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Reduce of Fermentation Time in Composting Process by Using a Special Microbial Consortium

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***Abstract*—**Composting is the process in which municipal solid waste (MSW) and other organic waste materials such as biosolids and manures are decomposed through the action of bacteria and other microorganisms into a stable granular material which, applied to land, as soil conditioner. Microorganisms, especially those that are able to degrade polymeric organic material have a key role in speed up this process. The aim of this study has been established to isolation of microorganisms with high ability to production extracellular enzymes for degradation of natural polymers that are exists in MSW for decreasing time of degradation phase. Our experimental study for isolation designed in two phases: in first phase we isolated degrading microorganism with selected media that consist a special natural polymer such as cellulose, starch, lipids and etc as sole source of carbon. In second phase we selected microorganism that had high degrading enzyme production with enzymatic assay for seed production. However, our findings in pilot scale have indicated that usage of this microbial consortium had high efficiency for decreasing degradation phase.

***Keywords*—** Biodegradation, Compost, Municipal Solid Waste, Waste Management.

I. Introduction

DUE to rapid increases in urban population, municipal solid waste (MSW) has increased dramatically in the past 20years. Environmental pollution caused by MSW has become a serious social problem which hinders urban development, especially for large cities in developing countries. It is critical that we find ways to effectively reuse such wastes and reduce their impact on the environment. Composting is a popular way to treat organic solid waste and has become the main method chosen for recycling municipal and agricultural wastes when the final aim is to obtain a product, that is, useful for agriculture. [1] - [16]

The addition of compost to the soil produces a general improvement of soil characteristics both from a physical and a biological point of view. On the one hand, properties such as cation exchange capacity, soil aeration and structure, buffer capacity or water holding capacity are enhanced when a soil is treated with compost. On the other hand, this product works as a source of organic matter, nutrients and living organism, as well as providing plant growth regulators and properties which contribute to the suppression of soil-borne pathogens. Nevertheless, the successful use of compost depends on its degree of maturity and stability, since the application of an immature product can induce anaerobic conditions or produce phytotoxic effects. Compost maturity refers to the decomposition of phytotoxic compounds produced in the earlier phases of the composting process and the proportion of stable humus formed as a consequence of the modification of organic matter both parameters are influenced by the nature of the refuse, its structure and composition, and the capacity of microorganisms to degrade the macromolecules that make up the residue. Thus, the success of the composting process and the usefulness of compost as an organic amendment are determined by microbial activity. Although, the microbial community naturally present in wastes usually carries out the process satisfactorily, the inoculation of residues with microorganisms that each of them produces one of several polymer degrading extra-cellular enzymes at high level is a strategy that could potentially enhance the way the process takes place or the properties of the final product. [16]

There are many factors that affect the composting process, such as the microbial diversity, proportions of the mixture, the aeration rate, oxygen consumption rates, compost recycling, moisture content, pH and C/N, and so on. [1]

Microorganisms that populate substrates during composting reflect the evolution and the performance of the composting process. Their metabolic paths lead to significant changes in the physical and chemical parameters of the composting substrate, and that, in turn, leads to changes in the microbial community structure. In addition, the microbial community structure is of interest because composting, if not properly managed, might sustain potential pathogenic factors and/or emit gases such as CH4 that contribute to the greenhouse effect. [5]

Microorganisms are the key factor in nutrient transformation. Microorganisms that involve in composting process excrete several extra-cellular enzymes include lignocelluloses, proteases, lipases and … that contribute in degradation of macro-molecules in organic wastes. Therefore inoculation of suitable microbial strains in initial organic wastes resulted in enrichment in the nutrient status of composts. [7]

The main aim of this study was to evaluate the capacity of microbial inoculants to improve the degree of major polymers decomposition in composting processes at pilot scale. Taking into account results described by other authors, in whom the success of inoculation is related to the nature of the refuse, four different raw material mixtures were assayed, being the

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wastes selected on the basis of its lignocelluloses content and its significance in the geographical area in which the study was performed.

II. MATERIALS AND METHODS

A. Sampling for Microbial Isolation

Since research has proven that the number and diversity of microorganisms that are involved in degradation of organic polymer materials are more in areas that these polymers are found abundantly, So for isolation of microorganisms that are able to degrade cellulose, hemicellulose, lignocellulose, starch, protein and lipid (the most significant organic polymers in MSW), we calculated various samples from different wastes such as paper mills wastes, oil factory wastes, Slaughterhouse animal waste and activated sludge in aeration pools of wastewater treatment plants in Isfahan.

B. Enrichment, Isolation and Purification

Collected samples of wastes (ca. 5 g or 5 ml) were inserted in 300 ml flasks, to which a substrate with a suitable carbon source as the only electron donor to the culture. For example whatman filter paper, Birchwood xylan, newspaper, soluble starch, mixture of different proteins and olive oil was added to the cultures as sources of cellulose, hemicellulose, lignocellulose, starch, and protein and lipid respectively. The flasks were shaken in a shaker for 1-7 day depends on rate of degradation and 120 rpm in order to assure aerobic conditions.

Next, from the culture, of 0.1 ml aliquots were spread on the surface of M9 agar medium with a suitable carbon sources. The cultures obtained were transformed on a liquid medium, shaken for 1-7 day and inoculated again on Petri dishes. This process was repeated several times, in order to purification and proves the ability of purified and isolated strains in break down of these polymeric compounds. Abiotic controls were established to test anaerobic chemical biodegradation for each compound.

C. Culture Condition

Since organic material are degraded first in a higher thermophilic temperature level (60-65°C) and then in a lower thermophilic temperature level (40-45°C) in the composting process, so to obtain a better result we decided to isolate degrading microorganisms in these two separate temperature range. We selected 62°C for isolation of high thermophilic microorganisms and 42°C for low thermophilic isolation. So we have isolated two different microbial consortia, one for each temperature range.

D. Media

M9 medium: (Na2HPO4 _ H2O – 0.134 g/L, KH2PO4 – 0.03 g/L, NaCl – 0.5 g/L, NH4Cl – 3.982 g/L, salts: MgSO4 _ 7H2O – 2.47 g/100 ml – 1 ml salt/100 ml medium, CaCl2 – 111 mg/100 ml – 1 ml salt/100 ml medium), minimal medium: (NH4Cl – 1.0 g/L), Davis medium: (K2HPO4 – 35 g/L, KH2PO4 – 10 g/L, MgSO4 _ 7H2O – 0.5 g/L, (NH4)SO4 – 2 g/L). Whatman filter paper (8 g/L), Birchwood xylan (10 g/L), newspaper (5 g/L), soluble starch (10 g/L), mixture of different proteins (10 g/L) and olive oil (2 ml/L) were added to the medium as the sole carbon source.

E. Microbial Identification and Sporulation

Morphology analysis, mobility capability, Gram stain reaction and spore staining were used as initial experiments to identify the isolated bacteria. In addition some biochemical experiments such as oxidase test, catalase test, glucose fermentation, gas production and OF test were used for identification in genus level.

F. Assay of Extracellular Enzyme Activities

In order to selection of the best strains in organic matter decomposition, all isolated strains were analyzed in enzyme production and activity. For this purpose, due to the high number of isolated strains, comparison of strains was performed in two stages. In first stage isolated strain were compared semi-quantitatively in enzyme production on special solid culture media such as skim milk agar, spirit blue agar, starch agar, cellulose and xylan agar that were included resazurin as a redox reagent. In this step we selected strains based on the zone diameter produced in around of colonies. In second stage selected strains from pervious stage were assessed in activity of produced enzymes. In this step amylase, CMCase and xylanase activities were analyzed by testing the generation rate of glucoses from enzymolysis under different incubation conditions. The amylase activity was measured using the modified method of Bernfeld. The CMCase and xylanase activity was determined according to Nakamura and Kitamura. The lipase activity was measured according Morgan method that was modified by Schinner and the protease activity was measured according to Lowry et al. [15]

G. Feedstock Preparation

MSW from an urban waste solid composting plant in Isfahan were used in this study. MSW was screened prior to entering the composting process, and biodegradable organic materials were separated non-compostable materials. After screening, compostable materials were mixed by appropriate amount of wood chips, sawdust and water. This mix was necessary to adjust C/N, porosity and moisture content of feedstock. Six composting piles were formed from above feedstock, three piles as control without inoculation and three as test piles with microbial inoculation. Dimensions of these piles were 2.5 m (width) * 1.5 m (height) * 20 m (length).The raw materials was first deposited for 7 days to increase their temperature over 80°C in order to destroy living microorganisms.

H.Microbial Inoculation

Simultaneous feedstock preparation, all selected microbial strains after enzymatic assay cultured in TSB or PDB medium to produce microbial biomass for inoculation to test piles. About 20 liters microbial biomass was produced for each isolated strain. After that, all of produced biomasses were mixed and sprayed to the test piles by a fresh sprayer.

I. Composting Method

Homogenization of the mixture is critical for aeration and uniform distribution of inoculated strains in test piles, so turned windrow method was used in these composting processes.

J. Sampling and Analyses

C/N is the most critical factor to control of composting maturation but other factor such as content moisture, content oxygen and temperature have to control in this process. Samples were taken every week. Each sample consisted of eight subsamples taken from different locations in the pile. One portion of each sample was air-dried and crushed into powder using a high speed miller, then sieved through a 2 mm sieve and stored for elemental analysis. Another portion was used for the NH4 +-N and pH analysis, and the rest of the sample was used for the analysis of moisture content and organic matter content. The compost temperature was monitored by using a stainless steel compost thermometer (19") twice a day until the termination of the composting trial. Moisture content (gravimetric, wet basis) was determined by drying at 105°C until constant weight was attained. The organic matter content (dry basis) was determined as sample weight loss (previously oven-dried at 105°C) upon ashing at 550°C for 2h in a muffle furnace. The sample was extracted with deionized water (solid to water ratio of 1:10 w/v) at 150rpm for 1h in a horizontal shaker at room temperature for pH analysis. The suspension was then centrifuged at 4000rpm for 30min and filtered through a 0.45 lm membrane. The pH was measured using a pH electrode. Ammonium was determined using the method that suggested by TMECC.

III. Result and Discussion

A. Microbiological Analysis

Since temperature control in composting process is very difficult and sometimes it increase dramatically, we attempt to isolate spore-forming microorganism firstly, so that they can tolerant high temperatures.

Therefore after purification step we isolated spore-producing bacteria which belonged to the Bacillus genus exclusively.

All of isolated strains were gram positive, bacillus form, spore- producing, positive motility, obligate aerobic and positive catalase and oxidase.

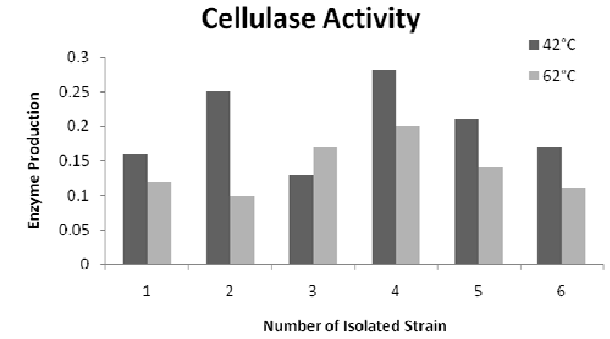
B. Enzymatic Assay Data

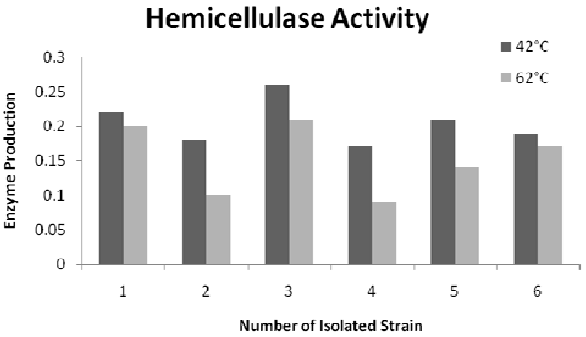
Isolated strains that have capability in spore production were evaluated in related enzyme production.

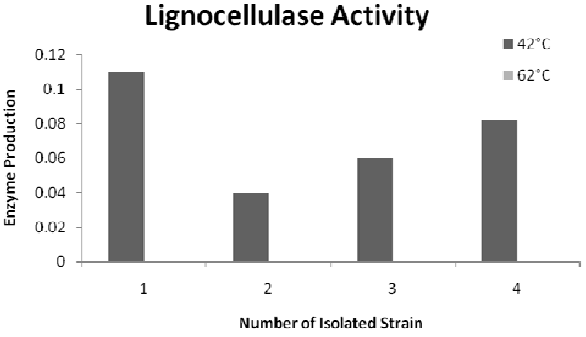
In each category (cellulose degrading bacteria, hemicellulose degrading bacteria, lignocellulose degrading bacteria and … in both range temperatures 42 and 62°C), the strains that show highest growth rate were evaluated.

Figure 1 show the result of enzymatic assay of best isolated strains in each category.

Ultimately the only species that show the highest enzyme production in each group were selected for inoculate production







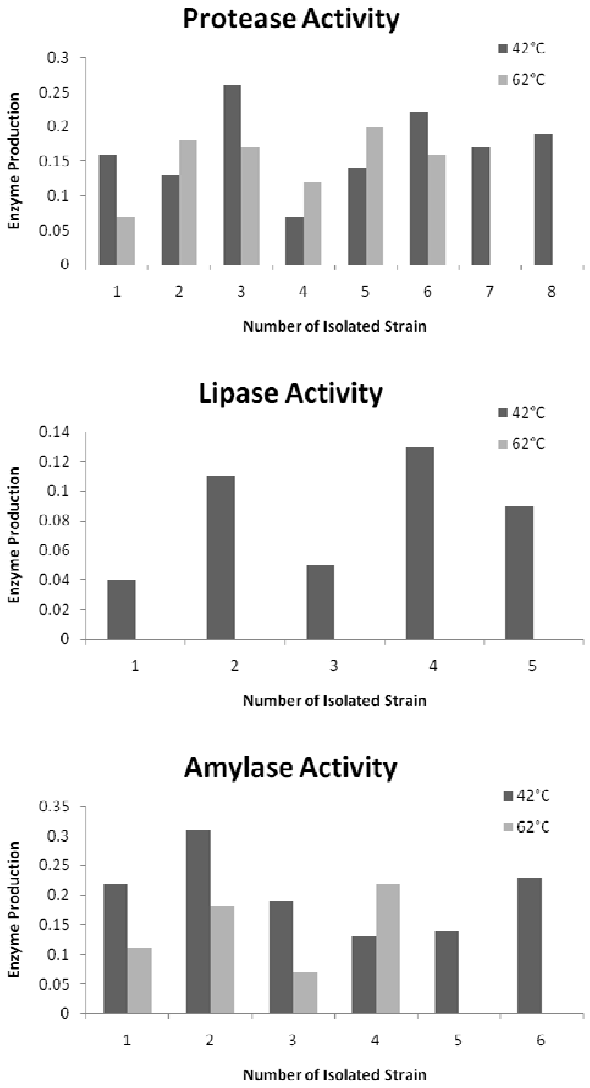


Fig. 1 Enzymatic assay analysis in selected strains in two point temperature for six organic polymeric substrate

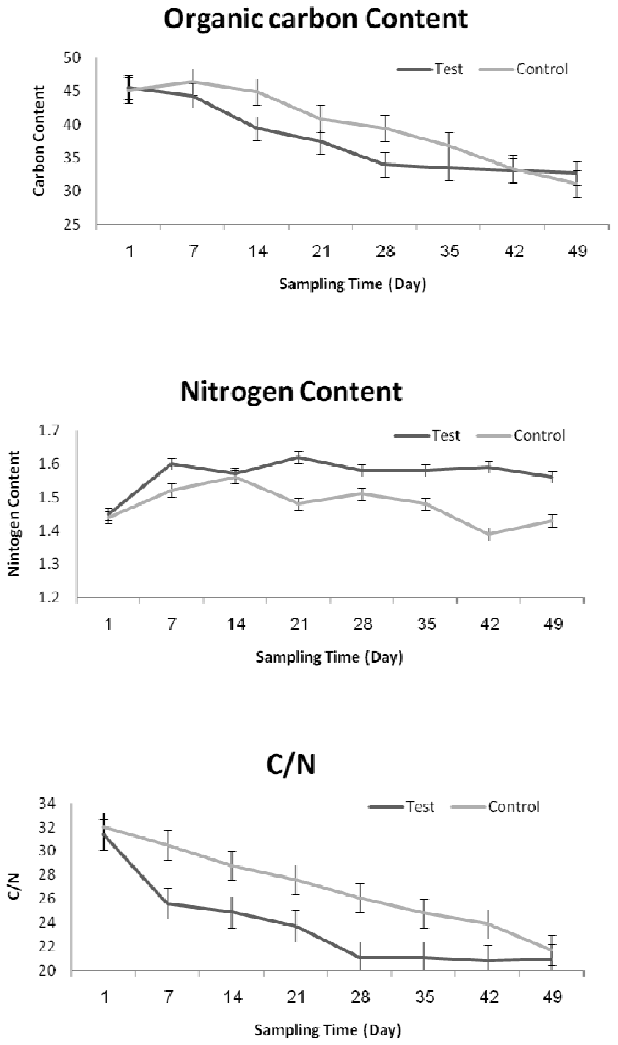


Fig. 2 Analysis organic carbon content, nitrogen content and C/N in control piles (without microbial inoculation) and test piles (inoculated piles) during compost process.

C. Composting Step Data

After inoculate production from selected strains in large amount, this inoculate was sprayed on three composting piles as test piles. Also we investigated three other piles without bacterial inoculation as control piles.

According to the composting references, the most important factors that can be show the maturation of composting piles in this process are organic carbon content, nitrogen content and C/N. Therefore we evaluated these factors fir this propose.

Figure 2 comprise the given data from these analysis for each factor.

IV. Conclusion

The use of inoculants to improve the composting process has been a controversial subject since scientists started to devote attention to this question. Some works describe the complete absence of effects of this kind of treatment (Golueke et al., 1954; Finstein and Morris, 1975; Lei and VanderGheynst, 2000), whereas others report the way that inoculation leads to the production of compost with better properties (Wani and Shinde, 1978; Requena et al., 1996). The results of our research reveal that the usefulness of inoculation in composting depends on the conditions in which the process is

carried out, in particular the characteristics of raw material and inoculants. Thus, it was possible to increase the decomposition of compost material whatever the characteristics of the waste.

Inoculants may therefore be a useful tool in composting processes when the capabilities of microorganisms are suitable for the characteristics of the waste to be composted.

REFERENCES

[1] L. Yanjun, W. Xingwu, G. Jifeng, "*Characteristics of municipal solid waste and sewage sludge co-composting*," Waste Managment. vol. 29, Sep 2009, pp. 1152-1157.

[2] M. Vargas-Garcia, F. Francisca, M. Lopez, J. Moreno, "*Influence of microbial inoculation and co-composting material on the evolution of humic-like substances during composting of horticultural wastes*," Process Biochemistry. vol. 41, 2006, pp. 1438–1443.

[3] C. Chroni, A. Kyriacou, I. Georgaki, T. Manios, M. Kotsou, K. Lasaridi, "*Microbial characterization during composting of biowaste*," Waste Management. vol. 29, 2009, pp. 1520–1525.

[4] P. Pramanik, G.K. Ghosh, P. Banik, "*Effect of microbial inoculation during vermicomposting of different organic substrates on microbial status and quantification and documentation of acid phosphatase*", Waste Management, vol. 29, 2009, pp. 574–578.

[5] Z. Wei, B. Xi, Y. Zhao, S. Wang, H. Liu, Y. Jiang, "*Effect of inoculating microbes in municipal solid waste composting on characteristics of humic acid*", Chemosphere, vol. 68, 2007, pp. 368–374.

[6] B. Xi, G. Zhang, H. Li, "*Process kinetics of inoculation composting of municipal solid waste*", Journal of Hazardous Materials, vol.124, 2005, pp.165–172.

[7] F. Lei, J.S. VanderGheynst, "*The effect of microbial inoculation and pH on microbial community structure changes during composting*", Process Biochemistry, vol. 35, 2000, pp. 923–929.

[8] Y. Lu, X. Wu, J. Guo, "*Characteristics of municipal solid waste and sewage sludge co-composting*", Waste Management, vol. 29, 2009, pp. 1152–1157.

[9] M. Taccari, M. Stringini, F. Comitini, M. Ciani, "*Effect of Phanerochaete chrysosporium inoculation during maturation of co-composted agricultural wastes mixed with olive mill wastewater,*" Waste Management, vol. 29, 2009, pp.1615–1621.

[10] D. Zhang, P. He, L. Yu, L. Shao, "*Effect of inoculation time on the bio-drying performance of combined hydrolytic–aerobic process*, Bioresource Technology, vol.100, 2009, pp.1087–1093.

[11] C.G. Golueke, B.J. Card, P.H. MaGauhey, *"A critical evaluation of inoculums in composting, "* Applied Microbiology, vol. 2, 1954, pp. 45–53.

[12] M.S. Finstein, M.L. Morris, *"Microbiology of municipal solid waste composting.* Advances in Applied Microbiology, vol. 19, 1975, pp.113–151.

[13] F.Lei, J.S. VanderGheynst,. "*The effect of microbial inoculation and pH on microbial community structure changes during composting,*" Process Biochemistry 35, 2000, 923–929.

[14] S.P. Wani, P.A. Shinde, "*Studies on biological decomposition of wheat.straw: II-Screeening of wheat-straw decomposing microorganisms under field conditions, "* Mysore Journal of Agriculture Science, vol. 12, 1978, pp.388–391.

[15] N. Requena, R. Azcon, M.T. Baca, "*Chemical changes in humic substances from compost due to incubation with ligno-cellulolytic microorganisms and effects on lettuce growth, "* Applied Microbiology and Biotechnology, vol. 45, 1996, pp.857–863.