CHAPTER-6

Bacterial consortiums and lignocellulosic waste degradability: A field-based assessment with crop refuge

6.1. Introduction

The persistent challenge of lignocellulosic waste (LCW) management, particularly agricultural residues such as field stubbles, poses significant environmental and economic concerns globally. The inefficient disposal methods, such as open burning, contribute to air pollution, soil degradation, and loss of valuable biomass that could otherwise be utilized for sustainable purposes [1]. The open burning of field stubbles releases harmful pollutants, including particulate matter (PM), carbon monoxide (CO), and volatile organic compounds (VOCs), exacerbating air quality issues and public health risks [2]. Consequently, there is an escalating need for innovative and eco-friendly approaches to decompose LCW efficiently.

One promising solution lies in the development of bacterial consortia tailored for the biodegradation of lignocellulosic materials. Bacterial consortia, comprising multiple bacterial strains with complementary metabolic capabilities, have demonstrated superior efficiency in breaking down complex organic compounds compared to single-strain cultures [3]. The synergistic interactions within these consortia can enhance the enzymatic degradation of cellulose, hemicellulose, and lignin, the primary constituents of LCW [4] Bacterial consortia produce a wide range of cellulases, hemicellulases, and ligninases, enabling comprehensive breakdown of LCW components [5]. Different bacterial species within a consortium can specialize in various degradation pathways, enhancing overall efficiency [6]. Studies have demonstrated that bacterial consortia can significantly accelerate the degradation of crop residues such as wheat straw and corn stover. For instance, a field study conducted by Sarma et al. (2022) [7] reported more than 30% increase in degradation rate when using a tailored bacterial consortium compared to natural microbial communities. Field assessments have shown that the application of bacterial consortia not only degrades LCW but also improves soil organic matter and nutrient content, promoting better crop growth [8]. Recent advances in microbiome research and biotechnological applications have facilitated identifying and optimizing bacterial strains with high LCW degradation potential. Studies have shown that microbial consortia,

developed through systematic screening and genetic engineering, can be tailored to specific substrates and environmental conditions [9]. The application of high-throughput sequencing technologies and metagenomics has allowed for a deeper understanding of microbial communities and their functional potential, developing more effective consortia [10]. Moreover, deploying such consortia in field trials has yielded promising results in reducing the environmental impact of agricultural residue management and improving soil health through the return of organic matter [11]. Field trials demonstrate that bacterial consortia can accelerate the decomposition of LCW, resulting in reduced residue accumulation and enhanced nutrient cycling in soils [12]. This approach mitigates the adverse environmental impacts and contributes to sustainable agricultural practices by enhancing soil fertility and structure. Field-based assessments are crucial for translating laboratory findings into practical applications. Direct application of bacterial consortia to crop residues in the field has shown promising results. For example, Mahapatra et al. (2024) [13] demonstrated that inoculating rice straw with a bacterial consortium led to a 40% reduction in biomass within 90 days. Utilizing bioreactors and composting methods with bacterial consortia has been effective in managing large volumes of LCW. These methods provide controlled environments that enhance microbial activity and degradation rates [14]. Field studies have highlighted the importance of optimizing environmental conditions such as temperature and moisture to maximize the efficiency of bacterial consortia. Understanding the interaction between introduced bacterial consortia and native soil microbiomes is critical. Research by Wu et al. (2023) [11] indicates that a balanced interaction can enhance overall soil health and degradation efficiency. The application of bacterial consortia for LCW degradation holds significant economic and environmental benefits. Utilizing bacterial consortia can reduce the need for chemical treatments and lower operational costs in waste management [15]. Enhanced degradation of crop residues reduces greenhouse gas emissions and mitigates the environmental impact of burning agricultural waste [16].

This study aims to develop robust bacterial consortia for the in vitro assessment of LCW degradation and subsequently evaluate their efficacy in on-field trials for decomposing agricultural stubbles. Integrating laboratory-based findings with field applications is expected to offer a scalable and sustainable solution to the LCW management problem, fostering a circular economy and mitigating adverse environmental impacts. By leveraging the synergistic potential of bacterial consortia, this research seeks

to provide a viable alternative to traditional LCW disposal methods, promoting environmental sustainability and agricultural resilience.

6.2. Materials and methods

6.2.1. Consortium development

As reported in previous chapters (Chapter 5), a total of 22 bacterial strains were initially isolated from vermicomposting and composting systems. Eight strains have exhibited multiple beneficial traits, including efficient biomass degrading potentials. These eight strains were primarily selected as consortium candidates (Table 6.1). As the rapidity of such a degradation process was important. Therefore, consortiums were developed using permutation and combination based on the cellulose-degrading potential of the isolates and their synergistic/antagonistic relationships. All isolated strains were first revived in nutrient broth media via 48 h incubation at 28 ^{o}C and eventually inoculated as inhibitors/promoters to each other following a modified overlay technique in nutrient agar media [17], where bacterial strains were used as antimicrobial agents to each other. The process has been illustrated with minute details in the flow-chart given below (Fig. 6.1).

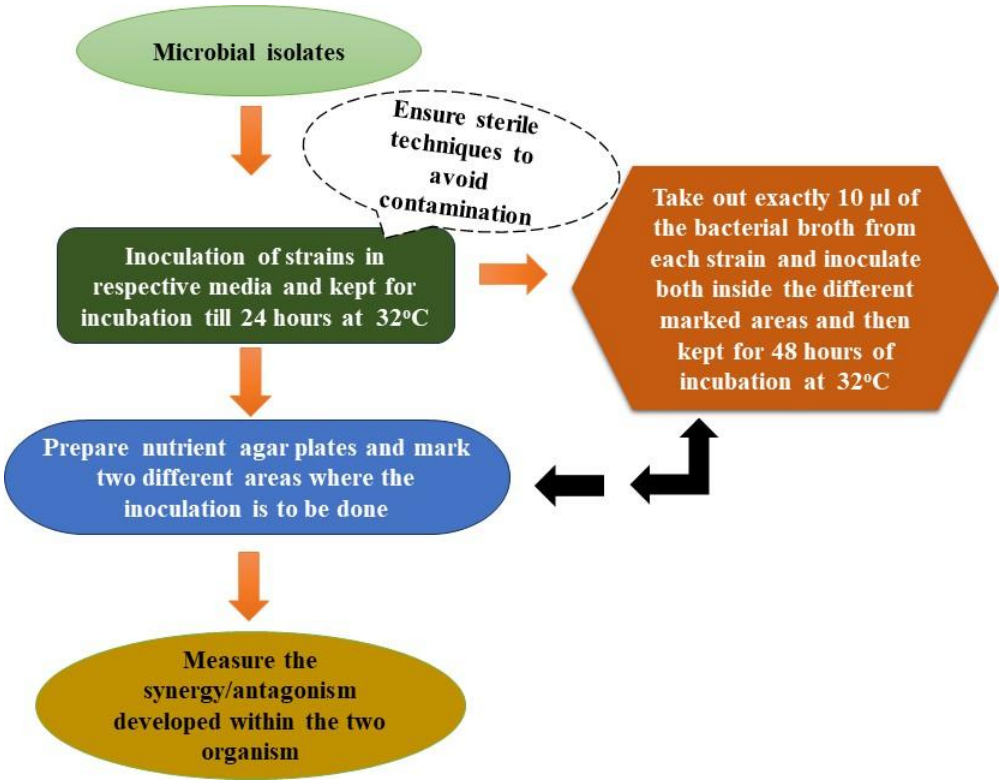

Fig. 6.1: Process flow chart for evaluating synergy and antagonism within microbial strains

The species-species interactions regarding synergy, antagonism, and mutualism were quantified using the aggressivity concept-based equation [18], as shown in equation 1. This equation is widely used to measure species-species interactions for intercropping situations in agriculture.

$$Aab = \frac{Dab}{Daa} \times Zab - \frac{Dba}{Dbb} \times Zba \ldots\ldots\ldots\ldots\ldots (1)$$

Where,

A_{ab}= Aggressivity of A compared to B.

D_{ab}= Colony area (mm^2) in combination with B in a mixture.

D_{aa}= Pure culture colony area (mm^2) of A.

Z_{ab}=Proportion of A with respect to B in the mixture.

D_{ba}= Colony area (mm^2) in combination with A in a mixture.

D_{bb}=Pure culture colony area (mm^2) of B.

Z_{ba}=Proportion of B with respect to A in the mixture.

The colony diameters were measured using a millimeter scale.

6.2.2. Media development for large scale application

The different treatment combination of efficient media was carried out for the production of the optimum microbial consortium. They include Rice Washed water (RWW), Rice starch (RS), Rice Washed water (RWW) + Rice starch (RS) (50:50) ratio, and Nutrient Broth (NB) (Positive Control). Eventually, selected strains-based consortiums were cultured in each media. After 24 hours of incubation, an OD reading at 280 nm was carried out. The best OD read culture media for all consortium formulations was selected for further experiments.

Table 6.1: Consortium candidates used for preparation of Consortium

Organism	Accession Number	Differential Staining	Percentage Identity	Isolated from	Property
Citrobacter freundii OS8	ON391669	Gram Negative	100	Earthworm gut	N-fixation, P-solubilization, K-solubilization, IAA, Siderophore production
Aeromonas hydrophila T3	OP107849	Gram Negative	100	Earthworm gut	Nitrogen fixation, Potassium solubilization, IAA, Siderophore production
Aeromonas Sp. OS2	ON386137	Gram Negative	100	Earthworm gut	Cellulose degradation, IAA
*Erwinia tasmaniensis PB*4	ON386145	Gram Negative	100	Vermicompost	Cellulose degradation, IAA
Bacillus aerius B6	OP107747	Gram Positive	100	Vermicompost	Potassium solubilization, Cellulose degradation, IAA
Serratia marcescens PB1	ON386148	Gram Negative	100	Vermicompost	Cellulose degradation, N-fixation, P-solubilization, K-solubilization, IAA, Siderophore production
Bacillus cereus B3	OP107744	Gram Positive	100	Vermicompost	IAA, Siderophore production, P-solubilization, K-solubilization, N-fixation.
Bacillus halotolerans B8	OP107754	Gram Positive	100	Vermicompost	K-solubilization, IAA, Siderophore production, P-solubilization, N-fixation.

6.2.3. Invitro determination of consortium-mediated lignocellulosic degradation

A total of 12 earthen tubs were used (according to the number of consortia used), wherein a 10:1 ratio of lignocellulosic waste mixed with consortium culture broth was added (100g of LCW mixed with 10 ml of broth). After an interval of every 20 days, carbon & nitrogen in 0-day and 20-day period were analyzed using the following methodology given below. Weight change after an interval of every two days was checked using a digital weighing balance till the 20^{th} day.

A. Determination of Total organic carbon (%)

1g of experimental sample was taken in a conical flask, and to it, 10 mL of potassium dichromate and 20 mL of concentrated sulphuric acid were added. The samples were heated for a while till bubbles occurred. It was then kept for some time for cooling, and then 200 mL of distilled water was added. Afterward, 1.5 mL of diphenylamine indicator was added, following the addition of 10 mL of orthophosphoric acid. It was then titrated with ferrous ammonium sulfate solution till the occurrence of dark green color.

Calculation:

$$\text{Total Organic carbon (\%)} = \frac{Vk \times \left(1 - \frac{vs}{vb}\right)}{W} \times Sk \times 0.3 \ldots\ldots\ldots\ldots\ldots (2)$$

Where,

V_k: Volume of $K_2Cr_2O_7$ Solution

V_s: Titrant reading

V_b: Blank reading

S_k: Strength of $K_2Cr_2O_7$ Solution

W: Weight of soil sample

For example,

Weight of the sample= 'W' g

Volume of $K_2Cr_2O_7$ solution= X mL

Titrant reading of the sample= A mL

Titrant reading of the sample= B mL

Strength of $K_2Cr_2O_7$ Solution= C

$$\text{Total organic carbon}(\%) = \frac{X \times \left(1 - \frac{A}{B}\right)}{W} \times C \times 0.003 \times 100 \ldots\ldots\ldots\ldots\ldots (3)$$

Where, 0.003= 1 ml of N Potassium dichromate equals 0.003 gm carbon.

B. Determination of total nitrogen (%)

The soil is digested in concentrated H_2SO_4 with a catalyst mixture to raise the boiling temperature and to promote the conversion from organic-N to NH^4-N. The NH^4-N from the digest is obtained by steam distillation, using excess NaOH to raise the pH. The distillate is collected in saturated H_3BO_3, and then titrated with dilute H_2SO_4 to pH 5.0. The method determines ammonium-N, most of the organic-N forms, and a variable fraction of nitrate-N in soil

Procedure:

Take 1 g soil sample, add 0.8 g $CuSO_4$ and7 g K_2SO_4. Then add 12- 15 mL conc. H_2SO_4. Digest the contents at 420 ^{0} C for 1 hr , keep it to cool and transfer the contents to distillation flask and add 80ml distilled water followed by 50 ml 40% NaOH until the appearance of black colour. Then, start distillation and the distillate is collected in a conical flask containing 20 mL 0.1 N H_2SO_4 and 3-4 drops of mixed indicator. Finally titrated with 0.1 N NaOH.

Calculation:

Total Nitrogen (%) = (Volume of H_2SO_4× strength – Volume of NaOH consumed ×strength) ×0.014×100) ÷ Weight of soil

6.2.4. On field trial: Field stubble degradation study

6.2.4.1. Description of Experimental Site

The experiment was conducted in a farmer's field near Tezpur Central University campus (26041N latitude and 92050E longitude). The field is located around 13 km away from Tezpur town in the Sonitpur district of Assam and at about 2 km from Tezpur University campus. The area falls under the North Bank Plain Agro climatic Zone (NBPAZ) of Assam. The soil is characterized by recent and old alluvial soils with clay-loam texture, class typic end aquepts and slightly to moderate acidic soil reaction.

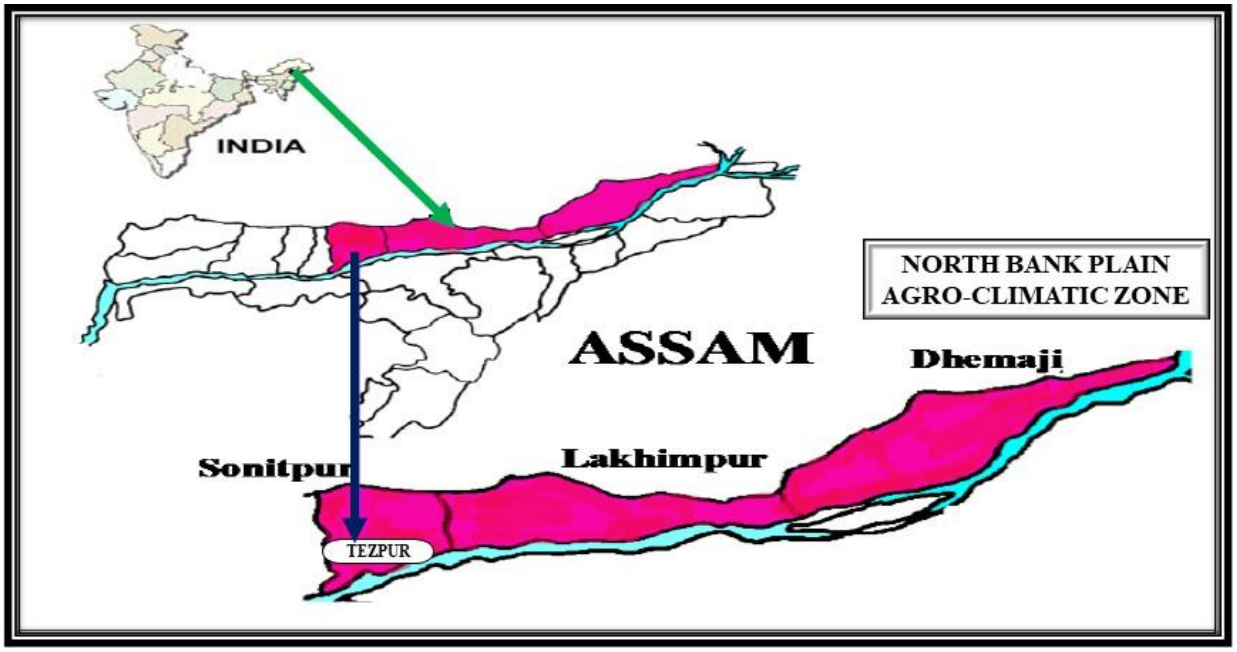

Fig. 6.2: Maps showing study area: North Bank Plain Agro climatic Zone (NBPAZ) of Assam, India

6.2.4.2. Climatic condition during the experiment

The details of the climatic condition during the experiment season of that year are presented in the table 6.2. The diurnal temperature in the study location was moderate throughout the experiment time with less rainfall and relative humidity. In general, the climate of the study location is distinguished by heavy precipitation with moderate summer and winter.

Table 6.2: Climatic conditions during the experiment (January, 2023)

Year- 2023		
Climatic condition	**Season**	**Reference**
	January	
Av. Rainfall (mm)	1.6 mm	[19]
Av. Max. Temp (°C)	25°C	[19]
Av. Min. Temp (°C)	12°C	[19]
Av. Morning Relative Humidity (%)	75%	[20]
Av. Evening Relative Humidity (%)	75%	[20]

6.2.4.3. Experimental design, treatment used and parameters

The experiment was laid following randomized block design (RBD) (Fig 6.3) principle with seven treatments and three replicates. The detailed treatment combinations are provided in Table 6.3. Based on the result analysis of the pilot-scale experiment, five prolific consortiums were selected for treatments in large-scale field experiment. They were scaled up through the augmentation of colonies. A total of 21 plots, uniformly covered by five cm thick stubble-cover, were prepared of size $4m^2$. In each plot 1 kg of straw was spread along uniformly. All the treatment combinations of consortiums were applied to each plots keeping the other management practices (weeding, cleaning etc.) identical during the experimental periods. A total of 1000 mL consortium was added for each plot except for the plot denoted by PC (Positive control); RB (Residue burned) and RR (Residue removed). In positive control, we have added a solution already available in the market known for their efficiency in degrading lignocellulosic wastes. The dose of positive control was same as was applied by the other treatments. The experiment was conducted for twenty days, 2023 during the winter season. This is the recommended harvesting season time for North Brahmaputra bank according to the package of practices for Kharif crops in Assam (2015) (Package of practices for Kharif crops of Assam, 2015) [21]. After every alternate day, 1000 mL of consortium culture was added, and then it went on till the 20th day. Below are the details of the study area, plot direction facing, plot initials and replicates. Layout of the experimental plan is given in Fig.6.2.

Table 6.3: Details of the treatment combinations applied during the experiment

Treatments	Combination of strains	Dosage and concentration percentage
S1	T3 + OS2 + B3 + PB4 + PB1 + B6 + OS8 + B8	10 ml consortia+ 990 ml water (1% consortia)
S2	T3 + B3 + B6 + B8 + OS8	10 ml consortia+ 990 ml water (1% consortia)
S7	PB4 + T3 + B6 + B3 + OS8	10 ml consortia+ 990 ml water (1% consortia)
S10	PB1 + PB4 + B6	10 ml consortia+ 990 ml water (1% consortia)
S11	T3 + PB1 + B8 + B6	10 ml consortia+ 990 ml water (1% consortia)
PC (Positive Control)		5 ml solution A + 5 ml Solution B + 990 ml water (1% Solution)
RB (Residue Burned)		
RR (Residue Removed)		

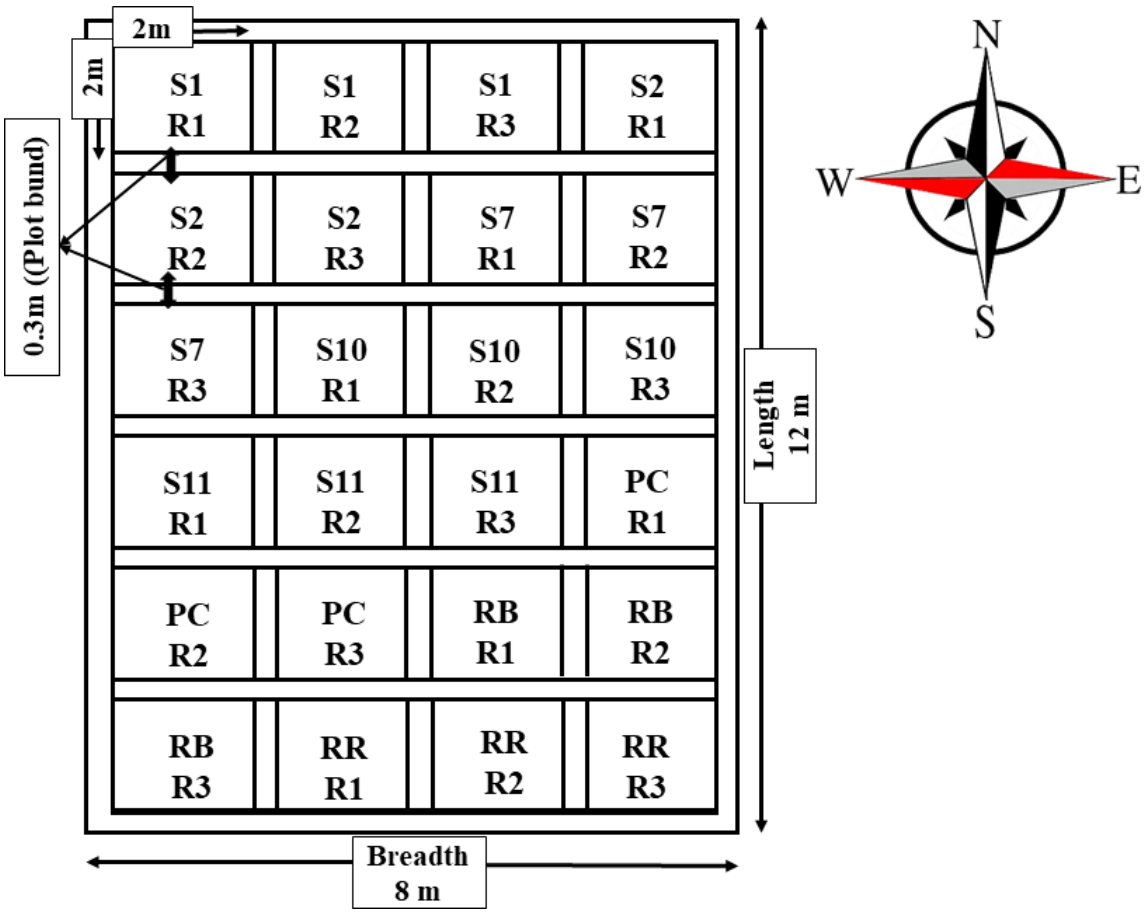

Fig. 6.3: Layout of the experimental plot, showing key plot locations, area measurement and boundaries.

On the 20th day the addition of consortium was stopped and final volume reduction was measured. Along with it, different physico-chemical analysis (pH, humic acid, fulvic acid, TKN, TOC and MBC) were carried out for both soil and biomass using the below given methodology.

6.2.4.4 Collection of experimental soil sample

Representative soil samples (0-15cm) were collected from different parts of experimental area before consortium application. Then a composite soil sample was prepared by mixing them. Three replicates of the representative composite sample were taken to obtain the initial status (pH, Humic acid, Fulvic acid, TOC, TKN and MBC) of the field. Further, after the experimental period soil samples were collected again from each plot, finally at 20th day. All the collected soil samples were air dried, ground to powder, sieved and stored in air tight bags for further analysis.

6.2.5 Physico- chemical analysis of soil samples

Analysis of various soil attributes such as pH, Humic acid (HA), Fulvic acid (FA), Total organic carbon (TOC), Total nitrogen (TN) and Microbial biomass Carbon (MBC) were performed following Page et al. [22].

A. Measurement of soil pH

Procedure:

About 10 g of sample was weighed in a beaker and 25 mL distilled water was added to it to prepare a suspension of 1:2:5. The solution was stirred intermittently with a glass rod about 1 hour. The pH of the suspension was recorded with the help of EuTech pH meter.

B. Total nitrogen (%)

The Total nitrogen from samples was estimated following the method of Page et al. [22]. The detail of the protocol was described in the section (6.2.3.2.)

C. Total organic carbon (%)

Total organic carbon was analyzed by the protocol described in earlier section (6.2.3.1)

D. Microbial biomass carbon

Principle: When moist sample is placed in an atmosphere containing chloroform vapor microorganisms are killed. A fraction of the cell constituents becomes soluble and can be extracted from the soil in potassium chloride solution. The nitrogen thus solubilized as amino acids and ammonium is estimated by reaction with ninhydrin and measured as a purple complex using a spectrophotometer. The amount of nitrogen measured is directly proportional to the biomass initially in the soil; only about one quarter of the biomass nitrogen is released, but the fraction is approximately constant for different soils provided standard conditions are used. Jenkinson, 1988 method was followed.

Reagents:

1. Ninhydrin reagent

2. Ethanol water

3. Potassium chloride solution (2N)

4. Chloroform

4. Nitrogen standard

Procedure:

10 gm moist sample (40% WHC) was taken for both fumigated and unfumigated, and incubated for 15 days at 25 ^{0}C. After 15 days, both sets were taken out and analyzed for unfumigated and fumigated carbon as given below:

Unfumigated process:

Sample taken in a conical flask, 40 mL of KCl solution added and shaken for 30 minutes and then filtered. 1 mL of filtrate taken in a test tube and 0.5 mL Ninhydrin reagent added slowly. Then the solution heated on a water bath until the colour develops and then cooled. 9.5 mL of ethanol water was added and thoroughly mixed. Finally, absorbance was measured at 570 nm with KCL solution as the blank.

Fumigated process:

Samples were placed in a vacuum desiccator preloaded with 10 g of soda lime, 25 mL of chloroform and boiling chips taken in a 250 mL beaker and placed along with the samples. Evacuated for 2 minutes till chloroform boils, and kept undisturbed for 24 hours. On the next day, the beaker containing chloroform was removed after repeated evacuation. Extraction process was same as discussed for unfumigated process.

Calculation:

MBC (µg/g oven dry sample) = 31* ninhydrin N

MBC = (Fumigated – Unfumigated) *4

E. Obstinate C fractions (Humic acid (%) and Fulvic acid (%))

Reagents:

a. Sodium pyrophosphate: 44.6g Sodium pyrophosphate was dissolved 1L distilled water with addition of 4g Sodium hydroxide.

b. 1N Potassium dichromate solution ($K_2Cr_2O_7$): 49.04g of Potassium dichromate was dissolved in 1L of distilled water.

c. 0.5 N Ferrous ammonium solution {$FeSO_4 \cdot (NH_4)_2SO_4 \cdot 6H_2O$}:196.1g of Ferrous iron solution was dissolved water followed by 20 mL of conc.H_2SO_4..

d. Diphenylamine indicator: 0.5g diphenylamine was dissolve in 20 mL of distilled water by the addition of 100 mL of conc.H_2SO_4.

e. 85 % Orthophosphoric acid (H_3PO_4)

f. Conc. Sulphuric acid (H_2SO_4)

Procedure:

Take 20 mL 0.1 N NaOH used to wash the precipitate and mix well the whole solution. Take 5 mL of the solution for Humic acid analysis following modified Walkley and Black method. Take the remaining solution and analyse at 465 nm and 665 nm in a UV spectrophotometer for both aliphatic and aromatic hydrocarbons to calculate the degree of humification.

Calculation:

$$\text{Total Organic carbon (\%)} = \frac{Vk \times \left(1 - \frac{vs}{vb}\right)}{W} \times Sk \times 0.3 \ldots\ldots\ldots\ldots\ldots (6)$$

Where,

V_k: Volume of $K_2Cr_2O_7$ solution

V_s: Titrant reading

V_b: Blank reading

S_k: Strength of $K_2Cr_2O_7$ solution

W: Weight of soil sample

$$\text{HA Carbon (\%)} = \frac{Vk \times \left(1 - \frac{vs}{vb}\right)}{W} \times Sk \times 0.3 \ldots\ldots\ldots\ldots\ldots\ldots (7)$$

$$\text{FA Carbon (\%)} = \frac{Vk\left(1 - \frac{Vs}{Vb}\right)}{W} \times \text{Sk} \times 0.3 \ldots\ldots\ldots\ldots\ldots\ldots\ldots (8)$$

Degree of Humification: Absorbance of aliphatic hydrocarbons taken at 465 nm/ Absorbance of aromatic hydrocarbons taken at 665 nm.

6.2.6. Biomass parameters

6.2.6.1. Biomass collection

At the initial days representative biomass samples (0-15 cm) were collected from different parts of experimental area before consortium application. Then a composite biomass sample was prepared by mixing them. Three replicates of the representative composite sample were taken to obtain the initial status (pH, Humic acid, Fulvic acid, TOC, TKN and MBC) of the lignocellulosic waste. Further, after the experimental period LCW samples were collected again from each plot at the 20th day. All the collected LCW samples were air dried, ground, and stored in air tight bags for further analysis.

A. Volume measurement (ft^3)

Volume measurement was done using the formula

$$Volume = Length \times width \times height \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots (5)$$

B. pH of the biomass sample

pH of the samples was analyzed using Eutech pH 700 following the method as described in the earlier section (6.2.5.1.).

C. Total nitrogen (%)

The Total nitrogen from the samples was estimated following the method of Page et al. [22]. The detail of the protocol was described in the section (6.2.3.2).

D. Total organic carbon (%)

Total organic carbon was analyzed by the protocol described in the section (6.2.3.1).

E. Microbial biomass carbon

Microbial biomass carbon was analyzed by the protocol described in earlier section (6.2.5.4.).

F. Obstinate C fractions (Humic acid (%) and Fulvic acid (%))

Humic acid and fulvic acid carbon was analyzed by the protocol described in earlier section (6.2.5.5.).

6.2.7. Statistical analyses

The biomass data from the phase 1 (Invitro) experiments were analyzed by two-way ANOVA. For Considering two factors (treatment and time) that might have influenced various biomass attributes, full factorial analysis of variance (ANOVA) was performed with a significance level of $P < 0.05$. One-way ANOVA was performed for all phase 2 (field) experiment soil and biomass attributes in SPSS 16 software. In addition, Least Significant Difference (LSD) was adopted to identify the competency of various treatment combinations.

6.3. Results and Discussion

6.3.1. Consortium development and Invitro efficacy assessment

6.3.1.1. Antagonism and Synergistic relationship of organism

According to McGilchrist (1965) [18], if the computed value of an aggressivity equation for 'A' vs 'B' (i.e., Aab) is positive, then the growth of A is more aggressive than B; if the value is negative, then B is more aggressive than A; and if the value is 0, then the relationship is mutualistic, which means no competition. High '+' or '-'values imply a big difference in competitive abilities between the tested species.

Table 6.4: Aggressivity of different strain combination for effective consortium development

Strain combination	Dab	Daa	Zab	Dab/Daa x Zab	Dba	Dbb	Zba	Dba/Dbb x Zba	Aab
T3 Vs B3	2.268	0.785	0.292	0.844	2.137	1.766	0.646	0.782	0.062
T3 Vs B8	3.15	0.785	0.365	1.465	2.302	1.815	0.828	1.050	0.414
T3 VS PB4	2.936	0.573	0.921	4.719	2.302	1.815	1.547	1.962	2.757
T3 Vs PB1	2.708	0.94	0.738	2.126	2.083	0.785	0.891	2.364	-0.238
T3 Vs B6	1.745	0.94	0.403	0.748	1.083	1.766	0.686	0.421	0.327
T3 Vs Os8	2.047	0.94	0.517	1.126	0.85	1.815	1.93	0.904	0.222
T3 Vs Os2	1.238	0.625	0.796	1.577	1.631	0.785	1.256	2.610	-1.033
B3 Vs B8	1.045	0.973	0.796	0.855	1.227	1.514	0.989	0.802	0.053
B3 Vs PB4	1.915	1.593	0.995	1.196	0.854	1.601	1.005	0.536	0.660
B3 Vs PB1	2.115	0.62	0.592	2.019	2.327	1.047	1.688	3.752	-1.732
B3 Vs B6	1.367	0.457	0.174	0.520	1.168	0.519	0.135	0.304	0.217
B3 Vs OS8	1.214	0.881	0.479	0.660	1.409	0.613	0.217	0.499	0.161
B3 Vs OS2	1.031	0.407	0.211	0.534	2.361	0.932	0.161	0.408	0.127
B8 Vs PB4	2.668	1.312	0.976	1.985	1.081	0.944	0.468	0.536	1.449
B8 Vs PB1	1.516	0.899	0.379	0.639	0.936	0.681	0.334	0.459	0.180
B8 Vs B6	2.117	0.583	0.302	1.097	2.031	1.616	0.319	0.401	0.696
B8 Vs OS8	3.241	0.856	0.412	1.560	2.577	1.638	0.715	1.125	0.435
B8 Vs OS2	1.525	0.784	0.316	0.615	1.293	0.521	0.236	0.586	0.029
PB4 Vs PB1	1.033	0.815	0.623	0.790	1.071	1.014	0.733	0.774	0.015
PB4 Vs B6	1.612	0.824	0.319	0.624	1.129	1.637	0.417	0.288	0.336
PB4 Vs OS2	1.151	0.232	0.172	0.853	2.143	0.594	0.061	0.220	0.633
PB4 Vs OS8	3.209	0.756	0.212	0.900	2.269	1.315	0.529	0.913	-0.013
PB1 Vs B6	1.508	0.443	0.171	0.582	0.991	1.718	0.628	0.362	0.220
PB1 Vs OS8	3.278	0.637	0.215	1.106	2.958	1.226	0.412	0.994	0.112
PB1 Vs OS2	1.812	1.336	0.748	1.015	0.693	1.051	1.035	0.682	0.332
B6 Vs OS8	1.217	0.418	0.189	0.550	2.016	0.731	0.153	0.422	0.128
B6 Vs OS2	0.918	0.103	0.081	0.722	1.047	0.357	0.061	0.179	0.543
OS8 Vs OS2	1.507	0.733	0.323	0.664	1.234	0.432	0.107	0.306	0.358

Table 6.5: Formulated consortium with combination of strains

Consortium	Combination of strains
S1	T3 + OS2 + B3 + PB4 + PB1 + B6 + OS8 + B8
S2	T3 + B3 + B6 + B8 + OS8
S3	B3 + OS2 + PB4 + B6 + OS8
S4	OS2 + PB4 + PB1 + B6 + OS8
S5	T3 + PB1 + B6 + OS8 + B8
S6	PB4 + B3 + B6 + OS2 + OS8
S7	PB4 + T3 + B6 + B3 + OS8
S8	OS2 + PB1 + B8 + B6
S9	T3 + B3 + B6
S10	PB1 + PB4 + B6
S11	T3 + PB1 + B8 + B6
S12	T3 + PB1 + B6

Aeromonas hydrophila T3 vs. *Erwinia tasmaniensis* PB4 shows the highest aggressivity value (2.757 mm^2), indicating a strong competitive interaction between these strains. The lowest aggressivity was found in *Erwinia tasmaniensis* PB4 Vs *Citrobacter freundii* OS8 (-0.013 mm^2), followed by *Aeromonas hydrophila* T3 Vs *Serratia marcescens* PB1(-0.238 mm^2). The data suggests that selecting strain combinations with lower aggressivity could lead to more effective and cooperative consortia. High-aggressivity combinations may inhibit growth and performance, while low-aggressivity combinations can foster balanced microbial communities [23].

6.3.2. Criteria for consortium formulations

The development of effective bacterial consortia for lignocellulosic waste (LCW) degradation relies on specific criteria, including aggressiveness, cellulose degradation capacity, and other beneficial traits. Each criterion plays a crucial role in ensuring the consortium's efficiency and adaptability in various environmental conditions. Aggressiveness is quantified using an aggressivity concept-based equation [18]. which measures how one strain affects another when grown together. An ideal consortium should balance these interactions to prevent dominance by any single strain, ensuring a stable and cooperative microbial community. This balance enhances the overall efficiency of LCW degradation, as it allows multiple strains to coexist and contribute their unique metabolic capabilities without outcompeting each other. In addition to aggressiveness and cellulose

degradation, bacterial strains are selected for traits such as nitrogen fixation, phosphorus solubilization, potassium solubilization, and the production of plant growth-promoting substances like indole-3-acetic acid (IAA) and siderophores. These traits contribute to soil health and plant growth by improving nutrient availability and promoting beneficial microbial activity.

Considering the outcome of interaction following aggressivity within the microbes, it was found that *Citrobacter freundii* OS8 demonstrates balanced interactions with other strains, preventing dominance. While, *Aeromonas hydrophila* T3 and *Aeromonas sp.* OS2 *Bacillus cereus* B3 and *Erwinia tasmaniensis* PB4 showed compatibility with other consortium members, maintaining a cooperative interaction within the consortium without outcompeting others. *Bacillus aerius* B6 and *Serratia marcescens* PB1 shows strong but balanced and stable interactions within the consortium. *Bacillus halotolerans* B8 was mostly, compatible with other strains.

Citrobacter freundii OS8 is not primarily a cellulolytic strain but supports overall consortium activity along with the ability of N-fixation, P-solubilization, K-solubilization, IAA production, siderophore production. Whereas, *Aeromonas hydrophila* T3, *Bacillus aerius* B6, *Bacillus cereus* B3 and *Bacillus halotolerans* B8 could show moderate cellulolytic activity. However, *Aeromonas hydrophila* T3 could fix nitrogen, solubilize potassium, and could produce IAA and siderophore. But *Bacillus aerius* B6 showed a higher efficiency for potassium solubilization and IAA production excluding N-fixation, P-solubilization and siderophore production. Interestingly, *Bacillus cereus* B3 and *Bacillus halotolerans* B8, showed positive response for all other traits including K-solubilization, IAA, siderophore production, P-solubilization, N-fixation. *Aeromonas sp.* OS2 and *Erwinia tasmaniensis* PB4 shows a strong cellulolytic activity. *Aeromonas sp.* OS2 shows IAA production excluding other beneficial traits like nitrogen fixation, potassium solubilization and siderophore production. Whereas, *Erwinia tasmaniensis* PB4 could only show IAA production as beneficial traits. *Serratia marcescens* PB1 also showed strong activity of cellulose degradation and other beneficial traits that includes N-fixation, P-solubilization, K-solubilization, IAA production, siderophore production.

Each of these strains brings a unique set of capabilities to the consortium, ensuring a comprehensive and efficient degradation process. By carefully selecting and balancing these strains, the consortium was developed for achieving optimal performance in LCW management and soil health improvement.

6.3.3. Invitro assessment of LCW degradation potential

Table 6.6: Temporal variation of weight change of LCW in the invitro consortium experiment at alternate day along with Mean±Stdev

	Days												
Treatments/Consortium	**Day 0**	**Day 2**	**Day 4**	**Day 6**	**Day 8**	**Day 10**	**Day 12**	**Day 14**	**Day 16**	**Day 18**	**Day 20**	**Total weight changed (g)**	**Mean±Stdev**
S1	100	99.1	99	97.7	96	95.87	95	95.09	95	94.16	94	6	96.45±2.13
S2	100	99.2	99.1	97	97	96.82	96.03	95.8	95	94.9	93.35	6.65	96.75±2.04
S3	100	98.5	98.22	97.9	97.7	97.5	97	96.07	96.04	96.2	96	4	97.38±1.27
S4	100	99.3	99.23	98.6	98.04	98	97.74	97.6	97.14	96.02	95.71	4.29	97.94±1.33
S5	100	99.1	98.67	97.5	97	97.03	96.67	96.3	96.01	95.76	95	5	97.19±1.52
S6	100	99.86	99.38	99.2	98.91	98.74	97.11	97	96.45	96.1	95.02	4.98	97.98±1.70
S7	100	99	97.9	96.3	96.1	96	95.04	95	94.3	94.1	91	9	95.89±2.49
S8	100	99.2	99.05	99.01	99	99.05	97	97.03	97	96.78	96.1	3.9	98.11±1.33
S9	100	98	97.14	96.9	96.8	96.15	96.03	96.68	96.09	96	95.8	4.2	96.87±1.22
S10	100	98.6	97.5	97.11	97	96.79	95	95.28	95.1	94.9	93.17	6.83	96.40±1.94
S11	100	99.15	98.93	96	96.04	95.51	95.3	94.33	94	93	92.5	7.5	95.89±2.51
S12	100	99.2	98.6	98.2	98	97.92	97.7	97.15	97.12	95.26	95.1	4.9	97.66±1.49
P Value													<0.01
LSD													2.239

Table 6.7: Temporal variation of weight change of LCW every 5th day in the invitro consortium experiment along with Mean±Stdev

	Days			
TREATMENTS	**5 DAYS**	**10 Days**	**15 Days**	**20 Days**
S1	97.72±0.02	95.88±0.03	95.04±0.05	93.03±0.04
S2	97.03±0.03	96.83±0.02	95.33±0.42	93.34±0.03
S3	97.94±0.03	97.54±0.03	96.04±0.01	96.03±0.04
S4	98.61±0.01	98.03±0.03	97.16±0.02	95.74±0.05
S5	97.53±0.04	97.04±0.01	96.04±0.03	95.04±0.04
S6	99.24±0.04	98.72±0.07	97.39±0.11	97.06±0.04
S7	96.34±0.03	96.04±0.04	94.32±0.03	91.03±0.03
S8	99.03±0.03	99.05±0.03	97.03±0.04	96.13±0.04
S9	96.95±0.02	96.15±0.02	96.08±0.02	95.84±0.05
S10	97.14±0.03	96.77±0.03	95.11±0.02	93.15±0.02
S11	96.04±0.04	95.55±0.04	94.03±0.04	92.53±0.04
S12	98.22±0.01	97.94±0.04	97.14±0.02	95.14±0.04
P(Treatment)		<0.01		
P(Time)		<0.01		
P(Treatment*Time)		<0.01		
LSD		0.25		

These findings illustrate that weight change varied significantly between treatments during the initial 15 days of the experiment, with this effect becoming non-significant by the 20th day. The general trend across all treatments was a decrease in weight over time. The highest reduction in weight was observed in treatment S7. The reduction in weight for S7 from the 5th day to the 20th day was 5.31 gm corresponding to 5.51% overall reduction. This was followed by S1 with a reduction of approximately 4.69 g, corresponding to 4.80%. The lowest reduction in weight was observed in treatment S9, approximately 1.11 g, corresponding to 1.14% weight reduction. Certain microorganisms might produce enzymes that break down organic matter, leading to a reduction in weight. Treatments like S7, S1, and S2 may contain more active or efficient microbial communities that accelerate the degradation process [24].

Table 6.8: Temporal variation of TKN (%) and TOC (%) in the given LCW treatment for 0 and 20 Days along with Mean±Stdev

		TKN (%)	TOC (%)
Time period	**Treatments**	**Mean±Stdev**	**Mean±Stdev**
0 D	**S0**	0.38±0.02	4.87±0.19
20 D	**S1**	2.14±0.03	17.38±0.29
	S2	2.33±0.02	14.47±0.30
	S3	2.28±0.02	16.38±0.36
	S4	1.33±0.03	12.54±0.27
	S5	1.34±0.03	22.56±0.30
	S6	1.07±0.06	16.49±0.36
	S7	1.07±0.03	16.48±0.27
	S8	0.82±0.02	13.25±0.36
	S9	0.75±0.05	18.51±0.30
	S10	2.19±0.02	14.36±0.40
	S11	1.08±0.02	17.20±0.30
	S12	0.67±0.04	12.31±0.41
P Value		<0.01	<0.01
LSD		0.043	0.389

The Total nitrogen increased drastically in S3 (2.28±0.02) followed by S10 (2.19±0.02) and S1 (2.14±0.03) in the 20th day. Contrastingly, the total organic carbon percentage in the final period of incubation was highest in S9 (18.51±0.30). The increase in TN can be attributed to microbial activity breaking down organic matter, releasing nitrogen in forms that contribute to TN [25]. As organic material decomposes, TOC levels initially rise due to the release of organic compounds. Over time, microbes convert these organic compounds into carbon dioxide and other gases, decreasing TOC levels [26]. Different treatments contain varying types of microbial consortia, affecting decomposition rates and nutrient release [24].

6.3.4. Field trial for assessing the efficacy of the consortiums to decompose field stubbles

Table 6.9: Temporal variation of field biomass volume reduction(ft^3) for 0 Days and 20 Days along with Mean±Stdev

	Time-period	
	0 D	**20 D**
Treatments	**Mean±Stdev**	**Mean±Stdev**
S1	27.36±0.13	13.56±0.11
S2	26.76±0.15	10.82±0.46
S7	26.92±0.10	7.85±0.18
S10	26.89±0.15	9.68±0.13
S11	27.39±0.10	15.38±0.07
PC	26.94±0.11	12.2±0.11
P Value	<0.01	<0.01
LSD	0.143	

The volume of the lignocellulosic biomass greatly reduced in all the treatments. The highest reduction in volume was found in S7 (7.85±0.18) approximately 70.84% reduction followed by S10 (9.68±0.13) approximately 63.99% reduction and S2 (10.82±0.46), approximately 59.74% reduction.

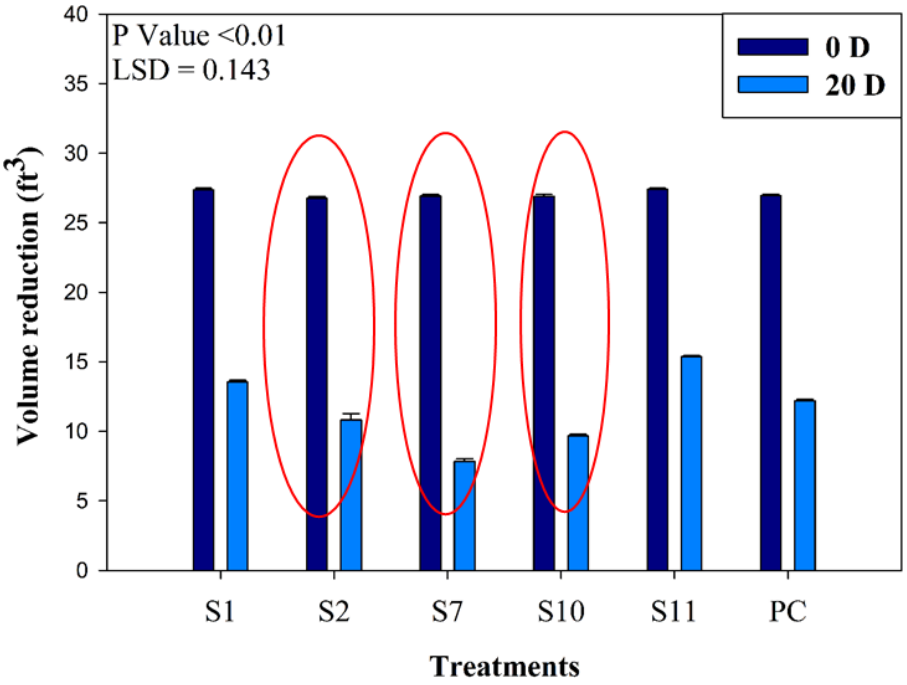

Fig 6.4: Temporal Variation of field biomass volume reduction(ft^3) for 0 Day and 20 Day time period along with P Value and LSD.

Treatment S11 exhibited the least reduction, approximately 43.85% reduction with a final volume of 15.38 ± 0.07 ft^3 at 20th day.

Table 6.10: Variation of lignocellulosic waste degradation attributes viz. pH, Humic acid, Fulvic acid, TOC, TKN and MBC activity under various treatment for 0 Days and 20 Days along with Mean±Stdev

	pH		Humic acid carbon (%)		Fulvic acid carbon (%)		TOC (%)		TKN (%)		MBC (µg g^{-1})	
	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days
Treatments	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**
S1	6.1±0.04	8.07±0.04	0.14±0.02	0.59±0.04	0.15±0.05	0.66±0.02	5.2±0.29	15.36±0.43	0.38±0.07	1.03±0.06	279.58±12.84	715.44±16.95
S2	6.3±0.05	7.42±0.05	0.22±0.05	0.47±0.03	0.27±0.03	0.51±0.01	4.9±0.20	18.43±0.86	0.39±0.03	0.81±0.01	315.75±18.29	1077.43±18.3
S7	6.11±0.06	7.72±0.08	0.16±0.01	0.83±0.06	0.17±0.02	0.53±0.03	5.1±0.22	17.95±0.87	0.42±0.02	1.21±0.03	514.19±11.15	1078.26±17.4
S10	6.27±0.07	7.87±0.01	0.12±0.03	0.43±0.02	0.13±0.06	0.48±0.07	5±0.18	18.52±0.87	0.33±0.03	0.81±0.02	624.13±17.48	875.56±12.8
S11	6.31±0.10	7.46±0.06	0.23±0.01	0.91±0.01	0.32±0.02	0.85±0.03	4.7±0.23	22.94±0.72	0.35±0.05	0.67±0.03	273.86±12.84	333.68±11.44
PC	6.5±0.02	7.02±0.03	0.21±0.02	0.44±0.03	0.18±0.04	0.46±0.06	5.4±0.27	18.43±1.03	0.33±0.06	0.64±0.04	201.38±15.70	582.13±15.47
P Value	<0.01											
LSD	0.113		0.46		0.069		1.47		0.079		6.11	

pH increased from acidic to slightly basic in all the treatments except for treatment S1(8.07±0.04), which is slightly higher basic then all the other treatments. The increase in pH may be attributed to the microbial activity and the breakdown of organic matter, which can release basic compounds into the soil [27]. Humic acid, fulvic acid carbon and total organic carbon percentage increased in all the treatments. The significant rise in humic acid, fulvic acid carbon and total organic carbon could be due to the decomposition of lignocellulosic waste, leading to the formation of humic substances [28]. The increment in total nitrogen percentage in all the treatments was significantly greater in the 20th day, highest being in S7 (1.21±0.03) followed by S1 (1.03±0.06). This suggests that in the two treatments mentioned viz; S1 and in S7 the presence of synergism of microbes was much better due to which the consortium strains augmented the nitrogen levels by accelerating the microbial activity [29]. Microbial biomass carbon increased in all the treatments. The final MBC was highest in S7 (1078.26±7.4) followed by S2 (1077.43±8.3) (P Value <0.01; LSD=6.11), it can be due to the fact that the consortium confronted the decay resistance of lignocellulosic macromolecules in the S7 and S2 dominated feedstocks by enhancing microbial loading.

Table 6.11: Variation of soil attributes viz. pH, Humic acid, Fulvic acid, TOC, TKN and MBC activity under various treatment for 0 Days and 20 Days along with Mean±Stdev

	pH		Humic acid carbon (%)		Fulvic acid carbon (%)		TOC (%)		TKN (%)		MBC (µg g-1)	
	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days	0 Days	20 Days
Treatments	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**	**Mean±Stdev**
S1	5.037±0.03	6.91±0.02	0.25±0.01	0.32±0.01	0.30±0.02	0.42±0.02	4.34±0.03	17.45±0.02	0.14±0.01	0.18±0.01	26.13±0.07	64.728±7.90
S2	5.005±0.01	6.57±0.01	0.16±0.04	0.19±0.04	0.37±0.03	0.55±0.03	3.96±0.02	14.42±0.04	0.13±0.05	0.15±0.02	26.29±0.03	160.54±5.60
S7	5.002±0.04	6.66±0.04	0.56±0.02	0.63±0.02	0.57±0.03	0.57±0.03	3.43±0.04	13.46±0.03	0.14±0.01	0.16±0.02	26.45±0.01	290.72±3.50
S10	5.104±0.06	6.77±0.07	0.08±0.06	0.18±0.06	0.19±0.06	0.25±0.06	5.75±0.02	15.41±0.05	0.16±0.03	0.17±0.04	26.38±0.09	230.64±7.60
S11	5.011±0.02	6.02±0.04	0.59±0.01	0.88±0.01	0.69±0.02	0.97±0.02	3.45±0.06	18.43±0.01	0.14±0.01	0.16±0.01	26.92±0.07	79.73±3.42
PC	5.017±0.05	6.78±0.05	0.19±0.07	0.37±0.07	0.30±0.02	0.43±0.02	3.88±0.03	17.40±0.06	0.14±0.05	0.15±0.02	26.07±0.08	160.47±7.60
RB	5.025±0.08	5.81±0.01	0.08±0.01	0.18±0.01	0.19±0.02	0.22±0.02	4.22±0.06	16.45±0.02	0.12±0.04	0.13±0.03	26.66±0.04	160.9±6.50
RR	5.026±0.03	5.10±0.01	0.19±0.03	0.29±0.03	0.30±0.02	0.45±0.02	4.96±0.05	6.42±0.025	0.14±0.02	0.15±0.04	26.51±0.07	80.37±8.78
P Value	<0.01											
LSD	0.71		1.64		0.074		0.061		0.09		1.02	

All treatments showed a significant increase in pH over 20 days. The largest increase was observed in treatment S1, where pH rose from 5.037±0.03 to 6.91±0.02 (+1.873). pH increased from acidic to near neutral in all the treated soil, S1 (6.91±0.02) being the highest increment in pH except for treatment RR (5.10±0.01) and RB (5.81±0.01) which has not much differed during the incubation period. However, humic acid, fulvic acid and total organic carbon increased across all treatments over 20 days. The most notable increase of humic acid was in S11, where it rose from 0.59±0.01 to 0.88±0.01 (+0.29). Whereas, in fulvic acid the highest increase was in S11, where it increased from 0.69±0.02 to 0.97±0.02 (+0.28). The most substantial increase was in treatment S1, where TOC rose from 4.34±0.03 to 17.45±0.02 (+13.11). The microbial consortium likely enhanced the decomposition of organic matter in the soil, converting it into carbon substances [30]. The increment in total nitrogen percentage in all the treatments was significantly greater in the 20th day. The highest nitrogen percentage in 20th day was found in S1 (0.18±0.01) followed by S10 (0.17±0.04). This suggests that the presence of microbes in the consortium augmented the nitrogen levels in the soil by accelerating the microbial activity [20]. MBC varied significantly among treatments, with some showing substantial increases. The most significant increase was in S7, where MBC rose from 26.45±0.01 to 290.72±3.52 (+264.27.26). The final MBC was highest in S10 treated soil (230.64±7.60) followed by RB soil (160.9±6.50) and S2 (160.54±5.60) (P Value <0.01; LSD=1.02). The conditions in treatment S7 may have been particularly favorable for microbial growth. The interaction between different microbial species in the consortium can have synergistic effects, enhancing overall microbial growth and biomass [31]. In both in vitro and field experiments, consortium S7 (*Erwinia tasmaniensis* PB4 + *Aeromonas hydrophila* T3 + *Bacillus aerius* B6 + *Bacillus cereus* B3 + *Citrobacter freundii* OS8) emerged as the top performer.

6.4 Conclusion

The present study reports the formulation of the different bacterial consortia from vermi-isolated cellulolytic bacteria strains and evaluate5d their potential utilities for LCW degradation particularly agriculture residues. The integration of advanced biotechnological tools and high-throughput sequencing has facilitated the development of 12 tailored consortia. The invitro experiment carried out through the development of five effective microbial consortia S1(*Aeromonas hydrophila* T3 + *Aeromonas Sp.* OS2 + *Bacillus cereus* B3 + *Erwinia*

tasmaniensis PB4 + *Serratia marcescens* PB1 + *Bacillus aerius* B6 + *Citrobacter freundii* OS8 + *Bacillus halotolerans* B8), S2(*Aeromonas hydrophila* T3 + *Bacillus cereus* B3 + *Bacillus aerius* B6 + *Bacillus halotolerans* B8 + *Citrobacter freundii* OS8), S7(*Erwinia tasmaniensis* PB4 + *Aeromonas hydrophila* T3 + *Bacillus aerius* B6 + *Bacillus cereus* B3 + *Citrobacter freundii* OS8), S10(*Serratia marcescens* PB1 + *Erwinia tasmaniensis* PB4 + *Bacillus aerius* B6) and S11(*Aeromonas hydrophila* T3 + *Serratia marcescens* PB1 + *Bacillus halotolerans* B8 + *Bacillus aerius* B6), illustrates their superior capability in breaking down lignocellulosic waste compared to individual microorganisms without any pretreatment. Based on various parameters of study for field experiment, the volume of the lignocellulosic biomass greatly reduced in all the treatments. The highest reduction in volume was found in S7 (7.85±0.18) approximately 70.84% reduction. The consortium S7 comprising of five bacterial strains (*Erwinia tasmaniensis* PB4 + *Aeromonas hydrophila* T3 + *Bacillus aerius* B6 + *Bacillus cereus* B3 + *Citrobacter freundii* OS8) was found most optimum to degrade rice straw under field conditions. The enhanced degradation rates observed in field trials highlight the practical applicability of this approach, not only in reducing wastes but also in improving soil health and fertility. Although the use of indigenously developed microbial consortium S7 has proved to be cost effective, there is still room for improvement in technology-related aspects.

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