INTERNATIONAL JOURNAL OF AGRICULTURE & BIOLOGY
1560–8530/2007/09–1–106–109
http://www.fspublishers.org

Enhancement of Rice Straw Composting by Lignocellulolytic Actinomycete Strains

HESHAM M. ABDULLA
Botany Department, Faculty of Science, Suez Canal University, Ismailia - 41522, Egypt
E-mail: hesham_abdulla@hotmail.com

ABSTRACT

Three cellulolytic actinomycete isolates, of the genera *Micromonospora*, *Streptomyces* and *Nocardiodes*, were used as inocula in combination with different organic amendments for rice straw composting and incorporation into soil. Results demonstrated that composting with thermally-treated municipal sludge amendment and actinomycetes inocula under aerobic conditions accelerated the straw decomposition process and reduced its bulk volume by 38.6 - 64%, after three months, compared to 13.6% in un-inoculated controls. The nutritional characteristics of the incorporated soil improved, particularly, in case of *Micromonospora* inoculation, as indicated by increase in organic matter to 34.9% and nitrates content to 0.59 mg/g, while those in the control reached 20% and 0.21 mg/g, respectively after the same incorporation time. Application of municipal sludge and *Micromonospora* combination may represent a rapid and environmentally friendly approach for disposal of rice straw. The value of this final product as bio-fertilizer is discussed.

Key Words: Ligncellulolytic actinomycetes; Straw incorporation; Composting; Bio-fertilizers; *Micromonospora*

INTRODUCTION

Rice cultivation produces large quantities of straw, as an agriculture waste, ranging from 2 to about 9 tons/ha globally. Components of rice straw are mainly cellulose and hemicellulose encrusted by lignin, in addition to a small amount of protein, which makes it high in C: N ratio. Therefore it is resistant to microbial decomposition compared to straw from other protein-rich grains such as wheat and barely (Parr *et al*., 1992). Egypt is the largest rice producer in the Near East region, where rice cultivation area occupies over 400,000 ha with an average farm yield of 8.2 tons/ha and an approximate straw production of 3 tons/ha (Sabaa & Sharaf, 2000). Currently, the major practice to eliminate such massive amounts of post-harvest rice residues is field open air burning. Although field-burning provides effective destruction of weed seeds and pathogenic microbial spores, the produced black smoke represents a threat to public health. The burning results in respiratory particles of < 10 µm size (PM_{10}, particulate matter less than 10 microns in diameter), which are a major cause of respiratory ailments such as asthma emphysema; it also introduces carbon monoxide and some nitrogen dioxide, which has statistically significant effect on asthma morbidity (Schwartz *et al*., 1993).

One promising alternative is straw incorporation into soil, where the actions of microbial enzymes transform the lignocellulose component of the straw into compost. However, direct incorporation into soil is limited by the great bulk of crop straw, slow bio-digestion in soil and harbouring of rice-stem diseases. Furthermore, the direct incorporation of rice straw into soil is known to reduce the availability of some important nutrients to growing plants by formation of organic complexes (Martin *et al*., 1978). In such cases, selective co-composting of straw with municipal sludge, animal manures or certain industrial wastes may provide a readily compostable mixture and higher quality product (Rynk, 1992). This hypothesis was adopted in the present study as rice straw has a high C: N ratio and is slow to decompose on its own. If it is incorporated into soil combined with a low C: N material such as cattle manure or municipal sludge and inoculated with active lingocellulose decomposing microorganisms, such as actinomycetes, a more favourable ratio is achieved for rapid decomposition and the produced material would have a higher nutrient content. Actinomycetes are able to degrade cellulose and solubilize lignin extensively as their primary metabolic activity, thus they are important agents of lignocellulose decomposition in soil (Ball *et al*., 1990; Tuomela *et al*., 2000), adding to their documented role in bio-control of plant diseases (El-Mehalawy *et al.*, 2004).

The aim of the present study is to provide a method to enhance decomposition of rice straw and humification of its residues in soil using combinations of lignocellulolytic actinomycetes strains with cattle manure and municipal sludge as organic amendments.

MATERIALS AND METHODS

Three actinomycetes isolates were used in the current investigation. Those were recovered from soil in a previous study, showed potential cellulolytic activities and were tentatively identified as *Micromonospora chalcea*, *Streptomyces roseflavus* and *Nocardiodes fulvus* (Abdulla &

El-Shatoury, 2006). The selected three isolates were refreshed from spore suspensions in baffled flasks containing 50 mL of Yeast extract-Dextrose broth (yeast extract, 10 g dextrose, 10 g & Distilled water1000 mL) and incubated at 100 rpm, 28^{o}C for 3 - 5 days. The actively growing mycelia were harvested by centrifugation, washed twice with phosphate buffer and used as inocula for the incorporation experiment.

Four sets of plastic pots, 17 x 12 x 7 cm, containing 400 g sand soils were prepared for the composting experiment. Each set consisted of duplicate pots with one of the following three treatments (in dry weight): 50 g thermally treated municipal sludge (to eliminate pathogens), 50 g cattle manure and the third was un-amended. Fifty grams of coarsely chopped rice straw were mixed thoroughly with the contents of all pots. Four grams of wet weight culture of each of the above mentioned actinomycetes were used to inoculate three sets of the pots. A mixture of these isolates was also prepared by mixing 1.3 g wet weight of each isolate and used to inoculate the fourth set of pots.

A control set of pots (un-inoculated) was included in the experiment. All sets were left open to the air and another copy of those sets were prepared with the same treatments and tightly covered; all the pots were kept at room temperature. The contents of all pots were mixed very well on daily basis to ensure sufficient aeration and water content was kept to be around 40 - 45% by water spraying; the covered pots were less frequently sprayed as they maintained a 45% moisture level.

Product evaluation was carried out, after three months, by determination of the gravimetric water content, soil pH, electric conductivity, salinity, organic carbon, organic matter, total phosphorus, nitrate and total nitrogen according to the methods proposed by Alf and Nannipieri (1995). Results were analysed using one-way ANOVA.

RESULTS

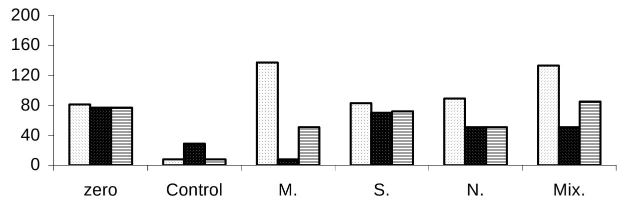
Measurements of carbon content of the final product with different actinomycetes inocula were significantly higher (P = 0.014) in sludge-amended pots compared to the corresponding manure-amended ones (Fig. 1a & b) Furthermore, higher carbon content was observed in the un-covered sludge-amended pots compared to the covered ones, although these differences were statistically non-significant (P = 0.15). Sludge-amended pots with *M. chalcea* inocula demonstrated the highest carbon levels under both un-covered and covered conditions, reaching 160 mg/g and 137 mg/g, respectively after 3 months of incubation.

Total nitrogen content at the end of experiment (Fig. 1c & d) has significantly increased in actinomycete-inoculated and sludge-amended pots compared to the un-inoculated control pots (P < 0.05) under both covered and un-covered conditions. However, the differences for covered and the corresponding un-covered pots were non-significant as showed by ANOVA (P > 0.05). The highest nitrogen content levels were observed in the sludge-amended un-covered pots inoculated with *Streptomyces* and *Micromonospora* strains, reaching 26.5 mg/g and 25 mg/g, respectively.

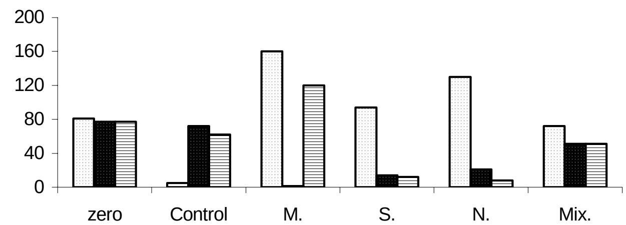
Fig. 1. Carbon and Nitrogen measurements after three months of rice straw incorporation into soil in covered and un-covered pots

[Incorporation pots were inoculated with different actinomycete strains and amended with sludge or manure as organic nutrient: *M*., *Micromonspora chalcea*; S., *Streptomyces roseflavus*; *N*., *Nocardioides fulvus*; Mix, mixture of the three strains. Zero, measurements at beginning of the experiment; Control, straw-incorporated soil without actinomycetes inocula; Soil unamended, straw-incorporated soil without organic amendment].

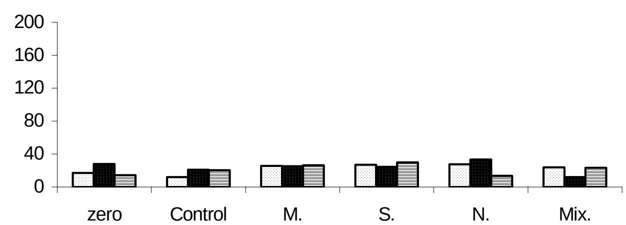
(a) Carbon (mg/g): covered pots



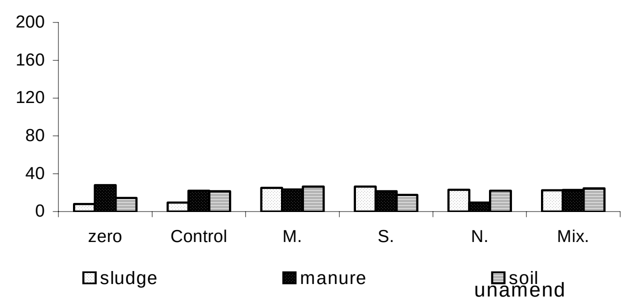
(b) Carbon (mg/g): un-covered pots



(c) Total Nitrogen (mg/g): covered pots



(d) Total Nitrogen (mg/g): un-covered pots



On the other hand, the differences between un-covered and corresponding covered pots, in terms of total volume reduction, were highly significant ($P < 0.01$); the greatest reduction in bulk volume being achieved in the un-covered pots with sludge amendment and actinomycete inocula (Fig. 2), ranging between 38.6 - 64%, compared to 13.6% in the un-inoculated sludge-amended control. Those pots (un-covered with sludge amendment & actinomycete inocula) were neutral in pH levels, ranging from 6.9 - 7.3. In contrast, the corresponding covered ones showed pH with an average of 5.5. The differences between covered and un-covered pots, in terms of pH values, were statistically significant ($P = 0.04$).

Quality of the final products from sludge-amended un-covered pots was further assessed as shown in (Table I). Different actinomycetes inocula did not significantly affect EC, salinity or total phosphorous of the composted straw ($P > 0.05$). On the other hand, final product containing *M. chalcea* inoculum showed higher organic matter (34.9%) compared to those containing other actinomycetes inocula (22.3 - 23.7%) and the un-inoculated control (20%). Similarly, pots inoculated with this *M.* strain showed higher nitrate content (0.59 mg/g) compared to 0.36 - 0.46 mg/g in actinomycetes-inoculated pots and 0.21 mg/g in the un-inoculated control pots. In all cases pH of the final product was within neutrality (pH 6.9 - 7.3).

Table I. Characteristics of the final product after rice straw incorporation into soil with sludge amendment and actinomycetes inocula for three months in uncovered pots

Character	M.[a]	S.[b]	N.[c]	Mix.[d]	Control	Std[e]
pH	7.3	6.9	6.9	7.3	7.3	<8
E.C. (mS/cm)	2.89	2.75	2.74	2.92	2.99	5.4
Salinity (mg/g)	2.94	2.94	2.73	3.1	3	NA[f]
Phosphates (mg/g)	16	15	15.5	16.5	15.5	>8
Nitrates (mg/g)	0.59	0.46	0.36	0.42	0.21	>0.25
% Organic matter	34.9	22.3	23.7	22.7	20	>30%
% Bulk volume reduction	64%	38.6%	60%	62.9%	13.6%	NA

[a], *Micromonspora chalcea*; [b], *Streptomyces roseflavus*; [c], *Nocardioides fulvus*; [d], mixture of the three species [e], Egyptian standards: Agricultural Research Centre, Central Laboratory of Organic Agriculture, Giza – Egypt (2003) [f], NA, no available standard.

Fig. 2. Pots of rice straw incorporated into soil with sludge amendment and *M. chalcea* inoculum at the beginning of the experiment (A) and after three month of incubation, uncovered at room temperature (B)

DISCUSSION

Rice straw can be decomposed in soil by microorganisms and transformed into safe bio-fertilizer that enhances soil quality. However, the decomposition process is ultimately slow, because of the characteristic wide C: N ratio in rice straw; in addition to the high lignin content (18%) physically encrusting its cellulose components, which greatly limit its decomposition and incorporation into soil (Howard *et al*., 2003). It is suggested that the incorporation process can be potentially enhanced by controlling the type of decomposing micro-organisms and certain abiotic factors such as air, temperature, moisture content and organic amendments (Parr *et al*., 1978).

Results of the present investigation demonstrate that rice straw incorporation into soil with sludge amendment was very effective, compared to manure amendment and seems to enhance microbial decomposition of straw due to the high content of essential nutrients in sludge. The carbon and nitrogen contents were always higher in the former case after three months of soil incorporation. Our results were in agreement with other studies (Shuval *et al*., 1981; Veeken *et al*., 2001), which have shown that straw incorporation with sludge amendment can increase organic matter and inorganic nitrogen content of soil.

Moreover, combining *M. chalcea* with sludge was superior to other actinomycetes inocula in the present study. It has improved the incorporation process, almost doubled the organic matter content of the final product and increased its nitrate content by three folds, compared to the un-inoculated sludge-compost. The high nitrogen content of this composted straw, adding to the slow-release character of nitrogen and phosphorous components in its residues may represent a good bio-fertilizer for plant growth and will reduce the use of chemical fertilisers as nitrogen source. Previous studies showed that commercially available bio-fertilizers can enhance crop yield by increasing available nitrogen and phosphorous and also improve soil tilth (Asad *et al*., 2004). They are cheaper, pollution free and based on renewable energy sources.

Results of incorporation under covered conditions also revealed better ability of *M. chalcea* to increase the carbon content of the final product, comparing to other actinomycete inocula. The acidic conditions, low evaporation rate and oxygen limitation conditions that were created in the covered pots are expected to result in semi-anoxic acidic conditions and may explain the better ability of *M. chalcea* to decompose the straw compared to other actinomycetes examined under these conditions. Micromonosporas are well documented, in previous studies, to dominate in aquatic habitats and to perform effective decomposition of lignocellulose material under both aerobic and microaerobic conditions (Sandrak, 1977; McCarthy & Williams, 1992; Wenzel *et al.*, 2002; Thawai *et al.*, 2005). The high nutritional value of the straw-incorporated soil, after three months of sludge amendment with *Micromonospora* inoculum, under aerobic conditions, indicates the effective role of this strain for decomposing the rice straw and transforming it into easily degradable material for further decomposition by the soil indigenous microflora. It is well established that, native lignocellulose degradation in actinomycetes is associated with their primary growth phase and that their enzymatic system is capable to depolymerise the lignocellulose material producing soluble lignocarbohydrates (Ball *et al*., 1990). Thus, actinomycetes of the current study may represent important agents for enhancing decomposition of rice straw and humification of its residues in soil.

In conclusion the role of actinomycetes in rice straw decomposition was quite evident in the present study especially when accompanied with thermally-treated municipal sludge. Due to the high macronutrient content of the obtained product it can be utilised as bio-fertiliser and soil conditioner, as an alternative to chemicals fertiliser, for newly reclaimed sandy soils. The final product characteristics were within the accepted levels for agriculture purposes according to the recommended Egyptian standards. Further detailed study is in progress to investigate the enzymatic system of the lignocellulolytic actinomycete *M. chalcea* and to optimise rice straw incorporation into soil using this strain.

REFERENCES

Abdulla, H. and S. El-Shatoury, 2006. Actinomycetes in rice straw decomposition. *Waste Manag.*, (In Press)

Alf, K. and P. Nannipieri, 1995. *Methods in Applied Soil Microbiology and Biochemistry.* Academic Press, London

Asad, S.A., A. Bano, M. Farooq, M. Aslam and A. Afzal, 2004. Comparative Study of the Effects of Bio-fertilizers on Nodulation and Yield Characteristics of Mung Bean (*Phaseolus vulgaris* L.). *Int. J. Agric. Biol.*, 6: 837–43

Ball, A.S., B. Godden, P. Helvenstein, M.J. Penninckx and A.J. McCarthy, 1990. Lignocarbohydrate solubilization from straw by actinomycetes. *Appl. Environ. Microbiol.*, 56: 3017–22

El-Mehalawy, A.E., H.M. Naziha, K.M. Hend, K.A. EL-Zahraa and Y.A. Youssef, 2004. Influence of Maize root colonization by the rhizosphere actinomycetes and yeast fungi on plant growth and on the biological control of late wilt disease. *Int. J. Agric. Biol.*, 6: 599–605

Howard, R.L., E. Abotsi, E.L. Jansen van Rensburg and S. Howard, 2003. Lignocellulose bio-technology: issues of bio-conversion and enzyme production. *African J. Biotechnol.*, 2: 602–16

Martin, J.P., R.L. Branson and W.M. Jarrell, 1978. Decomposition of organic material used in planting mixes and some effects on soil properties and plant growth. *Agrochemica*, 22: 248–61

McCarthy, A.J. and S.T. Williams, 1992. Actinomycetes as agents of bio-degradation in the environment - a review. *Gene*, 115: 189–92

Parr, J.F., G.B. Wilson, R.L. Chaney, L.J. Sikora and C.F. Tester, 1978. Effect of certain chemical and physical factors on the composting process and product quality. *In*: *Proceedings of the National Conference on Design of Municipal Sludge Compost Facilities,* Pp: 130–7. Hazardous materials control research institute. Silver spring, Maryland, U.S.A

Parr, J.F., R.I. Papendick, S.B. Hornick and R.E. Meyer, 1992. Soil Quality: attributes and relationship to alternative and sustainable agriculture. *American J. Altern. Agric*., 7: 5–11

Rynk, R., 1992. *On-farm Composting Handbook*. Northeast Regional Agricultural Engineering Service (NRAES-54), Cooperative Extension Service, Cornell University, Ithaca, New York, U.S.A

Sabaa, M.F. and M.F. Sharaf, 2000. Egyptian policies for rice development. *Cahiers Options Méditerranéennes*, 40: 25–36

Sandrak, K.A., 1977. Degradation of cellulose by Micromonospores. *Mikrobiologia*, 46: 478–81

Schwartz, J., D. Slater, T.V. Larson, W.F. Pierson and J.Q. Koenig, 1993. Particulate air pollution and hospital emergency room visits for asthma in Seattle. *American Rev. Respirable Diseases*, 147: 826–31

Shuval, H.I., C.G. Gunnerson and D.S. Julius, 1981. *Night Soil Composting: The World Bank*. Appropriate Technology for Water Supply and Sanitation, Research Monograph No. 10

Thawai, C., S. Tanasupawat, T. Itoh, K. Suwanborirux, K. Suzuki and T. Kudo, 2005. *Micromonospora eburnea* sp. nov., isolated from a Thai peat swamp forest. *Int. J. Syst. Evol. Microbiol.*, 55: 417–22

Tuomela, M., M. Vikman, A. Hatakka and M. Itavaara, 2000. Bio-degradation of lignin in a compost environment: a review. *Bioreso. Technol*., 72: 169–83

Veeken, A.H.M., F. Adani, K.G.J. Nierop, P.A. de Jager and H.V.M. Hamelers, 2001. Degradation of bio-macromolecules during high-rate composting of wheat straw-amended feces. *J. Environ. Qual.*, 30: 1675–84

Wenzel, M., I. Schonig, M. Berchtold, P. Kampfer and H. Konig, 2002. Aerobic and facultatively anaerobic cellulotic bacteria from the gut of the termite *Zootermopsis angusticollis*. *J. Appl. Microbiol.*, 92: 32–40

(Received 15 May 2006; Accepted 15 November 2006)