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Bioconversion of Some Agricultural Wastes and Associated Enzymes by *Trametes* species: A Wild Mushroom

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Abstract

The ability of *Trametes* speciesto degrade readily available agricultural wastes with associated enzymes under submerged fermentation was the aim of the study. Tissue culture technique was employed in obtaining active mycelium of the mushroom from its fresh fruiting body. Proximate analysis of the agricultural wastes after fermentation revealed that the percentage protein and moisture contents increased while the fat, crude fiber, ash and carbohydrate contents decreased. Analysis of the mineral contents of the wastes revealed a reduction with fermentation. Several types of agro-industrial wastes were evaluated as substrates for enzymes production by *Trametes* speciesin comparison to commercial substrates (control). The composition of the wastes was observed to affect the quantity as well as the activity of the enzymes assayed. Wheat bran had higher cellulase activity of 236.66µmol/min/mL between 48 and 72 h of fermentation.

***Keywords*:** *Trametes* species; Wild mushroom; Agricultural waste; Enzyme

1. Introduction

In recent years, there has been an increasing trend towards more efficient utilization of agro-industrial residues such as cassava, sugar beet pulp, wheat bran and apple pomase.

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Several processes have been developed that utilize these as raw materials for the production of bulk chemicals and value-added fine products such as ethanol, Single Cell Protein (SPC), mushroom, enzymes, organic acids, amino acids and biologically active secondary metabolites [27, 28]. Applications of agro-industrial residues in bioprocesses on the one hand provide alternative substrates and on the other hand help in solving pollution problems, which their disposal may otherwise cause. With the advent of biotechnological innovations, mainly in the area of enzyme and fermentation technology, many new avenues have opened for their utilization.

The potential of using microorganisms as biotechnological sources of industrially relevant enzymes has stimulated interest in the exploration of extracellular enzymatic activity in several microorganisms[5, 4]. The hyphal mode of growth and good tolerance to low water activity (aw) and high osmotic pressure conditions make fungi most efficient for bioconversion of solid substrates [7].

Lignocellulose is the major structural component of woody plants and non-woody plants such as grass and represents a major source of renewable organic matter. The chemical properties of the components of lignocellulosics make them a substrate of enormous biotechnological value [23]. Large amounts of lignocellulosic waste are generated through forestry and agricultural practices, paper-pulp industries, timber industries and many agro industries and they pose an environmental pollution problem. Sadly, much of the lignocelluloses waste is often disposed of by biomass burning, which is not restricted to developing countries alone, but is considered a global phenomenon. However, the huge amounts of residual plant biomass considered as waste can potentially be converted into various different value added products including biofuels, chemicals and cheap energy sources for fermentation, improved animal feeds and human nutrients. Lignocellulytic enzymes also have significant potential applications in various industries including chemicals, fuel, food, brewery and wine, animal feed, textile and laundry, pulp and paper and agriculture [18].

The ability of *Trametes* species to degrade readily available agricultural wastes with associated enzymes under submerged fermentation was the objective of the study.

2. Materials and Methods

study was conducted 2013 at the School of Science, Department of Microbiology, Federal University of Technology, Akure, Nigeria.

2.1. Pretreatment of substrates

Wheat bran, rice bran and palm kernel pericarp were procured from farm field or domestic sources while sawdust (white alfra) was obtained from saw mills. Each of these raw materials was dried and ground to pass through a 30 mm mesh sieve. The commercial substrates were used without pretreatment [7].

2.2. Preparation of media for bioconversion and enzyme assays

The basal medium for assay of enzymes had the following composition (g L^{-1}): NaCl 1.5, K_2HPO_4 1.5, KH_2PO_4 0.5, $(NH_4)_2SO_4$ 1.0, $MgSO_4$. $7H_2O$ 0.05, $CaCl.2H_2O$ 0.02 and yeast extract 0.5.Salts. The basal medium was

autoclaved at 121°C for 15 min. The carbon sources (wheat bran, rice bran, palmkernel pericarp and sawdust) were autoclaved separately and added to the basal medium to give a final concentration of 1% (w/v)[22].

2.3. Production of crude enzymes

Seventy Two hours old culture of *Trametes* species was inoculated into basal medium. The flask was incubated at 30±2°C for 96 h on a rotary shaker (Gallenkamp) at 120 rpm. At various intervals of 0, 24, 48, 72 and 96, 120, 148 and 160 h, respectively, samples were taken to assay for enzymes activities [22]. Sterile basal medium supplemented with commercial substrates served as the control.

2.4 .Enzyme assays

Cellulase activity was assayed by the method of Miller [24], amylase [13] and protease [21]. Enzyme activity is expressed as protein concentration, which is represented as umol/min/mL.

3. Results and Discussion

3.1 Proximate and mineral composition of selected wastes

The percentage protein and moisture contents increased with fermentation while a decrease in carbohydrate and mineral composition was observed (Table 1, 2).

3.2 Enzymes production and assay

Figure 1 to 4 show time course profiles of extra cellular enzymes (cellulase, alpha amylase, beta amylase and protease) production by *Trametes* species in submerged fermentation using different substrates.

Table 1: Proximate composition (%) of selected agro-industrial residues before and after use in submerged fermentation with T*rametes* species

SAMPLES		MOISTURE	ASH	FAT	FIBRE	PROTEIN	CARBOHYDRATE
WHEAT BRAN	a	8.79±0.02^{b}	6.32±0.02^{g}	2.38±0.01^{a}	3.18±0.02^{b}	19.54±0.09^{e}	59.77±0.06^{f}
	b	11.53±0.02^{e}	2.63±0.05^{d}	2.90±1.24^{b}	1.55±0.01^{a}	27.66±0.02^{g}	53.75±1.28^{g}
RICE BRAN	a	8.54±0.03^{a}	4.47±0.02^{f}	5.20±0.02^{b}	20.19±0.01^{d}	13.49±0.09^{c}	48.09±0.10^{e}
	b	10.32±0.00^{d}	2.27±0.02^{c}	2.86±0.02^{a}	16.11±0.00^{c}	23.21±0.11^{f}	45.23±0.14^{d}
SAW	a	9.09±0.01^{c}	0.19±0.01^{b}	5.60±0.01^{b}	69.77±0.02^{h}	10.96±0.10^{a}	4.73±0.50^{a}

DUST	b	13.97±0.02^{d}	0.12±0.01^{a}	2.44±0.02^{a}	65.33±0.00^{g}	12.86±0.01^{b}	5.29±0.05^{c}
PALM KERNEL	a	12.37±0.03^{f}	14.16±0.02^{h}	8.11±0.02^{d}	45.03±0.02^{f}	12.85±0.10^{b}	10.04±0.48^{b}
PERICARP	b	14.44±0.03^{h}	3.36±0.02^{e}	6.48±0.02^{c}	42.98±0.01^{e}	14.73±0.00^{d}	18.01±0.07^{c}

a: Sample before use (unfermented). b: Sample after use (fermented). Values are means of three replicates ±SD. The statistical significance was evaluated using Student's t-test and value of $p<0.05$ was considered to indicate a significant difference between the fermented and unfermented wastes

Table 2: Mineral composition of selected agro-industrial residues before and after use in submerged fermentation(ppm)

SAMPLES		Ca^{2+}	Mg^{2+}	Na^{+}	K^{+}	PHOSPHORUS
WHEAT BRAN	a	0.38±0.02^{d}	0.21±0.00^{c}	0.64±0.01^{f}	0.77±0.00^{d}	0.04±0.00^{a}
	b	0.33±0.02^{c}	0.17±0.02^{g}	0.34±0.04^{c}	0.72±0.00^{c}	0.03±0.00^{b}
RICE BRAN	a	0.25±0.01^{b}	0.06±0.01^{a}	0.27±0.01^{b}	0.65±0.01^{b}	0.16±0.00^{c}
	b	0.20±0.02^{a}	1.23±0.03^{h}	0.20±0.01^{a}	0.42±0.03^{a}	0.12±0.01^{b}
SAW DUST	a	1.32±0.02^{h}	0.46±0.00^{f}	1.20±0.02^{h}	2.94±0.00^{h}	1.57±0.02^{e}
	b	1.21±0.01^{g}	0.38±0.01^{d}	1.12±0.01^{g}	2.90±0.00^{g}	1.42±0.03^{d}
PALMKERNEL PERICARP	a	0.60±0.01^{f}	0.18±0.01^{b}	0.47±0.02^{e}	0.87±0.01^{f}	0.18±0.01^{c}
	b	0.54±0.02^{e}	0.42±0.03^{e}	0.42±0.00^{d}	0.81±0.01^{e}	0.16±0.00^{d}

a: Sample before use (unfermented). b: Sample after use (fermented). Values are means of three replicates ±SD. The statistical significance was evaluated using Student's t-test and value of $p<0.05$ was considered to indicate a significant difference between the fermented and unfermented wastes.

In all, enzymes production increased progressively with increase in incubation time until an optimum production was attained. Subsequent increase in incubation time beyond the optimum led to a decline in production.

Selected agro-industrial wastes were used for the production of extracellular enzymes which was in line with the methodology of [18, 20].They reported the use of agro-industrial wastes as substitutes for commercial known substrates for enzymes production. The biodegradation of wastes by associated enzymes was also reported in the research finding of [8]. The cultivation of edible mushroom with agro-industrial wastes is the value added process to convert these materials that are otherwise considered to be wastes, into human foods, biochemical

and enzymes. It represents one of the most biological ways by which these residues can be recycled [32, 3]. Alternative methods of utilizing the wastes are needed to correct environmental pollution problems associated with the disposal methods [10].

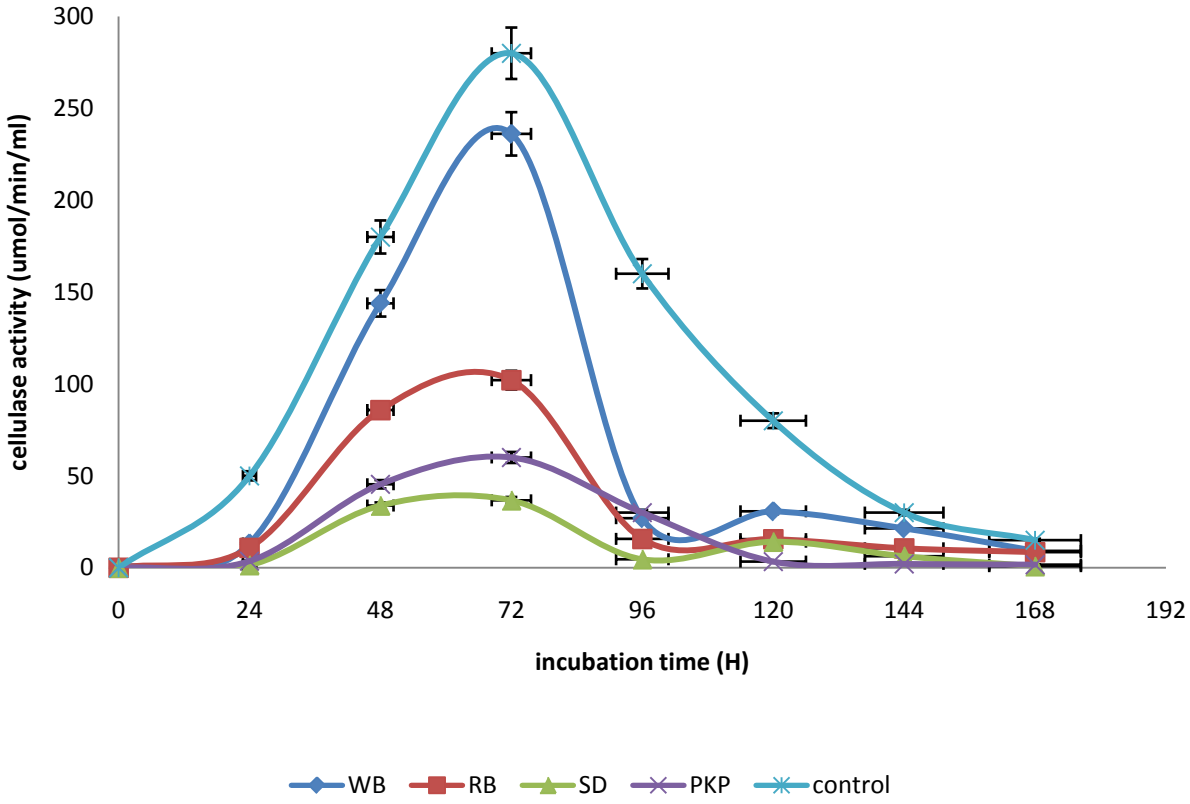

Fig. 1: Time course profile of cellulase production by *Trametes* species in submerged fermentation using different substrates. It shows cellulase activity of different agro-industrial residues at different time interval. Sawdust gave the highest cellulase activity of 236.66 µmol/min/mL at 72 h of fermentation but less than that obtained with carboxymethylcellulose (control) was 280 µmol/min/mL at 72 h while lowest was found with palm kernel pericarp

Bioconversion of these wastes help in increasing their nutritive values as well as their digestibility for animal feed [6, 14]. The use of low cost substrates for the production of industrial enzymes is one of the ways to greatly reduce production costs. This can be achieved using solid agricultural waste materials as substrates [31]. The amount of enzymes produced by each substrate differs depending on the amount carbon source utilized by the organisms. Wheat bran was observed to be better utilized than other substrates for enzyme production. The use of wheat bran as the best substrate for the production enzyme was reported in the research findings of [6, 7].

The result of proximate analysis revealed that wheat bran and rice bran contain considerable amount of carbohydrate which stimulate the cells to express many hydrolytic enzymes. This was supported by the findings of[2, 14] and 23]. In addition, it contains appreciable amounts of easily utilizable sugars which encourage growth initiation and protein, which serves as essential nitrogenous compounds.

The result of proximate composition of agro-industrial wastes before and after use in submerged fermentation with mushroom mycelia showed an appreciable increase in the protein content of fermented (used) agro-industrial wastes when compared with their unfermented samples. The increase in microbial biomass in the form of single cell protein can be one of the reasons for the increase in the protein content. This view was supported by the findings of [15, 3]. Protein content increase could also be as a result of hydrolysis of starch to glucose and its subsequent use by the same organism as a carbon source to synthesis fungal biomass rich in protein[11,17,19] and [3] also reported that protein increase may be due to secretion of certain extracellular enzymes which are proteinous in nature into the waste during their breakdown and its subsequent metabolism.

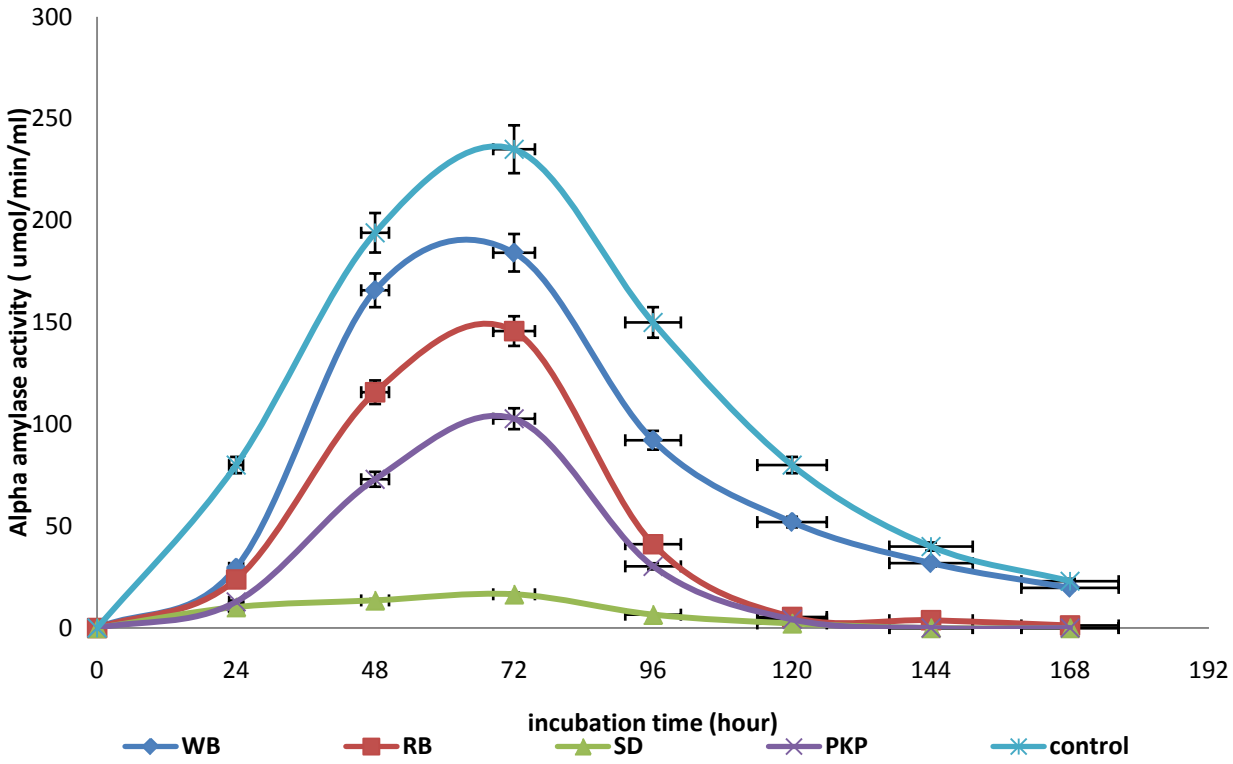

Fig. 2: Time course profile of alpha amylase production by *Trametes* speciesin submerged fermentation using different substrates. The highest alpha amylase production was recorded for soluble starch (control) with 235 µmol/min/mL between 48 and 72 h incubation time but there was sharp decrease with increase in incubation period. Of all the wastes used, wheat bran gave the highest alpha amylase activity of 184.2 µmol/min/mL at 72 h incubation time but less than that was obtained with soluble starch. The lowest activity was observed in palm Sawdust yielded 16.57 µmol/min/mL at 72 h of incubation time

The crude fibre content of the samples after fermentation was lesser than the unfermented samples. When *Trametes* species mycelial was used as culture, there was a general decrease in crude fibre content. This was supported by the findings of [3,9]. There were considerable changes in the fat and ash content. The ash content of the fermented samples showed slight decrease. The high value of ash content in the unfermented samples shows that agro-industrial wastes used might have a reasonable quantity of mineral elements. The ash content is always a rough measure of the inorganic mineral elements in the samples. It is therefore means that the

unfermented samples are likely to have more mineral elements when compared with the fermented samples. It is unlikely that the microorganisms might have used some of the minerals for their metabolic activities. This corroborated with the work of [16, 12].They reported that all living organisms required some mineral elements to maintain some metabolic functions. There was no appreciable decrease in the mineral composition of fermented agro-industrial residues. This could be attributed to a lot of factors including the type of substrate [26, 21]). Similar observation was reported by [25]. The reason for decrease in some of the mineral content in the fermented samples could be due to the fact that some of these metals could be part of some biological macromolecules which were released into the solution from such structures during fermentation or dewatering[30]. The gradual increase in moisture content observed in fermented samples may be due to the processes of forming slurry and activities and/or influence of *Trametes*spp which potentiate water retention within the fermentation medium[3,25]. There was appreciable decrease in the carbohydrate content of fermented samples. This observation might have occurred due the ability of *Trametes* sppto utilize carbohydrate in the production of cellulase and amylase.[18]also reported the ability of mushroom to hydrolyse carbohydrate in bioconversion process for various biochemical pathways.

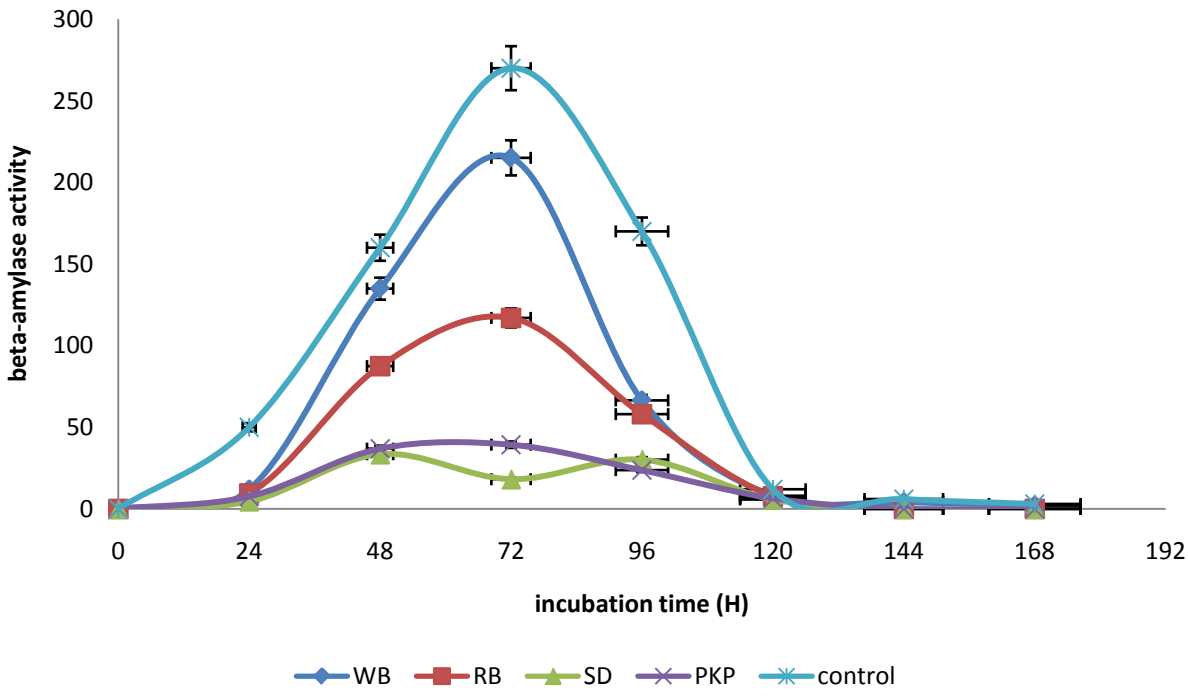

Fig. 3: Time course profile of beta amylase production by *Trametes* species in submerged fermentation using different substrates. The soluble starch produced the highest fold when compared with all the agro industrial wastes used. Of all the wastes used, wheat bran produced the highest beta amylase activity of 215.07mol/min/mL at 72 h incubation period followed by rice bran, while palm kernel pericarp gave least

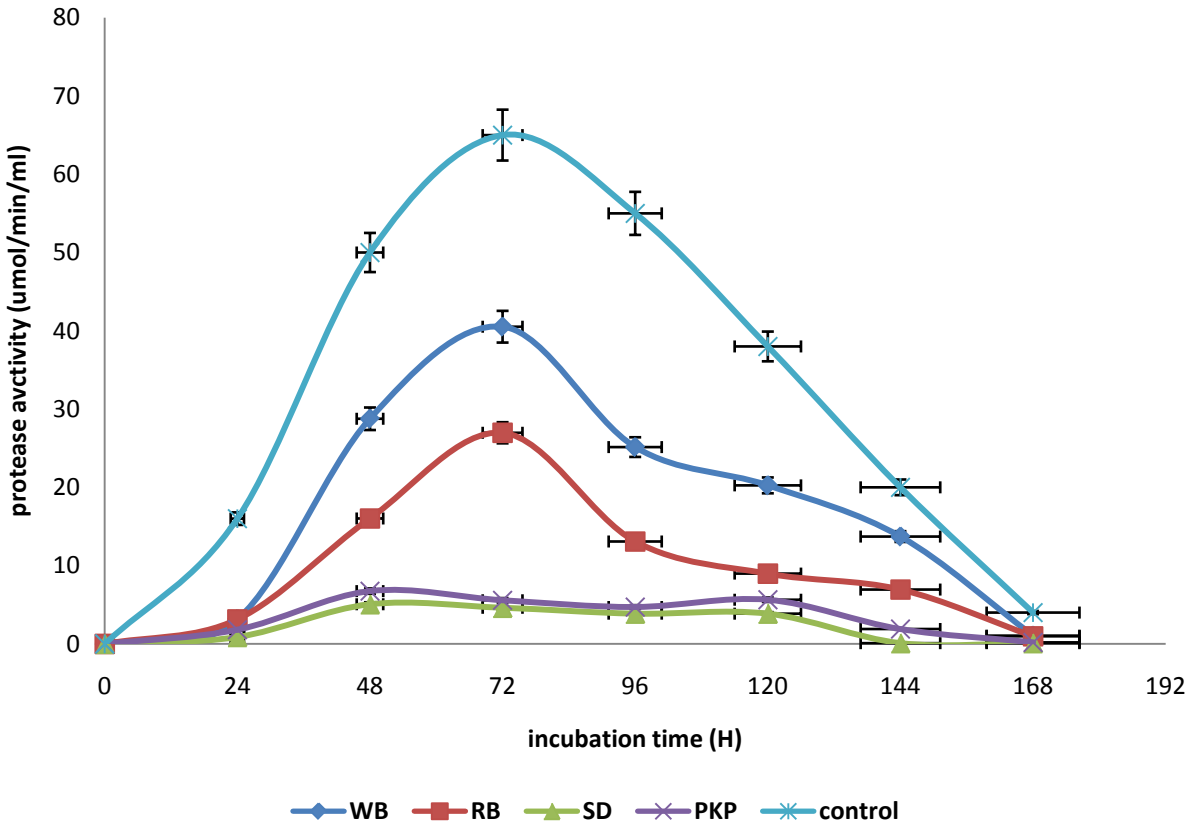

Fig. 4: Time course profile of protease production by *Trametes* sppin submerged fermentation using different substrates. The casein as the control was observed to produce highest protease activity when compared with wastes. Among all the wastes, wheat bran produced the highest protease activity of 40.52 µmol/min/mL at 72 h incubation time and other substrates used yielded low protease activity with all liberating more than 26.97 µmol/min/mL at 72 h incubation time but there was decline in activity with increase in incubation period.

4. Conclusion

The result obtained from the bioconversion process of selected wastes revealed the potential of extracellular enzymes produced by mushroom as a biotechnological tool for the transformation of wastes into biological products. The enzymes sourced from mushroom can be used as substitutes to chemicals used mostly for the treatment of pollutants which are deleterious to the environment. For many processes enzymes are preferred to acid or alkaline processes since they are specific biocatalysts, can operate under much milder reaction conditions, do not produce undesirable products and are environmentally friendly.

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