Original Research Article

Decomposition of Agriculture Farm Wastes by Cellulolytic Bacteria

Abstract

Microbial utilization of cellulose is the key factor for the utmost material flow in the biosphere. Despite this vast number of cellulase producers, there is a deficiency of microorganisms that can produce significant amount of the cellulase enzyme to efficiently degrade cellulose to fermentable products. Little emphasis has been given to cellulase production from bacteria despite their extremely high natural diversity, which endows them with the capability to produce stable enzymes. 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 per cent 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 mix culture followed by Pigeonpea straw and Soybean straw, while Wheat straw recorded minimum weight loss at 60 days of decomposition.

Keywords: Agricultural waste, cellulase producers, decomposition

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% (Thambirajah et al 2005). Lignin is an integral cell wall constituent, which provides plant strength and resistance to microbial degradation (Shibata et al 2008). 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.

The concept of organic matter decomposition is novel approach to utilize nutrient sources from the waste material. Indian soils are very deficient in organic matter and plant nutrient require for growth and development of the 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. (Bhuvaneshwari et al. 2019).Therefore, the production and improper disposal of agro wastes 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. 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 use to recycle the nutrients by way of efficient in-situ composting.

Material and Methods

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.

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 isolation. (Dhingra and Sinclair 1993 and Bahatkar 2022).

Isolation and Purification of cellulolytic bacteria

Cellulolytic bacterial strains were isolated from various samples by Dilution plate technique. Serial dilutions was done by weighing 1gm of sample in 9ml of distill 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) 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 culture was inoculated in CMC (Carboxy-methyl cellulose) medium supplemented with 1% CMC (Hi Media) and incubated at 30^0 C for 24 hours. (Bahatkar 2022)

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, K2HPO4 2.0, MgSO4.7H2O 0.3, (NH4)2SO42.5, 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. 1M NaCl was thoroughly used for counterstaining the plates. Clear zones were appeared around growing bacterial colonies indicating cellulose hydrolysis. The bacterial colonies having the clear zone were identified as cellulose degrading and selected for further studies. (Bahatkar 2022)

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_2 evolved

2. Loss in weight of substrate

Estimation of amount of CO_2 evolution

Estimation of CO_2 evolution during the process was carried out according to the method described by Pramer and Schmidt (1964) 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_2 evolved was calculated (according to [36]).

The amount of CO_2 evolved as

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

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.

Experiment Details

After CO_2 evolution studies the flask containing decomposed agricultural wastes were used to estimate the loss in weight. The content of these flask were sundried and after that 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. (Bhagat 2008)

Result and Discussion

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 media.

Generally microbial decomposer are carrier based ready to use live fungal or bacterial formulation, which on application to composting pits or substrates helps in mobilization of various nutrients by their biological activities. The product may be in liquid or carrier based formulation capable of holding very high population of specific microorganisms and it should be free from other contaminating microorganism. Isolation and identification of appropriate strain of a decomposer is a foremost important. Keeping in view twenty microbes were isolated from various sources. Organic material like forest litters, forest waste including infected plant, soil, wood logs and water samples were collected for obtaining efficient strains.

Screening of different microbial cultures

Screening of different microbial cultures were carried out by estimation of cellulase activity of isolated cultures 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, agrowaste etc, were isolated and screened on the basis of Congo red staining.

Bhagat (2008) 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. (2011) isolated cellulose degrading bacteria from various natural environments. Six bacterial isolates were isolated and comparatively analysed for effective production of cellulase enzyme. Among the six bacterial isolates, a bacterium F was found to be effective producer. It has the shown the clearing zone of 1.9 cm compared to next effective producer having clearing zone of 1.7 cm.

Pratima et al. (2012) isolated the 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. (2014) isolated cellulose degrading bacteria from mangrove soil of Mahanadi river delta, Odisha, India. Results showed that total 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 (2015) 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 Carboxymethylcellulose (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).

CO_2 evolution of each substrate

Cumulative amount of CO_2 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, it was observed that total amount of CO_2 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_2 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_2 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_2 evolution was highest from this treatment. Among all substrates, cotton straw released maximum amount of CO_2 (383.34 mg) and it was followed by pigeonpea stalk (341.82 mg), soybean stalk (338.86 mg), while minimum amount of CO_2 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_2 from different substrates. From all the substrates, cotton straw was found very effective in degradation process (350.72 mg CO_2) 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_2 was evolved i.e. 329.96 mg.

The combination of CDB 14 + CDB 19 was found third highest in releasing CO_2 from different substrates. Among all the substrates, maximum amount of CO_2 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_2.

In the present investigation, maximum CO2 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 CO2 in first week and reduced thereafter. Similarly, T. spiralis, C. globosum inoculated substrates evolved maximum CO2 during first week (Somani et al., 1979).

Similar lines of finding were reported by Potdukhe (1990) studied that T. viride and C. globosum were promising in CO2 evolution process during decomposition of cotton stalk, groundnut shells and sorghum stubbles. The highest CO2 evolved was from groundnut shells during two months of decomposition ie. 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. (2000) observed that the rate of CO2 evolution was maximum during 15 days and reported that maximum amount of CO2 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 (2001) studied the rate of CO2 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. (2004) 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 CO2 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 CO2 in soil, containing legume straw as compared to paddy straw. Similar findings were also reported by Neelay et al. (1991) and Schomberg et al. (1994).

Wankar (2005) 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 CO2 in the first week and reduced after subsequent weeks.

Bhagat (2008) studied the rate of decomposition by CO2 evolution, per cent loss in weight of substrate and C:N ratio. Evolution of CO2 was measured per day during the eight weeks of incubation as the quantum of CO2 released was directly proportional to the rate of decomposition of organic matter. Maximum rate of CO2 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 CO2 evolution, reducing the weight of the substrates, narrowed down the C:N ratio and increased total phosphorus content of the substrates after Vll th week of inoculation.

Estimation of loss in weight of substrate after CO2 evolution.

Moisture is one of the important factor that regulates the growth and activities of microorganisms in decomposition of organic material. Estimation of weight loss is an important factor for ascertaining the rate of degradation. After estimation of CO_2 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%).

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 (2008) 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 (2012) 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.

Conclusion

Among the 20 cultures isolated from different samples the efficient 9 isolates were selected as potential nine cultures which were used for decomposition of agricultural crop wastes *viz.*, cotton stalk, soybean stalk, pigeonpea stalk and wheat straw. The rate of decomposition of different substrates by selected cultures was evaluated by CO2 evolution and percent loss in weight of substrate. Higher amount of CO2 evolution was recorded within first week of incubation but it was declined to the extent of 50 percent during second week and later it was drastically reduced till sixth week of degradation. Cotton straw released maximum amount of CO2 followed by pigeonpea stalk soybean stalk and wheat stalk, which was inoculated with the combination of cultures i.e. CDB19+CDB20. 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. 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.

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Table 1. Total amount of CO_2 (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 CO2 evolution

Sr.No.	Treatments	(%) Percent weight loss of Substrate after CO_2 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

Plate 1. Results of CO2 evolution test

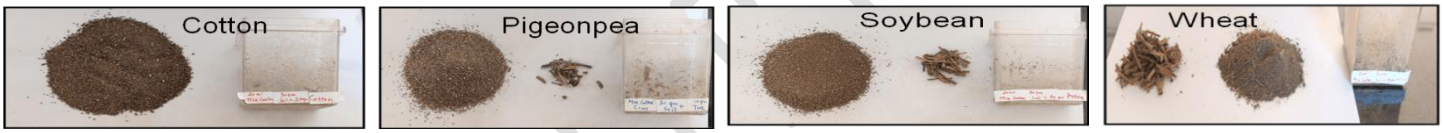

Plate 2. Percent loss in weight of Substrate by Microbial Cultures