



## Research Article

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# Wheat Straw Composting Through *Aspergillus oryzae*

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## Abstract

Wheat straw is an abundant by-product from wheat production. The average yield of wheat straw is 1.3-1.4 lb per lb of wheat grains. Composting is a cost effective and eco-friendly process to dispose abundant agricultural wastes. In our study we apply *Aspergillus oryzae* to enhance quality of compost. The parameters like pH, temperature, moisture, TS, total ash, TKN, TOC, organic matter, phosphorous and enzymatic properties like endocellulase, FPase, xylanase alkaline and acid phosphatase have been analyzed. The C: N ratio at 30 days was 17.41 as compared to natural composting which was 43.6. It increases to 9.588 and 10.52 at 60 days, respectively. Endocellulase activity increases upto 90 days that was 0.148 IU/g in bin 3 while in other two bins it was not detected. The data suggested that *A. oryzae* can degrade wheat straw maximum between 60-90 days. This data proves, locally isolated strain *A. oryzae* enhance degradation of wheat straw during composting.

**Key words-** Compost, Enzymatic profile, Physicochemical analysis

## Introduction

Owing to both rise in population and economic development an array of organic and inorganic by-products is generated from agricultural based industries, which are disposed-off according to their physical and chemical traits<sup>1</sup>. The disposal of large quantities of agro based industrial waste causes energy, economic and environmental problems. These wastes have a high content of organic matter and mineral elements; they can potentially be used to restore soil fertility<sup>2</sup>. Composting is useful for waste recycling and produces a chemically stable material that can be used as a source of nutrients and for improving soil structure<sup>3</sup>. Composting is a biological aerobic process managed under controlled conditions. It involves a complex transformation of the raw organic substrates, which are degraded and transformed into stable organic matter containing humic-like compounds. These substances positively affect soil ecology, structure, fertility and productivity and hence are considered a critical

factor for agricultural production<sup>4-7</sup>. The growing human population and concomitant increase in use of natural resources and generating a series of negative effects on ecosystems, such as pollution, loss of genetic diversity, soil fertility decline, climate changes, decline in yields, deforestation and desertification. Agriculture is asked to satisfy two apparent needs; to become more productive and at the same time more sustainable, without depleting renewable resources<sup>8</sup>. Agro industrial sector generates large amount of biodegradable wastes and by-products, which need to be suitably disposed-off in order to reduce their environmental impact. Best example of this is production of wheat which is very important crop for India. Wheat straw is an abundant by-product from wheat production. The average yield of wheat straw is 1.3-1.4 lb per lb of wheat grains<sup>9, 10</sup>. Based on data from FAO, 611 million metric tons of wheat was produced in world in 2007 (India production was 75 million metric tons)<sup>11</sup>. These wastes accumulate in the environment there by causing pollution problem,

because most of agricultural waste disposed by burning or slashing, a practice considered as major factor in global warming.

Composting cannot be considered a new technology, but it is an age long process. It takes 5 and 6 months to complete. Successful composting depends on number of factors like type of raw material, nutrient composition, moisture content, temperature, acidity or alkalinity and aeration which directly or indirectly influence activities of microorganisms<sup>12</sup>. Hemicellulose and cellulose of wheat straw may be the main substrates for microorganisms during maturation process<sup>13, 14</sup>. While lignin limits degradation and is recognized to be an important precursor of humic substances<sup>4, 15-18</sup>. During composting, the starting material is transformed through a variety of biological and biochemical processes in which enzymes play a key role<sup>19-22</sup>. Enzymes that catalyze the degradation of polymeric substances, such as cellulose, hemicellulose and lignin, are extracellular because the polymer is too large to be transported across the cellular membrane<sup>23, 24</sup>. Research has generally focused on the changes, with composting time, in total organic carbon and nitrogen<sup>25, 26</sup> and on molecular characteristics<sup>27</sup>, as well as colloidal humic like components<sup>3, 28</sup>. Less attention has generally been paid to the transformations that organic components undergo with the stabilization process.

The objective of this paper was characterization of organic matter transformation of compost material at different stages. On the other hand we tried to check effect of additional mass of previously isolated lignocellulolytic fungal strain *Aspergillus oryzae* by<sup>29</sup>, with cow dung. Here, we made a first attempt in compost technology to inoculate additional fungal biomass in direction, to reduce long time required for maturity of compost.

## Experimental

### Composting materials

Fresh buffalo dung and wheat straw were used as experimental materials collected locally from Sadra village of Gandhinagar District of Gujarat State, India (23.22 °N and 72.68 °E). Visual evaluation deemed the straw to be of good quality with no apparent signs of mould or decay. Immediately after transport to laboratory, the materials were analyzed (Table 1) and prepared for experiments. The straw was cut to the 3-5 cm size in length to allow uniform mixing of the buffalo dung and straw, and also facilitate sampling and analysis of composting solids.

**Table 1:** Physico-chemical characteristics of buffalo dung and wheat straw

Parameters	Buffalo Dung	Wheat straw
pH	8.50	NA*
TS (g %)	20(1) <sup>†</sup>	98(2)
TVS (g %)	13.94(0.5)	NA
Total Organic Carbon (%)	22.10(0.91)	2.66(0.14)
Organic Matter (%)	30.22(1.38)	4.59(0.36)
TKN (%)	1.79(0.06)	0.013(0.006)
Available Phosphorus (%)	0.29(0.01)	0.1(0.01)
Available Potassium (%)	0.49(0.02)	1.53(0.15)
Cellulose (g %)	NA	36
Hemicellulose (g %)	NA	42
Lignin (g %)	NA	20

<sup>†</sup> Values in parenthesis show standard deviation.

\* NA: Not Applicable

### Experimental set-up

Composting was carried out by pit composting method with modification described previously<sup>12</sup>. The experiment was set at a place where complete shadowing condition maintained to reduce moisture loss. The compost bin was prepared using bricks. Size of bin was 2'×2' and height was 1.6'. The working size was nearly 1.2'×1.5' feet. Bin was filled with buffalo dung and wheat straw on their dry basis. The buffalo dung and straw mixed manually to achieve better homogenization. There are three different mixtures (bin 1, 2 and 3) prepared with following composition: Bin 1 contain only wheat straw (100 %); Bin 2 contains mixture of buffalo dung and wheat straw in ratio of 1:5; and Bin 3 contains buffalo dung and wheat straw in 1:5 ratio which is inoculated with lignocellulolytic fungi *Aspergillus oryzae*.

### Fungal strain and culture condition

Isolates of *A. oryzae* maintained at 4°C on Potato Dextrose Agar (PDA) slants<sup>29</sup>. To achieve 6×10<sup>9</sup> spore count per mL, inoculum was prepared in salt medium, which contained 0.01% MgSO<sub>4</sub>, 0.1% (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, 0.2% KH<sub>2</sub>PO<sub>4</sub>, 0.7% K<sub>2</sub>HPO<sub>4</sub> and 0.05% Na-Citrate<sup>30</sup>. pH of the media was adjusted to 5.0± 0.2, using 0.1 N HCl or 0.1 N NaOH.

### Composting samples

The 100g samples were taken from pit after mixing the buffalo dung wheat straw, at 3, 30, 60 and 90 days as per the method described previously<sup>31</sup>.

### Moisture content

Moisture content was determined by dry weight in oven at 105°C for 24 h<sup>32</sup>. To maintain

moisture content of about 70-80% in composting material, sprinkling of water carried out as and when required.

### Temperature determination

Temperature was monitored using Immersion Zeal Co. thermometer. Process temperature was determined weakly by inserting the thermometer 25cm deep into compost pit.

### Physico-chemical analysis

Physico-chemical parameters such as pH, Total Solids, Total Volatile Solids, and total ash content were analyzed by standard method of APHA<sup>32</sup>. While total organic carbon, total kjldhal nitrogen, phosphorous and potassium analyzed by standard methods<sup>33-36</sup>.

### Enzymatic studies

5g sample from compost pit was extracted with 25 mL citrate buffer (pH 4.8; 50mM) by shaking the mixture at 180 rpm for 30 minutes at  $28 \pm 2$  °C and filter through Whatman No 2 filter paper. The filtrate was used for enzymatic assay like endocellulase, FPase, xylanase, MnP, acid and alkaline phosphatase following standard methods<sup>37-40</sup>.

### Statistical analysis

Results were analyzed for standard deviation<sup>41</sup>.

## Results and discussion

### Physico-chemical parameters

#### pH value

pH is an important parameter in composting. pH at initial stage in all 3 bin remain alkaline but it decreased at 60 and 90 days (Table 2). In bin 2 and 3 initial pH was 8.25 and 9.12 which was found 7.28 and 8.12 after 90 days. This indicates that during process organic acid were formed from organic waste degradation and decrease in  $\text{NH}_4^+$ <sup>42</sup>. After 60 days pH was, in bin 3 remains above 8.0. Same type of results found by Wang et al who use mixture of dairy manure- wheat straw, dairy manure- saw dust and pig manure- wood. They found pH above 8.50 I almost all set for more than 91 days<sup>43</sup>.

#### Temperature values

Temperature of compost bin reached  $>45^\circ\text{C}$  at first turning (15 days), reflecting the initiation

Table 2: Physicochemical characteristics of compost prepared by using *A. oryzae*

Parameters	30 Days			60 Days			90 Days		
	Control (WS)	A. oryzae (WS+CD)	Control (WS)	A. oryzae (WS+CD)	Control (WS)	A. oryzae (WS+CD)	Control (WS)	A. oryzae (WS+CD)	
pH	8.34	8.17	8.45	8.43	7.56	7.28	8.04	8.04	
Temperature (°C)	44	44	43	43	42	42	43	43	
TS (g %)	16.66(0.83)†	21.34(0.94)	26.20(1.04)	22(0.88)	24(0.93)	26(1.03)	24.2(0.96)	24.2(0.96)	
TVS (g %)	15(0.50)	14(0.43)	14(0.41)	14(0.38)	14(0.37)	22(0.61)	23(0.63)	23(0.63)	
Total Ash (g %)	85(3.48)	86(2.98)	86(3.40)	86(2.97)	86(3.09)	78(2.81)	67(2.09)	67(2.09)	
Moisture (%)	83.34(2.81)	78.66(2.76)	73.80(2.86)	78(2.50)	76(2.31)	74(1.99)	75.8(2.01)	75.8(2.01)	
TKN (%)	0.047(0.002)	0.088(0.004)	0.075(0.003)	0.50(0.003)	0.17(0.001)	0.20(0.001)	2.15(0.011)	2.15(0.011)	
TOC (%)	3.458(0.16)	3.764(0.18)	3.27(0.14)	4.794(0.21)	2.45(0.010)	1.957(0.009)	2.41(0.007)	2.41(0.007)	
Organic Matter	5.96(0.24)	6.49(0.27)	5.64(0.23)	8.27(0.31)	4.22(0.18)	3.37(0.12)	2.14(0.009)	2.14(0.009)	
Phosphorus (%)	0.30(0.011)	0.20(0.008)	0.80(0.021)	0.36(0.013)	1.0(0.035)	0.50(0.018)	0.12(0.006)	0.12(0.006)	
C:N Ratio	73.58	42.77	43.6	9.588	14.41	9.79	5.77	5.77	

† Values in parenthesis show standard deviation.

of composting process. From second turning (30 days) it remains below 46°C (Table 2). Highest temperature values measure at first turning, in bin 3. It might be probably due to addition of lignocellulolytic fungi. Yigitbasi et al was found highest temperature at second turning because addition of molasses at the 6th day of composting process<sup>44</sup>. Increased temperature at this stage is an indicator for a rapid and exothermic microbial activity<sup>44</sup>. These results are consist with previous findings<sup>2, 44-47</sup>. At the later stage, temperature did not increase further even after addition of water and turning. Temperature reached a plateau after 75 days. Same findings are also reported previously<sup>2</sup>.

### Moisture content

Moisture content has been referred to as a critical factor in optimizing composting systems<sup>48, 49</sup>, because the decomposition of organic matter depends on presence of water to support microbial activity. The initial moisture content was between 70 to 80% in all three bins and was maintain throughout composting process. Initial moisture content around 70% provides good condition for degradation of both easily and hardly degradable organic compounds<sup>50</sup>. It has been reported that initial moisture content for composting of poultry manure with wheat straw range from 65 to 80% on wet basis<sup>48, 51, 52</sup>. Optimum moisture content between 75 to 80% for farmyard manure and straw was reported<sup>48</sup>. While some reported that, for poultry manure and straw, 73-80% moisture content was optimum<sup>51</sup>. In our study moisture level remains above 75%. Very low moisture content causes dehydration, which produce physically stable but biologically unstable product<sup>53</sup>, where as high

moisture content can produce anaerobic condition as results of water logging<sup>54</sup>.

### Organic carbon, organic matter and C/N ratio

Organic matter is mineralized after composting, mostly due to degradation of easily degradable compounds, which are utilized by microorganisms as carbon and nitrogen sources. Rate of organic matter loss is an indicator of the overall composting rate<sup>49</sup>. While degrading organic compounds, microorganisms convert 60 to 70% carbon to carbon dioxide and remain 30-40% into their bodies as cellular components<sup>55</sup>. In our study organic matter content of materials decreased significantly in all bins during the process, but in bin 2 and 3 greatest reduction observed. In bin 2 and 3 C/N ratio at 90 days, was decreased to 9.79 and 5.77 from 42.77 and 17.41 respectively (Table 2). While in bin 1 C/N ratio was very high at 90 days with compare to bin 2 and 3. Same type of scenario was observed by various researchers<sup>31, 43, 50</sup>. Here in all bin wheat straw increased C/N ratio initially and provide degradable organic carbon<sup>49</sup>. The result of C/N ratio below 20 was indicated the maturity degree<sup>56</sup>. In our study result shows the C/N ratio was in range of maturity level in all 3 bins.

### Enzymatic parameters

Characterizing and quantifying the enzymatic activity during composting can reflects dynamics of the composting process in terms of the decomposition of organic matter and nitrogen transformation and may provide information about the maturity of composted product<sup>24</sup>. Enzyme activities during composting have been studied in the past<sup>19-22, 24, 57</sup>. Very few researchers have attempted to assess the changes in extracellular enzyme activities and link with composting

**Table 3.** Enzymatic characteristics of compost prepared by using *A. oryzae*

Parameters	30 Days			60 Days			90 Days		
	C1	C2	<i>A. oryzae</i>	C1	C2	<i>A. oryzae</i>	C1	C2	<i>A. oryzae</i>
Endocellulase (U/g)	ND	ND	ND	ND	ND	0.093	ND	ND	0.148
FPase (U/g)	ND	ND	0.37	0.041	0.032	0.546	0.021	0.015	0.045
Xylanase (U/g)	ND	ND	ND	11.1	4.95	1.389	5.6	2.98	ND
Acid Phosphatase1 (µg/g/h)	07.5	127.5	117.5	155	167.5	329.3	186	201	510
Alkaline Aosphatase (µg/g/h)	125	142.5	133.8	177.5	177.5	309.2	248.5	248.5	411

C1: Control (WS); C2: Control (WS+CD), ND: Not Detected

process<sup>24</sup>. Alkaline and acid phosphatase activities were high in all 3 bins from the beginning of the test and reaching of maximum activity by the end of testing period 90 days (Table 3). Similar type of results observed previously when using poultry manure and pig manure<sup>24</sup>. Alkaline phosphatase activity during paddy straw composting by thermophilic fungal consortium showed 530.07 µg PNP g<sup>-1</sup>h<sup>-1</sup> was analyzed<sup>58</sup>. Experimental results of other enzymes assay during composting were shown in table 3. Xylanase activity at 30 days not detected in any of the bin but at 60 days it was found 11.1, 4.95 and 1.389 IUg<sup>-1</sup> in bin 1, bin 2 and bin 3, respectively. Then, at 90 days it decreased to 5.6 and 2.98 IUg<sup>-1</sup> in bin 1 and bin 2 respectively, while in bin 3 xylanase activity not found. It indicates that at 60 days maximum xylan degradation was occurred. In bin 1 high xylanase activity observed may be due to high content of wheat straw. Similar type of results was obtained previously<sup>59</sup>. Agricultural waste wheat straw used in our experiment contains 36% cellulose content (Table 1). This cellulose is degraded by lignocellulolytic fungi by producing cellulase enzyme. Endocellulase production was higher at 90 days (0.148 IUg<sup>-1</sup>), which was initially not detected. But total cellulase activity in form of FPase found higher at 60 days which was 0.546 IUg<sup>-1</sup>. Then it was decreased to 0.045 IUg<sup>-1</sup> at 90 days.

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