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

K. Umsakul, Y. Dissara and N. Srimuang
Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand

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 Mittra, 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

Corresponding Author: Kamontam Umsakul, Department of Microbiology, Faculty of Science, Prince of Songkla University, Hat-Yai, Songkhla 90112, Thailand Tel/Fax: +66 74 446661

985
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 (Befia 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 (Kandelet 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; Bernto 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., 2010).
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. 1a-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

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

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

*nd*: Not detected

![Fig. 1: Changes in (a) Percentage moisture, (b) pH and (c) Temperature during composting](image-url)
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 25% 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 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 (Klumer and Baath, 1998). The population of fungi was highest at 4.4 log CFU g⁻¹ in the first week with only slight changes during the first 7 weeks and then a 1 log 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 database using the BLAST program. Results showed that B4 was a Bacillus sp. (Table 2).
Table 2: Identification results of strain B4

<table>
<thead>
<tr>
<th>Strain name</th>
<th>Closest sequence</th>
<th>Similarity (%)</th>
<th>Accession No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>B4</td>
<td>Bacillus sp. GGC-P1</td>
<td>481/481 (100%)</td>
<td>FJ348028</td>
</tr>
<tr>
<td></td>
<td>Length = 1385</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Score = 600 bits (962), Expect = 0.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Gaps = 0/481 (0%)</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Strand = Plus/Plus</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

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, corn cob 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.

ACKNOWLEDGMENTS

Financial support was provided by the research grant of the Faculty of Science, Prince of Songkla University. The authors are grateful to Dr. Brian Hodgson for his advice. We would like to thank the Marine and Coastal Resources Institute (MACORIN) for help with the composting process.

REFERENCES


