

## **Original Research Article**

### **Microbial inoculation influencing physical properties of composts produced from different agro-wastes**

#### **Abstract:**

A laboratory experiment was carried out by using three isolated cellulose degrading bacteria viz; CBG2, CBD4 and CBC9 with three organic substrates viz; rice straw, vegetable waste and maize stover. The physical properties after final product were examined. Irrespective of the strains inoculation the BD of rice straw was highest ( $0.82 \text{ Mg m}^{-3}$ ) followed by maize stock compost ( $0.74 \text{ Mg m}^{-3}$ ) and vegetable waste compost ( $0.73 \text{ Mg m}^{-3}$ ). Irrespective of the substrates the PD of the composts, was highest ( $1.55 \text{ Mg m}^{-3}$ ) in uninoculated control followed by CBC9 ( $1.02 \text{ Mg m}^{-3}$ ), CBD4 ( $0.97 \text{ Mg m}^{-3}$ ) and CBG2 ( $0.91 \text{ Mg m}^{-3}$ ). The porosity of the compost was highest (39 %) where CBG2 was inoculated followed by CBD4 (36%), CBC9 (35%) and uninoculated control (23%). The highest ( $42^{\circ}$ ) angle of repose was in CBG2 treated compost followed by CBD4 ( $39^{\circ}$ ), CBC9 ( $36^{\circ}$ ), and lowest ( $34^{\circ}$ ) was in uninoculated control. The tab density of compost from control was highest ( $1.15 \text{ Mg m}^{-3}$ ) followed by CBC9 ( $0.63 \text{ Mg m}^{-3}$ ), CBD4 ( $0.59 \text{ Mg m}^{-3}$ ) and lowest was in CBG2 ( $0.52 \text{ Mg m}^{-3}$ ). The highest (0.96) Hausner ratio (HR) was estimated in the uninoculated- control followed by CBC9 and CBD4 (0.95). The lowest (0.94) HR was in CBG2 inoculated compost.

**Key Words:** Agro-waste; Angle of repose; Composting; Hausner ratio; Physical properties

#### **Introduction:**

The production of required food to feed the burgeoning population of India is a great challenge now a days. The soil is the main factory for producing the required amount of food. The unbalanced and intensive cropping without taking proper care to soil is resulted into

degradation of quality. Maintaining good soil health is required for sustainable crop production. The application of organic fertilizer to the soil is essential to maintaining soil quality. In India the agricultural wastes generally used open dumping or in land filling. These practices result into green house gas emission and creates unhygienic environment (**Pandit et al. 2020**). In-situ crop residue is an important approach of crop residue management (**Pattanayak and Sethi 2022**). To produce compost from wastes several additives like microbes, swage sludge and animal waste are used (**Awasthi et al 2018**).

Comment [u1]: give a reference

Comment [u2]: give some references please

Crop residues, waste bedding materials, silage, manures, and farm wastes can be utilized as compost cover and base material materials (**Khater 2015**). The compost pile cannot be formed until the degradation has been completed, the type and thickness of the cover and base layer materials play a key role in influencing the biodegradation of carcasses, and the development and retention of heat that is necessary for pathogen inactivation (**Fonstad et al 2003**)

The production of organic fertilizers by composting is also an important and eco-friendly approach to manage the crop residue. The quality has great influence on soil properties. Apart from biological and chemical properties the physical properties are important parameter for enhancing quality of soil. The good quality of compost production should ensure adequate chemical and physical properties (**Inbar et al 2003**), as well as degree of stability and maturity (**Benito et al 2003**). The beneficial effects of compost on crop production and maintaining soil quality reported in literature (**Ho et al 2022**) are directly related to the physical, chemical and biological properties of the composts (**Khater 2015**). By considering above facts in this experiment we have tried to evaluate the physical properties of compost produced from different agro-waste inoculated with isolated cellulose degrading bacteria.

## Materials and Methods:

The experiment was conducted in the Soil Microbiology Laboratory of the Department of Soil Science and Agricultural Chemistry, College of Agriculture, Odisha University of Agriculture and Technology, Bhubaneswar. The substrates were collected from the nearby agricultural field. The substrates were selected as per the availability and production capacity. The fresh substrates were collected and chopped into small pieces. The cellulose degrading bacteria were isolated. The freshly prepared the growth medium of selected strains were inoculated with the substrates.

**Comment [u3]:** how was the isolation of the cellulose degrading bacteria done? with what method?

**Comment [u4]:** What medium?

The sample of final mature composts were collected after sieving through 4mm sieve. The bulk density as per **Zaki et al (2017)** and particle density was estimated by pycnometer method. The porosity was calculated by using the formulae (Eqn-1).

$$\text{Porosity (\%)} = 1 - \frac{BD}{PD} \times 100 \quad \text{Eqn. 1}$$

Maximum water holding capacity (%) was estimated by kinrhizoski box method and moisture content was estimated by keeping 100g of fresh compost in hot air oven at 70<sup>0</sup>c and the weight was measure after 5hrs till get a constant weight. Angle repose, and hushners ratio The tab density was measured by taking 250 ml plastic measuring cylinder containing 100g compost was hit on a rigid surface area @ 300 times per minute and at the end the volume of compost inside the beaker was measured and expressed in Mg m<sup>-3</sup> (**Zaki et al. 2017**). Angle of repose was measured by pilling method as described **Fraczek et al. (2007)**. The Hausner ratio (HR) was calculated by taking the ratio of tab density and bulk density (Eqn-2) (**Zaki et al 2017**).

$$\text{HR} = \frac{TB}{BD} \quad \text{Eqn. 2}$$

Statistics:

## Results and Discussion:

### Bulk density, particle density ( $\text{Mg m}^{-3}$ ) and porosity (%):

The bulk density is an important physical parameter for composts. The bulk density of compost indicates the compactness of the compost which directly reflects on the air space of the compost. The data regarding bulk density of composts has been presented in table 1. Irrespective of the substrates the bulk density of control was highest ( $1.2 \text{ Mg m}^{-3}$ ) followed by CBC9, CBD4, and CBG2 inoculated strains. The BD of CBC9 and CBD4 were statistically at par ( $P=0.05$ ). Whereas, uninoculated control and CBG2 were significantly different than other strains inoculation. Irrespective of the strains inoculation the BD of rice straw was highest ( $0.82 \text{ Mg m}^{-3}$ ) which was significantly different ( $P=0.05$ ) than other substrates. The BD of the vegetable waste compost ( $0.73 \text{ Mg m}^{-3}$ ) and maize stock compost ( $0.74 \text{ Mg m}^{-3}$ ) were statistically at par. The BD of rice straw compost ( $1.32 \text{ Mg m}^{-3}$ ) produced without inoculation of any strains was highest among all the composts followed by maize stock compost and vegetable waste compost produced from uninoculated strain ( $1.15 \text{ Mg m}^{-3}$ ). Among CBC9 inoculated strain the highest ( $0.69 \text{ Mg m}^{-3}$ ) BD was estimated in the maize stock compost followed by rice straw ( $0.68 \text{ Mg m}^{-3}$ ) and vegetable waste ( $0.63 \text{ Mg m}^{-3}$ ), respectively. A similar trend was observed in CBG2 strain inoculated composts. Among the CBD4 inoculated composts the highest ( $0.66 \text{ Mg m}^{-3}$ ) BD was estimated in rice straw compost followed by vegetable waste ( $0.61 \text{ Mg m}^{-3}$ ) and maize stock ( $0.60 \text{ Mg m}^{-3}$ ), respectively. According to the Fertilizer Control Order, India, all the composts produced by the inoculation of isolated strains could be utilized in agricultural purpose (FCO, 1985). Therefore, only uninoculated composts were unable to meet the standard of FCO, India. The microbial inoculation increases the degradation of organic matter so the bulk density was reduced. Therefore, we can assume that the bulk density of composts depends on the

substrate type and efficiency of the microbe inoculation. The microbe inoculation influences the overall properties of including physical properties of compost (Greff et al. 2022). According to Zhang and Sun, (2018) the bulk density of final composts produced from green wastes were around 0.4 (Mg m<sup>-3</sup>). The bulk density also has influence on other properties of composts (Jain et al. 2019). The bulk density of initial substrates also influencing the quality of the cucumber stalk compost (Chang et al. 2017). The BD reflects the looseness and porosity of the final product (Jain et al., 2018). The particle density (PD) of composts produced from different agro-wastes inoculated with cellulose degrading bacterial strains has been presented in table 1. The PD of the composts, irrespective of the substrates was highest (1.55 Mg m<sup>-3</sup>) in uninoculated control followed by CBC9 (1.02 Mg m<sup>-3</sup>), CBD4 (0.97 Mg m<sup>-3</sup>) and CBG2 (0.91 Mg m<sup>-3</sup>). The PD of control was significantly different (P=0.05) than other composts. The composts of CBD4 and CBG2 were statistically at par (P=0.05) similarly CBC9 and CBD4 were at par whereas CBC9 and CBG2 were significantly different. Irrespective of the strains inoculation the PD of the rice straw compost was highest (1.17 Mg m<sup>-3</sup>), followed by maize stock compost (1.09 Mg m<sup>-3</sup>), and vegetable waste compost (1.08 Mg m<sup>-3</sup>). The PD of the composts of different substrates are statistically nonsignificant (P=0.05). The highest PD (1.67 Mg m<sup>-3</sup>) was estimated in the compost where rice straw was composted without any strains followed by vegetable waste and maize stock. In CBC9 inoculated compost the highest PD was estimated in maize stock (1.04 Mg m<sup>-3</sup>), followed by rice straw (1.03 Mg m<sup>-3</sup>) and vegetable waste (0.98 Mg m<sup>-3</sup>). In CBD4 inoculated strains the highest was estimated in rice straw (1.01 Mg m<sup>-3</sup>), followed by vegetable waste (0.96 Mg m<sup>-3</sup>) and maize stock (0.95 Mg m<sup>-3</sup>). Likewise, in CBG2 the PD was highest in rice straw (0.97 Mg m<sup>-3</sup>), followed by maize stock (0.89 Mg m<sup>-3</sup>) and vegetable waste (0.87 Mg m<sup>-3</sup>). The variation in particle density of the compost depends on the nature and quality of compost substrate (Jain et al. 2019). The PD also depends on the ash and volatile solid

content during composting of organic substances (van Ginkel et al. 1999). Similarly, Mohee and Mudhoo, (2005) and van Ginkel et al. (1999) reported the variation of PD among the composts. The porosity of the composts was calculated and presented in table 1. Irrespective of the substrates the porosity of the compost was highest (39 %) where CBG2 was inoculated followed by CBD4 (36%), CBC9 (35%) and uninoculated control (23%). The porosity of the CBG2 compost was statistically at par (P=0.05) with CBD4 and significantly different than CBC9 and uninoculated control. Whereas the porosity of CBC9 and CBD4 were statistically at par (P=0.05). Among all the composts the highest (41%) was estimated in vegetable wastes inoculated with CBG2 strain and lowest (21%) was calculated in uninoculated rice straw compost. Irrespective of the strain inoculation the porosity of maize stock compost was highest (34%) and vegetable waste compost (34%). The lowest (32%) was calculated in rice straw compost. The porosity of the substrate composts was statically nonsignificant (P=0.05). The porosity of the compost depends on both the BD and PD. The porosity decreased with increase in BD (Khater et al. 2015). The total and air-filled porosity directly affect the availability of water and oxygen (O<sub>2</sub>) and are determining factors for the biological activity of the microorganisms involved in the composting process (Bernal et al. 2017).

**Table 1:** Influence of different cellulose degrading bacteria on bulk density, particle density (Mg m<sup>-3</sup>) and porosity (%) of composts produced from different agro-wastes.

<b>Bulk Density (Mg m<sup>-3</sup>)</b>					
Substrates	Control*	CBC9	CBD4	CBG2	Mean
Rice straw	1.32	0.68	0.66	0.62	0.82
Vegetable waste	1.15	0.63	0.61	0.52	0.73
Maize stock	1.15	0.69	0.60	0.54	0.74
Mean	1.20	0.67	0.62	0.56	
LSD (P=0.05): Strain-0.047; Substrate-0.041; Strain x Substrate-0.082 CV (%):7					
<b>Particle Density (Mg m<sup>-3</sup>)</b>					
Rice straw	1.67	1.03	1.01	0.97	1.17
Vegetable waste	1.50	0.98	0.96	0.87	1.08
Maize stock	1.50	1.04	0.95	0.89	1.09
Mean	1.55	1.02	0.97	0.91	
LSD (P=0.05): Strain-0.098; Substrate-0.085; Strain x Substrate-0.169 CV (%):9					

**Comment [u5]:** in the methodology the author of the article should at the beginning give us the characteristics of the identified bacterial isolates that were used to evaluate the quality of the compost. what are the inclusive criteria that allowed the choice of these bacterial strains?

In the results we do not understand the effect of the bacterial strains on the Bulk density, on the particle density and on the porosity.

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<b>Porosity (%)</b>					
Rice straw	21	34	35	36	32
Vegetable waste	23	36	37	41	34
Maize stock	23	34	37	40	34
Mean	23	35	36	39	
LSD (P=0.05): Strain-3.29; Substrate-2.85; Strain x Substrate-5.71					CV (%):6

\*Control: No strains

#### Maximum water holding capacity (%) and moisture content (%)

The data related to maximum water holding capacity (WHC) has been presented in table 2. The highest WHC was estimated in the vegetable waste compost inoculated with CBC9 (50 %) and CBG2 (50 %) followed by maize stock compost inoculated with CBG2 (49%), rice straw with CBC9 (47%), Maize stock with CBD4 (43%) and lowest was estimated in rice straw with CBD4 (25 %). Irrespective of the substrates the highest WHC was in CBG2 (43 %) and CBC9 (43%) followed by CBD4 (34 %) and lowest (32%) was in uninoculated compost. The WHC of CBC9 and CBG2 inoculated composts were at par (P=0.05) with each other and significantly different with CBD4 inoculated compost and uninoculated strains (control). Irrespective of the strains WHC of the vegetable waste compost was highest (42%) followed by maize stock (38%) and rice straw (35%) compost. The WHC of vegetable waste was significantly higher (P=0.05) than maize stock and rice straw compost. The WHC of maize stock and rice straw were statistically (P=0.05) at par. Among CBC9 inoculated compost highest (50%) WHC was estimated in vegetable waste followed by rice straw (47 %) and maize stock (31%). In CBD4 inoculated compost highest (43%) was estimated in maize stock followed by vegetable wastes (35%) and rice straw (25%). In CBG2 inoculated compost the highest (50%) was estimated in vegetable wastes compost followed by maize stock (49%) and rice straw (31%).

The moisture content of the composts has been presented in table 2. The highest moisture content (27%) was estimated in the vegetable wastes and maize stock compost where CBG2 was inoculated. The lowest (16) was in rice straw compost without any strain.

**Comment [u6]:** I would like to understand the performance of each bacterial strain chosen on The maximum water holding capacity (WHC)

Irrespective of the substrate types the compost from CBG2 inoculation was highest (27%) followed by CBD4 (25%), CBC9(24%) and lowest (16%) was in uninoculated compost. The moisture content of composts of all the isolated strains were at par. The moisture content is an essential parameter which influences the physico-chemical and biological properties of composts (Jain et al. 2019). The moisture content of compost could influence the quality but nonsignificant (Guo et al. 2012).

**Table 2:** Influence of different cellulose degrading bacteria on maximum water holding capacity (%) and moisture content (%) of composts produced from different agro-wastes

<b>WHC (%)</b>					
<b>Substrates</b>	<b>Control*</b>	<b>CBC9</b>	<b>CBD4</b>	<b>CBG2</b>	<b>Mean</b>
<b>Rice straw</b>	49	77	75	71	<b>68</b>
<b>Vegetable waste</b>	48	81	83	79	<b>73</b>
<b>Maize stock</b>	40	70	78	73	<b>65</b>
<b>Mean</b>	<b>46</b>	<b>76</b>	<b>79</b>	<b>74</b>	
LSD (P=0.05): Strain-6.70; Substrate-4.21; Strain x Substrate-8.42 CV (%):9					
<b>Moisture content (%)</b>					
<b>Rice straw</b>	16	24	25	26	<b>23</b>
<b>Vegetable waste</b>	17	24	24	27	<b>23</b>
<b>Maize stock</b>	17	23	25	27	<b>23</b>
<b>Mean</b>	<b>16</b>	<b>24</b>	<b>25</b>	<b>27</b>	
LSD (P=0.05): Strain-4.46; Substrate-3.86; Strain x Substrate-7.72 CV (%):8					

\*Control: No strains

**Angle of Repose ( $^{\circ}$ ) tab density ( $Mg m^{-3}$ ) and HR:**

The angle of repose of composts has been presented in table 3. The angle of repose was highest in the rice straw compost inoculated CBG2 ( $43^{\circ}$ ) followed by vegetable wastes ( $41^{\circ}$ ) and inoculated with CBG2 and maize stock ( $41^{\circ}$ ) inoculated with CBD4. The lowest ( $30^{\circ}$ ) was in vegetable wastes compost without any strain inoculation (control). Irrespective of the substrate types the highest ( $42^{\circ}$ ) was in CBG2 treated compost followed by CBD4 ( $39^{\circ}$ ), CBC9 ( $36^{\circ}$ ), and lowest ( $34^{\circ}$ ) was in uninoculated control. The angle of repose of CBG2 compost was statistically at par ( $P=0.05$ ) with CBD4 compost but significantly different than other isolated strains and control. Compost from CBD4 was significantly

different than control but statistically at par ( $P=0.05$ ) with CBC9 and CBG2. The highest ( $40^{\circ}$ ) angle of repose was measured in rice straw compost followed by maize stock ( $38^{\circ}$ ) and vegetable waste ( $35^{\circ}$ ).

The data related to tab density has been presented in table 3. Irrespective of the substrates type the tab density of compost from control was highest ( $1.29 \text{ Mg m}^{-3}$ ) followed by CBC9 ( $0.74 \text{ Mg m}^{-3}$ ), CBD4 ( $0.69 \text{ Mg m}^{-3}$ ) and lowest was in CBG2 ( $0.62 \text{ Mg m}^{-3}$ ). The tab density of control was significantly different ( $P=0.05$ ) than inoculation of isolated strains. The tab density of CBC9 was at par ( $P=0.05$ ) with CBD4 and significantly different than control and CBG2. The tab density of the CBG2 compost was significantly different than other isolated strains and control. Irrespective of the isolated strains inoculation the highest ( $0.88 \text{ Mg m}^{-3}$ ) tab density was estimated in rice straw compost followed by maize stock compost ( $0.82 \text{ Mg m}^{-3}$ ) and lowest was in vegetable waste compost ( $0.80 \text{ Mg m}^{-3}$ ). The tab density of each substrate was statistically at par ( $P=0.05$ ).

The Hausner ratio (HR) of the composts produced from different agro-wastes inoculated with isolated cellulose degrading bacteria has been presented in table 3. Among the strains inoculated composts the highest (1.13) HR was estimated in the CBG2 inoculated compost followed by CBC9 (1.12) and CBD4 (1.11) and the lowest (1.08) HR was in uninoculated compost. The HR of CBC9, CBG2 and CBD4 were statistically at par ( $P=0.05$ ) whereas it was significantly different ( $P=0.05$ ) than control. Irrespective of the isolated strains inoculation the HR of the vegetable waste was highest (1.13) which was statistically at par with maize stock (1.12) and significantly different ( $P=0.05$ ) than rice straw (1.08).

**Table 3:** Influence of different cellulose degrading bacteria on Angle of Repose ( $^{\circ}$ ) tab density ( $\text{Mg m}^{-3}$ ) and HR of composts produced from different agro-wastes

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Angle of Repose

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Substrates	Control*	CBC9	CBD4	CBG2	Mean
Rice straw	37	39	41	43	40
Vegetable waste	30	34	36	41	35
Maize stock	34	35	40	41	38
Mean	34	36	39	42	
LSD (P=0.05): Strain-4.61; Substrate-4.00; Strain x Substrate-8.00 CV (%):8					
Tab density (Mg m <sup>-3</sup> )					
Rice straw	1.43	0.72	0.71	0.67	0.88
Vegetable waste	1.22	0.71	0.67	0.61	0.80
Maize stock	1.23	0.78	0.69	0.59	0.82
Mean	1.29	0.74	0.69	0.62	
LSD (P=0.05): Strain-0.11; Substrate-0.09; Strain x Substrate-0.19					CV (%):6
HR					
Rice straw	1.09	1.05	1.08	1.09	1.08
Vegetable waste	1.07	1.16	1.10	1.19	1.13
Maize stock	1.08	1.14	1.16	1.11	1.12
Mean	1.08	1.12	1.11	1.13	
LSD (P=0.05): Strain-0.03; Substrate-0.04; Strain x Substrate-0.13					CV (%):9

\*Control: No strains

#### Conclusion:

Inoculation of cellulose degrading bacteria to agricultural wastes resulted into production of high-quality compost in terms of physical properties like bulk density, particle density, porosity, maximum water holding capacity, moisture content, angle of repose, tab density and Hausner ratio. Among the strains the CBG2 strain was found more efficient strains for composting than CBC9 and CBD4. Among the substrate the vegetable waste compost was most desirable compost for crop production. The inoculation of CBG2 with vegetable was found the best compost among all the tested combinations.

Comment [u7]: this conclusion is not seen in the discussion

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