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Production Kinetics of Xylanase and Carboxy-methyl Cellulase of *Schizophyllum commune* in Submerged Fermentation Technique

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Abstract— Tamarind kernel powder (TKP), a soluble agro-residue was used to evaluate the production of xylanase and CMCase in submerged culture of *Schizophyllum commune*, a white rot basidiomycetes fungi. Soluble TKP containing xyloglucan as the major polysaccharide induced significantly xylanase production but CMCase production was not significantly improved. The culture filtrate consisting of xylanase and CMCase at a ratio of 10:1 (approx) and 1.5:1(approx) in TKP and cellulose medium respectively. It may therefore, be indicative that inducer molecules released from TKP or cellulose were not identical and Tamarind kernel powder, although structurally similar to cellulose, therefore, was found to be the perfect inducer for xylanase production in submerged culture of *Schizophyllum commune.* Use of mixed substrate in a single set of fermentation reveals that addition of 0.5 % TKP in cellulose medium improved CMCase titer, whereas addition of 0.5 % cellulose in TKP medium did not increased the production level of xylanase. Therefore, the present study reports the successful economic utilization of TKP, an abundantly available for the production of xylanase, an enzyme of industrial importance.

Keywords— *Tamarind kernel powder, Schizophyllum commune, xylanase, CMCase*

I. INTRODUCTION

Enzyme production is a challenging field of biotechnology and the enzyme manufacturer focuses on the submerged fermentation technique since product recovery cost is the crucial point in industrial enzyme production. In recent years there has been an increasing interest in the effective utilization of crop residues for microbial transformation into a specific product of industrial importance. Agro-industrial residues such as sugarcane bagasse, wheat bran, rice bran, rice straw, corncobs, banana peels, cassava wastes, apple pomace etc are considered the best substrates for enzyme production. Carbon substrates used for the production of xylanase or Carboxy-methyl cellulase (CMCase) or polygalacturonase are mostly insoluble in nature and when present in fermentation medium invites many problems in large scale industrial enzyme production. Moreover, the particle size of lingo-cellulosic substances critically affects the enzyme production due to adsorption of extracellular enzymes to undigested particles of the substrate as well as mechanical resistance. The enzymatic hydrolysis of xylans and cellulose has been extensively reviewed and out of the all xylanolytic and cellulolytic enzymes, the *endo* 1,4 β-D xylanase (EC 3.2.1.3) and *endo* 1,4 β-D glucanase (EC 3.2.1.4) are the best characterized enzymes.

II. RELATED WORK

There are many reports available on the production of these enzymes by submerged fermentation or solid state fermentation using a wide variety of carbon substrates [1] although not much literature has been reported on xylanase and CMCase production by white rot fungi, *Schizophyllum commune.* Previous studies reported on the production efficiency of enzyme by *Scizophyllum commune* using insoluble carbon substrates [2, 3] and searching for a novel soluble carbon source, therefore a prerequisite for development of an effective enzyme bioprocess system. The aim of this present study was to utilize tamarind kernel powder (TKP), a soluble carbon substrate, an abundantly available agro-residue for the xylanase and CMCase production, which have an immense industrial importance.

III. METHODOLOGY

3.1 Microorganism and fermentation system

Mycelial culture of *Schizophyllum commune* (MTCC 1096), obtained from Institute of Microbial Technology, India was grown at 30 ± 1^0C for 5 days in medium containing 1 % glucose, 1 % malt extract, 10 % potato extract and 0.15 % KH_2PO_4 at pH 5.0. The enzyme production medium for solid

state fermentation contained (g/l): $NH_4H_2PO_4$- 24; $MgSO_4$, 7 H_2O- 0.5; $CaCl_2$, $2H_2O$- 0.37; H_3PO_4- 0.57; $FeSO_4$, 7 H_2O- 0.25; $MnCl_2$- 0.032, $NaMoO_4$- 0.032; yeast extract- 5 in combination with a carbon sources at different concentration and the pH of the medium was adjusted to 5.0. Submerged fermentation was carried out at 30 ± 1^0C for 9 days and culture filtrate was used as a source of extra-cellular enzymes.

3.2 Carbon substrates

Cellulose (Sigmacell,Type 50) was obtained from sigma Chemicals Company, USA. Tamarind kernel powder (TKP) was obtained from a local market. The powder was initially screened through 200 meshes to remove seed skin and the sieved mass was dried overnight at 60^0C.

3.3 Enzyme activity assays

CMCase (carboxy-methyl cellulase,) and xylanase activities of the culture filtrate were measured in terms of *endo* 1,4 β-D glucanase (EC 3.2.1.4) and *endo* 1,4 β-D xylanase (EC 3.2.1.3) activities using carboxymethyl cellulose and oat spelt xylan as substrates respectively [4]. Units of CMCase and xylanase activities were represented as µmoles of glucose or xylose equivalent liberated per minute under the assay conditions.

3.4 Data analysis

Experimental data were statistically analyzed for standard deviation and mean value of data obtained from 3 sets of experiments under identical conditions are represented in figures.

IV. RESULTS AND DISCUSSION

In an attempt to assess the optimal carbon substrate concentration for extracellular enzyme production in submerged fermentation of *Schizophyllum commune,* tamarind kernel powder (TKP) and Cellulose (Sigmacell, Type 50) were used as carbon source at varying concentration separately in the culture medium and data obtained from the experiment was represented in figure 1. The experimental findings revealed that with increasing conc. of TKP as carbon source, the extracellular xylanase and CMCase production were also increased although the enzymes production were not significantly increased after 2 % carbon substrate level. For example, in case of xylanase, activity was found as 120 U/ml at 0.5 % conc. while the enzyme activity reached as 400 U/ml at 2 % TKP level. On the other hand, CMCase activity was found as 40 U/ml at 0.5 % TKP level while 75 U/ml of CMCase titer was observed at 2 % TKP substrate conc. However, the maximum TKP conc. available in the culture media did not significantly increase both of the enzyme activity as compared to 2 % carbon source containing media. In case of culture media containing only cellulose as carbon substrate, CMCase activity was found significantly in higher yield as compared to TKP containing media in contrast to xylanase production, where the enzyme activity was higher in TKP media (Figure 1). Although *S. commune* produced more xylanase in TKP than cellulose medium, the activity of CMCase obtained from the cellulose medium was much lower. Though cellulose and xylanase were found to be co-produced by fungi, their relative production was significantly influenced by the carbon sources present in the fermentation media.

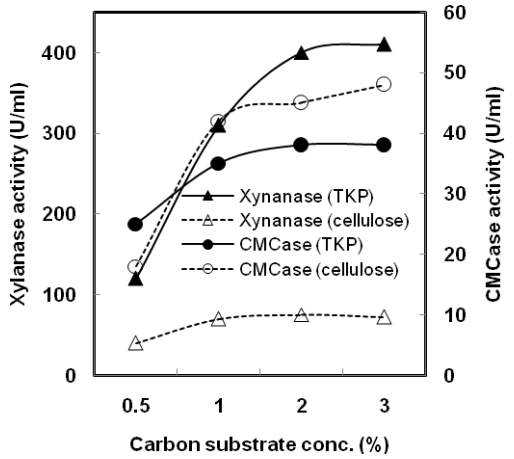

Figure 1: Production trend of enzyme titer (U/ml) as influenced by the level of carbon substrates by *S. commune* in submerged fermentation of 9 days. Data presented are the mean value of three identical sets of experiments and Solid line represents TKP culture medium and dotted line represents cellulose culture medium (Standard deviation for xylanase within ± 10% and for CMCase within ± 7%)

The role of cellulose in the co-production of CMCase and xylanase was found to be confusing. One group (*S. commune*) produces more xylanase than CMCase [5], while other group (*Penicillum pinophyllum*) produced more or similar CMCase than xylanase in cellulose medium [6].

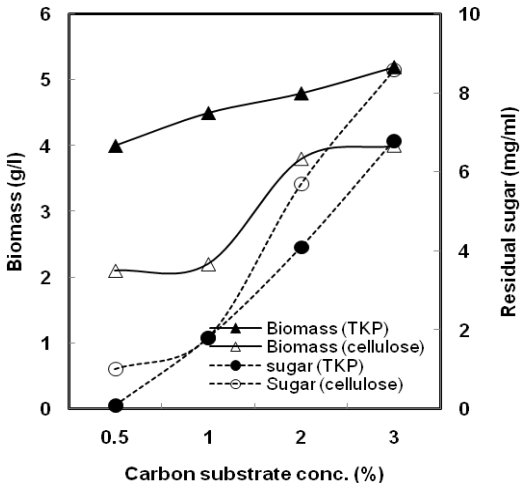

Figure 2. Biomass growth and substrate consumption as residual sugar present in the culture filtrate after 9th day of submerged fermentation of S. commune at different carbon substrates. Data presented are the mean value of three identical sets of experiments and Solid line represents fungal biomass and dotted line represents residual sugar (Standard deviation for biomass within ± 6 % and for residual sugar ± 5 %)

The results also supported by the works of Chatterjee et al., [7] in which the production ratio of xylanase to CMCase was higher (2:1) in cellulose medium compared to that (10:1) in xylan medium of submerged fermentation of *Termitomyces clypeatus*. The mechanism for the co-production of enzymes using different substrate remain unclear, possibly due to the absence of any unified regulatory system in the microorganism [7]. Using cellulose or cellulose rich substrate in culture media of wild strain of *S. Commune*, Haltrich and Steiner [8] reported that the synthesis of xylanase, cellulase and mannase activities is likely to be under common regulatory control and the inducers seems to be a small molecule derived from cellulose. Thus, release of soluble inducers from cellulose possibly resulted in dual induction while that released from xylan caused xylanse induction.

when the biomass in the submerged fermentation in respect to TKP and cellulose conc. is considered (figure 2), the results evinced that with increasing carbon substrate conc. in culture media, a constant increase of fungal biomass had continued to increase, though the maximum conc. of either TKP or cellulose did not increased significantly the biomass level. If the residual carbohydrate level was evaluated, it could be noted that at the end of the fermentation system, i.e., after 9th day of fermentation, the residual carbohydrate was present at a substantial level. For example, when initial 3 % conc. of either TKP or cellulose was used as a carbon substrate the residual carbon source was 6.8 mg/ml or 8.6 mg/ml in the culture filtrate in TKP or cellulose medium respectively. The results pointed out that fungi neither utilized the remaining carbon source or extracellular enzymes may be adsorbed on the undigested particles of carbon substrate. The enzyme yield coefficient value analysis of this fermentation technique for xylanase and CMCase revealed that concentration of carbon substrate (either TKP or cellulose) increased the value ($Y_{E/X}$) for both of the enzymes and the maximal value for xylanase (108.47 U/g) was obtained at 2 % TKP level where as 2 % cellulose media gave the maximal value of ($Y_{E/X}$) of 20.5 U/g of biomass for CMCase.

Tamarind kernel powder (TKP), obtained from Tamarind (*Tamaridus indica*) seeds, an agro-residue limited its use to jute and textile industries as a cheap seizing agent. The major polysaccharide present in TKP is xyloglucan, having β (1,4) linked D-glucose backbone with D-xylose, D-galactose and L-arabinose present in the side chain. the ratio of galactose, xylose and glucose in TKP is 1:2.5:2.8 and about 2-3 % is composed of minor polysaccharides containing branched (1,5) ɑ-L-arabinofuran and unbranched (1,4) β –D-glactopyranan [9]. On the other hand, cellulose, the most abundant biopolymer found in earth is a linearly arranged In β (1,4) linked D-glucose molecules. Thus the experiment may points out that TKP is a good option for the production of xylanase and CMCase in submerged fermentation, although the higher CMCase production was found in the cellulose containing medium. In our previous study, we have shown that TKP induced all cellulolytic and xylanolytic enzymes in either submerged fermentation [7] or in solid state fermentation [10] by *Termitomyces clypeatus*, an edible mushrrom. Gautam et al., [3] reported that maximum xylanase production from S. commune was found with rice straw (4288.36 IU/gds) as the carbon source under SSF conditions. Kolenova et al. [11] observed maximum xylanase

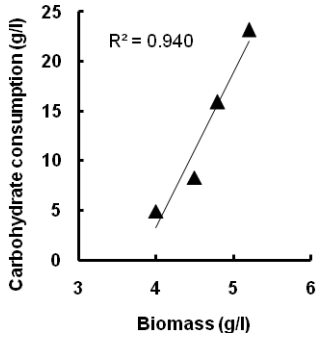

Figure 3A. Correlation analysis of fungal biomass and substrate consumption in TKP culture medium of *S. commune*, corresponds to a linear function of y = 15.65x – 59.33

activity (71.3 U/ml) by S. commune under submerged fermentation after 11th day of cultivation on cellulose medium. The correlation analysis (Figure 3) provided that substrate consumption and fungal biomass growth is highly correlated with a linear function as y = 15.65x – 59.33 for TKP media and y = 6.95x – 9.072 for cellulose culture media of *S. commune*.

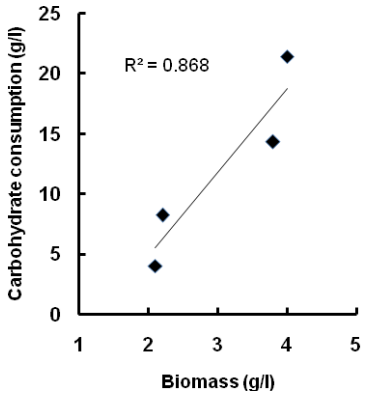

Figure 3B. Correlation analysis of fungal biomass and substrate consumption in cellulose culture medium of *S. commune*, corresponds to a linear function of y = 6.95x – 9.072

From the perspective of enzyme production, it may be concluded that tamarind kernel powder is a promising soluble carbon substrate in submerged fermentation of *S. commune*. Metreveli et al., [2] reported that both of the

enzymes i.e., xylanase and CMCase activities were significantly improved in culture filtrate when co-cultivation of *S. commune* with *Irpex lacteus* in a single set of fermentation. In the present study, co-cultivation was not

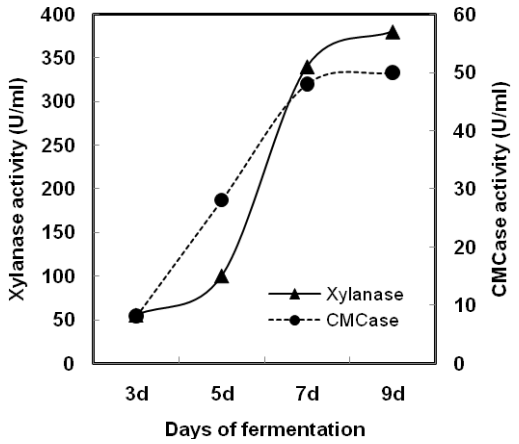

Figure 4. Production kinetics of enzymes in different types of carbon substrates. Solid line represents TKP culture medium and dotted line represents cellulose culture medium. (Standard deviation for xylanase within ± 8 % and for CMCase ± 5 %)

done and therefore, future studies should be focused on this aspect using TKP as carbon substrate. The kinetics of enzyme production in submerged culture of the fungi with either TKP or cellulose (Figure 4) revealed that the technique failed to able more significant increase if the fermentation was allowed to continue from 7th days to 9th days in both of the enzymes. It can be seen from the figure 4, that xylanase activity was 340 U/ml at 7th day as compared to 380 U/ml at 9th day, while in case of CMCase, enzyme titer was observed as 49.7 U/ml at 9th day in contrast to 48 U/ml at 7th day of culture in cellulose medium. However, a substantial carbohydrate was available in both of the culture medium. Kolenova et al., [11] also reported that xylanase titer was optimized under submerged fermentation of *S. commune* after 11th day of culture, which was similar to our present study. After critical analysis on the production behavior of CMCase and xylanase in a single fermentation, the use of mixed substrates in the culture media with varying conc. were experimented and the results shown as in Figure 5. The findings clearly showed that addition of 0.5 % TKP in cellulose medium improved CMCase titer (58 U/ml in contrast to 42 U/ml in cellulose medium), whereas addition of 0.5 % cellulose in TKP medium did not increased the production level of xylanase as observed in the presence of TKP only (Figure 1). Thus the presence of cellulose in TKP medium specifically improved CMCase production, although xylanase production was un-affected. Use of mixed substrate is reported in submerged fermentation of *Thecotheus pellelier* [12], in which both xylanase and CMCase production were improved. Addition of xylan however, reported to decrease xylanase production by *S. commune* [8]. From the experimental findings, it may be supposed that the inducer molecules liberated by TKP or cellulose were not identical and both of the carbon substrates induced CMCase and xylanase production independently as reported by Chatterjee et al., [7] in submerged fermentation of *Termitomyces clypeatus*. Tamarind kernel powder, although structurally similar to cellulose, therefore, was found to be the perfect inducer for xylanase production in submerged fermentation of *Schizophyllum commune.*

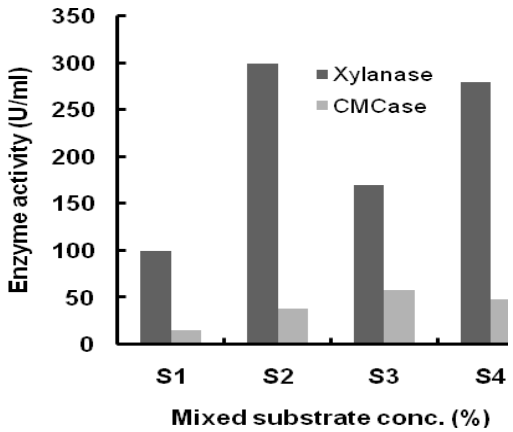

Figure 5. Production of xylanase and CMCase by S. commune in mixed substrate of TKP and cellulose. S1= 0.5% TKP + 0.5% cellulose, S2 = 1% TKP + 0.5 % cellulose, S3 = 0.5 % TKP + 1 % cellulose, S4 = 1% TKP + 1% cellulose (Standard deviation for xylanase within ± 8 % and for CMCase ± 6 %)

V. CONCLUSION AND FUTURE SCOPE

For the successful production of industrial enzymes, use of cheap agro-residues is a significant criterion since the cost of substrate plays a crucial role in the economics of enzyme production. From the experimental study, it moday be concluded that tamarind kernel powder induced xylanase production significantly in submerged fermentation of *S. commune*,a although it failed to improve the CMCase production in culture medium. Future studies therefore, would be necessary to clarify the catabolic repression observed in CMCase production and also characterization of xylanase and CMCase of *Schizophyllum commune.*

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