Bio-ethanol production from waste biomass of *Pogonatherum crinitum* phytoremediator: an eco-friendly strategy for renewable energy

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Abstract

In this study, we have described three steps to produce ethanol from *Pogonatherum crinitum*, which was derived after the treatment of textile wastewater. (a) Production of biomass: biomass samples collected from a hydroponic *P. crinitum* phytoreactor treating dye textile effluents and augmented with Ca-alginate immobilized growth-promoting bacterium, *Bacillus pumilus* strain PgJ (consortium phytoreactor), and waste sorghum husks were collected and dried. Compositional analysis of biomass (consortium phytoreactor) showed that the concentration of cellulose, hemicelluloses and lignin was 42, 30 and 17%, respectively, whereas the biomass samples without the growth-promoting bacterium (normal phytoreactor) was slightly lower, 40, 29 and 16%, respectively. (b) Hydrolysate (sugar) production: a crude sample of the fungus, *Phanerochaete chrysosporium* containing hydrolytic enzymes such as endoglucanase (53.25 U/ml), exoglucanase (8.38 U/ml), glucoamylase (115.04 U/ml), xylanase (83.88 U/ml), LiP (0.972 U/ml) and MnP (0.459 U/ml) was obtained, and added to consortium, normal and control phytoreactor derived biomass supplemented with Tween-20 (0.2% v/v). The hydrolysate of biomass from consortium phytoreactor produced maximum reducing sugar (0.93 g/l) than hydrolysates of normal phytoreactor biomass (0.82 g/l) and control phytoreactor biomass (0.79 g/l). FTIR and XRD analysis confirmed structural changes in treated biomass. (c) Ethanol production: the bioethanol produced from enzymatic hydrolysates of waste biomass of consortium and normal phytoreactor using *Saccharomyces cerevisiae* (KCTC 7296) was 42.2 and 39.4 g/l, respectively, while control phytoreactor biomass hydrolysate showed only 25.5 g/l. Thus, the amalgamation of phytoremediation and bioethanol production can be the truly environment-friendly way to eliminate the problem of textile dye along with bioenergy generation.

Keywords *Pogonatherum crinitum* · Phytoremediation · *Phanerochaete chrysosporium* · Enzymatic hydrolysis · Fermentation · Bioethanol

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Introduction

Recently the security of energy and environment has emerged as a primary concern of developing world. The decline of fossil fuels along with unprecedented rise in crude oil prices is going to lead the global energy crisis. Furthermore, environmental pollution is continuously growing problem due to various man-made activities. Therefore, greater attention is being focused to find out the secure alternative energy sources using low-polluting advanced technologies. It should, however, be noted that the developed renewable sources must be economically feasible and sustainable. This approach can positively contribute to the actualization of the United Nations Millennium Development Goals (Nzila et al. 2010; UN 2008). Plants maintain atmospheric carbon dioxide level by recycling about 10¹¹ tons of carbon through photosynthesis on the Earth's surface (Yeoman et al. 2010).

Plants are considered as the most abundant source of lignocellulosic biomass that can be used potentially for biofuels production. Thus, lignocellulosic biomass can be utilized as a clean and eco-friendly option for bioenergy production. It also reduces dependency on fossil fuels which are responsible for greenhouse gas emission. Hence, lignocellulosic biomass is gaining research interest due to its renewable nature (Anwar et al. 2014). The production of second generation biofuel is more desirable to grapple environmental and social crisis (Tollefson 2008).

Lignocellulosic biomass complex is a dense pack of cellulose and hemicellulose with a protective cover of lignin. Lignin provides the stability to biomass against enzymatic degradation. Therefore, the pre-treatment step becomes essential to enhance the accessibility of cellulose and hemicellulose to hydrolytic enzymes for the breakdown and removal of lignin from complex (Balat 2011). Conventional physical, chemical and thermal cellulosic biomass pre-treatment methods are mostly expensive as well as environmentally harmful (Mosier et al. 2005). Comparatively, biological pre-treatment methods for delignification of lignocelluloses are safer, environment-friendly and also offer some advantages such as minimized requirement of chemicals and energy (Binod et al. 2010). Enzymatic hydrolysis is the second important step for ethanol production which involves depolymerisation of cellulose and hemicellulose into hydrolysed product (Taherzadeh and Karimi 2008). The saccharification of biomass can be enhanced by taking benefits of both cellulolytic and hemicellulolytic enzymes in combination instead using single hydrolytic enzyme (Zhong et al. 2009). Previous studies have reported the utilization of different waste lignocellulosic biomasses such as sugarcane bagasse, banana waste, potato peels, pineapple peels, wheat straw for bioethanol production (Kshirsagar et al. 2016; Shu et al. 2015).

Effluent released from textile industries mainly contains textile dye stuff which contaminates water body and adversely affects aquatic ecosystem (Banat et al. 1996). These carcinogenic contaminants have a serious impact on human health, thus textile dyes present in effluent needs to detoxify (Kariminiaee et al. 2007). Phytoremediation is an ideal and eco-friendly approach for the removal of toxic contaminants present in textile effluent (Khandare and Govindwar 2015). This green technology is not only esthetically gratifying but also cost-effective and serves as a potential source for lignocellulosic biomass generation. This generated waste lignocellulosic biomass during phytoremediation can further be subjected as substrate for enzymatic saccharification and bioethanol production (Jagtap et al. 2014; Kagalkar and Govindwar 2010).

Watharkar et al. (2015) have revealed the advantage of the plant–bacterial consortia for enhanced decolorization and detoxification of real textile effluent using *Pogonatherum*

crinitum and *Bacillus pumilus* strain PgJ. The present study is the continuation of earlier work and deals with the subsequent enzymatic hydrolysis and bioethanol production from waste biomass of *P. crinitum* generated after phytoremediation of textile effluent. Concerns about management of plant biomass after phytoremediation are recurrently raised and is a matter of criticism. In this work, the phytoremediation candidate plant biomass has been utilized for bioenergy production which is an additional avenue of research. This study also gives an insight into the large-scale production of bioethanol using waste biomass generated in floating islands or constructed wetlands developed for phytoremediation of various wastewaters.

Materials and methods

Materials

The nonionic surfactant Tween-20 and dinitrosalicylic acid was procured from SRL, India. The commercial cellulosic material such as, carboxymethyl cellulose (CMC), avicel, birch wood xylan, 4-nitrophenyl β -D-glucopyranoside (PNPG), and Whatman filter paper No.1 were obtained from Sigma, Aldrich (USA). HPTLC plate silica gel 60 F₂₅₄ plates were obtained from Merck, India. All other chemicals used were of the highest purity available and of the analytical grade.

The hydroponic phytoreactor with *P. crinitum* was augmented with *B. pumilus* PgJ bacteria immobilized in Ca-alginate synthesized beads (Watharkar et al. 2015). Waste biomasses of *P. crinitum* were obtained from static hydroponic phytoreactors after treatment of real textile effluent with and without augmentation of plant growth promoting rhizobacterium *B. pumilus* PgJ and biotic control system, i.e., plants kept in tap water. Agricultural waste biomass of sorghum husk was collected from agricultural land near Kolhapur, Maharashtra, India and used as a substrate for the production of hydrolytic enzymes. The collected lignocellulosic biomasses of *P. crinitum* were washed with tap water and dried under sunlight. Dried biomasses were milled, sieved and stored under moisture-free conditions, until further study. The compositional analysis of waste biomass produced after phytoremediation was carried by AOAC methods (AOAC 1980).

Crude enzyme preparation

White-rot fungus *Phanerochaete chrysosporium* (MTCC 787) was cultured for 8 days on Dubos medium [NaNO₃, 0.5; K₂HPO₄, 1.0; MgSO₄·7H₂O, 0.5; KCl, 0.5; FeSO₄·7H₂O, 0.001 (g/l)] supplemented with 1% sorghum husk as carbon source. The supernatant of fermented broth separated by

centrifugation at 5000 rpm for 10 min and further filtered through 0.45 µm membrane filter. The filtrate obtained was used as the crude enzyme source for enzymatic hydrolysis for further studies.

Enzymatic analysis

Different enzyme activities of crude sample were determined as follows. Endoglucanase activity was determined using reaction mixture containing 1 ml of enzyme solution with 1 ml of 1% carboxymethylcellulose (CMC) in McIlvaine's buffer (0.1 mol/l citric acid–0.2 mol/l phosphate buffer; pH 5) and incubated at 50 °C for 30 min (Lo et al. 2009). Exoglucanase activity was determined by a reaction mixture containing 1 ml of enzyme solution with 0.5 ml of 1% avicel cellulose in McIlvaine's buffer and incubated at 50 °C, for 2 h. Product was estimated by adding 1 ml of dinitrosalicylic acid reagent (Lo et al. 2009). Filter paper (FPU) activity was determined by measuring the reducing sugars produced from Whatman no. 1 filter paper (50 mg, 1 × 6 cm) according to IUPAC recommendations. The reaction was carried out in 50 mM citrate buffer at pH 4.5, and incubated at 50 °C for 1 h (Adney and Baker 2008).

Xylanase activity was determined in a reaction mixture containing 1 ml of enzyme solution diluted in McIlvaine's buffer with 1 ml of an aqueous suspension of 1% xylan at 50 °C for 10 min (Saratale et al. 2010). Glucoamylase activity was determined in a reaction mixture containing 1 ml of enzyme solution appropriately diluted in McIlvaine's buffer with 1 ml of an aqueous suspension of 1% starch at 50 °C for 10 min (Anto et al. 2006). In these enzymes test, the reaction was terminated by adding dinitrosalicylic acid (DNSA) reagent and heating in boiling water bath for 10 min (Miller 1959). One unit of enzyme activity in each case was defined by the amount of enzyme that produces one microgram of reducing sugar from the substrate per min.

Phanerochaete chrysosporium also produces ligninolytic enzymes such as lignin peroxidase (LiP) and manganese peroxidase (MnP). Enzyme activities were determined on 8 days of the incubation period. One unit of LiP activity was defined as the amount of the enzyme that led to the production of 1 µmol veratryl aldehyde from the oxidation of veratryl alcohol per min (Liu et al. 2008). One unit of MnP activity was expressed as the amount of enzyme that led to the production of 1 µmol Mn³⁺ from the oxidation of Mn²⁺ per min (Rogalski et al. 2006).

Enzymatic hydrolysis of *P. crinitum* biomass

The crude enzyme of *P. chrysosporium* was used for enzymatic hydrolysis of *P. crinitum* plant biomasses obtained from phytoreactors with and without augmentation of *B. pumilus* and control system. Two separate experiments were

performed where 10% concentration of feedstock was incubated with crude enzyme dosage of 10 FPU/g of feedstock in 20 ml citrate buffer (50 mM, pH 4.8) in 100 ml Erlenmeyer flasks with and without supplementation of surfactant-like Tween-20 (0.2%, v/v) at 6th h of incubation. The flasks were kept for 48 h of incubation at 50 °C temperature under continuous shaking condition in shaker incubator (110 rpm). Hydrolysate samples were collected at 6 h time interval up to 48 h from the reaction mixture. The samples were centrifuged for the removal of unhydrolyzed biomass residue. The supernatant was heated for 5 min at 100 °C in water bath for deactivation of cellulolytic enzymes present in hydrolysate sample. The reducing sugar was determined using DNSA method (Miller 1959). The saccharification yield was calculated by Eq. 1 (Uma et al. 2010).

$$\text{Saccharification yield (\%)} = \frac{\text{Reducing sugar formed} \times 0.9 \times 100}{\text{Cellulose content of substrate}} \quad (1)$$

High performance thin layer chromatography analysis

High performance thin layer chromatography (HPTLC) was used for qualitative determination of sugars produced in hydrolysate after enzymatic hydrolysis of consortia plant biomass supplemented with Tween-20 (0.2%, v/v). In this study, HPTLC plate silica gel 60 F₂₅₄ (Merck) was activated by impregnating with 0.3 M KH₂PO₄. After activation of HPTLC plate, 10 µl of hydrolysate sample, sugar standards of glucose and xylose were applied on HPTLC plate by microsyringe using spray gas nitrogen sample applicator (Linomat V, CAMAG, Switzerland). The bandwidth of 8.0 mm was applied with track distance of 14.5 mm, a distance from the lower edge of 8.0 mm, and a distance from both edges of mostly 20 mm. Standard sugars, glucose and xylose were prepared in hydro-methanolic solution (1:9, v/v) with a final concentration of 0.2 µg/ml of sugars. Mobile phase n-propanol: water (17:3, v/v) was used for analysis of sugars. The plates were developed in the twin-trough chamber 10 × 10 cm (CAMAG) up to migration distance of 85 mm from the bottom edge. The developed HPTLC plate was derivatized by phosphomolybdic acid solution (20%, w/v) in ethanol for visual detection of sugars. Data processing was performed with software platform winCATS 1.4.4.6337 (CAMAG).

Surface characterization of *P. crinitum*

FTIR analysis

FTIR analysis was carried out for the detection of changes in functional groups caused due to enzymatic hydrolysis of different plant biomasses obtained after treatment of textile

effluent from consortium, normal and control phytoreactors. FTIR spectra were recorded by FTIR spectrometer (Shimadzu FTIR 8400S; Japan) in absorption band mode in the range of 650–4000 cm^{-1} with a resolution of 4 cm^{-1} and 32 scans.

XRD analysis

XRD analysis of residual *P. crinitum* biomasses from control, consortium and normal phytoreactors after enzymatic hydrolysis were done by XRD BRUKER (Germany), D2-Phaser set at 30 kV, 10 mA; with radiation of Cu K α (0.15418 nm) and grade range between 10° and 40° with a step size of 0.02°. Crystallinity index (CrI) of cellulose was calculated using Eq. 2 (Segal et al. 1959).

$$\text{CrI (\%)} = \frac{I_{002} - I_{\text{am}}}{I_{002}} \times 100, \quad (2)$$

where CrI is the crystallinity index, I_{002} is the maximum intensity at $2\theta = 22.5^\circ$, and I_{am} is minimum intensity corresponding to amorphous content at $2\theta = 18.0^\circ$.

The degree of crystallinity (X_c) was calculated using Eq. 3 as follows (Zhou et al. 2005).

$$X_c = \frac{F_c}{F_a + F_c} \times 100, \quad (3)$$

where F_c and F_a are the area of crystalline and non-crystalline regions, respectively.

Fermentation for ethanol production

Saccharomyces cerevisiae (KCTC 7296) cultured separately in medium containing malt extract, 3.0; glucose, 10; yeast extract, 3.0 and peptone, 5.0 (g/l) in deionized water. Freshly grown yeast cells were collected and washed with 0.1% peptone water for the removal of residual media. The enzymatic hydrolysate of *P. crinitum* was concentrated to reducing sugar concentration of 5% by evaporation at 80 °C and supplemented with yeast extract (50 g/l), $(\text{NH}_4)_2\text{SO}_4$ (100 g/l), KH_2PO_4 (45 g/l) and $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$ (10 g/l).

S. cerevisiae (5%, v/v) was aseptically inoculated to filter and sterile hydrolysate samples produced from Tween-20 supplemented enzymatic hydrolysis of *P. crinitum* biomasses from control system as well as phytoreactors with and without augmented *B. pumilus*. The inoculated hydrolysate broths of corresponding plant biomasses were incubated for fermentation at 30 °C for 48 h under anaerobic condition. Fermented broths were distilled using Borosil distillation assembly after termination of fermentation. The estimation of ethanol in distillates was done using $\text{K}_2\text{Cr}_2\text{O}_7$ method (Williams and Reese 1950). The ethanol yield for

plant biomasses from control and test phytoreactors was calculated using Eq. 4 described by Yoswathana et al. (2010).

$$\text{Ethanol yield} = \frac{\text{Ethanol produced (g) in fermented broth}}{\text{Initial reducing sugar in fermented broth} \times 0.511} \times 100. \quad (4)$$

Statistical analysis

Data have been analyzed using one-way analysis of variance (ANOVA) with Tukey–Kramer multiple comparison tests with the help of GraphPad InStat version 3.06 software.

Results and discussion

Production of biomass

Watharkar et al. (2015) had already developed the phytoreactor using *P. crinitum* plants for the decolorization and detoxification of real textile effluents with augmentation of immobilized *B. pumilus* cells. This consortia system was found to be more efficient and applicable when compared to individual reactors of plants and bacteria. The synergistic system of plant–bacteria decreased ADMI, COD, BOD, conductivity, turbidity, TDS and TSS of real effluent to harmless levels, i.e., 93, 78, 70, 4, 90, 13 and 70%, respectively, within 12 days. The treated effluent exerted less toxic effects on the seed germination of *Phaseolus mungo* and *Sorghum vulgare* as well as on tessellated darter fish (Watharkar et al. 2015). Plant biomasses obtained from these phytoreactors were further utilized for bioethanol production.

Composition analysis of *P. crinitum* biomass

Compositional analysis exhibited the variations among the waste biomasses harvested from phytoreactors with and without consortium as well as control after phytoremediation of real textile effluent. Dried powder of plant biomass from consortium phytoreactor showed higher concentrations of cellulose, hemicelluloses and lignin up to 42, 30 and 17%, respectively. While, plant biomass from phytoreactor devoid of consortium constituted cellulose, hemicelluloses and lignin concentration up to 40, 29 and 16%, respectively. Comparatively, control plant biomass contributed slightly less amount of cellulose (39%), hemicelluloses (28%) and 15% of lignin (Table 1). The overall increase of these components was observed in plant biomass of consortium phytoreactor than that of phytoreactor without consortium and control system. Jagtap et al. (2014) have reported similar results about increase in biomass of *P. densiflora* during microbial consortium assisted phytoremediation of diesel-contaminated soil. This might have taken place because

Table 1 Composition of *P. crinitum* biomass obtained after phytoremediation

Component	<i>P. crinitum</i> biomass (control)	<i>P. crinitum</i> biomass (normal phytoreactor)	<i>P. crinitum</i> biomass (consortium phytoreactor)
Cellulose	39.2 ± 0.4	40.8 ± 0.1	41.9 ± 0.3
Hemicellulose	28.4 ± 0.2	29.5 ± 0.3	30.1 ± 0.4
Lignin	15.3 ± 0.1	16.1 ± 0.2	17.4 ± 0.2

Unit of cellulose, hemicellulose and lignin is in % weight

Values are mean of three experiments, SEM (±), and by one-way ANOVA with Tukey–Kramer multiple comparisons test

of abiotic stress, release of phytohormones by augmented potent degrader and augmentation of plant growth promoting rhizobacteria *B. pumilus* strain PgJ during bacterial assisted phytoremediation. *P. crinitum* in the control and normal phytoreactor showed comparatively less biomass synthesis as plants were merely exposed to tap water and real textile effluent, respectively, without any nutrients and augmentation.

Hydrolysate (sugar) production

Hydrolytic enzymes production by *P. chrysosporium*

Activities of different hydrolytic enzymes were determined from a crude sample of *P. chrysosporium*. Activities of cellulolytic enzymes such as endoglucanase and exoglucanase were observed to be 53.25 and 8.38 U/ml, respectively, while hemicellulolytic enzymes glucoamylase and xylanase showed 115.04 and 83.88 U/ml, respectively. Different ligninolytic enzymes such as LiP (0.972 U/ml) and MnP (0.459 U/ml) from the crude sample also played a significant role in the removal of lignin from the waste biomass during enzymatic hydrolysis. Filter paperase activity (FPU) of crude enzyme was 1.038 U/ml. However, lignocellulolytic enzymes produced by *P. chrysosporium* might be because of carbon deficient condition in the culture medium (Table 2). This analysis confirmed the presence of required cocktail of hydrolytic enzymes in the crude source. Govumoni et al. (2015) have reported production of the lignocellulolytic enzyme from *P. chrysosporium* (MTCC 787) utilizing agricultural waste biomass.

Enzymatic hydrolysis of *P. crinitum*

Enzymatic hydrolysis is a crucial and second step for the production of lignocellulosic ethanol. Waste plant biomasses of *P. crinitum* remained after phytoremediation of textile effluent were further subjected to enzymatic hydrolysis. The lignin and hemicelluloses in lignocellulosic plant biomass act as strong adsorbents for cellulase enzyme. This non-specific binding of cellulase to these components hampers the process of cellulose hydrolysis (Berlin et al. 2005; Hall

Table 2 Enzymes produced by *P. chrysosporium* (MTCC 787) on 8 days of incubation

Enzyme activity (U/ml)	
Cellulolytic enzymes	
Endoglucanase	53.25 ± 0.88
Exoglucanase	8.38 ± 0.43
FPU	1.038 ± 0.23
Hemicellulolytic enzymes	
Glucoamylase	115.04 ± 2.54
Xylanase	83.88 ± 0.97
Ligninolytic enzymes	
Lignin peroxidase	0.972 ± 0.094
Manganese peroxidase	0.459 ± 0.026

Values are mean of three experiments, SEM (±), and by one-way ANOVA with Tukey–Kramer multiple comparisons test

et al. 2010). Thus, delignification of lignocellulosic biomass through ligninolytic enzyme is necessary. This increases accessibility of cellulose in lignocellulosic plant biomass for the action of cellulase during enzymatic hydrolysis which ultimately leads higher yield of reducing sugars.

The supplementation of Tween-20 in early stage of enzymatic hydrolysis of plant biomasses was found effective to increase reducing sugars concentration in hydrolysate samples (Fig. 1). The reducing sugar concentration released after 48 h in hydrolysates of Tween-20 treated plant biomasses with and without consortium as well as from control system were 0.93, 0.82 and 0.79 g/l, respectively, while reducing sugars produced in absence of Tween-20 were 0.34, 0.30 and 0.29 g/l, respectively (Fig. 1a, b). Seo et al. (2011) suggested that Tween-20 increases adsorption of cellulose effectively on available cellulase surface area. The reducing sugar released after saccharification of consortium biomass was found to be higher than that of reported for *Pinus densiflora* biomass using enzymes from *Fomitopsis pinicola* (70.9 mg/g) (Lee et al. 2008).

The saccharification yield of biomasses after enzymatic hydrolysis showed noteworthy enhancement in the presence of nonionic surfactant Tween-20. Saccharification yields of biomasses in the presence of Tween-20 from phytoreactor

Fig. 1 Reducing sugars produced after enzymatic hydrolysis of waste *P. crinitum* biomasses generated after phytoremediation, **a** without Tween-20 pre-treatment and, **b** with Tween-20 (0.2%, v/v) pre-treatment. Where, *P. crinitum* from (1) Control, (2) Normal phytoreactor, (3) Consortium phytoreactor

Table 3 Saccharification yield of biomasses of *P. crinitum* obtained from phytoremediation reactor by enzymatic hydrolysis using crude enzyme of *P. chrysosporium* and effect of Tween-20

Biomass	Saccharification yield (%)	
	Control	Tween-20
<i>P. crinitum</i> (control)	6.22 ± 0.8	16.96 ± 1.4
<i>P. crinitum</i> (normal phytoreactor)	6.44 ± 0.5	17.61 ± 1.1
<i>P. crinitum</i> (consortium phytoreactor)	7.30 ± 0.9	19.97 ± 2.1

Values are mean of three experiments, SEM (±), and by one-way ANOVA with Tukey–Kramer multiple comparisons test

with and without consortium and control system were 20, 18 and 17%, respectively, which were higher than that of reported for beech wood hydrolysis (9.5%) (Sawada et al. 1995). While in the absence of Tween-20, saccharification yields of biomasses were 7.3, 6.4 and 6.2%, respectively

(Table 3). These results endorsed the significance of Tween-20 surfactant supplementation for the enhancement of enzymatic hydrolysis as it avoids non-productive binding of cellulase enzyme to lignin content by masking lignin and enabling maximum cellulase to act on cellulose (Eriksson et al. 2002). Interestingly, higher reducing sugars released during enzymatic hydrolysis of waste biomass of consortium phytoreactor might be due to biostimulative and stress sharing nature of plant growth-promoting rhizobacteria *B. pumilus* used in consortium for textile effluent phytoremediation (Gutierrez-Maneroa et al. 2001; Khaliq et al. 2013). The enhanced enzymatic degradation in presence *B. pumilus* might be because of the production of surfactant by this bacterium (Oliveira and Cruz 2013).

Qualitative determination of reducing sugars by HPTLC

Reducing sugars in different hydrolysate samples produced using control and test plant biomasses were qualitatively determined by HPTLC. After derivatization of HPTLC plate by phosphomolybdic acid, sugars became visible with dark greenish color. HPTLC analysis of hydrolysate confirmed the production of glucose and xylose. The R_f value of glucose and xylose produced during enzymatic hydrolysis were 0.48 and 0.59, respectively (Fig. 2). This difference in R_f values of sugar might be due to complexity variation. Similar R_f values of glucose and xylose have been reported by Adachi (1965) using mobile phase *n*-propanol:water (17:3). Sugarcane bagasse hydrolysate has already been reported by Waghmare et al. (2014) for the production of reducing sugars like glucose and xylose.

Surface characterization of *P. crinitum* biomass

FTIR analysis FTIR analysis performed before and after enzymatic hydrolysis of various *P. crinitum* biomasses have revealed the structural changes (Suppl. Figure 1). Suppl. Figure 1A showed some common IR bands in unhydrolysed samples of *P. crinitum* biomasses powder from control, normal and *B. pumilus* augmented phytoreactors such as 2919 cm^{-1} assigned to C–H stretching of alkanes, 2854 cm^{-1} assigned to C–H stretching of aldehydes, 1630 cm^{-1} assigned to C=O stretching of ketones, 1599 cm^{-1} correspond to C=O stretching of acetyl group of aldehyde, 1510 cm^{-1} assigned to C=C stretching of the aromatic rings of lignin, 1480 cm^{-1} assigned to C–H deformation, 1459 cm^{-1} assigned to C–H deformation of alkane with asymmetric CH_3 , 1374 cm^{-1} assigned to alkane– CH_3 , 1320 cm^{-1} assigned to C–O stretching, 1242 cm^{-1} assigned to O–H bending, 1159 cm^{-1} assigned to C–O–C stretching at β (1–4) glycoside linkages, 1032 cm^{-1} assigned to C–O stretching at C–6.

Fig. 2 HPTLC profile of reducing sugars produced in hydrolysate of *P. crinitum* biomass of consortium phytoreactor after enzymatic hydrolysis with Tween-20 (L1-Glucose, L2-Xylose, L3-Hydrolysate)

The control biomass residues after enzymatic hydrolysis showed the presence of new IR bands when compared with those of before hydrolysis viz. 1649 cm^{-1} assigned to C=O vibration of primary amide, 1595 cm^{-1} correspond to carbonyl stretching (C=O) for acetyl groups in hemicelluloses. While 1630 cm^{-1} stretching corresponds to ketones was found to be eliminated after enzymatic hydrolysis (Suppl. Figure 1B (i)). In case of biomass residue from phytoreactor devoid of consortium, new IR bands were found to arise after enzymatic hydrolysis such as 1735 cm^{-1} corresponds to C=O stretching of saturated aliphatic aldehydes, 1595 cm^{-1} corresponds to carbonyl stretching (C=O) for acetyl groups in hemicelluloses, 1450 cm^{-1} corresponds to aliphatic C-H deformation, 1431 cm^{-1} corresponds to C-H deformation of ketones. Whereas IR bands like 1599 cm^{-1} and 1374 cm^{-1} , 1320 cm^{-1} were found to be eliminated (Suppl. Figure 1B (ii)). FTIR spectra of hydrolyzed plant biomass from consortium phytoreactor showed new IR bands of 1411 cm^{-1} correspond to C-H deformation of alkenes and 1341 cm^{-1} corresponds to O-H deformation of primary and secondary alcohols. While IR bands such as 1630 , 1599 , 1480 , 1459 , 1374 , 1320 cm^{-1} were eliminated during enzymatic hydrolysis (Suppl. Figure 1B (iii)). Interestingly, the removal of lignin during enzymatic hydrolysis was supported by the elimination of IR band of 1510 cm^{-1} from all biomass residues.

The functional group changes occurred in biomass residues indicates degradation of cellulose and hemicellulose by crude enzyme during hydrolysis. Along with above functional group changes, FTIR profile also showed an increase in absorbance intensity of hydrolyzed biomass

residue obtained from plant–bacteria consortium phytoreactor as compared to unhydrolyzed biomass residue.

XRD analysis The crystallinity of cellulose is a major factor determining the enzymatic hydrolysis efficiency. Thus, CrI is used as an important parameter since more than 50 years for determination and interpretation of structural changes occurred in cellulose after cellulase action. Factors which affect the accessibility of cellulose for enzymatic hydrolysis are crystallinity, lignin and hemicelluloses content, distribution, particle size, and porosity of lignocellulosic material, etc. (Park et al. 2010). The cellulose component of lignocellulosic biomass consists of two regions as crystalline and amorphous. It has been documented that cellulolytic enzyme easily degrades amorphous region than the crystalline region of cellulose (Park et al. 2010).

CrI was determined by XRD analysis and provided for the qualitative and semi-quantitative evaluation of amorphous and crystalline cellulosic components in control and test plant biomasses residues of *P. crinitum* (Suppl. Figure 2). The CrI of hydrolyzed plant biomasses from the control, normal and consortium phytoreactors were comparatively less such as 44, 38 and 32%, respectively, than those of before enzymatic hydrolysis, i.e., 46, 40 and 39%, respectively. These results highlight the significance of biological enzymatic hydrolysis in decreasing CrI of biomasses (Table 4). Similarly, the degree of crystallinity gets reduced after enzymatic hydrolysis of biomasses from the control, normal and consortium phytoreactors, i.e., 52, 50 and 28%, respectively, while those before enzymatic hydrolysis were 55, 53 and 34%, respectively (Table 4). These results indicate the decrease in degree of crystallinity of biomasses due to

Table 4 Crystallinity index and degree of crystallinity of cellulose present in *P. crinitum* biomass before and after enzymatic hydrolysis

Parameters	Biomass before enzymatic hydrolysis			Biomass after enzymatic hydrolysis		
	Control	<i>P. crinitum</i> (normal phytoreactor)	<i>P. crinitum</i> (consortium phytoreactor)	Control	<i>P. crinitum</i> (normal phytoreactor)	<i>P. crinitum</i> (consortium phytoreactor)
Crystallinity index (%)	46	40	39	44	38	32
Degree of crystallinity (%)	55	53	34	52	50	28

Table 5 Fermentation of hydrolysates of *P. crinitum* waste biomasses using *S. cerevisiae*

Biomass	Ethanol (g/l)	Ethanol yield (g/g)	Volumetric ethanol productivity (g/l/h)
<i>P. crinitum</i> (control)	25.5	0.41	0.53
<i>P. crinitum</i> (normal phytoreactor)	39.4	0.44	0.82
<i>P. crinitum</i> (consortium phytoreactor)	42.23	0.57	0.87

biological enzymatic hydrolysis. These results also indicate the noteworthy action of cellulase from the crude sample on crystalline zone than the amorphous zone of cellulose present in *P. crinitum* biomass during enzymatic hydrolysis. The decrease in the CrI of cellulose after degradation by an enzymatic treatment has been reported earlier by Janardhan and Sain (2011).

Ethanol production

Fermentation of *P. crinitum* hydrolysate

The hydrolysates of *P. crinitum* waste biomasses obtained after enzymatic hydrolysis were further subjected to fermentation. The released reducing sugars in hydrolysates were utilized by yeast strain *S. cerevisiae* during fermentation. Ethanol yield was estimated from the distillate samples collected after completion of fermentation. Considerably higher amount of ethanol was produced due to fermentation of microbe augmented plant biomass hydrolysate (42.2 g/l) when compared to other hydrolysates of plant biomasses of normal phytoreactor (39.4 g/l) and control phytoreactor (25.5 g/l). Previous reports are also available on ethanol production using *S. cerevisiae* from different biomasses such as sugarcane bagasse (66 g/l), rice straw (49 g/l), wheat straw (34 g/l), corn stover (16.8 g/l), deinked newspaper (14.8 g/l), barley straw (10.4 g/l) and palm kernel press cake (12.5 g/l) (Belkacemi et al. 2002; Cervero et al. 2010; Kuhad et al. 2010; Öhgren et al. 2007). The maximum ethanol yield of 0.57 g/g was determined in the case of fermentation of consortium phytoreactor biomass hydrolysate than that of

hydrolysates from normal phytoreactor biomass (0.44 g/g) and control phytoreactor biomass (0.41 g/g). In the present study, higher volumetric ethanol productivity (0.87 g/l/h) was observed for the fermentation of consortium phytoreactor biomass hydrolysate when compared to without consortium (0.82 g/l/h) and control (0.53 g/l/h) (Table 5). Öhgren et al. (2007) have also reported 0.70 g/l/h volumetric ethanol productivity during fermentation of corn stover by *S. cerevisiae*.

Conclusion

The waste biomass of *P. crinitum* generated after phytoremediation of real textile effluent can further be utilized as a substrate for bioenergy production. This study also investigated additional advantage of bacterial assisted phytoremediation for effective treatment of real effluent along with substrate biomass increase for biofuel production. The increase in plant biomass serves beneficial for high reducing sugars production after enzymatic hydrolysis and finally for higher ethanol yield. This study informs that amalgamation of phytoremediation and bioethanol production is a truly environment-friendly way to eliminate the problem of dye along with biofuel synthesis in future. The present study is a preliminary attempt in bioenergy field, a more detailed study on fermenter level is underway to scale up the bioethanol production from biomass generated after phytoremediation of real textile effluent.

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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