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Chemical, Physical and Microbiological Changes during Composting of the Water Hyacinth

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Abstract: An investigation of the physical, chemical and microbial population changes that occurred during the composting of water hyacinth was carried out. After 11 weeks of composting, the compost turned black, had decomposed and had no smell. The pH was 7 and the highest temperature reached, of 40°C occurred in the first week. The initial carbon/nitrogen ratio was 17.61 and this increased to 18.12 by the end of the composting. The coliform population declined greatly from 8.11 to 5.85 MPN (log)/g and fecal coliforms and *Escherichia coli* were not detected in the final product. Bacteria were the dominant microbes in the compost followed by actinomycetes and fungi. Mesophilic microorganisms were present in higher numbers than thermophilic microorganisms throughout the composting. The highest cellulase and xylanase activities in the compost of 6.67 and 10.24 U/kg DW, respectively were detected in the second week which was related to the temperature. *Bacillus* sp. strain B4 was isolated and investigated for cellulase and xylanase using agro-industrial residues as substrates during Solid-State Fermentation (SSF) processes. Corn cob and rice straw were good substrates for the production of the enzymes with a maximum cellulase of 1.19 U/gDW and xylanase activity of 2.54 U/g DW, respectively. The activities of both enzymes were stable and maximum at 50°C. This study indicated that agro-industrial residues should be mixed with water hyacinth for composting to facilitate the development of a thermophilic phase during the composting process and to improve the product. *Bacillus* sp. strain B4 can be used as a starter strain.

Key words: *Bacillus*, cellulase, xylanase, solid state fermentation, agro-industrial residues

INTRODUCTION

The water hyacinth (*Eichhornia crassipes*), a native aquatic weed of Brazil and possibly other central South American countries (Center, 1994) is an invasive aquatic macrophyte with a potential for particularly rapid growth and dispersal (Cook, 1990). It has been spread around the world by humans (Gopal, 1987) and can cause considerable damage to local environments, human health and economic development (Fernandez *et al.*, 1990; Epstein, 1998). Lately the water hyacinth has invaded many tropical lakes rivers and canals especially in Thailand and has had a big impact on local communities. Its rapid growth has reduced the supply of clean potable water, caused difficulties in water extraction, blocked irrigation canals, increased transportation costs, reduced fish catches, decreased available landing sites and depleted the major nutrients present in normal non potable water sources (Gunnarsson and Petersen, 2007; Malik, 2007).

In Thailand, with the favorable conditions for growth, water hyacinth causes major problems in many irrigation systems and because of the high cost of removal and its rapid growth this weed mostly remains unharvested and unutilized. Although water hyacinth is considered to be one of the world's most environmentally unfriendly aquatic plants, there have been many efforts to try to devise useful applications such as for use as a raw material for better value products including paper, wine, hand crafts and biogas. One of the easiest ways to utilize water hyacinth is to subject it to composting. Water hyacinth compost has been shown to have positive effects on plant growth (Sharma and Mitra, 1990; Abdel-Sabor and Abo El-Seoud, 1996; Chukwuka and Omotayo, 2009). The use of composted organic wastes as amendments to improve soil organic matter levels and long term soil fertility and productivity is gaining importance. Compost is one of the key factors in producing organic foodstuffs. Haug (1980) defined composting as the biological decomposition and

stabilization of organic substrates under conditions that allow development of thermophilic temperatures as a result of biologically produced heat, with a final product sufficiently stable for storage and application to land without any adverse environmental effect. It has always been a widely used method for disposal of organic wastes (Goyal *et al.*, 2005). Mesophilic and thermophilic microorganisms are involved in the composting and their succession is important for the effective management of the composting process (Beffa *et al.*, 1996; Ishii *et al.*, 2000). The heat generated during composting helps to destroy potential pathogens and controls the relationships between microorganisms (Feinstein *et al.*, 1986; Schlegel and Jannasch, 1992). Different hydrolytic enzymes are released by the different microorganisms and they cause depolymerization of the many different waste organic polymers (Kandeler *et al.*, 1999; Marx *et al.*, 2001). Important enzymes involved in the composting process include different cellulases and xylanases, other glycosidases, phosphatases and proteases. High levels of cellulase and xylanase activities have been detected throughout the active phase of composting (Cunha Queda *et al.*, 2002; Mondini *et al.*, 2004) and many of these enzymes are thermostable.

Composts prepared from different organic wastes differ in their quality and stability. This mainly depends upon the composition of the raw material used for the composting process (Gaur and Singh, 1995; Ranalli *et al.*, 2001). Compost quality is closely related to its stability and maturity. As many different chemical and biological changes occur during composting, the choices of raw materials and the composting method have made it difficult to agree on methods for the practical assessment of maturity (Itavaara *et al.*, 2002; Benito *et al.*, 2003; Wang *et al.*, 2004). Various parameters have been used to assess the quality and maturity of composts such as CO₂ production, the C:N ratio of the finished product, presence of potential pathogens such as coliform bacteria, moisture, temperature and pH of the finished compost (Garcia *et al.*, 1992; Huang *et al.*, 2001; Wu and Ma, 2002; Al-Turki, 2010).

Water hyacinth compost has been used widely in Thailand but there has been little study in terms of its microbiological changes. This present study was conducted to monitor the physical, chemical and microbiological changes during composting of the water hyacinth and also to select bacteria with the potential to produce high levels of cellulase or xylanase activities.

MATERIALS AND METHODS

Composting and sampling protocol: Water hyacinth was collected from the Pakphanang River, Nakhonsithammarat

in January, 2008. It was dried by sunlight for 2 weeks and then mixed with cattle dung and coconut shell (9:3:1). The material was shaped into 2m x 2m x 70 cm pile and allowed to decompose for a period of 3 months. During composting the moisture content was measured every week and kept at a level of 60%. The compost was turned every 2 weeks. Compost samples were drawn from three different points of the pile every week to investigate for microorganisms, pH, moisture content and enzyme activities. All analysis was performed in triplicate.

Physio-chemical and biological analysis: Total carbon was analyzed by dynamic flash combustion and total nitrogen was estimated by N/protein analysis (Horwitz, 1997). The pH of the compost was determined in distilled water with a 1:10 (w/v) compost: water ratio. The moisture content was measured by drying to constant weight in an oven at 105°C. Changes in the temperature of the compost pile were recorded using a mercury thermometer. The mean of the pile temperature at two monitoring points is reported. Cellulase and xylanase activities were estimated using carboxymethyl cellulose and xylan as substrates respectively. The reducing sugars released by enzymes were determined by the method of Nelson-Somogyi (Somogyi, 1952). One unit of enzyme activity was defined as the amount of enzyme required to liberate 1 µmole of glucose per minute and was expressed as U.

Coliforms, fecal coliforms and *E. coli* present in the first and final weeks of composting were determined by the most probable number method (MPN) (APHA, AWWA, WPCF, 2005). The number of bacteria, fungi and actinomycete were counted by the diluted plate count method on TSA (tryptic soy agar), Rose bengal medium and actinomycete isolation agar, respectively. The incubation temperature was 35°C for mesophilic and 50°C for thermophilic microorganisms. Random bacterial colonies on TSA were selected and tested for cellulase and xylanase enzyme production on CMC and xylan agar respectively. In order to detect cellulase or xylanase activity, plates were flooded with 0.1% (w/v) Congo red solution for 30 min and then rinsed several times with 1 M NaCl (Chen *et al.*, 2004). This procedure revealed distinct hydrolysis regions. Isolates producing a high degree of hydrolysis were then further investigated for enzyme production and identity.

Enzyme assay: Enzyme production was investigated by a solid state fermentation using different agricultural wastes including water hyacinth, coconut shells, rice straw and corncob. Each substrate was cut into 1 cm pieces and oven-dried. Seventy grams of each substrate was mixed with 150 mL of Berg's mineral salts medium (Berg *et al.*,

1972) and the moisture content was adjusted to 70% by adding distilled water. Substrates were autoclaved before use. Bacteria were grown in Berg's mineral salts medium at 35°C for 24 h. Cell suspensions were adjusted to the 0.5 McFarland standard and 1 mL of culture was inoculated into substrate. Cultures were incubated at 35°C for 13 days. Cellulase and xylanase were assayed everyday.

Effect of temperature on activity and stability of enzymes:

Bacterial isolates were grown with water hyacinth mixed with coconut shells (7:1) and 150 mL of Berg's mineral salts medium. The moisture content was adjusted to 70%. After 6 days of incubation at 35°C, enzyme activities were determined at 40, 50, 60, 65, 70 and 80°C to study the effect of temperature on enzyme activity. For the thermal stability test, the enzyme extract was incubated with substrates at 50, 60, 65, 70, 80 and 90°C for 30 min before determining enzyme activity as mentioned.

Bacterial identification: Isolates with high ability of enzyme production were identified by a partial 16s rDNA analysis and their morphological and physiological properties.

RESULTS AND DISCUSSION

Changes in temperature, pH and moisture at various stages of decomposition are shown in Fig. 1 a-c; the pH of the compost remained stable at 7 during the 11 weeks of the process. An initial temperature of 28°C was recorded and the highest temperature, of up to 40°C, was observed at 7 days of composting and then declined gradually. It has been previously reported that the increase of temperature in the early phase of composting may be the result of a thermophilic phase of composting. The temperature rises as a consequence of the rapid breakdown of organic matter, mostly readily available substrates rich in carbon, by microbial metabolism (Kutzner, 2001). Although studies of Inbar *et al.* (1993) and Tiquia (2002) suggest that in the thermophilic phase the temperature could rise up to 60-70°C, in this study an increase of only to 40°C was observed. This could be attributed to the differences of material used and the composting process. The moisture content was high throughout the process at 80% in the first week and 70% in the final week.

The initial C: N ratio of the water hyacinth compost was low at 17.61. An unexpected increase in C: N ratio to 18.12 was found at the end of composting (Table 1). The initial content of carbon however gradually decreased as the decomposition progressed. The decrease in total N content was due to conversion of organic nitrogen to ammonia and the subsequent loss of ammonia. This of course depends upon the type of material being composted and its C:N ratio. The increase of C:N ratio may be due to a low initial C:N ratio that results in more N losses than in high C:N ratio wastes (Sanchez-Montero *et al.*, 2001). This agreed with the findings of the study done by Goyal *et al.* (2005) that an initial low C/N ratio of water hyacinth led to a nitrogen loss compared to other substrates with high C/N ratio.

It has been reported that a lower C:N formulation exhibited a slower increase in temperature and was an indication of poorer decomposition (Huang *et al.*, 2004; Erickson *et al.*, 2009). C/N is among one of the

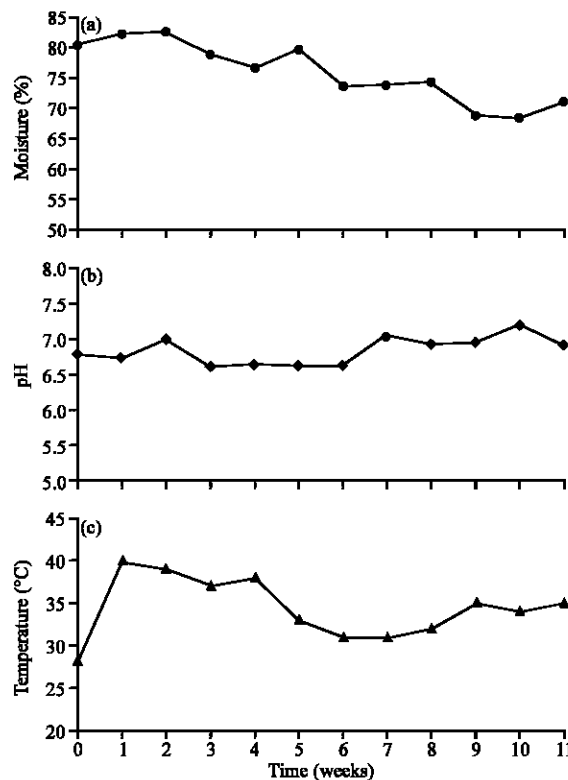


Fig. 1: Changes in (a) Percentage moisture, (b) pH and (c) Temperature during composting

Table 1: Carbon and nitrogen content, C:N ratio and the amount of coliform bacteria and *Escherichia coli* of the compost

Weeks of composting	Carbon (%)	Nitrogen (%)	C:N ratio	Coliforms MPN (log)/g	Fecal coliforms MPN (log)/g	<i>Escherichia coli</i> MPN (log)/g
0	33.29	1.89	17.61	8.11	7.04	6.70
11	29.71	1.64	18.12	5.85	nd*	nd*

nd*: Not detected

important factors affecting compost quality (Michel *et al.*, 1996). It is recommended to start composting with C/N at 25-30 as this is considered to be the optimum ratio (Huang *et al.*, 2004). Only a 10% carbon decrease was detected in this study compared to Atkinson *et al.* (1996) who found about a 29% reduction during the composting of poultry litter with saw dust. The loss was due to the release of carbon dioxide. Even though the C:N ratio did increase, any value of below 20 can be considered satisfactory for use in the field (Kutzner, 2001; Raut *et al.*, 2008).

The presence of pathogens in the compost was investigated by determining the numbers of coliforms, fecal coliforms and *Escherichia coli* at the beginning and the end of the process. Coliform bacteria reduced by more than 2 log MPN/g (Table 1). High amounts of fecal coliforms and *E. coli* present at the start with 7.04 and 6.7 log MPN/g, due to the use of cattle dung, were reduced to non detectable levels. Even though there is no criterion for an allowable number of pathogen in compost in Thailand, the determination of indicator microorganism is essential. In this study, we investigated coliforms, fecal coliforms and *E. coli* only as indicator organisms. Other pathogens may be less affected by biodegradation and could survive for much longer times. Other resistant pathogens must be considered with respect to handling safety.

Various hydrolytic enzymes control the rate at which various substrates are degraded. Enzymes are the main indicators of various degradative processes (Tiquia *et al.*, 1996; Tiquia, 2002). Therefore the changes in the activities of two important enzymes; cellulase and xylanase which are responsible for hydrolysis of cellulose and xylan, respectively, were studied to understand the degradation of organic wastes. The cellulase and xylanase activity increased during decomposition and was maximum at 2 weeks (6.67 and 10.24 U/kg DW, respectively) followed by a decline (Fig. 2). The results agree with those described by Goyal *et al.* (2005) in that the cellulase activity increased during decomposition, reached a maximum at 30 days and declined further at 60 and 90 days. The reports of Raut *et al.* (2008) and Castaldi *et al.* (2008) also showed that the production of hydrolytic enzymes was maximized within 2 weeks of composting. These results indicate the importance of the initial phase of composting. Because of the levels of xylanase and cellulase in the compost they also most probably have important roles in the composting processes of water hyacinth.

Microbial succession is known to play a key role in the composting process and the appearance of some microorganisms reflects the quality of the maturing compost (Ishii *et al.*, 2000). The population of mesophilic

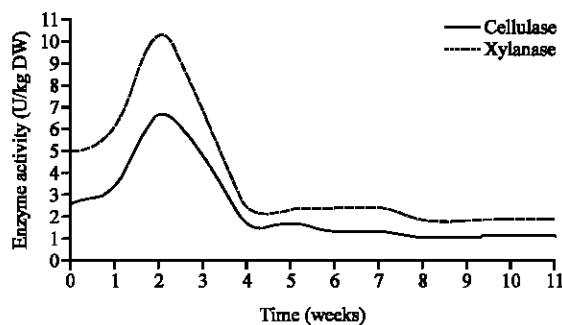


Fig. 2: Changes in cellulase and xylanase activity during composting

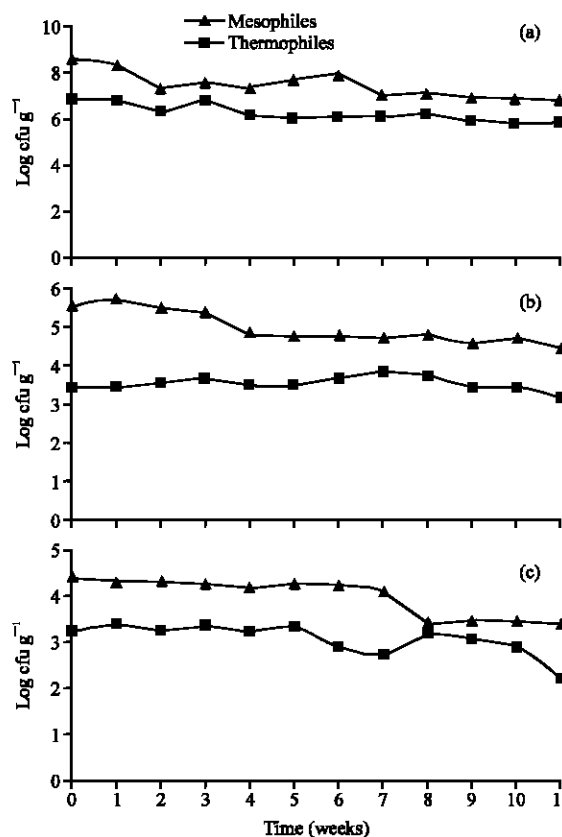


Fig. 3: Population of (a) Bacteria, (b) Actinomycetes and (c) Fungi during composting; mesophiles and thermophiles

and thermophilic bacteria, fungi and actinomycetes at various stages of the composting process was determined. Bacteria were the dominant group throughout the process at more than 2 log CFU g⁻¹ higher than for fungi and actinomycetes. Mesophilic bacteria and fungi were found to be at their highest numbers during the first week of composting (Fig. 3a-c) with numbers of 8.6 and 5.7 log CFU g⁻¹, respectively and then both declined.

Fungi have been reported to be an important group in the early phase of composting (Klamer and Baath, 1998). The population of fungi was highest at $4.4 \log \text{CFU g}^{-1}$ in the first week with only slight changes during the first 7 weeks and then a $1 \log \text{CFU g}^{-1}$ decline was observed. The thermophilic groups were lower than the mesophilic groups and changed only slightly compared to the mesophilic groups. The lower numbers and changes in the thermophilic microorganisms correlated with the low temperature of the compost. This indicated that the poor decomposition resulted in low temperature rises so there was little selection for any thermophilic microorganisms.

The development of mesophilic and thermophilic microorganisms during composting is related to the mesophilic and thermophilic stages of the composting process (Diaz-Ravina *et al.*, 1989; Davis *et al.*, 1991; Ishii *et al.*, 2000; Riddech *et al.*, 2002), thus as the maximum temperature reached was only 40°C at day 7 of the composting there was no observed succession from a mesophilic to a thermophilic microflora.

Isolation of bacteria with an ability to produce good levels of cellulase and xylanase, led to the selection of isolate B4. The investigation of enzyme production was performed using 4 different agricultural substrates, water hyacinth, coconut shells, rice straws and corncob. All substrates produced xylanase activity that was more than two fold higher than the cellulase. Cellulase was maximum at day 5 with 1.19 U/g DW from corncob while xylanase was highest at day 9 with 2.54 U/g DW from rice straws (Fig. 4a, b). Surprisingly, water hyacinth produced the lowest activity for both enzymes. The reason for this could be that water hyacinth has the lowest C/N ratio compared to other substrates therefore not so much excess of carbon.

Both enzymes exhibited maximum activity (Fig. 5) and stability at 50°C (Fig. 6) and declined above this temperature although the activity of both enzymes was still at 80% at 60°C (Fig. 5). The cellulase and xylanase retained 20% of activity at 80°C . The stability of xylanase declined more rapidly when the temperature rose.

Agricultural wastes in the form of cellulose and xylan are the most abundant renewable resources in the biosphere and can be used in the production of valuable value added products. In this study, rice straws, corncob, coconut shells and water hyacinth have shown to be used as substrates to produce high amounts of enzymes. These results highlight the industrial potential of these substrates as possible raw materials for cellulase and xylanase enzyme production by microorganisms.

The isolate strain B4 was initially characterized to be a yellow-white colony, Gram-positive, rod shaped and produced endospores. The resulting 16s rDNA partial sequence were compared with those in the GenBank

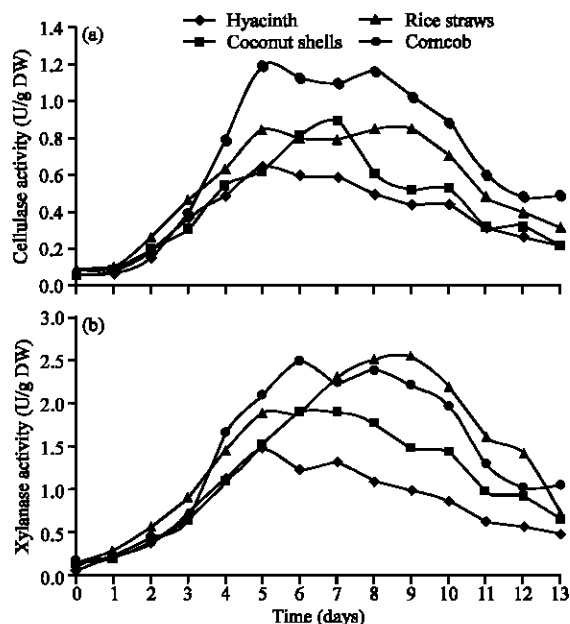


Fig. 4: Time profile for cellulase (a) and xylanase (b) production from strain B4 using 4 different substrates; water hyacinth, coconut shells, rice straws and corncob at 35°C for 13 days

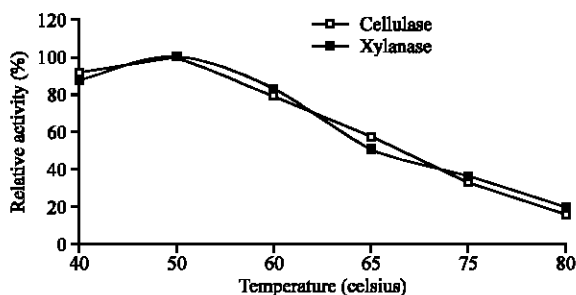


Fig. 5: Effect of temperature on the relative activity of cellulase and xylanase from strain B4

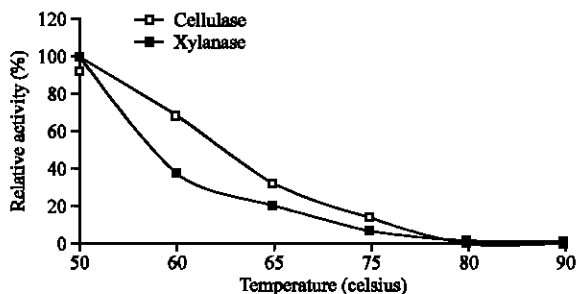


Fig. 6: Effect of temperature on the stability of cellulase and xylanase from strain B4

database using the BLAST program. Results showed that B4 was a *Bacillus* sp. (Table 2).

Table 2: Identification results of strain B4

Strain name	Closets sequence	Similarity (%)	Accession No.
B4	<i>Bacillus</i> sp. GGC-P1 Length = 1385 Score = 868 bits (962), Expect = 0.0 Gaps = 0/481 (0%) Strand = Plus/Plus	481/481 (100%)	FJ348028

CONCLUSIONS

Water hyacinth is a potential unlimited resource for composting in Thailand. In this study the composting process was performed in the way that local people make their water hyacinth compost. We found some problems with their process. Since water hyacinth can readily absorb water, it is essential to first dry it and ensure that the moisture content at the start is not more than 70%. Chemical, physical and microbiological changes during composting of various organic wastes have indicated that there is a succession of microbial populations that depend on the temperature reached during the composting process. In this process there was no clear microbial succession because the maximum temperature attained was only 40°C. It is crucial in the first month of composting to control the right conditions for microorganisms. Most parameters do not change much after 6 weeks so the time for production can be significantly reduced. The water hyacinth compost used here starts with a low C/N ratio of 17.61 which is well below the optimum ratio of 25 to 30. This presumably is also the reason for the low maximum temperature as microbial metabolic rates are limited. However the final C:N ratio of 18.12 indicates that it might certainly be a good product to improve the fiber content of soil and this might offset its low N content. Additional agricultural wastes with very high organic carbon content added at the start should increase the C/N ratio. Rice straw, corncob and coconut shells are cheap residues that can be used as additional substrates for composting and also for increasing polysaccharide hydrolases such as cellulase and xylanase enzyme production and also temperatures to help with sterility. The isolate, *Bacillus* sp. B4, was shown to produce reasonable amounts of cellulase and xylanase activity. It will now be used as a starter strain for the composting process.

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REFERENCES

- Abdel-Sabar, M.F. and M.A. Ebo El-Seoud, 1996. Effects of organic-waste compost addition on sesame growth, yield and chemical composition. *J. Agric. Ecosyst. Environ.*, 60: 157-164.
- Al-Turki, A.I., 2010. Quality assessment of commercially produced composts in Saudi Arabia market. *Int. J. Agric. Res.*, 5: 70-79.
- APHA, AWWA, WPCF, 2005. *Standard Methods for the Examination of Water and Wastewater*. 21st Edn., American Public Health Association, Washington, DC, USA.
- Atkinson, C.F., D.D. Jones and J.J. Gauthier, 1996. Biodegradability and microbial activities during composting of poultry litter. *Poult. Sci.*, 75: 608-617.
- Beffa, T., M. Blanc, L. Marilley, J.L. Fischer, P.F. Lyon and M. Arangno, 1996. Taxonomic and Metabolic Microbial Diversity During Composting. In: *The Science of Composting*, De Bertoldi, M., P. Sequi, B. Lemmes and T. Papi (Eds.). Chapman and Hall, London, pp: 149-161.
- Benito, M., A. Masaguer, A. Moliner, N. Arrigo and R.S. Palma, 2003. Chemical and microbiological parameters for the characterization of the stabilizing and maturing of pruning waste compost. *Biol. Fertil. Soils*, 37: 184-189.
- Berg, B., B.V. Hofstan and B. Petterson, 1972. Growth and cellulase formation by *Cellulibrio fulvus*. *J. Applied Bacteriol.*, 35: 201-214.
- Castaldi, P., G. Garau and P. Melis, 2008. Maturity assessment of compost from municipal solid waste through the study of enzyme activities and water soluble fractions. *Waste Manage.*, 28: 534-540.
- Center, T.D., 1994. Biological Control of Weeds: Water Hyacinth and Water Lettuce (Chapter 23). In: *Pest Management in the Subtropics: Biological Control a Florida Perspective*, Rosen, D., F.D. Bennett and J.L. Capinera (Eds.). Intercept Publishing Co., Andover, UK., pp: 481-521.
- Chen, P.J., T.C. Wei, Y.T. Chang and L.P. Lin, 2004. Purification and characterization of carboxymethyl cellulose from *Sinorhizobium fredii*. *Bot. Bull. Acad. Sinica*, 45: 111-118.
- Chukwuka, K.S. and O.E. Omotayo, 2009. Soil fertility restoration potentials of tithonia green manure and water hyacinth compost on a nutrient depleted soil in South Western Nigeria. *Res. J. Soil Biol.*, 1: 20-30.
- Cook, C.D., 1990. *Origin, Autoecology and Spread of some of the World's Most Troublesome Aquatic Weeds: The Ecology and Management of Nuisance Aquatic Vegetation*. Oxford University Press, Oxford, UK.

- Cunha Queda, A.C., G. Vallini, M. Agnolucci, C.A. Coelho, L. Campos and R.B. Sousa, 2002. Microbiological and Chemical Characterization of Composts at Different Levels of Maturity with Evaluation of Phytotoxicity and Enzymatic Activities. In: *Microbiology of Composting*, Insam, H., N. Riddech and S. Klammer (Eds.). Springer, New York, pp: 345-355.
- Davis, C.L., S.A. Hinch, C.J. Donkin and P. Germishuizen, 1991. Changes in microbial population numbers during composting of pine bark. *Bioresour. Technol.*, 39: 85-92.
- Diaz-Ravina, M., M.J. Acea and T. Carballas, 1989. Microbiological characterization of four composted urban refuses. *Biol. Wastes*, 30: 89-100.
- Epstein, P., 1998. Weeds bring disease to the east African waterways. *Lancet*, 351: 577-586.
- Erickson, M.C., J. Liao, L. Ma, X. Jiang and M.P. Doyle, 2009. Inactivation of *Salmonella* spp. in cow manure composts formulated to different initial C:N ratios. *Bioresour. Technol.*, 100: 5898-5903.
- Feinstein, M.S., F.C. Miller and P.E. Strom, 1986. Waste Treatment Composting as a Controlled System. In: *Biotechnology Microbial Degradation*, Rhelm, H.J. and G. Reed (Eds.). Vol. 8, VCH Publishers, New York, pp: 363-369.
- Fernandez, O.A., D.L. Sutton, V.H. Lallana, M.R. Sabbatini and J.H. Irigoyan, 1990. Aquatic Weed Problems and Management in South and Central America. In: *Aquatic Weeds-the Ecology and Management of Nuisance Aquatic Vegetation*, Charudattan, R. (Ed.). Oxford University Press, New York, pp: 406-425.
- Garcia, C., T. Hernandez, F. Costa and M. Ayuso, 1992. Evaluation of the maturity of municipal waste compost using simple chemical parameters. *Commun. Soil Sci. Plant Anal.*, 23: 1501-1512.
- Gaur, A.C. and G. Singh, 1995. Recycling of Rural and Urban Wastes Through Conventional and Vermicomposting. In: *Recycling of Crop, Animal, Human and Industrial Wastes in Agriculture*, Tandon, H.L.S. (Ed.). Fertilizer Development and Consultation Organization, New Delhi, pp: 31-49.
- Gopal, B., 1987. *Water Hyacinth: Aquatic Plant Studies*. Elsevier, Amsterdam, pp: 741.
- Goyal, S., S.K. Dhull and K.K. Kapoor, 2005. Chemical and biological changes during composting of different organic wastes and assessment of compost maturity. *Bioresour. Technol.*, 96: 1584-1591.
- Gunnarsson, C.C. and C.M. Petersen, 2007. Water hyacinths as a resource in agriculture and energy production: A literature review. *Waste Manage.*, 27: 117-129.
- Haug, R.T., 1980. *Compost Engineering Principles and Practice*. Ann Arbor Science Publishers, Inc., Ann Arbor, MI.
- Horwitz, W., 1997. Thermo Finnigan Flash 1112 Series, Method 972.43. In: *Official Method of Analysis of AOAC International*, Latimer, G.W.J. (Ed.). 16th Edn., AOAC, Virginia, USA.
- Huang, G.F., M. Fang, Q.T. Wu, L.X. Zhou, X.D. Liao and J.W.C. Wong, 2001. Co-composting of pig manure with leaves. *Environ. Technol.*, 22: 1203-1212.
- Huang, G.F., J.W.C. Wong, Q.T. Wu and B.B. Nagar, 2004. Effect of C/N on composting of pig manure with sawdust. *Waste Manage.*, 24: 805-813.
- Inbar, Y., Y. Hadar and Y. Chen, 1993. Recycling of cattle manure: The composting process and the characterization of maturity. *J. Environ. Qual.*, 22: 857-863.
- Ishii, K., M. Fukui and S. Takii, 2000. Microbial succession during a composting process as evaluated by denaturing gradient gel electrophoresis analysis. *J. Applied Microbiol.*, 89: 768-777.
- Itavaara, M., O. Venelampi, M. Vikman and A. Kapanen, 2002. Compost Maturity Problems Associated with Testing. In: *Microbiology of Composting*, Insam, H., N. Riddech and S. Klammer (Eds.). Springer Verlag, Heidelberg, pp: 373-382.
- Kandeler, E., M. Stemmer, S. Palli and M.H. Gerzabek, 1999. Xylanase, Invertase and Urease Activity in Particle Size Fractions of Soils. In: *Effect of Mineral-Organic-Microorganism Interactions on Soil and Fresh Water Environments*, Berthelin, J., P.M. Huang and J.M. Bollag (Eds.). Kluwer Academic/Plenum Publishers, New York, London, pp: 275-286.
- Klamer, M. and E. Baath, 1998. Microbial community dynamics during composting of straw material studied using phospholipid fatty acid analysis. *FEMS Microbiol. Ecol.*, 27: 9-20.
- Kutzner, H.J., 2001. Microbiology of composting. *Biotechnology*, 11: 35-100.
- Malik, A., 2007. Environmental challenge vis a vis opportunity: The case of water hyacinth. *Environ. Int.*, 33: 122-138.
- Marx, M.C., M. Wood and S.C. Jarvis, 2001. A microplate fluorimetric assay for the study of enzyme diversity in soils. *Soil Biol. Biochem.*, 33: 1633-1640.
- Michel, F.C., L.J. Forney, A.J.F. Huang, S. Drew, M. Czuprendski, J.D. Lindeberg and C.A. Reddy, 1996. Effects of turning frequency, leaves to grass mix ratio and windrow vs. pile configuration on the composting of yard trimmings. *Compost Sci. Util.*, 4: 126-143.

- Mondini, C., F. Farnasier and T. Sinicco, 2004. Enzymatic activity as a parameter for the characterization of the composting process. *Soil Biol. Biochem.*, 36: 1587-1594.
- Ranalli, G., G. Botturea, P. Taddei, M. Garavni, R. Marchetti and G. Sorlini, 2001. Composting of solid and sludge residues from agricultural and food industries. Bioindicators of monitoring and compost maturing. *J. Environ. Sci. Health*, 36: 415-436.
- Raut, M.P., S.P.M. Prince-William, J.K. Bhattacharyya, T. Chakrabarti and S. Devotta, 2008. Microbial dynamics and enzyme activities during rapid composting of municipal solid waste: A compost maturity analysis perspective. *Bioresour. Technol.*, 99: 6512-6519.
- Riddech, M., S. Klammer and H. Insam, 2002. Characterization of Microbial Communities during Composting of Organic Wastes. In: *Microbiology of Composting*, Insam, H., N. Riddech and S. Klammer (Eds.). Springer Verlag, Heidelberg, pp: 43-52.
- Sanchez-Montero, M.A., A. Roig, C. Paredes and M.P. Bernal, 2001. Nitrogen transformation during waste composting by the Rutgers system and its effects on pH, EC and maturity of the composting mixtures. *Bioresour. Technol.*, 78: 301-308.
- Schlegel, H.G. and H.W. Jannasch, 1992. Prokaryotes and their Habitats. In: *The Prokaryotes*, Balows, A., H.G. Truper, M. Dworkin, W. Harder and K.H. Schleifer (Eds.). 2nd Edn., Springer-Verlag, New York, pp: 76-125.
- Sharma, A.R. and B.N. Mitra, 1990. Response of rice to rate and time of application of organic materials. *J. Agric. Sci.*, 114: 249-252.
- Somogyi, M., 1952. Notes in sugar determination. *J. Biol. Chem.*, 195: 19-23.
- Tiquia, S.M., N.F.Y. Tam and I.S. Hodgkiss, 1996. Microbial activities during composting of spent pig manure saw dust litter at different moisture contents. *Bioresour. Technol.*, 55: 201-206.
- Tiquia, S.M., 2002. Evolution of extracellular enzyme activities during manure composting. *J. Applied Microbiol.*, 92: 764-775.
- Wang, C.M., C.M. Changa, M.E. Watson, W.A. Dick, Y. Chen and H.A.J. Hoitink, 2004. Maturity indices of composted dairy and pig manures. *Soil Biol. Biochem.*, 36: 767-776.
- Wu, L. and L.Q. Ma, 2002. Relationship between compost stability and extractable organic carbon. *J. Environ. Qual.*, 31: 1323-1328.