Recovery of Lignocellulolytic Enzymes and Valorization of Spent Mushroom Substrate

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ABSTRACT

Spent Mushroom Substrate (SMS) comprises sugarcane bagasse, coconut coir, chicken manure, and paddy straw; inoculated with and farmed for *Agaricus bisporus*. At present, the waste generation at a mushroom cultivation plant in Goa is 40 tons/day (15,000 tons annually). Valorization of this waste has been explored in terms of extracting lignocellulolytic enzymes and briquette production. SMS was screened for the presence of lignocellulolytic enzymes and then was used to make briquettes. The enzymes found in SMS were cellulase and laccase, which were further concentrated via tangential flow filtration (TFF). Enzyme activity for Cellulase increased by four-fold (from 255.34±1.30 U/mL increased to 1022.21±4.84 U/mL) and Laccase increased by three-fold (from 4.83±0.02 U/mL to 13.21±0.05 U/mL). The concentrated enzyme cocktail was used to decolorize congo red dye. After only eight hours of enzymatic treatment at pH 4.8 on congo red, approx. 40-49% decolorization was accomplished. The color removal was due to the presence of the laccase enzyme. After enzyme extraction, all the residual SMS was utilized to generate briquettes with an initial reduction in its moisture content from 50% to 10%. The resulting briquette gave a Gross Calorific Value of 4,143 Kcal/kg with 12.60% ash content. Thus, SMS proves to be a valuable source for recovering enzymes and a cost-effective material for briquette production rather than going into landfills.

1. INTRODUCTION

The common button mushroom, *Agaricus bisporus*, is cultivated for commercial purposes in a controlled environment. *Agaricus bisporus* is grown on a biomass substrate consisting of clay, sugarcane bagasse, coconut coir, chicken feed, and paddy straw mixed in a predetermined proportion. At Tropical Mushrooms Pvt. Ltd. (Goa) India, approximately 15,000 tons of Spent Mushroom Substrate (SMS) is generated annually. Currently, after the mushroom is harvested at the farm, the biomass substrate is disposed of in the landfill. Three significant environmental impacts that occur due to the landfill are: (a) Landfill construction-can damage the ecology; (b) Landfill gas-gaseous emission consists mainly of CO_2 and CH_4, which contributes to global warming; (c) Leachate-can invade to groundwater causing water pollution and also, metals (from groundwater) retained by the soil absorb by plants and then enters in the food chain (Danthurebandara et al., 2013). Therefore, landfill is not a feasible way of waste management, which originates the need to explore a sustainable waste management solution.

SMS is rich in lignocellulosic content. The fungal mycelium inherently present in the SMS consumes these lignocellulosic content and flourish (Fen et al., 2014). The microorganisms utilize these contents (by degradation) for their growth with the help of extracellularly secreted lignocellulolytic enzymes (Singh et al., 2003). Therefore, SMS will be a rich source for extracting these enzymes, which are of commercial interest.

The extensive industrial application of lignocellulolytic enzymes makes them valuable, mainly for the food and textile industries. In particular, cellulase and xylanase are used for biofuel production, as they possess the ability to saccharify biomass. The lignin-degrading laccase is a versatile enzyme as it also

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has a vital role in the treatment of dye wastewater/textile industry effluent (Thurston, 1994; Abadulla et al., 2000; Lim et al., 2013). Laccase possesses the ability to decolorize dyes by oxidizing the aromatic rings; also, it is much less specific because it can work on a vast range of synthetic dyes such as Remazol brilliant blue R, Congo red, Indigo, etc. which are extensively used in industries these days (Thurston, 1994; Abadulla et al., 2000; Lim et al., 2013).

Residues generated from the agricultural industry have a very high potential for resource recovery and reuse as the substrate for fermentation, biogas, biofuel, briquette, etc. (Demirbas, 1999; Sadh et al., 2018). Agro-wastes, which can be used for briquettes production are corn straw, leaves of various species, coconut husk, bagasse, sawdust, etc. (Tamilvanan, 2013; Garrido et al., 2017). The SMS biomass generated after mushroom harvesting and recovery of enzymes can be converted into high-quality biofuel products by compacting into a high-density product called briquettes - a source of green energy (Garrido et al., 2017). Briquetting is a densification process that produces a compact material with higher energy per unit volume (Garrido et al., 2017). Low-cost production and with increased access to consumers, briquettes can be chosen over coal and other non-renewable burning fuel for domestic and agro-industries usage. The advantages of briquetting - higher heat intensity, uniform clean and stable fuel, convenience in use, and relatively smaller space requirements for storage, and reduced transportation costs (Garrido et al., 2017). Briquetting without the binder makes the process convenient but increases cost and sophistication in drying equipment and briquette press, thus making the process less attractive for a developing country like India. Briquetting with a binder like paper, wood chips, etc., is suitable for developing countries, as it decreases the production cost and requirement of more skilled labor. Preliminary research showed that the briquettes produced with binders like cassava starch and palm oil sludge result in smoky briquettes. Generally, briquettes are made from locally available materials, which include sawdust, cowpea chaffs, corncobs, and water hyacinth (Olorunnisola, 2007). The waste used as raw materials for briquette production is oil palm residue, paper waste, plastic waste, coconut husk and wheat straw (Demirbas, 1999; Olorunnisola, 2007; Sing and Aris, 2013; Garrido et al., 2017). The key significant factors for making briquetting are low production cost, waste material management, and conversion of waste to revenue.

It is proposed here to utilize the SMS for enzyme recovery and manufacturing of fuel briquettes. The study is focused on waste management at the mushroom farm by enzyme recovery followed by briquette production. Enzymes such as cellulase and laccase were recovered. Tangential Flow Filtration (TFF) technique was applied to crude enzyme extract (CEE) to increase the enzyme concentration. The recovered lignocellulolytic enzymes were tested for their dye decolorization ability. Briquetting from reusing the SMS material post mushroom production and enzyme recovery with and without a binding agent (wood chips) was also demonstrated.

2. METHODOLOGY

2.1 Chemicals

Carboxymethylcellulose (CMC) and Congo red (CR) were procured from HiMedia, India. 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was purchased from Roche Diagnostic, Germany. Mira cloth of 22-25 µm pore size was bought from Merck Millipore.

2.2 Crude enzyme extraction

A mushroom bed has two distinct layers; the top layer is the casing layer (3.8 cm height, 2.0 kg weight) and at the bottom is the compost layer (16.5 cm height, 8.5 kg weight). The extraction of the enzyme from SMS was carried out by removing the casing layer from the mushroom bed. SMS (300 g) was added in 500 mL of sterile distilled water and agitated at 300 rpm for 60 min at 4°C. Antunes (2020) found that, using a solvent like water, recovery of enzymes (with notable activity) from SMS is possible. The mixture was filtered through Mira cloth (Lim et al., 2013), the filtrate was then centrifuged at 7,000 rpm for 10 min at 4°C to obtain a clear supernatant. The supernatant containing crude enzymes was retained and stored at 4°C until enzyme activity analysis was carried out (Lim et al., 2013). Crude enzyme extract (CEE) was concentrated by passing the CEE through the Krosflo® KR2i TFF system from Spectrum Labs, using the NMCW-30kDa column at operating conditions of 2.5 psi feed pressure and feed flow rate was 140 mL/min, these conditions were maintained throughout the process. All the extraction procedures were performed in triplicate.

2.3 Screening of enzyme

The CEE samples were analyzed for the presence of various enzymes, namely cellulase, laccase, xylanase, amylase, and invertase, by agar plate diffusion assay. The assay was performed in duplicate. Substrates used for cellulase, laccase, xylanase, amylase, and invertase were CMC, ABTS, xylan, starch, and sucrose, respectively. Agar plates (1%) containing 0.5% CMC, 0.2 mM of ABTS, 0.5% xylan, 0.5% starch, and 0.5% sucrose were poured. The media for all the enzymes were prepared with one substrate only to get the specific activity. In each agar plate, a well of approximately 5 mm diameter was made. CEE was then added into the wells, following which the plates were incubated at 37°C (for cellulase, xylanase, amylase, and invertase activity) and 25°C (for laccase activity) (More et al., 2011). Cellulase activity on the plates was obtained by staining with CR dye (1 g/L, 1% ethanol) followed by destaining with 1 M NaCl.

2.4 Enzyme activity

The CEE, TFF-retentate, and TFF-permeate samples were analyzed for cellulase and laccase activity. The cellulase and laccase activity was determined in duplicates by the DNS method (using CMC solution) and ABTS oxidation, respectively. CMCase activity was estimated (employing 0.5% CMC as substrate) by the assay described by Isikhuemhen and Mikiashvili (2009). The reaction mixture, composed of enzyme sample (0.5 mL), CMC (0.5 mL), and 0.05 M sodium citrate buffer (pH 4.8), was held at 50°C (Isikhuemhen et al., 2009) for 60 min. Following the reaction, the amount of reducing sugar was determined by the DNS method (Miller, 1959). One unit of enzyme activity is defined as 1 µmol of glucose equivalent molecules released per minute under the given conditions.

Laccase activity was determined by measuring oxidized ABTS at 420 nm, as described earlier (Shin and Lee, 2000; Suwannawong et al., 2010; Lim et al., 2013). The reaction mixture, consisting of enzyme sample (0.3 mL), 0.2 mM ABTS (0.7 mL) and 0.1 M sodium acetate buffer (pH 4.5), was incubated at 25°C for 15 min. One unit of enzyme activity is defined as the amount of enzyme required to oxidizing 1 µmol of ABTS per minute under the given conditions.

2.5 Dye decolorization

CR dye was the substrate employed to determine the decolorization ability of CEE at the lab scale. The stock solution of CR (1.0%) was prepared in sterile distilled water with 1.0% ethanol. The working solution concentration was 0.01%. Enzyme concentration, ranging from 0.25% to 5.0% (0.25%, 0.50%, 0.75%, 1.0%, 2.5% and 5.0%) was mixed with 0.01% CR in 0.1 M sodium citrate buffer (pH 4.8) and 0.1 M sodium acetate buffer (pH 6.0). The experiment was carried out at 28±2°C. Decolorization was determined spectrophotometrically at 592 nm using Spectroquant® Prove 100 from Merck. The effect of agitation on decolorization rate was also tested with an experimental set up with 0.01% CR, and 1% enzyme concentration at pH 4.8 kept at 28±2°C for 24 h, one set was shaken, and the other was in a stationary state. All experiments were carried out in triplicate.

2.6 Briquetting process

The SMS left after the enzyme extraction, along with the rest of the mushroom bed, were used to produce briquette, a clean and green energy source. SMS consists mainly of bagasse, wheat husk, coconut husk, etc. The material was in bags of ~20 kg each, contained 40-60% moisture, and existed as lumps. The moisture content of the raw materials for briquette manufacturing should be in the range of 10-15%. Therefore, the SMS was dried in a rotary drum drier to decrease the moisture down to 10%, making it suitable for the briquetting process. The input temperature of the drier was kept at 250°C, and the output temperature was monitored to be 125°C. The rotation cycle of the drum drier was set at 15-20 rpm to give SMS enough exposure to the heat.

For the briquetting process, the material was fed into the briquetting press, which has a 75HP motor to drive its ram and two 1,200 kg flywheels. Since the ram has a diameter of 90 mm, this translates into a pressure of 8.79 MPa. Alternatively, SMS was mixed with wood chips (WC, size 8 mm with moisture level 15-20%) as binding material. Thus, three different types of briquettes were prepared: (a) only SMS; (b) 65% SMS and 35% WC; (c) 50% SMS, and 50% WC. All final products were tested for calorific value, moisture content, ash content, volatile matter, carbon content, bulk density, size, ignition test, and flow ability test.

3. RESULTS AND DISCUSSION

3.1 Lignocellulolytic enzyme profile in SMS

Bioactive compounds like lignocellulolytic enzymes have been previously extracted and characterized from mycelium, fruiting body, SMS,

and fermentation broth (Antunes et al., 2020). The lignocellulolytic enzymes involved in breaking down lignin and cellulosic material are cellulases, laccases, xylanases proteases, and amylases (Antunes et al., 2020). The lignocellulolytic enzymes, namely cellulase, laccase, xylanase, amylase, and invertase, were screened qualitatively for their presence in CEE from SMS by plate diffusion assay. The predominant presence of cellulase and laccase was observed, while amylase activity was below the limit of detection. No xylanase and invertase activity were seen. Cellulase activity was confirmed by the formation of a yellow halo zone (4.50±0.25 mm diameter of zone of activity) around the well. The yellow halo zone formation confirms the cellulose degradation by cellulase enzyme to monosaccharides. CR is unable to stain monosaccharides which causes yellow halo zones to form (Figure 1(a)). Laccase activity was due to ABTS oxidation, which turns into a green color. This green color formed around the well confirmed the presence of laccase; the diameter of the activity zone was 9.0±0.5 mm (Figure 1(b)). The enzymatic unit of cellulase and laccase was found to be 255.34±1.30 U/mL and 4.83±0.02 U/mL, respectively (Figure 2(a) and 2(b)). Based on several reports, the range of cellulase and laccase activity achieved in the extract from was 0.4-133.0 U/mL (Ryu and Mandels, 1980; Mtui, 2012; Saravanan et al., 2013; Saroj et al., 2018) and 0.14-0.89 U/mL (Sahay et al., 2008; Mtui, 2012; Haripriya et al., 2014), respectively. Comparison of enzymes activity in our study with the reported literature revealed higher cellulase and laccase activity recovered from lignocellulosic waste. To enhance the laccase activity, concentration of the enzyme is essential for rendering recovered enzymes from SMC for potential industrial application. After the enzyme was extracted from SMS, various value-added products also have been reported to be obtained from SMS, such as biogas, organic fertilizer, animal feed supplements, fertilizer, cosmeceuticals, bio-remediation, bio-based materials, and energy (Sadh et al., 2018; Antunes et al., 2020).

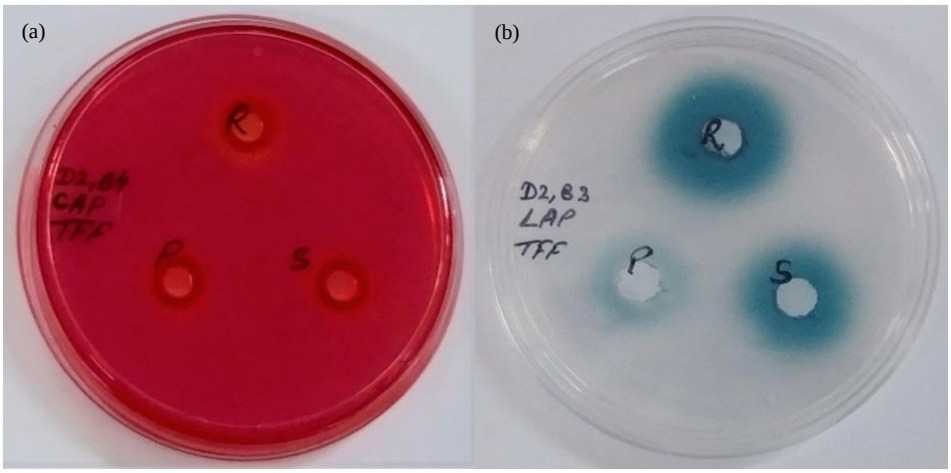

Figure 1. Agar plate diffusion assay: (a) cellulase assay; (b) laccase activity. (R=retentate, P=permeate, S=initial sample)

3.2 Lignocellulolytic enzyme concentration

TFF was employed to concentrate the crude enzyme extract. The filtration of CEE via the TFF process resulted in two streams, permeate and the retentate. The enzymatic activity determination of both the streams showed that the retentate stream had a highly considerable amount of enzyme activity. During qualitative analysis, the diameter of activity noted in the retentate was 14.50±0.25 mm and 22.75±0.89 mm; and in permeate 2.00±0.50 mm and 1.00±0.50 mm, for cellulase and laccase, respectively (Table 1). The cellulase and laccase activity increased by 3.2 times and 2.25 times, respectively. The activities recorded in the quantitative analysis were 1022.21±4.84 U/mL (Figure 2(a)) for cellulase and 13.21±0.05 U/mL for laccase (Figure 2(b)). Cellulase and laccase in permeate were nearly the same as in non-concentrated CEE were 251.30±1.56 U/mL and 2.95±0.08 U/mL, respectively. The increase in activity observed was 300.34% for cellulase and 173.56% for laccase. As expected, the cellulase and laccase activity in the permeate stream decreased by 1.58% and 38.84%, respectively, compared to the feed enzyme activity. In comparison to other techniques like ultra-centrifugation etc., TFF is a robust, scalable, time-efficient, and reproducible method, especially for large volume samples as experienced during our study (Busatto et al., 2018). Narra et al. (2016) and Rajeeva

and Lele (2010) found the recovery of cellulase and laccase via TFF to be 79-84% and 97%, respectively. Recovery rate of CEE from the optimized process during the present study was found to be 98%, with a processing time of 10 min/L. Hence, concentrating enzyme by TFF is an efficient method in terms of efficiency, consistency, reliability, and stability. The concentrated enzyme cocktail post TFF has the potential to be used for various industrial applications like anaerobic digestion, textile industry, food industry, etc. However, economic feasibility is the study that should be focused on in the future.

Table 1. Qualitative analysis: Measured diameter of the enzyme activity of the samples before and after TFF concentration

Sample	Activity diameter (mm)	
	Cellulase	Laccase
Initial	1.2	2
Retentate	14	25
Permeate	0	0

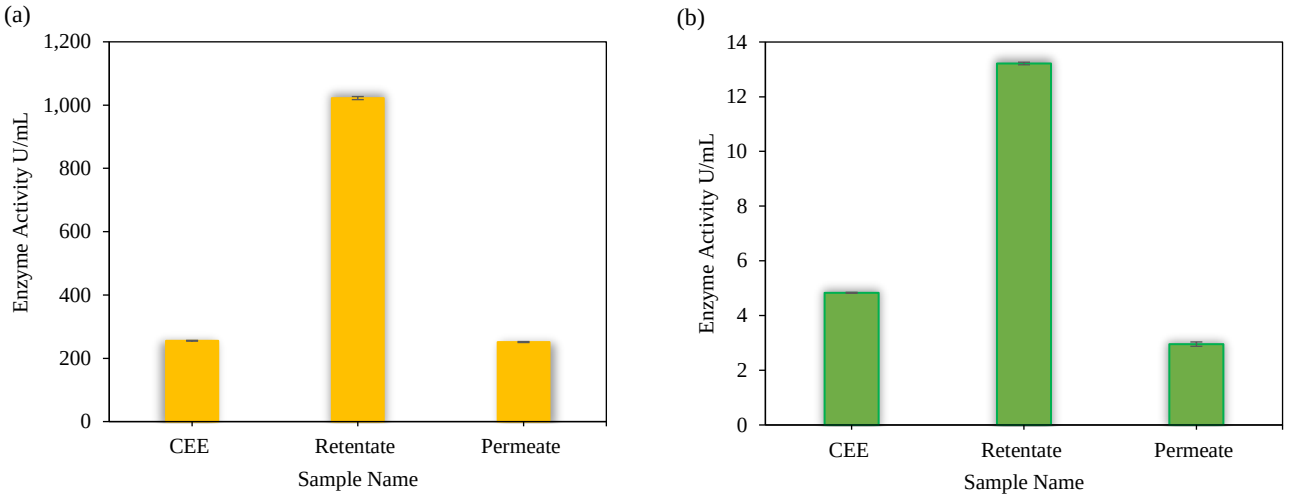

Figure 2. Quantification of enzyme activity using, (a) CMCase-DNS method, (b) by oxidation ABTS; comparing activity between CEE, retentate, and permeate

3.3 Dye decolourization

Laccase is a versatile enzyme as its applications range from lignin degradation to bioremediation. In this study, the ability of CEE to decolorize CR was explored. It is the most extensively used dye in the textile industry, making it the major constituent of the effluent. Hence, the removal of CR dye is a crucial step in treating textile industry wastewater. Laccase proves to be the most efficient in dye decolorization, where the pH of the reaction mixture plays a vital role during the CR decolorization process.

From the graph shown in Figure 3(a), it can be seen that there was an exponential increase in the dye removal rate at pH 4.8 as compared to that achieved at pH 6.0. On the contrary, Suwannawong et al. (2010) found the optimum pH for decolorization was 6.0-7.0 (Suwannawong et al., 2010). The percentage of dye removal achieved at pH 4.8 with enzyme solution concentration being 1% was around 50% after 8 h incubation, while the removal reached 25% after 40 h incubation at pH 6.0. The possible reason is that the optimum pH at which activity is maximum for laccase is acidic pH ranging pH 3.0 to 5.0 (Heinzkill et al., 1998; Madhavi and Lele, 2009), and pH >6.5 inhibits decolorization rate (Asses et al., 2018). From the graph shown in Figure 3(b), it can be seen that with the increase in the enzyme concentration from 0.25% to 0.5%, the maximum dye removal percentage after 70 h incubation increases (i.e., from 24% to 35%) at pH 6.0. However, a further increase in the enzyme concentration to 0.75% and 1% does not cause any further increase in the maximum dye removal percentage. Therefore, from Figures 3(a) and 3(b), it can be concluded that pH 4.8 is the optimal pH for achieving better removal rates. Figure 3(c) and Figure 4 shows the results of dye removal experiments carried out at pH 4.8 at different CEE concentrations. From the graph, it can be seen that the removal trend with respect to time for all the enzyme solution concentrations is the same. The maximum removal is achieved after 24 h. However, in comparison, there is no significant difference in the removal achieved after 16 h. Further, increase in the enzyme concentration (0.25-2.5%) increases the enzyme removal rate up to a concentration of 5%. Further increase in the enzyme concentration does not have any effect on the dye

removal percentage. Also, the faster removal rate is achieved after 8 h at 2.5% enzyme concentration. Figure 3(d) illustrates that with agitation decolorization rate of 49% attained in eight hours, which is approximately double compared to the set kept without agitation, which showed only 23% of decolorization in eight hours. The dye removal percentage achieved in our study was significant and higher than that reported by Das et al. (2016) which was 36.84% decolorization after 20 h incubation at 35±2°C (Das et al., 2016). Thus, from the obtained results, it can be inferred that pH 4.8 is the ideal pH for decolorization of congo red. Also, the optimum pH for laccase enzymatic activity is 5.0 (Kumar et al., 2016). Therefore 4.8 pH is ideal for the enzymatic mediated decolorization process.

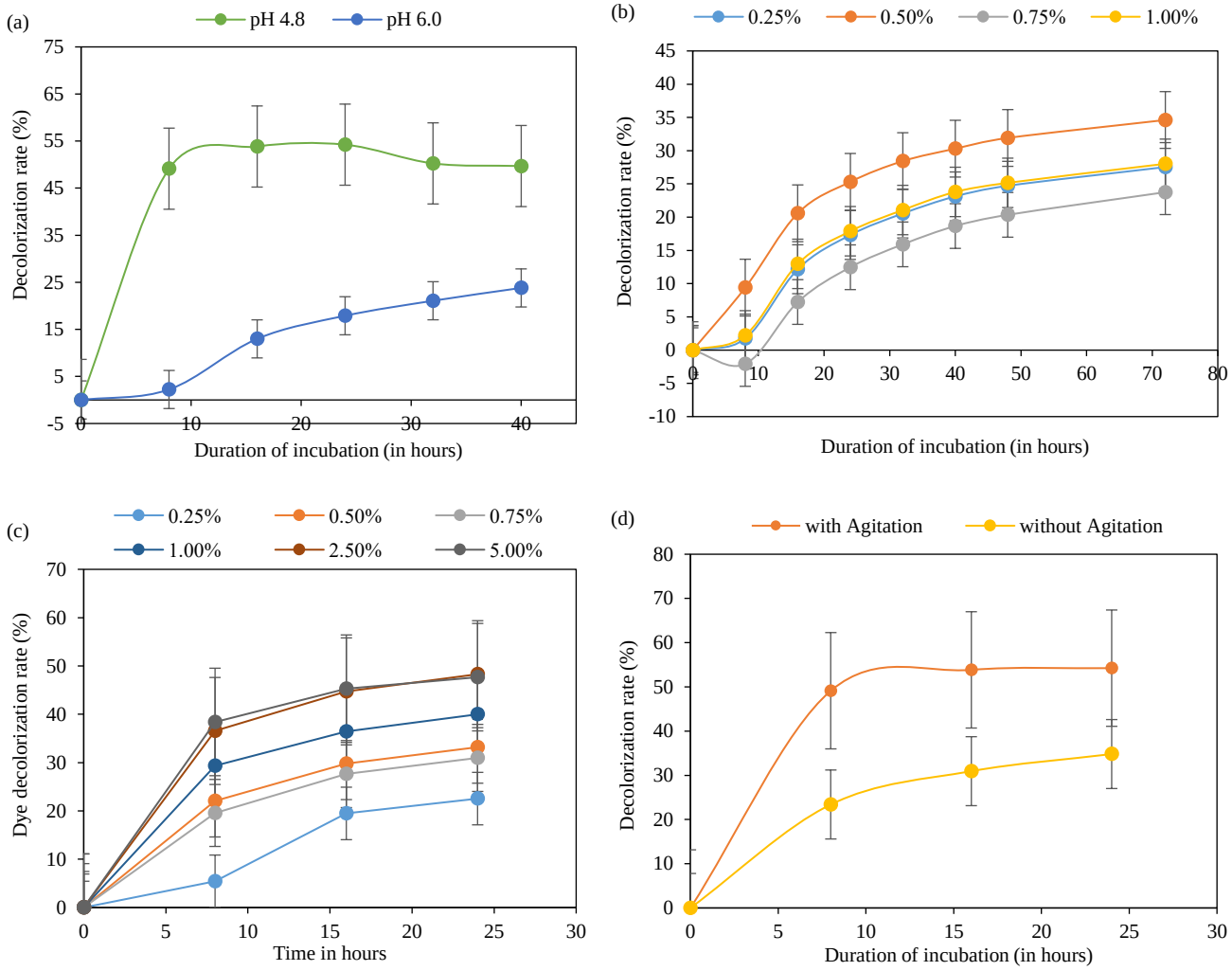

Figure 3. (a) 1% concentrated enzyme (TFF retentate) used for dye decolorization of 0.01% congo red at pH 4.8 and pH 6.0; (b) Dye decolorization rate (%) of 0.01% congo red at pH 6.0. Concentrated or TFF retentate enzyme sample was added at 0.25%, 0.50%, 0.75% and 1.00% different concentration; (c) Dye decolorization rate (%) of 0.01% congo red at pH 4.8. SMC CEE TFF-R is the concentrated or TFF retentate enzyme sample. A range of 0.25% to 5.00% - different concentrations of enzyme used; (d) Effect of agitation on decolorization rate of 0.01% congo red using 1.0% SMC CEE TFF-R enzyme in both cases

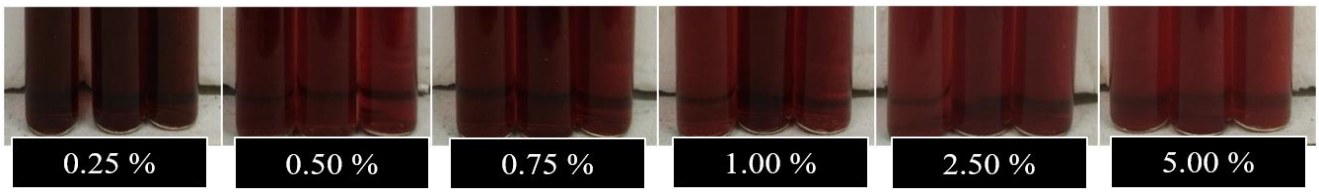

Figure 4. Different concentrations of SMC CEE TFF-R enzyme showing congo red decolorization at pH 4.8 after 8 h of incubation at room temperature

As congo red is a diazo dye, the degradation or decolorization is a complex process. Chakravarthi et al. (2021), demonstrated that the CR is attacked by laccase enzyme by asymmetric cleavage of azo bonds which is then followed by oxidative cleavage, desulfonation, deamination, and demethylation, yielding the end product of benzene dicarboxylic acid and CO_2.

3.4 Briquettes from mushroom bed

The characterization of SMS is summarized in Table 2. As mentioned, SMS consist of 47% moisture, which needs to be decreased to a minimum of 10% before using it as a raw material for briquette production.

Table 2. SMS characterization

Parameter	Value
Moisture on a wet basis (%)	47
Ash (dry) (%)	14
Volatile (dry) (%)	66
Fixed carbon (dry) (%)	21
Ash fusion (°C)	1,100
Bulk density (kg/m^3)	140
Size (mm)	>3 mm 66%
	<3 mm 34%
Ignition test	Burns easily
Flow ability test	Flows easily
Calorific value on dry basis (kcal/kg)	4,000

The presence of high moisture content makes SMS less suitable for briquette production until now. A drying step has been added here before starting briquette production. For moisture reduction, two parameters that play essential roles are the temperature and rotation speed of the drum.

The lignin within the material (SMS) gets activated at a high temperature. The material temperature rises due to the friction caused by the material with the carbon steel die on the application of the pressure by the ram. This raises the temperature inside the die, and the lignin binds the material from outside. The die has to be cooled with circulating water in its shell to keep the material from charring. With proper moisture levels, the briquetting happens, and the ultimate output was little on the harder side.

WC was mixed with SMS to decrease ash and volatile matter content, which increases the calorific value and lignin content of the final product, i.e., briquette. The briquettes with 65% SMS and 35% WC are the finest among others, as it showed the highest calorific value of 4,143 Kcal/kg and the lowest ash content of 12.60% compared to briquettes prepared with only SMS, and 50% SMS and 50% WC (Table 3). Increasing the percentage of WC for making briquette showed an increment in moisture content and production cost. Briquette from SMS and WC prove to be superior in terms of calorific value compared to routinely used raw materials like sawdust, paper, wood, plastic, coal, rice husk, and others (Table 4). Some of the different briquettes, along with their calorific value in comparison with the SMS briquette, are listed in Table 4 (Demirbas, 1999; Yaman and Kuc, 2001; Olorunnisola, 2007; Kers et al., 2010; Sing and Aris, 2013; Garrido et al., 2017).

Apart from the competent physical attributes of SMS briquettes, they are cheap and cost-effective also. The cost of briquette production with SMS is 3,500 INR per ton, which is lesser than 50% of the cost of briquettes made of rice husk, wheat straw, cocopeat, wood dust sawdust that comes to 8,000 INR per ton (Source: Kushi Bio Fuel Briquettes, Goa (India)-commercial company).

Table 3. Comparison between different types of briquettes produced from SMS

Parameter	Only SMS	65% SMS and 35% WC	50% SMS and 50% WC
Moisture (%)	5	10.81	12.32
Ash content (%)	17	12.60	15.59
Volatile matter (%)	66	72.04	70.81
Gross calorific value (Kcal/kg)	3,600	4,143.46	4,244.47

Table 4. Calorific value comparison between different types of briquettes

Raw material for briquetting	Calorific value (MJ/kg)	References
Palm kernel shells	19.38	Sing and Aris (2013)
Palm fiber	18.08	Sing and Aris (2013)
Coal/lignite	16.28	Sing and Aris (2013)

Table 4. Calorific value comparison between different types of briquettes (cont.)

Raw material for briquetting	Calorific value (MJ/kg)	References
Coconut husk	18.80-20.80	Olorunnisola (2007)
Straw	17.30	Demirbas (1999)
Waste paper	16.40	Demirbas (1999)
Rice husk (biocoal)	17.58-18.13	Demirbas (1999)
Lignite	19.80	Yaman and Kuc (2001)
Wood, carton, paper, plastic and textile	26.14	Kers et al. (2010)
Automotive shredder residues	18.75	Garrido et al. (2017)
Print circuit board	11.08	Garrido et al. (2017)
Sawdust	18.90	Garrido et al. (2017)
Palm trunk	21.00	Garrido et al. (2017)
Cover (wire)	13.15	Garrido et al. (2017)
Insulation (wire)	10.40	Garrido et al. (2017)
Molasses	17.50	Yaman and Kuc (2001)
Olive refuse	21.40	Yaman and Kuc (2001)
Pine cone	18.10	Yaman and Kuc (2001)
Paper mill waste	13.00	Yaman and Kuc (2001)
Cotton refuse	17.50	Yaman and Kuc (2001)
Sawdust	16.70	Yaman and Kuc (2001)
Rice husk, wood dust and saw dust	10.46	Kushi Bio Fuel Briquettes, Goa (India)
Spent mushroom substrate (SMS)	15.06	Zycon ReNew Energy, Goa (India)
SMS and wood chips	17.33	Zycon ReNew Energy, Goa (India)

3.4 Mushroom farm waste management

Annual waste production of 15,000 tons at the mushroom farm can be utilized for enzyme recovery and produce 2,034 L/day of enzyme solution possessing 1,022 U/mL of cellulase and 13.21 U/mL of laccase. The remaining biomass can be used to generate 24,000 kg of briquettes per day.

4. CONCLUSION

The study presents the potential direct and indirect use of SMS, which would otherwise be disposed of in a landfill. The extraction of lignocellulolytic enzymes was achieved and application of the extracted enzymes were demonstrated in terms of decolorization of CR dye. The crude extract consisting mainly of cellulase and laccase enzymes showed lower activities and hence was concentrated by TFF. TFF enhanced the cellulase and laccase enzyme activity by 4.0 fold and 2.74 fold, respectively. The concentrated CEE achieved decolorization of congo red dye exhibiting its potential application. The removal percentage achieved by 2.5% enzyme solution after 8 h incubation was 40%. Agitation enhanced the removal to 49% even at lower concentration enzyme solution (1%). The direct use of the SMS after enzyme extraction was also demonstrated. The briquette obtained from SMS post enzyme recovery with wood chips as binding material was found to have a calorific value of 4,143 Kcal/kg, making it a potential green energy fuel. The low production cost of 3,500 INR per ton to the briquette economic option of fuel too. In the future, the CEE can be studied for its ability to treat textile industry wastewater. Overall, the study proposes a process to obtain value-added products from the SMS.

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