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Research article

Conversion of lignocellulosic biomass: Production of bioethanol and bioelectricity using wheat straw hydrolysate in electrochemical bioreactor

Akansha Shrivastava, Rakesh Kumar Sharma *

Department of Biosciences, Manipal University Jaipur, Jaipur 303007, Rajasthan, India

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ABSTRACT

The present study evaluated efficiency of wheat straw (WS) hydrolysate obtained through fungal pre-treatment to produce ethanol and electricity in an electrochemical bioreactor. Three white rot fungi *Phanerochaete chrysosporium, Phlebia floridensis* and *Phlebia brevispora* were used to degrade WS for hydrolysate preparation, Lignocellulolytic enzyme production was also monitored during the pretreatment. Yeast *Pichia fermentans* was allowed to ferment all three hydrolysates up to 12 days. The yeast showed maximum electrochemical response as open circuit voltage (0.672 V), current density 542.42 mA m^{-2}, and power density of 65.09 mW m^{-2} on 12th day in the hydrolysate prepared using *Phlebia floridensis*. Maximum ethanol production of 9.2% (w/v) was achieved on 7th day with a fermentation efficiency of about 62.1%. Further, the coulombic efficiency improved from 0.06 to 1.46% during 12 days of the experiment. Thus, the results indicated towards the possible conversion of lignocellulosic biomass into bioethanol along with bioelectricity generation.

1. Introduction

The unceasing usage of fossil fuels has led to a worldwide energy crisis and increase in CO_2 emission. Therefore, quest towards sustainable alternative energy sources is solicited. In this regard, sustainable plant biomass is an emerging feedstock for biofuel production, such as biodiesel, biobutanol, bioethanol, and power generation medium [1]. However, most of the production is achieved through sugar and starch containing first generation biomass such as sugarcane and grains. Bio-based product market expansion is being driven by an overall shift towards green and sustainable products with little or no environmental impact. This will increase the market share of lignocellulosic biomass feedstock to 25% by 2030 for the production of bio based products [2]. Some countries such as United states of America, India, China, Russia, German, Brazil, among others have framed a target of generating bioenergy products for the implementation of the programme on Energy from urban, agricultural, and industrial waste residues recently [3]. The Indian National Biofuel Policy-2018 offers an indicative goal of achieving 20% (E20) ethanol blending in petroleum by 2030 [4].

Recently, the plant-based lignocellulosic biomass (LCB) has appeared as carbon-free emission and renewable energy source in nature [5]. The biomass is easily degraded into its constituent sugars through pretreatment methods. The produced sugars from lignocellulosic hydrolysates are further used as a substrate to produce organic acids, biofuels, and power generation from Microbial Fuel Cells (MFCs) [6]. These fuels are produced from renewable sources and currently researched to provide alternatives over

* Corresponding author.,
E-mail addresses: rakeshkumar.sharma@jaipur.manipal.edu, r.18sharma@gmail.com (R.K. Sharma).

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petrochemical fuels. Therefore, as an alternative source of energy, utility of bioethanol is continuously increasing. However, the issue of 1st generation based bioethanol production competing with food has sparked a serious debate on its sustainability [7], particularly because sugarcane cultivation necessitates highly fertile soils with high rainfall and/or irrigation. As a result, the most abundant source of biomass is lignocellulosic waste, which has been highlighted as a potential substrate for biofuels, biochemical, and bioelectricity generation. LCB is mostly made up of lignin, hemicellulose, and cellulose. The lignocellulose is a compact, polymers-based partially crystallized structure interconnected by covalent and non-covalent bonds. The cellulose supports crystalline structure attainability. The hemicellulose polymer consists of arabinose, mannose, glucose, galactose, and xylose while organic acids include acetic acid, ferulic, p-coumaric acid, and glucuronic acid [8]. The presence of hemicelluloses increases the overall stability of lignocellulose. Whereas, the lignin is composed of phenylpropane units, comprising coniferyl, sinapyl, and coumaryl alcohol. Its chemically stable and complex structure of biomass makes it hard to utilize it in a natural form.

The complex structure consists of cellulose, hemicellulose, lignin and recalcitrant nature of LCB is the primary impediment to its valorisation into bioethanol and other products of value [9,10]. The effectiveness of a bioconversion process depends on the pre-treatment, hydrolysis, and fermentation techniques employed. Most of the traditional pretreatment methods such as alkali, acids, steam explosion, ionizing radiations, microwave, or some other combined processes required special instruments and require a lot of energy and generate inhibitors which affects the hydrolysis and fermentation processes [11]. On contrary, biological pretreatment naturally uses metabolite of microorganisms for enhancing saccharification rate sustainably by consuming less energy and less environmental damage [12]. During this method, no toxic chemicals are used, which again does not include another step of recycling chemicals without release of any toxic compounds into the environment.

Natural degradation of LCB is carried out by several bacteria and fungi. Fungi specifically white rot fungi (WRF) are the best producers of selective ligninolytic enzymes i.e. laccase, lignin peroxidase (Lip), manganese peroxidase (MnP), along with some cellulolytic enzymes. Degradation of LCB by WRF results in physicochemical changes, which may enhance its nutritional value as animal feed [13]. Thus, these fungi are the most suitable candidates for hydrolysate preparation from LCB. WRF are known as selective lignin degraders. However, these fungi also produce cellulolytic enzymes along with lignin degrading enzymes. WRF were the choice in this case because the cellulose, which is protected by the lignin-hemicellulose matrix, can be degraded by this fungus and simultaneously the cellulolytic enzymes can also degrade cellulose. Another important point is that this 'one fungal pre-treatment' can solve both the problems i.e., lignin degradation and the production of monosaccharides and other soluble sugars, which may further be used by the yeast. Thus, it completely avoids the use of any harsh chemical treatment for lignin removal being completely eco-friendly.

LCB hydrolysate contains pentose and hexose sugars along with some phenolics and amino acids. Hexose sugars are quite easily fermented by different yeast strains. On the contrary, pentose sugar utilization and fermentation, requires a specific yeast or bacterial strains. Temperature and pH also play a vital role in providing the required conditions for fermentation. Other factors including yeast growth rate, stability, tolerance towards inhibitors, and stress, along with ethanol production. Microorganisms i.e. *Saccharomyces cerevisiae* and *Zymomonas mobilis* have demonstrated high ethanol tolerance, consume hexose sugars, and exhibit high rates of fermentation, however, at the same time unable to ferment pentose [14]. Moreover, some yeasts e.g. *Pichia* spp., *Pachysolen* spp., and *Candida* spp. can ferment pentose and hexose sugars with high ethanol yield. Thus, *Pichia fermentans* being an exoelectrogenic, non-pathogenic and wine producing organisms was used in the experiment.

Over recent years, MFCs have emerged as a promising technology for harnessing energy from organic matter. MFCs are enthralling biological fuel cells which utilizes microbial metabolism to convert chemical energy into electrical energy from biomass. Electron transfer from the microbial cells to the anode is either direct through cytochromes and presumed nanowire structures or indirect through soluble redox active mediators [15]. Moreover, MFCs could also be used to generate electricity from cellulosic sugars. Sugars are the solitary carbon source widely employed in bioelectricity generation [16]. The presence of fermentative and exoelectrogenic microorganisms or mixed culture may help in biofuel production and bioelectricity generation. Bacterial strains such as *Shewanella* spp., *Pseudomonas* spp., *Geobacter* spp., *Clostridium* spp., *Aeromonas* spp., *Enterococcus* spp., are among the exoelectrogens that are efficiently involved in electricity generation in MFCs [17].

In this regard, electrons released during the fermentation process are harvested and co-production of electricity is achieved during bioethanol production could improve the energy efficiency of sugar-containing hydrolysate or biomass utilization. This is a cost-effective, green, and sustainable energy recovery technology. *Phanerochaete chrysosporium* and *Phlebia* spp., can produce more complete ligninolytic enzyme complex activity for oxidoreductive reactions, conversions, and lignin degradation. Thus, the present study demonstrates fungal pre-treatment of wheat straw by three different WRF as primary fermentation. All the three hydrolysates were produced from WS during the primary fermentation and utilized as substrate or fuel for electricity generation and ethanol production during secondary fermentation in an electrochemical reactor.

2. Materials and methods

2.1. Microorganisms and substrate

Wheat straw was obtained from agricultural areas of Bagru, Jaipur, India. The oven-dried WS (60 °C for 48 h) was milled and stored for further use. The fungal cultures *Phlebia floridensis* (HHB-7157), *Phanerochaete chrysosporium* (BKM-F-1767), and *Phlebia brevispora* (HHB-7024) were obtained from the Centre for Forest Mycology Research (USDA), Madison, Wisconsin, USA. These cultures were grown and maintained on malt extract agar as previously done [18]. Yeast culture *Pichia fermentans* (MTCC 189), was obtained from Microbial Type Culture Collection, Chandigarh, India and maintained at 30 °C on yeast peptone dextrose agar.

2.2. Preparation of wheat straw (WS) hydrolysate

An Erlenmeyer flask of 250 ml volume containing 2.5 g of dried WS, and 50 ml distilled water were closed with cotton plug and sterilized (at 1.06 kg cm^{-2} stream pressure) for 15 min. The flasks were then inoculated with 4 agar discs (6 mm diameter) containing mycelia of *P. brevispora, P. floridensis*, or *P. chrysosporium.* An uninoculated flask served as control. All the flasks were incubated (static condition) at 28 °C for 15 days. Each flask was then added with 50 ml of sterile distilled water and kept for 4 h at 200 rpm in a shaking incubator. After incubation, the content from the flasks was filtered using sterile Whatman filter paper No. 1 under aseptic conditions. The filtrate thus obtained called as hydrolysate. Whereas, the dried residues of degraded WS (65 °C for 48 h) were weighed to estimate the amounts of remaining biomass and referred as Total organic matter (TOM) and analyzed for its contents through sequential fractionation.

2.3. Analytical methods

2.3.1. Sequential fractionation

Sequential fractionation of degraded and control WS was carried out as described earlier [19]. After pre-treatment, 1 g of WS was added in 50 ml distilled water and treated at 100 °C in water bath for 1 h, then cooled at room temperature. The contents were filtered and the left behind residual biomass that remained was dried at 60 °C. Weight loss in the content represents water-soluble fraction. Again, the same dried residue was treated for 1 h at 100 °C with 50 ml of 0.5 M H_2SO_4. The contents of the flasks were filtered, cooled to room temperature, and then dried. The loss in this step was considered as hemicellulose fraction. After this, dried residues from the previous step were suspended in 5 ml of 72% (v/v) H_2SO_4 and kept in a water bath for 30 °C for 1 h. The content was then made up to 4% (v/v) of H_2SO_4 and kept in steam under pressure (1.06 kg cm^{-2}) in an autoclave for about 20 min. Then the content was again allowed to cool, filtered, dried, and weighed as mentioned in previous steps. The residue was presented as lignin and the loss in this step was considered as cellulose.

2.3.2. Activity detection of crude enzyme

The laccase (EC 1.10.3.2) estimation for delignification was assayed using guaiacol as substrate and was expressed as colorimetric units per ml (CU ml^{-1}) at 450 nm of absorbance [20]. The lignin peroxidase (EC 1.11.1.14) assay was estimated by the oxidation of Azure B in the presence of H_2O_2. The enzyme activity was expressed in 0.1 Unit min^{-1} ml^{-1} as an absorbance decrease. The CMCase or carboxymethyl cellulase assay for cellulase (EC 3.2.1.4) estimation was carried out by using the DNS method [19,21].

2.3.3. Total phenols and protein estimation

The total phenolic contents were estimated using Folin-Ciocalteu (FC) reagent, while gallic acid was used to compare [22]. The protein content was evaluated by following Lowry's method and using Bovine Serum Albumin as standard [19].

2.3.4. Gas chromatography (GC) characterization

The compositional analysis of WS hydrolysate was carried out using Shimadzu GCMS-QP2020. N-Methyl-N-(trimethylsilyl)trifluoroacetamide (MSTFA) and methoxyamine hydrochloride are used as derivatization reagents [23]. The gas flow rate was kept 1.17 ml min^{-1} and linear heating (temperature rise; 4 °C min^{-1}) from 40 °C to 290 °C was selected for the oven program (to confirm no residuals retention), proceeded with a hold at 290 °C for 5 min.

2.4. Single chambered experimental setup

A glass flask with a working volume of 150 ml was used to construct a single-chambered MFC setup. Three different hydrolysates (prepared from WS degraded by *P. chrysosporium, P. floridensis*, and *P. brevispora*) were inoculated with 24 h old 10 ml culture of *P. fermentans* (1 OD_{600}). Bow-shaped carbon fiber was used as an anode, while circular stainless steel wire was used as the cathode. The electrodes were sterilized by using ethanol (70% v/v) and washed with sterile distilled water and then dried under sterile air flow and UV. Both the electrodes were aseptically inserted in MFCs. All the setups were incubated for 12 days at 30 °C ± 2 °C. All setups were in triplicate and repeated with adequate controls.

2.5. Electrochemical evaluation

The electrochemical response was recorded regularly across various external resistors (R_{ex}) extending from 100 Ω to 820 kΩ using a digital multimeter. The difference in electrical potential between two terminals of a fuel cell without external load was referred as open-circuit voltage (OCV). The polarization curves were prepared at varied time intervals against the external resistors (R_{ex}). The polarization curve depicts the fuel cell output voltage for a particular current density loading. Current (I) was calculated using formula V=IR, where V stands for cell voltage and R stands for external resistance. The current and power densities were calculated using anode area (2.2 cm^2) whereas, the power was evaluated by formula $P = VI$. The Coulombic efficiency (CE) was calculated using $CE = CE_x \times 100/C_{TH}$ [24]. Internal resistance (R_{int}) was calculated from $R_{int} = R\,(E/V - 1)$, where R stands for external resistance, E stands for OCV or voltage without any resistance, and V is output voltage with resistors. All of the data in the publication is presented as an average of three replicates, along with the standard error.

2.6. Total reducing sugar and ethanol estimation

The concentration of total sugars available in hydrolysate was routinely estimated by following the dinitrosalicylic acid (DNSA) method [25]. The sugar concentration was calculated from glucose as standard and was stated as mg ml^{-1} sample. Ethanol production was monitored by using Agilent HPLC 1260 II INFINITY with auto-sampler and C 18 column (5 μm; 25 × 0.46 cm) and the output was recorded daily as described earlier [26].

2.7. Thermogravimetric analysis (TGA)

Thermal decomposition of untreated and pretreated WS was carried out using a DTG60H thermogravimeter (Shimadzu, Japan). The WS samples were placed under heating in an alumina crucible for temperatures ranging from 30 to 900 °C [27]. An increase in heating rate as 10 °C min^{-1} was maintained throughout under nitrogen atmosphere during the entire process.

3. Results & discussion

3.1. Wheat straw degradation and hydrolysate preparation during primary fermentation

3.1.1. Lignocellulolytic enzyme production

WRF are prominent producers of different lignocellulolytic enzymes. Laccase, lignin peroxidase, and cellulase production by *P. floridensis*, *P. brevispora,* and *P. chrysosporium* was monitored during 15 days of incubation. *P. chrysosporium* did not show laccase production under the experimental conditions. On the other hand, the maximum laccase (0.433 CU ml^{-1}) was produced by *P. floridensis* on the 5th day of incubation, which further showed a gradual decrease from 0.349 to 0.252 CU ml^{-1} during further incubation. In the case of *P. brevispora*, it gradually increased from 0.171 (5th day) to 0.233 CU ml^{-1} (10th day) and decreased slightly down to 0.182 CU ml^{-1} on the 15th day (Fig. 1a).

All the three fungi showed the lignin peroxidase (Lip) activity. The Lip production showed a gradual increase up to 10th day by all the three fungi, which remained constant thereafter (Fig. 1b). These results indicated that these enzymes may be involved in lignin depolymerization, even at the secondary growth phase. Earlier, fungal pre-treatment of corn stover using different WRF demonstrated efficient ligninolytic enzymes production and reported maximum laccase production of 29.22 U g^{-1} and cellulase production of 15.50 U g^{-1} of dry biomass [28]. An earlier study evaluated the complete extraction and concentration of ligninolytic enzymes secreted by selected fungi during solid-state fermentation. Maximum per unit laccase (14.44 U g^{-1}) production was observed in *Fusarium verticillioides* TERIDB16, while *Alternaria gaisen* TERIDB6 produced the most LiP (137.42 U g^{-1}) [29]. The distillate was then used to test the efficacy of its decolorization of undiluted distillery effluent. In the present study, all three WRF demonstrated significant production of lignocellulolytic enzymes, with *P. floridensis* exhibiting the highest enzyme production efficiency. In 2013, a study was conducted on switchgrass in which the fungus *Pycnoporus* sp. SYBC-L3 produced ligninolytic enzymes. The study revealed maximum laccase and Lip production of approximately 8.8 U g^{-1} and 1.6 U g^{-1}, respectively [30].

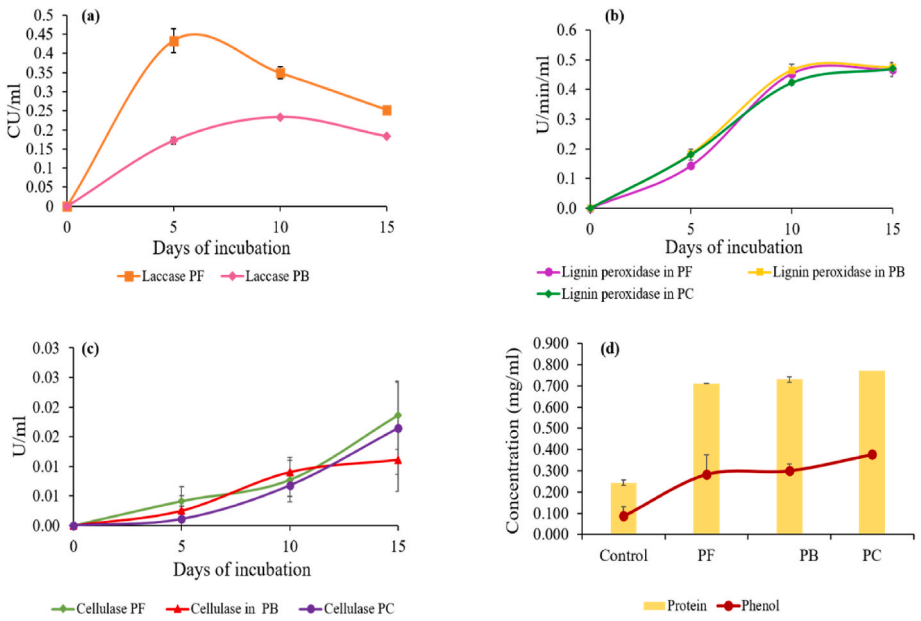

Fig. 1. (a–c) Laccase, lignin peroxidase, and cellulase production by *P. floridensis* (PF), *P. brevispora* (PB), *P. chrysosporium* (PC) during pre-treatment and, (d) phenol and protein content after 15 days of incubation.

CMCase production was observed with a gradual increase up to the end of the pre-treatment (15th day) (Fig. 1c). This enzyme was maximally produced by *P. floridensis* on the 15th day followed by *P. brevispora* and *P. chrysosporium*. As cellulase hydrolyse β-1,4-glycosidic linkage of cellulose to form glucose [31]; this trend also confirms that ligninolytic enzymes were most active during initial phase of degradation, while cellulolytic enzymes were active during secondary phase of degradation. It also supports the selective ligninolytic potential of selected fungi.

3.1.2. Total phenols and protein contents

A gradual increase in phenolic compounds were observed during the experimental period. The maximum phenolic content was released by *P. chrysosporium* (0.377 mg ml^{-1}) followed by *P. brevispora* 0.300 mg ml^{-1} and *P. floridensis* 0.283 mg ml^{-1} (Fig. 1d). The total protein content in the pre-treated wheat straw hydrolysate was found maximum in *P. chrysosporium* 0.770 mg ml^{-1}, *P. brevispora* (0.730 mg ml^{-1}), and *P. floridensis* (0.710 mg ml^{-1}), respectively. The pretreatment method and the feedstock have a substantial impact on the production of by-products. The cellulose, hemicellulose, and lignin in lignocellulosic biomass are often broken down during pretreatment, producing pentose and hexose sugars, sugar acids, aliphatic acids, furan aldehydes, and other phenolic group chemicals [32]. The ligninolytic enzymes during the pretreatment of lignocellulosic biomass oxidize lignin to form various phenolic compounds. The increase in phenolic content reveals that ligninolytic enzymes degraded the complex lignin polymer and released several smaller phenolic units. The presence of phenolic compounds in the hydrolysate boosts power generation and conversion efficiency. The positive effect was most likely due to phenolics aromatic ring structure, which can accept an extra electron and thus shift between oxidised and reduced states [33]. This enhancement in total phenolic content can be correlated with the power output and internal resistance reduction as their involvement in the electron transport is well-known [34]. These phenolic units may act as a mediator in the peroxidase enzyme system during LCB degradation. Further, in hydrolysate, this mediator system facilitates the transport of electrons to anode and the oxidation of complex substrate and non-phenolic compounds [35].

3.1.3. Sequential fractionation

Total organic matter (TOM) obtained after WS pre-treatment for 15 days consisted of a complex mixture of degraded LCB along with fungal biomass. Since TOM loss during saccharification of straw is mainly attributed to the fungal decomposition of lignocellulose. Therefore, the decrease in TOM can reflect the degree of WS degradation. Sequential fractionation of control (undegraded WS) revealed that it contained 6% water solubles, 10% hemicellulose, 58% cellulose, and 26% lignin. The carbohydrate contents of WS consists of hemicellulose and cellulose, which is collectively termed as holocellulose. Agricultural holocellulose contains quite a high amount of hemicellulose as compared to forestry holocellulose. During the fungal pretreatment for hydrolysate preparation, hemicellulose was converted to fermentable sugars such as galactose, arabinose, mannose, and xylose. Cellulose polymer consists of many glucose units having β-1, 4-glycosidic linkages. The hydrolysis of such polymers results in the sugar molecule glucose and oligosaccharides [36]. All three fungi demonstrated their selective delignification and thereby enhanced the cellulose content in TOM. This residual biomass has been suggested to have higher nutritive values and digestibility to be used as animal feed [13].

About 46–50% of WS was degraded by all different fungi (Fig. 2). Sequential fractionation of degraded WS was performed to estimate different biomass contents i. e. cellulose, hemicellulose, lignin, and water-soluble part in degraded WS. Loss or degradation of these constituents were calculated by comparing the residual values from undegraded (control) WS (Fig. 2). *P. floridensis* degraded 50.4% of WS (17% of cellulose, 12.5% of hemicellulose, 18% of lignin and 3% of water-soluble part). Thus, a collective degradation of 29.5% in holocellulose, which potentially have been contribute into production of reducing sugars (17.2%), as estimated by using DNS method. *P. brevispora* degraded 50.8% of WS (16% of cellulose, 15% of hemicellulose, 17% of lignin and about 2% of water soluble). Out of 31% of holocellulose degraded, and 16% was available as reducing sugars in the hydrolysate. Similarly, *P. chrysosporium* degraded 45.6% of WS by degrading almost equal amount (15%) of hemicellulose and lignin, while 12% of cellulose. Out of 27% of

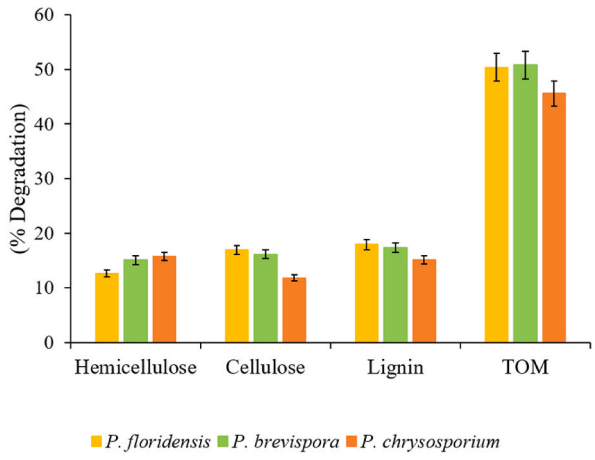

Fig. 2. Degradation of different components of wheat straw biomass by *P. floridensis*, *P. brevispora*, and *P. chrysosporium*.

holocellulose degraded, 15.2% was available in hydrolysate as reducing sugars. *P. floridensis* had the highest saccharification efficiency (reducing sugars released/carbohydrate content of biomass *100) of about 25.3% compared to *P. chrysosporium* and *P. brevispora*, which had efficiencies of 23.5% and 22.3%, respectively. In comparison to selective lignin degrading WRF used in the present study, non-selective WRF readily degrades a significant amount of cellulose polymer, causing the pretreated substrate to be enriched in more recalcitrant cellulose. Whereas, the selective lignin-degrading WRF well-preserved most of the cellulose by removing the cellulose-encapsulating components e.g. xylan, lignin etc.

The holocellulose degradation may result in the release of reducing and non-reducing sugars and oligosaccharides. GC- MS characterization of hydrolysate also revealed that it contained xylose, arabinose, lactose, and talose like sugars. Apart from this, the presence of organic acids i.e. acetic acid, oxalic acid, gluconic acid along with ethylene glycol and glycerol was also confirmed by GC-MS.

3.1.4. Thermogravimetric analysis (TGA)

All the degraded WS samples along with the control were dried before the TGA and then processed. The change in weight loss was examined at a constant rate for its thermal stability and fraction of volatile components. This technique is a useful tool to analyse the thermal behaviour of biomass. It provides detailed information about the pyrolysis and breakdown of the lignocellulosic biomass along with its reaction kinetics [37]. Fig. 3 shows the TGA curves for an untreated and pretreated wheat straw with *P. floridensis*, *P. brevispora*, and *P. chrysosporium*. The thermogravimetric curve is mainly distributed into three diverse phases distinct by percentage loss in the biomass section. Thus, TGA curves could be divided into three diverse phases marked by variation in percentage loss [38].

The wheat straw treated with the *Phlebia brevispora* and *Phanerochaete chrysosporium* might contained some chemicals compounds (proteins, phenolics, enzymes etc.), which are highly reactive in oxidation process and ultimately leads to slight increase in weight during initial phase. The 1st phase of minor biomass loss in the case of *P. floridensis* was observed till 200 °C which inferred to the mass loss mainly due to the dehydration and elimination of volatile constituents. The major fraction of biomass damage was perceived in the 2nd phase ranged from 250 to 450 °C. This major phase marked the loss in two sub-divisions: 1st was between 250 and 350 °C with hemicellulose loss and 2nd between 350 °C and 450 °C with cellulose loss [39]. The 3rd phase of loss in biomass started from 500 °C and continued to 900 °C that could be attributed to a loss in lignin and other heavy components. The complete weight loss was achieved in the control sample which was not achieved by any degraded sample even up to 850 or 900 °C, which point towards the higher ash content in the degraded sample. It was also observed earlier that the ash content in the degraded biomass always increases as that is not degraded by the fungus [13].

The use of fungi to pre-treat lignocellulosic biomass reduces the crystallinity of cellulose, resulting in considerable sugar release during the hydrolysis process [40]. In the present study, it was observed that the reaction rate of *P. floridensis* pretreated WS was higher than the untreated WS (control). Depolymerization allows an accelerated primary reaction in LCB, resulting in the production of primary volatile compounds that certainly escape from the solid phase. However, results also suggest that this also depends on the fungal species used for the pretreatment as the fungal growth pattern, their cell-wall constituents, metabolites, and degradation by-products can influence the results.

3.2. Operation of microbial fuel cell for ethanol production using hydrolysate during secondary fermentation

3.2.1. Electrochemical response

Three different hydrolysates were prepared from all the pretreated wheat straw samples and were inoculated with yeast *P. fermentans* for bioelectricity and bioethanol production. The yeast *P. fermentans* grew under the experimental conditions in all three hydrolysates. The electrochemical performance of all the fuel setups containing hydrolysate showed a gradual increase after 24 h of inoculation. In all the hydrolysates the performance was examined up to 12 days of incubation along with suitable control without yeast. No significant OCV was recorded in the control MFC, which ranged from 15 to 50 mV OCV throughout the experiment. Maximum OCV 0.672 ± 0.014 V was observed for *P. fermentans* in *P. floridensis* hydrolysate on 12th day (Fig. 4). A power density of

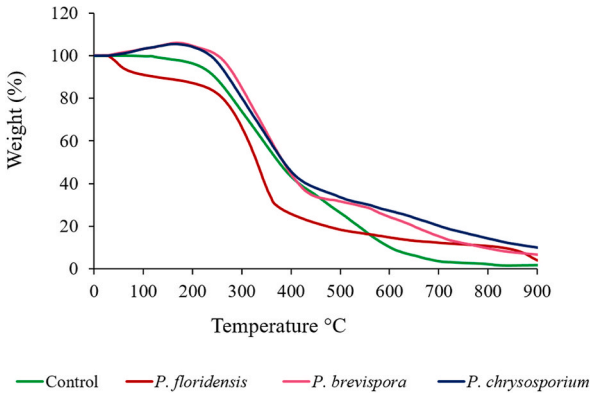

Fig. 3. TGA curve of wheat straw (control) and wheat straw degraded by *P. floridensis, P. brevispora*, and *P. chrysosporium* for 15 days.

65.09 mW m^{-2} was observed in the same setup on same day (Fig. 5a), while the current density was observed from 115.15 mA m^{-2} (1st day) to 542.42 mA m^{-2} (12th day) (Fig. 5b). In *P. brevispora* hydrolysate, *P. fermentans* showed a maximum OCV of 0.594 ± 0.025 V the 3rd day and remain constant, which slightly declined to 0.392 ± 0.040 V on day 9 (Fig. 4). On reaching the 12th day it improved up to 0.549 ± 0.038 V and the maximum power density (50.75 mW m^{-2}) was achieved on 5th day (Fig. 5c). The current density was recorded from 87.87 mA m^{-2} (1st day) to 478.78 mA m^{-2} (5th day), which also decreased and then increased thereafter (Fig. 5d). The maximum OCV of *P. fermentans* in *P. chrysosporium* hydrolysate was observed 0.571 ± 0.024 V on the 6th day, which slightly declined up to 0.452 ± 0.091 V on the 8th day. Whereas, the power density showed its maximum value as 14.25 mW m^{-2} on the 5th day of incubation (Fig. 5e). The current density for the same was from 46.97 mA m^{-2} (day 1) to 427.27 mA m^{-2} (10th day), which slightly declined up to 365.15 mA m^{-2} (Fig. 5f).

The internal resistance (R_{int}) in *P. floridensis* hydrolysate remained similar against various R_{ex} from 100 Ω to 1 kΩ, while it increased on day 1 from 10 kΩ to 100 kΩ and then decreased from 2nd day to 12th day (Fig. 6a). For *P. brevispora* hydrolysate the same pattern was followed against R_{ex} from 100 Ω to 10 kΩ on day 1 and further decreased from the 2nd day (Fig. 6b). In the case of *P. chrysosporium* hydrolysate, internal resistance increased on day 1 and then decreased (Fig. 6c).

A slight increase in internal resistance was observed at 180 kΩ on the 7th day of the study. On the 12th day, a slight increase in internal resistance against external resistance 270 Ω was observed. The coulombic efficiency increased from day 1 and continued increasing till the end of the experiment in all the combinations of hydrolysates with *P. fermentans*. The maximum coulombic efficiency was observed in *P. floridensis* hydrolysate (0.06–1.46%) than in *P. brevispora* (0.04–0.91%) and *P. chrysosporium* (0.01–0.78%).

Though various LCBs have been utilized proficiently for MFC such as rice straw, raw corn stover, barley straw, wheat straw, miscanthus, etc. The MFC efficiently harnesses the energy stored in the biomass through catalytic reactions by microorganisms and converts the chemical energy into electrical energy. The unique electrogenic property of microorganisms enables them to degrade the waste biomass with various metabolic pathways to release electrons and pass them to the anode [41]. The electrons are then transferred to the cathode to generate electric current. This way the biomass directly gets converted to electrical energy in MFC. The biological conversion of chemical energy to electrical avoids the Carnot cycle that limits the energy efficiency [42]. Interestingly, this conversion could be implemented in mild conditions for various other promising applications.

A successful report was published comprising rapeseed hydrolysate as a potential feedstock for electricity generation followed by hydrothermal pretreatment [43]. The maximum power density was observed as 54 mW m^{-2} along with the increased coulombic efficiency. In the present study, the observed maximum power density showed that the yeast *P. fermentans* efficiently utilized the WS hydrolysate for electricity generation. The exoelectrogenic property of *P. fermentans* has also been observed in our previous study with glucose. Another recent study reported the electrogenic activity of *Shewanella marisflavi* BBL25 fuelled with barley straw, pine, and miscanthus hydrolysates [44]. The maximum current density and power density were observed as 6.850 mA cm^{-2} and 52.80 mWcm^{-2} for barley straw hydrolysate. The study further revealed the role of outer membrane-bound cytochromes and redox mediators for electron transfer mechanism. A sludge complemented with LCB such as corn bran, banana peel, and palm oil effluent was studied in a single-chambered MFC. Amongst all, banana peels resulted in a maximum power density of 23.75 mW m^{-2} followed by corn bran (12.65 mW m^{-2}) and palm oil (22.03 mW m^{-2}) [45].

3.2.2. Total reducing sugar consumption and ethanol production

The total reducing sugar utilization and ethanol production by *P. fermentans* using WRF pre-treated hydrolysate were studied in a 100 ml electrochemical bioreactor setup. The reducing sugar in hydrolysate was assessed to be 17.2% (absolute concentration 0.43 g from 2.5 g of WS) and the non-reducing sugars (soluble polysaccharides and oligosaccharides) to be 12.2% (as derived from the results of residual biomass sequential fractionation, based on theoretical calculations), respectively. The total reducing sugar in the hydrolysate prepared using *P. floridensis* was used up during the early days as well as subsequent days of incubation. After 24 h, the amount of sugar-reduced from ~17% (w/v) to 14 ± 0.06% (w/v) in the cell setup with yeast *P. fermentans*, while at the end of the experiment it remained 0.52 ± 0.02% (w/v). A maximum amount of ethanol of 9.2% (w/v) was obtained on day 7 (Fig. 7a) and then it decreased on succeeding days.

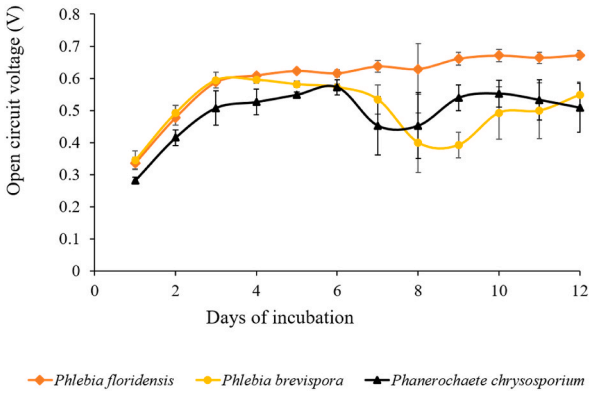

Fig. 4. Open circuit voltage of single chambered setup with *Pichia fermentans* in different hydrolysates.

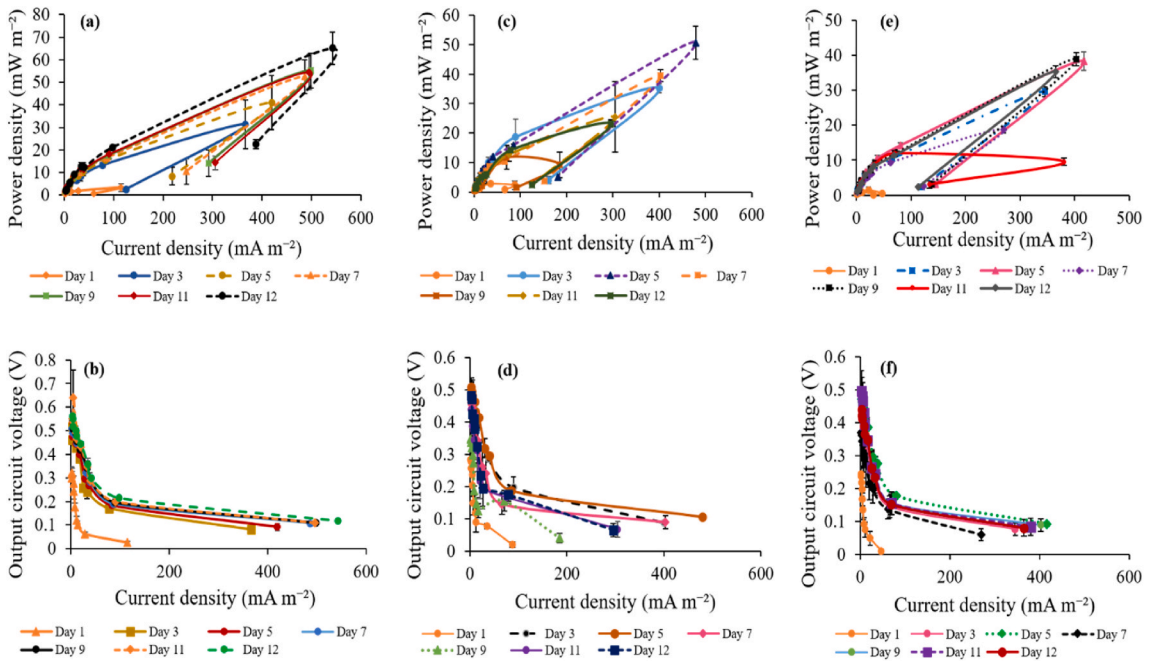

Fig. 5. The maximum power density, output voltage, and current density curves by *Pichia fermentans* (a) and (b) in *P. floridensis* WS hydrolysate; (c) and (d) in *P. brevispora* WS hydrolysate; (e) and (f) in *P. chrysosporium* WS hydrolysate.

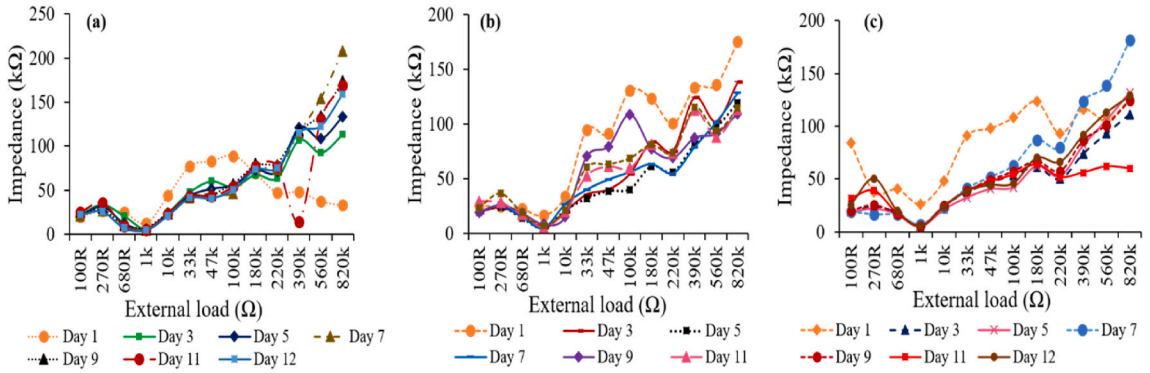

Fig. 6. The internal resistance of cell setup in all the hydrolysates with P. fermentans; (a) in *P. floridensis*, (b) in *P. brevispora*, and (c) in *P. chrysosporium* across different external loads.

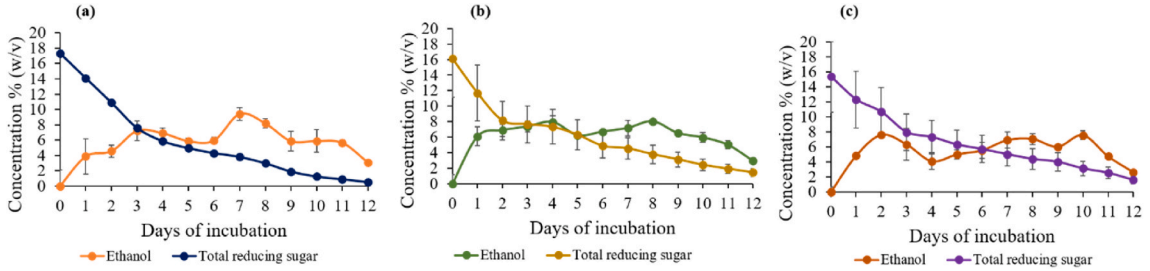

Fig. 7. Total sugar utilization and production of ethanol by *P. fermentans* in WS hydrolysates prepared by: (a) *P. floridensis*, (b) *P. brevispora*, and (c) *P. chrysosporium*.

In the case of WS hydrolysate from *P. brevispora*, the total reducing sugar was also utilized gradually by the yeast. The reducing sugar in hydrolysate was assessed to be 16.1% (absolute concentration 0.40 g from 2.5 g of WS) and the non-reducing sugars (soluble polysaccharides and oligosaccharides) to be 15.1% (as derived from the results of residual biomass sequential fractionation). The sugar concentration reduced from 16 to 11 ± 5% (w/v) in the reactor after 24 h, which remained 1.4 ± 0.4% (w/v) at the end of the experiment. A maximum amount of ethanol of 8% (w/v) was detected on day 8 (Fig. 7b) and then it decreased on the following days. In the case of *P. chrysosporium* WS hydrolysate, the yeast gradually absorbed the whole reducing sugar. The reducing sugar in hydrolysate was assessed to be 15.3% (absolute concentration 0.40 g from 2.5 g of WS) and the non-reducing sugars (soluble polysaccharides and oligosaccharides) to be 12.2% (as derived from the results of residual biomass sequential fractionation). The sugar concentration reduced to 12 ± 3% (w/v) with the yeast *P. fermentans,* and at the end, it was about 1.5 ± 0.4% (w/v) (Fig. 7c). A maximum concentration of 7.5% (w/v) ethanol was produced during the initial days of incubation, which remained almost constant up to 10 days and then decreased thereafter. Conversion of lignocellulosic biomass into fermentable sugars is considered a major technological bottleneck in the production of biofuels [46]. It was observed that the cellulose and hemicellulose obtained from fungal degradation, consist of 29.6% total sugar (theoretical yield), which contained reducing sugar and non-reducing sugar along with other oligosaccharides being 17.2% and 12.4%, respectively. The schematic representation of maximum ethanol production and power generation on 29% (w/v) of total sugar obtained from 100 g of WS was presented in Fig. 8. An initial increase in ethanol production was recorded during the fermentation in all the three hydrolysates. During polysaccharide degradation, the cellulolytic enzymes act on the internal bonds of cellulose by endogluconase, cellobiohydrolases, β-glucosidase or cellobiases. An endogluconase enzyme cut cellulose polymer randomly within the amorphous region. Whereas, the exoglucanase or cellobiohydrolases I and II produce cellobiose with reducing and non-reducing ends. Cellobiases hydrolyzes cellobiose and some oligomers consists up to six glucose molecules [47]. Thus, non-reducing sugar along with the reducing sugar in the hydrolysate might also be utilized and an increase in initial ethanol production was observed.

Different detoxification methods have been used to detoxify sugar beet pulp hydrolysate for ethanol fermentation by *P. stipitis*. The ethanol produced was in the range between 0.108 and 0.122 g g^{-1} [48]. Another recently published study reported bioethanol production by *P. stipitis* NCIM3498 using rice straw hydrolysate. Yeast consumed ~90% of sugars present in hydrolysate and resulted in an ethanol yield of 8.8 g L^{-1} [49]. In our study, *P. fermentans* utilized maximum sugars present in WS hydrolysate and resulted in a maximum ethanol yield of 9.2%. Though it was lower compared to other efficacious studies, it comparatively showed prominent results along with power generation. This showed that non-conventional yeasts are vitally efficient in utilizing sugars present in LCB. However, a recombinant of *P. pastoris* produced a maximum ethanol yield of 0.42 g g^{-1} in WS-based LCB, which was comparatively lower than our study [50]. Earlier, different fungal strains i.e. *Trichoderma reesei*, *Aspergillus niger*, *A. fumigates, A. acculeatus*, and *Fusarium Solani* have been employed for the biological pretreatment of rice straw and *P. stipitis* was used for bioethanol fermentation, which resulted in a maximum ethanol yield of 25.3 g L^{-1} using 55.6 g L^{-1} sugar of rice straw [51].

The xylose-fermenting yeast *Pichia* spp. naturally utilize pentose sugars and ferment them into ethanol. The yeast is considered to be a promising agent as it ferments a varied range of sugars including cellobiose. Previously, improved bioethanol production from WS was obtained using mutant strains of *P. stipitis* and *C. shehatae*, which produced 12.15 ± 0.57 and 9.55 ± 0.47 g L^{-1} of ethanol, respectively [52]. Co-culture of *S. cerevisiae* and *P. fermentans* demonstrated maximum power density of 77.5 mW m^{-2} along with ethanol generation up to 8.7% (w/v) in a single vessel electrochemical bioreactor using wheat straw hydrolysate [18]. Although the recalcitrant biomass contains furfural, (5-hydroxymethylfurfural) HMF, and acetic acid that might affect the fermentation process. The inhibitors mainly include organic acids, sugar derivative compounds, and delignification by-products. These inhibitors mainly formed during the pretreatment stage of lignocellulosic biomass. Literature suggests that these factors negatively affected the overall fermentative efficiency of cell growth, ethanol yield, and sugar utilization rate by yeast. *Pichia* spp. are capable to metabolize furfurals and HMF into less inhibitory compounds by consuming available sugars in hydrolysate [53].

It is hypothetical that the MFC performance will not get affected by the fermentation process when operated under proper experimental conditions. The power generation and ethanol formation occur simultaneously along with the sugar metabolization in a yeast fuel cell. Yeast adherence to carbon-fiber anode may enhance ethanol production and electricity generation [54]. Numerous studies suggest that under anaerobic conditions, 1 mol of glucose produces 2 mol of ethanol along with 2 mol of electrons. Compared to *Geobacter* and *Shewanella*, yeasts are highly inadequate in the direct electron transfer. This study, thus focused on concurrent bioethanol and bioelectricity generation for substantial benefit. The electron transfer efficiency and internal resistance are mostly affected by dead yeast cells. A large number of sugars, phenolic compounds, and amino acids are formed during pretreatment may act as a mediator system in the hydrolysate. The mediator system behaves as a shuttle system to enable electrochemical reaction. The degradation-derived phenolics exploit the electrons, which may be transported by the anode to facilitate the oxidation of non-phenolic groups [55].

Beside ethanol and electricity, the process may be used to produce hydrogen gas along with electricity. However, it requires the integration of waste treatment process with biohydrogen production as a co-culture based system (*Clostridium acetobutylicum* and *Enterobacter aerogenes*) has been studied for simultaneous hydrogen production and electricity generation [56]. Similarly, a electrochemical cell with cellobiose as carbon source produced 0.93 mmol L^{-1} $h^{-1}$of hydrogen, when voltage of 0.8 V is applied [57].

4. Conclusion

Pretreatment with all the three white rot fungi demonstrated WS hydrolysate production. Among these fungi, *P. floridensis*, was the most efficient fungus in terms of releasing maximum sugar in hydrolysate. Besides converting sugars present in the wheat straw hydrolysate into ethanol, *Pichia fermentans* also demonstrated electricity generation in proposed electrochemical bioreactor during the

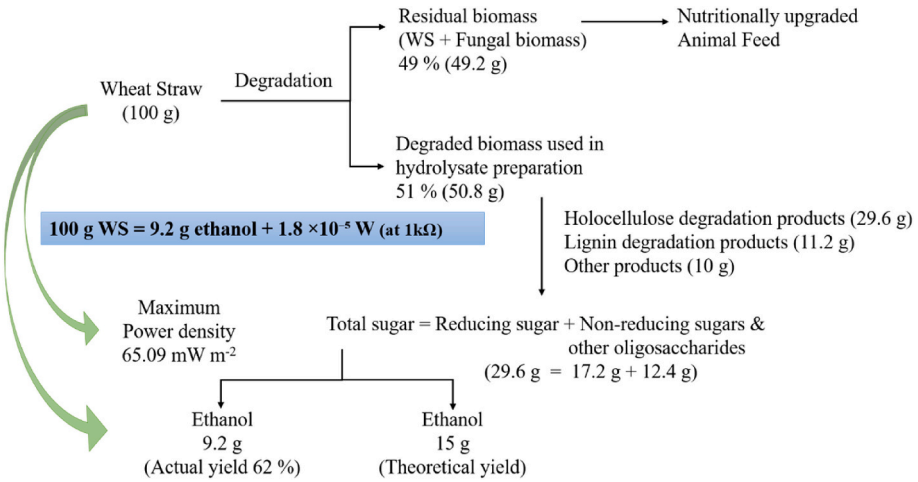

Fig. 8. Schematic representation showing wheat straw conversion into electricity and ethanol.

fermentation process. The system thus developed demonstrated about 9 g of ethanol along with 65.09 mW m^{-2} power density was generated from 100 g of wheat straw, while about 50% degraded or residual biomass was obtained, which can be used for other applications. The process requires no additional energy or catalysts, thus making the system sustainable and environmentally friendly. This technology strengthens the waste to bioenergy process that possesses considerable interest and further opens the possibility for using renewable energy sources. Further, utilization of complex residues provides an option for the management of abundantly available plant biomass. It is possible to use the process for bioethanol and bioelectricity production by upscaling the technology on a commercial scale in the future.

Author contribution statement

Akansha Shrivastava: Performed the experiments; Analyzed and interpreted the data; Wrote the paper.

Rakesh Kumar Sharma: Conceived and designed the experiments; Contributed reagents, materials, analysis tools or data; Wrote the paper.

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Data availability statement

Data will be made available on request.

Declaration of interest's statement

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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