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EFFECTS OF AMINO ACIDS AND METAL IONS ON DEGRADATION OF LIGNOCELLULOSIC BIOMASS BY *SPOROTHRIX CARNIS* CPF-05

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ABSTRACT
Cost-effective saccharification of cellulose and hemicelluloses to fermentable sugars is of critical importance to bioconversion of lignocellulosic feedstocks to biofuel. Therefore, it is expedient to investigate the roles of medium components in optimizing lignocellulose biodegradation process. In this study, corn cob was exposed to degradation by *Sporothrix carnis* CPF-05 under submerged fermentation. Influence of amino acids and metal ions on biodegradation efficiency, yield of fermentable sugars and activities of degrading enzymes was investigated. It is highly remarkable that degradation of lignocellulosic biomass by *S. carnis* CPF-05 improved in the presence of tyrosine, glutamic acid, aspartic acid, Mn^{2+} and Cu^{2+} with biodegradation efficiency in the range of 116% to 132% compared with control. Impressive yield of glucose and xylose of about 3.0 fold increase over control was obtained in media supplemented with polar amino acids with improved production of hydrolytic and oxidative enzymes (215% - 281% relative activities). Mn^{2+} and Cu^{2+} enhanced 3.5 and 3.0 fold increase in yield of glucose and xylose, respectively. Decrease in biodegradation efficiency was recorded in the presence of non-polar amino acids (leucine, tryptophan and valine), Zn^{2+} and Fe^{2+}. Results revealed that polar amino acids serve as alternatives to complex nitrogenous substrates in enhancing lignocellulose biodegradation.
Keywords: Amino acids; biodegradation; lignocellulose; metal ions; *Sporothrix carnis*

INTRODUCTION

Lignocellulosic biomass is a major raw material for forestry, pulp and paper industry, and the emerging second generation biofuel production. It consists of cellulose, hemicellulose and recalcitrant lignin (Sweeney and Xu, 2012). Among cellulose and hemicellulose, lignin is a major component of lignocellulosic biomass which is largely responsible for its strength (Li *et al.*, 2011).

Lignocellulose is an insoluble polymer due to its recalcitrant lignin component. This feature therefore makes the initial steps in biodegradation of lignocellulose extracellular with secretion of hydrolytic and oxidative enzymes (Gupta *et al.*, 2015). Corn cob which contains about 45% cellulose, 35% hemicellulose and 20% lignin is a cheap and good substrate for microbial growth and production of extracellular lignocellulolytic enzymes (Olajuyigbe and Ogunyewo, 2016a). Cellulose biodegradation is a synergistic process involving endo-1,4-glucanase, exoglucanases and β-glucosidase. The internal glycosidic bonds in the β-1-4 chain are first hydrolyzed by endoglucanases to generate free non-reducing ends, from which the exoglucanases then remove cellobiose units that are converted to glucose by β-glucosidase (Shah *et al.*, 2015). Hydrolysis of xylan mainly requires the action of endo-1,4-xylanase and β-xylosidase. However, presence of other accessory enzymes is required to hydrolyze substituted xylans (Gaur *et al.*, 2015). Due to the hydrophobicity of lignin and complex random structure that lacks the regular hydrolysable bonds, lignin is poorly degraded by most microorganisms (Levin *et al.*, 2008).

Organisms known to extensively degrade lignin are fungi and, to a lesser extent, certain

actinomycetes and bacteria (Li *et al.*, 2011). A wide range of microorganisms including bacteria and fungi are capable of producing cellulases and hemicellulases but only a limited number of these microorganisms are capable of producing lignin degrading enzymes (Raghukumar *et al.*, 2008). Among these microorganisms, filamentous fungi appear to be the most efficient producers of lignocellulose degrading enzymes (Jorgensen, 2003). The ability of these fungi to produce specific enzymes for degradation of different carbon and nitrogen sources is due to the diverse habitats in which they are found (Jorgensen *et al.*, 2007). White-rot fungi (WRF) are unique in their ability to completely degrade all components of lignocellulosic materials. The ability of WRF to degrade lignin has been attributed to their extracellular nonspecific and non-stereoselective enzyme system (Fernández-Fueyo *et al.*, 2014). This enzyme system is made up of laccase, lignin peroxidase and manganese peroxidase, which function together with H_2O_2-producing oxidases (Woolridge, 2014).

The understanding of physiological mechanisms regulating enzyme synthesis in bioconversion of lignocellulose is very crucial for improving growth of microorganisms and biodegradation process. In optimizing the synthesis of lignocellulolytic enzymes for bioconversion of lignocellulose by WRF, it is worth noting that the activity of these enzymes depends not only on the fungal physiology but also on the composition of culture medium. Some earlier studies have proved that both the nature and the concentration of some macromolecules are essential for regulating synthesis of lignocellulolytic enzymes by WRF (Johnsy and Kaviyarasan, 2011; Olajuyigbe and Ogunyewo, 2016b). Few supplements have been reported to possess stimulatory effects on the synthesis of lignocellulolytic enzymes when added to culture medium (Cristica *et al.*, 2012; Elshafei *et al.*, 2012; Liao *et al.*, 2014). However, there is dearth of information on the effects of amino acids and metal ions on biodegradation of lignocellulose and influence of supplements on interaction of lignocellulolytic enzymes.

Interactions between hydrolytic and oxidative enzymes produced by white rot fungus, *S. carnis*, during degradation of corn cob under different process parameters has recently been reported (Ogunyewo and Olajuyigbe, 2016). In an attempt to optimize degradation of lignocellulosic biomass by *S. carnis* CPF-05, effects of supplements such as amino acids and metal ions on biodegradation, yield of fermentable sugars and activities of degrading enzymes were investigated.

MATERIALS AND METHODS

Materials

Carboxymethyl cellulose, birch wood xylan, 2,2'azino-di-[3~ethylbenzothiazoline-6-sulphonic acid] (ABTS), sodium - potassium tartarate, dinitrosalicyclic acid, bovine serum albumin (BSA), manganese sulphate, copper sulphate, cellobiose, avicel, xylose, tryptophan, aspartic acid, glutamic acid, tyrosine, leucine, hydrogen peroxide and medium components were products of Sigma-Aldrich (St Louis, MO, USA). All other chemicals used were of analytical grade. Corn cob was purchased from a local market (Oja Oba) in Akure, South West Nigeria. The corn cob was sun dried and powdered into fine particles and used as carbon source in the basal medium. Further processing of the powdered corn cob was carried out using standard sieve with an average size of 1 mm.

Microorganism

The microorganism used was a white rot fungus, *Sporothrix carnis* CPF-05 obtained from the culture collection of Enzyme and Microbial Technology Laboratory, Department of Biochemistry, Federal University of Technology, Akure, Nigeria as previously described (Olajuyigbe and Fatokun, 2017; Olajuyigbe and Ogunyewo, 2016a).

Inoculum preparation, degradation of corn cob and production of lignocellulolyticenzymes by *S. carnis* CPF-05

Seed culture was prepared by growing a loopful of slant culture in 30 mL culture medium containing glucose (10.0 g/L), ammonium nitrate (2.0 g/L), KH_2PO_4 (0.8 g/L), K_2HPO_4 (0.2 g/L), $MgSO_4.7H_2O$ (0.5 g/L), yeast extract (2.0 g/L) with pH adjusted to 6.0. The culture was incubated at 30 °C for 72 h at 160 rpm in a shaking incubator (Stuart, UK). The 3 day old seed culture was used as inoculum for the production medium. Seed inoculum of 1.25 mL (constituting 2.5% v/v) was transferred into a 50 mL corn cob based (CCB) medium which comprised corn cob (10 g/L), ammonium nitrate (2.0g/L), KH_2PO_4 (0.8 g/L), K_2HPO_4 (0.2 g/L), $MgSO_4.7H_2O$ (0.5 g/L), $CuSO_4.5H_2O$ (0.25g /L), yeast extract (2.0 g/L) and $MnSO_4.7H_2O$ at pH 6.0. This was incubated at 30 °C for 144 h at

160 rpm. At the end of the period, the fermented broth was filtered and the total biomass consisting of fermented substrate and mycelia was measured using Whatman filter paper to evaluate the degradation of corn cob in the medium. The filtrate was thereafter centrifuged at 4 ^{o}C and 6,000 rpm for 15 min. Glucose and xylose concentrations in culture supernatants were determined using the method of Miller (1959). The supernatant obtained was used to assay for the activities of lignocellulolytic enzymes (cellulase, xylanase, laccase and total peroxidase) following standard assay procedures. The degradation efficiency was calculated as earlier reported (Ogunyewo and Olajuyigbe, 2016). Statistical analysis was done using SPSS version 16.0 (SPSS Institute Inc., Cary, NC).

Determination of glucose and xylose liberated during degradation of lignocellulosic biomass

The concentration of glucose liberated in the fermentation medium was determined spectrophotometrically at the end of the 144 h period using the culture supernatant samples according to the method described by Miller (1959). The reaction mixture constituted 300 μL of the supernatant and 700 μL of dinitrosalicylic acid (DNSA) solution, which was boiled at 100 °C for 5 min. This reaction mixture was cooled under water and absorbance was taken at 575 nm. The amount of glucose liberated during the biodegradation period was estimated using glucose standard curve. Xylose concentration in culture supernatant samples was determined using D-xylose assay kit (Megazyme) according to the manufacturer's protocols.

Assays for hydrolytic enzymes

Determination of cellulase activity

Cellulase activity was determined using the method described by Wood and Bhat (1998) with some modifications. One hundred and fifty microliter (150 µL) of enzyme extract was added to 450 µL of 1% (w/v) carboxylmethyl cellulose (CMC) in 50 mM sodium acetate buffer (pH 4.8) in an Eppendorf tube and incubated at 40 °C for 20 min. The reaction was terminated with the addition of 400 µL of dinitrosalicylic acid (DNSA) and boiled at 100 °C for 5 min. The absorbance was recorded at 575 nm against blank. One unit of CMCase activity was expressed as 1 µmole of glucose liberated per minute under standard assay conditions.

Determination of xylanase activity

Xylanase activity was determined according to the method of Saha (2002) with slight modification. The reaction mixture comprised 400 µL of 1% (w/v) solution of birch wood xylan in 50 mM Tris-HCl buffer pH 9.0 incubated with 100 µL of culture supernatant for 15 min at 40 °C. The released reducing sugar was assayed using the DNSA method (Miller, 1959). One unit of xylanase activity was defined as the amount of the enzyme that liberated 1 µmol of xylose equivalent per minute under the standard assay conditions.

Assays for oxidative enzymes

Determination of laccase activity

Laccase activity was determined according to a modified method of Bourbonnais and Paice (1990). This was done by monitoring spectrophotometrically the change in absorbance at 420 nm (A_{420}) related to the rate of oxidation of 1 mM 2,2'-azino-bis-[3-ethylbenzthiazoline-6-sulfonate] (ABTS) in 50 mM sodium acetate buffer (pH 3.8). Assays were performed in 1 mL cuvettes at room temperature with 750 µl ABTS and 250 µl of enzyme extract. One unit of laccase activity was defined as the amount of enzyme that leads to the oxidation of 1 µmol of ABTS per minute with a molar extinction for the ABTS radical cation (the reaction product) of $\varepsilon_{420\ nm} = 36000\ M^{-1}\ cm^{-1}$.

Determination of peroxidase activity

Total peroxidase activity was assayed according to the method of Hunter *et al*. (2003). Peroxidase activity was determined via oxidation of 0.24 mM 2,2'azino-di-[3~ethyl benzothiazoline-6-sulphonic acid] (ABTS) buffered with 50 mM sodium acetate buffer pH 5.0 in the presence of 5 mM H_2O_2 at 414 nm for 5 min in a UV/Visible spectrophotometer (Unico). The reaction mixture (750 µl) contained equal volume of ABTS, culture supernatant and H_2O_2. One unit (U) of peroxidase activity was defined as the amount of enzyme oxidizing 1 µmol ABTS per minute at pH 5.0 and 25 ^{o}C with a molar extinction coefficient for the ABTS radical cation (the reaction product) of $\varepsilon_{414nm} = 31100\ M^{-1}\ cm^{-1}$.

Determination of protein concentration and specific activity of enzyme

Protein concentration was determined by the method of Bradford (1976) using bovine serum

albumin (BSA) as standard. Diluted dye reagent (200 µL) was added to 10 µL of sample solution. The mixture was incubated at room temperature for 15 min to allow proper colour development. The absorbance was measured at 595 nm against blank. The specific activity of enzyme was expressed as U/mg protein.

Effect of polar and non-polar amino acids on degradation of corn cob and production of lignocellulolytic enzymes by *S. carnis* CPF-05

The effect of polar and non-polar amino acids on degradation of corn cob and production of lignocellulolytic enzymes by *S. carnis* CPF-05 was evaluated by supplementing the CCB medium with some amino acids which included tryptophan, valine, leucine, isoleucine, tyrosine, glutamic acid and aspartic acid (0.3% w/v). CCB medium without any supplement served as control. Each CCB medium containing 2.5% inoculum of *S. carnis* CPF-05 was incubated at 160 rpm for 144 h at 30 ^{o}C. At the end of 144 h, the fermented broths were filtered. The total biomass consisting of fermented substrate and mycelia, was measured using Whatman filter paper to evaluate the degradation of corn cob in the medium. The degradation efficiency was calculated as earlier reported (Ogunyewo and Olajuyigbe, 2016). The filtrate was centrifuged at 4 ^{o}C and 6,000 rpm for 15 min. Glucose and xylose concentrations in culture supernatants were determined using the method of Miller (1959). The supernatants were used to assay for activities of lignocellulolytic enzymes in the presence and absence of amino acid supplements following standard assay procedures earlier described. All experiments were done in triplicates.

Effect of metal ions on degradation of corn cob and production of lignocellulolytic enzymes by *S. carnis* CPF-05

Effect of some metal ions on degradation of corn cob and production of lignocellulolytic enzymes by *S. carnis* CPF-05 was evaluated by supplementing the CCB medium with some divalent metal ions (Zn^{2+}, Fe^{2+}, Mg^{2+}, Mn^{2+} and Cu^{2+}) using their respective chlorides at 0.3% w/v. CCB medium without any metal ion was used as control. Each CCB medium containing 2.5% inoculum of *S. carnis* CPF-05 was incubated at 160 rpm for 144 h at 30 ^{o}C. At the end of 144 h, the degradation efficiency was evaluated as earlier described. The fermentation broths were centrifuged and the supernatants were used to assay for the activities of lignocellulolytic enzymes in the presence and absence of the metal ions according to standard assay procedures earlier described. The experiments were done in triplicates.

RESULTS AND DISCUSSION

Degradation of corn cob, liberation of fermentable sugars and production of hydrolytic and oxidative enzymes by *S. carnis* CPF-05 under basal conditions

Degradation studies showed that 71% of corn cob was degraded by *S. carnis* CPF-05 at the end of 144 h of fermentation. Yields of glucose and xylose from biodegradation of lignocellulosic substrate were 16.11 µmol/mL and 12.27 µmol/mL, respectively under basal conditions (Table 1). Hydrolytic enzymes were produced in higher yield than oxidative enzymes. The yields of cellulase and xylanase were 214.66 U/mL and 184.08 U/mL, respectively while the yields of laccase and peroxidase were 98.06 and 178.75 U/mL, respectively (Table 2). These values served as controls for yields obtained from fermentation media supplemented with amino acids and metal ions.

Effect of polar amino acids on degradation of corn cob, production and interaction of lignocellulolytic enzymes by *S. carnis* CPF-05

Results showed that the degradation efficiency increased with inclusion of polar amino acids as supplement in the fermentation medium when compared with control (Fig. 1). Glutamic acid supported the highest degradation with relative degradation efficiency of 129.7% followed by aspartic acid (124%). Similarly, tyrosine favoured the degradation process with relative degradation efficiency of 116% (Fig. 1). It is very striking that increase in yield of glucose and xylose in the presence of polar amino acids was almost 3 fold compared with control (Table 1). Most fungi while growing possess adequate machineries to synthesize all amino acids required for protein synthesis which could enhance metabolic processes. However, supplementation of culture medium with some amino acids promotes rapid growth of fungi (Guedes *et al.*, 2011) which was demonstrated in improved biodegradation of corn cob in the presence of polar amino acids. Fig. 1 shows that polarity of culture medium enhanced the

degradation process and production of both hydrolytic and oxidative enzymes (Fig. 1).

Table 1: Concentration of glucose and xylose liberated during the degradation of corn cob by *S. carnis* CPF-05 in the absence and presence of supplements

Supplements	Glucose concentration (µmol/mL)	Xylose concentration (µmol/mL)
Amino acids		
Control	16.11 ± 0.07^{e}	12.27 ± 0.60^{c}
Leucine	19.66 ± 0.12^{d}	11.78 ± 0.27^{d}
Tryptophan	14.82 ± 0.39^{f}	9.57 ± 0.12^{e}
Valine	14.18 ± 0.53^{g}	9.33 ± 0.08^{e}
Isoleucine	16.76 ± 0.65^{d}	11.41 ± 0.45^{d}
Tyrosine	45.27 ± 0.52^{b}	28.84 ± 0.49^{b}
Aspartic acid	38.32 ± 0.50^{c}	28.67 ± 0.26^{b}
Glutamic acid	48.01 ± 0.28^{a}	29.29 ± 0.29^{a}
Metal ions		
Control	16.11 ± 0.07^{d}	12.27 ± 0.60^{d}
Zn^{2+}	15.40 ± 0.59^{e}	11.56 ± 0.14^{e}
Mg^{2+}	22.55 ± 0.30^{c}	19.88 ± 0.50^{c}
Fe^{2+}	15.95 ± 0.30^{e}	9.45 ± 0.15^{f}
Mn^{2+}	54.13 ± 0.19^{a}	42.83 ± 1.50^{a}
Cu^{2+}	47.39 ± 0.49^{b}	38.39 ± 0.84^{b}

*Values represent mean ± standard deviations of triplicate determinations. Means with different superscript are significantly different ($P < 0.05$) across the column while means with same superscript are not significantly different ($P < 0.05$) across the column. Data were evaluated by one way ANOVA and Duncan's multiple range test with SPSS version 16.0 (SPSS Institute Inc., Cary, NC)

Table 2: Production of lignocellulolytic enzymes by *S. carnis* CPF-05 at 144 h fermentation period under basal conditions

Lignocellulolytic enzymes	*Enzyme activity (U/mL)	Protein concentration (mg/mL)	Specific activity (U/mg protein)
Cellulase	241.664 ± 0.219^{a}	0.236 ± 0.116^{a}	1024 ± 0.171^{a}
Xylanase	184.08 ± 0.173^{b}	0.236 ± 0.116^{a}	780 ± 0.241^{b}
Laccase	98.058 ± 0.119^{d}	0.236 ± 0.116^{a}	415.5 ± 0.116^{c}
Peroxidase	178.7464 ± 0.284^{c}	0.236 ± 0.116^{a}	757.4 ± 0.209^{d}

*Enzyme activity was used as a measure of enzyme production

Values represent mean ± standard deviations of triplicate determinations. Means with different superscript are significantly different ($P < 0.05$) across the column. Data were evaluated by one way ANOVA and Duncan's multiple range test with SPSS version 16.0 (SPSS Institute Inc., Cary, NC)

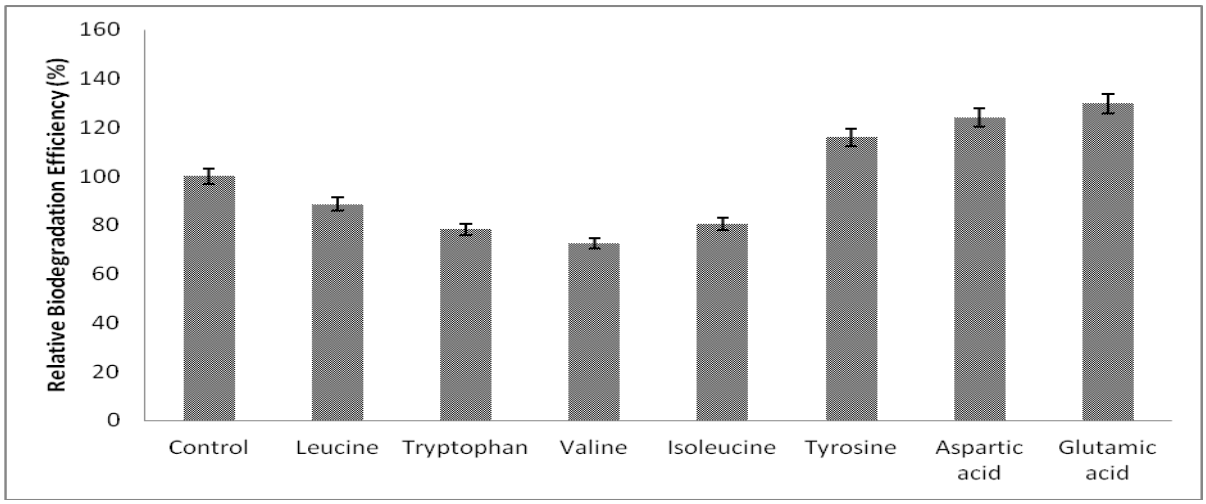

Figure 1: Effect of amino acid supplements on degradation of corn cob by *S. carnis* CPF-05 (Error bars represent mean values and standard deviation of triplicate determination)

Remarkably, yields of the hydrolytic enzymes were higher than the yields of the oxidative enzymes in culture media supplemented with glutamic acid. A synergistic effect was observed in production of all lignocellulolytic enzymes in the medium supplemented with glutamic acid (Fig. 2). The results showed that glutamic acid enhanced the production of cellulase and xylanase with relative activities of 298% (790.15 U/mL) and 239% (439.4 U/mL), respectively. This enhancement could be responsible for the simultaneous increase in activity of the oxidative enzymes, laccase (439.4 U/mL) and peroxidase (417.56 U/mL) corresponding to relative activities of 259% and 233.6%, respectively compared with control. Increased enzyme production favoured the biodegradation process of lignocellulosic biomass with higher relative degradation efficiency of 129.7%. Enzyme production by white-rot fungi has been reported to be strongly affected by the presence of amino acids in culture media (Viswanath *et al*., 2014**)**. It could be inferred that the rapid increase observed in the degradation efficiency with glutamic acid as supplement, over control is linked to the generation of many open ends through cellulose and xylan hydrolysis. The hydrolysis enhanced accessibility to the recalcitrant lignin component of the corn cob that triggered synthesis of the oxidative enzymes by *S. carnis* CPF-05.

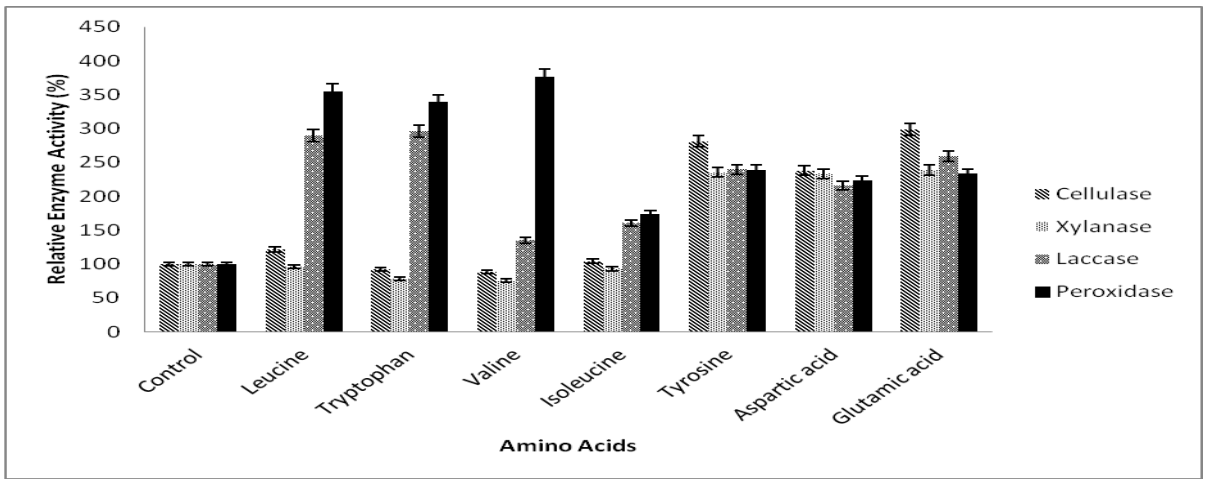

Figure 2: Effect of amino acid supplements on production of lignocellulolytic enzymes by *S. carnis* CPF-05 during degradation of corn cob (Error bars represent mean values and standard deviation of triplicate determination)

Similarly, another synergistic effect was found in the fermentation media supplemented with aspartic acid and tyrosine. The supplementation caused a simultaneous increase in the production of the hydrolytic and oxidative enzymes in the media. It was observed that the synergy helped to improve the degradation efficiency (Fig. 1). Relative activities of 237.8% and 233.6% for cellulase (574.67 U/mL) and xylanase (430.01 U/mL), respectively were obtained in the medium supplemented with aspartic acid (Fig. 2). Enhanced production of the hydrolytic enzymes obtained with this supplement had a stimulatory effect on the production of the oxidative enzymes. This suggests that the mechanism of action of the oxidative enzymes in the degradation of lignocellulose depends more on the action of the hydrolytic enzymes (Ogunyewo and Olajuyigbe, 2016). Relative activities of the oxidative enzymes obtained with aspartic acid as supplement were 215.4% and 222.9% for laccase (211.22 U/mL) and peroxidase (398.43 U/mL), respectively compared with control (Fig. 2). In same manner, the yield of hydrolytic enzymes in the culture medium supplemented with tyrosine was higher than the yield of oxidative enzymes. Relative activities of 281% and 235% were recorded for cellulase (679.06 U/mL) and xylanase (432.59 U/mL), respectively under this condition with relative degradation efficiency of 116%.

Tyrosine supported about 239% increase in production of the oxidative enzymes over control. It was observed that yield of the oxidative enzymes were 234.85 U/mL and 426.5 U/mL for laccase and peroxidase, respectively in the presence of tyrosine as supplement. These indicate that polar amino acids had substantial effect on the production of both hydrolytic and oxidative enzymes responsible for degradation of lignocellulose. In addition, polar amino acids have been found as essential constituents that must be included in culture medium towards achieving optimum degradation of lignocellulose by fungi. Increase in activities of the hydrolytic and oxidative enzymes by *S. carnis* in the presence of amino acids as supplements indicates that the fungus had suitable amino acid permeases which allowed uptake of amino acids required for protein synthesis in the cell (Wiebe *et al.,* 2001). Some earlier studies have shown that supplementation of fermentation medium with certain amino acids enhance enzyme production by complementing the bioavailability of nitrogen source in the fermentation medium for fungal growth (Dong *et al.,* 2005; Elisashvili *et al.,* 2010).

Glutamine has been reported to stimulate an increase in production of oxidative enzymes by the fungus *Penicillium martensii* NRC (Elshafei *et al.,* 2012). Similarly, asparagine and glutamic acid enhanced the production of hydrolytic enzymes from *Trichoderma reesei* while methionine inhibited synthesis of the enzymes (Cristica *et al.,* 2012).

Effect of non-polar amino acids on degradation of corn cob, production and interaction of lignocellulolytic enzymes by *S. carnis* CPF-05

It is very surprising that results showing effects of non-polar amino acids on the degradation of corn cob by *S. carnis* CPF-05 revealed a decline in the degradation efficiency over control. Decrease in degradation efficiency between 11.4% and 31.6% were obtained in culture media supplemented with the non-polar amino acids studied (Fig. 1).There was a decline in yield of xylose in the culture supernatants of media supplemented with the non-polar amino acids (leucine, tryptophan, valine and isoleucine) under study relative to control (Table 1). Similar trend was recorded for yield of glucose in the presence of all the non-polar amino acids except leucine which improved yield of glucose by 1.25 fold (Table 1). In addition, tryptophan, valine, leucine and isoleucine inhibited the production of hydrolytic enzymes. It is quite interesting that the non-polar amino acids enhanced production of oxidative enzymes (Fig. 2).

Relative activities of the hydrolytic enzymes obtained in the presence of tryptophan were 92% and 78%, for cellulase (222.33 U/mL) and xylanase (143.58 U/mL), respectively. The low degradation efficiency of 78.3% obtained in the medium supplemented with tryptophan could be attributed to the 8% and 22% decrease in the yield of cellulase and xylanase which play initial role in the degradation of lignocellulose obtained under this condition. Remarkably, higher yield of oxidative enzymes was obtained with tryptophan as supplement. About 296% and 339% increase in relative activities of laccase (290.26 U/mL) and peroxidase (605.96 U/mL), respectively were obtained. Increase in laccase and peroxidase activities obtained in this study with tryptophan as supplement is contrary to previous report in which tryptophan was reported to repress laccase production by

Penicillium martensii (Elshafei *et al.*, 2012). The increase in laccase and peroxidase production by *S. carnis* CPF-05 cultivated on medium supplemented with tryptophan could be related to the impressive enzyme producing ability of *S. carnis* as previously reported (Olajuyigbe and Ogunyewo, 2016a).

Decrease of 11.4%, 27.5% and 19.6% in degradation efficiency was recorded in the presence of leucine, valine and isoleucine, respectively. About 12% and 24% decrease in production of cellulase and xylanase were recorded in culture medium supplemented with valine. Under the condition of using valine as supplement, cellulase and xylanase yields were 212.66 U/mL and 139.90%, respectively. Similarly, about 4% and 7% reduction in production of xylanase was observed in the media supplemented with leucine and isoleucine, respectively. The slight inhibition in activity of xylanase could be responsible for the decline in the degradation efficiencies of 88.6% and 80.4% obtained with leucine and isoleucine as supplements (Fig. 1). It could be inferred that in the degradation of lignocellulose the hydrolytic enzymes must act cooperatively to drive the degradation process.

Results on production of oxidative enzymes by *S. carnis* CPF-05 in media supplemented with valine, leucine and isoleucine showed that the yield of peroxidase was higher than the yield of laccase. Relative peroxidase activities of 376%, 355% and 173.7% were obtained with valine, leucine and isoleucine, respectively as supplements. Relative activities of laccase obtained with valine, leucine and isoleucine as supplements were 135%, 290% and 160.3%, respectively. Addition of some non-polar amino acids has been reported to enhance the production of laccase by *Cyathus bulleri* while maximum production of extracellular peroxidase (55 U/mL) was obtained when alanine was used as supplement by *Lentinus kauffmanii* (Dhawn and Kuhad, 2002; Johnsy and Kaviyarasan, 2011). Higher yield of peroxidase over laccase obtained in this study suggest that peroxidases play more prominent role in the degradation of lignin present in lignocellulose than laccase. This might be an indication that laccase is a secondary enzyme required in the degradation of lignocellulosic biomass.

Although, the yields of the oxidative enzymes were relatively higher than the yield of hydrolytic enzymes when non-polar amino acids were used as supplements, the degradation process was affected adversely. The decline in degradation efficiencies with non-polar amino acids could be attributed to the low yield of hydrolytic enzymes in the presence of the supplements. These results therefore suggest that the hydrolytic enzymes drive the cooperative enzymatic interaction responsible for the degradation process.

Effect of metal ions on degradation of corn cob, production and interaction of lignocellulolytic enzymes by *S. carnis* CPF-05

Degradation efficiencies obtained in fermentation media supplemented with Mn^{2+} and Cu^{2+} were 131.8% and 122%, respectively (Fig. 3). These two metal ions also improved yield of glucose and xylose compared with control with about 3.5 fold increase. Mg^{2+} enhanced glucose and xylose yields with 1.5 fold increase over control (Table 1). Lower yields of glucose and xylose were obtained in the presence of Zn^{2+} and Fe^{2+} as supplements for bioconversion. In addition, Mn^{2+} and Cu^{2+}supported higher production of both hydrolytic and oxidative enzymes. Enhanced production of cellulase and xylanase triggered a corresponding increase in production of laccase and peroxidase. Relative enzyme yields in the presence of Mn^{2+} were 336%, 349%, 237% and 266%, for cellulase (811.98 U/mL), xylanase (642.44 U/mL), laccase (232.4 U/mL) and peroxidase (475.48 U/mL), respectively over control. Similarly, the relative enzyme yields obtained with Cu^{2+} as supplement were 294.2%, 312.8%, 226% and 257.4% for cellulase (710.96 U/mL), xylanase (575.8 U/mL), laccase (221.61 U/mL) and peroxidase (460.1 U/mL), respectively over control (Fig. 4).

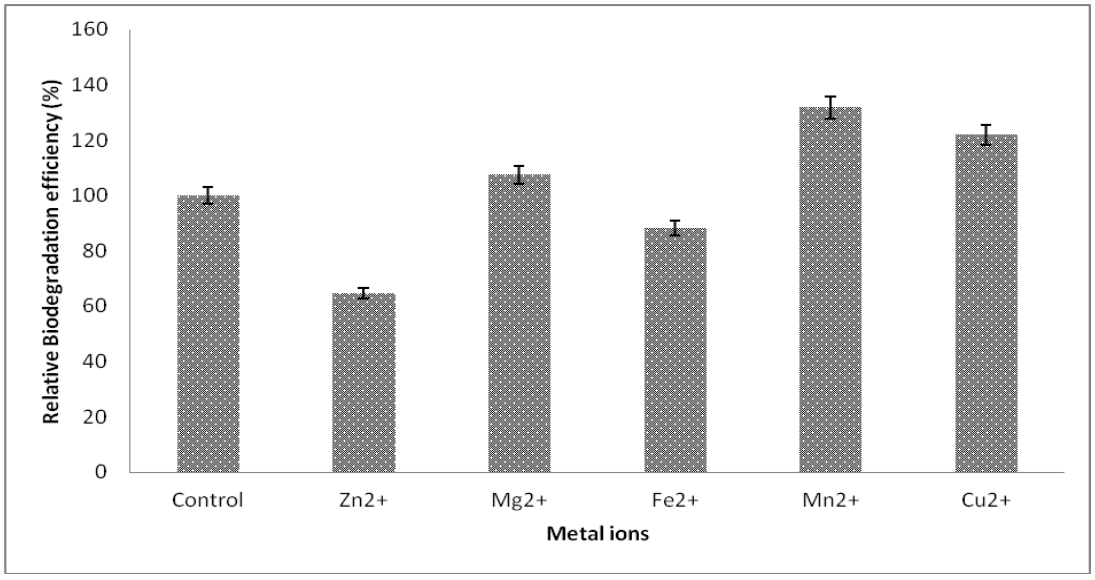

Figure 3: Effect of metal ion supplements on degradation of corn cob by *S. carnis* CPF-05. (Error bars represent mean values and standard deviation of triplicate determination)

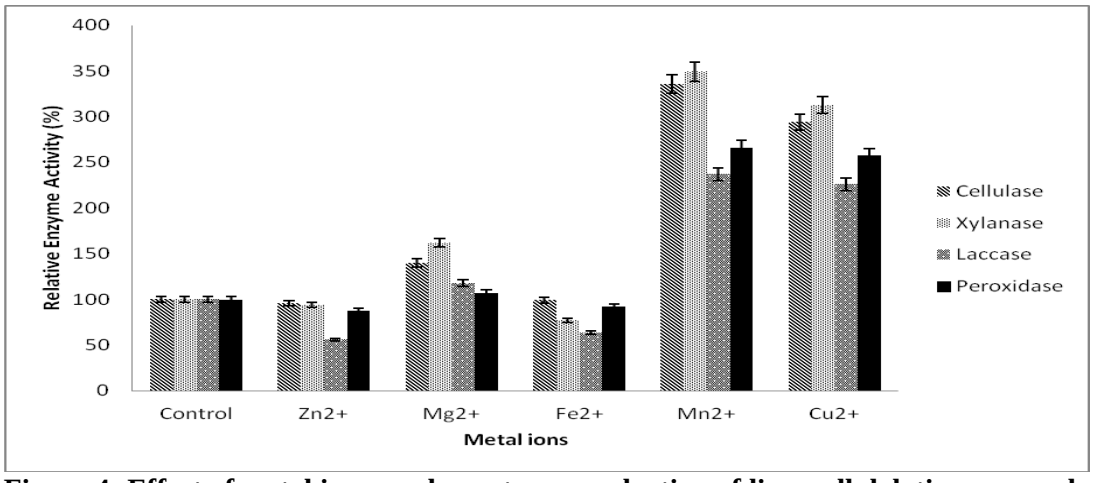

Figure 4: Effect of metal ion supplements on production of lignocellulolytic enzymes by *S. carnis* CPF-05 during degradation of corn cob (Enzyme activity was used as a measure of enzyme production; error bars represent mean values and standard deviation of triplicate determination)

Mg^{2+}enhanced the production of cellulase and xylanase with yields of 338.3 U/mL and 298.2 U/mL, respectively. The 140% and 162% increase in cellulase and xylanase production stimulated increase in production of laccase (115.71 U/mL) and peroxidase (191.13 U/mL). However, the degradation efficiency was not as high as that obtained with Mn^{2+} and Cu^{2+}. There was only about 7% increase in degradation efficiency over control (Fig. 4). Different strains and species of Basidiomycetes have been reported to differ in their sensitivity towards metals during their growth on lignocellulosic substrates (Satthiya *et al.*, 2007). Enhanced production of the hydrolytic and oxidative enzymes from *S. carnis* in the presence $Mn^{2+,}$ Mg^{2+} and Cu^{2+}as supplements helped in the improvement of the biodegradation process. Mg^{2+} and Mn^{2+} have previously been reported to enhance cellulase and xylanase production by *Aspergillus niger* NS-2 (Bansal *et al.*, 2012) while production of extracellular peroxidase by the white rot fungus strain L-25 was enhanced in the presence of Mn^{2+} as supplement (Fujihara *et al.*, 2010).

Decrease of 11.7% and 35.32% in degradation efficiencies was obtained in media supplemented with $Fe^{2+}$and Zn^{2+}, respectively when compared with control (Fig. 3). The decline in degradation efficiencies obtained with these metal ions could be attributed to low production level of both hydrolytic and oxidative

enzymes by *S. carnis* CPF-05 when $Fe^{2+}$and Zn^{2+} were used as supplements (Fig. 4). Cellulase and xylanase yield in the presence of Fe^{2+} as supplement were 239.24 U/mL and 141.74 U/mL, respectively. The 23% decrease in the production of xylanase by *S. carnis* CPF-05 with this supplement could be responsible for the slight decrease in cellulase production by *S. carnis* CPF-05 under this condition. Similarly, the yields of cellulase and xylanase when Zn^{2+} was used as supplement were 231.03 U/mL and 173.40 U/mL, respectively.

Production of oxidative enzymes by *S. carnis* CPF-05 in the presence of Fe^{2+} and Zn^{2+} as supplements revealed that the yield of peroxidase was higher than the yield of laccase. Peroxidase yields with Fe^{2+} and Zn^{2+} as supplements were 164.45 U/mL and 157.3 U/mL, respectively which were 8% and 12% lower than the control, respectively. About 36% and 44.1% decrease in laccase production was recorded in Fe^{2+} (62.76 U/mL) and Zn^{2+} (54.82 U/mL) supplemented media, respectively compared with control. These results demonstrated that inhibition of enzyme production by *S. carnis* CPF-05 with Fe^{2+} and Zn^{2+} as supplements resulted in low degradation efficiency. Low yield of oxidative enzymes obtained in this study with Fe^{2+} and Zn^{2+} is consistent with some previous reports in which synthesis of laccase was inhibited by Fe^{2+}, Zn^{2+} and Ag^{+} (Couto *et al.*, 2005; Selim *et al.*, 2013; Zavarzina *et al.*, 2004). It was suggested that these ions may have interrupted the electron transport system of laccase and substrate conversion which consequently affected the degradation process adversely (Kim and Nicell, 2006). Findings in this study clearly indicate that metal ions play important role in the biodegradation process of lignocellulose.

CONCLUSION

This study has provided deeper insights into the roles of amino acids and metal ions in degradation of lignocellulose by *S. carnis* CPF-05. Biodegradation of corn cob was greatly enhanced by supplementing the fermentation medium with polar amino acids, $Mn^{2+}$and Cu^{2+}. It was further revealed that hydrolytic enzymes drive the cooperative enzymatic interaction required for biodegradation of lignocellulosic biomass.

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