the different isiolates may belong to the conditions of growth which was optimmum for *Pseudomonas fluorescens* more than the other types ie *Bacillus subtilus* and *E. coli*. As Bakare *et al*. (2005) (Muhammad Irfan, 2012) who found that the cellulase enzyme produced by *Pseudomonas fluorescence* was activated at 30 to 35''C showing the optimum temperature at 35''C. While optimum temperature for maximum growth of *Bacillus subtilis* was 40''C (Bakare, 2005). Therefore the future studies should take the side of physical and chemical conditions very seriously.

Table 4. The diameter of zone of clearance indifferent isiolates

Pseudomonas fluorescens	10-15mm
Bacillus subtilus	5-10mm
E. coli	5mm

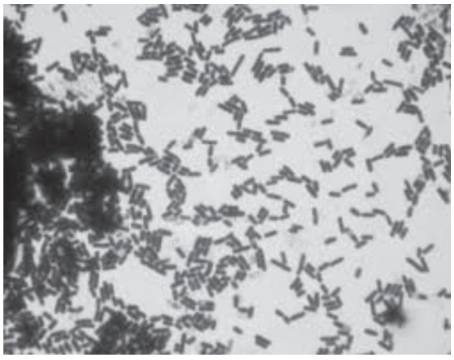

Fig. 3. *Bacillus subtilus* after gram staining

The lowest zone of clearance can be explicated as the complexity of paper components which are consist of fibers of cellulose (are fibers made with

ether or esters of cellulose these fibers are compound of hemicellulose and lignin) (Free Online Encyclopedia, 2014) .

CONCLUSION

The aim of the present work was to found a cheap and safe way to revenge up waste paper. *Pseudomonas fulorescens* among *E. coli* and *Bacillus subtilis* produced maximum yield of cellulases. The optimum temperature was determined as 35°C.

Further studies were in advancement in the purging and use of cellulase in diverse business fields. The filtered cellulase can be utilized for different purposes as a part of cleanser commercial ventures, nourishment businesses, and pharmaceutical industries. The high movement and soundness of cellulase proteins between unbiased to antacid pH and high temperature will be useful in different modern and biotechnological application.

ACKNOWLEDGEMENT

Authors are very thankful to the unit of Environment in college of science whose valuable comments help to improve this manuscript.

REFERENCES

Alexander, M. 1994. *Biodegradation and Bioremediation*, San Diego CA Academic press.

Andro, T., Chambost, J.P., Kotoujansky, A., Cattano, J., Barras, F., *et al*. 1984. Mutants of Erwinia chrysanthemi defective in secretion of pectinase and cellulase. *J Bacteriol.* 160:1199-1203.

Bahkali, A. H. 1996. Influence of various carbohydrates on xylanase production in *Verticillium tricorpus. Bioresource Technology*. 57 (3) : 265-268.

Bennet, J.W. , Wunch, K.G. and Faison, B.D. 2002. Use of fungi biodegradation. *Manual of Environmental Microbiology* , 2nd ed., ASM Press: Washington, D.C., 960-971.

BakareI, M.K., Adewale, O., Ajayi, A. and Shonukan, O.O. 2005. Purification andcharacterization of cellulase from the wildtype and two improvedmutants of *Pseudomonas fluorescens. AfricanJournal of Biotechnology*. 4 (9) : 898–904.

Cowan ST, Steel KJ. Manual for the identification of medical bacteria 3rd edn. 150-152 Cambridge University press, 1993; pp. USA.

Cullimore, D.R. 2000. *Practical Atlas for Bacterial Identification*. Lewis Publishers, Boca Raton, London, New York. Pp. 209.

Cherry, J.R. and Fidants, A.L. 2003. Directed evolution of industrial enzymes: an update. *Current Opinion in Biotechnology*. 14 (4) : 438–443.

Das, A., Bhattacharya, S. and Murali, L. 2010. Production of cellulase from a thermophilic *Bacillus sp* isolated from cow dung. *American. Eurasian J Agric Environ Sci*. 8 (6) : 685-691.

Free Online Enciclopedy "Cellulose Fiber - definition of cellulose fiber in the ". The Free Online Dictionary. Retrieved December 7, 2014.

Immanuel, G., Dhanusha, R., Prema, P. and Palavesam, A. 2006. Effect f different growth parameters on end oglucanase enzyme activity by bacteria isolated from coir retting effluents of estuarine environment. *International Journal of Environmental Science and Technology*. 3 (1) : 25–34.

Ivanen, D.R., Rongjina, N.L., Shishlyannikov, S.M., Litviakova, G.I., Isaeva-Ivanova, L.S., Shabalin, K.A. and Kulminskaya, A.A. 2009. Novel precipitated fluorescent substrates for the screening of cellulolytic microorganisms. *J Microbiol Methods*. 76 (3) : 295–300.

Jarvis, M. 2003. Cellulose stacks up. *Nature*. 426 (6967) : 611–612.

Koomnok, C. 2005. Selection of cellulase producing thermophilic fungi. In: *Proceedings of the 31st Congress on Science and Technology of Thailand of Technology*, Suranaree University, October 2005.

Kujawa, R. 2002. *Decentralized Sanitation and Reuse: Concepts, Systems and Implementations* (eds, Lens, P., Zeeman, G and lettinga G) Iwa Publishing (http// www.iwaponline.com/wri/2002/04).

Marinescu, M., Dumitru, M. and Lacatusu, A. 2009. Biodegradation of Petroleu Hydrocarbons in aArtificial Polluted Soil. *Research Journal of Agricultural Science*. 41(2).

Magnelli, P. and Forchiassin, F. 1999. Regulation of the cellulase complex production by Saccobolus saccoboloides: induction and repressionby carbohydrates. *Mycologia*. 91 (2) : 359–364.

Muhammad Irfan, Asma Safdar, Quratulain Syed and Muhammad Nadeem, 2012. Isolation andscreening of cellulolytic bacteria from soil and optimization of cellulase production and activity Yayýn tarihi 30 Eylül, 2012 ©TurkJBiochem.com.

Nakamura, K. and Kppamura, K. 1982. Isolation and identification of crystalline cellulose hydrolyzing bacterium and its enzymatic properties. *Journal of Fermentation.* 343–348.

Ray, A.K., Bairagi, A., Sarkar Ghosh, K. and Sen, S.K. 2007. Optimization of fermentation conditions for cellulase production by *Bacillus subtilis* CY5 and *Bacillus circulans* TP3 isolated from fish gut. *Acta Ichthyologica et Piscatoria.* 37 (1) : 47–53.

Shin, C.S., Lee, J.P., Lee, J.S. and Park, S.C. 2000. Enzyme production of Trichoderma reesei rut C-30 on various lignocellulosic substrates. *Applied Biochemistry and Biotechnology A*. 84–86 : 237–245.

Sonia Sethi, Aparna Datta, B. Lal Gupta, and Saksham Gupta, 2013. Optimization of Cellulase Production from Bacteria Isolated from Soil Departmentof Biotechnology , Dr. B. Lal Institute of Biotechnology,

Malviya Industrial Area, Malviya Nagar, Jaipur, India.

Tomme, P., Warren, R.A.J. and Gilkes, N.R. 1995. Cellulose hydrolysis by bacteria and fungi. *Advances in Microbial Physiology*. 37 : 1–81.

Wang, C.M., Shyu, C.L., Ho, S.P. and Chiou, S.H. 2008. Characterization of a novel thermophilic,cellulose-degrading bacterium *Paenibacillus* sp. strain B39. *Lett Appl Microbiol*. 47 (1) : 46–55.

Zhangand, Y.H.P. and Lynd, L.R. 2004. Toward an aggregated understanding ofenzymatichydrolysis of cellulose: Noncomplexed cellulase systems. *Biotechnology and Bioengineering*. 88 (7) : 797–824.