Production of cellulase and xylanase using food waste by solid-state fermentation

Mengmeng Tian[a], Alvan Wai[b], Tuhin K Guha[b], Georg Hausner[b], Qiuyan Yuan[a,*]

[a]Department of Civil Engineering, University of Manitoba, Winnipeg, MB, R3T 5V6, Canada

[b]Department of Microbiology, University of Manitoba, Winnipeg, MB, R3T 2N2, Canada

[*]Corresponding author

E-mail address: Qiuyan.Yuan@umanitoba.ca (Q. Yuan)

Tel: 2044748604

Fax: 2044747513

ABSTRACT

Fifteen fungal strains were compared with regards to their ability to produce cellulase and xylanase from food waste by solid-state fermentation (SSF). The fungi were isolated from six different types of composts and they were identified based on rDNA internal transcribed spacer sequence data. The Congo red test was performed for the preliminary screening of fungi for cellulase and xylanase production. After the initial screening, the fungi that showed cellulase and xylanase producing ability were further tested on the enzymatic activities in food waste through solid-state fermentation. The effects of different parameters including moisture content of the substrate, incubation temperature, inoculum level, and incubation period on cellulase and xylanase production were also evaluated. Preliminary results indicated that all the fungi, except for strain F1-20-35A, had cellulase and xylanase production activities. During SSF process, the strain F2-20-44A showed the highest level of extracellular cellulase and xylanase activities, which is 17.37 ± 3.76 U/g ds and 189.24 ± 2.96 U/g ds, respectively. Moreover, treatment with the strain at normal moisture content (77.67%), 0.5 mL inoculum level at 25 ℃ incubation temperature for 6 days were the most efficient conditions for cellulase and xylanase production (28.81 ± 0.67 U/g ds and 213.47 ± 10.66 U/g ds, respectively), which was later identified within the Genus *Aspergillus*. This study demonstrated that strain *Aspergillus niger* can be used potentially for enzyme production and proposes a new and economical method to produce high value enzymes with food waste by SSF, which could potentially alleviate environmental issues caused by food waste.

Keywords: Food waste; Cellulase; Xylanase; Solid-state fermentation; Optimization

1. Introduction

Food waste means the discard or loss of foodstuff, which mainly contained unsold food, food processing leftovers and uneaten food from residences and commercial establishments such as restaurants and supermarkets [1]. Food waste is produced in every process of the food supply chains. Figure 1 shows the percentage of waste created throughout the food supply chain in Canada [2]. Organic materials account for approximately 40% of the municipal solid waste in Canada [3]. In Canada, more than $30 billion of food waste is thrown out every year and almost four-fifth of that food waste is perfectly edible [2]. Similarly, the single largest composition of municipal solid waste is food wasted in the US [4]. Only in retail and consumer levels, Americans threw away approximately $161.6 billion dollars of food in 2010 [5]. With the rapidly increasing urban populations and economic growth combined with a swiftly expanding catering industry [6], alternative food waste disposal technologies have become a major concern in recent years. Food waste is a global environmental challenge for the waste management, as it has high moisture content (around 80%) and is difficult to handle. Currently, landfilling with other municipal solid wastes is the predominant method for food waste disposal in North America. However, this approach is facing more and more regulations and environmental pressures.

Solid-state fermentation (SSF) provides an extraordinary way to separate organic waste from municipal solid wastes and thereby reducing the amount of waste sent to the landfills. SSF is a technology that produces biomolecules from microorganisms in solid substrates without free-flowing liquid [7]. The nutrient-rich organic waste could be potentially used as a substrate in SSF for generating value-added biomolecules, such as enzymes [8,9]. Lignocellulolytic enzymes such as cellulase and xylanase have many applications in various industries. Cellulase is an enzyme that can hydrolyze cellulose. It can be used for industrial food processing, such as in coffee, textile industry, laundry detergents, pulp and paper industry, and also in the pharmaceutical industry. Xylanase was used in animal feed initially and then more recently applied in food, textile, as well as in the paper industries. Tsai et al. [10] reported that it is feasible to use xylanase as feed additives for wheat-based diets or feed that requires high arabinoxylan.

Cellulase and xylanase are produced by fungi, bacteria, and actinomycetes, but the most common producers are fungi. The high cost of cellulase is mainly due to the substrates used in production, and also the slow growth rate of fungi. According to Polizeli et al. [11], cellulases, xylanase, and pectinase contributed towards one-fifth of the world enzyme market and most of cellulase and xylanase are produced through submerged culture. However, SSF has some advantages over the submerged fermentation, e.g. low energy consumption, high production rate, high production yield, and simpler extraction process [12,13]. Therefore, there is an increasing interest in using SSF to produce cellulase and xylanase.

Cellulase and xylanase have been produced from agro-industrial substrates, such as olive pomace, wheat bran, oil palm trunk, and corncob residue [14,15,16,17]; however, no research study has attempted their production using food waste as the substrate. Therefore, the objectives of this study were: 1) to evaluate the potential of locally isolated fungi strains for the production of extracellular cellulase and xylanase, 2) to investigate the feasibility of using food waste as the substrate to produce cellulase and xylanase during SSF, and 3) to optimize the parameters that influence the maximization of cellulase and xylanase production.

2. Materials and methods

2.1. Strain isolation and inoculum preparation

A total of 15 fungal strains were isolated from six different types of composts (including various combinations of plant debris, worm castings, and wood chips) based on methods described by Ottow [18] and Malloch [19]. Fungal strains were identified in part based on the morphological observations and by the utilization of rDNA internal transcribed spacer sequence data [20]. The fungi were kept in potato dextrose agar (39 g/L PDA) plates and maintained by periodic transfer to fresh media. They were stored at 4 ℃ until further experiments. In order to prepare inoculum, sporulating PDA plates were flooded with 10 mL of sterile distilled water and spores were collected by dislodging with inoculation needle. The spore suspensions with appropriate dilutions (1×10^7 spores/mL) were used as the inoculum.

2.2. Fungi identification

Nucleic acid extraction protocols and amplification protocols for the fungal rDNA ITS regions have been previously described in Hausner et al. [21] and Hausner and Wang [22], respectively. Whole cell DNA was used as the template for amplifying DNA fragments of interest using the OneTaq® DNA polymerase system (New England Biolabs, MA, USA). Primers SSUZ and LSU4 [23] were used to amplify the ITS regions. The PCR primer sequences, amplification conditions, sizes of the expected PCR products, and preparation of sequencing templates for fragments have been previously described [22]. Amplicons were prepared for sequencing with the aid of the Promega Wizard SV Gel and PCR clean-up system (Promega, Madison, WI). Purified PCR products were sequenced in both directions using cycle-sequencing protocols and automated Fluorescent DNA sequence analysis (The Manitoba Institute of Cell Biology DNA sequencing facility, Winnipeg, MB). The NCBI resources such as BLASTn combined with alignment and distance tree options were utilized to find matches for rDNA sequences obtained from strains analysed in this study.

2.3. Food waste preparation and characterization

The substrate was a simulated food waste that was obtained based on the typical United States diet as compiled from the USDA [24]. A predetermined amount of food waste was crushed by a blender. The resulting mixed paste was used as the substrate in SSF process. Total solids (TS), volatile solids (VS), and total Kjeldahl nitrogen (TKN) were analyzed using standard methods described by the American Public Health Association [25]. The carbon content of the food waste was determined by the percentage of volatile solids divided by 1.83 [26]. The oven-drying method was used for determination of the moisture content of the food waste. One g of food waste (dry weight) was mixed with 5 mL of distilled water and the suspension was shaken at 180 rpm at room temperature for 20 minutes. The pH of the mixture's supernatant was taken as the pH of the food waste. All measurements were conducted in triplicate. Table 1 shows the characteristics of the food waste together with standard deviations. TS and VS values are based on wet weight while total carbon and TKN are based on dry weight.

2.4. Preliminary screening of cellulase and xylanase production by Congo Red Test

The Congo red test served as a preliminary screen for cellulase and xylanase production. More specifically, the fungi were inoculated at the center of the agar plates, which contained Mandel Weber salts

medium and used carboxymethylcellulose (CMC) and xylan as the source of carbon for cellulase and xylanase producers, respectively [14]. The agar plates were incubated for 5 days at 25 ℃ to allow the fungi to secrete enzyme(s). After the incubation period, the agar plates were stained for 15 minutes with 10 mL aqueous solution of Congo red (0.1% w/v). The Congo red solution was then discarded and the plates were further rinsed by 10 mL of 1 M NaCl. The clear hydrolysis zone around the fungal colony indicated the production of cellulase and xylanase by the fungus. For each fungal strain, two replicates of the Congo red test were performed.

2.5. Solid-state fermentation

SSF process was conducted in the 500 mL Erlenmeyer flask and each flask contained 20 g of food waste (wet weight). The wet substrate in the flask was autoclaved at 121 ℃ for 40 minutes. Then 1.0 mL spore suspension of the fungus was inoculated to the substrate until the substrate cooled down to the room temperature. Subsequently, the flasks were incubated for 6 days at 25 ℃. The SSF process for every strain was conducted in triplicate.

2.6. Optimization of cellulase and xylanase production

The production of cellulase and xylanase was optimized by following 'one-variable-at-a-time' approach to study the effects of different factors on the growth of *Aspergillus niger*. The effect of various factors including incubation temperature (20, 25, 30, 35, and 40 ℃), incubation period (2, 4, 6, 8, and 10 days), initial moisture content (40%, 50%, 60%, 70%, and 80%), and inoculum level (0.5, 1.0, 1.5, 2.0, and 2.5 mL) were examined individually.

2.7. Crude cellulase and xylanase extraction and analytical methods

The crude enzymes were extracted as described by Tian and Yuan [36], mixing the fermented matter with 25 mL distilled water containing 2% $CaCl_2 \cdot 2H_2O$ on the rotary shaker and shaking at 200 rpm for 1 hour at room temperature. The mixture was then centrifuged at 4700 rpm for 0.5 hour at 4 ℃. The supernatant was used for enzyme analysis.

The activity of cellulase and xylanase was determined based on a standard procedure that measures the amount of reducing sugars released by crude enzymes through the dinitrosalicylic acid method as described by Miller [27]. In brief, cellulase was determined by incubating 0.9 mL 2% (w/v) CMC in sodium acetate buffer (50 mM, pH 4.7) with 0.1 mL suitably diluted crude enzyme at 50 ℃ for 40 minutes. Xylanase activity was determined under the same condition as cellulase; however, 2% (w/v) birchwood xylan was used as the reaction substrate. After incubation, the reaction was interrupted by DNS solution (1%). Then the tubes were placed in boiling water for 10 minutes and subsequently cooled for 5 minutes in the ice bath. The absorbance was measured spectrophotometrically (BioTek® Instruments, Winooski, USA) at 540 nm. The production of the reducing sugar was deduced from glucose and xylose standard curves. The standard curves were prepared by 5, 10, 15, 20 and 30 mmol/L of the glucose and xylose in acetate buffer, respectively. One unit of enzyme activity was defined as the amount of enzyme required to release 1 μmol of glucose reducing sugar equivalents per minute from 2% (w/v) CMC under 50 ℃ and pH 4.7. The activity of cellulase and xylanase activity was expressed in U per gram of dry substrate.

2.8. Statistical analysis

SPSS (Version 22.0, Armonk, NY, IBM Corp.) was used for statistical analysis and one-way ANOVA was applied for determining the significance of different conditions for the enzyme production. For the multiple comparisons, S-N-K test was used. Treatments were reported to have a significant influence on the result when the P value was less than 0.05 (95% confidence level).

3. Results and discussion

3.1. Fungi identification

Fungi originally isolated from compost materials were identified with the aid of molecular sequences derived from the rDNA ITS region. The ITS region was amplified and sequenced from fourteen isolates; for one isolate DNA could not be obtained that was suitable for PCR. Various primer combinations were applied to increase the chances of obtaining ITS amplicons (Table 2). The ITS sequences were used as queries in BLASTn to find similar or identical sequences in the database. Based on sequences extracted from GenBank, isolates were assigned to genera and in some cases to species level (Table 2). The collection of 15 strains

based on cultural and molecular variations could be assigned to a range of different families belonging to either the Ascomycota (Genera: *Aspergillus*, *Penicillium*, *Podospora*, *Fusarium*, *Aureobasidium*, *Trichoderma*) or the Zygomycota (Genera: *Absidia*, *Lichtheimia*, *Umbelopsis*). In many instances, Genus level designation was possible but species designations in some cases were challenging; for example, different species of Trichoderma or Penicillium share identical ITS regions. This has been previously noted by Visagie et al. [20], Yilmza et al. [28] and Jaklitsch [29].

3.2. Preliminary screening of fungi for enzyme production by Congo red test

The preliminary screening was performed to observe the growth of fifteen fungal strains on agar plates that contained CMC or xylan as the carbon source. The fungi that produced visible clear zones due to hydrolysis in CMC or xylan plates indicate cellulase or xylanase activity, respectively. In addition, the bigger the ratio of the hydrolysis zone diameter to the colony diameter, the higher the expected cellulase or xylanase activity of the fungus. The results of the preliminary screen are depicted in Table 3. Among the fifteen fungal isolates, only one strain (F1-20-35A) did not show cellulase and xylanase activity after five days of incubation. Therefore, all the fungi except F1-20-35A were selected for SSF.

3.3. Screening for cellulase and xylanase producers by solid-state fermentation

The production of cellulase and xylanase in SSF is gaining interest since it is a cost-effective technology with low substrate cost and high enzyme yields [15, 30]. SSF was conducted to assess the enzyme productivity of the fungi that were selected from the preliminary screening and to test the feasibility of using food waste as the substrate for producing cellulase and xylanase. The cellulase and xylanase production from different fungal strains are presented in Table 4. The strain F2-20-44A showed the highest amount of extracellular cellulase and xylanase activities, which were 17.37 ± 3.76 U/g ds and 189.24 ± 2.96 U/g ds, respectively. The enzymatic activities in some strains were undetectable, which is probably because the enzyme activities produced from these strains were below the detection limit. Moreover, it was observed that xylanase activity was 10.9-fold higher than cellulase activity. The reason is that the structure of xylan is considered “weak” and can be easily hydrolyzed [16]. Bansal et al. [15] showed that individual kitchen waste such as carrot peelings, orange peelings, pineapple peelings, and potato peelings pre-treated with H_2SO_4 and

NaOH can be used as the substrate for cellulase production in SSF. In this SSF study, the results demonstrated that the strain F2-20-44A was the most efficient cellulase and xylanase producer on food waste. Therefore, F2-20-44A was selected for further optimization in SSF.

3.4. Optimization of cellulase and xylanase production in solid-state fermentation

Initial moisture content of the food waste was adjusted to 40%, 50%, 60%, 70%, and 80% with the oven-drying method or moistened with distilled water. Cellulase and xylanase activities obtained at different moisture content are shown in Fig. 2 and they are significantly ($P < 0.05$) affected by moisture content of the food waste. Generally speaking, the enzyme productivity increased dramatically as the moisture content increased. The activities of the cellulase were undetectable when the initial moisture content of the food waste was 50% and 60%. Similar cellulase activities (10.98 ± 3.63, 11.46 ± 3.52, and 11.33 ± 3.63 U/g ds) were observed at the other three moisture content treatments (40%, 70%, and 80%, respectively). For xylanase activities, the moisture content of 70% and 80% showed 160.94 ± 8.29 and 159.89 ± 7.62 U/g ds, respectively (Fig. 2b). As compared with high initial moisture content (70% and 80%), xylanase activities (6.76 ± 2.53, 3.43 ± 1.99 and 13.69 ± 7.65 U/g ds) decreased significantly when the initial moisture content was low (40%, 50%, and 60%). This reduction was mostly likely due to the decrease in the solubility of nutrients in the substrate [9, 31], which consequently affected the growth of the microbial agent and led to the poor production of the enzyme. Besides, moisture content can also interfere with the decomposition rate of the organic matter in the substrate [32] and therefore affect the enzymes productivity in SSF. The cellulase and xylanase activities observed from the moisture content of 70% and 80% were lower than the enzyme activities obtained at the normal moisture content in the previous step (17.37 ± 3.76 and 189.24 ± 2.96 U/g ds for cellulase and xylanase, respectively). This indicated that the normal moisture content (77.67%) of the food waste was the optimum condition for the enzyme production.

Incubation temperature is another important factor in SSF as it ultimately influences the growth of fungus, the formation and germination of the spore, and metabolic activities such as enzyme production [9]. The results for the effect of incubation temperature on cellulase and xylanase activity are shown in Fig. 3. Maximum cellulase production occurred at 30 ℃ with a yield of 13.86 ± 1.03 U/g ds, while cellulase yield at

20 ℃ was not detectable (Fig. 3a). According to the results of the multiple comparisons (S-N-K test), there were no significant differences for cellulase production at 25, 30, 35, and 40 ℃. In addition, low temperature means lower energy requirement in the SSF process, which is more economical. Therefore, 25 ℃ was the economical choice in subsequent experiments for cellulase production. For xylanase production, the activities (206.23 ± 4.95 U/g ds) obtained at 25 ℃ was significantly ($P < 0.05$) higher than the xylanase yield at other temperatures. The xylanase productivity decreased when the incubation temperature increased and the lowest xylanase activity (10.79 ± 4.09 U/g ds) was observed at 40 ℃. Bansal et al. [15] reported that high temperatures can alter the constituent of the cell membrane, stimulate protein catabolism, and induce cell death. Therefore, 25 ℃ was again selected as the incubation temperature for further experiments. The optimum temperature of 25 ℃ for cellulase and xylanase production is in agreement with other reports that showed the optimum temperature for SSF ranges from 25 to 30 ℃ [30,33].

Substrates were inoculated with different amounts of spore suspension (0.5, 1.0, 1.5, 2.0, and 2.5 mL) to investigate the effect of inoculum size on enzyme production in SSF. Figure 4 shows the cellulase and xylanase production with different inoculum levels. The highest cellulase activity (18.81 ± 11.40 U/g ds) was achieved at the inoculum level of 2 mL. Higher or lower inoculum levels resulted in a decrease in cellulase activity (Fig. 4a). In addition, this study showed that 1.5 mL inoculum level resulted in the highest xylanase production and xylanase production was gradually decreased with an increasing volume of the inoculum. The reason for this could be explained by the imbalanced ratio of the nutrients in the substrate to the amount of biomass [34]. The high amount of inoculum size means a large volume of initial biomass competing for nutrients and oxygen in the substrate. Therefore, low cellulase and xylanase production were obtained at high inoculum levels [35]. A one-way ANOVA analyses revealed no significant difference ($P > 0.05$) for the effect of inoculum level in the cellulase production or in the xylanase production. Thus, 0.5 mL inoculum size was used in further experiments. Tian and Yuan [36] also reported that inoculum level had no significantly influence on enzyme productivity in SSF. However, Bansal et al. [15] investigated the influence of different inoculum sizes for cellulase production on wheat bran by SSF and concluded that the optimum condition is the medium inoculum size.

The inoculated flasks were incubated for different time periods ranging from 2 to 10 days. Cellulase and xylanase activities in the substrate were tested at every second day. Cellulase and xylanase activities were significantly affected by fermentation period (Fig. 5). The results showed that SSF with 6 days and 10 days incubation significantly increased cellulase production. The maximum cellulase activity occurred after 10-day incubation with the cellulase yield of 36.07 ± 12.43 U/g ds and increases 3.64-fold as compared with 2-day incubation (9.92 ± 2.07 U/g ds) (Fig. 5a). However, the cellulase production obtained at 6 days and 10 days does not have a significant difference ($P > 0.05$), which indicated that longer fermentation time does not result in a significant improvement in cellulase production. Similarly, longer incubation periods resulted in poor xylanase activities (Fig. 5b) and 6 days incubation period showed the maximum amount of xylanase production (213.47 ± 10.66 U/g ds). For longer incubation periods, the xylanase production decreased at 8 days and 10 days, which were significantly ($P < 0.05$) lower than the xylanase yield at 6 days of incubation. The observation that enzyme yield decreased at longer incubation periods is in agreement with findings of Bansal et al. [15] and dos Santos et al. [37]. Besides, Mrudula and Murugammal [31] reported that cellulase activity increased steadily at the beginning but longer incubation period reduced the enzyme production after it reached maximum activity at 72 h of incubation. In general, a longer incubation period can result in a decrease in enzyme production due to the reduction of nutrients in the substrate, the release of proteases, and the drop in the pH in the substrate [15, 36].

4. Conclusions

This study highlighted a strain isolated from compost, *Aspergillus niger*, which could produce high-levels of cellulase and xylanase from food waste by SSF. Maximum cellulase and xylanase production (28.81 ± 0.67 and 213.47 ± 10.66 U/g ds, respectively) were achieved using normal moisture content (77.67%), 0.5 mL inoculum level at 25 ℃ for 6 days incubation. Therefore, food waste from municipal solid waste can be used as a potential substrate for cellulase and xylanase production. This technique could reduce the enzyme production cost by lowering the substrate cost in the enzyme industry and potentially alleviate environmental issues caused by food waste.

Acknowledgments

This work was financially supported by Green Manitoba (WRAPP 14-028) and Natural Sciences and Engineering Research Council of Canada (RGPIN-2014-05510).

Conflict of interest

The authors declare that there is no conflict of interest.

References

[1] Fisgativa, H., Tremier, A., Dabert, P.: Characterizing the variability of food waste quality: A need for efficient valorisation through anaerobic digestion. Waste Manage. 50, 264-274 (2016)

[2] Gooch, M., Felfel, A., Marenick, N.: Food waste in Canada: Opportunities to increase the competitiveness of Canada's agri-food sector, while simultaneously improving the environment. Value Chain Management Center (2010)

[3] Environment Canada, Technical document on municipal solid waste organics processing. Canada (2013) https://www.ec.gc.ca/gdd-mw/3E8CF6C7-F214-4BA2-A1A3-163978EE9D6E/13-047-ID-458-PDF_accessible_ANG_R2-reduced%20size.pdf

[4] US EPA, Municipal solid waste in the United States: 2007 facts and figures. Office of Solid Waste and Emergency Response, Washington, DC (2008) http://large.stanford.edu/publications/coal/references/docs/msw07-rpt.pdf

[5] Jean, C.B., Hodan, F.W., Jeffrey, H.: The estimated amount, value, and calories of postharvest food losses at the retail and consumer levels in the United States. United States Department of Agriculture (2014) https://www.ers.usda.gov/webdocs/publications/eib121/43680_eib121.pdf

[6] Li, Y., Jin, Y.: Effects of thermal pretreatment on acidification phase during two-phase batch anaerobic digestion of kitchen waste. Renewable Energy 77, 550-557 (2015)

[7] Pandey, A.: Recent process developments in solid-state fermentation. Process Biochem. 27(2), 109-117 (1992)

[8] Pandey, A., Soccol, C.R., Mitchell, D.: New developments in solid state fermentation: I-bioprocess-products. Process Biochem. 35(10), 1153-1169 (2000)

[9] Pandey, A.: Solid-state fermentation. Biochem. Eng. J. 13(2), 81-84 (2003)

[10] Tsai, T., Dove, C.R., Cline, P.M., Owusu-Asiedu, A., Walsh, M.C., Azain, M.: The effect of adding xylanase or β-glucanase to diets with corn distillers dried grains with solubles (CDDGS) on growth performance and nutrient digestibility in nursery pigs. Livestock Sci. 197, 46-52 (2017)

[11] Polizeli, M.L.T.M., Rizzatti, A.C.S., Monti, R., Terenzi, H.F., Jorge, J.A., Amorim, D.S.: Xylanases from fungi: properties and industrial applications. Appl. Microbiol. Biotechnol. 67(5), 577-591 (2005)

[12] Chen, H.: Modern Solid State Fermentation, Theory and Practice. Springer, Heidelberg (2013)

[13] Pandey, A., Selvakumar, P., Soccol, C.R., Nigam, P.: Solid-state fermentation for the production of industrial enzymes. Curr. Sci. 77, 149-152 (1999)

[14] Leite, P., Salgado, J.M., Venâncio, A., Domínguez, J.M., Belo, I.: Ultrasounds pretreatment of olive pomace to improve xylanase and cellulase production by solid-state fermentation. Bioresour. Technol. 214, 737-746 (2016)

[15] Bansal, N., Tewari, R., Soni, S.K.: Production of cellulases from Aspergillus niger NS-2 in solid state fermentation on agricultural and kitchen waste residues. Waste Manage. 32(7), 1341-1346 (2012)

[16] Ang, S.K., Shaza, E.M., Adibah, Y., Suraini, A.A., Madihah, M.S.: Production of cellulases and xylanase by Aspergillus fumigatus SK1 using untreated oil palm trunk through solid state fermentation. Process Biochem. 48(9), 1293-1302 (2013)

[17] Xia, L., Cen, P.: Cellulase production by solid state fermentation on lignocellulosic waste from the xylose industry. Process Biochem. 34(9), 909-912 (1999)

[18] Ottow, J.C.G.: Rose bengal as a selective aid in the isolation of fungi and actinomycetes from natural sources. Mycologia 64, 304-315 (1972)

[19] Malloch, D.: Moulds, their isolation, cultivation and identification. University of Toronto Press., Toronto, Canada (1981)

[20] Visagie, C.M., Houbraken, J., Frisvad, J.C., Hong, S.B., Klaassen, C.H.W., Perrone, G., Seifert, K.A., Varga, J., Yaguchi, T., Samson, R.A.: Identification and nomenclature of the genus Penicillium. Stud. Mycol. 78, 343-371 (2014)

[21] Hausner, G., Eyjólfsdóttir, G.G., Reid, J., Klassen, G.R.: Two additional species of the genus *Togninia*. Can. J. Bot. 70, 724-734 (1992)

[22] Hausner, G., Wang, X.: Unusual compact rDNA gene arrangements within some members of the Ascomycota: Evidence for molecular co-evolution between ITS1 and ITS2. Genome 48, 648-660 (2005)

[23] Hausner, G., Iranpour, M., Kim, J. -J., Breuil, C., Davis, C.N., Gibb, E.A., Reid, J., Loewen, P.C., Hopkin, A.A.: Fungi vectored by the introduced bark beetle *Tomicus piniperda* in Ontario, Canada, and comments on the taxonomy of *Leptographium lundbergii*, *Leptographium terebrantis*, *Leptographium truncatum*, and *Leptographium wingfieldii*. Can. J. Bot. 83, 1222-1237 (2005)

[24] Kim, M., Chowdhury, M.M.I., Nakhla, G., Keleman, M.: Characterization of typical household food wastes from disposers: fractionation of constituents and implications for resource recovery at wastewater treatment. Bioresour. Technol. 183, 61-69 (2015)

[25] APHA, Standard methods for the examination of water and wastewater. American Public Health Association, Washington (2005)

[26] Barrington, S., Choinière, D., Trigui, M., Knight, W.: SE-Structures and environment: compost airflow resistance. Biosystems Eng. 81(4), 433-441 (2002)

[27] Miller, G.L.: Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem. 31(3), 426-428 (1959)

[28] Yilmaz, N., Visagie, C.M., Houbraken, J., Frisvad, J.C., Samson, R.A.: Polyphasic taxonomy of the genus *Talaromyces*. Stud. Mycol. 78, 175-341 (2014)

[29] Jaklitsch, W.M.: European species of *Hypocrea* Part I. The green-spored species. Stud. Mycol. 63, 1-91 (2009)

[30] Singhania, R.R., Sukumaran, R.K., Pillai, A., Prema, P., Szakacs, G., Pandey, A.: Solid-state fermentation of lignocellulosic substrates for cellulase production by Trichoderma reesei NRRL 11460. Indian J. Biotechnol. 5, 332-336 (2006)

[31] Mrudula, S., Murugammal, R.: Production of cellulase by Aspergillus niger under submerged and solid state fermentation using coir waste as a substrate. Brazilian J. Microbiol. 42(3), 1119-1127 (2011)

[32] Gautam, P., Sabu, A., Pandey, A., Szakacs, G., Soccol, C.R.: Microbial production of extra-cellular phytase using polystyrene as inert solid support. Bioresour. Technol. 83(3), 229-233 (2002)

[33] Gautam, S.P., Bundela, P.S., Pandey, A.K., Khan, J., Awasthi, M.K., Sarsaiya, S.: Optimization for the production of cellulase enzyme from municipal solid waste residue by two novel cellulolytic fungi. Biotechnol. Res. Int. (2011). doi:10.4061/2011/810425

[34] Abdullah, J.J., Greetham, D., Pensupa, N., Tucker, G.A., Du, C.: Optimizing cellulase production from municipal solid waste (MSW) using solid state fermentation (SSF). Fundam. Renewable Energy Appl. 6(3), 206 (2016). doi:10.4172/2090- 4541.1000206

[35] Haq, I., Iqbal, S.H., Qadeen, M.A.: Production of xylanase and CMC cellulase by mold culture. Pak. J. Biotechnol. 4, 403-409 (1993)

[36] Tian, M., Yuan, Q.: Optimization of phytase production from potato waste using Aspergillus ficuum. 3 Biotech 6(2), 256 (2016)

[37] dos Santos, T.C., Gomes, D.P.P., Bonomo, R.C.F., Franco, M.: Optimisation of solid state fermentation of potato peel for the production of celluloytic enzymes. Food Chem. 133(4), 1299-1304 (2012)

352 **Figure legends**

353 **Fig.1** Percentage of waste created throughout the food supply chain in Canada [2]

354 **Fig. 2** Effect of moisture content by *A. niger* on the **a** cellulase production and **b** xylanase production; Letters
355 shared in common between or among the groups indicate no significant difference according to S-N-K test at
356 the significance level of 0.05; error bars are standard deviations of three replicates

357 **Fig. 3** Effect of incubation temperature *A. niger* on the **a** cellulase production and **b** xylanase production;
358 Letters shared in common between or among the groups indicate no significant difference according to S-N-K
359 test at the significance level of 0.05; error bars are standard deviations of three replicates

360 **Fig. 4** Effect of inoculum level by *A. niger* on the **a** cellulase production and **b** xylanase production; error bars
361 are standard deviations of three replicates

362 **Fig. 5 a** Effect of incubation period *A. niger* on the **a** cellulase production and **b** xylanase production; Letters
363 shared in common between or among the groups indicate no significant difference according to S-N-K test at
364 the significance level of 0.05; error bars are standard deviations of three replicates

365

366 **Table 1**

367 Characteristics of the food waste

Characteristics	Unit	Average value
pH	-	5.05 ± 0.02
Total solids	mg/g w.w.[a]	223.27 ± 0.01
Volatile solids	mg/g w.w.[a]	220.97 ± 0.01
Moisture content	%	77.67 ± 0.06
Total carbon	% d.w.[b]	52.12 ± 0.03
TKN	mg/g d.w.[b]	33.28 ± 5.50
C/N ratio	-	15.93 ± 2.44

368 [a] On wet weight basis; [b] On dry weight basis.

369

Table 2

Identification of various compost fungi used in this study

Strain number	SSU3/LSU2	ITS1/ITS4	ITS1/LSU2	Identity: Genus (Order)
F1-20-32B		√	√	*Penicillium* sp. (Eurotiales)[A]
F1-20-35A		√		*Absidia* sp. (Mucorales)[Z]
F1-37-40A	√			*Penicillium* sp. (Eurotiales)[A]
F2-20-43A	√			*Lichtheimia corymbifera* (Mucorales)[Z]
F2-20-44A	√			*Aspergillus niger* (Eurotiales)[A]
F2-37-31A	√			*Lichtheimia corymbifera* (Mucorales)[Z]
F3-37-58B*				Unknown Ascomycete
F3-37-60A	√			*Penicillium adametzii* (Eurotiales)[A]
F3-37-62A	√			*Aureobasidium* sp. (Dothedialeas)[A]
F4-20-52B	√	√		*Podospora* sp. (Sordariales)[A]
F4-20-53B	√			*Umbelopsis* sp. (Mucorales)[Z]
F5-20-10B	√			*Fusarium* sp. (Hypocreales)[A]
F5-20-12B	√	√	√	*Fusarium* sp. (Hypocreales)[A]
F6-37-1B	√			*Trichoderma* sp. (Hypocreales)[A]
F6-37-4B	√			*Trichoderma* sp. (Hypocreales)[A]

Primers used to amplify the ITS region: SSU3 = 5′- GTC GTA ACA AGG TCT CCG -3′ and LSU2 = 5′- GAT ATG CTT AAG TTC AGC G -3′;
ITS1 = 5′- TCC GTA GGT GAA CCT GCG G -3′ and ITS4 = 5′- TCC TCC GCT TAT TGA TAT GC -3′.
*Sample failed to yield DNA that could be used as a PCR substrate. √ Indicate successful amplification and DNA sequence determination. [A] = Genus belonging to the Ascomycota; [Z] = Genus belonging to the Zygomycota.

379 **Table 3**

380 Preliminary screening of fungi to verify the ability to produce extracellular cellulase and xylanase

Strain number	Cellulase	Xylanase	Fungi number	Cellulase	Xylanase	Fungi number	Cellulase	Xylanase
F1-20-32B	+	+	F2-37-31A	+	+	F4-20-53B	+	+
F1-20-35A	-	-	F3-37-58B	+	+	F5-20-10B	+	+
F1-37-40A	+	+	F3-37-60A	+	+	F5-20-12B	+	+
F2-20-43A	+	+	F3-37-62A	+	+	F6-37-1B	+	+
F2-20-44A	+	+	F4-20-52B	+	+	F6-37-4B	+	+

381 + Positive result; - negative result.

382

383 **Table 4**

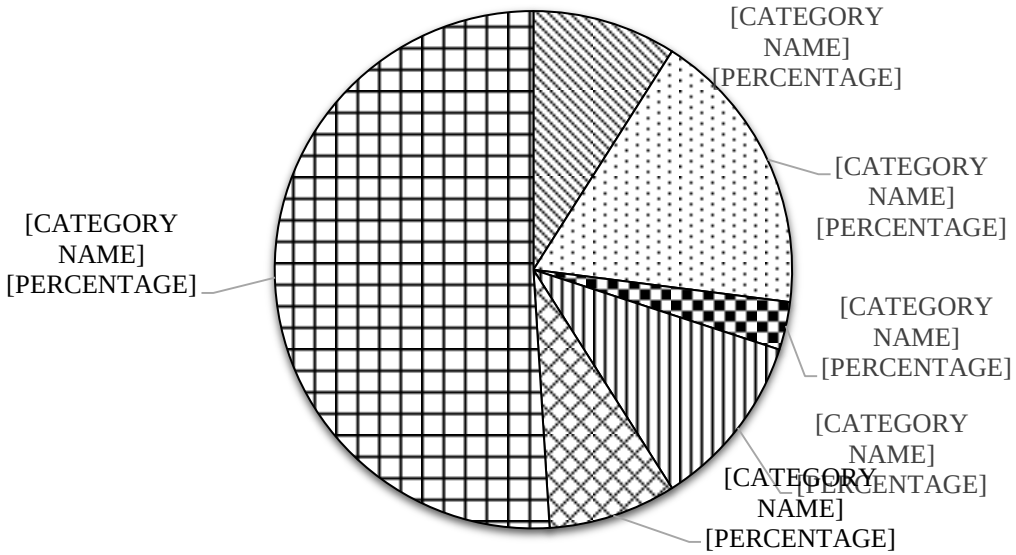
384 Enzyme screening by solid-state fermentation

Fungi number	Cellulase activity (U/g ds)	Xylanase activity (U/g ds)	Fungi number	Cellulase activity (U/g ds)	Xylanase activity (U/g ds)
F1-20-32B	-	10.08	F3-37-62A	-	-
F1-37-40A	2.60	8.52	F4-20-52B	0.21	5.34
F2-20-43A	-	-	F4-20-53B	0.86	0.28
F2-20-44A	17.37	189.24	F5-20-10B	-	2.95
F2-37-31A	-	-	F5-20-12B	1.53	13.89
F3-37-58B	1.95	45.6	F6-37-1B	14.56	82.20
F3-37-60A	6.17	14.54	F6-37-4B	-	11.90

385 - Undetectable

386

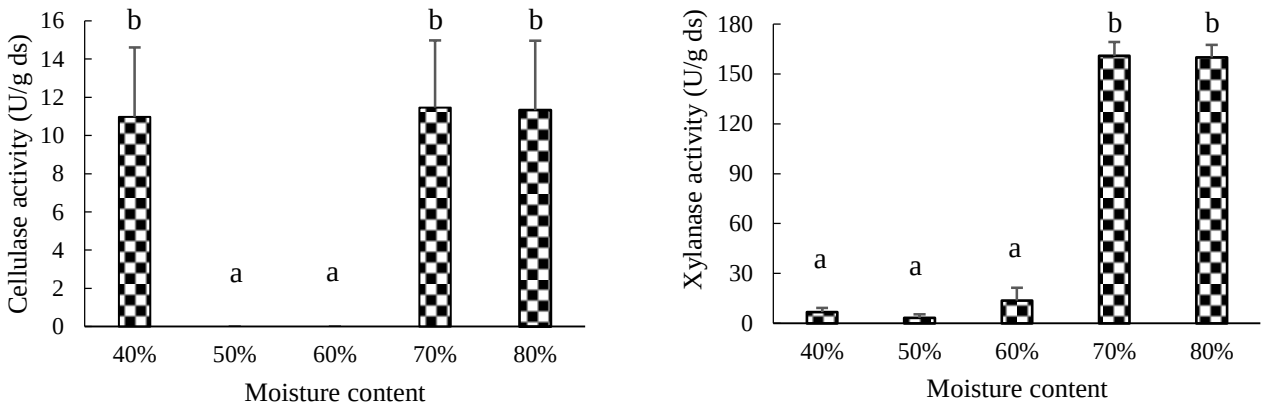
387



388

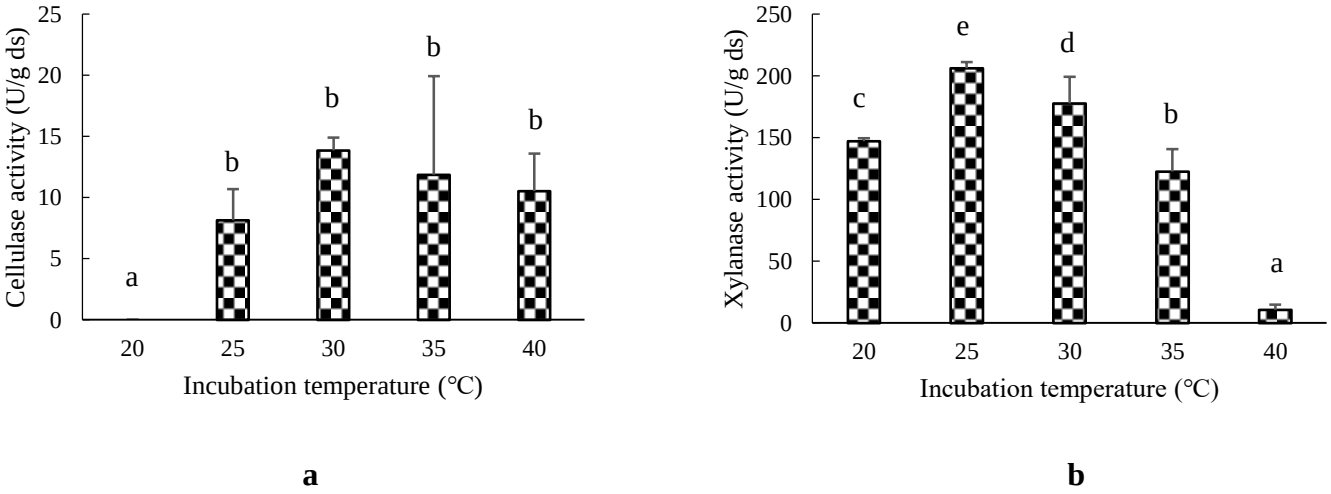
389 **Fig.1** Percentage of waste created throughout the food supply chain in Canada [2]

390



a **b**

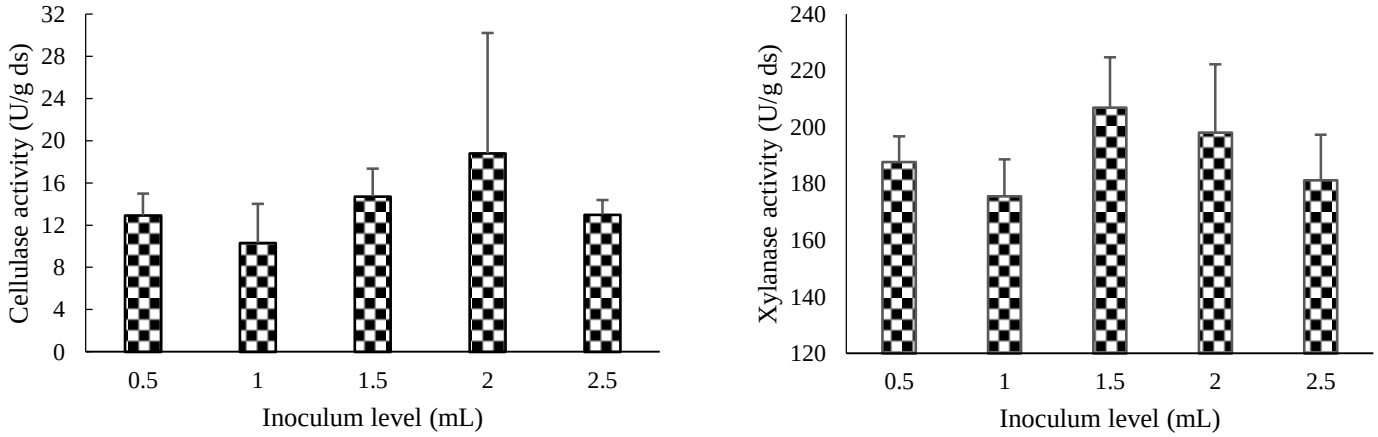
Fig. 2 Effect of moisture content by *A. niger* on the **a** cellulase production and **b** xylanase production; Letters shared in common between or among the groups indicate no significant difference according to S-N-K test at the significance level of 0.05; error bars are standard deviations of three replicates



a **b**

Fig. 3 Effect of incubation temperature *A. niger* on the **a** cellulase production and **b** xylanase production; Letters shared in common between or among the groups indicate no significant difference according to S-N-K test at the significance level of 0.05; error bars are standard deviations of three replicates

401



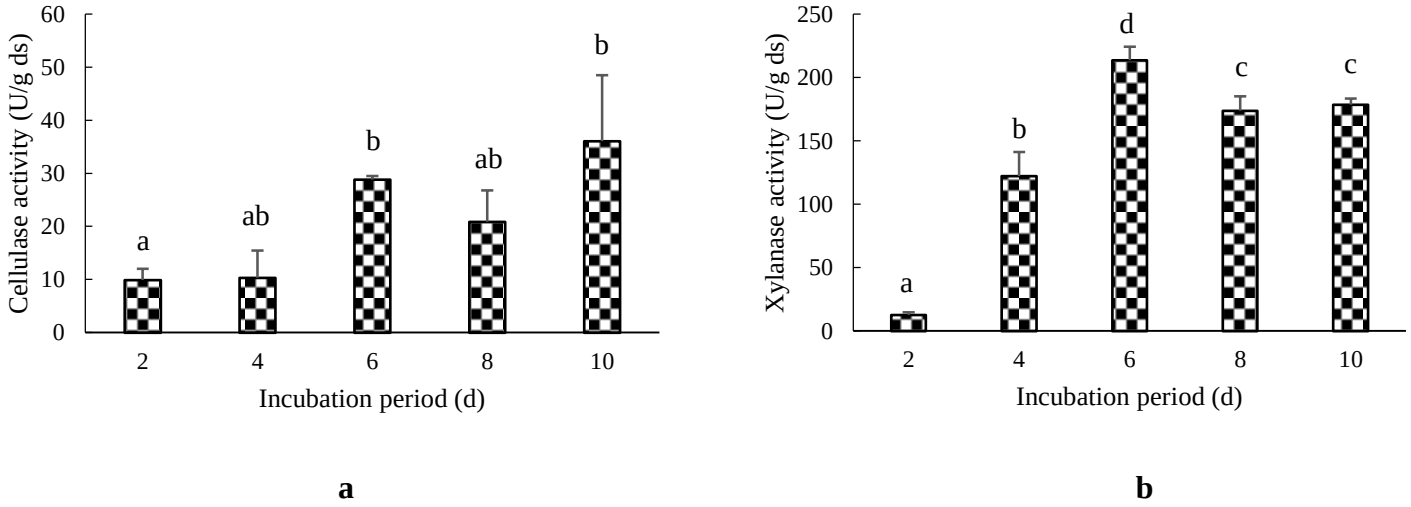
a **b**

402 **Fig. 4** Effect of inoculum level by *A. niger* on the **a** cellulase production and **b** xylanase production; error bars

403 are standard deviations of three replicates

404

405



a **b**

406 **Fig. 5 a** Effect of incubation period *A. niger* on the **a** cellulase production and **b** xylanase production; Letters
407 shared in common between or among the groups indicate no significant difference according to S-N-K test at
408 the significance level of 0.05; error bars are standard deviations of three replicates

409