International Journal of Emerging Technology and Advanced Engineering

Volume 3, Special Issue 3: ICERTSD 2013, Feb 2013, pages 58-65

An ISO 9001:2008 certified Int. Journal, ISSN 2250-2459, available online at www.ijetae.com

FUNGAL ISOLATES FROM LOCAL ENVIRONMENT: ISOLATION, SCREENING AND APPLICATION FOR THE PRODUCTION OF ETHANOL FROM WATER HYACINTH

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ABSTRACT

Water hyacinth biomass is a potential substrate for the production of ethanol which can be conveniently used as a gasoline additive as an octane booster and improve the vehicle emissions. The present study is focused on isolating and screening indigenous fungi for the hydrolysis of water hyacinth in the lignocellulosic conversion process. The initial cellulose activity was measured as I_{CMC} (carboxymethyl cellulose) index which was found to be max at pH=5. Crude enzymes extracted after solid state fermentation (SSF) in an effort to reduce the cost of ethanol production, which needs lesser infrastructure and relatively less skilled manpower besides being able to use cheaper raw materials for enzyme production. The cellulytic and xylanytic activity was monitored using water hyacinth as substrate. Out of several isolates screened, best result was recorded up to 131.995 mg/g of reducing sugar and xylose yield up to 210.4 mg/g of WHB. Concentrated enzymatic hydrolysate of WHB containing reducing sugars and xylose was used for ethanol fermentation. The maximal ethanol yield was 2.217 g/l using *Pichia stipitis*, 2.163 g/l using *Candida shehatae* and 1.925 g/l using *Saccharomyces cerevisae*. Fungal isolate F6 provided the best yield as well as total extracellular enzyme production in comparison to other fungal strains, so identification of the fungal sample to its nearest species was undertaken. Fungal strain F6 (*Alternaria alternate)* was sequenced and the genomic DNA information was submitted to gene bank NCBI and an unique accession no JQ781696 was assigned.

Keywords: Isolation, Ethanol Production, Water hyacinth, Lignocellulosic biomass.

1. INTRODUCTION

At present the world is facing a major energy threat which needs to be solved by virtue of alternative energy sources. Biomass forms a major contributor as future supply of renewable energy along with benefits like environment protection and economic development [1]. Bioethanol has received considerable attention in the transportation sector because of its utility as an octane booster, fuel additive, and even as a neat fuel [2]. Most of the fuel ethanol produced in the world is currently sourced from starchy biomass or sucrose (rice straw, wheat straw, molasses, sugarcane juice, corn, etc. or other food crops), however, due to their primary utility as food and feed, these crops cannot meet the global demand for ethanol production as an alternative transportation fuel. For avoiding this competition to food crops for arable land resources, aquatic biomass water hyacinth (*Eichhornia crassipes*) [3] adds an advantage and can be used as a supplement for alternative sources.

Lignocellulosic biomass is projected as a virtually eternal source of raw material for fuel ethanol production. Hydrolysis of lignocellulosic biomass to reducing sugars for the production of bio-ethanol is a promising strategy for efficient utilization of renewable resources. Lignocellulosic biomass, which includes agricultural residues, wood and paper industry wastes etc., is inexpensive and abundantly available resource composed of up to 75% carbohydrates, and in the near future it will become an essential source for fermentable carbohydrate. Apart from these feed stocks, lignocellulosic biomass comprises mostly of cellulose (20–50%), hemicellulose (20–35%), and lignin (10–35%), is an alternative feed stock for bioethanol production being the earth's most prevalent renewable organic materials available for microbial or other conversions.

Plant biomass/lignocellulosic feedstock is composed primarily of cellulose, hemicelluloses and lignin and smaller amounts of pectin, protein,

Presented at International Conference on Energy Resources and Technologies for Sustainable Development, 07-09 February 2013, Howrah, India.

ISSN 2250-2459, *Volume 3, Special Issue 3: ICERTSD 2013, Feb 2013, pages 58-65*

extractives and ash. Distribution of cellulose, hemicelluloses and lignin as well as the content of the different sugars of the hemicelluloses varies significantly between different plants. Cellulosic materials are renewable natural biological resources and generation of bioenergy from such resources is important for the conservation of the environment and also important in reducing our dependency on the fossil fuels [4].

Lignocellulosic structure is complex. Carbon is locked in lignocellulosic structure in the form of different types of sugars. The major carbon flow from fixed carbon sink to atmospheric CO_2 is through cellulose biodegradation. Cellulases are specialized enzymes for degradation of cellulose and among microorganisms, filamentous fungus like *Trichoderma reesei* are considered to be the most efficient cellulase producer which has a long history in the production of hydrolytic enzymes and has been widely used in the food and feed industries, textile, pulp and paper industries.

Cellulose, the most abundant constituent of the plant cell wall, is a homo-polysaccharide composed entirely of D-glucose linked together by β-1,4-glucosidic bonds and with a degree of polymerization of up to 10 000 or higher. Therefore, hexose sugars from cellulose and pentose sugars from hemicellulose can be used for fermentation to ethanol, which is an attractive route to energy feedstock that supplements the depleting stores of fossil fuels.

It has been reported that the biomass of water hyacinth has about 48% hemicelluloses, 18% cellulose 3.5% lignin [5]**.** Though there is a significant amount of variability in composition reported by different labs, but in general the biomass is considered to be rich in hemicelluloses and with very less lignin content. The biomass productivities of this plant is very high [6] and there is abundant availability of this plant in certain parts of the world making it a suitable feedstock for distributed ethanol production. The possibility of converting water hyacinth to biogas or fuel ethanol is currently established in a number of developing countries, mainly in India.

The cellulose and hemicellulose content of biomass material can be hydrolyzed chemically or enzymatically. Processing lignocellulosic into bioethanol consists of four steps: pretreatment, hydrolysis (enzyme saccharification), fermentation and distillation. Pretreatment process is the most important step, which reduce cellulose crystallinity, increase porosity of materials and digestibility of the lignocellulosic materials improving enzyme access to cellulose. Among various pretreatment process enzymatic hydrolysis (crude enzyme) of lignocellulose can help cut cost, reduce environmental toxicity and hydrolyze both cellulose and hemicellulose for efficient release of the sugars for fermentation. Novel fungal strains having high hydrolysing activity on the feedstock have been studied. Such enzymes play vital role in depolymeriztaion of lignocellulosic feedstock. Therefore in this study fungi having cellulolytic and xylanolytic activity have been isolated and screened. The isolation was done from different natural samples like decaying wood, humus, leaf litter etc. The fungal strains were monitored for presence of endoglucanase enzymes by CMCase assay and release of pentoses was measured using Trinder method [7]. The enzyme assays were performed to check the enzyme activity.

2. MATERIALS AND METHODS

2.1 Biomass Preparation

Water hyacinth plants were collected from a local unpolluted pond within the CSIR- Central Mechanical Engineering Research Institute campus. Only the shoots and leafy parts of the plants were used and the roots were discarded. The shoots and leaves were first reduced to a particle size of about 2-3cm and then dried at 106 ˚C for 6 h. After drying particle size was further reduced to 1- 2mm by grinding. This grinded Water hyacinth Biomass (WHB) was used for the experiments. It was stored in air tight containers at room temperature.

2.2 Dilute Acid and Alkali Treatment

The dried WHB was pretreated with dilute sulfuric acid (5%) with soaking time 1h and treatment time 10min at 130˚C and also with sodium hydroxide solution (5%) with soaking time 1 h and treatment time at 150˚C. Biomass loading was 10% (w/v) as that was the maximum possible loading. The acid treated and alkali treated WHB was then washed thoroughly under running water until pH was neutralized. The washings were then dried at room temperature and stored in air tight container till further use.

2.3 Medium and Culture Conditions for Isolation of Lignolytic Fungal Isolates

Three separate mediums were prepared in order to differentiate cellulose-utilizing microorganisms. Enrichment and selective media were used for isolation of fungal strains and help in plate screening methodology for fungal isolates. Potato Dextrose Agar (PDA) (g/l) consisted of potato infusion -200, dextrose-20 and 2% agar. The medium was autoclaved for 20 min at 121°C, dispensed into sterile petri-dishes and cooled. This was used as enrichment media for fungal cultivation. Further selection of fungal strain was done on Mandel's Medium (g/l) containing NH_2SO_4-1.4, KH_2PO_4-2.0, Urea-0.3, $CaCl_2$-0.3, $MgSO_4$-0.3, $FeSO_4.7H_2O$-0.005, $MnSO_4.H_2O$-0.0016, H_2O-0.0014, $CoCl_2$-0.002, Carbon Source-1-2% and pH-6.0. This media was used for cultivation and selection of cellulolytic fungi utilizing lignocellulosic substrate like CMC [8]**.** Another selective media used during this study was Basal Media (g/l) containing different macro- and micro-nutrient for fungal cultivation, Carboxy methyl Cellulose-10, $NaNO_3$-6.5, K_2HPO_4-6.5, Yeast Extract-0.3, KCl-6.5, $MgSO_4.7H_2O$-3.0, Glucose-0.65, Agar-17.5 and

ISSN 2250-2459, *Volume 3, Special Issue 3: ICERTSD 2013, Feb 2013, pages 58-65*

pH-8.0-9.0. After the incubation period the fungal colonies exhibited zones of clearing and was used for identifying Index of relative enzyme activity (I_{CMC}). Other media like Malt extract medium (g/l) containing Malt extract-12.5, Glucose-2.5, Glycerol-1ml and Peptone-2.6, and Czapek's Dox Agar (g/l) having sucrose-30.00, sodium nitrate-3.00, di-potassium phosphate-1.00, magnesium sulphate-0.50, potassium chloride-0.50 and ferrous sulphate-0.01, and the final pH-7.3 were used for colony differentiation and cultivation of diverse form of fungal strains. During fungal screening all above given media were incubated at 25°C with variability in incubation period. The isolates were stored on potato dextrose agar slants for their further use during experimentation.

2.4 Sample Collection and Dilution Method

In nature, fungi are found abundantly in soil, usually exist in the organic upper layers (humus and topsoil), although some species have also been found in underlying rocky layers i.e. the subsoil. Collection of samples for isolation of cellulolytic fungi was done from tree plantations and some softwood indigenous trees, wet dead wood surfaces, from the live trees and wet tree stumps and some others were collected from the decaying humus in the soil, decaying wood, leaf litter and other decomposed organic matter .20gms of each sample was suspended in 200ml NaCl solution (0.75% w/v) and mixed for 15 minutes on a rotary shaker (120 rpm). Two dilutions (10^{-5} and 10^{-4} CFU/ml) were used for analysis of total units of fungal population present in the sample and were further evaluated for isolation of cellulolytic fungi [9]**.**

2.5 Isolation of Colonies

The colonies observed during initial screening on potato dextrose media as shown in Fig. 1. were used for selection on mineral media like Mandel's and Basal media.

Fig.1. PDA plates showing colony morphology.

Fungal isolates were screened on Mandel's mineral salts solution [8]**,** with addition of 2% agar and carbon source used as CMC. This step enhanced the proliferation of fungal isolates which could utilize cellulose as their carbon source and help in determining initial cellulase activity of isolated colony from PDA plates. The selective media plates were incubated for 4-7 days at 25°C after which clear zones could be observed only around colonies of active fungal strains. Basal medium was used to further test cellulase activity of fungal isolates. Isolates showing positive results on Mandel's medium were inoculated on Basal medium plates at three different pH (5.0, 7.0 and 9.0). The plates were inoculated exactly at the center so that a clear circular colony could be formed so as to get clear zone.

2.6 CMCase Assay

For the screening of cellulase production CMC Congo red plate technique was used. The isolates were grown on Carboxymethyl Cellulose Basal medium. Plates were incubated at 25 °C for 2-3 days and then stained with 1% Congo Red dye for 10-15 min followed by distaining with 1 M NaCl for 20 min. Then 0.1M HCl was applied on the plates so a distinct zone of hydrolysis is obtained. A zone of clearance was immediately visible (Fig. 2).

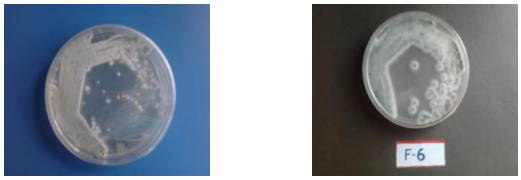

Fig. 2. Fungal colonies showing clearance zone on CMC plate after dyeing with Congo Red dye for Screening Cellulolytic and xylanolytic Activity of Fungal Isolates on Lignocellulosic feedstock

The Index of Relative Enzyme Activity (I_{CMC}) was recorded as clear zone ratios as shown in Table 1.

$$I_{CMC} = \frac{\text{clear zone diameter}}{\text{colony diameter.}}$$

Table1 : I_{CMC} Index values for the cellulose degrading fungi and the pH on which it was found to be maximum.

Sample No	I_{CMC}	pH
F1	1.99	5
F3	1.6	7
F4	1.7	7
F6	2.15	5
F8	1.9	5
F14	1.82	5
X1-A	1.76	5
X2-A	1.5	7
X3-3B	1.619	5
X3-2B	1.667	5
X2-B	1.333	7
X2-A	1.2	5
X3-4A	1.437	5
X3-3C	1.288	5
X4-A	1.318	7
X3-1B	1.25	7
X3-3D	1.321	5

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2.7 Screening of Cellulolytic and Xylanolytic Activity of Fungal Isolates on Lignocellulosic Feedstock

The potential strains screened from CMCase assay were later tested on WHB used as lignocellulosic feedstock. Fungal beads were produced in Basal medium by inoculating spore suspension, kept for 4-5 days which were then centrifuged at 5000 rpm for 10 min and supernatant was discarded. The pellet obtained was inoculated in conical flask containing Mandel's media with 5% acid, alkali and untreated WHB, autoclaved at 121^{o}C and 15psi for 15 min. The shake flask cultures were incubated at 25°C for 10 days at 125 rpm and sugar estimation was monitored as reducing sugars and xylose, for the enzymatic activity check of fungal strains on lignocellulosic feedstock.

2.8 Crude enzyme production from Solid State Fermentation and measurement of enzyme activity

For individual enzyme assay of cellulase, xylanase and β-glucosidase; the fungi were grown on water hyacinth under solid state fermentation (SSF) conditions. The properly sporulated fungal plate cultures were taken and untreated dried WHB was used as the only carbon source. 5 g of untreated WHB was taken and the rest media components were supplemented with Mandel media's mineral components. The moisture content was adjusted to 60%. The incubation was done at 28°C for 7 days. For extraction of crude enzyme, the contents in each flask were suspended in 100 ml cold citrate buffer (0.05M, pH- 4.8) and put on a rotary shaker for 10 min. The contents were then centrifuged at 10,000 rpm at 4°C for 10 min to separate the biomass. The supernatant was collected and four parts of ice cold acetone was added. The mixture was again centrifuged at 10,000rpm at 4°C for 15 min. The supernatant was discarded and the aqueous layer of crude enzyme obtained was collected. This crude enzyme was then suspended in citrate buffer (0.05M, pH- 4.8) and stored at 4°C until further use.

2.9 Analytical methods

Estimation of reducing sugar in the hydrolysate of biomass was done by DNS method [10] and the estimation of xylose was done by Trinder method [7]. At regular interval samples from incubated flasks were collected for determining sugars released in the media due to hydrolysis of lignocellulosic feedstock by the fungal activity. The samples collected were filtered using Whatman no. 1 filter paper. The filtrate was then used for estimation of sugars. To determine the enzyme productivities, total protein content in the culture supernatants was measured by the Lowry method [11] using bovine serum albumin as standard.

2.10 Enzyme assays

Cellulase was measured according to the IUPAC methods[12] using Whatmann filter paper no.1 as substrate and glucose as standard. Xylanase and β-glucosidase was estimated according to [13], [14]. Xylan was used as substrate for xylanase and xylose as standard for xylanase. Salicin was used as substrate and glucose as standard was used for β-glucosidase [15]. All the values were expressed in terms of IU/min/ml (Table. 2).

Table 2: Enzyme activity present in isolated strains (Enzyme activity is expresses in terms of IU/min/ml)

Sample No	Cellulase*	Xylanase
F-1	0.512	1.673
F-6	0.229	1.512
F-14	0.165	1.204

*Values were too less to be expressed as FPU/ml. Hence, one unit of enzyme activity is one μmole of glucose released per minute per ml of enzyme.

2.11 Fermentation and Ethanol Estimation

Pichia stipitis (NCIM 3500) and *Candida shehatae* (NCIM 3497) were used for pentose fermentation, obtained by the courtesy of NCIM, Pune. The medium used for inoculum preparation contained (g /L): D-xylose, 50; glucose, 5; yeast extract, 3; malt extract, 3; peptone, 5; pH 5.0. The media were sterilized by autoclaving at 120 °C for 15 min. *Saccharomyces cerevisae* (MTCC 181) was obtained by the courtesy of I.M. Tech., Chandigarh. The medium used for inoculum preparation contained (g/L): yeast extract 10, peptone 20, D-glucose 10. To prepare the inoculum, a 250 mL Erlenmeyer flask containing 50 mL medium was inoculated from a fresh culture plate, and incubated at 30°C on rotary shaker at 250 rpm. The culture was grown again for 18 hr for *S. cerevisae* and 20 h for *P. stipitis* and *C. shehatae* at rotatory shaker at 125 rpm, 28^{0}C and the broth was centrifuged at 10,000rpm for 10 min. The cell pellet was washed and suspended in sterile distilled water. The hydrolysate supplemented with (g/l) NH_4Cl 0.5, KH_2PO_4 2.0, $MgSO_4 \cdot 7H_2O$ 0.5, yeast extract 1.5, $CaCl_2 \cdot 2H_2O$ 0.1, $FeCl_3 \cdot 2H_2O$ 0.1, $ZnSO_4 \cdot 7H_2O$ 0.001 was inoculated with 10% (v/v) inoculum of each strains at pH 5.0 and incubated at 28^{0}C with 125rpm of agitation. Samples were withdrawn at regular intervals of time for the determination of ethanol and residual sugar concentration. Ethanol estimation was done spectrophotometrically by potassium dichromate method. Reagents:Acid dichromate solution (0.1M $Cr_2O_7^{2-}$ in 5M H_2SO_4): 7.5 g of potassium dichromate was dissolved in dilute ulphuric acid and final volume was adjusted to 250 mL. Ethanol stock solution (10 mg/ml) [16].

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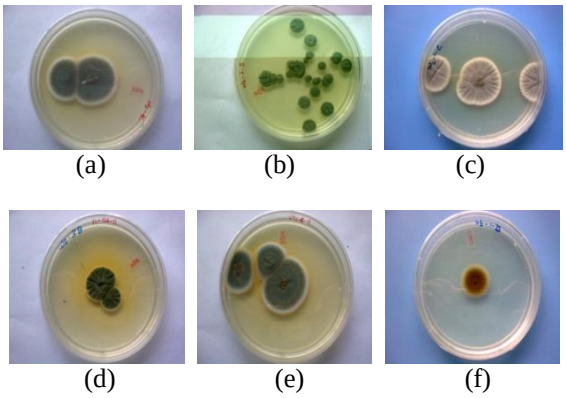
3. RESULTS & DISCUSSION

3.1 Fungal Isolates: Characteristics and Diversity

The fungal strains isolated from natural sources showed a variety of phenotypic characteristics. The isolated colonies from dilutions, collected from natural sources were mainly selected for further screening on the basis of their colony characteristics on Potato Dextrose Agar. A large variety of fungi were distinguished on the basis of morphological characteristics, which ranged in colony size, color, surface characteristics, appearance and shape. Most of the colonies were found to be mold-like in appearance and circular in shape (Fig. 3a). The color of such colonies ranged from light olive green to blackish olive green. Most were also found to have granular appearance and spores with various shades of green in color (Fig. 3b). Some were also observed to have cottony and fury appearance (Fig. 3c). A few of these isolates have turned the media in the plate yellow to slightly orange in color which may have been due to production of secondary metabolites in the media (Fig. 3d). Also there were isolates, which had granular appearance with spores ranging in color from bluish grey to bluish green/black in color and white edges (Fig. 3e & f). Most colonies were found to have dark green colored spores and some were dark green colored molds with grooves on present on their surfaces.



(a) (b) (c)

(d) (e) (f)

Fig.3. Morphological characteristics of the fungal colonies isolated grown on PDA plates a) mold-like in appearance and circular in shape, b) colonies ranged from light olive green to blackish olive green, c) cottony and fury appearance, d) Yellowish pigment (secondary metabolite) produced in the media, e) & f) granular appearance with bluish grey to bluish green/black spores.

3.2 Index of Relative Enzyme Activity

After screening of fungal strains on enrichment media, the isolated fungal strains were evaluated for cellulolytic activity and for this selective media like Mandel's media and basal media were used [17], [18], [19]. During screening process fungal colony characteristics and their diameter were observed. Colony diameter varied among fungal isolates grown at pH 5.0, 7.0 and 9.0 Further to ascertain the cellulolytic characteristics of the strains and to identify the endoglucanase activity (CMCase) of the strain, indices of relative enzyme activity (I_{CMC}) was determined on CMC supplemented basal medium. Table 1 represents the preliminary I_{CMC} activity of the fungal isolates on three different pH. The isolates having the index (I_{CMC}) equal to or greater than 1.5 were used for further screening. Potential strains with higher I_{CMC} were later investigated on the shake flask culture using lignocellulosic feeds as carbon source.

3.3 De-polymerization of lignocellulosic feedstock at Shake Flask

After evaluation of the CMCase activity of different fungal strains, inoculums of these strains were cultivated on shake flask with water hyacinth as lignocellulosic feedstock, during the cultivation, sugar content of the media were measured by Trinder method [7] for xylose estimation and DNS method for reducing sugars [10]. Enzymatic activity of whole cell biocatalyst released reducing sugars and xylose, which is an indication of cleavage of cellulose and xylan molecules in the water hyacinth. It was observed that during incubation, xylose sugar yield was at its maximum on the 4th day and then reduced from thereafter as shown in Fig. 4 and Fig. 5. Also the total reducing sugar yield was found to be maximum in and around 4^{th} to 5^{th} day as indicated in Fig .4. It is also observed that the reducing sugar yield reaches maximum after 5-6 days of incubation when using whole cell biocatalyst systems. Total reducing sugars as glucose and pentose sugar estimation as xylose are indicative of cellulase and xylanase production by the screened fungal strains as there was only water hyacinth used in the mineral medium as carbon source. Out of several strains, two strains, F-6 and F-1, showed the maximum yield of reducing sugar and xylose yield. The isolate F6 gave a reducing sugar yield of 329.67 mg/g and isolate F1 gave 302.34 mg/g when alkali pretreatment was given to WHB. But when acid pretreatment was done prior to biological hydrolysis, the reducing sugar yield was reduced to 138.01 mg/g and 114.305 mg/g for F6 and F1 respectively. The maximum xylose yield was 110.09 mg/g for F6 and 101.15 mg/g for F1, when alkali pretreated WHB was used. There was very less difference in the amount of reducing sugars released with acid pretreated WHB, with 103 mg/g and 92.6 mg/g for F6 and F1, respectively. Thus, alkali pretreatment of WHB is more effective for the overall maximization of biomass saccharification by whole cell biocatalysts because during acid pretreatment, there might have been considerable loss of cellulose during acid hydrolysis.

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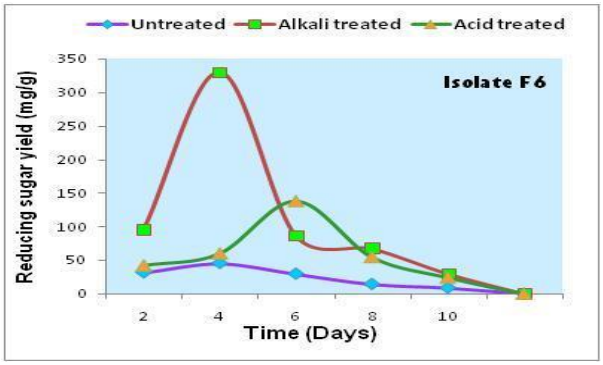


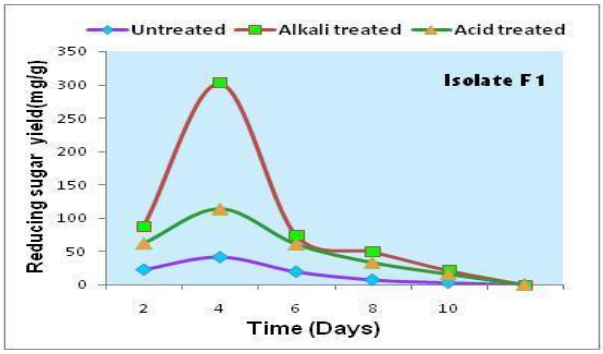
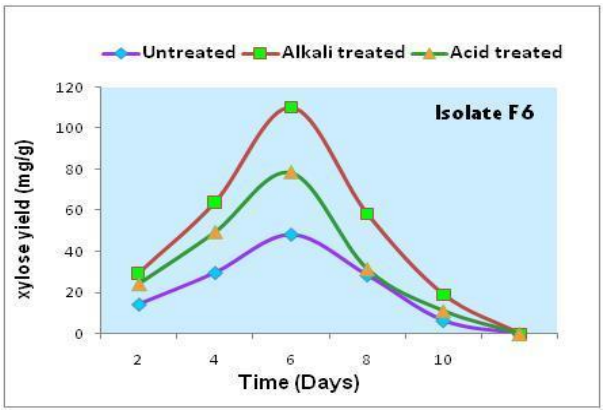


Fig. 4. Effect of time on reducing sugar yield for untreated, acid treated & alkali treated WHB for fungal isolation



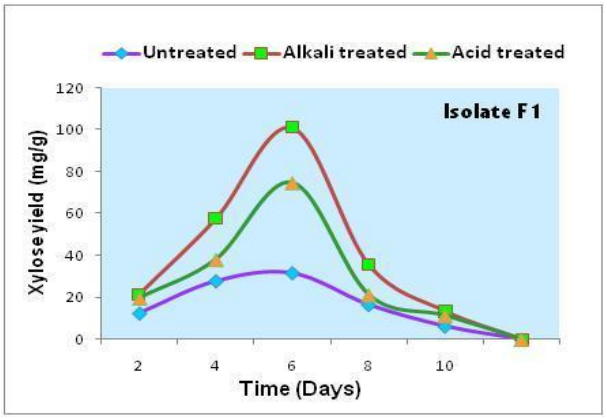
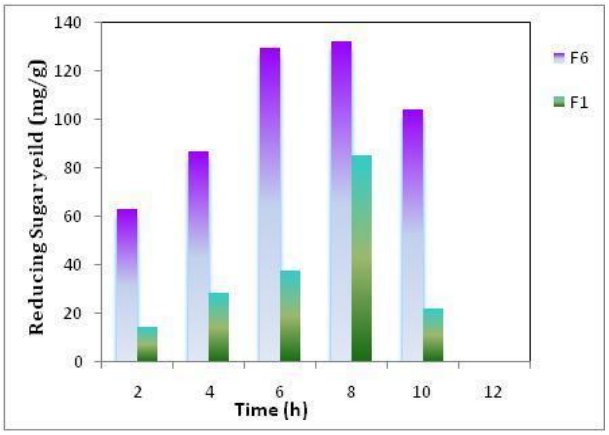


Fig.5. Effect of time on xylose yield for untreated, acid treated & alkali treated WHB for fungal isolation

3.4 Saccharification of alkali treated WHB using crude enzyme extracts from the Fungal Isolates

A significant part of the cost for lignocellulosic ethanol production is the cost of cellulose and xylanase enzyme used for biomass hydrolysis. On site production of enzymes for saccharification can reduce the cost of enzyme production and that of transportation. Crude preparations of enzymes extracted after solid-state fermentation (SSF) of untreated WHB were used in this study in an effort to reduce the cost of ethanol production. SSF needs lesser infrastructure and relatively less skilled manpower besides being able to use cheaper raw materials for enzyme production. All these add to the economic advantage of this mode of fermentation for enzyme production. SSF also produces a more concentrated product, which in this case is very much advantageous [20]. As evident from Fig. 6, the amount of sugars released by using crude enzyme extract from F6 was observed to be highest. Xylose yield and reducing sugar yield were observed to be 210.4 and 131.995 mg/g of dry Alkali treated WHB. Xylose yield was seen to have been more than reducing sugar yield. This is because the crude enzyme extracted from strain F6 has more xylanase activity than cellulase.



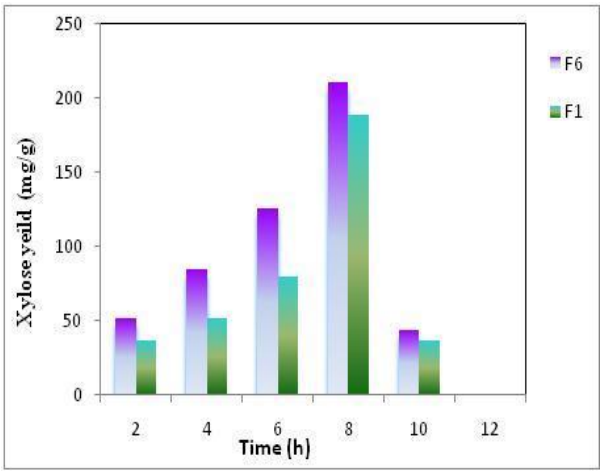


Fig. 6. Saccharification using crude enzyme preparations

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3.5 Production of Ethanol

Concentrated enzymatic hydrolysate of alkali, acid and untreated WHB was used as substrate for ethanol fermentation. From the studies maximum ethanol yield was recorded from the hydrolysate of alkali treated WHB. From the results of ethanol fermentation study shown in Fig. 7, it becomes clear that the efficiencies of ethanol production from the WHB hydrolysate are less and the maximal ethanol yield was 2.217 g/L when *P. Stiptis* was used, followed by 2.163 and 1.927 g/L for *C. shehatae* and *S. cerevisae*. *Saccharomyces cerevisiae* can ferment only hexoses, probably accounting for the low ethanol production. The overall low ethanol production may have aroused due to the initial sugar loss during the hydrolysis step. Pretreatment of lignocellulosic biomass may produce degradation products with an inhibitory effect on the fermentation process.

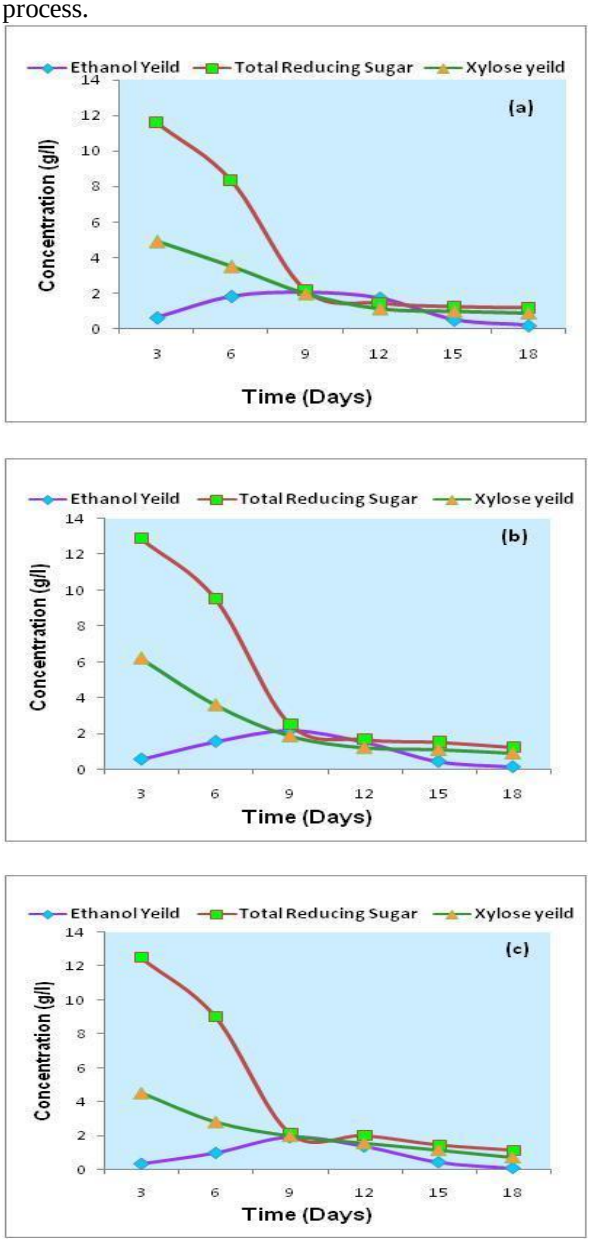

Fig. 7. Ethanol production from WBH hydrolysate by (a) *C. shehatae* (b) *P. Stipitis* and (c) *S. cerevisae*

3.6 Characterization of isolate F-6

As fungal isolate F6 provided the best yield of total reducing sugar and xylose yield as well as total extracellular enzyme production as compared to other fungal strains, we chose to identify the fungal sample to its nearest species. The strain F6 was characterized by M/s Chromous Biotech Pvt. Ltd, Bangalore. It was found to be closest to *Alternaria brassicae* (NCBI Acc No: JN108912.1). The next closest homologue was found to be *Alternaria arborescens* (NCBI Acc No JN108912.1). The GenBank entry was done as *Alternaria alternata* CMERI-F6 and the accession number is JQ781696.

5. CONCLUSIONS

As evident from the graphs, it was observed, that the amount of sugar release increased with the increase in the production of extracellular enzymes. Thus, it substantiates that there is production of cellulolytic and xylanolytic enzymes which are responsible for saccharification of water hyacinth biomass. Also it was observed that xylose is more than that of reducing sugars in case of saccharification using crude enzyme. It may be because of the fact that water hyacinth is richer in hemicelluloses content than that of cellulose content. The yield of sugar release can be increased if the water hyacinth biomass is first chemically treated with either acid or alkali. It was also concluded that water hyacinth can be used as a potential source for bioethanol production. The production of ethanol can further be enhanced by using both hexose and pentose fermenting yeast simultaneously. The fungal sample F6 which gave the maximum yield was obtained from decaying plant matter in CMERI, Durgapur was sequenced and the information was submitted in GeneBank, NCBI and a unique accession number JQ781696 was assigned.

ACKNOWLEGEMENTS

Authors are grateful to Director, CSIR_CMERI for his kind support to carry out this research work and permission for the publication.

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