PRELIMINARY INVESTIGATION OF CELLULOLYTIC ACTIVITY OF SOME SELECTED FUNGI ON SAW DUST

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INTRODUCTION

Among the widely used sources of non-renewable energy, fossil fuels play a major role. The demand for fossil fuels is ever increasing and is expected to exceed the supply in the near future. Hence, the need for alternate, renewable energy sources is a global requirement.

Lignocelluloses is the most abundant renewable natural resource and substrate available for conversion to fuels. Amongst available cellulosic feed stocks from agriculture and other sources maize is one of the largest crops which is potentially available for fuel production (Somerville *et al*., 2010). Furthermore, tremendous amounts of cellulose are available as municipal and industrial wastes which contribute to our pollution problems at present. Thus, there is great potential in the use of cellulosic biomass as a renewable source of energy *via* breaking down to sugars that can then be converted to ethanol. Industrially ethanol is produced from corn and sugar which is used as an alternative fuel (Bhat and Bhat, 1997).

The potential quantity of ethanol that could be produced from cellulose is over an order of magnitude larger than that producible from corn. In contrast to the corn-to-ethanol conversion, the cellulose-to-ethanol route involves little or no contribution to the greenhouse effect and has a clearly positive net energy balance (five times better). As a result of such considerations, microorganisms that metabolize cellulose have gained prominence in recent years (Bhat and Bhat, 1997, Tomme *et al*., 1995). Some examples of fungi used for the degradation of cellulose are *Fusarium sp., Aspergillus sp*., *Trichoderma sp., Pencillium sp*., etc (Gunatilake *et al*., 2013)

METHODOLOGY

***General experimental procedures*:** Sterilization of all glassware, media, and other solutions was carried out at 15 psi and 121ºC using the Gemmy SA-300VL autoclave. Absorbance of test solutions and spore solutions were measured using the WPA S104 analogue UV-Vis spectrophotometer. Saw dust was ground and sieved (500 μm) prior to use in all experiments

***Preparation of working cultures*:** Stock cultures of five species of fungi from the available OUSL fungal collection and another two species of soil fungi collected from OUSL, all of which have shown cellulolytic activity, were revived on yeast malt agar (YMA) and potato dextrose agar (PDA) media at 34.5 ºC to prepare working cultures for the experiments. Cellulolytic ability of all the seven species was confirmed using the filter paper assay (FPA) technique. Identification of these fungi was attempted by studying the morphological characters.

***Filter paper assay (FPA)*:** In this test, the only available source of carbon for the microorganism was provided by cellulose on the filter paper. Spore solutions of different fungi sample (1.1 mL) were inoculated into test tubes which contained 10 mL of mineral salt medium with 1cm x 14 cm strip of filter paper. About half of the filter paper was allowed to project above the surface of the liquid. They were incubated at 34.5 ºC and were examined daily. The degree of degradation of the filter paper was observed as a measure of the cellulolytic activity.

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***Pre-treatment of saw dust*:** *Heat pre-treatment* was carried out by heating the feedstock for 1 h in an oven at 160 ºC. *Steam pre-treatment* was carried out by exposing the feedstock to steam in a large vessel for 1 h.

***Determination Cellulolytic activity of selected fungi on saw dust*:** Mineral salt medium (8.00 mL) [containing $(NH_4)_2SO_4$ 6.0 g, KH_2PO_4 1.0 g, K_2HPO_4 1.0 g, $MgSO_4$ 0.1 g, $CaCl_2$ 0.1 g, yeast extract 0.5 g, $FeCl_3.6H_2O$ 16.5 mg, $ZnSO_4.7H_2O$ 0.18 mg, $CuSO_4.5H_2O$ 0.16 mg, EDTA 20.1 mg and $CoCl_2$ 0.18mg in 1L of sterile water] was added to a flat bottle containing sieved saw dust (2.0 g) along with 2 ml of spore suspension having similar spore concentration (spore suspension was prepared taking into account a specific number of spores using a haemocytometer and adjusting the number of spores per unit volume in order to have, approximately, the same concentration in all spore suspensions) and incubated at room temperature (30 ºC). This experiment was repeated with each of the seven (7) fungal cultures separately as well as in pairs (1 ml each of the two spore suspensions were added), using sieved saw dust under three different conditions [pre-treated (heat or steam) and non-pretreated] and subjected to incubation periods of 14, 21 and 28 days. Saw dust without fungus served as the control. After specific time duration the saw dust samples were analyzed for their reducing sugar contents as follows. Each sample was mixed with a small amount of distilled water, sonicated for 5 min, filtered through celite and the final volume of filtrate was adjusted to 10.00 ml. A sample (3.00 mL) of this filtrate was analyzed for reducing sugar content by dinitrosalicylic acid (DNS) method (Miller, 1959) as described below and the absorbance was measured at 575 nm. The concentration of reducing sugars in terms of D-glucose was then determined using the standard curve. The filtrates from each of the control were used as the blanks in measuring the absorbance. These experiments were carried out in duplicate.

***Determination of the Glucose concentration using DNS method*:** From a stock solution of D-glucose (0.005 M), a series of dilutions (0.0006 to 0.0028 M) was prepared. Dinitrosalicylic acid (DNS) reagent (3.00 ml) was added to 3.00 mL portions of each of these solutions, mixed well and heated to 90 ºC in order develop the reddish orange/brown color. To stabilize the color, 40% potassium sodium tartarate buffer solution (1.00 ml) was then added to each of the hot solutions and rapidly cooled to room temperature in an ice bath. The blank solution was also prepared using water (3.00 ml) instead of glucose solution. A standard curve of *Absorbance vs Concentration* was constructed by measuring the absorbance of these solutions at a wavelength (λ) of 575 nm.

RESULTS AND DISCUSSION

Screening of fungal library for the cellulolytic activity using FPA resulted in selection of seven fungal cultures having potential for hydrolyzing cellulose. Total reducing sugar contents in terms of W/W percent of glucose are given in Table 1.

Table 1: Percentage of glucose (W/W) produced by the action of seven fungi on saw dust under different conditions**.**

Fungus	Concentration of sugars in terms of glucose (percent W/W)								
	Non-pretreated			Heat pre-treatment			Steam pre-treatment		
	14 days	21 days	28 days	14 days	21 days	28 days	14 days	21 days	28 days
Aspergillus sp. (A)	0.090	0.080	0.068	0.086	0.063	0.063	0.153	0.144	0.135
Monilia sp. (B)	0.054	-	-	0.090	0.068	0.063	0.135	0.108	0.100
Aspergillus sp. (C)	0.072	0.054	-	0.072	0.054	0.054	0.100	0.090	0.081
Aspergillus sp. (D)	-	-	-	0.054	0.054	0.063	0.081	0.072	0.072
Aspergillus sp. (E)	-	-	-	0.063	0.054	-	0.081	0.072	0.072
Unidentified sp. (F)	0.086	0.077	0.063	0.050	0.045	0.054	0.144	0.135	0.117
Aspergillus sp. (G)	0.109	0.095	0.072	0.090	0.072	-	0.162	0.144	0.144

It appeared that among the seven fungal cultures tested, two *Asperigillus* sp. (A) and (G) and, an unidentified fungal species (F), have produced relatively high yield of glucose from saw dust. It was also observed that the maximum sugar content is produced at 14 days duration. The reduction of sugar content after 14 days may be accounted for the use of soluble carbohydrates by the fungus for its metabolism.

Out of the 21 possible pairs of the seven fungi, three combinations (A+C, A+F and A+G) have shown marked increase in glucose content (See Table 2). It was found that incubation of pairs of fungi too have produced the maximum sugar content from steam pretreated saw dust in 14 days. Since the results indicate that there is a synergistic effect of fungi in hydrolysing naturally occurring complex carbohydrates, a consortium of microorganisms may be more effective in degradation of cellulose.

Table 2: Percentage of glucose (W/W) produced by the action of three combinations of two fungi species on saw dust under different conditions.

Fungus	Concentration of sugars in terms of glucose (percent W/W)								
	Non-pretreated			Heat pre-treatment			Steam pre-treatment		
	14 days	21 days	28 days	14 days	21 days	28 days	14 days	21 days	28 days
A+C	0.178	0.162	0.157	0.211	0.189	0.167	0.211	0.205	0.178
A+F	0.211	0.205	0.173	0.227	0.205	0.173	0.227	0.216	0.208
A+G	0.300	0.262	0.224	0.324	0.262	0.224	0.410	0.367	0.300

CONCLUSIONS/RECOMMENDATIONS

The foregoing results clearly show that steam pre-treated saw dust over 14 day incubation period produced the highest glucose content. The use of combination of fungi species (pairs in this case) has shown a further increase in cellulolytic activity, with the combination A+G providing the most promising results amongst them. The trend shown clearly indicates the potential for use of above fungi in converting cellulolytic bio mass (saw dust) to reducing sugars. Further investigations are needed to be carried out in terms of optimising the time duration and steam pre-treatment while other waste material also should be tested for the production of sugars.

REFERENCES

Bhat, M. K., and S. Bhat. (1997). Cellulose degrading enzymes and their potential industrial applications. *Biotechnol. Adv.* 15: 585-620.

Gunathilake, K.M.D, Ratnayake, R.R, Kulasooriya, S.A, Karunaratne, D.N (2013) Evaluation of cellulose degrading efficiency of some fungi and bacteria and their biofilms. *J. Natn.Sci.Foundation Sri Lanka*, 41(2): 155-163.

Miller GL. 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. *Anal. Chem.* 31:426-428.

Somerville C., Youngs, H., Taylor, C., Davis, S. C. and Long, S.P. (2010). Feedstocks for lignocellulosic biofuels. *Science*, 329: 790-792.

Tomme, P., Warren, R. A. and Gilkes. N. R. (1995). Cellulose hydrolysis by bacteria and fungi. *Adv. Microb. Physiol*., 37: 1-81.

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