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Solid state fermentation of *Lathyrus sativus* and sugarcane bagasse by *Pleurotus sajor-caju*

Md. Rezaul Karim[1], Zinat Mahal[2], Safia Iqbal[1], Harun or Rashid[2], M. Abu Hena Mostofa Jamal[1], Md. Azizul Islam[1], Md. Mafizur Rahman[1*]

[1]*Department of Biotechnology and Genetic Engineering, Islamic University, Kushtia, Bangladesh*

[2]*Institute of Food and Radiation Biology, Atomic Energy Commission, Bangladesh*

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Abstract

Lathyrus sativus (Khesari plant) and sugarcane bagasse are considered as agro wastes. Khesari plants are generally grown in fields as weeds and they have to be removed prior to cultivation. Taking this view in mind, we have investigated the conversion of these lignocellulosic agro-waste as an enriched feed stock for cattle via by solid state fermentation using a cellulolytic fungus, *Pleurotus sajor-caju*. The strain required 8 weeks to complete the fermentation on both the untreated and treated (with alkali, lime and presoaked) substrates at 30°C. Higher amounts of reducing sugar and soluble protein were found in each of the lime treated substrates than untreated substrates. Results also indicated that presoaked substrate contain higher amounts of reducing sugar and soluble protein than unsoaked substrate. Among the substrates, mixed substrate (khesari plant + sugarcane bagasse) was found to accumulate higher amount of sugar, 22.15 mg/g and protein, 22.80 mg/g than those of khesari plant in the 5th week of fermentation. The treatments that augmented the level of sugar and protein were also found to enhance the cellobiase, carboxy methyl cellulase and avicelase activity of crude culture extracts. These results suggest that lime treatment and presoaking seem to increase the digestibility of the substrates by the fungal cellulolytic enzymes. During eight weeks of fermentation, relatively higher cellobiase activity was found as compared to that of carboxymethylcellulase and avicelase at 30^{o}C for the fungul strain. The results of the present study clearly indicate that fungal conversion with pretreatment transform these lignocellulosic agro-wastes to a nutritionally enriched animal feed.

* **Corresponding Author:** Md. Mafizur Rahman ✉ shilon83@gmail.com

Introduction

In developing agro-based countries like Bangladesh, farmers use cattle for farming. Due to financial constraints, farmers are not always able to provide their cattle with high quality feed. Consequently, most of them suffer from nutritional deficiency that give rise to different diseases. To tackle this issue it is imperative to transform a cattle feed into rich nutrients source (Hossain *et al*., 2009). Beside this, globally, an estimated 15% of total waste consists of agro-waste which is 998 millions tones in a year (Hsing *et al,* 2004). It was projected that waste generation will be simply doubled by 2025. Therefore, worldwide, this receives an important consideration in the waste management strategy. The alternate approaches of using this waste are a valued and appreciated practice. Currently these are uses in paper making pulp, energy and fuel, heavy metal removal and as fertilizer and animal feed.

Agricultural crop residues and/or byproducts are staple energy substrate of ruminant livestock in developing countries (Tripathi *et al*., 2008). In Bangladesh, *Lathyrus sativus* (Khesari plant) and sugarcane bagasse are two agricultural wastes mainly consisting of lignin, cellulose and hemicellulose. Even though these two crop wastes contain enough cellulose to make them excellent sources of energy for ruminants, they are poor quality feeds due to low digestibility, poor palatability, low protein content and bulkiness (Umasaravanan *et al*., 2011). Sugarcane bagasse and Khesari plant are mainly utilized as fuel. Conversion of these wastes by biotechnological approaches seems to be practical and promising alternative for transforming them into animal feed, increasing their nutritional value and thus producing a value-added product.

For this, we could exploit some bacteria (such as *Clostridium thermocellum*) and fungi (like *Trichoderma*) which are among the mostly utilized sources of cellulase enzyme being convert cellulose to glucose. Among these, fungi have been studied extensively. *Pleurotus sajor-caju* (edible fungi) obtain their energy by decomposing wood especially sapwood and heartwood of broad-leaved or rarely coniferous trees (Hossain *et al*., 2009). However, making cellulose accessible to the enzymes is an essential factor to increase the rate of hydrolysis. Chemical pretreatment like alkaline pretreatment can increase the enzymatic effect on cellulose. This pretreatment not only removes lignin but also acts as a swelling agent, which enhances surface area of the substrate accessible for enzymatic activity (Rodhe *et al*., 2011). Different pretreatment like physical, chemical, and biological types have been employed to enhance the rate of hydrolytic degradation but among these lime treatment of cellulose is the best known chemical method to increase digestibility of cellulosic materials (Fan *et al*., 1981; Gharpuray *et al*., 1983). This treatment loosens the hemicelluloses seals and removes lignin from the substrates, so as to make substrate more susceptible to enzymatic attack (Choudhury *et al.,* 1980 and Gray, 1978). This treatment is so effective that the percentage of lignin in sugarcane bagasse comes to a value of 10.10% (Banarjee and Mukhopadhayay, 1990).

Solid state fermentation of lignocellulose materials by fungi has received attention, primarily because of low energy consumption, process simplicity, superior enzyme productivity, low capital investment, negligible liquid waste product and ease in product recovery (Reid and Seifert, 1982; Mukhopadhyey and Nandi, 1999).In addition, there is little literature on the use of fermented compound feed manufactured according to solid-state fermentation. This type of feed may have the potential to be efficient in promoting animal health and growth (Hu *et al*., 2008). This process can greatly improve the nutritional value of cellulosic substrates like straw, bagasse etc (Hossain *et al*., 2009).Inoculating agro-wastes with useful microorganisms, subsequently *Pleurotus sajor-caju* synthesized cellulases were used for saccharification of agro wastes. Moreover, maximum saccharification was achieved within the range of 30-45 °C coinciding with the characteristic of mesophiles (Baig *et al*, 2004), increasing their nutritional values by fermentation further improve their quality as animal feed processing and to rupture

the cell wall can greatly improve the feeding value of Khesari plant and bagasse by *Pleorotus sajor-caju*. Thus it may help in both establishing animal feed industry and commercial livestock firm and may save a huge amount of foreign currency, which is now being used for importing expensive animal feeds.

In case of liquid state fermentation, the protein content can be increased in a short time but it is difficult to increase the substrate concentration because of the occurrence of substrate precipitation. To overcome this problem, solid state fermentation is a better option for the production of animal feed, where it is expected to produce the good feeds with homogenous and high protein content. Further research, however, is necessary to develop strategies for industrial scale production of enriched animal feed from so called agro-wastes. Therefore, the aim of this study was to evaluate the nutritional quality of the upgraded feed through solid state fermentation via a white rot fungi *Pleurotus sajor-caju*.

Materials and methods

This study was done in the research laboratories of the Department of Biotechnology and Genetic Engineering, Islamic University and Institute of Food and Radiation biology, Atomic Energy Commission; Bangladesh from January 2011 to December 2011.

Biological Samples and Treatment

Two natural cellulosic agro-wastes were selected for the present study. Khesari plant alone or in combination with sugarcane bagasse were used as a substrate. Khesari plant and sugarcane bagasse were collected from local area of Navaron near Jessore district of Bangladesh. Collected cellulosic materials were first cleaned off from dirt and unwanted materials. Then, these were cut manually into tiny pieces (1.5cm), washed manually with water to clean dirt and unwanted materials, sun dried and then grounded by passing through a pulverizer (BICO Inc., USA).

The pure *P. sajor-caju* culture was used for fermentation. *P. sajor-caju* was routinely sub-cultured and maintained on potato dextrose agar (PDA) slants and was stored at 4°C.

Experimental design

In this study mixed substrate (mixture of sugarcane bagasse and khesari plant) are compared with khesari plant. Initially the grounded khesari plant and the mixture of sugarcane bagasse and khesari plant (50:50) were divided into three groups. One of them was lime treated, another group was alkali treated and the remained one was kept untreated. Now each of the three groups was further divided into two subgroups. One of the each subgroup was soaked overnight in distilled water (100ml/25gm) at room temperature and the other one kept unsoaked. Soaking was done to find out how this water treatment affects the final result. All the six subgroups were subjected to fermentation by *P. sajor-caju* followed by determination of reducing sugars, soluble proteins and enzyme activity.

Lime pretreatment

500g of untreated substrate were taken and then soaked with a lime solution (10g $CaCO_3$ in 3.75 liter distilled water). The substrates were left in soaking condition overnight. Then the lime solution was drained out by tap water (Awang *et al.*, 1994). This process was repeated until the pH of the final wash water reached 7.2). Treated substrate was then spread over aluminum foil and allowed to dry overnight at 60°C.

Alkali pretreatment

Untreated substrates were treated with sodium hydroxide (0.1 g/g of substrate) (Varga *et al.*, 2002). Substrate (50 g) was taken into a two liter conical flask containing 5 g sodium hydroxide in one liter distilled water. The flask containing substrate and sodium hydroxide solution was heated in boiling water (100°C) bath for 1hour. The flask was cooled under running tap water. The alkali treated substrate was separated from the solution by filtering with nylon cloth. Excess alkali was removed by repeated washing with tap water until the pH was neutral. Complete removal of alkali was checked by pH paper.

The nylon cloth containing substrate was squeezed to remove excess water and washed substrate spread over aluminum foil and allowed to dry overnight at 60°C.

Fermentation of substrates

P. sajor-caju was subcultured from stock PDA slant to PDA plate. After two weeks of incubation at 30°C, three pieces of mycelial growth (about 1 cm in diameter) were taken with two loops full of a hollow borer for inoculation in the fermentation medium in 100 ml conical flask in each containing 50 ml PDA broth. Then each flask was shaked in shaker for one week. Each 50 ml inoculums was then transferred to presoaked substrates (i.e. 20g substrate soaked in 110 ml H_2O for overnight) and unsoaked substrates (i.e. 20g substrate) and incubated in thermostat incubator at 30°C for 8 weeks. Controls for both presoaked and unsoaked substrate groups were maintained at same incubation conditions without *P. sajor-caju* inoculums. Both treated and untreated substrates were inoculated by fungi *P. sajor-caju* and incubated at temperature 30°C for 8 weeks as stated above.

Estimation of reducing sugar

Preparation of Standard Curve for reducing sugar estimation:

At first, 0.5 g glucose was dissolved in 1L distilled water and made the stock glucose solution. Then various concentration of glucose (0.4 g/L, 0.3 g/L, 0.2 g/L, 0.1 g/L) were made from the stock glucose solution. After that 1 ml sample were taken from the various concentration of glucose in a test tube. Next 3 ml DNS reagent was poured in each test tube and mixture was heated in boiling water bath for 15 minutes and cool down. Finally Reagent blank were made for O.D comparison. Optical density was taken for different concentration of glucose at 540 nm by using spectrophotometer.

Determination of Reducing Sugar

Reducing sugar in fresh fermented and non-fermented (just before fermentation taking all preparation for fermentation and supplemented) substrates and in substrates at their various stages of fermentation were determined by the dinitrosalicylic acid (DNS) method (Miller, 1959). 1ml of appropriately centrifuged and diluted samples was mixed with 3.0ml DNS reagent. The mixtures were heated in a boiling water bath for 15 minutes and then allowed to cool down. The absorbance of the colored samples was taken against a reagent blank at 540 nm. A standard curve using 0.0-0.05 g/100 ml glucose was prepared with each experiment.

Estimation of soluble protein

Preparation of Standard Curve for Soluble Protein estimation:

At first, 0.04 g Bovine Serum Albumin (BSA) was dissolved in 100 ml distilled water and made the stock protein solution. Then various concentration of protein (400 µg/ml, 300 µg/ml, 200 µg/ml, and 100 µg/ml) was made from the stock protein solution. After that 1ml sample were taken from the various concentration of protein in a test tube. Then 5 ml reagent C was poured in each test tube, mixed and allowed to stand for 10 minutes at room temperature. Next 0.5 ml reagent F were added, mixed and allowed to stand for 30 minutes for full color development. Finally Reagent blank were made for O.D comparison. Optical density was taken for different concentration of glucose at 550nm by using spectrophotometer. Standard curve was made by computer.

Determination of Soluble Protein

The concentrations of extra cellular protein in the fresh, non-fermented and fermented substrates in substrates at their various stages were determined by the Lowry *et al method* (1951), using FCR reagent. Appropriately centrifuged and diluted 1.0 ml sample was mixed with 5 ml of freshly prepared reagent C and allowed to stand at room temperature for 10 minutes. 0.5 ml Reagent F was then added with immediate mixing and the tubes were allowed to stand for 30 minutes for full color development. The absorbance readings of colored samples were determined against a reagent blank at 550 nm. Bovine Serum Albumin (BSA) was used as a standard calibration protein for the Lowry assay. The standard

curve was linear in the concentration range 100-400 µg/ml.

Preparation of Standard Curve for Estimation of Enzyme Activity

At first, 0.5 g glucose was dissolved in 1L distilled water and made the stock glucose solution. Various concentration of glucose (100-500 µg/ml) was made from the stock glucose solution. 1 ml sample were taken from the various concentration of glucose in a test tubes. Then 3 ml DNS reagent was poured in each test tube and allows to mixture was heated in boiling water bath for 15 minutes and cool down. Finally Reagent blank were made for O.D comparison. Optical density was taken for different concentration of glucose at 540nm by using spectrophotometer.

Determination of enzyme activity

The fermented substrates were pressed to get extra cellular crude enzymes for enzyme assay. The activities of different cellulose components were measured using the method of Mandels and Sternberg (1976). 0.05 M citrate buffer at pH 4.8 was used throughout the enzyme assay. Three types of enzymes activity were measured such as Cellobiase, Carboxymethylcellulase (CMCase) Activity & Avicelase Activity.

Results

Effect of lime, alkali and presoaking treatment in accumulation of reducing sugar and protein

Before pretreatment, sugar (reducing sugars constituted of both cellulose and hemicellulose) and protein content were 5.12 mg/g and 0.9 mg/g respectively in mixed substrate. After alkali treatment, sugar and protein content increased slightly (5.50 mg/g and 0.93 mg/g respectively). After lime treatment, sugar and protein content increased up to 5.89 mg/g and 1.06 mg/g respectively.

When, khesari plant was fermented, the highest amount of reducing sugar was found at 6th week of fermentation in presoaked lime treated substrate (17.05 mg/g) and in the following weeks, it was decreased. In lime treated unsoaked khesari plant, the highest amount of sugar was found at 6th week of fermentation (13.01 mg/g). In the case of lime treated presoaked alkali, the amount of the reducing sugar was found at 5th week of fermentation 12.26 mg/g while in case of unsoaked alkali treated substrate the amount of reducing sugar was found 12.68 mg/g at 6th week of fermentation, and for presoaked untreated it was 11.74 mg/g. In addition, unsoaked untreated substrate had 10.87 mg/g of reducing sugar at 6th week of fermentation (Figure 1A). Similar pattern can be observed when the substrate was mixed (khesari plant + sugarcane bagasse), the highest amount of reducing sugar was found at 6th week of fermentation in presoaked lime treated substrate (22.15 mg/g) and gradually decreased in unsoaked lime treated (17.84 mg/g), presoaked alkali treated (15.91 mg/g), unsoaked alkali treated (15.16 mg/g), presoaked untreated (raw) (14.83 mg/g) and unsoaked untreated (13.06 mg/g) substrates respectively (Figure 1B).

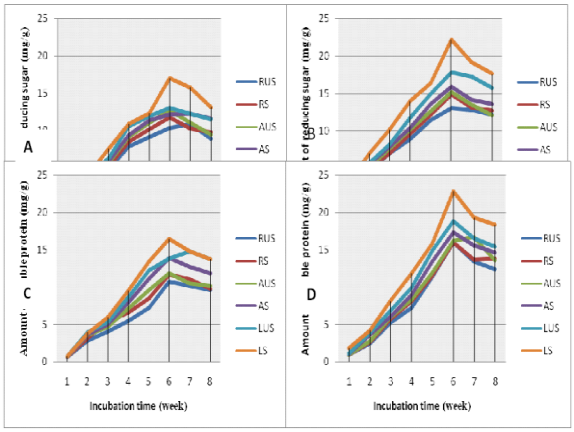

Fig. 1. A. Reducing sugar accumulation pattern in differently treated khesari plant during 8 weeks of fermentation. B. Reducing sugar accumulation pattern in differently treated mixed substrate (khesari plant + sugarcane bagasse) during 8 weeks of fermentation. C. Soluble protein accumulation pattern in differently treated khesari plant during 8 weeks of fermentation. D. Soluble protein accumulation pattern in differently treated mixed substrate (khesari plant+ sugarcane bagasse) during 8 weeks of fermentation (RUS=Raw unsoaked, RS=Raw soaked, AUS=Alkali treated unsoaked, AS=Alkali treated soaked, LUS=Lime treated Unsoaked and LS=Lime treated soaked mixed substrate).

In case of khesari plant, the highest amount of soluble protein was found 16.42 mg/g at 6th week of fermentation in presoaked lime treated substrate and gradually it was decreased in presoaked alkali treated (13.92 mg/g), unsoaked alkali treated (11.81 mg/g), presoaked untreated (raw) (11.68 mg/g), unsoaked untreated (10.73 mg/g) substrates respectively and the highest amount of soluble protein was reported in unsoaked lime treated at 6th week (13.83 mg/g) (Figure 1C).

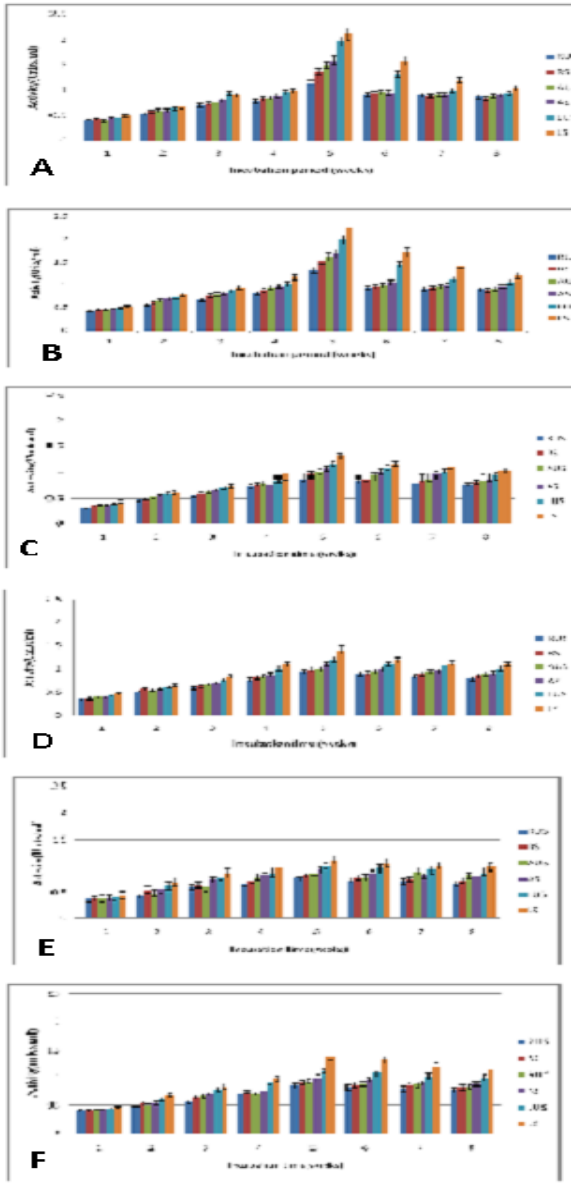

Fig. 2. A. Activity of cellobiase of differently treated khesari plant during 8 weeks of fermentation. **B.** Activity of cellobiase of differently treated mixed substrate (khesari plant+ sugarcane bagasse) during 8 weeks of fermentation. **C.** Activity of CMCase of differently treated khesari plant during 8 weeks of fermentation. **D.** Activity of CMCase of differently treated mixed substrate (khesari plant+ sugarcane bagasse) during 8 weeks of fermentation. **E.** Activity of avicelase of differently treated khesari plant during 8 weeks of fermentation. **F.** Activity of avicelase of differently treated mixed substrate (khesari plant+ sugarcane bagasse) during 8 weeks of fermentation (RUS=Raw unsoaked, RS=Raw soaked, AUS=Alkali treated unsoaked, AS=Alkali treated soaked, LUS=Lime treated unsoaked and LS=Lime treated soaked mixed substrate).

Therefore, the same pattern has been observed when the substrate was mixed (khesari plant + sugarcane bagasse), the highest amount of soluble protein was found at 6th week of fermentation in presoaked lime treated substrate (22.80 mg/g) and gradually decreased in unsoaked lime treated (18.87 mg/g), presoaked alkali treated (17.35 mg/g), unsoaked alkali treated (16.23 mg/g), presoaked untreated (raw) (15.99 mg/g) and unsoaked untreated (15.96 mg/g) substrates respectively (Figure 1D).

Activity of crude cellulolytic enzymes in the fermented substrates

Activity of cellobiase

Cellobiase activity was found to increase swiftly from 1st to 5th week of incubation and then tend to decrease. Cellobiase activity in presoaked lime treated khesari plant was found highest (2.10 units/ml), and a gradual decrease was observed in other substrates. The activity of unsoaked lime treated, soaked alkali treated, unsoaked alkali treated, soaked untreated, and raw unsoaked khesari plant were 1.96 units/ml, 1.58 units/ml, 1.49 units/ml, 1.36 units/ml and 1.12 units/ml respectively.(Figure 2A).

In case of mixed substrate (khesari plant + sugarcane bagasse), the activity was increased in a very similar pattern. Highest cellobiase activity was observed in 5th week, was found in presoaked lime treated mixed substrate (2.22 units/ml) and gradually decreased. The enzymatic activity for unsoaked lime treated on mixed substrate was found to be 1.98 units/ml, while soaked alkali treated, unsoaked alkali treated, soaked

untreated substrate activity was found to be 1.68 units/ml, 1.61 units/ml, 1.54 units/ml respectively and for untreated unsoaked the activity was 1.29 units/ml.(Figure 2B).

Activity of Carboxymethylcellulase (CMCase)

Activity of CMCase also increased gradually from 1st to 5th week and then decreased slightly. In case of khesari plant substrate, CMCase activity in presoaked lime treated was found highest, 1.30 units/ml and a gradual decrease was recorded in other substrates. In case of unsoaked lime treated, soaked alkali treated, unsoaked alkali treated, soaked untreated khesari plant the values were 1.14 units/ml, 1.04 units/ml, 0.97 units/ml, 0.94 units/ml and the activity of raw unsoaked was 0.89 units/ml (Figure 2C). In addition, the activity of mixed substrate (khesari plant + sugarcane bagasse) was increased in a very similar pattern. Highest CMCase activity was at 5th week mostly. Best CMCase activity was found in presoaked lime treated mixed substrate, 1.41 units/ml and a gradual decline was found in other substrates. Unsoaked lime treated, soaked alkali treated, unsoaked alkali treated, soaked untreated mixed substrate presented 1.21 units/ml, 1.10 units/ml, 0.99 units/ml, 0.98 units/ml CMCase activity for untreated unsoaked mixed substrate was 0.94 units/ml (Figure 2D).

Activity of Avicelase

Activity of avicelase also rose slowly from 1st week to the 5th week of solid-state fermentation of differently pretreated cellulosic agro-wastes and then decreased slightly. The avicelase activity was found to shoot up drastically up to 5th week of incubation then decreased slightly by 8th week when khesari plant was used as substrate for solid-state fermentation. All the unsoaked and presoaked differently treated substrates followed similar pattern but a higher activity was observed in case of soaked lime treated khesari plant. Highest avicelase activity was 1.10 units/ml, in presoaked lime treated khesari plant which was greater than others. There were some results for unsoaked lime treated khesari plant, 0.99 units/ml, soaked alkali treated, 0.91 units/ml, unsoaked alkali treated, 0.87units/ml, soaked untreated, 0.82 units/ml and for raw unsoaked substrate, the activity was 0.75 units/ml (Figure 2E).

In case of mixed substrate (khesari plant+ sugarcane bagasse), the activity was found in a very similar pattern. Highest avicelase activity was at 5th week mostly during the 8 weeks of fermentation. The best avicelase activity was found in presoaked lime treated mixed substrate, 1.37 units/ml and there was a gradual decrease in other substrates. For unsoaked lime treated, soaked alkali treated, unsoaked alkali treated, soaked untreated mixed substrate those were 1.11 units/ml, 0.98 units/ml, 0.92 units/ml, 0.90 units/ml respectively. Finally, the activity for untreated unsoaked was 0.84 units/ml (Figure 2F).

Discussion

The increase in reducing sugar and soluble protein content and the activities of the cellulolytic enzymes namely cellobiase, CMCase and avicelase were determined for a period of eight weeks. Initially, the raw substrates contained small amount of reducing sugars and soluble proteins. At different stages of solid state fermentation, reducing sugar and protein content of each of the treated substrate gradually increased with the duration of fermentation by *P. sajor-caju*. The solid state fermentation was carried out for 8 weeks. It was observed that the reducing sugar content increase rapidly up to 5th week in all substrates and after that tend to slow down. It could be due to the fact that the rate and extent of biomass hydrolysis are inextricably linked to biomass structural characteristics (Zhu, 2005).

In the present study, reducing sugar level concentration has decreased at the end of the fermentation process. It was found that the excess fungal growth lead to the consumption of some of these sugars as their carbon source for metabolic activity (Qiu *et al.*, 2003).

The lime treated khesari plant and mixed substrate (khesari plant + sugarcane bagasse) contained higher amount of reducing sugar than untreated and alkali treated substrates (Figure 1A and 1B). Among them

the highest amount of reducing sugar was found in lime treated presoaked mixed substrate. Thus, it can be said that lime treatment is responsible for structural modification of the substrates leading to their increased digestibility which in turn increases the reducing sugar content. Pretreatment may reduce the indigenous microflora which could positively influence solid state fermentation and fungal growth (Tengerdy and Szakacs, 2003). Rani *et al.* (2006) reported that pretreatment of substrate increased the yields of cellulase upto 33%. The presence of lignin in cellulosic substrates hinders the saccharification into monomeric sugars. Alkali treatment dissolves the lignin by breaking the linkages (Lee, 1997). Sodium hydroxide is a typical alkali used in alkaline pretreatment as used in this study (Varga *et al.*, 2002). It has been established by Valchev *et al.*, (2009) that the enzyme treatment on raw material as first stage treatment can be inefficient, probably the impeded access of the enzyme molecules to the amorphous sections of the carbohydrate chains.

The pattern for soluble proteins in all the treated substrates were found very similar as the reducing sugars (Figure 1C and 1D). Both presoaked and lime treated khesari plant and mixed substrate (khesari plant + sugarcane bagasse) contained higher amounts of soluble proteins compared to alkali treated, untreated and unsoaked substrates. Similar results were also obtained in case of enzyme activity. da Silva *et al.*, (2005) showed a very low CMCase activities when substrate grown on liquid medium. However, when fungus was grown by solid state fermentation this enzyme production increased. The findings in this paper justify the increase of CMCase activity at 5^{th} week of fermentation. This may be due solid state fermentation provides the fungus with an environment closer to its natural habitat (wood and decayed organic matter), which stimulates this strain to produce more cellulolytic enzymes. In this study best CMCase activity was found in presoaked lime treated mixed substrate, 1.41units/ml and a gradual decrease in other substrates after 5^{th} week. Similar pattern were obtained for the maximum cellulases productivity by *P. ostreatus* and *P. sajor-caju* cultivated on leaf biomass and pseudo-stem biomass of banana (Reddy *et al.*, 2003). Alkali treatment might be another cause of higher enzymatic activity because alkaline condition might increase accessibility of the enzyme to the substrate for enzymatic attack by removal of lignin seal (Ahmed *et al.*, 2012). Boonmee (2009) showed some fungal isolates produced either CMCase or cellobiase, when other produces both enzymes at the same level. Our study showed different level of CMCase or cellobiase activity (1.41 units/ml and 2.22 units/ml respectively). These extracellular enzymes produced by the *P. sajor-caju* could degrade the cell wall components of the substrates (Naraian *et al.*, 2010).

Conclusion

Finally, it can be said that, khesari plant and mixed substrate (khesari plant + sugarcane bagasse) can be upgraded to enriched animal feed by chemical pretreatments especially lime treatment and solid-state fermentation using the edible fungus, *P. sajor-caju,* as the fermented products contained high amount of reducing sugar and soluble protein.

Conflict of interest

The authors declare that they have no conflict of interest.

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