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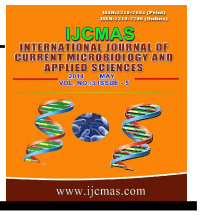

Original Research Article

Comparative study of saccharification of fruit waste by mono and mixed cultures of cellulolytic fungi

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A B S T R A C T

The present study aims to the use of the fungal consortia for the degradation of the fruitwaste by solid state fermentation for cellulase production. Three fungal strains were tested to find their ability to produce cellulases, which catalyze the degradation of cellulose, which is a linear polymer made of glucose subunits linked by -1, 4 glycosidic bonds. T All three fungal strain was noticed to show maximum zone of hydrolysis on carboxy-methyl cellulose agar .The three fungal mixture (consotia) produce higher activities of the cellulases and it were determined by Filter paper assay (FPA), Carboxy-methly cellulase assay (CMCase) and -β-glucosidase assay respectively. The production patterns of these enzymes were studied during the growth on the organisms for a period of 21 days. Fungal consortia exhibited high level of enzyme activities and saccahrification. Maximum specific activities of enzymes were obtained between 7 to 15 days of culture growth at 30^0C, 5.5 pH and 3 to 5% of substrate concentration.

Keywords

Fruit waste, Saccharification, Mixed culture, Cellulase production, Optimization

Introduction

Cellulose is considered as one of the most important sources of carbon in this planet and its annual biosynthesis by both land plants and marine algae occurs at a rate of 0.85×10^{11} tonnes per annum .Cellulose, a polymer of glucose residues connected by β-1,4 linkages, being the primary structural material of plant cell wall, is the most abundant carbohydrate in nature (Chandra, M., A *et al*., 2009).

It has become considerable economic interest to develop processes for effective treatment and utilization of cellulosic wastes as inexpensive carbon sources. Enormous amounts of agricultural, industrial and municipal cellulosic wastes have been accumulating or used inefficiently due to the high cost of their utilization processes (Chinedu, S.N *et al.*, 2010). Cellulases provide a key opportunity for achieving tremendous benefits of biomass utilization.

Cellulase is the enzyme that hydrolyzes the β-1, 4-glycosidic bonds in the polymer to release glucose units (F. T. Fan, M. M *et al.*, 19987). Cellulase is a multi enzyme system composed of several enzymes with numerous isozymes, which act in synergy.

The basic enzymatic process for the depolymerisation of cellulose requires three types of enzymes: Endoglucanase (EG or CX), hydrolyses internal β-1,4 glucan chain of cellulose at random, primarily within amorphous regions and display low hydrolytic activity toward crystalline cellulose; Exoglucanase i.e., exoacting cellobiohydrolases (CBH), removes cellobiose from the non-reducing end of cello-oligosaccharide and of crystalline, amorphous and acid or alkali treated cellulose; Cellobiase or β-glucosidase (BGL) (Jahromi, M.F *et al.*, 2010) hydrolyses cellobiose to yield two molecules of glucose which completes the depolymerisation of cellulose.

Cellulases have enormous potential in industrial applications. Glucose produced from cellulosic substrate by hydrolysis could be further used as substrate for subsequent fermentation or other processes which could yield valuable end products such as ethanol (Louime, *et al*., 2008), butanol, methane, amino acid, single-cell protein ,organic acids (Kuhad, R.C *et al.*, 1937) etc. (Penttila *et al.*, 2004), feed preparation, (Ishikuro, 1993) waste-water treatment, detergent formulation (Mekala. N.K *et al*., 2008), textile production and in other areas(Van-Wyk e*t al.*, 2003).Additional potential applications include the production of wine, beer and fruit juice. Nevertheless, all these uses are of rather small magnitude compared with cellulase requirements for bioconversion of lignocellulosic biomass to fuel ethanol.

One major obstacle facing the development of lignocellulosic biofuel is the cellulose hydrolysis. Generally speaking, there is a lack of efficient microorganisms which can produce sufficient amounts of all three types of cellulases to efficiently breakdown crystalline cellulose to glucose. Moreover, the bio refining process remains economically unfeasible due to a lack of biocatalysts that can overcome costly hurdles (Mekala. N.K *et al*., 2008) Recently, the cost of ethanol production from cellulosic material is US$1.8 per gallon. However, development of enzymatic processing can decrease the ethanol cost as low as US$0.2 per gallon (Mekala. N.K *et al*., 2008). Therefore, the chance to obtain cheap ethanol will depend on the successful screening of novel cellulose producing strain.

Cellulases are produced by large number of microorganisms and are either cell-bound or extracellular. Fungi and bacteria are the main natural agents of cellulose degradation. The cellulose utilizing population includes aerobic and anaerobic mesophilic bacteria, thermophilic, alkaliphilic bacteria, actinomycetes, certain protozoa and filamentous fungi. However, fungi are well known agents of decomposition of organic matter (Lynd *et al*., 2002). Although a large number of fungi can degrade cellulose, only a few of them produce significant quantities of free enzyme capable of completely hydrolyzing crystalline cellulose. Therefore, there has been much research aimed at obtaining new microorganisms producing cellulase enzymes with higher specific activities and greater efficiency.

In this study, efficient cellulase producing microorganisms were isolated from different natural sources like compost soil, decayed wood and lignocellulosic waste. The purpose was to identify and characterize those isolates displaying the greatest cellulase activity for the possible use in large scale bio refining and producing cellulase enzyme and reducing

sugar using mono and mixed cultures of these fungi.

Materials and Methods

Isolation and screening of cellulolytic microbes

Isolation of microbes is important in order to obtain pure culture. Isolation method involves serial dilution method, by exposing CMC agar in to environment which is rich in decayed cellulosic material and scrapings were taken from surfaces with visible fungal growth or an accumulation of organic material.

Serial dilution and spread plating was performed with soil and compost samples .The soil sample and compost were serially diluted and spread plated on a CMC agar.The plates were incubated for 7 days at room temperature and observed for clear zone around colony. To visualize the hydrolysis zone, the plates were flooded with an aqueous solution of 0.1% Congo red for 15 min and washed with 1 M NaCl Bradner *et al*., 1999).To indicate the cellulase activity of the organisms, diameters of clear zone around colonies on CMC agar were measured. Single colony from these CMC agar plates were sub cultured on fresh CMC plate. These plates were used as master plate.

Identification of Fungal Isolates

Isolated cellulolytic fungi were identified based on colony morphology, cultural characteristics and, especially, on the morphology of their sporulaing structures. The morphology of the isolates, stained with lacto phenol-cotton blue, was studied using a light microscope. Fungus was inoculated in Potato dextrose agar and incubated at 28ºC for 7 days and these cultures were used for fungal staining. A wet mount of 7 day old culture fungus was prepared by suspending some of the culture from the PDA plate in a few drops of Lacto phenol Blue and observe under the microscope.

Cellulase Enzyme production

The Carboxy methyl cellulose (CMC) broth containing 0.2% (w/v) CMC as sole carbon/energy source was used for enzymes production. One ml of fungal culture was inoculated into the 50ml of the sterile CMC broth. The cultures were incubated in shaking for 7 days at room temperature (25ºC – 30ºC). Cultures were harvested by centrifugation at 6000xg for 15 minutes and the cell free culture supernatants used as crude enzyme source.

Cellulase activity assay

The cellulase activity of each culture was measured by determining the amount of reducing sugars liberated by using a dinitrosalicylic acid (DNS) method (Miller, 1959). One unit of FP-ase, CMC-ase was defined as the amount of enzyme, which released μmole of reducing sugar measured as glucose per min under the assay conditions.

Filter paper assay (FPase) for total cellulase activity in the culture filtrate was determined according to the standard method. 0.5ml of culture filtrate as enzyme source was added to Whatman No. 1 filter paper strip (1 x 6 cm; 50 mg) immersed in 1ml of 0.5 M Sodium citrate buffer of pH 5.0. After incubation at 50 ^{0} C for 1h, the reducing sugar released was estimated by dinitrosalicylic acid (DNS) method (T. K. Ghose., 1987) One unit of filter paper (FPU) activity was defined as the amount of enzyme releasing 1 μmole

of reducing sugar from filter paper per ml per min. CMCase activity was measured using a reaction mixture containing 1 ml of 1% carboxymethyl cellulose (CMC) in 0.5 M citrate acetate buffer of pH 5.0 with 0.5ml of enzyme supernatant filtrate (Wood and Bhat, 1988). The reaction mixture was incubated at 50^0C for 30 min and the reducing sugar produced was determined by DNS method

Molecular identification of fungal isolate

Molecular characterization, particularly DNA sequence analyses of 18S rDNA was used to confirm the identity of isolated cellulolytic fungi. 18S rDNA sequence analysis was performed to identify the phylogeny of the fungi. Fungal material was scraped from pure cultures and DNA extracted using CTAB method .The 18S rDNA sequences were amplified using primers ITS-4: TCCTCCGCTTAT TGATATGC and ITS-5: GGAAGTAAA AGTCGTAACAAGG. Since, fungal ITS sequences generally provide greater taxonomic resolution than sequences generated from coding regions (Anderson *et al.,* 2003). PCR amplification was done in a MJ Research PTC Mini-cycler with the following protocol: 95^0C for 5 min; 30 cycles of 94^0 C for 1 min, 55^0C for 1 min, 72^0C for 1 min followed by a final extension step of 72^0C for 1 min. Each 50-µl reaction mixture contained 30 mM Tris (pH 8.4), 50 mM KCl, 1.5 mM $MgCl_2$, 50 mM concentrations of each deoxynucleotide triphosphate, 10 pmol of each primer, and 1 U of *Taq* polymerase.

Cellulase enzyme production using fruit waste as substrate

10 g of fruit waste was moistened with Mandel and Reese medium to get initial moisture content 50% and autoclaved at 1210C for 15 min at 15lbs pressure. After cooling sterilized flask were inoculated with mono cultures ,cocultures and mixed cultures. For inoculating monoculture 3 ml of fungal spores were inoculated in to 10 g of sterilized substarte, For co cultures (mixture of two fungi) 1.5 ml of each fungal cultures added and for mixed cultures (mixture of three fungi) 1ml of each culture was added .The final spore concentration was maintained as $3x10^6$ spores /ml. The content in the flask were mixed thoroughly to ensure uniform distribution of inoculums and flask were incubated at 30^0C for 21 days.The sample were withdrawn after 5^{th}, 7^{th}, 15^{th}, 21th day and estimated endoglucanase, exoglucanase and beta glucosidase activity. Reducing sugars were determined with the DNS method (Miller *et al*, 1960) using glucose as standard. For protein determinations BSA (fraction V, Sigma) was used as a standard (Lowry *et al*, 1951).

Optimization of enzyme production

To select the suitable temperature, pH, incubation period and substrate concentration for saccharification of paper by mono and mixed fungal strain were cultivated with varying temperatures of 20°C-60°C, pH range 3-6.5, incubation period range of 2-30 days, and substrate concentration 1% - 10% by keeping all other parameters constant for 10 days.

Solid state fermentation Using Fruit waste as substrate

Solid-state fermentation was employed for production of cellulase enzyme by fungal consortium on fruit waste. 25 ml of fermentation medium was transferred to the 250 ml cotton plugged Erlenmeyer

flasks containing 10 g fruit waste . After autoclaving at 15 lb/inch2 pressure and 121ºC for 15 min., and cooling the medium at room temperature, 3.0 ml of spore inoculum containing 2x 10^7 spores was transferred to each flask aseptically. The flasks were then incubated at 30±1ºC in an incubator for 15 days. All experiments were run parallel in duplicates. Spore count was measured with the help of hemocytometer .Protein concentration and reducing sugar concentration was determined.

Determination of endoglucanase, exoglucansae and β-glucosidase activity

Before determining the hydrolysis of paper waste with the supernatants, cellulolytic activity was determined. For endoglucanase activity, the study employed a 50 µl substrate of Carboxymethyl cellulose (CMC), 0.8% (w/v) in a sodium acetate buffer pH 5.0 with 500 µl of supernatant from mixed fungus, according to Ghose (1987). For the exoglucanase determination, a substrate was used of 15 µl of *p*-nitrophenyl-β-D-cellobioside (pNPC) at 1% (w/v) as a substrate under the same conditions and 500 µl of supernatant. Both reactions were incubated at 50^{o}C for 1 h and 4°C for 10 min. Then, 2ml of the solution was mixed with 2ml of a DNS solution (Ghose 1987) mixed well and performed DNS assay for determination of released reducing sugars. The mixture was incubated for 5 min at 90°C and then cooled 10 min at 4°C.

Determination of β-glucosidase activity

180 µL of *ρ*-nitrophenyl-β-D-glicopiranosido (PNPG) was used as a substrate and 50 µL of supernatant. Following incubation with the substrate, 20 µL of this solution were added with 80 µL of 2% sodium carbonate and 180 µL water. We measured the release of *p*-nitrophenol as the increase in absorbance at 405 nm and used pNitrofenol (10, 20, 30 and 50 µg mL-1) for the calibration curve of β-glucosidases (Sadana and Patil, 1988). In allcases, the tests were performed in triplicate.

Statistical analysis

Analysis of variance (ANOVA) was performed on all data using the SAS (1985) statistical package. The mean values were compared by the least significant difference (LSD) test at 5% level of confidence.

Results and Discussion

Isolation and Screening of the cellulase producing fungi

The cellulase producing fungi were isolated from natural environment by different isolation method. A novel method of isolation was adopted in this study. CMC agar were exposed in lignocellulosic rich environment The isolates were numbered with NASC. Carboxymetyl cellulose agar is a selective media and selectively supports the growth of the cellulolytic fungi because cellulase producing organisms can only utilize cellulose as the carbon source.

The screening of the cellulolytic fungal isolate was performed based on the diameter of clearing zone surrounding the colony on the CMC medium. Congo red intercalate between the cellulose. The diameter of clearing zone for each isolate are shown in Table 1.The appearance of the clear zone around the colony after the addition of Congo red solution was strong

evidence that the fungi produced cellulase in order to degrade cellulose (Figure 1). Among the 50 isolates,5 isolates have shown the higher cellulase activity.These isolate has been used for further studies into the enzyme production and their ability to degrade cellulose. Results showed that isolated (NAS3) has highest enzyme activity among total isolates. Depending of clear zone diameter NASC3 used for further studies.

The isolates were identified based on the colony morphology and microscopic observation .The isolated fungi was purified by repeated Sub-culturing on the Potato Dextrose Agar medium at regular intervals and incubated at 28°C. Isolate numbers NASC1, NASC2 and NASC5 were identified as *Aspergillus* sp, NASC3 was identified as *Penicillium* sp and NASC5 and NASC6 identified as *Mucor* sp as well as Trichoderma sp (Table 1). NASC3,NASC2 and NASC 6 was used for molecular identification because of its high capacity to produce cellulase enzyme.

Cellulase enzyme assay

Cellulases production was quantitatively determined for 6 fungal isolates grown in the CMC broth media**.** Enzyme activities of fungal isolates are presented in Figure 2. The FP-ase and CMC-ase activities of *Penicillium* sp NASC3 was higher than other isolates, suggesting that these isolates which have appreciable cellulolytic activity are valuable in the bioconversion process of cellulolytic materials.

Molecular characterization based on 18S rRNA gene

The isolated hyper producing fungi were identified by molecular characterization technique in which genomic DNA was isolated and internal transcribed spacer (ITS) was amplified with suitable primers and phylogenetic analysis was done. *Penicillium* sp NAS3 showed high cellulolytic activity, so these fungi was molecularly identified. The genomic DNA was isolated and checked on agarose gel, was found to be of high molecular weight and intact. The spectrophotometric analysis of the DNA showed that the DNA had an A260/A280 ratio of 1.80. Amplification of genomic DNA from NAS3 was conducted through polymerase Chain Reaction (PCR). The PCR of 18S rRNA gene was carried out by using universal primer (ITS4 and ITS5) specific to 18S rRNA genes. The gel electrophoresis was performed to analysis the PCR product. The PCR product has a size in a range between 900 to 1500bp proved that the 18S RNA gene of NASC3,2 and 6 were successfully amplified and after purification it was used for sequencing. The obtained sequence of 18srRNA fragment from NASC 3 showed 92% similarity with target sequence to the closely related fungal sequence *Penicillium citrinum*. The fungal isolates, NASC3 was identified as *Penicillium citrinum (GenBank.No.:KF129059)*, NASC 2 identified as *A.oryzae (GenBank No: KF 129058)*and NASC 6 Identified as *T.viride.*

Cellulase enzyme production on Fruit waste in SSF by monocultures

Table 1,2 & 3 reflects the saccharification of fruit waste and CMC by *P. citrinum, A. oryzae and T.viride* as well as mixture of them. Fruit waste showed the strongest susceptible for hydrolysis by *mixed cultures* followed by CMC. To increase the degree of saccharification fugal cultures were mixed and incubated with all

cellulosic materials (Figure 1). An equal mixture (2:2:2) resulted in the highest increase in saccharification with all substrates. Similar tendencies with cellulases from *P. funiculosum* and *Trichoderma reesei* on these cellulose materials were observe (Van Wyk, 1998).
The rate of saccharification can be increased by optimizing conditions for microbial growth. During growth of microorganisms in media containing paper waste, they utilize cellulose in paper as Carbon source. These organisms produce and secrete cellulase enzyme for the degradation of cellulose and release glucose. The process of saccharification of paper can be increased by optimizing the conditions like Temperature, pH, incubation time and substrate concentration etc. The saccharifying media was prepared and cultures were inoculated in 2:2:2 ratio.

Fig. 1 shows the effect of incubation time on saccharification of fruit waste by mono and mixed fungal cultures on media containing fruit waste as carbon source. The Saccharification increased with the increase in incubation period and reached maximum after 15 days of incubation for monocultures where as the maximum saccharification was observed at 10^{th} day in mixed cultures. Further increase in the incubation period however, resulted in the gradual decrease in the saccharification. Therefore, incubation period of 15 days for monoculture and 10 days for mixed culture was found to be optimal for saccharification.

The optimization of the time course is of prime importance for saccharification by fungi (Khud & Sing, 1993). The decrease in the saccharification by monocultures and mixed cultures after 10 to 15 days of incubation period might be due to the depletion of the nutrients and accumulation of other byproducts or catabolic repression of cellulase enzyme by released glucose.

The effect of incubation temperature (20-55ºC) on the saccharification of fruit waste by mono and mixed cultures of fungi is shown in Fig. 2. There was a gradual increase in saccharification as the temperature was increased. But it showed maximum yield at 30ºC i.e., reducing sugar concentration for monocultures1.5 to 2.5 $\pm$ 2 mg/ml and for mixed cultures 4.5$\pm$ 2 mg/ml. As the temperature was further increased, there was a gradual reduction in the saccharification. This may be due to the fact that higher temperature denatures the saccharifying enzymes mainly cellulase (Solomon, B.O.1999). High temperature may also lead to inhibition of microbial growth. Mekala *et al*., (2008) showed that cellulases production and thus saccharification was maximum in flasks incubated at 33^{O}C and decreased with high temperature.

The effect of initial pH (3.0-6.5) of the culture medium on the saccharification by fungal cultures was studied (Fig. 3). At the pH value of 4.5, there was very little saccharification of fruit waste 1 to 2.4 mg/ml by mono cultures and mixed cultures, however, it started to increase as the initial pH of the growth medium was increased and reached maximum at pH 5.5.

Further increase in pH resulted in a gradual reduction of saccharification by the organism. Hence, pH of 5.5 was optimized for the maximum saccharification by fungi. After pH value of 5.5, the production of cellulases decreased which might be due to the fact that cellulase are acidic proteins and are greatly affected by the neutral pH values (Chandra *et al*., 2009).

Figure.1 Screening of fungal isolated on CMC agar after treatment with Congo Red showing Clear zone around the colonies

Table.1 The clearing zone of cellulase activities of fungi isolates used in the cellulolytic screening study

Isolates	Fungal isolates	Diameter of clear zone (mm)
NASC1	*Aspergillus* sp	20
NASC2	*Aspergillus* sp	35
NASC3	*Penicillium* sp	44
NASC4	*Aspergillus* sp	19
NASC5	*Mucor* sp	22
NASC6	*Trichoderma sp*	27

Table.2 Total reducing sugar produced from fruit waste and Carboxy methyl cellulose at the optimum ratio mixture of *P. citrinum A. oryzae and T.viride* relative to the monoculture action.

Substrate	**Total reducing sugar concentration (mg/ml) produced by mono and mixed cultures of cellulolytic fungi**				
	P.citrinum	**A.oryzae**	**T.viride**	**Mixed culture 1**	**Mixed culture 2**
Fruit waste	1.3	1.455	1.0	1.88	2.56
CMC	0.78	0.99	0.58	1.20	1.52

CMC-Carboxymethyl cellulose, Mixed culture 1-1:1:1 ratio of three culture, Mixed culture 2-2:2:2 ratio of cultures

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Table.3 Cellulase enzyme produced during Saccharification of fruit waste and Carboxy methyl cellulose by monoculture and mixed cultures of cellulolytic fungi

Substrate	Cellulase enzyme activity (U/ml) relative to individual cellulase action				
	P.citrinum	A.oryzae	T.viride	Mixed culture 1	Mixed culture 2
OP	1.2	1.36	0.98	1.59	2.17
CMC	0.63	0.83	0.45	1.36	2.09

CMC-Carboxymethyl cellulose, Mixed culture 1-1:1:1 ratio of three culture, Mixed culture 2-2:2:2 ratio of cultures

Table.4 Protein produced during Saccharification of fruit waste and Carboxy methyl cellulose by monoculture and mixed cultures of cellulolytic fungi

Substrate	Protein concentration (mg/ml) relative to individual cellulase action				
	P.citrinum	A.oryzae	T.viride	Mixed culture 1	Mixed culture 2
Fruit waste	1.5	1.4	1.15	2.12	3.26
CMC	0.79	0.7	0.71	1.121	1.13

CMC-Carboxymethyl cellulose, Mixed culture 1-1:1:1 ratio of three culture, Mixed culture 2-2:2:2 ratio of cultures

Figure.1 Effect of incubation time on saccharification of fruit waste by *Trichoderma viride*, *Aspergillus oryzae*, *Penicillium citrinum* and mixed culture

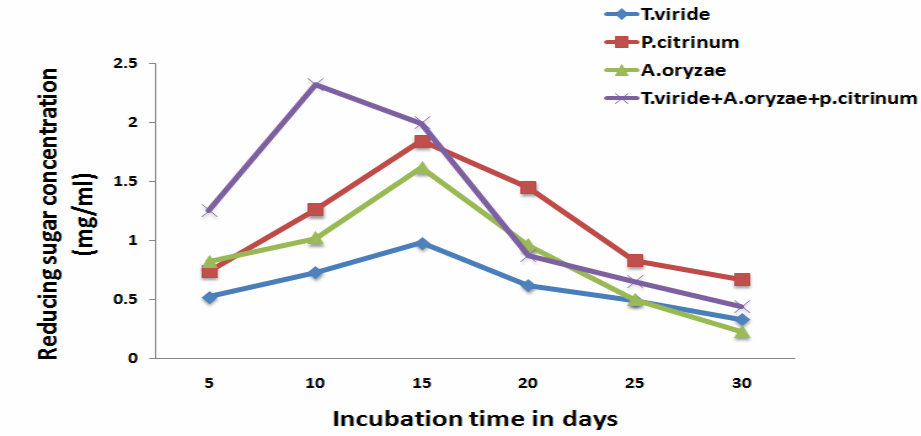


Figure.2 Effect of Temperature on saccharification of fruit waste by *Trichoderma viride*, *Aspergillus oryzae*, *Penicillium citrinum* and mixed culture incubated for 15 days.

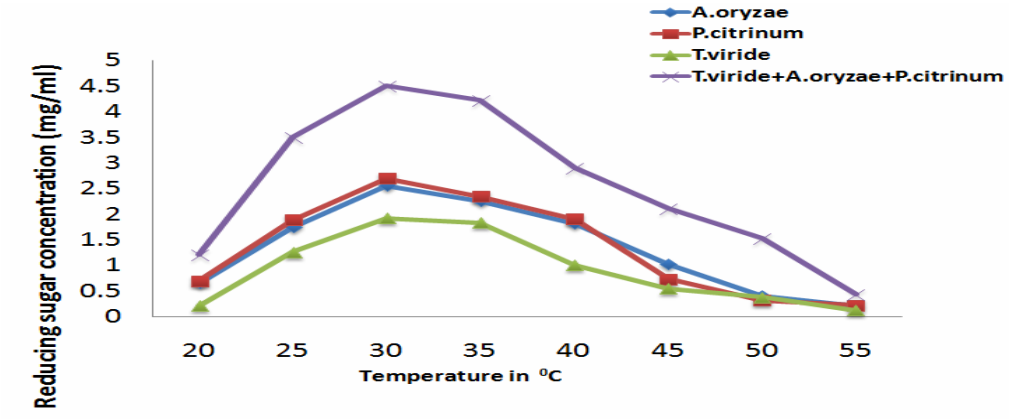


Figure.3 Effect of pH on saccharification of Fruit waste by *Trichoderma viride*, *Aspergillus oryzae*, *Penicillium citrinum* and mixed culture incubated at 30°C for 15 days

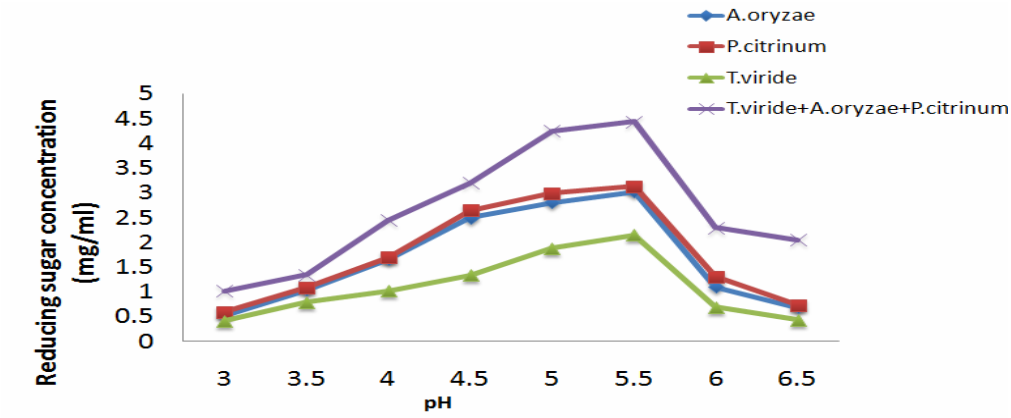


Figure.4 Effect of substrate concentration on saccharification of Fruit waste by *Trichoderma viride*, *Aspergillus oryzae*, *Penicillium citrinum* and mixed culture incubated at 30°C, pH 5.5 for 15 days.

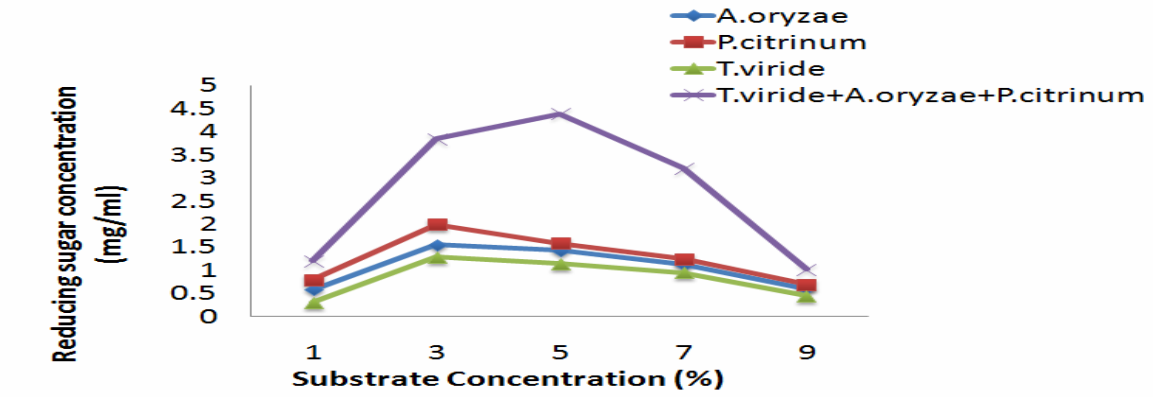
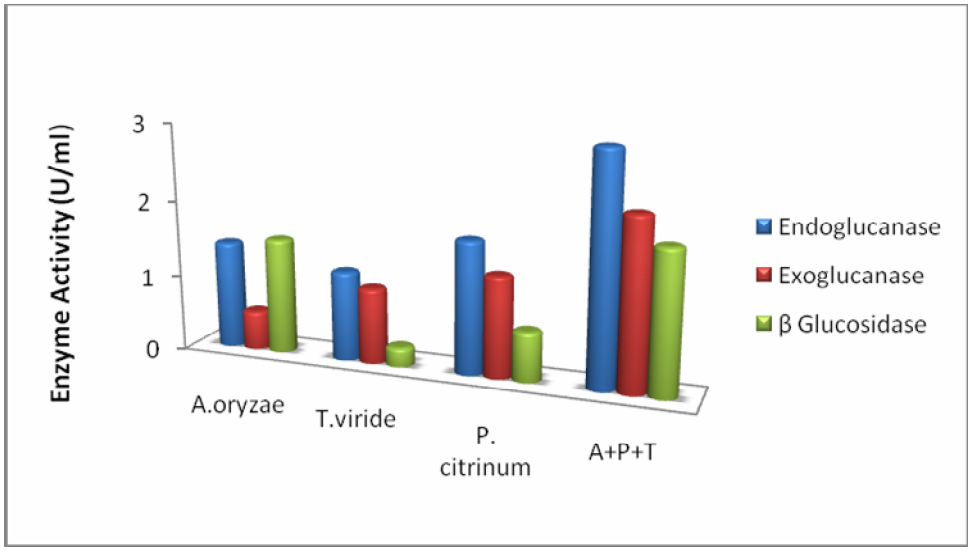


Figure.5 Enzyme activity of mono and mixed cultures of fungi on 3% of fruit waste incubated at 30°C,pH 5.5 for 15 days



Effect of substrate concentration on saccharification of fruit waste by mono and mixed cultures were carried out (figure 4). It was observed that the highest saccharification was carried out 3 % of substrate for monocultures where as mixed cultures highest saccharification obtained at 5 %.This may be due to mixed culture contain all the enzyme complex of cellulase enzyme hence , they can convert high concentration of paper in to sugars.

It revealed that *P.citrinum (NASC3*) produced more endoglucanase (1.73± 0.1 U/ml) exoglucanase (1.32 U/ml) and very less β glucosidase activity (0.65± 0.12 U/ml) when compared to other two fungi. *A.oryzae (NASC2)* produced more β – glucosidase (1.52 ± 0.21 U/ml) and endoglucanase (1.42 U/ml) but less exoglucanase (0.52 ± 0.2). When compared to other two fungi, *T.viride* (NASC6) enzyme activity was very less.It secreted more endoglucanse (1.18 ± 0.2 U/ml) and less exoglucanase (0.99 ± 0.2U/ml) and β-glucosidase activity(0.25 ± 0.2U/ml).(Figure 5).

The substrate used for determining the activity of each enzyme is indicated in the materials and methods section, carboxymethyl cellulose (CMC) for endoglucanase activity, ρ-nitrophenyl-β-D-cellobioside (pNPC) and ρ-nitrophenyl-β-D - glicopiranósido (PNPG) for β-glucosidase and exoglucanases respectively.

The advantage of mixed cultures is more pronounced in SSF condition, because the colonization of the substrate may be accomplished better in symbiotic association.It is find out that cellulase from different organism are closely related to each other (Ahmadi, et al 2010). The endoglucanase from one fungal isolate can operate with other component of cellulase produced by other isolates. The possible mechanism behind the enhanced production of cellulase by mixed cultures is the secretion of all components of cellulase enzyme in appreciable amount in the media while in the monocultures,one or more of the enzyme components may be missing or produed in insufficient

amount (Baker, J.O., et al 1998).as a result there will be more degradation of lignocellulosic waste with mixed cultures than monocultures.

In this research work, from above findings it may be concluded that strains *A. oryzae*, *T.viride* and *P.citrinum* showed good performance for the saccharification of fruit waste. Fruit waste was the best inducer of cellulase enzyme and found to be the best for releasing maximum reducing sugar and saccharification. The maximum saccharification was obtained with mixed cultures of fungi in 2:2:2 ratio and temperature 30°C, pH 5.5 , incubation time 10 days and substrate concentration 50 %.The reducing sugar produced from fruit may be used in future for ethanol production or others. This research may be meaningful both in the conversion and utilization of renewable biomass, and in the reduction of environmental pollution.

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