



# Decomposition of Agriculture Farm Wastes by Cellulolytic Bacteria

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## Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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## ABSTRACT

The most important component of the greatest material flow in the biosphere is the microbial consumption of cellulose. Despite the abundance of cellulase manufacturers, there aren't enough microorganisms that can effectively create enough of the enzyme to effectively break down cellulose into fermentable compounds. Despite the fact that bacteria have a very high level of natural variety and the potential to manufacture stable enzymes, little attention has been paid to their ability to produce cellulase. The present study aimed at the isolation and selection of cellulose degrading bacteria isolated from different samples for agriculture waste decomposition. Bacterial cultures were applied on agriculture waste material comprising soybean straw, pigeonpea straw; wheat straw and cotton stalk to investigate their percentage loss in weight. Among all the cultures, CDB 19 has shown the highest weight loss of the substrate (99.99%) followed by CDB 20 (99%), CDB5 (94.2%), CDB2 (92.8%) and CDB14 (88.6%). It was also observed that maximum weight loss of cotton straw (99.99%) was recorded by mixed culture followed by Pigeonpea straw and Soybean straw, while Wheat straw recorded minimum weight loss at 60 days of decomposition.

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**Keywords:** *Agricultural waste; cellulase producers; cellulose degrading bacteria (CBD); decomposition.*

## 1. INTRODUCTION

“Agricultural wastes contain a high proportion of cellulosic matter which is easily decomposed by a combination of physical, chemical and biological processes. The bunch consists of 70% moisture and 30% solid; of which holocellulose accounts for 65.5%, lignin 21.2%, ash 3.5%, hot water-soluble substances 5.6% and alcohol-benzene soluble 4- 1%” [1]. Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation [2]. “The recognition that environmental pollution is a worldwide threat to public health has given rise to a new massive industry for environmental restoration. Biological degradation, for both economic and ecological reasons, has become an increasingly popular alternative for the treatment of agricultural, industrial, organic as well as toxic waste. These wastes have been insufficiently disposed off leading to environmental pollution” [3,30-34].

“The concept of organic matter decomposition is a novel approach to utilize nutrient sources from the waste material. Indian soils are very deficient in organic matter and plant nutrient are required for growth and development of crop. In different agro-ecological regions of India, a wide range of crops are cultivated across the vast majority of land with significant quantity of crop residue (non-economical plant parts) that is left in the field after harvest. After being used in competitive alternatives such as cattle feed, animal bedding, organic manure etc., nearly 500 Million tons (Mt) of crop residue per year on an average is generated in India according to the Indian Ministry of New and Renewable Energy (MNRE) Out of this, 110 Mt of wheat, 122 Mt of rice, 71 Mt of maize, 26 Mt of millets, 141 Mt of sugarcane, 8 Mt of fiber crops (jute, cotton) and 28 Mt of pulses. However, there is still a surplus of 140 Mt out of which 92 Mt is burned each year” [4]. Therefore, the production and improper disposal of agrowastes has become a major pollution issue round the globe. Thus, biological decomposition of farm waste is the most important and effective way to remove these compounds from the environment [22-25]. But most of the farm waste is utilized by burning it in the farm leading to loss of economical soil microflora, therefore farm waste can be used to recycle the nutrients by way of efficient in-situ composting [26-29].

## 2. MATERIALS AND METHODS

### 2.1 Agricultural Waste

Agricultural waste without rain touch such as cotton stalk, wheat stalk, pigeonpea stalk and soybean stalk were obtained from different Research Units of Dr. Panjabrao Deshmukh Krishi Vidyapeeth, Akola.

### 2.2 Sample Collection for Isolation of Cellulolytic Bacteria

“Samples were collected from Melghat Forest region and were stored at 4°C in sterile containers until inoculation. Tenfold serial dilutions of each sample were prepared in and diluted sample was spread on Carboxymethyl cellulose medium for bacterial isolation” [5,6].

### 2.3 Isolation and Purification of Cellulolytic Bacteria

Cellulolytic bacterial strains were isolated from various samples by plate dilution technique. Serial dilutions were done by weighing 1gm of sample in 9ml of distilled water in a test tube (1: 10). After that 1ml of suspension was transferred from first test tube to second test tube containing 9 ml of sterile distilled water (1: 100) and from second test tube to third test tube containing 9 ml of sterile distilled water (1: 1000). Similar dilution process was continued as per requirement. Bacterial cultures was inoculated in CMC (Carboxy-methyl cellulose) medium supplemented with 1% CMC (Hi Media) and incubated at 300 C for 24 hours [6].

### 2.4 Determination of Cellulase Producing Activity of the Bacterial Isolates

The medium used for determination of cellulase producing activity of the bacterial isolates was carboxymethyl cellulose agar (CMC agar) with the following composition (g/l): peptone 10.0, carboxymethyl cellulose (CMC) 10.0, K<sub>2</sub>HPO<sub>4</sub> 2.0, MgSO<sub>4</sub>·7H<sub>2</sub>O 0.3, (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub> 2.5, gelatin 2.0 and agar 15, pH was adjusted at 6.8-7.2, and the plates were incubated at 35 °C for 24hours. After incubation for 24 hours, CMC agar plates were flooded with 0.1- 0.2% Congo red and allowed to stand for 15 min at room temperature. One molar (1M) NaCl was thoroughly used for counter-staining the plates. Clear zones which appeared around growing bacterial colonies indicated cellulose hydrolysis. The bacterial

colonies having the clear zone were identified as cellulose degrading and selected for further studies [6].

## 2.5 Selection of Efficient Organism and its Validation for Degrading Efficiency

The efficient organism was selected on the basis of rate of decomposition of substrate which was measured by following method.

1. Amount of CO<sub>2</sub> evolved
2. Loss in weight of substrate

## 2.6 Estimation of Amount of CO<sub>2</sub> Evolution

Estimation of CO<sub>2</sub> evolution during the process was carried out according to the method described by Pramer and Schmidt [7] with little modification as mentioned below.

Twenty grams of finely chopped (2-3 cm in length) substrate was added in each flask (2000 ml capacity). Moisture level was maintained at 60 per cent water holding capacity. Two agar discs (6 mm) of previously grown cultures were inoculated in flask.

A vial containing 10 ml N/10 of sodium hydroxide (NaOH) solution was hung in each flask. These flasks were then corked tightly and sealed with parafin wax and incubated at room temperature (i.e. 27 ± 2°C) and further observations were recorded.

The observations were recorded and the amount of CO<sub>2</sub> evolved was calculated (according to Pramer and Schmidt, [7]).

The amount of CO<sub>2</sub> evolved as

NaOH consumed (c) = Volume of NaOH taken (x) - Volume of HCl consumed (y)

## 2.7 Estimation of Rate of Decomposition of Substrate by Microbial Culture

The efficient organism was selected on the basis of rate of decomposition of substrate and percent loss in the weight of substrate by the microbial cultures.

## 2.8 Experiment Details

After CO<sub>2</sub> evolution studies, the flask containing decomposed agricultural wastes were used to

estimate the loss in weight. The content of these flask were sun-dried and after being air dried under hot air oven at 50°C for 72 hrs and weighed so as to calculate the loss in weight of substrates during the process of decomposition. The loss in weight was determined by subtracting the final weight from the initial weight [8].

## 3. RESULTS AND DISCUSSION

### 3.1 Collection and Isolation of Sample

Sixteen different samples were collected from different places of Melghat forest region. Twenty different bacterial cultures were isolated from sixteen different collected samples by dilution plate technique and pure bacterial colonies were obtained by repeated streaking on CMC medium.

When applied to composting pits or substrates, microbial decomposers typically consist of carrier-based, ready-to-use live fungal or bacterial formulations that aid in the biological mobilization of various nutrients [35-41]. The product should not contain any other contaminating microbes and may come in a liquid or carrier-based formulation capable of holding very high populations of certain germs. The most crucial step is isolating and identifying the right strain of a decomposer. 20 microorganisms were isolated from various sources, keeping that in mind. For the purpose of developing effective strains, organic material such as forest litter, waste from the forest, including sick plants, soil, wood logs, and water samples were gathered.

### 3.2 Screening of Different Microbial Cultures

Screening of different microbial cultures were carried out by estimation of cellulase activity of isolated bacteria in CMC agar plate through halo zone formation.

The 20 cultures isolated from various different samples were tested for their cellulolytic activities by culturing pure cultures of bacterial isolates on CMC agar plates. The experiment was performed in three replications and after 15 days of incubation, cultures showing clear zones around growing bacterial colonies were considered as cellulolytic indicating cellulose hydrolysis.

All the cultures were found at par however, CDB 12 found significantly superior over others and

recorded maximum HC value (4.4) followed by CDB9 (3.75), CDB5 (3.4), CDB15 (03) and CDB10 (2.8).

Several lines of evidence also supports the present observations that cellulose degrading bacteria from different samples like soil, wood log, agro-waste etc, were isolated and screened on the basis of Congo red staining [46-51].

Bhagat [8] carried out "cultural test for cellulolytic activity of different fungi and bacteria by quantitative method and reported that among bacteria, *C. bibula* showed maximum clearance zone i.e. 11.50 mm followed by *B. polymyxa* (5.33mm) while *P.striata* (4.33 mm) and *Lactobacillus sp.* (3.00mm) exhibited minimum clearance zone".

Ponnambalam et al. [9] "isolated cellulose degrading bacteria from various natural environments. Six bacterial isolates were isolated and comparatively analyzed for effective production of cellulase enzyme. Among the six bacterial isolates, a bacterium F was found to be effective producer. It was observed that the clearing zone of 1.9 cm compared to next effective producer having clearing zone of 1.7 cm".

Pratima et al. [10] "isolated cellulose degrading bacteria (CDB) by enriching the basal culture medium with filter paper as substrate for cellulose degradation. To indicate the cellulose activity of the organisms, diameter of clear zone around the colony and hydrolytic value on cellulose Congo red agar media was measured. CDB-8 and CDB-10 exhibited the maximum zone of clearance around the colony with diameter of 45 and 50 mm and with the hydrolytic value of 9.0 and 9.8, respectively".

Behera et al. [11] "isolated cellulose degrading bacteria from mangrove soil of Mahanadi river delta, Odisha, India. Results showed that total of fifteen cellulose degrading bacteria were isolated based on their halo zone formation on Congo red agar medium. Their maximum CMC hydrolysis capacities (HC value) ranged from 1.18 to 2.5 cm".

Abedin [12] isolated "cellulose degrading bacteria from soil samples collected from National parliament area & BRAC nursery. The five isolates were screened for cellulolytic activity using Congo red stain on Carboxymethyl cellulose (CMC) agar plates among which CBD -

3, CDB - 4 and CDB-5 showed largest clear zone and HC value i.e. 2.4mm, 3.6mm and 2.0mm".

Lingling Ma et al. (2020) carried out "isolation of cellulose degrading bacteria from five rotten wood samples, a total of 81 strains were isolated based on diameters ratio between clear zone and strain by Congo red method. Out of selected 55 cellulolytic strains, *B. subtilis* 1CJ1 and *Bacillus sp.* 1CJ4 had shown the largest diameters of clear zone more than 25mm, and the largest value of diameters ratio between clear zone and strain was 3.71 which belonged to *Bacillus sp.* 3AJ7".

Bhimani et al. (2021) performed "screening and characterization of cellulolytic bacteria isolated from soil. Forty nine isolates were selected on the basis of clear zone produced greater than or equal to 7mm. Cellulolytic activity test showed that isolate AII3, AI3 and CIII5 has the largest cellulolytic index (4.0, 2.0 & 2.0) isolate BI2 & isolate DI1 has the smallest cellulolytic index (0.9 & 0.4)".

### 3.3 CO<sub>2</sub> Evolution of Each Substrate

Cumulative amount of CO<sub>2</sub> evolved during six weeks is presented in Table 1 and Plate 1, which might be efficient to degrade the substrate at faster rate.

From the Table 1, it was observed that total amount of CO<sub>2</sub> evolution was maximum with cotton straw i.e. 332.54 mg by CDB 20, followed by pigeonpea stalk (302.88 mg), soybean stalk (299.92 mg) and wheat stalk (296.96 mg).

CDB 19 has released highest amount of CO<sub>2</sub> with cotton straw i.e. 317.72 mg, followed by pigeonpea stalk (308.82 mg), soybean stalk (305.86 mg) and wheat stalk (302.88 mg) also released maximum CO<sub>2</sub> and was quite efficient in degradation process.

First in the list (Table 1), combination of CDB 19 + CDB 20 was found to be best as the total amount of CO<sub>2</sub> evolution was highest from this treatment. Among all substrates, cotton straw released maximum amount of CO<sub>2</sub> (383.34 mg) and it was followed by pigeonpea stalk (341.82 mg), soybean stalk (338.86 mg), while minimum amount of CO<sub>2</sub> released by wheat straw (335.88 mg).

The combination of CDB 2+ CDB 19 was found very effective as it released more amount of CO<sub>2</sub>

from different substrates. From all the substrates, cotton straw was found very effective in degradation process (350.72 mg CO<sub>2</sub>) followed by the second best substrate i.e. pigeonpea stalk (335.88 mg) and soybean stalk (332.92 mg) while from wheat straw least amount of CO<sub>2</sub> was evolved i.e. 329.96 mg.

The combination of CDB 14 + CDB 19 was found third highest in releasing CO<sub>2</sub> from different substrates. Among all the substrates, maximum amount of CO<sub>2</sub> was evolved from cotton straw (329.96 mg) followed by pigeonpea stalk (318.09 mg), Soybean stalk (312.16 mg) and wheat straw (309.20 mg) which released least amount of CO<sub>2</sub>.

In the present investigation, maximum CO<sub>2</sub> evolution was during first week of incubation and subsequently gradually declined within second week in all agricultural wastes. The results are in agreement with the observation of Pande (1978). He also observed that *C. lagopus* and *M. echinata* inoculated farm wastes viz. cotton stalk, mug trash and tur stalks evolved maximum CO<sub>2</sub> in first week and reduced thereafter. Similarly, *T. spiralis*, *C. globosum* inoculated substrates evolved maximum CO<sub>2</sub> during first week [13].

Similar lines of finding were reported by Potdukhe [14] studied that *T. viride* and *C. globosum* were promising in CO<sub>2</sub> evolution process during decomposition of cotton stalk, groundnut shells and sorghum stubbles. The highest CO<sub>2</sub> evolved was from groundnut shells during two months of decomposition i.e. 1093.60 mg by *Penicillium funiculosum* followed by *Trichoderma viride* (1077.90 mg) from cotton stalks, 778.88 mg and 814.68 mg by *Penicillium funiculosum* and *T. viride*, respectively and from sorghum stubble 684.32 mg and 695.62 mg by *Penicillium funiculosum* and *T. viride*, *C. globosum* were also promising.

Ravankar et al. [15] observed that the rate of CO<sub>2</sub> evolution was maximum during 15 days and reported that maximum amount of CO<sub>2</sub> was evolved from groundnut husk (156.2 mg) in first 15 days and after 30 days of incubation and lowest was obtained from parthenium, due to low carbon content in the material.

Gathe [16] studied the rate of CO<sub>2</sub> evolution of five organic matter viz., cotton stalk, groundnut husk, sorghum waste pigeonpea waste and soybean waste by using six fungi of which *Trichoderma harzianum*, *T. viride* and

*Chetomium globosum* were more promising in decomposition process.

Gupta et al. [17] studied the organic matter degrading capacity of various beneficial microbes' viz., *Trichoderma viride*, *Bacillus polymyxa*, *Pseudomonas striata* and *Azospirillum spp.* which were inoculated in soil containing 2 per cent paddy straw and legume straw. The extent of degradation was measured in terms of cumulative amount of CO<sub>2</sub> evolved during different period of incubation (1st to 5th week) and found *Bacillus polymyxa* and *Trichoderma viride* were the most efficient, as they release higher amount of CO<sub>2</sub> in soil, containing legume straw as compared to paddy straw. Similar findings were also reported by Neelay et al. [18] and Schomberg et al. [19].

Wankar [20] also observed that *T. viride*-3, *A. niger*-1 and *T. harzianum*-1 when inoculated in vegetable wastes viz., cabbage waste, spinach waste, coriander waste and brinjal waste alone and in combination, evolved maximum amount of CO<sub>2</sub> in the first week and reduced after subsequent weeks.

Bhagat [8] studied the rate of decomposition by CO<sub>2</sub> evolution, per cent loss in weight of substrate and C:N ratio. Evolution of CO<sub>2</sub> was measured per day during the eight weeks of incubation as the quantum of CO<sub>2</sub> released was directly proportional to the rate of decomposition of organic matter. Maximum rate of CO<sub>2</sub> evolution was found during first week and reduced thereafter, during decomposition period. All the cultures effectively decomposed the substrates at different rates. While combination of cultures treatment i.e. *T. spiralis* + *C. globosum* + *T. viride* was found more effective in maximum CO<sub>2</sub> evolution, reducing the weight of the substrates, narrowed down the C:N ratio and increased total phosphorus content of the substrates after VII th week of inoculation.

### 3.4 Estimation of Loss in Weight of Substrate after CO<sub>2</sub> Evolution

Moisture is one of the important factor that regulates the growth and activities of microorganisms in decomposition of organic material [42-45]. Estimation of weight loss is an important factor for ascertaining the rate of degradation. After estimation of CO<sub>2</sub> evolution during decomposition of different substrates by various treatment, the content of the flask were first dried in air and finally in oven. The oven dried substrates from individual flasks were then

weighed and per cent loss in weight was calculated (Table 2 and Plate 2).

Maximum loss in weight of wheat stalk was observed by CDB 19- CDB 20 (74.88%) and was

found significantly superior to all the treatment but at par with CDB 2- CDB 19 (74.66%) and CDB 14- CDB 19 (74.44%) whereas, minimum loss in weight was obtained by CDB 15 (63.88%).

**Table 1. Total amount of CO<sub>2</sub> (mg/week) evolved from each substrate by different treatments**

| Sr. No. | Treatments | Cotton | Pigeonpea | Soybean | Wheat  |
|---------|------------|--------|-----------|---------|--------|
| 1.      | CDB 2      | 294.00 | 282.12    | 276.20  | 273.24 |
| 2.      | CDB 5      | 296.96 | 285.09    | 279.16  | 276.20 |
| 3.      | CDB 10     | 282.12 | 273.24    | 267.30  | 264.34 |
| 4.      | CDB 12     | 279.16 | 270.26    | 264.34  | 261.37 |
| 5.      | CDB 14     | 288.06 | 279.16    | 273.24  | 270.26 |
| 6.      | CDB 15     | 276.20 | 267.30    | 261.37  | 258.40 |
| 7.      | CDB 16     | 285.09 | 276.20    | 270.26  | 267.30 |
| 8.      | CDB 19     | 317.72 | 308.82    | 305.86  | 302.88 |
| 9.      | CDB 20     | 332.54 | 302.88    | 299.92  | 296.96 |
| 10.     | CDB 2-10   | 315.12 | 306.24    | 300.30  | 297.34 |
| 11.     | CDB 2-14   | 318.09 | 309.20    | 303.26  | 300.30 |
| 12.     | CDB 2-19   | 350.72 | 335.88    | 332.92  | 329.96 |
| 13.     | CDB 2-20   | 321.06 | 312.16    | 306.24  | 303.26 |
| 14.     | CDB 10-14  | 306.24 | 297.34    | 291.40  | 288.43 |
| 15.     | CDB 10-19  | 327.00 | 315.12    | 309.20  | 306.24 |
| 16.     | CDB1 10-20 | 309.20 | 300.30    | 294.37  | 291.40 |
| 17.     | CDB 14-19  | 329.96 | 318.09    | 312.16  | 309.20 |
| 18.     | CDB 14-20  | 312.16 | 303.26    | 297.34  | 294.37 |
| 19.     | CDB 19-20  | 383.34 | 341.82    | 338.86  | 335.88 |

**Table 2. Percent loss in weight of substrate after CO<sub>2</sub> evolution**

| Sr. No. | Treatments     | (% ) Percent weight loss of Substrate after CO <sub>2</sub> evolution |               |               |               |
|---------|----------------|---|---------------|---------------|---------------|
|         |                | Cotton  | Soybean       | Pigeon Pea    | Wheat         |
| 1.      | CDB 2          | 71.04 (57.79)   | 69.38 (56.37) | 69.82 (56.65) | 68.72 (55.96) |
| 2.      | CDB 5          | 71.26 (57.92)   | 69.60 (56.51) | 70.04 (56.79) | 68.94 (56.10) |
| 3.      | CDB 10         | 70.38 (57.37)   | 67.40 (55.15) | 69.16 (56.24) | 67.84 (55.42) |
| 4.      | CDB 12         | 68.84 (56.42)   | 68.72 (55.96) | 67.62 (55.29) | 66.30 (54.48) |
| 5.      | CDB 14         | 70.82 (57.65)   | 69.16 (56.24) | 69.60 (56.51) | 68.50 (55.83) |
| 6.      | CDB 15         | 68.40 (56.15)   | 66.96 (54.89) | 67.18 (55.02) | 63.88 (53.03) |
| 7.      | CDB 16         | 70.60 (57.51)   | 68.94 (56.10) | 69.38 (56.37) | 68.06 (55.56) |
| 8.      | CDB 19         | 73.38 (59.27)   | 70.04 (56.79) | 70.48 (57.06) | 69.38 (56.37) |
| 9.      | CDB 20         | 72.70 (58.20)   | 69.82 (56.65) | 70.26 (56.92) | 69.16 (56.24) |
| 10.     | CDB 2- CDB10   | 74.88 (59.89)   | 74.22 (59.46) | 74.66 (59.75) | 73.34 (58.88) |
| 11.     | CDB 2- CDB14   | 75.10 (60.04)   | 74.44 (59.60) | 74.88 (59.89) | 73.56 (59.03) |
| 12.     | CDB 2- CDB 19  | 76.98 (61.62)   | 75.32 (60.18) | 75.76 (60.48) | 74.66 (59.75) |
| 13.     | CDB 2- CDB 20  | 75.32 (60.18)   | 74.66 (59.75) | 75.10 (60.04) | 74.00 (59.31) |
| 14.     | CDB 10- CDB 14 | 70.92 (57.34)   | 70.26 (56.92) | 70.70 (57.20) | 69.60 (56.51) |
| 15.     | CDB 10- CDB 19 | 75.54 (60.33)   | 74.88 (59.89) | 75.32 (60.18) | 74.22 (59.46) |
| 16.     | CDB 10- CDB 20 | 72.90 (58.60)   | 72.46 (58.32) | 72.68 (58.46) | 71.80 (57.90) |
| 17.     | CDB 14- CDB 19 | 76.76 (61.48)   | 75.10 (60.04) | 75.54 (60.33) | 74.44 (59.60) |
| 18.     | CDB 14- CDB 20 | 73.34 (58.88)   | 72.90 (58.60) | 73.12 (58.74) | 70.70 (57.20) |
| 19.     | CDB 19- CDB 20 | 77.20 (61.77)   | 75.54 (60.33) | 75.98 (60.62) | 74.88 (59.89) |
|         | F test         | Sig.  | Sig.          | Sig.          | Sig.          |
|         | C.D.           | 0.528   | 0.523         | 0.526         | 0.519         |
|         | SE(m)±         | 0.184   | 0.182         | 0.183         | 0.180         |



In cotton stalk, maximum loss was recorded by CDB 19- CDB 20 (77.20%) and was found significantly superior to all the treatment but at par with CDB 2- CDB 19 (76.98%) and CDB 14- CDB 19 (76.76%), while CDB 15 (68.40%) recorded minimum weight loss of substrate after decomposition.

In soybean stalk, combination of CDB 19- CDB 20 had shown maximum degrading activity by reducing the substrate weight to 75.54 per cent and found significantly superior to all treatments but found at par with CDB 2- CDB 19 (75.32%) and CDB 14- CDB 19 (75.10%), while CDB 15 (66.96%) recorded minimum weight loss of substrate after decomposition.

Maximum loss in weight of pigeonpea stalk was observed by CDB 19- CDB 20 (75.54 %) and found significantly superior to all the treatment but at par with CDB 2- CDB 19 (75.32 %) and CDB 14- CDB 19 (75.10 %) whereas, minimum loss in weight was obtained by CDB 15 (66.96%).

These results were found to be comparable to the findings of Gade et al (2010) performed application of fungal and bacterial cultures on agriculture waste material comprising soybean straw, pigeonpea straw, wheat straw, cotton stalk and weed to investigate their per cent loss in weight, C:N ratio as well as their effect on the development of soil microflora i.e. fungi, bacteria and actinomycetes. Treatment *Trichoderma*+*Trichurus*+*Cellulomonas* (T7) gave maximum per cent wt. loss of substrate during all three years (56.52%) followed by *Trichoderma*+*Trichirus* (T4) (54.97%).

Bhagat [8] carried out decomposition of agricultural wastes like cotton stalk, sorghum stalk, pigeonpea stalk, sugarcane trash, wheat straw, parthenium and weeds These substrate were treated with four efficient cultures, i.e. *T. spiralis*, *C. globosum* and *T. viride* alone and in combination and *C. bibula* separately. The efficiency of degradation of different substrates was also measured by its per cent weight loss of substrates. She reported that as the decomposition period increased, the weight loss of the substrates also increased, while maximum weight loss was found with *T. spiralis* + *C. globosum* + *T. viride* in parthenium and it was followed by wheat straw, weeds, sorghum stalk, etc.

Kadarmoidheen et al. [21] studied the effects of cellulolytic fungi on the biodegradation of cellulosic wastes at the periodical interval of 15,

30 and 45 days. Among the three fungal isolates studied *Trichoderma viride* was found to be the most efficient in degrading the cellulosic wastes viz., paddy straw, sugarcane baggase and banana stalks decreasing the cellulose content by 53.70, 51.59 and 55.28 per cent respectively. This was followed by *Aspergillus niger* and *Fusarium oxysporum* in their efficiency to degrade the different cellulosic wastes.

#### 4. CONCLUSION

This research demonstrated isolation and selection of cellulose degrading bacteria for agriculture waste decomposition. Among the 20 isolated cultures i.e CBD1 to CBD20, the 09 efficient isolates i.e CDB2, CDB5, CBD 10, CDB12, CDB14, CBD15, CDB16, CBD19 and CDB20 were selected as potential cultures which were used for decomposition of agricultural crop wastes viz., cotton stalk, soybean stalk, pigeonpea stalk and wheat straw. Among the 09 effective cellulolytic bacteria isolated from different samples, CBD 19 and CBD 20 were the predominant strains that exhibited a highest cellulolytic activity. The combination of CDB19+ CDB20 gave highest percent loss in weight of cotton straw and it was followed by pigeonpea stalk soybean stalk and wheat stalk. Cotton straw released maximum amount of CO<sub>2</sub> followed by pigeonpea stalk soybean stalk and wheat stalk, which was inoculated with the combination of cultures i.e. CDB19+CDB20. These selected potential nine bacterial cultures are capable of lignocellulosic biomass degradation. This study might be potentially useful candidates for efficient cellulosic biomass conversion and can be used as inoculants for microbial composting to enhance the degradation of cellulose of which the agricultural waste is composed of.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Thambirajah JJ, Zulkafli MD, Hashim MA. Microbiological and biochemical changes during the composting of oil palm empty fruit bunches. Effect of nitrogen supplementation on the substrate. Bioresource Technology 2005; 52: 133-134.
2. Shibata M, Varman M, Tono Y et al. Characterization in chemical composition

- of the oil palm (*Elaeisguineensis*). J Jap Inst.Energy 2008; 87: 383-388.
3. Fabiyi LL, Ogunfowora A. Economics of production and utilization of organic fertilizer in the Nigerian agriculture. Proceedings of a National Organic Fertilizer Seminar, Mar. 26-27, Zaria, Niger, pp: 138-145. 2011.
  4. Bhuvaneshwari S, Hettiarachchi H, Meegoda JN. Crop Residue Burning in India: Policy Challenges and Potential Solutions, Int. J Environ. Res. Public Health. 2019;16:832.
  5. Dhingra OD, Sinclair JB. Basic plant pathology methods. CBS Publisher New Delhi; c1993. p. 179-180.
  6. Bahatkar BP, SJ Gahukar, AA Akhare, DR Rathod, AM Charpe and YV Ingle. 2022. Isolation, screening and identification of cellulose-degrading bacteria from different types of samples. The Pharma Innovation Journal; 11(12): 2500-2507.
  7. Parmer C and A. Schmidt, 1964. Organic matter. In: Methods of soil analysis, Part-I. C A Black (ed.). American Society of Agronomy Madison, USA. 1395-1397.
  8. Bhagat D. Enrichment of Compost through Microbial Inoculants and Chemical Amendments Ph.D. Thesis (Unpub.) PKV, Akola; 2008.
  9. Ponnambalam AS, Deepthi RS, Ghosh AR. Qualitative Display and Measurement of Enzyme Activity of Isolated
  10. Pratima Gupta, Kalpana Samant, Avinash Sahu. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential, International Journal of Microbiology. 2012, (5) Article ID 578925.
  11. Behera BC, Parida S, Dutta SK, Thatoi HN. 2014. Isolation and Identification of Cellulose Degrading Bacteria from Mangrove Soil of Mahanadi River Delta and Their Cellulase Production Ability. American Journal of Microbiological Research. 2; 2 (1):41-46.
  12. Abedin. Isolation and Identification of Cellulose Degrading Bacteria from Soil Sample; 2015.
  13. Somani RB, Wangikar PD, Bhagwat VY, Raut BT. Study on decomposition of Agricultural waste by soil fungi. Food Farming and Agri. 1979;12(6):140-143.
  14. Potdukhe, S. R. 1990. Use of cellulolytic fungi in degradation of agricultural wastes. Ph.D. Thesis (Unpub.) Dr. PDKV, Akola.
  15. Ravankar, H. N., Rita Patil and R. B. Puranik. 2000. Decomposition of different organic residues in soil. PKV Res. J. 24(1): 23-25.
  16. Gathe, A. G., 2001. Studies on cellulolytic fungi in disposal of agricultural wastes. M.Sc. (Agri.) Thesis (Unpub.), Dr. PDKV, Akola.
  17. Gupta P, Samant K, Sahu A. Isolation of Cellulose-Degrading Bacteria and Determination of Their Cellulolytic Potential. International Journal of Microbiology; 2012.
  18. Neelay, C. L., M. H. Beare, W. L. Hangrove and D. C. Coleman, 1991. Relationship between fungal and bacterial substrate induced respiration, biomass and plant residue decomposition. Soil Biol.Biochem. 23(10): 947-954.
  19. Schomberg, H. H., J. L. Steiner and P. W. Unger, 1994. Decomposition and nitrogen dynamics of crop residue quality and water effects. Soil Sci. Soc. America. 58(2): 372-381.
  20. Wankar, L. 2005. Studies on fungi responsible for decomposition of vegetable market wastes. M.Sc. (Agri.) Thesis (Unpub.), Dr.PDKV., Akola.
  21. Kadarmoidheen M, P Saranraj , D Stella. Effect Of Cellulolytic Fungi On The degradation Of Cellulosic Agricultural wastes. International Journal of Applied Microbiology Science 2012; 1(2): 13- 23.
  22. Ashjaraan A, Sheybani S. Drug Release of Bacterial Cellulose as Antibacterial Nano Wound Dressing. International Journal of Pharmaceutical Research and Allied Sciences. 2019; 8 (3):137-143.
  23. Batubara UM, Mardalisa M, Suparjo S, Maritsa HU, Pujianto E, Herlin M. 2021. Isolation and Characterization of Cellulolytic Bacteria Diversity in Peat land Ecosystem and Their Cellulolytic Activities, Earth and
  24. Bhagat SA, Kokitkar SS. Isolation and identification of bacteria with cellulose-degrading potential from soil and optimization of cellulase production, Journal of Applied Biology and Biotechnology. 2021; 9(06):154-161.
  25. Bharti, Sandhu. Partial Purification of Cellulase Produced By A Bacterium Isolated from Wood Decompost, Int. J Pure App. Biosci. 2015;3(4):208-215
  26. Bhowmick, Sengupta. Enumeration of Soil Inhabiting Cellulolytic Bacteria as Plant

- Growth Promoter International Journal of Life Sciences. 2015;9(6):50-55.
27. Bremner JM. Total organic carbon in methods of soil analysis Part- 2 chemical and micro-biological properties. Page, A. L. (ed). II Edn. Amer. Soc. Agron. Inc. and Soil Sci. Amer. Inc. Madison, Wisconsin, USA; c1970. p. 475-594.
  28. Cheng Q, Wang J, McNeel JF, Jacobson PM. Water Retention Value Measurements of Cellulosic Materials Using a Centrifuge Technique. Bio Resources. 2010;5(3):1945-1954.
  29. Gomashe AV, Gulhane PA, Bezalwar PM. Isolation and screening of cellulose degrading microbes from Nagpur region soil. International Journal of Life Science. 2013;1:291-293.
  30. Gupta, S. B., D. K. Tamrakar, M. P. Thakur, K. Tedia, A. Tomar, and P. K. Deshry, 2004. Effect of crop beneficial microbes on decomposition rate of different crop residues. J. Soils and Crops. 14(1): 1-4.
  31. Haripriya R, Thirumalaivasan P. Isolation of cellulolytic bacteria and production of cellulase from coir pith, Int. J Res. Ins. 2017;4(1):13-23.
  32. Irfan Muhammad, Asma Safdar, Quratulain Syed, Muhammad Nadeem. Isolation and screening of cellulolytic bacteria from soil and optimization of cellulase production and activity, Turkish Journal of Biochemistry–Turk J Bio chem. 2012; 37(3):287-293.
  33. Kamara AH, Kamar S, Kamara MS. Effect of rice straw biochar on soil quality and the early growth and biomass yield of two rice varieties. Journal of Agricultural Sciences. 2015;6:798-806.
  34. Khaleel HL, Abd AN, Ali KM. Preparation of Nano-Cellulose from Industrial Waste by Ultrasonic Device. Journal of Biochemical Technology. 2018;9(1):35.
  35. Kiiro IK, Mulaa F, Jackim Wamalwa B, Munyali, Edward KM. Isolation and Characterization of a Thermos table Cellulase from *Bacillus licheniformis* Strain Vic Isolated from Geothermal Wells in the Kenyan Rift Valley. The Open Biotechnology Journal. 2016;10:198-207.
  36. Krieg NR, Dobereiner J. Genus *Azospirillum* In: J. G. Holt and N. R. Krieg (Eds.) *Bergey's Manual of Systematic Bacteriology* Williams and Wilkins Baltimore, MD. 1984;1:94-104.
  37. Kumar. Aerobic and Anaerobic Digestion of Agricultural Waste Followed by Vermicomposting and Enrichment; 2016.
  38. Li X, Gao P. Isolation and partial properties of cellulose-decomposing strain of *Cytophaga* sp. LX-7 from the soil. J Appl. Microbiol. 2008;82:73-80.
  39. Loow YL, Wu TY, Md Jahim J, Mohammad AW, Teoh WH. Typical conversion of lignocellulosic biomass into reducing sugars using dilute acid hydrolysis and alkaline pretreatment. Cellulose. 2016;23:1491-1520.
  40. Maki M, Leung KT, Qin W. The prospects of cellulase-producing bacteria for the bioconversion of lignocellulosic biomass. Int. J Biol. Sci. 2009;5:500-516.
  41. Maki ML, Broere M, Tin Leung K, Wensheng Q. Characterization of some efficient cellulases producing bacteria isolated from paper mill sludges and organic fertilizers. International Journal of Biochemistry and Molecular Biology. 2011;2(2):146-154.
  42. Miller GL. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 1959;31:426-428.
  43. Ojumu T, Solomon V, Bamidele O, Betiku E, Layokun SK. Cellulase Production by *Aspergillus flavus* Linn Isolate NSPR 101 fermented in sawdust, bagasse and corncob. African J Biotechnol. 2003;2:150-152.
  44. Pande VS. Recycling of organic wastes by fungi. M.Sc. (Agri.) Thesis (Unpub.). Dr. PDKV, Akola; c1978.
  45. Piper CS. Soil chemical analysis Hans Publications, Bombay; c1966.
  46. Pointing SB, Buswell JA, Jones EG, Vrijmoed LL. Extracellular cellulolytic enzyme profiles of five lignicolous mangrove fungi. Mycological research. 1999 Jun 1;103(6):696-700.
  47. Saini JK, Saini R, Tewari L. Lignocellulosic agriculture wastes as biomass feed stocks for second-generation bioethanol production: concepts and recent developments. Biotech. 2015;5:337-353. [https:// doi.org/10.1007/s13205-014-0246-5](https://doi.org/10.1007/s13205-014-0246-5) PMID: 28324547
  48. Shinde VS, Agrawal T, Kotasthane AS. Molecular Characterization of Cellulolytic Bacteria Derived From Termite Gut and Optimization of Cellulase Production, International Journal of Current Microbiology and Applied Sciences. 2017;6(10):2474-2492

49. Vimal J, Akhil V, Jini J. Isolation and Identification of Cellulose Degrading Bacteria and Optimization of the Cellulase Production, International Journal of Research in Biosciences. 2016;5(3):58-67.
50. Zaghoud L, Gouamid M, Benmenine A, Khanblouche A. Kinetic and Thermodynamic of Gentian Violet Removal by 2, 3-Dialdehyde Nano cellulose. Journal of Biochemical Technology. 2019;10(2):38-42.
51. Somani RB, Wangikar PD. Cellulolytic activity of some soil fungi. Food Farming and Agri. 1979;12(4):96-98.

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