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Use of *Pleurotus sajor-caju* in upgrading green jute plants and jute sticks as ruminant feed

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ABSTRACT

In this study, superfluous jute plants and jute stick were converted into upgraded animal feed by solid state fermentation (SSF) using a cellulolytic fungus, *Pleurotus sajor-caju*. Prior to fermentation, substrates were subjected to several pretreatments such as soaking with water overnight and alkali or lime pretreatment. SSF was carried out with 20 g of substrate in 100 ml conical flask and was incubated at 30°C for 8 weeks. In all treatments, the highest amount of reducing sugar, soluble protein as well as the cellulolytic activities of three enzymes viz. cellobiase, carboxymethyl cellulase and avicelase were obtained at 6th week of fermentation. Compared to raw, unsoaked substrates, soaking treatment alone could produce 10% more soluble protein in both substrates whereas reducing sugar increment was 5% and 6% in jute sticks and jute plants, respectively. From all treatments, combination of soaking and lime treatment in green jute plants yielded higher value than jute sticks in terms of reducing sugar, soluble protein and enzymatic activity. The radiation doses at 20, 30 and 40 kGy appeared to have no effect on sugar and protein accretion. During eight weeks of fermentation, relatively higher cellobiase activity was found compared to that of carboxymethyl cellulase and avicelase. The present investigation indicates that fungal conversion with pretreatment can turn these lignocellulosic agro-wastes to a nutritionally enriched animal feed by increasing the crude protein and reducing sugar content. However, further research is necessary to develop strategies for industrial scale production to overcome the crisis of nutritionally improved animal feed.

Key words: agro-wastes, solid state fermentation, animal feed, *Pleurotus sajor-caju*, soluble protein, reducing sugar

Introduction

Globally, an estimated 15% of total waste consists of agro-waste, which is 998 millions tones in a year (Hsing et. al., 2004). Agro-waste consists of animal waste, food processing material, residual chemicals used in cultivation and crop waste. Among these, crop wastes are used as animal feed all over the world. Crop waste mainly composed of lignocellulosic residues. Lignocellulose is the three-dimensional polymeric composites formed by plants as structural material, an excellent source of fuel for energy (Ingram et al., 1999). The chemical components of lignocellulose can be divided into four major components e.g. cellulose, hemicelluloses, lignin and extractives (Brown, 2003). The inadequate utilization of lignocelluloses is due to lignin which surrounds and protects the cellulose from enzyme hydrolysis (Teferedegne, 2000).

Jute is a lignocellulosic bast fibre consisting up to 69% of cellulose cemented by non-cellulosic materials i.e., pectin, lignin, hemicelluloses etc. (Begum et al., 2007). Jute sticks are produced in the large quantities throughout the world, including Bangladesh and are normally considered as agro-

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wastes. During jute cultivation, some green jute plants are also weeded out and removed from field for better growth (Leng, 2001).

The weeded jute plants have been used as animal feed for a long time in Bangladesh, but this confers less nutrition to the animals because of the lack of proper utilization of the nutrients contained in them. On the other hand, jute sticks are mostly used for household cooking and are not currently being used as animal feed because of its relatively low nutritive value with regards to the protein content and extreme low digestibility**.** By suitable biological treatments (e.g., solid state fermentation), these agro-wastes can be converted into value added feedstuff for ruminant. Therefore, jute is considered as an ideal substrate for bioconversion into upgraded animal feed in both industrial and biotechnological point of view.

A diverse spectrum of lignocellulolytic microorganisms, mainly fungi (Falcon et al., 1995; Baldrian & Gabriel, 2003) and bacteria (McCarthy, 1987; Vicuňa, 1988; Zimmermann, 1990) have been isolated and identified over the years and this list still continues to grow rapidly. Among these, fungi have been studied extensively because of their ability to degrade lignocellulosic materials by complex mixtures of cellulases, hemicellulases and ligninases (Weng et al., 2008). White rot fungus such as *Pleurotus spp*. are excellent bio-catalytic systems for bioconversion processes e.g., bioconversion of lignocellulosic materials into value-added products including nutritious animal feed. The hydrolytic enzymes can be produced either by solid-state fermentation (SSF) or submerged culture of the enzyme producing microorganisms (Ishida et al., 2006). SSF offers potential advantages over submerged fermentation such as low energy consumption, process simplicity, superior enzyme productivity, low capital investment, negligible liquid waste product and ease in product recovery (Gupte & Madamwar, 1997).

The present study was undertaken to develop a process for producing animal feed from lignocellulosic materials such as jute sticks and green jute by a white rot fungi *Pleurotus sajor-caju* through solid state fermentation. The effects of chemical pretreatment on the nutritional quality of the fermented substrates were also assessed.

Materials and Methods

Two natural cellulosic agro-wastes, Jute sticks and green jute plants, were used in the present study. Collected cellulosic materials were first cleaned off from dirt and unwanted materials. Then, these were cut into tiny pieces, washed with water, sun dried and then ground by passing through a pulverizer (BICO Inc., USA) to made the particle size smaller than 1 mm.

Pleurotus sajor-caju was used for fermentation. The culture was maintained on potato dextrose agar (PDA) slants and was stored at 4°C.

Pretreatment

After washing and drying, the grounded jute sticks and green jute plants (jute stick with fiber) were divided into three batches. One batch was treated with lime solution (10 g $CaCO_3$ in 3.75 liter distilled water for 500 g substrate) then washed and dried in 60°C overnight. Another one was treated with alkali (boiled one hour with 0.1 g/g substrate NaOH solution and then washed until neutral pH gained and then dried in 60°C overnight) and the remaining was kept untreated. Each batch was further divided into two subgroups. One of the each subgroup was soaked overnight with distilled water (100 ml/ 25g) at room temperature and the other one kept unsoaked. For each substrate, all the twelve treatments were subjected to solid state fermentation (SSF) by *Pleurotus sajor-caju*. Finally, lime treated substrates were irradiated by ^{60}Co source (50000 ci). The dose rate was 340 Gy/h and the radiation doses used ranged from 20 to 40 kGy. The overall experimental plan is shown in Figure 1.

Fermentation

Pleurotus 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 10 mm in diameter) were taken for inoculation in 100 ml conical flasks containing 50 ml potato dextrose broth. Then each flask was incubated in a shaker at 30°C and after a week, the globular patches of mycelia were used to inoculate 25 g each of the different pretreated substrates in 100 ml flasks. Flasks were incubated at 30°C for 10 weeks.

The percentage of soluble protein and reducing sugar was determined by Lowry et al. (1951) and Miller (1959), respectively. The activities of three types of cellulose enzymes (cellobiase, carboxymethylcellulase /CMCase/ & avicelase) were measured using the method of Mandels & Sternberg (1976). 0.05 M citrate buffer at pH 4.8 was used throughout the enzyme assay as preservative.

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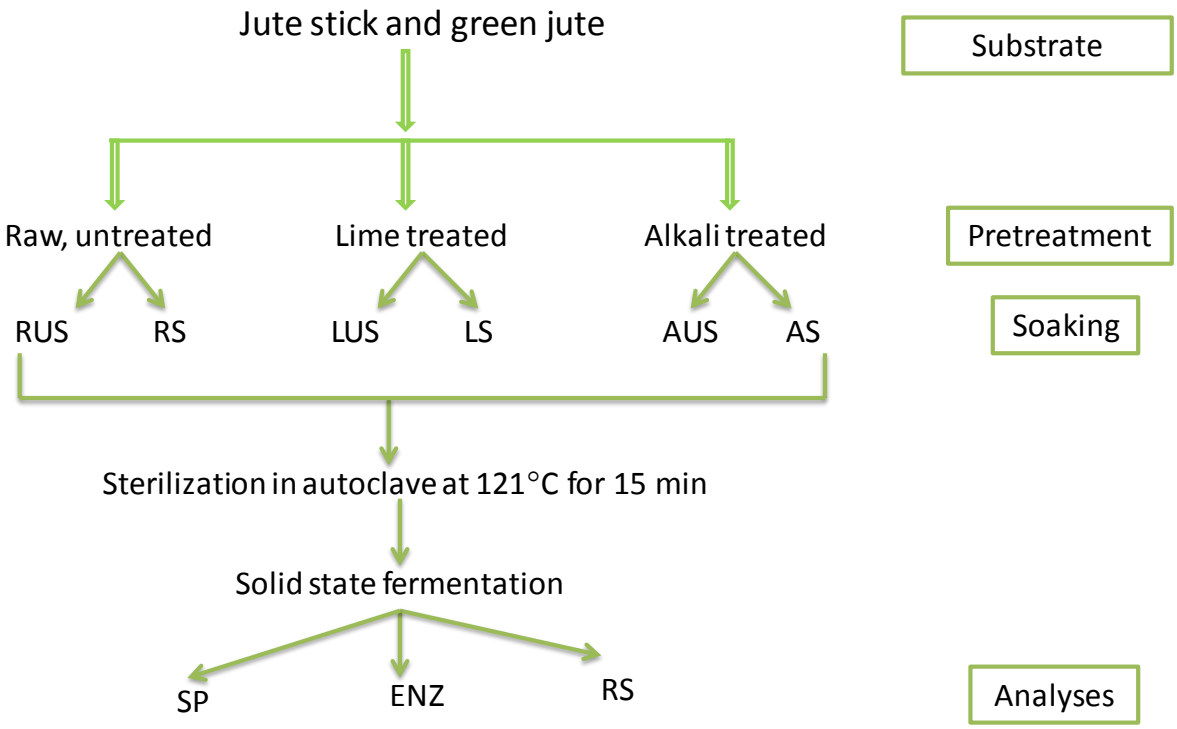

Figure 1. *Pretreatment of substrate and experimental protocol used in this study. R - raw, US - unsoaked; L - lime; A - Alkali; SP - soluble protein; ENZ - enzymatic activity and RS - reducing sugar.*

Results and Discussion

The amount of reducing sugar, soluble protein and cellulolytic enzyme contents of the different cellulosic substrates were found to increase with the increase in the incubation period. The release of reducing sugar content followed a regular pattern from 1st week to 8th week of fermentation mostly with highest increment at 6th week and then tends to leveled off or decrease.

Effect on reducing sugar and soluble protein accumulation

Figure 2 shows the reducing sugar accumulation pattern in differently treated jute sticks. The highest amount of reducing sugar was detected at 6th week of fermentation in presoaked lime treated substrate (LS-14.52 mg/g) followed by 12.22 mg/g in unsoaked lime treated (LUS) and 11.26 mg/g in presoaked alkali treated (AS) jute sticks. Soaking alone resulted in the release of 10.37 mg/g reducing sugar, whereas untreated sample had 9.90 mg/g reducing sugar at 7th week of fermentation.

In case of jute plants, similar trend was noticed where maximum amount of reducing sugar was attained at 6th week of fermentation in the order of presoaked lime treated substrate > unsoaked lime treated > presoaked alkali treated > unsoaked alkali treated > presoaked untreated > unsoaked untreated samples (Figure 3). In both cases, combination of lime and soaking enhanced the release of reducing sugar compared to untreated raw and alkali treated substrates.

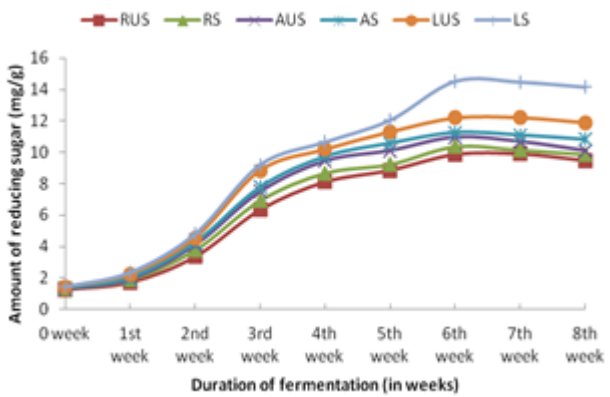

Figure 2. *Accumulation of reducing sugar content in jute sticks during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

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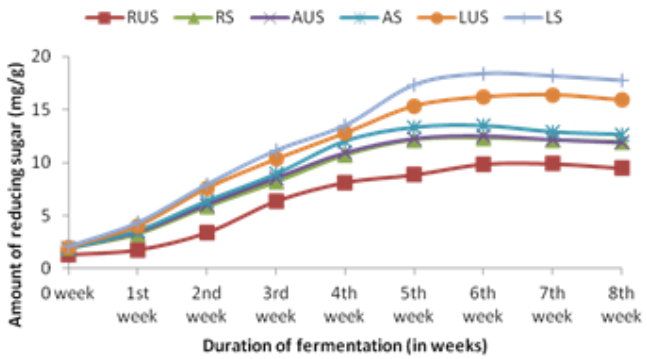

Figure 3. *Accumulation of reducing sugar content in green jute during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

Lime treatment is responsible for structural modification of the substrates leading to their increased digestibility, which in turn increases the reducing sugar content. Alkali (NaOH) treatment, on the other hand, causes disruption in the lignin seal followed by hydration and swelling, which decreases its crystallinity (Dunlap et al., 1976). Results showed that the increase in the reducing sugar content was relatively higher in presoaked substrates compared to unsoaked. This may be due to the structural swelling of substrates in aqueous medium, which might increase the accessibility of the substrate to enzyme attack. Regardless of treatment, the amount of reducing sugar accumulation was higher in the green jute than jute sticks because of the presence of highly cellulosic fibre along with the stick (Bashiruzzaman et al., 1964).

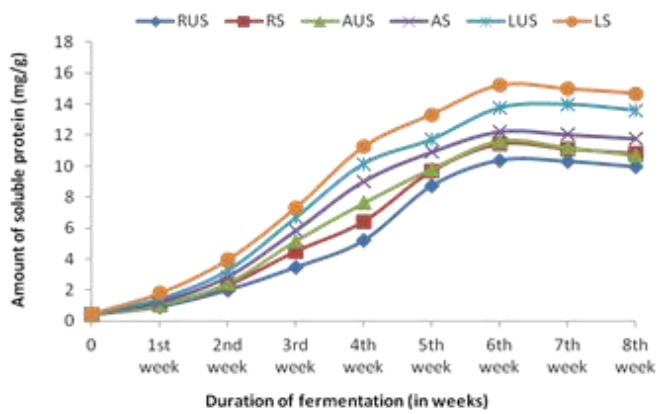

Figure 4. *Accumulation of soluble protein content in jute sticks during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

Figures 4 and 5 show the soluble protein accumulation pattern in differently treated jute sticks and green jute during fermentation. After 6th week of fermentation, the highest amount (15.20 mg/g) of soluble protein was found in presoaked lime treated jute sticks followed by unsoaked lime treated (13.98 mg/g) and presoaked alkali treated (12.23 mg/g) substrate. Lowest release of soluble protein was 10.38 mg/g in the raw material without any treatment (Figure 4).

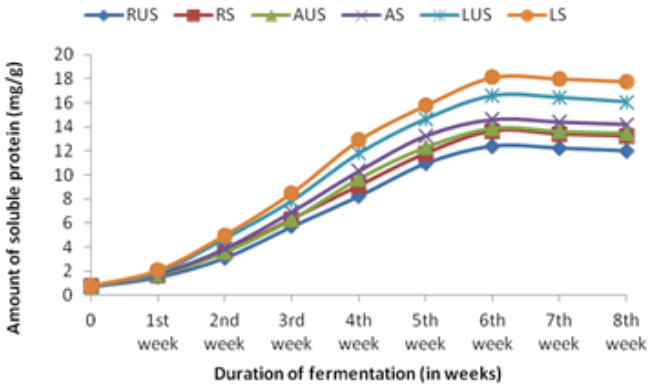

Figure 5. *Accumulation of soluble protein content in green jute during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

In case of green jute, the highest amount of soluble protein was detected at 6th week of fermentation in soaking and lime-treated substrates (LS-18.13 mg/g). Lime treatment alone could release 16.60 mg/g of soluble protein whereas alkali treatment alone results in the release of 13.88 mg/g soluble protein. Combination of soaking and alkali treatment results in the release of 14.61 mg/g soluble protein during fermentation. Similar to jute plants, substrate without soaking or any treatment yielded lowest amount of soluble protein (12.40 mg/g) (Figure 5). Changes in substrate without inocula were also ignorable.

The pattern of the increase of soluble proteins in all the differently treated substrates was found very similar as the reducing sugar. From the result, it seems that the increase in soluble protein could be attributed to the increased excretion of extracellular cellulolytic enzymes by *Pleurotus sajor-caju*, involved in the hydrolysis of cellulosic agro-wastes. Cellulases, which are known as inducible enzymes, need the presence of cellulose for production of different enzyme components for cellulose breakdown (Moubasher & Mazen, 1991). Cellulase enzymes played the key role in the animal feed upgradation process. Lime treatment and presoaking

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seem to be a better inducer for this purpose, which supported the better yields of fermentation i.e. higher growth, higher increase of reducing sugar and soluble protein.

In general, cellobiase activity in jute sticks was highest at 6th week till 9th week of fermentation for all treatments. Highest activity of cellobiase was around 1.03 units/ml in presoaked lime treated jute sticks followed by 0.97 units/ml in unsoaked lime treated jute sticks, 0.92 units/ml in soaked alkali treated, 0.85 units/ml in unsoaked alkali treated and 0.86 units/ml in soaked untreated samples from 6th week onwards. The enzyme activity in raw unsoaked jute sticks was 0.82 units/ml (Figure 6).

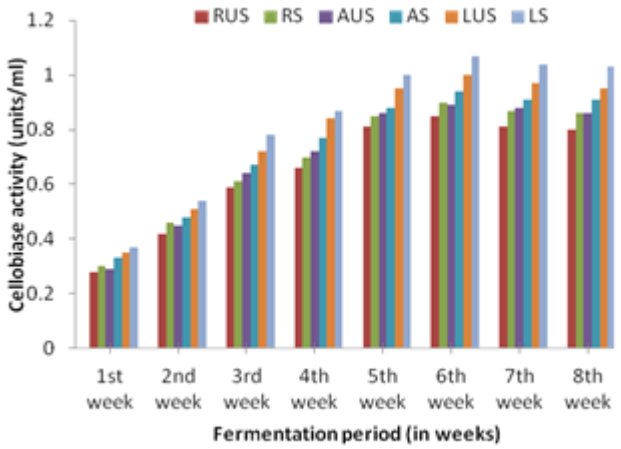

Figure 6. *Cellobiase activities in differently treated jute sticks during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

In green jute, the cellobiase activity showed similar pattern. Cellobiase activity was highest at 6th week mostly. The highest cellobiase activity was found in presoaked lime treated green jute - 1.15 units/ml followed by unsoaked lime treated green jute - 1.06 units/ml, soaked alkali treated - 0.98 units/ml, unsoaked alkali treated - 0.92 units/ml and soaked untreated- 0.92 units/ml. The enzyme activity for untreated, unsoaked sample was 0.86 units/ml (Figure 7).

Almost similar type of activities was found for the carboxymethylcellulase (CMCase). In the presoaked lime treated jute sticks the activity was 1.07 units/ml. For unsoaked lime treated jute sticks - 1.00 units/ml, soaked alkali treated - 0.94 units/ml, unsoaked alkali treated - 0.89 units/ml, soaked untreated - 0.90 units/ml and raw unsoaked the activity was 0.85 units/ml (Figure 8).

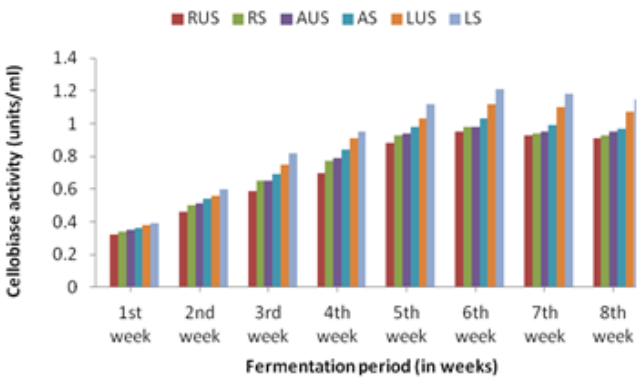

Figure 7. *Cellobiase activities in differently treated green jute during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

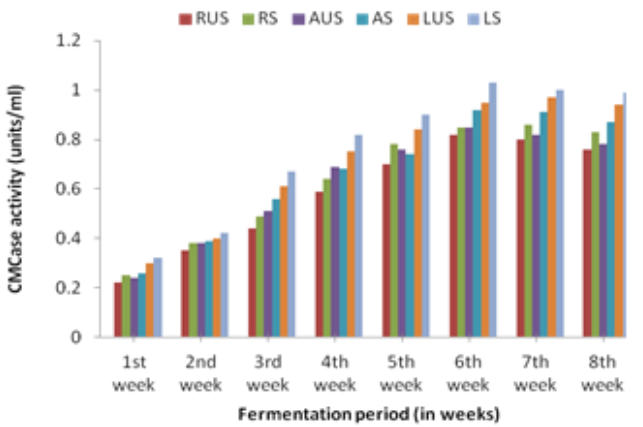

Figure 8. *CMCase activities in differently treated jute sticks during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

Lime pretreatment and/or presoaking was found to enhance CMCase activity in green jute as well. The highest CMCase activity was found in presoaked lime treated green jute - 1.21 units/ml and a gradual decrease in other substrates. For unsoaked lime treated green jute the activity was 1.12 units/ml, for soaked alkali treated - 1.03 units/ml, for unsoaked alkali treated - 0.98 units/ml, for soaked untreated - 0.98 units/ml and for untreated unsoaked - 0.95 units/ml and that were highest for them (Figure 9).

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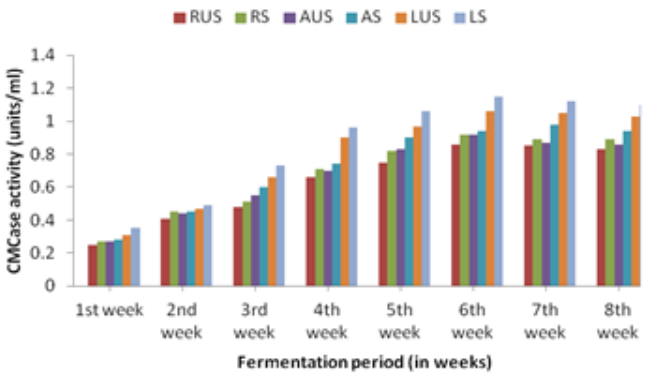

Figure 9. *CMCase activities in differently treated green jute during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

The avicelase activity was found to increase quickly up to 6th week of incubation and remained almost unchanged up to the 8th week when jute stick was used as substrate for solid-state fermentation. As shown in Figure 10, all the unsoaked and presoaked differently treated substrates followed similar pattern, but a higher activity was observed in case of soaked lime treated jute sticks. Highest avicelase activity (1.00 units/ml), was found in presoaked lime treated jute sticks, which was greater than others. For unsoaked lime treated jute sticks it was 0.95 units/ml, for soaked alkali treated - 0.88 units/ml, for unsoaked alkali treated - 0.91 units/ml, for soaked untreated - 0.77 units/ml and for raw unsoaked the activity was 0.70 units/ml. These values are the highest for each (Figure 10).

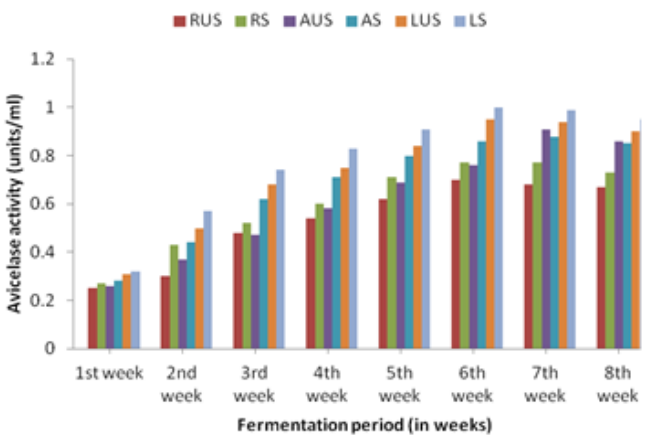

Figure 10. *Avicelase activities in differently treated jute sticks during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

And in green jute the activity increased in a very similar pattern. Highest avicelase activity was at 6th week mostly. Best avicelase activity was found in presoaked lime treated green jute (1.12 units/ml) and a gradual decrease in other substrates. For unsoaked lime treated green jute it was 1.03 units/ml, for soaked alkali treated - 0.92 units/ml, for unsoaked alkali treated - 0.84 units/ml, for soaked untreated - 0.86 units/ml and for untreated unsoaked the activity was 0.81 units/ml (Figure 11).

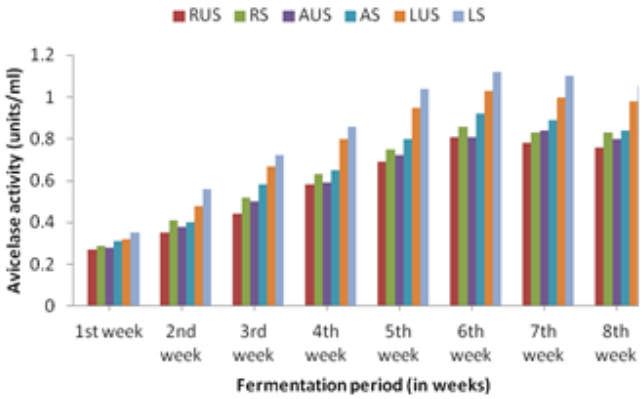

Figure 11. *Avicelase activities in differently treated green jute during 8 weeks of SSF by Pleurotus sajor-caju (R=Raw, A=Alkali treated, L=Lime treated, US=Unsoaked, S=Soaked).*

Effect of radiation

As the lime treated soaked green jute was found best for accumulation of reducing sugar and protein during fermentation, radiation doses of 20 kGy, 30 kGy and 40 kGy were applied to the green jute and then both the raw and lime treated green jute samples were subjected to fermentation. It was found that radiation doses have no effect on the reducing sugar and protein accumulation in either raw or lime treated substrates. This is in line with the report of Hossian et al. (2009), where the amount of reducing sugar and soluble protein were found to be almost the same in 5 and 10 kGy irradiated and non-irradiated substrates. The radiation doses have no notable effect on the enzyme activities as well.

Conclusion

In conclusion, it can be said that green jute plants and jute sticks can be upgraded to enriched animal feed by chemical pretreatments especially lime treatment and solid-state fermentation using the edible fungus (*Pleurotus sajor-caju*)

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as the fermented products contained high amount of reducing sugar and soluble protein. Further research is necessary to develop strategies for industrial scale production of enriched animal feed from so called agro-wastes. The present investigation may contribute to overcome the crisis of nutritionally improved animal feed and may save a huge amount of foreign currency, which is now being used for importing expensive animal feeds.

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