www.ThePharmaJournal.com

The Pharma Innovation

ISSN (E): 2277-7695
ISSN (P): 2349-8242
NAAS Rating: 5.23
TPI 2023; 12(2): 729-733

www.thepharmajournal.com
Received: 26-12-2022
Accepted: 29-01-2023

Jagadeesh U
PhD Scholar (Agricultural Microbiology), Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Muthuraju R
Associate Professor, Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Nalini BS
Research Associate, Natural Farming Project, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Kadalli GG
Professor, Department of Soil Science and Agricultural Chemistry, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Yogananda SB
Professor, Department of Agronomy, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Corresponding Author:
Jagadeesh U
PhD Scholar (Agricultural Microbiology), Department of Agricultural Microbiology, University of Agricultural Sciences, GKVK, Bengaluru, Karnataka, India

Evaluation of lignocellulolytic cultures on *In-vitro* degradation of paddy straw

Jagadeesh U, Muthuraju R, Nalini BS, Kadalli GG and Yogananda SB

Abstract
This study was carried out to select the potential combination of lignocellulolytic cultures using rice straw substrate for degradation. Efficient lignocellulolytic cultures were used to assess their degradation potential using paddy straw. On the basis of all the qualitative assays and quantitative assays, the most efficient lignocellulolytic microorganisms were selected for assessment of degradation potential under greenhouse conditions. Four most efficient cultures consisting of two fungi, one bacterium strain and one actinobacterial strain which were identified as *Phanerochaete chrysosporium* UASBLCF_01, *Purpureocillium lilacinum* UASBLCF_02, *Bacillus inaquosorum* UASBLCB_03 *and Streptomyces viridosporus* UASBLCA_04, were selected for experiment and the isolates were found to be compatible with each other. and exhibited significant paddy straw decomposition. Steady decrease in Neutral Detergent Fiber (NDF) and ADF (Acid Detergent Fiber) was observed instead of rapid decrease. For the first sampling from pots of decomposing paddy straw at 15 DAC, T_{13} containing paddy straw and all the four cultures showed maximum decrease in NDF to 45.54 percent as compared to 68.81 percent of control and ADF to 30.43 percent as compared to 46.14 percent of control. There was a significant decline in NDF and ADF of paddy straw pre-treated with different combinations of potential lignocellulolytic cultures.

Keywords: Lignocellulolytic cultures, neutral detergent fiber (NDF) and ADF (Acid detergent fiber), paddy straw

Introduction
Lignocellulosic waste is considered as a chief component of renewable biomass on the earth, with an annual output of about 1,500 million tons (Xing *et al*., 2020) [23]. It is a product associated with agricultural production and straw is one of the primary sources of lignocellulose. India, the second largest agro-based economy with year-round crop cultivation, generates a large amount of agricultural waste, including crop residues. The leading crop residue producing crops are maize, rice, sugarcane, wheat, soybean and barley contributing almost 85 percent of the global crop residue production (FAO, 2022) [7]. Major crop residue accumulates for paddy straw 1135.12 MT, maize stover 1162.35 MT and sugarcane trash 467.43 MT (Reshma *et al*., 2022) [15].

Farmers burn crop residue *in-situ* huge quantities due to the advent of mechanized harvesting causing loss of nutrients and soil organic matter. Burning or incineration of agricultural wastes may reduce the volume of wastes but has an adverse effect on the environment. Combustion releases greenhouse gases such as carbon dioxide (CO_2), nitrous oxide (N_2O), which have the capability of depleting the ozone layer, thereby contributing to global warming (Bhat *et al*., 2018) [3]. Retention of paddy straw residue on- field benefits soil health, soil water conservation, soil productivity and environment. Many off field options for utilization of paddy straw residue are composting, biogas production, substrate for edible mushroom cultivation and as packaging material for transportation (Subhendu, 2020, Bhuvaneshwari *et al*., 2019) [19, 4].

Paddy straw is the most abundant ligno-cellulosic waste on earth. Rice plant approximately contains cellulose (30-40%), hemicelluloses (20-24%), Lignin (10-13%), Ash (5%) and Silica (13%) (Soest, 2006) [18]. The cellulose content in maize stover is 39.3 percent, 38.1 percent of hemicellulose and 12.6 percent of lignin (Woźniak *et al*., 2021) [22]. Lignin content of sugarcane trash is 21.7 percent, the cellulose content is 39.7 and 40.8 percent of hemicellulose (Franco *et al*., 2013) [8]. The enzymatic activity plays important role in the turnover of carbon polymers like cellulose, hemicelluloses and lignin. Cellulolytic microorganisms produce enzymes, which are responsible for hydrolysis of cellulosic materials.

Enzymatic hydrolysis involves a series of enzymes, which act in a synergistic way to completely hydrolyze cellulose to simple sugars. These enzymes are highly specific for their substrates. The product of one enzyme acts as a substrate for another enzyme and in this way cellulose system completely degrades cellulose to simple sugars (Shi *et al.*, 2009) [17].

Microorganisms can naturally degrade lignin and produce lignin-degrading enzymes, including fungi, actinomycetes, and bacteria (Li *et al*., 2020; Bohacz and Kowalska, 2020) [11, 5]. They can secrete extracellular lignin degradation oxidases, which are primarily divided into lignin peroxidase (EC 1.11.1.14), manganese peroxidase (EC 1.11.1.13) and laccase (EC 1.10.3.2) (Adarsh and Chandra, 2020) [1].

Paddy straw can be utilized as a source of nutrients for succeeding paddy crop by incorporating paddy straw into soil along with urea, bio-mineralizer and cow dung slurry which resulted improved nutrient uptake of available nutrients and crop yield in contrast to incorporation of paddy straw (Vijayaprabhakar *et al*., 2020) [21]. Kumar *et al.* (2019) [10] showed that besides causing air pollution, burning of paddy straw leads to the loss of soil organic matter and essential nutrients, pH variation, reduces microbial activities and the land more vulnerable to soil erosion and suggested that paddy straw retention improves soil physico-chemical properties, increases microbial population and microbial activity, as compared to burning residues.

The enzymatic degradation of lignocellulosic biomass is important for sustainable utilization of agricultural residues. Hence, production of efficient lignocellulolytic enzymes including cellulase, xylanase and glycosyl hydrolases, has received a great attention. Therefore, the main aim of this investigation is to select the best combination of isolated novel microbial strains of bacteria, fungi and actinobacteria from various sources and screening their lignocellulolytic ability and also determining the enzyme activity for assessing their biodegradation capability of paddy straw which can be the cost-effective method for bioconversion of lignocellulosic biomass.

Material and Methods

An experiment was conducted to study the efficacy of the isolated strains of fungi, bacteria, actinobacteria individually and their consortium for decomposition of paddy straw at Department of Agricultural Microbiology, University of Agricultural Sciences, Gandhi Krishi Vigyana Kendra, Bangalore. Paddy straw was obtained from college of Agriculture Vishweshwaraiah Channel Farm (V. C Farm), Mandya and was cut into 2-3 cm long.

Potential lignocellulolytic microorganisms which were screened from qualitative and quantitative lignocellulolytic assays were subjected for preparation of inoculum individually and in different combinations after the compatibility test. The organisms screened were two fungi, one bacterium strain and one actinobacterial strain which were identified as F1: *Phanerochaete chrysosporium* UASBLCF_01, F2*: Purpureocillium lilacinum* UASBLCF_02, B1: *Bacillus inaquosorum* UASBLCB_03 *and* A1: *Streptomyces viridosporus* UASBLCA_04.

In this experiment, 39 numbers of 10 kg capacity pots (13 treatments × 3 replications for each substrate) were taken. Thirteen treatments which included two fungi (F1 &F2), one bacterium (B1) and one actinobacterium strains individually and in combinations with one control (no microbial strain) were imposed along with paddy straw. In each pot 1000 g of chopped paddy straw of 2–3 cm size were added (Plate 1). One litre of liquid fungal broth was comprised of freshly prepared spore solution with 10^6 –10^7 spores mL^{-1}. Similarly, 1 L of liquid bacterial and actinobacterial broth contains 10^6 – 10^8 viable cells mL^{-1}. Finally, these liquid broths were used as single inoculum and also prepared the consortia by mixing thoroughly. Then the broths were transferred to jaggery solution for rapid multiplication of microbes for 5–7 days and 200 mL of jaggery based microbial inoculum applied to every pot containing substrate according to the treatments. Optimum moisture content was maintained during the decomposition process (15–20%). The samples were collected during straw decomposition from different treatments at fifteen days interval for estimating the loss in cellulose and lignin fiber content *i.e*, Neutral Detergent Fiber (NDF) and ADF (Acid Detergent Fiber) content.

The proximate chemical composition of paddy straw was estimated (AOAC 2000) [2] with different lignocellulolytic microorganisms. Neutral Detergent Fiber (NDF) and ADF (Acid Detergent Fiber) was determined according to the procedure outlined by Goering and Vansoest (1970) [9] and Vansoest *et al*. (1991) [20].

The data obtained from the experiments were subjected to statistical analysis for evaluating treatment effects. Analysis was carried out by completely randomized design with the help of Microsoft excel sheet (2019 version) as well as OPSTAT online statistical analysis software. Experiments with two factors were analyzed using CRD one factor analysis in OPSTAT, Critical difference were values calculated and used to identify the best treatments (Duncan, 1995) [6].

Results and Discussion

Efficient lignocellulolytic cultures F1: *Phanerochaete chrysosporium* UASBLCF_01, F2*: Purpureocillium lilacinum* UASBLCF_02, B1: *Bacillus inaquosorum* UASBLCB_03 *and* A1: *Streptomyces viridosporus* UASBLCA_04 were used to assess their degradation potential using paddy straw under greenhouse conditions. Characteristics of substrate straw were estimated which are presented in the Table 1.

Table 1: Characteristics of substrate used for *in-vitro* evaluation of degradation potential

Substrate	C (%)	N (%)	C:N	NDF (%)	ADF (%)
Paddy straw	48.26	0.57	84.66	73.25	51.74

The majority of the structural elements in plant cells, including lignin, cellulose, and hemicellulose, are measured by NDF. A neutral detergent that dissolves plant pectins, proteins, sugars, and lipids is used to measure NDF concentration. As a result he fibrous components including cellulose, lignin and hemicellulose are left behind.

Steady decrease in NDF and ADF was observed instead of rapid decrease (Fig. 1 and Fig. 2). For the first sampling from pots of decomposing paddy straw at 15 DAC, T_{13}: $F_1 + F_2 + B_1 + A_1$ showed maximum decrease in NDF to 45.54 percent as compared to 68.81 percent of control and ADF to 30.43 percent as compared to 46.14 percent for control. Non-significant results were obtained between T_7, T_8, T_9 and T_{10} during sampling at 15, 30 and 45 DAC in case of NDF, followed by significant variation during 60, 75 and 90 DAC. Whereas, significant difference between treatments was observed in between all the treatments in case of ADF during

all the samplings. T_{11}: $F_1 + F_2 + B_1$ showed sharp decrease in NDF to 23.59 percent and 18.60 percent ADF by 90DAC with gradual breakdown of the fiber content compared to control *i.e* 41.19 percent NDF and 31.19 percent ADF at 90 DAC. T_{13}: F1 + F2 + B1 + A1 showed the least value of NDF and ADF *i.e* 20.20 percent and 15.40 percent respectively at 90 DAC which was a sharp decrease in NDF as well as ADF compared to all the treatments which is presented in the Table 2 and Table 3 respectively.

Tendency of reduction in ADF and NDF at every interval of paddy straw degradation was observed with highest reduction of ADF (4.8%), NDF (10.78%) after 6 days of incubation period (Md *et al*., 2021) [14]. Ma *et al*. (2022) [12] pre-treated paddy straw and resulted hydrolysis of 70.16–63.79 percent NDF and 44.29–39.21 percent ADF contents of rice straw to a greater extent in combined treatment of corn steep liquor with urea-alkali and fungal consortium than alone. Mak-Mensah *et al*. (2022) [13] observed ADF of maize stover cultivated on the biochar-amended soil increased by 10.74% compared with the no-biochar application, NDF increased by 6.04 percent compared with the control.

Treatment of urea increased crude protein (p<0.001) while decreased NDF (p<0.01) contents of rice straw. The ADF and lignin contents were similar between the control and urea-treated rice straw. Proportion of degradable fraction and degradation rate of rice straw were enhanced due to urea treatment (Ridla *et al*., 2021) [16].

Table 2: Effect of microbial pre-treatments on percent NDF with increased incubation period of paddy straw

Treatments	NDF (%)					
	15 DAC	30DAC	45DAC	60DAC	75 DAC	90DAC
T_1	68.81^{a}	65.62^{a}	62.61^{a}	53.57^{b}	44.83^{b}	41.19^{b}
T_2	56.48^{d}	54.71^{e}	47.96^{d}	40.08^{d}	31.52^{g}	28.09^{e}
T_3	59.26^{c}	56.76^{d}	45.65^{e}	37.18^{f}	35.52^{d}	30.19^{d}
T_4	65.43^{b}	59.62^{c}	50.47^{c}	45.17^{c}	40.83^{c}	38.49^{c}
T_5	68.41^{a}	67.29^{b}	60.40^{b}	56.27^{a}	51.24^{a}	48.59^{a}
T_6	48.82^{f}	48.58^{h}	38.73^{g}	30.48^{i}	27.32^{k}	24.19^{hi}
T_7	53.20^{e}	51.64^{g}	45.65^{e}	40.28^{d}	32.32^{f}	27.19^{f}
T_8	53.79^{e}	52.77^{f}	46.76^{de}	38.78^{e}	30.52^{h}	26.19^{g}
T_9	52.10^{e}	51.34^{g}	42.24^{f}	37.08^{f}	32.22^{f}	26.89^{f}
T_{10}	52.90^{e}	52.16^{fg}	45.65^{e}	36.48^{f}	33.42^{e}	28.19^{e}
T_{11}	48.03^{f}	46.63^{i}	38.93^{g}	31.88^{h}	28.32^{j}	23.59^{i}
T_{12}	47.33^{fg}	46.02^{i}	37.32^{h}	33.18^{g}	29.22^{i}	24.39^{h}
T_{13}	45.54^{g}	44.18^{j}	36.02^{h}	29.48^{i}	25.62^{l}	20.20^{j}

Note: Each column's mean values for the same superscript do not significantly differ from one another at P=0.05 level by DMRT
Incubation period- 3 months
DAC- Days after composting

T_1: Control	T_5: A1	T_9: F2 + B1	T_{13}: F1 + F2 + B1 + A1	F1: *Phanerochaete chrysosporium* UASBLCF_01
T_2: F1	T_6: F1 + F2	T_{10}: F2 + A1		F2: *Purpureocillium lilacinum* UASBLCF_02
T_3: F2	T_7: F1 + B1	T_{11}: F1 + F2 + B1		B1: *Bacillus inaquosorum* UASBLCB_03
T_4: B1	T_8: F1 + A1	T_{12}: F1 + F2 + A1		A1: *Streptomyces viridosporus* UASBLCA_04

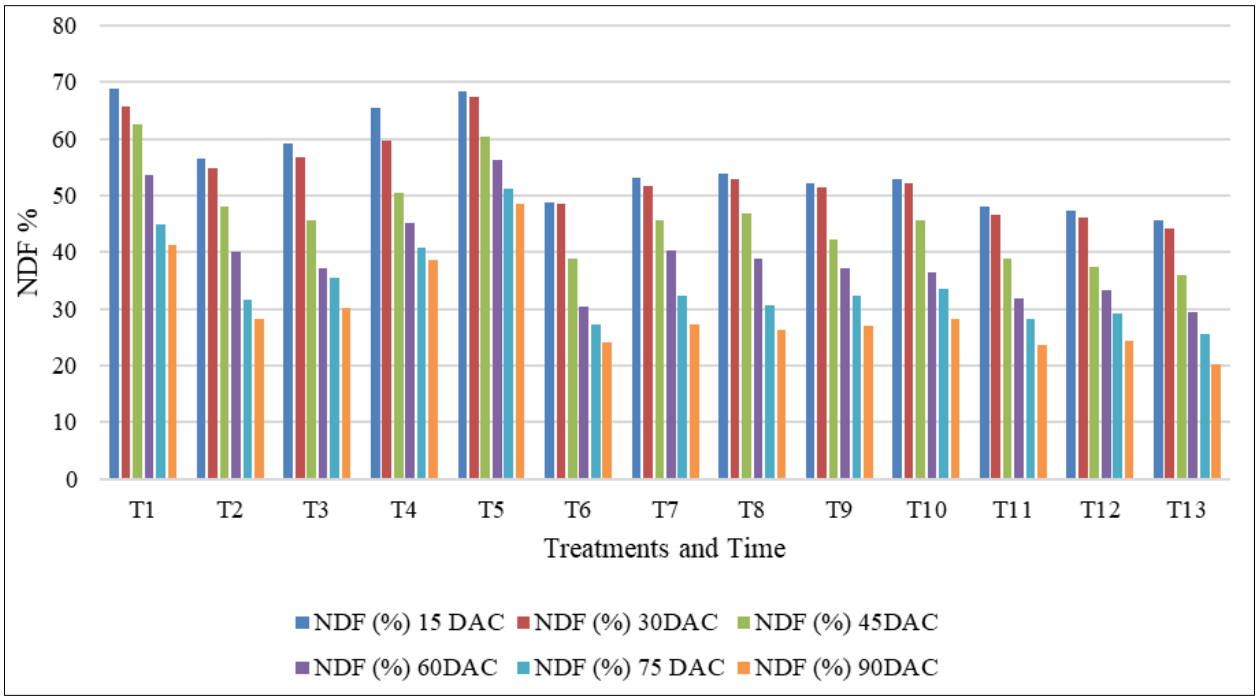

Fig 1: Effect of microbial pre-treatments on percent NDF with increased incubation period of paddy straw

Table 3: Effect of microbial pre-treatments on percent ADF with increased incubation period of paddy straw

Treatments	ADF (%)					
	15DAC	30DAC	45DAC	60DAC	75 DAC	90DAC
T_1	46.14^{a}	44.59^{a}	42.04^{a}	39.58^{a}	33.62^{a}	31.19^{a}
T_2	37.98^{d}	37.63^{d}	35.52^{d}	33.38^{c}	27.42^{d}	23.19^{d}
T_3	37.69^{d}	36.41^{e}	34.31^{e}	31.88^{d}	26.12^{e}	22.49^{e}
T_4	39.87^{c}	38.96^{c}	36.62^{c}	33.58^{c}	28.32^{c}	25.69^{c}
T_5	42.26^{b}	41.21^{b}	38.73^{b}	37.18^{b}	32.62^{b}	29.39^{b}
T_6	34.40^{g}	32.83^{g}	28.60^{j}	25.19^{i}	22.12^{j}	18.60^{j}
T_7	35.99^{ef}	34.87^{f}	32.31^{gh}	29.38^{fg}	23.62^{h}	20.20^{h}
T_8	36.99^{de}	35.38^{f}	33.01^{fg}	30.48^{e}	24.22^{g}	21.79^{f}
T_9	37.88^{d}	36.61^{e}	33.61^{ef}	30.88^{e}	24.62^{g}	21.10^{g}
T_{10}	36.59^{de}	34.57^{f}	31.81^{h}	29.58^{f}	23.12^{i}	20.50^{h}
T_{11}	34.30^{g}	32.32^{g}	28.70^{j}	26.78^{h}	25.22^{f}	18.60^{j}
T_{12}	35.00^{g}	33.03^{g}	30.70^{i}	28.78^{g}	21.52^{k}	19.40^{i}
T_{13}	30.43^{h}	21.68^{h}	25.38^{k}	22.49^{j}	17.21^{l}	15.40^{k}

Note: Each column's mean values for the same superscript do not significantly differ from one another at P=0.05 level by DMRT Incubation period- 3 months

DAC- Days after composting

T_1: Control	T_5: A1	T_9: F2 + B1	T_{13}: F1 + F2 + B1 + A1	F1: *Phanerochaete chrysosporium* UASBLCF_01
T_2: F1	T_6: F1 + F2	T_{10}: F2 + A1		F2: *Purpureocillium lilacinum* UASBLCF_02
T_3: F2	T_7: F1 + B1	T_{11}: F1 + F2 + B1		B1: *Bacillus inaquosorum* UASBLCB_03
T_4: B1	T_8: F1 + A1	T_{12}: F1 + F2 + A1		A1: *Streptomyces viridosporus* UASBLCA_04

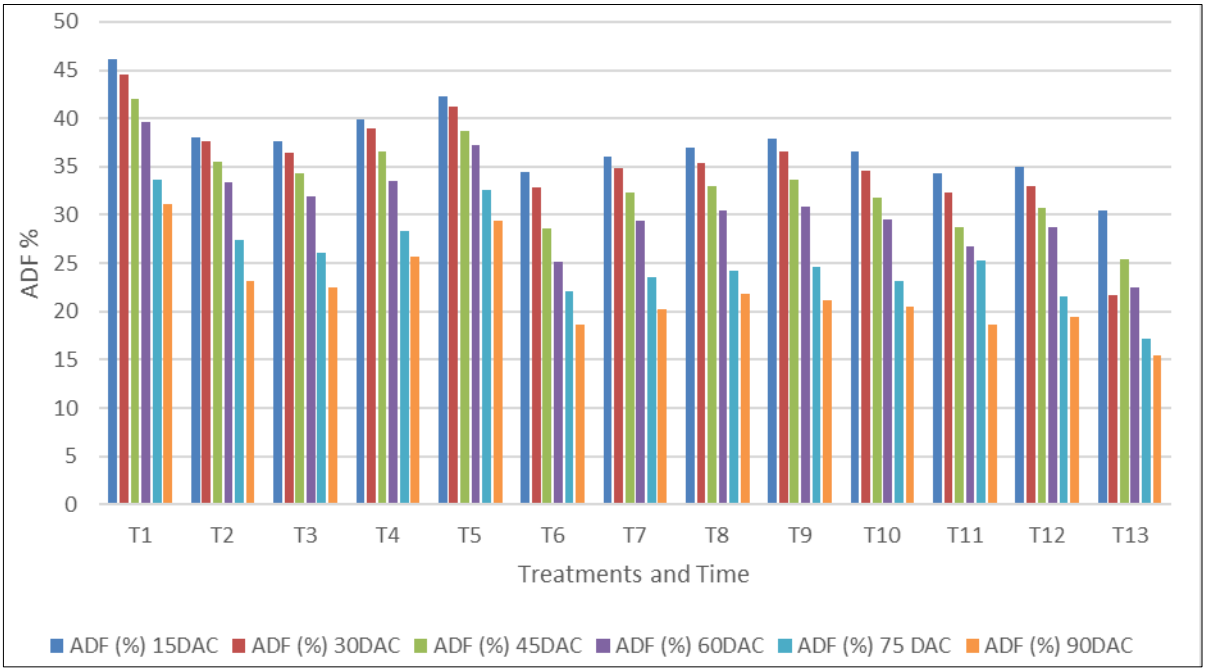

Fig 2: Effect of microbial pre-treatments on percent ADF with increased incubation period of paddy straw

Conclusion

This study confirms that T_{13} *i.e.* the combination of four cultures which includes fungi, bacteria and actinobacteria *viz.* F1: *Phanerochaete chrysosporium* UASBLCF_01, F2*: Purpureocillium lilacinum* UASBLCF_02, B1: *Bacillus inaquosorum* UASBLCB_03 *and* A1: *Streptomyces viridosporus* UASBLCA_04 as best combination of lignocellulolytic cultures which degraded chopped paddy straw significantly compared to individual cultures and combinations with significant reduction in NDF and ADF to 20.20 percent and 15.40 percent respectively at 90 DAC which was a sharp decrease in NDF as well as ADF compared to all the treatments. This work demonstrates that combined bioaugmentation of synergistic microbes has a significant advantage in reducing paddy straw biomass and facilitating hydrolysis. This strategy may also be adapted to work on degradation of different lignocellulosic biomasses.

Acknowledgement

This work was supported by Department of Agricultural Microbiology, UAS, GKVK, Bengaluru-65, Department of Agronomy, College of Agriculture, VC Farm, Mandya, Department of Soil Science & Agricultural chemistry, UAS, GKVK, Bengaluru-65.

References

1. Adarsh K, Chandra R. Ligninolytic enzymes and its mechanisms for degradation of lignocellulosic waste in environment. Heliyon. 2020;6(2):170-178.
2. AOAC. Association of official analytical chemists,

official methods of analysis, 17th edition, Maryland, USA; c2000.
3. Bhat RA, Dar SA, Dar DA, Dar GH. Municipal solid waste generation and current scenario of its management in India. Int. J. Adv. Res. Sci. Eng. 2018;7(2):419-431.
4. Bhuvaneshwari S, Hiroshan H, Jay NM. Crop Residue Burning in India: Policy Challenges and Potential Solutions. Int. J. Env. Res. Pub. He. 2019;16(832):1-19.
5. Bohacz J, Kowalska KT. Modification of post-industrial lignin by fungal strains of the genus *Trichoderma* isolated from different composting stages. J Environ. Manage. 2020;266:3-11.
6. Duncan DB. New multiple range and multiple F-test. Biometrics. 1995;11(1):1-42.
7. FAO. OECD-FAO Agricultural Outlook; c2022-2031.
8. Franco HCJ, Pimenta MTB, Carvalho JLN, Magalhães PSG, Rossell CEV, Braunbeck OA, *et al*. Assessment of sugarcane trash for agronomic and energy purposes in Brazil. Scientia Agricola. 2013;70:305-312.
9. Goering HK, Vansoest PJ. Forage fiber analysis. USA, Department of Agriculture Research Services. Agriculture Handbook; c1970. p. 379.
10. Kumar A, Kushwaha KK, Singh S, Shivay YS, Meena MC, Nain L. Effect of paddy straw burning on soil microbial dynamics in sandy loam soil of Indo-Gangetic plains. Environ. Technol. Innov. 2019;16:1-10.
11. LI J, Wang P, Salam N, LI X, Ahmad M, Tian Y, *et al*. Unraveling bacteria-mediated degradation of lignin-derived aromatic compounds in a freshwater environment. Sci. Total Environ. 2020;749:1-40
12. MA Y, Chen X, Khan MZ, Xiao J, Liu S, Wang J, *et al*. Biodegradation and hydrolysis of rice straw with corn steep liquor and urea-alkali pretreatment. Front. Nutr. 2022;9:1-15.
13. Mak-Mensah E, Zhao W, Zhou X, Zhang D, Zhao X, Wang Q, Obour PB. Influence of tied-ridge with maize straw biochar on Alfalfa fodder yield, nutritional quality, and production economics. J Soil Sci. Plant Nutr. 2022;51:1-12.
14. MD RI, MD MR, Shilpi I, Shabbir A, Mueena J, Sulav IP, *et al*. Degradation of lignocellulosic content of rice straw using aerobic cellulolytic bacteria isolated from forest soil of Bangladesh. Afr. J. Microbiol. Res. 2021;15(3):161-170.
15. Reshma S, Dhirendra KS, Prabhakar M, Chandra SS, Sushanta KN, Nandkishore T, *et al*. Management of crop residues with special reference to the on-farm utilization methods: A review. Ind. Crops Prod. 2022;181:1-10.
16. Ridla M, Dewi M, Laconi EB, Jayanegara A. Urea treatment of rice straw: A modelling approach of its degradation kinetics in the rumen. IOP Conf. Ser.: Earth Environ. Sci. 2021;788(1):1-4.
17. Shi J, Shivappa RR, Chinn M, Howell N. Effect of microbial pretreatment on enzymatic hydrolysis and fermentation of cotton stalks for ethanol production. Biomass. Bioener. 2009;33(1):88-96.
18. Soest VPJ. Rice straw, the role of silica and treatments to improve quality. Anim. Feed. Sci. Technol. 2006;130(3):137-71.
19. Subhendu BG, Ramyajit M, Sanjib KM. Crop residue management options in rice–rice system: A review. Archiv. Agron. Soil. Sci. 2020;66(9):1218-1234.
20. Vansoest PJ, Robertson JB, LEWIS BA. Methods for dietary fiber, neutral detergent fiber and Nonstarch polysaccharides in relation to animal nutrition. J. Dairy Sci. 1991;74(10):3583-3598.
21. Vijayaprabhakar A, Nalliah D, Hemalatha M, Joseph M. Study on rice residue management options on growth parameters and growth indices of rice crop. J. Exp. Agric. Int. 2020;42(1):56-63.
22. Woźniak M, Ratajczak I, Wojcieszak D, Waśkiewicz A, Szentner K, Przybył J, *et al*. Chemical and structural characterization of maize stover fractions in aspect of its possible applications. Materials. 2021;14(6):1527.
23. Xing BS, Han Y, Cao S, Wen J, Zhang K, Yuan H, *et al*. Co-substrate strategy for enhancing lignocellulose degradation during rumen fermentation *in-vitro*: Characteristics and microorganism composition. Chemosphere. 2020;250:1-13.