ISSN: 2347-4688, Vol. 11, No.(1) 2023, pg. 125-136

Current Agriculture Research Journal

www.agriculturejournal.org

Conversion of Lignocellulosic Wastes into Biofertilizer using Bacterial Consortium

ASMITA GAIKWAD, KAVITA JADHAV and SHUBHADA NAYAK*

Department of Microbiology, Rayat Shikshan Sanstha's Karmaveer Bhaurao Patil College, Vashi, Maharashtra, India.

Abstract

Lignocellulosic biomass abundantly and ubiquitously occupies the earth. However, their complex molecular structure prevents their use as a source of organic material for fermentable sugars and nutrients to be used as foods, fertilizers and biofuels. For an efficient carbon cycle, microbial enzymes play a key role in slow biodegradation of lignocellulosic wastes in nature. Microbiological applications can enhance the rate of biodegradation to utilize agro-industrial and organic municipal solid wastes, containing up to 50% lignocellulose substrates, as an inexpensive and sustainable source of plant nutrients. With this hypothesis, the current study was carried out to prepare a consortium of lignocellulose degrading bacteria and use it to convert lignocellulosic substrates in garden, sugarcane, rice, cotton and fruit waste into biofertilizer. Overall, 7-14% reduction in cellulose and 3-6% reduction in lignin content, along with decrease in pH was observed on treatment of above wastes with microbial consortium in 42 days. In spite of the low conversion rates observed in our study, better root, shoot as well as leaf development was observed in moong seedlings grown in soil amended with biofertilizer (3:1 ratio) as compared to controls. Another interesting observation was the biofertilizers with low pH prepared from sugarcane wastes (pH 3.1) and fruit wastes (pH 3.6) supported plant growth more efficiently as compared to other biofertilizers (pH 5.0 to 5.7). Thus, in addition to feasible conversion of lignocellulosic wastes into biofertilizer, our study further suggests the use of selective wastes as raw material depending on the preference of plants for slightly acidic to neutral soil pH for growth.

Article History

Received: 08 January 2023

Accepted: 10 April 2023

Keywords

Agrowastes;
Biofertilizer;
Consortium;
Lignocellulose;
Municipal Solid Wastes.

Introduction

Lignocelluloses are complex organic carbohydrates that represent over 50% of the global biomass fixed with photosynthetic activity.[1] This accounts to the production of over 155 billion tons of dry lignocellulosic biomass produced every year.[2]

CONTACT Shubhada Nayak shubhadanayak@kbpcollegevashi.edu.in Department of Microbiology, Rayat Shikshan Sanstha's Karmaveer Bhaurao Patil College, Vashi, Maharashtra, India.

Doi: http://dx.doi.org/10.12944/CARJ.11.1.11

The plant cell wall contains 40-60% cellulose, 20-40% hemicellulose and 10-25% lignin along with relatively small amounts of pectin, proteins, transporter molecules and minerals.[1] This composition varies with factors like plant age, variety, season and soil characteristics.[3] Cellulose is an insoluble, highly crystalline and non-uniform linear polysaccharide with β-acetyl linkage groups, consisting of 2000 to 14,000 residues. Hemicellulose is a heterogeneous polysaccharide consisting of either mannose, D-glucose, D-xylose, L-arabinose or D-galactose.[4] They are linked to cellulose by hydrogen bonds and lignin by covalent bonds. Lignin consists of phenyl propane units joined together by different types of linkages giving it a unique and complex chemical structure. All three components form a complex in the cell wall of plants to prevent their biodegradation.[5, 6,7]

Although, it is a perfect strategy of nature to prevent plants from degradation, it poses significant challenge to utilize or dispose large quantities of lignocellulosic materials like hardwood, dead trees, branches, grasses and agro-industrial wastes like bagasse, wheat and paddy straws, husks, hulls, stalks, shells and cobs.[8,9] To maximize the use of these wastes in bio-refineries, they are categorized as agricultural, forestry, urban and industrial wastes, or energy crops.[10] This segregation aids in developing a proper system to optimally benefit from lignocellulosic wastes. So far, the energy crops including cassava, cereal straws, sweet sorghum and bagasse are subjected to pre-treatment and biodegraded to produce bioethanol, biobutanol, bio-hydrogen, and biogas.[11,12,13] They are considered for their economic benefits and also regarded as second generation biofuel stocks due to their high sugar content.[14] Similarly, forestry wastes are also in high demand for their energy value. In contrast, the urban and industrial wastes are either added to compost piles or discarded in land-fills. In compost piles, they take a very long time to decompose without any pretreatment. Lignin containing organic compounds in compost piles may take few years for complete decomposition under natural conditions.[15] Hence, although many studies have indicated the use of lignocellulosic substrates as slow release organic compost to enrich soil health,[16,17,18] they are rarely considered for use as biofertilizer.[19]

The lignocellulosic wastes require physicochemical pre-treatment to disrupt the complex matrix and expose the polysaccharides to microbial or chemical enzymatic hydrolysis. These treatments are expensive, consume extensive energy and often produce toxic intermediate compounds.[14] On a positive side, numerous bacteria, fungi, actinomycetes and cyanobacteria have been reported to degrade lignocellulosic wastes.[20, 21] More commonly, fungal strains capable of depolymerizing lignin and producing cellulase enzymes is reported in literature.[21] Considering the above factors, an attempt was made in this study to convert lignocellulosic substrates into biofertilizer with the help of microbial consortium consisting of 5 bacterial strains capable of degrading both lignin and cellulose. The effect of this biofertilizer was observed on moong plants and evaluated based on morphological parameters like root and shoot length, total number of leaves, fresh and dry weight of the seedlings after 10 days of growth. The biofertilizer characteristics like moisture holding capacity, pH and temperature changes were also noted up to 42 days.

Materials and Methods

Screening of Lignin and Cellulose Degrading Bacteria

A compost sample was purchased from a nursery and soil samples were collected from forests, local gardens and agricultural farms in Navi Mumbai. All soil samples were collected from approximately 10cm below the ground level and stored in polythene zip lock bags. They were transferred immediately to the laboratory and used for screening of lignin and cellulose degrading bacteria. For isolation, 1 g of soil was suspended in 10 ml of saline suspension. Various dilutions of this suspension were plated onto the surface of Carboxy-Methyl Cellulose (CMC) agar media [composition in g/l: CMC (10), tryptone (2), KH_2PO_4 (4), Na_2HPO_4 (4), $MgSO_4.7H_2O$ (0.2), $CaCl_2.2HO$ (0.2), $FeSO_4.7H2O$ (0.001), agar (15), pH 7] and incubated at Room Temperature (RT; ~27°C) for 3 days. After incubation, a single colony was re-isolated on CMC agar plates to ensure purity of isolate, and maintained on CMC agar slants at 4°C. The cellulose degrading bacteria thus obtained were further screened for their ability to degrade lignin using selective Lignin Basic Medium (LBM) agar plates [composition in g/l: Na_2HPO_4 (2.4), K_2HPO_4 (2), MH_4NO_3 (0.1), $MgSO_4$ (0.01), Lignin (1), agar (15), pH 7].

Confirmation of Lignin and Cellulose Degradation
The ability of isolated bacteria to degrade lignin and cellulose was confirmed based on qualitative staining tests and quantitative assays.

Fresh LBM and CMC agar plates were prepared for qualitative analysis of lignin and cellulose degraders respectively. The test isolates (5 µl suspension) were spot inoculated on above plates. The CMC plates were incubated for 48 h and LBM plates for 5-10 days. Observation of clear zones around the spot inoculum after flooding the LBM plates with 1% ferric chloride and potassium ferricyanide solutions confirmed lignin degradation.[22] The CMC plates were stained with 0.1% Congo red dye and constantly revolved for 15 mins. This was followed by addition of 1M NaCl. The plates were revolved again for ~15 min until clear zone was observed, which indicated cellulose degradation.[23]

Quantitative enzyme assays for estimation of cellulase from the cell free supernatant of growth medium (after 48 h incubation) was performed using standard DNSA method and absorbance was measured at 560 nm. One unit of cellulase activity was expressed as 1 µmol of glucose liberated per ml of enzyme per min.[24] Quantitative enzyme assays for estimation of lignin degrading enzyme activity was performed using a protocol described by Sahadevan and co-workers.[25] Precisely, the cell free supernatant (250 µl) of growth medium (after 7 days incubation) was mixed with 2.5 ml phosphate buffer (pH 7.6) and absorbance was measured at 280 nm using a UV-visible spectrophotometer.

Identification of Potential Lignin and Cellulose Degrading Bacteria
The isolates were gram stained and their morphological characteristics were noted. The potential isolates were identified using an automated microbial identification system, VITEK2 [Biomeruex].

Analysis of Agro/Industrial Wastes as Lignocellulose Substrates
Five different substrates including garden wastes and byproducts obtained on processing of sugarcane, rice, cotton and fruits were collected in clean polythene bags. They were sundried for few days and any remaining moisture was removed by dehydrating them in hot air oven. They were then cut in small pieces and ground to fine powder. Analysis of lignin and cellulose composition was done by using the method described by Chesson *et al.*[26] For this purpose, 1g (a) of dried substrates was mixed with 150 ml of aquadest and heated in a boiling water bath at 90-100°C for 1 h. After heating, the mixture was filtered, washed with 300 ml of hot water and then dried in a hot air oven until constant weight (b) was obtained. It was then mixed with 150 ml of 1N sulphuric acid (H_2SO_4) and heated in a boiling water bath at 90-100°C for 1 h. After heating, the mixture was filtered, washed with 300 ml of aquadest and then dried in a hot air oven until constant weight (c) was obtained. Next day, the residue was soaked in 10 ml of 72% H_2SO_4 at room temperature for 4 h. To this mixture, 150 ml of 1N H_2SO_4 was added and refluxed using soxhlet apparatus for 1 h. The semi-solid mass thus obtained was washed with 400ml of aquadest and heated in oven at 105°C until a constant weight was obtained (d). The solid mass was heated again until it turned into ash and then weighed (e). Cellulose and lignin content was calculated using the formula represented below.

Cellulose content (%)=(c-d)/a x 100

Lignin content (%)=(d-e)/a x 100

Pre-Treatment of Lignocellulose Wastes
Five substrates used in study were garden waste, sugarcane waste, rice waste, cotton waste and fruit waste. Approximately 200 g of these substrates were pretreated with hot water at temperatures between 140°C and 240°C. During the pre-treatment, the hemicellulose components are depolymerized and the products dissolve in the liquid phase whereas the cellulose is retained in the solid phase. The lignin component of substrates is simultaneously depolymerized and then re-polymerized, due to its glass transition temperature in aqueous conditions between 80°C and 100°C. Insoluble lignin is retained in solid phase.[27]

Preparation of Bacterial Consortium and Biofertilizer
A consortium of potential lignin and cellulose degrading bacteria were prepared using a modified approach reported by Zhang *et al.*[28] The selected bacterial isolates were grown in bulky quantities on nutrient agar by spread plate technique. They were harvested in 10 ml saline to prepare dense suspensions. Equal volume (2 ml) of each isolate

was mixed well, vortexed and used as a consortium for degradation of lignocellulose substrates. This consortium was added to a 2L conical flask along with 100g substrate and 250ml mineral salt medium. Similarly, a control flask was prepared by replacing the consortium with equal volume of distilled water. The flasks were incubated at RT for 42 days and changes in visual characteristics and physical parameters (pH and temperature) were noted daily. The remaining lignin and cellulose content of the biofertilizer was determined at an interval of 7 days using the Chesson method26 described earlier in this section. After 42 days, the moisture content and water holding capacity of the biofertilizer was also determined using the formula.

Moisture content (%)=(Wet weight-dry weight)/ (Wet weight) x 100

Efficiency of Prepared Biofertilizer

The growth promoting ability of prepared biofertilizer using each substrate was studied on moong plants. The garden soil was collected and autoclaved to minimize its normal microbial flora. The soil and biofertilizer were added to the pots in 3:1 ratio. The moong seeds were purchased from local market and soaked in the water overnight. These seeds were placed deep in soil and the pots were watered with sterile distilled water. A control pot without biofertilizer was also maintained similarly. The pots used in our study had small holes to prevent water retention in pots. All the test and control pots were kept in the sunny area and watered daily for 10 days. Morphological parameters were measured on regular basis. Root length, shoot length, a total number of leaves, fresh weight, and dry weight of the mature plants was noted for both controls as well as test plants. The fresh weight of plants was noted after 10 days of growth. For dry weight determination, plant parts were separated and dried in a hot air oven at 80°C for 48 h.

Results and Discussion

Screening and Identification of Potential Lignocellulose Degrading Bacteria

In the present study, 34 isolates were obtained on CMC agar plates (Figure 1). These isolates showed zone of clearance in the range of 8.9 to 67.5 mm on CMC plates. Among the same isolates, 19 were identified as lignin degraders based on the observed zone of clearance in the range of 8.0 to 24.0mm on LBM plates (Table 1). The 5 isolates showing best lignin as well as cellulose degrading potential were selected based on quantitative analysis and used to prepare a consortium. The details of these isolates are represented in Table 2.

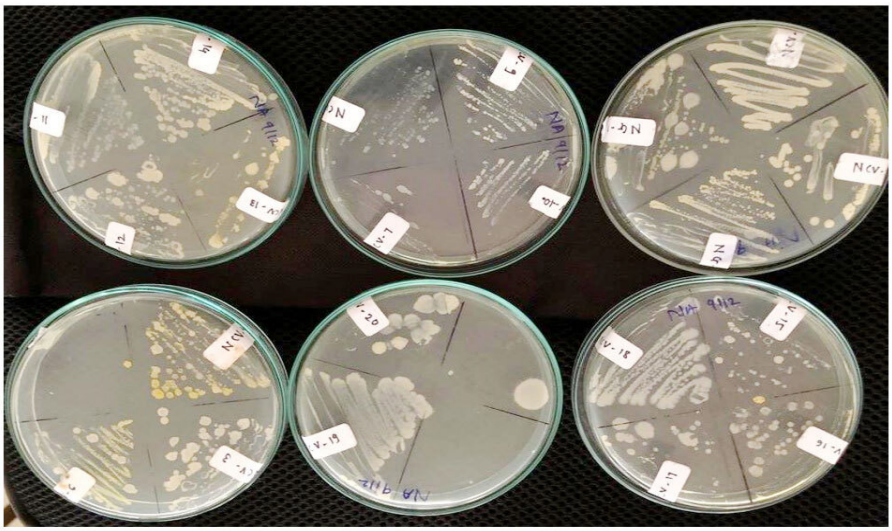

Fig. 1: Representative cellulose degrading isolates obtained on CMC agar plates

Table 1: Qualitative analysis of lignin and cellulose degradation

Sr. No.	Isolate	Zone size on CMC (in mm)		Zone size on LBM (in mm)	
		Before staining	After staining	Before staining	After staining
1	NCV-1	10	25.5	-	-
2	NCV-2	13	39	13	19
3	NCV-3	35	47	-	-
4	NCV-4	11	31	8	15.5
5	NCV-5	0	0	-	-
6	NCV-6	34.5	62.5	12	24
7	NCV-7	29.5	52.5	9	13
8	NCV-8	9.5	31	12	15
9	NCV-9	22.5	38	-	-
10	NCV-10	40	53	-	-
11	NCV-11	11.5	32	-	-
12	NCV-12	41	43	13	24
13	NCV-13	31.5	42	-	-
14	NCV-14	10	20.5	8	13
15	NCV-15	38.5	55.5	-	-
16	NCV-16	38	57.5	-	-
17	NCV-17	14.5	19.5	-	-
18	NCV-18	47.5	67.5	9	12
19	NCV-19	25	28	9.25	9.75
20	NCV-20	30	37	11.15	15
21	BAS-3	13.5	18.5	13.24	18.85
22	BAS-8	9.5	25	7	15
23	BAS-10	12.5	27	8	8.1
24	BAS-21	10	14	9	9.5
25	KM-2	10	24.5	9.2	9.5
26	KM-5	9	22.5	9.2	9.5
27	KM-6	8.5	8.9	-	-
28	KM-7	10	15	-	-
29	KB-4	18.5	21.5	-	-
30	KB-10	13	14.5	8	8.34
31	KB-11	9.5	18.5	9.5	11.5
32	KB-3	17.5	21.5	7	10
33	NG-1	11	25.5	-	-
34	NG-2	41	45	-	-

Lignocellulose substrates have complex structures which resist degradation by organic acids and non-specific microbial hydrolytic enzymes. The degradation of cellulose, lignin and hemicellulose by various microorganisms have been reported in literature. For instance, Hussain *et al.* (2019) isolated 4 cellulase producing Bacillus strains from soil samples that produced up to 40 U/mg of enzyme in 24h under aerated conditions.[29] Broeker *et al.*, (2018) have reported 20 new enzymes, in addition to the 50 known glycoside hydrolases, for the bioconversion of hemicellulose from *Clostridium stercorarium* DSM 8532.[30] Precisely, both cellulose and hemicellulose is depolymerized with extracellular enzymes and then degraded intracellularly.[29,31] However, hemicellulose is easily depolymerized and degraded compared to cellulose.[32] Degradation of lignin is considerably more complex than both these compounds.

Saprotrophic (white rot, brown rot and soft rot) fungi are reported to be most efficient in decomposing lignin since they practically thrive on all dead organic matter.[33] However, only white rot fungi are capable of completely degrading lignin to carbon dioxide and water.[34] Among bacteria, many species of α- and γ-Proteobacteria are known for their lignin degrading potential.[33] They rarely produce lignin peroxidase or specific lignin degrading enzymes.[35] Instead, their lignin degrading activity can be attributed to production of non-specific enzymes like lignin peroxidase, manganese peroxidase, catalase and laccase.

Table 2: Potential lignocellulose degraders used in consortium

Sr. No.	Isolate	Identification	Gram nature	Concentration of reducing sugar (µg/ml)	Wavelength range 200-400 nm
1	NCV-2	*Staphylococcus pseudintermedius*	Gram positive	334.125	281.25
2	NCV-4	Unidentified	Gram positive	409.125	280.97
3	NCV-6	Unidentified	Gram positive	621.625	280.98
4	BAS-8	Unidentified	Gram negative	409.125	280.91
5	NCV-12	*Granulicatella elegans/ Kocuria varians*	Gram positive	384.125	280.86

Degradation of lignocellulose Substrates by Consortium

The reduction in lignin and cellulose content of substrates, on treatment with bacterial consortium over a period of 42 days, is represented in Table 3. Visually, the size of wastes were reduced within 7 days and continued to reduce until 21st day. After that, it remained the same up to 42nd day. Figure 2 represents the changes in pH and temperature during degradation of lignocellulosic substrates. Except for sugarcane wastes, other substrates showed minor decrease in pH over 42 days as compared to controls. Also, a slight and gradual rise in temperature of all treated wastes was observed in our study (Fig. 2).

In nature, complex microbial ecosystems prevail and flourish due to synergistic activities between different microorganisms, and between plant roots and microorganisms.[36] In the present study, we specifically intended to mimic natural environmental conditions and evaluate the efficacy of bacterial consortium to degrade lignocellulosic substrates. Hence, 5 potential strains that produced both lignin and cellulose degrading enzymes were selected, and after treatment the substrates were kept at RT under static conditions. Overall, 7-14% reduction in cellulose and 3-6% reduction in lignin content was observed in our study.

Both sugarcane and fruit wastes contain higher amount of simple sugars as compared to garden, rice and cotton wastes. As expected, sugarcane wastes showed a significant drop in pH during treatment compared to control. The saccharification of lignocellulose biomass releases sugars which can be fermented by microbial strains to produce organic acids.[37] Hence, considering the above observation, it is possible that sugarcane wastes contained higher concentration of readily degradable sugars. Alternatively, it is also possible that sugarcane wastes were more effectively degraded by bacterial consortium in our study. The pH of degraded substrates in sugarcane and fruit wastes was 3.1 and 3.6 respectively. Compared to these, garden, rice and cotton substrates showed higher pH in the range of 5.0 to 5.7 on treatment with consortium. This may indicate that the above substrates were degraded using non-specific enzymes with or without fermentation that produced very low amount of organic acids.

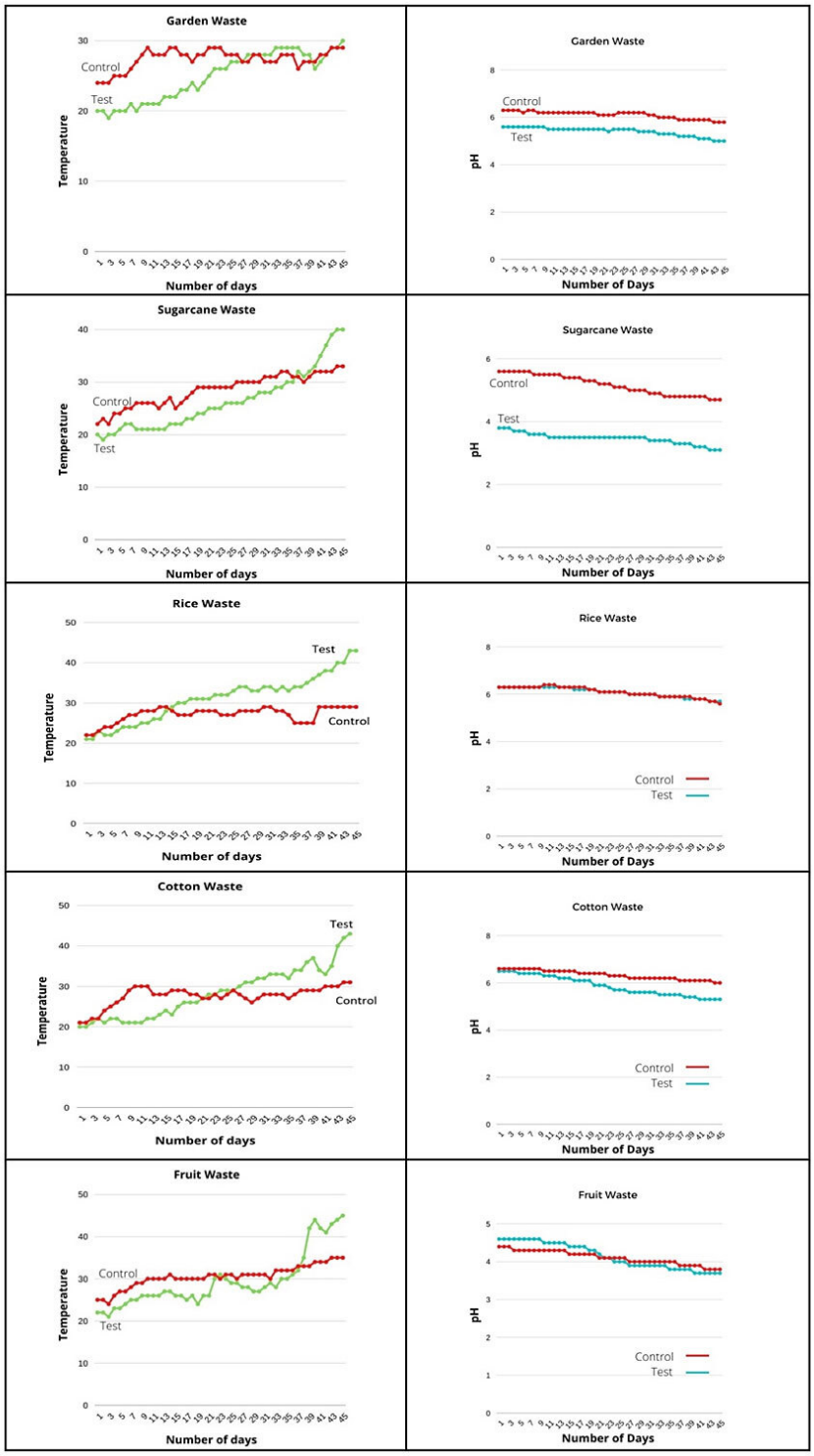

Fig. 2: Changes in pH and temperature of substrates during preparation of biofertilizers

Table 3: Lignin and cellulose content of substrates

Sr. No.	Substrate	Garden waste	Sugarcane waste	Rice waste	Cotton waste	Fruit waste
DAY- 1	Cellulose	43.79%	44.61%	37.85%	40.07%	27.43%
	Lignin	6.55%	7.38%	8.46%	14.10%	6.30%
DAY – 7	Cellulose	41.34%	42.12	37.12	38.97%	26.01%
	Lignin	6.02%	7.10%	7.90%	13.11%	5.97%
DAY-14	Cellulose	38.97%	38.43	36.43	37.67%	24.96%
	Lignin	5.96%	6.56%	6.98%	12.54%	4.87%
DAY-21	Cellulose	35.87%	36.23	34.98	36.92%	23.12%
	Lignin	5.23%	5.92%	6.56%	11.76%	4.34%
DAY-28	Cellulose	32.65%	35.12	34.12%	36.12%	21.56%
	Lignin	4.98%	5.56%	6.23%	11.12%	4.21%
DAY-35	Cellulose	31.73%	35.49%	32.81%	35.33%	20.54%
	Lignin	4.65%	5.04%	6.16%	10.51%	3.92%
DAY-42	Cellulose	29.00%	33.56%	29.98%	33.54%	18.12%
	Lignin	4.02%	4.65%	5.87%	9.23%	3.20%

Fig. 3: Representative pictures showing growth of moong seedlings after 4 days (left) and 10 days (right) in soil amended with biofertilizers

Efficiency of Degraded Lignocellulose Substrate as Biofertilizer

The efficiency of degraded lignocellulose substrates as biofertilizer was evaluated based on growth of moong seedlings (Fig. 3). The morphological and growth characteristics of seedlings are indicated in Table 4. All seedlings showed better growth in soil amended with biofertilizer as compared to control. These characteristics were distinctly better in soil amended with sugarcane and fruit waste biofertilizers. Maximum root and shoot dry weight was observed in seedlings grown in fruit waste biofertilizer. The moisture holding capacity was highest in biofertilizer prepared from sugarcane waste (70%), followed by garden (68%) and fruit waste (64%). Comparatively low moisture holding capacity was observed in biofertilizer prepared from rice (55%) and cotton (50%) wastes.

Table 4: Morphological and growth characteristics of seedlings

Sr. No.	Bio ferti lizers	Obser vation	No. of leaves	No. of shoots	Average shoot length (cm)	Fresh weight (g)	Dry weight (g)	Root dry weight (g)	Shoot dry weight (g)	No. of root hairs
1	Garden	Control	11	7	8	3.2	1.33	0.35	0.98	7
		Test	64	33	10	13.95	6.97	1.99	4.98	30
2	Sugar	Control	11	6	8	3.4	1.35	0.31	1.04	8
	cane	Test	52	27	12	11.52	4.56	1.99	2.57	25
3	Rice	Control	10	6	8	3	1.28	0.3	0.98	6
		Test	22	13	15	7.32	3.32	1.34	1.98	12
4	Cotton	Control	12	6	7	2.9	1.28	0.31	0.97	6
		Test	60	32	11	15.55	6.56	2.34	4.22	30
5	Fruit	Control	12	7	8	3.12	1.34	0.32	1.02	7
		Test	70	35	16	17.23	8	3.21	4.97	32

Moong plant prefers a soil pH between 6.2 and 7, and soil moisture between 60 and 70% for optimum growth.[38] However, slightly acidic soil conditions (~pH 6.2) improve the nutrient uptake capacity of plants.[39] In the present study, the soil and biofertilizer was used in 3:1 ratio. Hence, the small quantity of degraded substrates (sugarcane and fruit waste) with low pH slightly reduced the soil pH to provide optimum growth conditions. In addition, sugarcane as well as fruit wastes provided sufficient moisture to soil. Hence, soil amended with these biofertilizers showed better morphological and growth characteristics of seedlings (Table 4).

Many studies have suggested the use of lignocellulosic wastes as biofertilizers. To the best of our knowledge, this is the first study proposing the potential of bacterial consortium, capable of degrading both lignin as well as cellulose, to convert a range of lignocellulosic wastes into biofertilizer. Previously, Chandran *et al.*[40] suggested the use of bagasse as carrier material for phosphate biofertilizer with the help of phosphate solubilizing *Bacillus megaterium*. In another study, a biofertilizer produced after hydrogen dark fermentation of food wastes was suggested for growth of *Raphanus sativus*. Although significant improvement in growth was observed on its application, it was found to be toxic at higher concentrations.[41] In comparison with published studies, we report a safe, simple and direct application of lignocellulosic biomass as biofertilizer on treatment with bacterial consortium.

Conclusion

The results reported in this study suggest an environmental friendly and quick application of microbial consortium to convert lignocellulose into biofertilizer. Many advanced protocols requiring high energy inputs are reported in literature for conversion of lignocellulose to useful products like glucose, fructose and biofuel. However, for

large scale implementation of any application, it is necessary to adopt simple strategies that can be practiced without unnecessary technological inputs. This is especially true in remediating large scale organic wastes including lignocellulosic substrates. Besides, at present, we face environmental issues of depleting bioenergy sources like fossils and sustainable alternatives is in high demand. In such a scenario, the bioconversion of lignocellulose to useful products is not only a practical strategy but also a necessity. In agriculture, the use of organic fertilizers is highly encouraged. Hence, converting one of the most abundant global waste (especially agro-industrial waste) into fertilizer can complement the growth of organic farms cost effectively. Besides, there will still remain enough lignocellulosic biomass for production of other valuable products of bio-refinery industries.

Acknowledgement

The author would like to thank, Department of Microbiology, Rayat Shikshan Sanstha's Karmaveer Bhaurao Patil College, Vashi, Maharashtra, India. for their guidance and support to complete this article.

Funding

The author(s) received no financial support for the research, authorship, and/or publication of this article.

Conflict of Interest

The authors do not have any conflict of interest.

References

1. Zoghlami A, Paës G. Lignocellulosic biomass: understanding recalcitrance and predicting hydrolysis. *Front Chem*. 2019; 7: 874.
2. Guragain YN, Vadlani PV. Renewable biomass utilization: a way forward to establish sustainable chemical and processing industries. *Clean Technol.* 2021; 3(1): 243-259.
3. Frei M. Lignin: characterization of a multifaceted crop component. *Scientific World J*. 2013; 2013: 436517.
4. Lynd LR, Weimer PJ, van Zyl WH, Pretorius IS. Microbial cellulose utilization: fundamentals and biotechnology. *Microbiol Mol Biol Rev.* 2002; 66(3): 506-577.
5. Somerville C, Youngs H, Taylor C, Davis SC, Long SP. Feedstocks for lignocellulosic biofuels. *Science*. 2010; 329(5993): 790-792.
6. Li S, Bashline L, Lei L, Gu Y. Cellulose synthesis and its regulation. *Arabidopsis Book.* 2014; 12: e0169.
7. Vanholme R, Demedts B, Morreel K, Ralph J, Boerjan W. Lignin biosynthesis and structure. *Plant Physiol.* 2010; 153(3): 895-905.
8. Antunes FAF, Chandel AK, Terán-Hilares R, *et al.* Overcoming challenges in lignocellulosic biomass pretreatment for second-generation (2G) sugar production: emerging role of nano, biotechnological and promising approaches. 3 *Biotech.* 2019; 9(6): 230.
9. Zheng B, Yu S, Chen Z, Huo YX. A consolidated review of commercial-scale high-value products from lignocellulosic biomass. *Front Microbiol.* 2022; 13: 933882.
10. Narisetty VRR, Maitra S, *et al.* Waste-derived fuels and renewable chemicals for bioeconomy promotion: A sustainable approach. *Bioenergy Res.* 2022; 1-17.
11. Devi A, Bajar S, Kour H, Kothari R, Pant D, Singh A. Lignocellulosic biomass valorization for bioethanol production: a circular bioeconomy approach. *Bioenergy Res*. 2022; 1-22.
12. Guo Y, Liu Y, Guan M, *et al.* Production of butanol from lignocellulosic biomass: recent advances, challenges, and prospects. *RSC Adv.* 2022; 12(29): 18848-18863.
13. Dutta N, Usman M, Ashraf MA *et al.* Methods to convert lignocellulosic waste into biohydrogen, biogas, bioethanol, biodiesel and value-added chemicals: a review. Environ Chem Lett (2022). https://doi.org/10.1007/s10311-022-01511-z
14. Wagner AO, Lackner N, Mutschlechner M, Prem EM, Markt R, Illmer P. Biological pretreatment strategies for second-generation

lignocellulosic resources to enhance biogas production. *Energies (Basel).* 2018; 11(7): 1797.

15. Mukai S, Oyanagi W. Decomposition characteristics of indigenous organic fertilisers and introduced quick compost and their short-term nitrogen availability in the semi-arid Ethiopian Rift Valley. *Sci Rep*. 2019; 9(1): 16000.
16. Saritha M, Arora A, Lata. Biological pretreatment of lignocellulosic substrates for enhanced delignification and enzymatic digestibility. *Indian J Microbiol.* 2012; 52(2): 122-130.
17. Heiss-Blanquet S, Fayolle-Guichard F, Lombard V, *et al.* Composting-like conditions are more efficient for enrichment and diversity of organisms containing cellulase-encoding genes than submerged cultures. *PLoS One.* 2016; 11(12): e0167216.
18. Shi F, Yu H, Zhang N, *et al.* Microbial succession of lignocellulose degrading bacteria during composting of corn stalk. *Bioengineered*. 2021; 12(2): 12372-12382.
19. Moreno-Bayona DA, Gómez-Méndez LD, Blanco-Vargas A, *et al.* Simultaneous bioconversion of lignocellulosic residues and oxodegradable polyethylene by *Pleurotus ostreatus* for biochar production, enriched with phosphate solubilizing bacteria for agricultural use. *PLoS One*. 2019; 14(5): e0217100.
20. Janusz G, Pawlik A, Sulej J, Swiderska-Burek U, Jarosz-Wilkolazka A, Paszczynski A. Lignin degradation: microorganisms, enzymes involved, genomes analysis and evolution. *FEMS Microbiol Rev.* 2017; 41(6): 941-962.
21. Kumar A, Chandra R. Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. *Heliyon.* 2020; 6(2): e03170.
22. Pointing SB. Qualitative methods for the determination of lignocellulolytic enzyme production by tropical fungi. *Fungal Divers.* 1999; 2: 17–33.
23. Teather RM, Wood PJ. Use of Congo red-polysaccharide interactions in enumeration and characterization of cellulolytic bacteria from the bovine rumen. *Appl Environ Microbiol.* 1982; 43: 777–780.
24. Kim YK, Lee SC, Cho YY, Oh HJ, Ko YH. Isolation of cellulolytic *Bacillus subtilis* strains from agricultural environments. *ISRN Microbiol.* 2012; 2012: 650563.
25. Sahadevan LDM, Misra CS, Thankamani V. Characterization of lignin-degrading enzymes (LDEs) from a dimorphic novel fungus and identification of products of enzymatic breakdown of lignin. 3 *Biotech*. 2016; 6(1): 56.
26. Chesson A, Gordon AH, Lomax JA. Substituent groups linked by alkali-labile bonds to arabinose and xylose residues of legume, grass and cereal straw cell walls and their fate during digestion by rumen microorganisms. *J Sci Food Agric.* 1983; 34: 1330-1340.
27. Kucharska K, Rybarczyk P, Hołowacz I, Łukajtis R, Glinka M, Kamiński M. Pretreatment of lignocellulosic materials as substrates for fermentation processes. *Molecules.* 2018; 23(11): 2937.
28. Zhang W, Ren X, Lei Q, Wang L. Screening and Comparison of Lignin Degradation Microbial Consortia from Wooden Antiques. *Molecules*. 2021; 26(10): 2862.
29. Hussain AA, Abdel-Salam MS, Abo-Ghalia HH, Hegazy WK, Hafez SS. Optimization and molecular identification of novel cellulose degrading bacteria isolated from Egyptian environment. *J Gen Eng Biotechnol.* 2017; 15(1): 77-85.
30. Broeker J, Mechelke M, Baudrexl M, Mennerich D, Hornburg D, Mann M, Schwarz WH, Liebl W, Zverlov VV. The hemicellulose-degrading enzyme system of the thermophilic bacterium *Clostridium stercorarium:* comparative characterisation and addition of new hemicellulolytic glycoside hydrolases. *Biotechnol Biofuels.* 2018; 11: 229.
31. Aarti C, Arasu MV, Agastian P. Lignin degradation: A microbial approach. *Indian J Biol Sci.* 2015; 1: 119–127.
32. Zamocky M, Ludwig R, Peterbauer C, *et al*. Cellobiose dehydrogenase--a flavocytochrome from wood-degrading, phytopathogenic and saprotropic fungi. *Curr Protein Pept Sci.* 2006; 7(3): 255-280.
33. Janusz G, Pawlik A, Sulej J, Świderska-Burek U, Jarosz-Wilkołazka A, Paszczyński A. Lignin degradation: microorganisms,

enzymes involved, genomes analysis and evolution. *FEMS Microbiol Rev.* 2017; 41(6): 941–962.

34. Rytioja J, Hilden K, Yuzon J *et al.* Plant-polysaccharide-degrading enzymes from Basidiomycetes. *Microbiol Mol Biol R.* 2014; 78: 614–649.
35. Barr DP, Shah MM, Grover TA, Aust SD. Production of hydroxyl radical by lignin peroxidase from Phanerochaete chrysosporium. *Arch Biochem Biophys.* 1992; 298(2): 480-485.
36. Moënne-Loccoz Y, Mavingui P, Combes C, Normand P, Steinberg C. Microorganisms and Biotic Interactions. *Env Microbiol Fund Applicat.* 2014; 11: 395-444.
37. Chacón MG, Ibenegbu C, Leak DJ. Simultaneous saccharification and lactic acid fermentation of the cellulosic fraction of municipal solid waste using *Bacillus smithii. Biotechnol Lett.* 2021; 43(3): 667-675.
38. Hossainr MM, Islam MT, Prodha AKMA. Effect of soil moisture levels on growth and yield of winter mungbean. *Bangladesh J. Train. Dev.* 2006-07; 19/20(1&2):87-93.
39. Li B, Wang S, Zhang Y, Qiu D. Acid soil improvement enhances disease tolerance in citrus infected by Candidatus Liberibacter asiaticus. *Int J Mol Sci.* 2020; 21(10): 3614.
40. Chandran AM, Subashini A. Production of phosphate biofertilizer using lignocellulosic waste as carrier material. *Asian J Chem.* 2014; 26(7): 2065-2067.
41. Tashyrev OB, Matvieieva NA, Hovorukha VM, Tashyreva HO, Bielikova OI, Havryliuk OA, Duplij VP. Application of lignocellulosic substrate obtained after hydrogen dark fermentation of food waste as biofertilizer. *Ind Biotechnol.* 2018: 315-322.