Sustainable Waste Managementby growing mushroom *(Pleurotusflorida)*on anaerobically digested waste and agro residues

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ABSTRACT

Improper disposal of agro waste and digestates often results in contamination of water bodies and soil. The impacts of these wastes on the economy cannot be ignored and managing them has become a major problem .A large portion of nutrients is dissolved in the digestates resulting from anaerobic digestion (AD) of organic wastes. Direct application of digestates to field is not suitable, as it would lead to a significant release of ammonia because in the digestatetotal- N will be in the form of NH_4-N. The higher content of NH_4-N will lead to increased risk of ammonia emission as well as leaching which causes environmental pollution and adversely affects the global nitrogen cycle. Thus, stabilizing the product prior to application to the field was proposed, and a series of experiments were conducted to evaluate the appropriateness of this approach. The study was conducted using the digestate from plug flow type biogas plant. The digestate material along with agricultural residues such as coir pith, paddy straw were used as substrates for the cultivation of Pleurotusflorida and it was investigated to reduce the nutrients in acceptable range.

The outcome of the investigation showed the bioconversion of agro residues through cultivation of mushroom. This study revealed the significant reduction of lignin, C: N ratio. Maximum reduction of C: N ratio from initial value of 115 to 60 was observed in paddy straw, lignin reduction of 64% was observed in paddy straw. Maximum mushroom yield of 2.3 kg of pleurotusflorida/kg of substrates was observed in paddy straw supplemented with anaerobic digestates compared to only paddy straw when used as substrates.

Mushroom cultivation offers the opportunity to utilize renewable resources in the production of edible, protein-rich food that will sustain food security for people in developing countries. The technology can also limit air pollution associated with burning agriculture wastes.

1. INTRODUCTION

Most agricultural residues are rich in lignocellulosic compounds whose handling and disposal are often problematic, due to their chemical structure and decomposition properties. Cotton wastes including stem-leaf residues and gin trash, wheat straw, peanut shells, coir pith, maize stalks and sorghum stalks are of particular interest for the agricultural economy of temperate and subtropical countries, since they are produced in large quantities and their post-harvest treatment is mainly accomplished through burning or incorporation into the soil.

In India, there are about 3.67 million biogas units [15] however due to shortage of dung in rural areas several small scale biogas plants operating on non-dung based feeds such as biomass are being developed for rural use [6] Biomass has been defined as organic matter formed by photosynthetic capture of solar energy and stored as chemical energy which includes, animal wastes, forest and mill residues, wood and wood wastes, livestock operation residues, aquatic plants, fast-growing trees and plants, and municipal and industrial wastes

Anaerobic digestion (AD) is a process in which microorganisms break down biodegradable material in the absence of oxygen. Anaerobic digestion creates two main types of output: biogas which is rich in energy and digestate which is rich in nutrients. These digestate obtained after anaerobic digestion is commonly used as a fertilizer in agricultural fields and one of the drawback in using it as a fertilizer is the application at times of the year when there is less plant uptake (e.g. autumn and winter), this can result in nutrient leaching and runoff into ground and surface waters (e.g. of N and P) and the utilization percentage of N in digestate should be equivalent to the share of ammonium. However, when digestate is applied to

a field surface some ammonia volatilization will take place after application. As a result, the utilization percentage will decrease. Therefore it pollutes the environment and valuable nutrients would be lost. As a consequence it is important to minimize this nutrient loss by recovering these nutrients. Mushrooms such as Pleurotus and various other varieties are a promising possibility to recycle nutrients as they have been used for centuries to produce protein rich food from solid waste.

In the present study different agricultural wastes along with spent biomass were examined for rapid degradation by fungal consortia (Pleurotus species) and check the feasibility of these substrates for growing mushrooms. Parameters like, yield, moisture content, organic carbon, available nitrogen, cellulose content, and lignin were determined to evaluate the rate of biodegradation process.

2. MATERIALS AND METHODS

2.1 Substrate Selection

The potentials of various locally available lignocellulosic substrates were explored to select the best supporter for the production of Pleurotus spp. The substrates used for the experiment were as follows:

- Paddy straw
- Coir pith
- Anaerobically digested waste

2.2 Collection of the substrates

Paddy straw, coir pith used in the experiment as substrates were obtained from a village in Hassan district. Anaerobically digested waste was recovered from a plug flow type biomass based biogas plant, operating on leafy biomass, the feed material was mainly comprised of banana leaf (Musa sp).

2.3 Source of Spawn

The spawn ofPleurotusflorida were obtained from Indian Institute of Horticultural Science (IIHR), Bangalore.

2.4 Pre-Treatment of Substrate

The substrates thus collected were dried in oven at 90°C for 24hrs. The dried paddy straw were chopped into 5 to 7 cm length, the chopped material was filled in gunny bags and soaked in fresh tap water for 12 h. Excess water was then allowed to drain off and substrates was pasteurized by dipping in hot water at 70^0C for about 20 to 30 minutes, steam sterilization was carried out for coir pith and digestate because of the problem of dis integration of substrates during pasteurization. Later this pasteurized substrate was dried under sun to remove the excess moisture. Spawning was done when the moisture content of the substrates was around 50%.

2.5 Mushroom Cultivation

The mushrooms were cultivated by the perforated polythene bag method given by Bano and Srivastava with minor modifications. After the pretreatment of the substrates, controlled and combined methods were adopted for the investigation. The controlled experimental method was with respect to independent agro wastes and anaerobic digested waste like:

- Paddy straw (PS)
- Coir pith(CP)
- Anaerobically digested waste(DW)

The combinations were prepared by mixing the agro wastes and anaerobically digested waste in the proportions of 7:3 (w/w) i.e.

- Paddy straw + anaerobically digested waste(PS+DW)
- Coir pith + anaerobically digested waste (CP+DW)

These bags were incubated in simple humid chamber covered with jute cloth and it was maintained at room temperature under dark condition for period up to completion of spawn run. After incubation, the bag was cut open and was hanged in the chamber using nylon mesh. The humidity inside the room was maintained between 80 to 90 percent by moistening the jute cloth. The beds were given diffused light during day time. Three replicates were used for each growing trial for each substrate.

2.6 Sampling of Substrates

A composite sample of 100 g was collected from different bags at different intervals i.e., before spawning, 10th day after spawning, after complete mycelia colonization, at the first harvest and final harvest. The sample was dried in hot air oven at 90°C. The dried material was powdered and samples were stored in paper bags. These samples were used for variousestimations in order to analyze nutrient status of the substrates.

2.7 Chemical Analysis of Samples

2.7.1 Organic Carbon (OC) Estimation

The OC in the samples are estimated by using dry ashing procedure [2]. The known sample is weighed and evaporated to dryness in hot air oven at 150°C. Thereafter, the dried solids are cooled and weighed. The material in the crucible represents the total solids. The Total Solids in a pre-weighed silica crucible is taken. The samples are kept in a Muffle furnace at a temperature of 600°C for 2 hours. The crucibles were later transferred to desiccators, cooled and immediately weighed to a constant weight (ash weight). Volatile Solids in percent is calculated by taking difference of dry weight of samples and Ash weight of the sample. Then organic carbon was calculated by dividing the Volatile solids percent by the factors 1.724 [2].

2.7.2Total Kjeldhal Nitrogen (TKN)

Total Kjeldhal nitrogen was determined by kjeldhal method as outlined by [12]. 0.5g of dried sample is digested using 10ml of concentrated sulphuric acid in presence of 1g of catalytic mixture (K_2SO_4, $CUSO_4$ and Selenium powder) in the ratio 50:10:1 in order to raise the boiling temperature of the digestion mixture and to shorten the digestion time. There is a color change from dark brown to black. After 5minutes of digestion, 5ml of Hydrogen peroxide is added in the micro kjeldahl digestion unit to destroy the organic material and to minimize foaming. Following the addition of H_2O_2 there is a color change from black to very light yellow. The digest obtained is diluted with distilled water and distilled after addition of sufficient quantity (10ml) of 40 percent NaOH to make the digest alkaline. The evolved ammonia was absorbed in 10 ml of 4 percent boric acid and titrated with 0.02 N sulphuricacid using mixed indicator. The Nitrogen content is calculated from the volume of acid consumed.

$$\text{Percent nitrogen} = \frac{T.V*0.00007*100*100}{0.5*5}$$

2.7.3 Determination of Lignin content

0.5g of dry sample was ground and added to crucibles, weights of these crucibles were noted down, 50ml of water was added and it was placed in hot refluxing apparatus. It was heated to boil for 3hrs.Crubilcles filled with hot water which was then filtered using vacuum. The crucibles were dried at 105°C for 8hrs and the next day weights of the crucibles were taken. The difference in weights gives the amount of water soluble fraction present in the sample. The same procedure was carried but instead of adding water, ammonium oxalate solution was added to find out the ammonium oxalate soluble fraction and 0.5M H_2S0_4 was added to find out the amount of cellulose and hemicellulose present. To the crucibles containing the residue 72% H_2S0_4 was added and stirred with glass rod to smooth paste. The paste was left to stand for 1hr.The rod was then rinsed with hot water and the contents was left to boil for 2hrs and then the crucibles were cooled and the contents were filtered using vacuum to obtain the lignin. The residue present in the crucible was scraped and were then placed in muffle furnace set at 550°C .the furnace was then switched of and the residue is left to cool down to room temperature, the difference in weights of the residue gives the amount of ash present in the sample.

2.8 Chemical Analysis of Mushrooms

The mushroom samples were analyzed for moisture and crude protein, as explained under.

2.8.1 Crude protein

The total nitrogen was estimated by micro Kjeldhal method using dried mushroom powder. Protein was obtained by multiplying the total Kjeldhal nitrogen by the factor 6.25.

2.8.2 Yield and Biological efficiency

Total weight of all the fruiting bodies harvested from all the three pickings were measured as total yield of mushroom. The biological efficiency (yield of mushroom per kg substrate on dry wt. basis) was calculated by the following formula Chang et al., (1981).

$$\text{B.E. (\%)} = \frac{\text{Fresh weight of mushroom}}{\text{Dry weight of substrate}} \times 100$$

3. RESULTS AND DISCUSSION

3.1 Nutrients Utilization from Substrates

The analysis of nutrients in the original as well as combination of various substrates and after the experimentation in spent substrates are investigated and presented in the following sections.

3.2 Effecton C: N Ratio

Sustainable oyster mushroom production can be achieved by employing cultural practices which optimize and integrate nutrient management. Agricultural residues used for oyster mushroom cultivation provide most of the nutrients and vitamins for growth. Carbon is readily available from cellulose, hemicellulose and lignin from agricultural wastes, but nitrogen occurs mainly in a bound form and is not available until it is enzymatically released. Various workers have also reported that Pleurotusspp have the capability to fix atmospheric nitrogen [10] but this has not been proved conclusively. In the cultivation of Pleurotus species the addition of protein rich supplements is a common practice, which indicates that either the compost is deficient in nitrogen or the bacterial proteins present in the compost are inadequate. Various workers have used supplements from animal and plant origins, including protein-rich, carbohydrate-rich or oil-rich substances, for oyster mushroom cultivation.

Mushrooms also have a great impact on nitrogen transformations, by enhancing nitrogen mineralization, so that mineral nitrogen was retained in the nitrate form, loss of carbon as carbon dioxide in the process of respiration and production of enzymes enhances the level of nitrogen, which lowers the C: N ratio in the feeds during mushroom harvesting and the observation are presented in Table1.

Table 1:Effect of P florida growth on C: N ratio of substrates

Agricultural waste	C:N ratio	
	Substrate	Spent Substrate
PS	114.69	60.98
PS+DW	76.16	37.01
CP	78.31	34.45
CP+DW	70.06	46.13

The changes in C/N ratio showed that the C/N ratios for all supplemented and unsupplemented substrates declined rapidly. One of the main problems with organic residues having high C: N ratio is that, higher C: N ratios will lead to immobilization of N and cause slow microbial decomposition of Organic Matter. When the agro wastes are directly discarded to soil without any treatment it would cause serious environmental concern, the slow decomposition of coir pith was expected to result in net N immobilization and accumulation of carbon in the soil. Therefore by reducing the C: N ratio of organic residues nitrogen immobilization can be reduced. It is evident from the above table that C/N ratio of substrate was decreased by fungal treatment. Biological treatment of agricultural wastes with fungi reduces the environmental pollution and these treated substrates can also be used as animals feed.

With an increase in nitrogen component of the substrate, a proportionate decrease in carbon was observed. These results are in accordance with those reported by Shetty and Moorthy (1981) [11], Thilagavathy et al. (1991)[14] and Theradimani and Marimuthu (1991)[13]. They reported that the C: N ratio of coir pith could be narrowed down due to cultivation of mushroom. Jadhav et al., (1998) [7] also reported that the concentration of nitrogen in spent substrates increased with reduction in C: N ratio and thus showed possibility of using spent mushroom as a quality cattle feed.

3.3 Degradation Pattern of Lignin

A variety of agricultural crops are grown in India. There are enormous potential of agro wastes in India like crop residues, tree wastes, aquatic weeds etc. They form the potential renewable resources. Several methods have been adopted for the better exploitation of agro wastes. One such method issolid state fermentation of the agro wastes through mushroom cultivation. Mushroom cultivation is an eco-friendly method of solid waste management. It is obvious that mushroom cultivation opens the dead lock in the biological degradation of natural resources. Recognizing the potential of edible mushroom, the Govt. of India also included “Edible Mushroom Cultivation” as one of the trades under the TRYSEM (Training for rural youth and self-employment) project during the eighth five year plan. The main function of agricultural residue is to provide a reservoir of cellulose, hemicellulose and lignin, which is utilized during the growth of spawn and during fructification.

Materials with higher lignin concentrations (> 15%) lead to slow decomposition and N immobilization. Because lignin is an aromatic, branched and complex compound, it will require a longer time before being broken down by soil microorganisms. Lignin contributes to the recalcitrance of agro wastes to decomposition by occluding more easily decomposable polysaccharides. Hence the higher the lignin concentration, the slower the decomposition and N mineralization rate of the residue. All the residues

had very high concentrations of carbon. The bulk of litter or residue comprises structural components of plant cell walls (cellulose, lignin and hemicelluloses) and hence carbon is always in much larger concentration than other nutrients.

Pleurotus species are known to produce a wide range of hydrolytic and oxidative enzymes that enable them to successfully colonize, degrade and bioconvert many lignocellulosic substrates [3]. Such degradation of lignocellulosic materials results from the concerted and synergistic action of many enzymes: endoglucanases, exoglucanases, laminarinases, b-glucosidases, xylanases, laccases and polyphenol oxidases [4].

The decomposition pattern of lignin by P. florida on different supplemented and unsupplemented substrates is presented in Figure 1 and 2. The present study points out a decline lignin content of the spent substrates this may be because of laccase activity, indicating the lignocellulolytic nature of the pleurotus species. The efficient degrading capacity of the fungi demonstrated their potential use in the conversion of agricultural wastes into commercially more valuable biomass like mushrooms. One of the goals of biological delignification of agricultural wastes using white-rot fungi is to make as much possible of the digestible substrate carbohydrate and reduce environmental hazard [1]

Degradation of macromolecules composition was noticed from an early stage. Data, in general, revealed that the cellulose content decreased as the days of spawning increased, for P. florida in all the three supplemented and unsupplemented substrates. The extent of cellulose degradation being 50-70% of the dry weight in both supplemented and unsupplemented substrates of coir pith, paddy straw and sugarcane trash. The decrease in hemicellulose was relatively greater than cellulose in substrates such as coir pith and sugarcane trash supplemented with spent biomass implying that the fungi utilized a greater percentage of hemicellulose than cellulose.

The lignin content was more (40-50%) in coir pith supplemented with spent leafy biomass and coir pith alone than in other substrates. The percentage of lignin degradation varies from 10–20% on the tenth day and around 50–60% on the 40th day when compared to the initial lignin content for all the substrates. The production of lignin degrading enzymes by the inoculated microbes during mushroom cultivation might have accelerated the degradation process. This indicates that pleurotusflorida was more effective in degrading lignocellulosic content of agricultural residue.

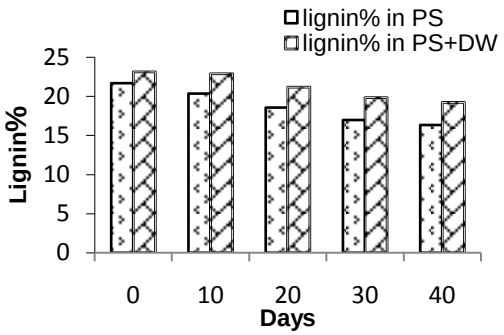

Figure 1. Effect of P.florida growth on lignin content of PS and PS+DW

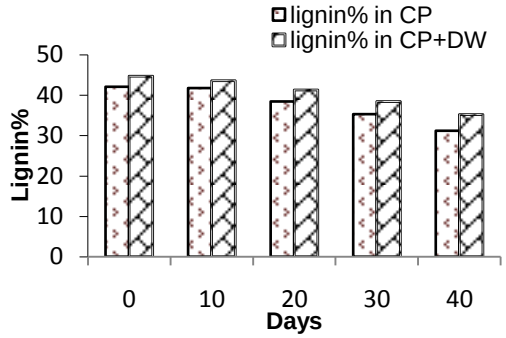

Figure 2. Effect of P.florida growth on lignin content of CP and CP+DW

3.4 Growth Characteristics and Biological Efficiency

The mushrooms are cultivated on different agricultural waste amended with digested leafy biomass. The fruiting bodies of mushrooms are produced in all the substrates were harvested and weighed, to know the yield performance of the substrates. Also the other yield parameters like days for pin head formation, days for fruiting body formation of pleurotusflorida are presented in Table 2. Analysis of the substrate showed that all substrates supplemented with spent biomass had higher moisture than the agricultural wastes when used as substrate. This could be due to fast mycelia growth in supplemented substrates was due to the high moisture content which may have been conductive for the mycelia. Spent biomass provide mushroom with a sufficient amount of easily metabolizable organic matter and non-assimilated carbohydrates favor the growth and yield of the mushroom.

Table2. Effect of different substrates on number of days taken for pin head formation

Substrates	Pleurotusflorida
Paddy straw	18
Paddy straw + Digested waste	15
Coir pith	19
Coir pith+ Digested waste	16
Digested waste	-

Results presented in Table 3 clearly indicate that spent biomass was very effective in increasing yield of mushroom irrespective of the substrate used for mushroom cultivation. Productivity of Pleurotusflorida was found maximum in this experiment when agricultural residues was supplemented with spent biomass from biogas plant. Maximum biological efficiency of 231.93% for P. florida were observed with paddy straw(Figure 4) supplemented with spent biomass whereas the substrate without this supplementation produced less yield. Fruiting initiation did occur in spent biomass after 20 to 25 days of spawning but the pinheads withered and could not develop in to mature fruiting bodies, thus rendering them unsuitable for growing P. florida(Figure3).Figure 5 shows the Pinhead formation of P.florida on coir pith amended with spent biomass.

The varied production potential of different substrates might have been due to the variations in their physical properties and nutritional composition. The poor growth and low yield of mushroom in these substrates might have been related to their bulk density, aeration, water holding capacity etc. Proper gaseous exchange in the basal substrate is essential for the mycelium to acquire more oxygen and remove respiratory gases and harmful volatiles. Such conditions would be favoured by moist and solid substrate with a high degree of pore space. The unsuitability of digested waste or spent biomass might have been due to its high nitrogen and lignin content.

Table3. Effect of different substrates on yield and biological efficiency (B.E) of P florida

Substrates	Yield(g/kg of substrates) P. florida	B.E% P. florida
PS	1453.39	145.34
PS + DW	2319.27	231.93
CP	1209.02	120.90
CP + DW	1483.09	148.31

This suggests that the amendment of substrate with spent biomass from biogas plant could be beneficial as a nutrient supplement as well as providing moisture to the growing system. Thus, mushroom cultivation proves to be a highly economical method for disposing of the agricultural residues, such as paddy straw and coir pith, along with providing by-product in the form of manure for field application. In this regard, Kakkar&Dhanda (1998)[8] compared wheat and paddy straws for the growth of Pleurotus and obtained a much higher yield of fruiting bodies with wheat straw than with paddy straw.

The results of nutritional analyses of mushroom viz., protein and moisture contents are presented in Table 4. From the cited data, it is clearly evident that supplementation of spent biomass improved the protein content in mushroom in comparison to agricultural residue when used as substrate. Therefore, the results indicated that there was a favourable change in the nutritional quality of mushroom [9]. So, from overall evaluation, it is evident that supplementation of spent biomass were effective in improving nutritional quality of oyster mushroom in terms of protein content.

Table4. Nutrient composition of Pleurotusflorida on different substrates (dry weight basis)

Substrates	Moisture (%)	Crude protein (%)
Paddy straw	89.64	22.5
Paddy straw + Digested waste	90.4	23.67

Coir pith	88.42	20.25
Coir pith+ Digested waste	88.97	21.33

Figure3. Mycelia formation on spent biomass

Figure 4. Extensive production of P. florida on paddy straw amended with spent biomass.

Figure 5.Pinhead formation of P.florida on coir pith amended with spent biomass.

4. CONCLUSIONS

The present investigation is carried out to recover the nutrients from spent biomass and different agricultural waste for growing mushroom. From the study carried out, the following conclusions can be drawn. Chemical composition of substrates indicated the significant variation in all chemical constituents of the substrates used. The lignin, C: N showed a significant reduction during growth of fungus. Among the two substrates used for mushroom cultivation, maximum amount of lignin reduction of 64%was observed in coir pith. Reduction in C: N ratio was observed in all the combination of substrates and among that maximum reduction of C: N ratio from initial value of 115 to 60 was observed in paddy straw. Maximum biological efficiency of mushroom was obtained for paddy straw supplemented with spent biomass from biogas plants for both Pleurorusflorida (231.93%).When spent biomass alone is used as substrates mushroom yield was less because of its high lignin and nitrogen content. During mushroom cultivation increase in protein content of mushroom was observed in substrates supplemented with spent biomass. Spent mushroom compost obtained from substrates paddy straw and coir pith and its combination during mushroom cultivation can be used as a bio fertilizer or soil conditioner because of its optimum C: N ratio. Utilization of agro wastes helps in reducing the wastes, converting them into mushroom protein and vitamins.Thus the use of wastes can provide more food, more jobs, better family income, and improved living standard, curb global warming and clear up the crop residues on road sides and forest margins.

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