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Production of cellulase and xylanase by *Trichoderma reesei* (QM 9414 mutant), *Aspergillus niger* and mixed culture by solid state fermentation (SSF) of water hyacinth (*Eichhornia crassipes*)

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Studies on cellulase production by *Trichoderma reesei* QM 9414 mutant (*T. reesei M*) and *Phanerochaete chrysosporium* in flask culture indicated suitability of water hyacinth (WH) as a substitute for conventional wheat bran (WB) medium by Solid-State Fermentation (SSF). Maximum cellulase production was obtained with *T. reesei M* on WH substrate with Toyoma Ogowa (TO) medium at liquid /solid ratio of 2.5, and incubation period of 10 days. However, *T. reesei M* failed to produce β-glucosidase on WH medium. Further experiments on cellulase and xylanase production were performed in Solid State Cabinet Fermenter (SSCF) to resemble conditions in tray fermenter. As compared to the flask culture, cellulase production by *T. reesei M* was more in SSCF, and supplementation of TO medium with whey (40%) and peptone (0.15%) further enhanced production of both cellulase complex and xylanase by 2-3 fold. When *Aspergillus niger* was used in combination with *T. reesei M*, production of both cellulase and xylanase was enhanced considerably. The synergistic effect may be attributed to the β-glucosidase production by *A. niger* which could eliminate the inhibitory effect of cellobiose.

Keywords: Cellulase, Xylanase, Water hyacinth, Solid State Fermentation, *Trichoderma reesei* (QM9414 mutant), *Phanerochaete chrysosporium, Aspergillus niger,* Mixed culture fermentation

Water hyacinth (*Eichhornia crassipes)* is one of the fastest growing aquatic weeds[1]. It causes huge economic loss by impeding water flow, accelerating water evaporation, increasing mosquito breeding and destroying rice fields. Solid State Fermentation (SSF) is described as cultivation of microorganisms on solid particulate substrate, with just enough water to solubilise the nutrients[2]. Present investigations consider utilizing this aggressive weed as substrate for cellulase and xylanase production in Solid State Fermentation (SSF). Substrates used for cellulase production such as cotton, solka floc, avicel or sulphite pulp are extremely expensive and contribute around 48-52% to overall cost of cellulase production[3]. Considering the important biotechnological applications of cellulase and xylanase and the economical constraint of using purified cellulose, natural lignocellulosic substrate *Eichhornia crassipes* (water hyacinth) is an attractive alternative. The weed is abundantly available almost at negligible cost throughout the country. The approach of utilizing water-hyacinth for cellulase and xylanase production is expected to serve the twin objectives of utilization of otherwise nuisance weed as well as production of hydrolytic enzymes at low cost. Reduction in enzyme cost would, in turn, reduce conversion cost of cellulose to glucose and hemicellulose to xylose that can be used for producing bioethanol and variety of value added chemicals and other products.

Experimental Procedure

Substrates

Water hyacinth (WH) collected from local pond was chopped to 2-3 cm size and oven dried at 70ºC for 48 h. It was pulverized and the material retained on 80-mesh sieve was used as substrate. Wheat bran was oven dried at 70ºC for 48 h. Wood straw (20 g) was dispersed into 1000 mL of NaOH (2% *w/v*), autoclaved for 15 min at 15 psi and cooled to room

temperature. After draining away the alkali, delignified wood straw was repeatedly washed with running acidic water till *p*H of water was around 5 to 6 and was oven dried at 70ºC for 48 h.

Elemental analysis of water hyacinth

Uniform, finely ground dry water hyacinth was analyzed for carbon, hydrogen, nitrogen and sulphur content by the CHNS-O Elemental Analyzer, Carlo Erba Model 1108 attached with DP200, under operation conditions: left furnace temperature, 1020ºC; right furnace temperature, 500ºC; oven temperature, 60ºC; and filament temperature, 190ºC with Helium as the carrier gas with a flow rate of 100-120 mL/min on GC column porapack QS and thermal conductivity detector.

Microorganisms

Trichoderma reesei QM 9414 mutant (*T. reesei M*) developed by Gadgil[4] using ultraviolet light and sodium nitrite (45 min, 0.5 mg mL^{-1}) at National Environmental Engineering Research Institute (NEERI), Nagpur, was maintained on Potato Dextrose Agar (PDA) slants. *Aspergillus niger* and *Phanerochaete chrysosporium* MTCC-787, procured from Institute of Microbial Technology, Chandigarh, were maintained on PDA and Malt Extract Agar (MEA) slants respectively. All cultures were subcultured every four weeks, incubated at 30ºC for 7 days and subsequently stored at 4ºC for inoculum preparation.

Enzyme assay

Filter Paper Activity (FPA) was determined as described by Mandels *et al.*[5]. Carboxy methyl cellulase (CMCase) and β-glucosidase activites were determined by the method of Saddler[6]. Reducing sugar liberated in reaction mixture was measured by DNS method[7]. Xylanase activity was determined under similar conditions by using 1% xylan solution (1 mL) in place of 1% carboxymethyl cellulose. One unit of FPA, CMCase, and β-glucosidase activity was expressed as one μmol glucose equivalent released $min^{-1}mL^{-1}$ under specified experimental conditions. One unit of xylanase activity was expressed as one μmol of xylose released $min^{-1}mL^{-1}$. When glucose is used as standard, the xylose values are found to be 15% high on a weight basis[8], hence the xylanase acivity was calculated accordingly. Protein content was estimated according to the modified method of Lowry *et al*.[9].

Cellulase production in flask culture

Initial studies in flask culture were carried out for production of cellulase by *T. reesei M* and *P. chrysosporium,* using Toyoma Ogawa (TO) medium[10] and whey as nutrient supplement.

Inoculum

Spores scraped out from 7 days old slants were dispersed in desired quantity of sterile distilled water containing 0.1%Tween 80 and vortexed. Spore count was measured with haemocytometer and adjusted to 2×10^6 spores / mL by adjustment of optical density.

Solid- State Fermentation (SSF)

Fermentation was carried out under SSF[11] using water hyacinth (WH), wheat bran (WB), wood straw (WS) and their combinations as substrates. Solid substrate (10 g) was mixed with nutrient supplement (*p*H 4.5) at 1:2.5 ratio in 250 mL conical flasks to obtain a 1-2 cm layer of mixture without free liquid. The flasks were sterilized by autoclaving, cooled, shaken thoroughly to break the mass. Spore suspension (20×10^6/ mL) was transferred @ 2% (*v*/*w*) per flask. The flasks were incubated at 30ºC under static conditions for 10 days.

Enzyme extraction

Enzyme was extracted with 0.1 N saline containing 0.1%Tween 80 at 1:15 (*w*/*v*) ratio of moldy bran: extractant, selected by preliminary trials. Moldy bran was mixed with one third quantity of extractant and held for one hour with occasional mixing and shaking. Thereafter, it was squeezed through muslin cloth and residue was extracted with two more equal portions in a similar way. Pooled extract was centrifuged at 8,000 rpm for 20 min and was made up to fixed volume to give crude enzyme solution (CES).

Cellulase and xylanase production in Solid State Cabinet Fermenter (SSCF)

Considering limitations of flask fermentation with respect to control of crucial fermentation parameters such as moisture, humidity, temperature and aeration, a Solid State Cabinet Fermenter (SSCF) resembling a tray fermenter was used for further studies. This type of fermenter provides more space and larger surface area to the substrate and better control of the fermentation parameters. Laboratory studies for enhancement of production of cellulase and xylanase by mono and mixed cultures of *T. reesei M* and *A. niger* were carried out in SSCF.

The solid state cabinet fermenter (SSCF) was an incubator (dimensions 3.4'×2.20'×2.90') with SS interior, two stainless steel racks dividing the cabinet into three compartments, and a glass door. The cabinet was fumigated with saturated formaldehyde vapour prior to the fermentation. Drying of substrate was avoided by keeping a thermostat controlled water bath in the lowest chamber to continuously generate water vapour and maintain 90-95% RH in the cabinet. A portable exhaust fan cum blower assembly mounted inside the cabinet ensured uniform circulation of humid air inside the cabinet and removal of gases produced during fermentation.

Fermentation of WH by *T. reesei* M, *A. niger* and mixed culture in SSCF

WH was mixed with liquid nutrient supplement in 1:3 proportions to compensate for comparatively higher evaporation losses due to more surface area available in SSCF compared to flask culture. The substrate was sterilized, cooled to room temperature, and was spread to appox. 5 mm depth on sterile aluminium sheets in presterilised SSCF. It was inoculated with spore suspension (2.0×10^6/mL) @ 2% (*v/w*) prepared separately from a 7 days old malt extract agar slant in case of mono cultures of *T.* reesei *M* and *A. niger*. In case of mixed culture, *A. niger* mycelial inoculum was added to the *T. reesei* fermented substrate after 36 h. The fermentation was carried out at 30±2ºC and 95% RH and enzyme activity in the CES was determined on alternate day for 15 days. Effect of nutrient supplements on cellulase and xylanase production by *T. reesei M* was carried out by combining different nitrogen sources and inducers with whey/TO. Selected nutrient supplement was used for fermentation with individual and mixed culture.

Results and Discussion

Table 2–Effect of nutrient supplements on cellulase production by *T. reesei M* and *P. chrysosporium* on different substrates under SSF conditions

Micro organism	Substrate	Enzyme activities in CES		
		CMCase (IU/mL)	β-glucosidase (IU/mL)	FPA (FPU/mL)
T. reesei	WH (10 g) + TO (25 mL)	1.35 (0.84)	ND	0.95 (0.59)
T. reesei	WH (10 g) + Whey (25 mL)	0.51 (0.51)	ND	0.15 (0.15)
P. chrysosporium	WH (10 g) + TO (25 mL)	0.84 (0.67)	ND	0.64 (0.51)
P. chrysosporium	WH (10 g) +Whey (25 mL)	0.40 (0.26)	ND	0.14 (0.09)
T. reesei	WB (10 g) + TO (25 mL)	1.30 (1.30)	0.35 (0.35)	0.41 (0.41)
P. chrysosporium	WB (10 g) + TO (25 mL)	1.25 (1.19)	0.31 (0.29)	0.35 (0.33)
T. reesei	WS (10 g) + TO (25 mL)	0.65 (0.65)	ND	0.35 (0.35)
P. chrysosporium	WS (10 g) + TO (25 mL)	0.51 (0.52)	ND	0.30 (0.31)

NB: Values in brackets indicate Sp activities; ND: Not detected

Composition of water hyacinth (WH)

Being an aquatic weed, WH has 95% moisture content. Analysis of dried water hyacinth (Table 1) is comparable to that of some of the known agricultural residues commonly being used for solid-state fermentation e.g. wheat bran and straw[12]. Ash content in WH was found to be 12.1% which is comparable with 10% ash content reported in agricultural residue[13]. Elemental composition of water hyacinth was found conducive for fermentation.

Enzyme production in flask culture

Preliminary studies on liquid/solid ratio for water hyacinth for cellulase production indicated maximum production at ratio 1:2.5 in flask fermentation.

Results in Table 2 indicated that *T. reesei M* produced higher cellulase levels compared to *P. chrysosporium*. Both *T. reesei M* and *P. chrysosporium* produced 2-2.5 times more cellulase on water hyacinth substrate with TO medium in comparison to whey supplement. Better performance of TO medium was also observed in case of wheat bran and wood straw. Rao *et al.*[14] have reported similar observations for cellulase production on various substrates using *Pestalotiopsis versicolor*. It was further observed that *T. reesei M* and *P. chrysosporium* on WH gave higher FPA than the corresponding values on WS and WB. CMCase production by *T. reesei M* was not altered on WB but decreased on WS. *P. chrysosporium* produced more CMCase on WB. Both the organisms produced β-glucosidase only on WB medium. FPA production by *T. reesei M* on WH medium (0.950 FPU/mL) is found to be more than the FPA reported by Peitersan[15] from *T. reesei* QM 1123 grown on alkali-treated barley straw (0.28 FPU/mL) and by Tangu *et al.*[16] from 1% and 2% alkali-treated corn stover (0.12 and 0.28 FPU/mL). However, it is less than the value of 1.74 FPU/mL reported by Wen *et al.*[17] on the substrate dairy manure. Dhillon *et al.*[18]obtained negligible quantities of cellulase but appreciable quantities (19.28 IU/mL) of extracellular thermophilic xylanase with *Bacillus circulans* AB 16 isolated from a garbage dump when grown on 0.3% xylan.

Cellulase production on mixed substrates (WH:WB and WS:WB) using nutrient supplements whey/TO indicated higher cellulase production by *T. reesei M* compared to *P. chrysosporium* on mixed substrate (Table 3) though the specific activity did not differ significantly. Saddler[6] recorded similar observations for *T. reesei* (C30, QM 9414) which indicated that a highly degradative fungi such as *P. chrysosporium*, capable of reducing the weight of a wood block by 50% after 3 months growth, was not necessarily a high cellulase producer, whereas *T. viride* produced more cellulase with more than 4% weight loss of wood blocks. CMCase and FPA obtained with the

Table 1–Analysis of water hyacinth

Constituents	% (dry weight basis)
Cellulose	21.50
Hemi-cellulose	33.90
Fat	01.65
Crude protein	13.75
Ash	12.10
Lignin	07.01
Total solids	05.01
Carbon	45-50
Hydrogen	5.30-5.50
Nitrogen	1.80-3.20
Sulphur	0.25-0.35

Table 3–Effect of combination of substrates on Cellulase Production by *T. reesei M* and *P. chrysosporium* by SSF in flask culture

Substrate	Nutrient supplement (25 mL)	Enzyme activities of *T. reesei* CES			Enzyme activities of *Pchrysosporium* CES		
		CMCase (IU/mL)	β-glucosid (IU/mL)	FPA (FPU/ mL)	CMCase (IU/mL)	β-glucosidase (IU/mL)	FPA (FPU/ mL)
WH (8 g) + WB (2 g)	TO	0.760 (0.50)	ND	0.223 (0.15)	0.600 (0.49)	N D	0.158 (0.13)
WH (7.5 g)+ WB (2.5 g)	TO	0.763 (0.50)	ND	0.230 (0.15)	0.605 (0.49)	N D	0.160 (0.13)
WH (5 g) + WB (5 g)	TO	0.851 (0.53)	0.349 (0.22)	0.247 (0.16)	0.610 (0.53)	0.302 (0.26)	0.160 (0.14)
WH (8 g) + WB (2 g)	Whey	0.592 (0.46)	ND	0.180 (0.14)	0.398 (0.27)	N D	0.138 (0.095)
WH (7.5 g) + B (2.5 g)	Whey	0.595 (0.42)	ND	0.201 (0.14)	0.445 (0.26)	N D	0.194 (0.11)
WH (5 g) + WB (5 g)	Whey	0.650 (0.45)	0.289 (0.20)	0.221 (0.15)	0.451 (0.36)	0.205 (0.16)	0.154 (0.12)
WS (8 g) + WB (2 g)	TO	0.650 (0.45)	ND	0.190 (0.13)	0.510 (0.46)	N D	0.100 (0.091)
WS (5 g) + WB (5 g)	TO	0.740 (0.51)	0.290 (0.20)	0.210 (0.14)	0.690 (0.62)	0.210 (0.19)	0.130 (0.12)

NB: Values in brackets indicate Sp activities; ND: Not detected

substrate ratio WH:WB (50:50) was higher than that obtained with other combinations for similar nutrient supplements for both *T. reesei M* and *P. chrysosporium*. Definite enhancement of β-glucosidase production was recorded with addition of 50% wheat bran which is also reported by Shamala and Sreekantiah[19]. Improvement in production of xylanases by *Chaetomium globosum Ch.g./5* and *Aspergillus niger A.n.33/2/8* in solid-state fermentation using combinations of different carbon sources-wheat bran, wheat straw, beet pulp and apple pomace was also reported[20]. The best xylanolytic activities from both organisms were obtained on a medium containing wheat bran (75%), beet pulp (20%) and apple pomace (5%). On this medium Ch.g./5 produced 17·5 and 20·0 XU/mL and A.n.33/2/8 produced 24·0 and 45 XU/mL of the extract in laboratory and pilot-plant scales, respectively.

Effect of nutrient supplement on cellulase and xylanase production by *T. reesei M* in SSCF

Maximum cellulase and xylanase activity was obtained on 9th day (Fig. 1). Slight reduction in cellulase activity (FPA) was observed after 9 days, whereas xylanase activity (XA) was stable till 11th day and decreased subsequently. Therefore, incubation period of 9 days was considered optimum for enzyme production. Reduction in enzyme levels upon prolonged incubation may be attributed to its irreversible adsorption to lignin/cellulose, resulting in non-availability, inactivation of the enzyme proteins, or to the repression effect of cellobiose on cellulase production. This incubation time is comparable with the optimum incubation time reported by Milagres *et al.*[21] who obtained high level of thermostable xylanase (1597 U/g) after 10 days of solid-state fermentation in

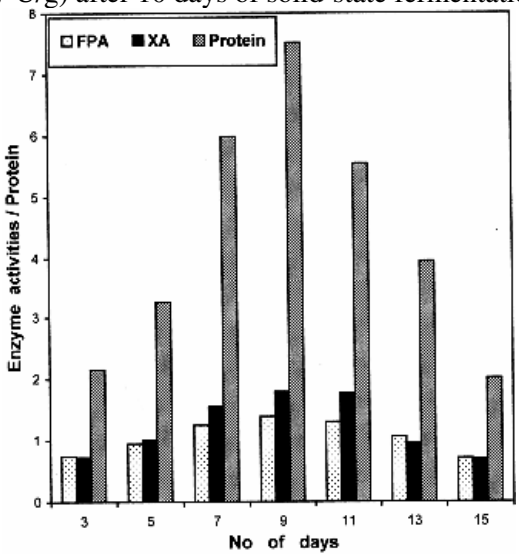

Fig. 1–Determination of optimum incubation period for enzyme production by *T. reesei M* on water hyacinth in Solid State Cabinet Fermenter (SSCF)

glass-column reactor with forced aeration using *Thermoascus aurantiacus* ATCC 204492 on sugar cane bagasse as a substrate. The effects of different airflow rates [0, 3.0, 6.0 l/ (h g) bagasse] and initial mass of bagasse (8, 12.5, 17 g) on the production of xylanase were investigated by the team using a statistical experimental design.

The composition of various nutrient supplements used for production of cellulose and xylanase by *T. reesei* M on WH by SSCF is given in Table 4. Effect of addition of nutrient supplements on enzyme production in SSCF (Table 5) shows that TO medium produced 8-9 fold increase in cellulase and xylanase production compared to whey, but β- glucosidase was produced only on whey medium. Incorporation of whey in TO medium improved β-glucosidase production, without much effect on xylanase production, whereas replacing TO with other nitrogen sources resulted in lower enzyme levels (D, E, F supplements in Table 4). Combination of TO, whey and peptone (Medium I) showed maximum production of all enzymes. It is reported by Prasertsan *et al.*[22] that although nitrogen has profound effect on enzyme production, proper C/N ratio promotes good fungal growth and consequently higher enzyme production. Excess nitrogen lowers C/N ratio and is presumably the reason to hinder enzyme production. Production of β-glucosidase on whey medium is due to lactose, which is known to be an inducer for cellulase production[23]. Increase in β-glucosidase production by *T. reesei* on increasing peptone is consistent with the reports of Doppelbauer *et al.*[24]. Nutrient supplement **'I'** combined the inducer action of lactose with appropriate nitrogen level, enhanced the production of cellulase and xylanase, along with β-glucosidase. Positive or negative effect of nutrient supplements was also studied by Virupakshi *et al.*[25] who observed maximum production of xylanase (3644 U/g DBB) by *Bacillus* sp. JB-99 on rice bran moistened with mineral salt solution (MSS_3) at a substrate-to-moisturizing agent ratio of 1:2.0 (*w*/*v*) at 50°C for 72 h. Yeast extract, beef extract and xylan enhanced enzyme production, while glucose, lactose and fructose strongly repressed the production process. Ghanem *et al.*[26] adopted Plackett–Burman

Table 4–Composition of nutrient supplements for production of cellulase and xylanase by *T. reesei M* on WH by SSCF

Medium	Liquid component (%)			Additional nutrients (%)						Inducers (%)	
	Water	Whey	TO	Peptone	Beef**/ Yeast* extract	Malt Extract	Wheat Bran	NH_4PO_4	KH_2PO_4	CMC	Tween 80
Whey	-	100	-	-	-	-	-	-	-	-	-
TO	-	-	100	-	-	-	-	-	-	-	-
A	-	15	85	-	-	-	-	-	-	-	-
B	-	25	75	-	-	-	-	-	-	-	-
C	-	35	65	-	-	-	-	-	-	-	-
D	75	25	-	1.310	-	-	-	0.30	0.052	0.05	-
E	75	25	-	1.310	-	-	-	0.30	-	0.05	-
F	90	10	-	1.000	0.05**	-	-	0.41	-	-	0.20
G	-	-	100	0.300	-	-	-	-	-	0.30	-
H	-	25	75	0.125	1.00*	2.00	2.85	-	-	-	-
I	-	40	60	0.150	-	-	-	-	-	-	-

Table 5–Effect of addition of nutrient supplements on cellulase and xylanase production by *T. reesei M* on WH in SSCF

Substrate	Enzyme activities in CES				Protein (mg/mL)	Sp. activity			
	CMCase (IU/mL)	β-glucosidase (IU/mL)	FPA FPU/mL	Xylanase (IU/mL)		CMCase (IU/mL)	β -glucosidase (IU/mL)	FPA FPU/mL	Xylanase (IU/mL)
WH+whey	0.504	0.024	0.476	0.210	2. 38	0.210	0.010	0.200	0.088
WH+TO	4.000	N/D	1.400	1.800	8.00	0.500	-	0.175	0.225
WH+A	1.269	0.200	0.790	0.870	4.53	0.280	0.045	0.174	0.192
WH+B	2.167	0.349	1.043	0.970	6.99	0.310	0.050	0.130	0.149
WH+C	2.522	0.422	1.230	0.992	8.13	0.310	0.052	0.150	0.120
WH+ D	0.690	0.120	0.460	0.260	3.00	0.236	0.040	0.150	0.090
WH+ E	0.650	0.120	0.440	0.230	3.09	0.210	0.040	0.140	0.074
WH+ F	0.750	0.047	0.710	0.790	4.69	0.160	0.010	0.150	0.170
WH+ G	1.781	0.290	0.810	0.910	6.09	0.292	0.048	0.148	0.133
WH+ H	0.700	0.096	0.660	0.730	4.82	0.145	0.020	0.140	0.150
WH + I	6.950	0.995	2.490	5.100	12.00	0.580	0.083	0.210	0.430

experimental design when best xylanase activity was obtained using *Aspergillus terreus* cultivated on finely ground wheat straw (10 g) in solid-state fermentation moistened with a concentrated nutrient salt solution and incubated for 4 days at 30°C.

Comparison of cellulase and xylanase production in flask fermentation and SSCF

Comparison of FPA level produced by *T. reesei M* on water hyacinth substrate with TO supplement in flask culture and SSCF revealed that the enzyme production in SSCF was nearly 1.5 fold. This may be attributed to better distribution of moisture, humidity, temperature and aeration in the new design in the form of solid state cabinet fermenter (SSCF). Production of cellulase as well as xylanase was further improved 2-3 fold with supplementation with medium I (Table 6).

Comparison of cellulase and xylanase production by *T. reesei M, A. niger* and mixed culture in SSCF

From Fig. 2 it may be noted that the cellulase production obtained by *T. reesei M* / *A. niger* mixed culture were substantially higher than corresponding levels obtained individually by *T. reesei M* and *A. niger*. They are also higher than the levels reported by Gadgil[4] in flask culture. In mixed culture, β-glucosidase activity was nearly 4 folds and 2.0 folds higher than that of monocultures of *T. reeei M* and *A. niger*. *A. niger* produced higher levels of β-glucosidase (1.50 units/mL) than *T. reesei M* (0.995 units/mL) although the CMCase and FPA values for *A. niger* were lower than those for *T. reesei M*. This observation is in agreement with literature reports that although *T. reesei* is one of the potent producers of cellulase enzyme system[27], the enzyme complex is deficient in β-glucosidase[28]. For proper saccharification of lignocellulosics, β-glucosidase and hemicellulases are required along with cellulase system at an optimum level[29]. Deficiency of β-glucosidase in the culture filtrate of *Trichoderma* species can be overcome by co-culturing it with high β-glucosidase producers *Aspergillus* species[30,31]. Enhancement of cellulase production is the result of synergism between *Trichoderma M* and *A. niger*. Since *A. niger* cannot degrade lignocellulose alone due to lower cellulase production, it must rely on cellulose hydrolysis by *T. reesei* to obtain reducing sugars for it's growth[32]. Sugar consumption by the *Aspergillus* relieves the feedback effects of cellobiose on the

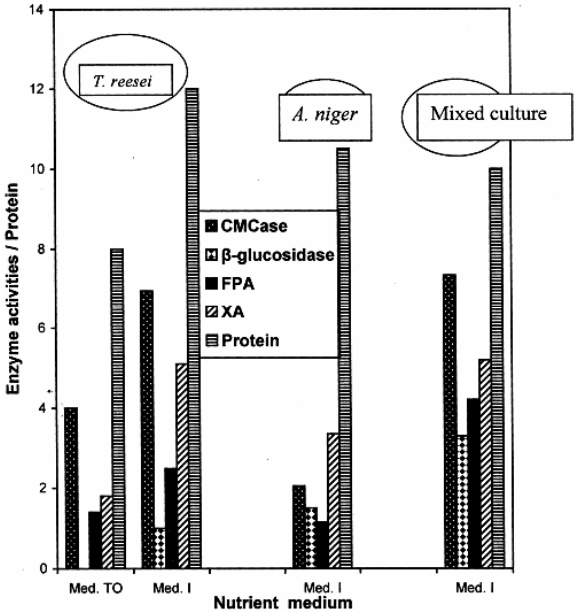

Fig. 2–Cellulase and xylanase production by *T. reesei M, A. niger* and mixed culture on water hyacinth (WH) substrate in Solid State Cabinet Fermenter (SSCF)

Table 6–Comparison of cellulase and xylanase production on water hyacinth (WH) in flask culture and Solid State Cabinet Fermenter (SSCF)

Paramaeter	*T. reesei M* flask culture with TO		*T. reesei M* SSCF with TO		*T. reesei M* SSCF with Supplement I		*A. niger* SSCF with Supplement I		Mixed culture SSCF with Supplement I	
	FPA	Xylanase	FPA	Xylanase	FPA	Xylanase	FPA	Xylanase	FPA	Xylanase
Activity units /mL of CES	0.95	1.9	1.4	1.8	2.49	5.10	1.15	3.35	4.21	5.20
Sp. activity (Activity / mg protein)	0.144	0.29	0.175	0.225	0.21	0.43	0.11	0.32	0.42	0.52
Yield/ g dry water hyacinth substrate (FPU or xylanase IU $g^{-1)}$	10.45	20.90	11.0	19.8	27.39	56.1	12.65	36.85	46.31	57.20
Yield / g dry Cellulose/ Hemicellulose (FPU or xylanse IU $g^{-1)}$	48.60	61.65	71.5	91.98	127.4	165.5	58.84	108.7	215.4	168.73

WHS: Water Hyacinth Substrate; FPA: Filter Paper Activity; FPU: Filter Paper Units

cellulase production by *Trichoderma*. Apparently, *Aspergillus* boosted the cellulase production of the *Trichoderma M* and the *Trichoderma M* boosted the biomass and β-glucosidase production of the *Aspergillus*. Both the enzyme activities per unit weight of substrate are increased in SSCF (Table 6). The cellulase activity for mixed culture (215.4 FPU/g) is comparable with the value of 213.4 FPU/g cellulose in 500 mL flask and 222.8 FPU / g cellulose in 30 m^3 by *T. reesei* ZU-O2 in stirred fermentor used for large scale fermentation of corn cob[33] and is higher than FPase activity 19.5 FPU/ g in 4 days on rice straw in SSF by *A. niger KK2* reported by Kang *et al.*[34]. Production of xylanase on mixed culture (168.73 IU/g) is significantly higher than the levels of 14 -15 IU/g dry weight reported by Tengerdy[35] in SSF of sugar cane bagasse but less than the activity of 5200-5600 IU/g reported by Mohana *et al.*[36] in solid state fermentation using distillery spent wash by *Burkholderia* sp. DMAX strain. Hairong *et al.*[37] reported a total xylanase activity of about 1350 IU/mL on mixture of Oat husk hydrolysate and lactose in simple fed batch trials and Battan *et al.*[38] reported production of 5407 IU/mL cellulase-free, thermostable xylanase by newly isolated strain of *Bacillus pumilus* under submerged fermentation in a basal medium supplemented with wheat bran (2%, *w*/*v*).

Conclusion

The investigations suggest that the nuisance weed water hycinth can be a potential substrate for production of cellulase and xylanase by SSF. Incorporation of whey and co-culturing *T. reesei QM9414 mutant* with β-glucosidase producing *A. niger* enhances the levels of cellulase and induces β-glucosidase production due to synergistic action by reducing the feed back inhibition of cellulase production by cellobiose.

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