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Lignocellulose degradation of rice straw by thermophilic microbial consortium isolated from mature compost of sugarcane industry

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Abstract

Lignocellulose describes namely cellulose, hemicelluloses and lignin. Cellulose and hemicellulose are degraded into sugars, which are further conversion by fermentation, biocatalytic, and chemocatalytic processes to value-added products, including biofuels. In this study, the thermophilic bacterial consortium was isolated from mature compost of sugarcane industry. The thermophilic bacterial consortium showed efficient degradation on rice straw as compare to the previous reported microbial consortium isolated from rice straw compost. The lignocellulose degradation ratio by isolated thermophelic bacterial consortiun was 76.92 % after 7 days of incubation at 50 ºC. The percentage of lignin was 15.38 % in PCS medium after 7 days of incubation at 50 ºC.

Keywords: lignocellulose, thermophilic, bacterial consortium, cellulose and hemicelluloses. 1

I. INTRODUCTION

Lignocellulosic plant biomass is an important renewable carbon resource for the biorefinery industry. The plant biomass is considering a sustainable and environmentally friendly alternative to the current petroleum platform (Kamm *et al.*, 2004). Lignocellulosic biomass, such as agricultural residues and herbaceous energy crops, consists mainly of three different types of biopolymers i.e. cellulose (35–50%), hemicellulose (25–30%) and lignin (25–30%). The composition of lignocelluloses depends not only on the species but also on the growth conditions and different parts of the plant with their age (Jorgensen, 2003). The abundance of lignocellulosic materials make them potentially inexpensive and readily available natural resources for the manufacturing of high value compounds and biofuels production in a sustainable environment. The increasing energy demands have focused worldwide attention on the utilization of renewable resources. Several workers from the world have reported successful conversion of waste materials to useful compost. The use of these materials involves a separation of the polymeric compounds - cellulose and hemicelluloses. The spontaneous degradation of these compounds are extremely slow, but there are microorganisms in soil and in the rumen of ruminants, capable of degrading them into sugars, which can be utilized as energy and carbon source by various microorganisms for the production of different products. These organisms are capable of growing on lignocellulosic materials and thus produce a wide range of enzymes that could be of scientific or industrial importance. Utilization of lignocellulose from forestry or non-food energy crops, or agricultural and forestry waste products, represent as large resource available for energy and chemical feedstock production (Cullen, 1992). Lignocellulose degrading bacteria has an important role in energy supply for ruminants. In the present study our aim was to biologically degrade the lignocelluloses material under thermophilic conditions because most of the time in India hot environmental condition remains in the plain area.

II. MATERIALS AND METHODS

2.1. Chemical, lignocellulosic materials and pretreatment of rice straw

The compost sample was collected from Sir Shadilal sugarcane industry, Shamli (U.P., INDIA). The rice straw was obtained from local field of rice near Kurukshetra University. All the chemicals and reagents used in the present investigation were of high purity and analytical grade. The chemicals and reagents were procured from Hi Media and CDH. The rice straws were taken and cut into equal pieces of 3 cm then washed properly with water. The pretreatment of rice straw pieces were done by putting these straw pieces in 1 % NaOH solution for a period of 24 hours. These were rinsed properly with distilled water for at least thrice. After washing these pieces were dried properly in oven at 50 ºC.

2.2. Isolation of lignocellulolytic thermophilic bacterial consortium

The lignocellulose degrading thermophilic microbial consortium was isolated from the compost of sugarcane industry as described by (Haruta *et al.* 2002) with some modification. The 5 g of compost sample was poured into the 100 ml PCS medium (0.1 % yeast extract, 0.5 % peptone, 0.5 % CaCO3, 0.5 % NaCl, 1 % Rice straw, pH 7.0) with a filter paper strip (0.3 g) as an indicator for cellulase activity. The flasks were incubated at 50 ºC under static conditions. Once the strip of filter paper was completely degraded and rice straws become softened, 5 ml enriched culture was transferred into fresh enrichment medium. This process was repeated 10 times at 50 °C. The enrichment process was continued for 6 month. The enriched culture was used for the isolation of bacterial consortium by using spread plate method on nutrient agar plates at 50 ºC. The colonies with distinct morphologies were picked, streaked twice for purification. The isolated microbial consortium was stored on the PCS medium for further study.

2.3 Optimization of lignocellulosic degradation under different environmental and nutritional conditions

To obtained high degradation of lignocellulose following factors were optimized i.e. pH, temperature and carbon source. The effect of carbon source on degradation efficiency was studied by adding (1% glucose, 1% fructose and 1% maltose) in lignocellulosic medium inoculated with thermophilic bacterial consortium at 50 ºC for 7 days under static condition. The effect of pH on degradation efficiency was studied at different pH (5.0, 6.0, 7.0, 8.0 and 9.0) of lignocellulosic medium inoculated with thermophilic bacterial consortium at 50 ºC for 7 days under static condition. The effect of temperature on degradation efficiency was studied at different temperature (25 ºC, 37 ºC and 50 °C) in lignocellulosic medium for 7 days under static condition. The effect incubation time on degradation efficiency was checked at time intervals (3, 5, 7 days) in lignocellulosic medium at 50 ºC for 7 days under static condition.

2.4. Synergistic effect

The thirty different combinations of six isolated thermophilic bacterial strains (LDB1, LDB2, LDB 3, LDB 4, LDB 5, and LDB 6) were made to check their effect on degradation of lignocellulose. This synergic effect of these combination was checked in PCS medium (0.1 % yeast extract, 0.5 % peptone, 0.5 % CaCO3, 0.5 % NaCl, 1 % rice straw, pH 7.0) with a filter paper strip (0.3 g) as an indicator for cellulase activity.

2.5. Analysis of lignocelluloses degradation

2.5.1. Cellulose

The microbial consortium was cultured in PCS medium containing 1 % cellulosic material (rice straw and filter paper) for 7 days at 50 ºC under static condition. The uninoculated PCS medium was

used as a control. When the strip of filter paper was degraded into fiber fragments, the culture was filtered. The solid filtrate was obtained and suspended in to 100 ml acetic acid/nitric acid reagent, then heated at 100 ºC for 30 min to remove the biological cells. The acetic acid/nitric acid treated suspension was filtered again. The remaining cellulosic material was washed three times with using 100 ml of distilled water each time. After washing and filtration, the filtered solids were dried at 80 ºC and determined gravimetrically (Feng *et al.*, 2010). The weight loss of cellulosic materials was calculated by subtracting the weight of the residual substrates from the total weight of cellulosic materials before degradation. The reactions were performed in triplicate and the degradation ratio was calculated as followed equation, where M_t is total weight of the cellulosic materials before degradation and M_r is the weight of the residual substrates after degradation.

$$\text{Degradation ratio (\%)} = (Mt - M_r/M_t) \times 100$$

2.5.2. Lignin

In 1 g of dried sample, 70 ml (1.25 % sulphuric acid) was added. The mixture was refluxed for 120 min, filtered and washed with water. After washing 30 ml (72 % sulphuric acid) was added and the material was allowed to stand for 4 h with occasional stirring. It was then filtered, washed and dried at 100 ºC (Material A) and incinerated at 540 ºC (Material B). Lignin content was determined by using the method (Van Soest and Wine 1967). The experiment was carried out in triplicates.

$$\text{Percentage of Lignin} = (\text{Material A}) \times (\text{Material B})/\text{Weight of the sample} \times 100$$

III. RESULTS

3.1. Isolation of lignocellulose degrading thermophilic bacterial consortium

The isolation of thermophilic microbial consortium was done by using the enrichment technique at 50 °C for six months. After the enrichment procedure, six morphologically different bacterial cultures were isolated by using spread plate method on nutrient agar plates. A code was given to all purified thermophilic bacterial cultures as (LDB1, LDB 2, LDB 3, LDB 4, LDB 5 and AS6). The qualitative degradation of lignocellulose was observed in PCS medium at 50 ºC. The cellulosic materials (filter paper and rice straw) were degraded into very small fragments in 7 days of incubation in flask inoculated with microbial consortium at 50 °C and no degradation was observed in uninoculated flak as shown in Fig. 1.

3.2. Analysis and synergistic effect of lignocellulose degradation

The lignocellulose (cellulose and lignin) degradation by thermophilic bacterial consortium was analyzed as shown in Table 1. All cellulosic materials were degraded efficiently under the experimental conditions (pH 7.0 and 50 ºC) in 7 days. When whole consortium was used, the maximum degradation ratio of cellulose was found 76.92 % [M_t =1.3 gm and M_r =0.3 gm (apporx.)] and percentage of lignin was found 15.38 % in PCS medium after 7 days of incubation at 50 ºC. The thirty different types of combinations of six isolated thermophilic bacterial strains were used to check their effect on degradation of lignocellulose as shown in Table 2. The degradation of lignocellulosic content was observed only, when combination of all six isolated bacterial cultures were inoculated in PCS medium after 7 days of incubation and rest all 29 different combinations used were not showing even qualitative degradation of lignocellulose.

3.3. Optimization of lignocellulose degradation by thermophilic bacterial consortium

The pH of the medium had significant effect on degradation activity by thermophilic bacterial consortium as shown in Table 3. The degradation was least in medium having pH 5.0 i.e. (53.7 %). The degradation increases with increasing pH and maximum degradation of lignocellulose was found 74.6 % at pH 7.0, with 0.34 gm residual weight of lignocellulose. The further increase in pH (8.0) of the medium leads to decrease in degradation ratio (70.7%). At pH 9.0 the degradation ratio was further decrease to 56.1 %. The optimum temprature for degradation was 50 °C at which degradation ratio was 76.92 %. At 30 °C and 25 °C degradation was no observed in the PCS medium. There was no degradation observed in the medium even up to 2nd day of incubation. The softening of rice straw was observed in the medium and the degradation ratio was found 44.61 % at 3rd of incubation. At 5th day of incubation filter paper (indicator of cellulose) was completely degraded in the medium and degradation ratio was found 63.07 %. The maximum degradation was observed in the medium after 7 days of incubation which was 75.38 % as shown in Table 4. The different carbon sources (1% glucose, fructose and maltose) were used in the PCS medium for the optimization of lignocellulose degradation as shown in Table 8. It was found that degradation ratio decreases in the presence of the carbon sources i.e. D-glucose, fructose and maltose. In case of D-glucose degradation was 67.6 % with 0.42 gm residual lignocellulose content after 7 days of incubation at 50 °C. In case of fructose, the degradation ratio was 63.6 % with 0.47 gm residual lignocellulose content. The degradation ratio was found 61.5 % with 0.5 gm residual lignocellulose content when maltose was added into the medium.

IV. DISCUSSION

The lignocellulose degrading microbial consortium was isolated at high temperature from rice straw compost by (Haruta *et al.* 2002). They obtained a structurally stable and complex lignocellulolytic microbial consortium from successive enrichment culture from rice straw compost with a high activity on various cellulosic materials, including rice straw, paper, and cotton. In our study the isolation of thermophilic microbial consortium was done by using an enrichment technique with repeated change of the enriched medium at 50 °C for six months. After enrichment by using spread plate method, six morphologically different bacterial cultures were isolated. Wongwilaiwalin *et al*. (2010) used same above method with some modifications, by using enrichment method they have isolated 8 different types of bacterial cultures which were capable to degrade lignocellulose content sufficiently. The degradation of lignocellulosic content was observed only, when combination of all six isolated bacterial cultures were inoculated in PCS medium after 7 days of incubation. Whole consortium was showing the lignocellulose degradation and rest all 29 different combinations used were not showing even qualitative degradation of lignocelluloses. In our study all cellulosic materials were degraded efficiently under the experimental conditions in 7 days. The degradation ratio was found to be 76.92 % after 7 days of incubation at 50 ºC. The percentage of lignin was 15.38 % in PCS medium after 7 days of incubation at 50 ºC when whole consortium was used in enriched medium. Umasaravanan *et al.* (2011) reported the lignocelluloses degradation in the sugarcan bagasse and rice straw. The cellulose and lignin content in rice straw containing medium was 56.2 % and 17.4 % respectively and in sugarcane bagasse the values were 41.4 % and 4.4 % respectively.

The optimization of lignocellulose degradation was done under different environmental and nutritional conditions. The maximum degradation of lignocellulose content was found in the medium containing glucose as carbon source in PCS medium at 7th day. The degradation ratio was maximum 67.6 % when glucose was added in the medium, but this value was less than 76.92 % (when glucose was not used in medium). This incidates the presence of glucose reduces the efficiency of lignocellulose

degradation. Odier and Roch (1983) reported that the degradation of poplar wood was stimulated by reduced glucose concentration. Abdullah *et al.* (1999) indicated that lignin hydrolysis was best at a marginal carbon/energy source for maintenance of metabolism. The decomposition of lignin was reported to take place after profuse microbial growth. It was further reported that no microorganism could grow on carbohydrate-free medium and decomposition of lignin was difficult in the absence of readily degradable, high-energy source (Costa *et al*., 2002). Wang *et al.* (2011) isolated 14 aerobic and anaerobic bacteria at thermophillic range i.e. 50 °C, which can degrade 99.0 % of filter paper, 76.9 % of cotton and 81.3 % of rice straw under static conditions, which suggest that some microorganism have ability to degrade lignocellulose at higher temperature which will be further useful for large scale industrial processes.

V. CONCLUSION

In this study a thermophilic lignocellulose degrading bacterial consortium was isolated. The thermophilic bacterial consortium has shown efficient degradation on rice straw with the overall lignocellulose degrading capability comparable to the previously reported microbial consortium isolated from rice straw compost. The degradation efficiency of the consortium was found high with less incubation time used in our experiment as compared to previously reported microbial consortium. The synergistic effect of isolated thermophilic bacterial consortium was also studied under the defined experimental conditions (pH 7.0, 50 °C). The optimization of lignocellulose degradation under different nutritional condition was checked and it was observed that the presence of glucose reduces the efficiency of lignocellulose degradation in the PCS medium. At different incubation time (3, 5 and 7 days) degradation of lignocellulose was checked. It was observed that the consortium was capable to degrade lignocellulose substrates within 7 days. The analysis of a thermophillic lignocellulosic degrading bacterial consortium was also estimated. The degradation ratio was 76.92 % after 7 days of incubation at 50 ºC. The percentage of lignin was 15.38 % after 7 days of incubation at 50 ºC. This work is considered a significant contribution to the research on lignocellulosic biomass degradation by complex microbial community with potential for further biotechnological applications, especially the degradation of biomass-based biorefinery feedstocks and after the degradation of lignocellulose biomass into sugars, we can further develop various different values added products including bio fuels, chemical, cheap energy sources for fermentation.

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Fig 1. Qualitative degradation of lignocellulose by thermophilic bacterial consortium.

Table 1. The % degradation of lignocellulose by thermophilic bacterial consortium.

Material	Degradation ratio
Cellulose	76.92 % [M_t=1.3 gm and M_r =0.3 gm (apporx.)]
Lignin	15.38 %

Table 2: The synergetic effect of isolated microbial consortium on lignocelluloses degradation

Combinations of isolated bacteria	Results
LDB 1, LDB 2, LDB 3 , LDB 4, LDB 5, LDB 6, LDB 1 + LDB 2,LDB 1 + LDB 3, LDB 1 + LDB 4, LDB 1 + LDB 5, LDB 1 + LDB 6, LDB 2 + LDB 3, LDB 2 + LDB 4, LDB 2 + LDB 5, LDB 2 + LDB 6, LDB 3 + LDB 4, LDB 3 + LDB 5, LDB 3 + LDB 6, LDB 4 + LDB 5, LDB 4 + LDB 6, LDB 5 + LDB 6, LDB 1 + LDB 2 + LDB 3, LDB 1 + LDB 5 + LDB 6, LDB 2+ LDB 3 + LDB 4, LDB 3 + LDB 4 + LDB 5, LDB 4 + LDB 5 + LDB 6, LDB 1 + LDB 2 + LDB 3 + LDB 4, LDB 3 + LDB 4 + LDB 5 + LDB 6, LDB 1 + LDB 2 + LDB 5 + LDB 6	Lignocellulose degradation was absent
LDB 1 + LDB 2 + LDB 3 + LDB 4 + LDB 5 + LDB 6	Residues of rice straw left in powder form

Table 3: The degradation of lignocellulose by thermophilic bacterial consortium at different pH

pH	Degradation ratio
5.0	53.07 % (Where M_r = 0.61gm approx and M_t = 1.3gm)
6.0	68.4 % (Where M_r = 0.42gm approx and M_t = 1.3gm)
7.0	74.6 % (Where M_r = 0.34gm approx and M_t =1.3gm)
8.0	70.7 % (Where M_r = 0.38gm approx and M_t = 1.3gm)
9.0	56.1 % (Where M_r = 0.57gm approx and M_t = 1.3gm)

Table 4 Effect of incubation time on degradation of lignocellulose

Appearance	% degradation
No change in rice straw appearance on first and second day of incubation	0 %
Soften of rice straw on third day of incubation	44.61 %
Filter paper was completely degraded on fifth day of incubation	63.07 %
Residues of rice straw left in the form of powder on seven day of incubation	75.38 %

Table 5 Effect of Different carbon sources on degradation of lignocellulose

Carbon source	Degradation ratio (%)
Glucose	67.6 % (Where M_r = 0.42gm approx and M_t = 1.3gm)
Fructose	63.6 % (Where M_r = 0. 47gm approx and M_t = 1.3gm)
Maltose	61.5 % (Where M_r = 0.5gm approx and M_t = 1.3gm)