5th International Conference of the International Commission of Agricultural and Biosystems Engineering (CIGR)

Hosted by the Canadian Society for Bioengineering (CSBE/SCGAB)
Virtually from Québec City, Canada – May 11-14, 2021

LABORATORY ASSESSMENT OF MICROBIAL DEGRADATION RESISTANCE FOR BIOMASS CHAR MADE FROM CORN RESIDUES CONVERTED BY MILD PYROLYSIS

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CSBE21807

ABSTRACT Biomass char may be seen as a value-added product with respect to raw biomass due to improved properties, such as the potential resistance to biodegradation. This resistance to biodegradation property might be of interest for two practical biomass char applications, i.e.: bioenergy, for the storage of the solid energy feedstock and biochar, as a soil amendment where carbon stability of the biomaterial would be a major asset. This research was conducted in a perspective of a step taken along the development of a laboratory method to assess the microbial degradation resistance of biomass char; especially low severity char pyrolysis production such as mild pyrolysis (torrefaction). Along this research, corn residues and charred corn residues, which were produced by mild pyrolysis at 300 ± 10 °C for 15 minutes at this temperature, were both inoculated with a consortium of *Trichoderma reesei* fungi and *Candida* yeast in aqueous conditions at 25 °C into 250 mL individual flasks. Degradation of the inoculated samples were followed with respect to non-inoculated samples. Incubation time effect was studied for 7, 14 and 21 days incubation period. Biodegradation of biomaterials was followed by dry mass balance, by analyzing protein content within soluble content, by elemental analysis as well as quantifying *Candida* yeast development along community forming units method on potato dextrose agar (PDA) and tryptone soya agar (TSA). Significant differences were observed among samples analyses and are presented and discussed along this study.

Keywords: biodegradation resistance assessment method, bio-char, *Trichoderma reesei*, *Candida*, mild pyrolysis, torrefaction.

INTRODUCTION Biomass chars obtained from thermochemical conversions, depending on properties that have been conferred to the charred material, have the potential to be used for applications, such as: bioenergy, soil amendment, carbon storage and water treatment (Uslu et al. 2008, Lehmann 2007, Ioannidou and Zabaniotou 2007). Pretreatment such as torrefaction (mild pyrolysis) is reported to be a manner to confer improved storage attributes compared to raw biomass, due to its microbiological degradation resistance (Medic et al. 2012). Improving resistance to microbial activity could also mean reduction of work environmental issues related to spores development, such as situations where more than 10^7 spores per m^3 of air may be met in conventional untreated biomass handling (Jirjis 2009). The improvement in resistance to microbial activity could also lead to reduction of storage off-gassing issues resulting from heating humid biomass under microbial and chemical activity and subsequently leading in decay losses or heating and auto-ignition possible issues. Resistance to microbial decay could be related to "the elimination of hemicellulose and an increase in hydrophobicity, but also to the formation of sugar and lignin degradation products toxic to microorganisms, such as furan and phenol derivatives that are trapped in the pores of torrefied material" (Medic et al. 2012).

Along mild pyrolysis (or torrefaction) technological developments, process equipments are designed and operate within a wide range of conditions. Chemical change of biomaterial leading to biodegradation resistance may then vary according to conversion degree and operating conditions. As a carbon sequestration application, the assessment of biomass char regarding its stability is a necessity and is still in development (Leng et al. 2019). Laboratory conditions have been investigated for the biochar as a soil amendment, especially for the study of its carbon sequestration potential (Hamer et al. 2004, Cheng et al. 2006, Zimmerman 2010, Leng et al. 2019). Mild pyrolysis (torrefaction) of agricultural and woody biomass char resistance to microbial degradation for biorenewable productions are also investigated (Medic et al. 2012, Esteves and Pereira 2009). Biomass chars have also been laboratory tested for chemical degradations of specific biomass chars properties, such as surface chemistry, surface area, pore volume, morphology or adsorption properties (Liu et al. 2013, Kawamoto et al. 2005).

Trichoderma reesi is a fungus which produces enzymes degrading hemicelluloses and cellulose (Juhász et al. 2005). Pandya and Albert (2014) used *Trichoderma reesi* and some white rot fungi to bio-bleach paper. Clayton and Srinivasan (1981) reported the biodegradation of Lignin by *Candida* spp.. Specific *Candida* spp. such as the *Candida utilis*. were shown to be able to metabolize cellulose and lignin polymers (Villas-Bôas et al. 2002). Salam and Das (2014) isolated the *Candida* VITJzN04 from contaminated soil and the yeast strain was shown to produce enzymes such as lignin peroxidase and manganese peroidases capable of degrading lignin (Placido and Capareda 2015). *Candida* yeast type was studied in order to verify its development following soil amended with biochar (Marks et al. 2014).

In a perspective of developing a laboratory method for quantifying the recalcitrance to biodegradation of biomass and biomass char, especially biomass chars produced at

lower temperature severity conditions (at temperatures around 300 °C), the following study was conducted. Within this temperature range, mostly hemicelluloses and parts of lignin are thermally decomposed (Yang 2006). During this study, corn residues biomass and biomass char from corn residues torrefaction were inoculated with the consortium of *Trichoderma reesei* and a *Candida* yeast. Corn stover residues were used for this study, because of the wide availability of residues from this major agricultural crop production. Microbiological development and potential biodegradation tendencies were followed along 21 days of incubation. Twenty one days of incubation was chosen for practical time limitation of a laboratory method application.

MATERIAL AND METHODS

Microbial consortium The consortium consisted of a mix of the *Trichoderma reesei* fungus Rut-C30 ATCC 56765® and a *Candida* yeast genus. The culture, containing approximately $4X10^8$ spores of *Trichoderma reesei* and about $9.3x10^5$ CFU/mL of *Candida* yeast, was transferred to a 1L Erlenmeyer flask containing 200 mL of distilled water and 4.8 g of Potato Dextrose Broth (PDB) medium both previously sterilized. The cultivation was performed in an incubator-agitator at 25 °C, 200 rotations per min (rpm) for 48h before used as an inoculum for biodegradation tests.

Corn residues biomass Corn stover residues used to produce biomass char were obtained from Ferme Olivier and Sébastien Lépine of Agrosphère Co (Saint-Alexis de Montcalm, Canada). Corn residues containing corn cobs, stems and leaves were dried under forced air conditions and milled using a hammer mill. This corn residues mixture was composed at 45.7 % of cellulose, 25.4 % of hemicelluloses and 4.6 % of ash (Larose 2012).

Biomass char production Biomass char was prepared from the corn residues in a closed aluminum container. Gases produced were expulsed by a top square hole of 6x6 mm. From that same hole, a K-type thermocouple (6mm diameter) was inserted in the reactor, within feedstock material. Desired temperature level was set for the muffle furnace, but reaction temperature and reaction residence time were followed from the perspective of the thermocouple in the reactor (Fig. 1). Biomass char was prepared in batches. For each production batch, 25 g of biomass of corn residues was inserted within the reacting chamber, yielding to 11.3 ± 0.4 g (48.4 ± 1.9 %) of char. Feedstock heating rate to reacting temperature was evaluated at 17 °C/min. Residence time at 300 ± 10 °C was around 15 minutes (Fig. 1). Chars obtained from three batches under same conditions were well mixed together in order to obtain a homogenized sample for further experiments. Raw and charred corn residues were both sieved (using Ro-Tap® shaker) prior to biodegradation experiments and only material between 0.25 mm and 2 mm was kept for the experiments.

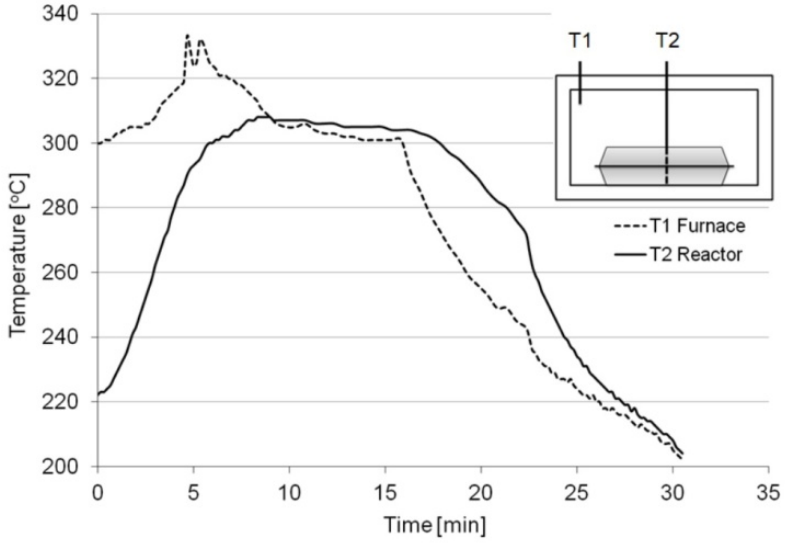

Figure 1. Example of temperature profile within muffle furnace and reacting chamber during biomass char production; presented with schematic representation of reacting chamber in the muffle furnace. Legend: T: Thermocouple.

Biodegradation test Biodegradation tests were conducted in 250 mL Erlenmeyer flasks which were firstly washed, dried (105 °C for 24h) and weighted before to be sterilized. For each samples' preparation, the raw corn residues and char feedstocks were both dried at 105 °C for 24h in a oven where their respective Erlenmeyer container was closed just before to be transferred directly to biological hood to avoid any biological contamination. The experimental plan of the biodegradation test is presented in Fig. 2. According to the plan, the corn residues and the char were inoculated with the microbial consortium. The corn residues (M), char (CM) and inoculated corn residues (iM) and char (iCM) were incubated at 25 °C and 150 rpm of agitation in batches of 7, 14 and 21 days. Under biological containment hood, 0.25 g of dry feedstock (105 °C for 24h) was transferred in each Erlenmeyer and suspended with 25 mL sterile saline water (0.85 % NaCl in demineralised water). A 5 % v/v (1.25 mL) of the inoculum was added to the Erlenmeyer. In order to follow samples gravimetrically (on a weekly basis; Fig. 2), Equation 1 was used. Where $m_{initial\ material}$ corresponds to initial dry sample (dried at 105 °C) before treatment without the inoculation and $m_{final\ sample}$ [expressed in mass % or in $g_{final\ sample}/100g_{initial\ sample}$] corresponds to sample after incubation. Mass of dry solid of strain initially added ($m_{inoculation}$) was determined by drying 3 mL of strain solution at 105 °C for 24h (in triplicates) and converted on a per mL basis. Amount of NaCl (m_{NaCl}, at 0.85 % concentration) within the 25 mL of sample solutions and the inoculum was also taken into account accordingly (Equation 1).

$$m_{final\ sample} = \frac{m_{dry\ flask+final\ sample} - m_{dry\ flask} - m_{NaCl}}{m_{initial\ material} + m_{inoculation}} * 100 \quad (1)$$

For each experimental batch obtained at 7, 14 and 21 days, the viable cell counts were determined by plate count technique. Inoculated samples were serially diluted. All samples were plated on potato dextrose agar (PDA) for *Candida* yeast cells count. All

samples were also plated on tryptone soya agar (TSA) for *Candida* yeast cells count and to follow up on the development of contaminated bacteria in case it happens beside the *Candida* cells count. These samples plated on PDA and TSA were incubated at 35 °C during 24h. Counts were reported as colony forming units (CFU) per mL of solution samples. Presence of *Trichoderma reesei* was also followed by observation of its filamentous form on PDA.

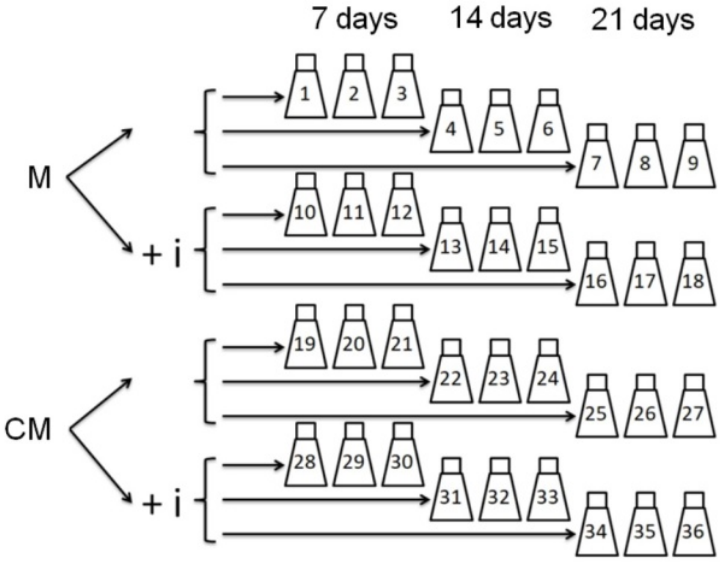

Figure 2. Schematic representation of the experimental plan. N.b.: numbers on Erlenmeyer bottles schematics represent samples identification numbers. Legend: M: raw maize (corn stover) residues, CM: char maize residues and i: inoculated.

Chemical analysis Chars, corn residues and every sample from biodegradation experiment were all characterized for ash content based on ASTM E1755-01 method with 575 °C maximum ashing temperature. Organic elemental composition C, H and N of corn residues and chars was conducted using EA 1108 Fisons apparatus for analysis. This involved completely oxidizing the biomass (about 2 mg) at a temperature of 1021 °C. The combustion gases (CO_2, H_2O, N_2) were transported to the chromatographic column using helium (He) as the carrier gas. They were then separated and detected by a thermal conductivity meter, which produced a signal proportional to the concentration of each component: C, H and N.

Soluble protein content of the samples was also determined following Sigma-Aldrich, Bicinchoninic Acid Protein method BCA1 AND B9643. One mL of the samples was centrifuged at 10 000 x g for 10 min to separate supernatants for protein measurement.

Statistical analysis For every condition, triplicates of corn residues and corn residues char, both inoculated and non-inoculated, were prepared. Inoculation in corn residues

and corn residues char was conducted in a completely randomized order. One factor ANOVA was used to determine significant differences among samples.

RESULTS AND DISCUSSION

Biodegradation of biomass char in terms of mass Before to compare the effect of torrefaction-pyrolysis treatment on biodegradability of inoculated samples, it is possible to observe that even the non-inoculated feedstocks (M and CM) have lost up to about 8-9 % of their initial respective masses (Fig. 3). The highest loss was observed for the longest incubation treatment (Fig. 3), almost at equal levels between M and CM at 21 days (8.8 and 8.3 % of mass losses respectively; with no significant difference from each other, for F = 0.05). These losses could have been generated from volatile organic compounds fragments potentially lost during drying step at 60 °C before final mass determination (Wang et al. 2019). Concerning char, since torrefaction at temperature employed (300 °C) is known to affect mostly hemicelluloses (Yang et al. 2006), fragments of hemicelluloses remaining in the feedstocks of CM may have been hydrolyzed or solubilised along the incubation period in water at 25 °C (agitation at 150 rpm) and/or during water heating for the drying step at 60 °C. Pilon and Lavoie (2013) produced chars at 300 °C containing extractable compounds (e.g.: benzene derivatives, furan derivatives, etc.), which are known as potential volatile organic compounds (Borén et al., 2017 and Leff and Fierer 2008); extractable compounds of these or other types remaining in char material could have been lost during drying at 60 °C.

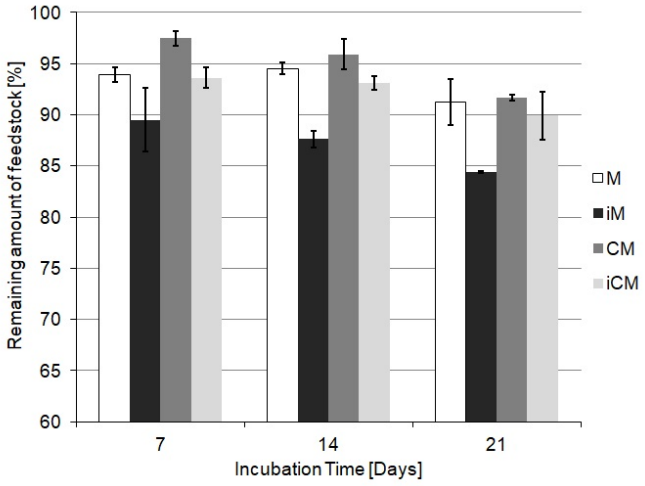

Figure 3. Biodegradation levels of maize and char maize material. Legend: M: raw maize (corn stover) residues, CM: char maize residues and i: inoculated

From the mass basis comparison, as shown on Fig. 3, it is possible to observe that iM were more degraded compared to M at all incubation times. This behaviour is significant at 14 and 21 days (*P* values of 0.0003 and 0.006 respectively). Char was also more degraded when it was inoculated (iCM) compared to when it was not (CM) (Fig. 3; 93.6 vs. 97.5 %, 93.1 vs. 95.9 % and 89.9 vs. 91.7 % at 7, 14 and 21 days respectively). This latter observation appears to be significant at 7 and 14 days (*P* = 0.006 for both). On the other hand, there are no major difference between inoculated or non-inoculated

samples for maize and char maize residues respectively, among the C-H-O organic elemental and ash content analyses at 21 days incubation treatment (Table 1).

Compared to iM, iCM got significantly less degraded at 14 and 21 days (F = 0.05; *P* = 0.0008 and 0.01, respectively, Fig. 3). These latter results correspond to about 5 % lesser biodegradation for iCM compared to iM at each incubation time (Fig. 3). This recalcitrance to biodegradation of biomass char is in accordance with Medic et al. (2012), noting lesser biodegradation for torrefied biomaterials (corn stover torrefied at 250 and 300 °C), which could be explained by the presence of remaining secondary tar compounds in the char structure inhibiting microbial development. Such tarry compounds (eg.: phenols or furan derivatives) were also observed by Pilon and Lavoie (2013) during mild pyrolysis conditions (at 300 °C as well).

The reduction in remaining amount of feedstock with respect to time either for iM or iCM from microbiological organisms development would eventually produce CO_2 which would account in the mass balance. Nonetheless, carbon organic elemental composition of feedstocks obtained at 21 days of incubation do not present any significant change with respect to i vs. non-i samples (Table 1). The dissolved and non-dissolved solids may both account in the final solid product in the dried samples. Nonetheless, the microorganisms could be expected more within the dissolved fraction. For further mass balance verification using organic elemental composition, analysis with respect to specific dissolved and non-dissolved fractions could provide more information.

In Table 1, it is also possible to observe a major difference between carbon content and ash content of non-incubated maize and charred corn residues and their respective incubated feedstocks at 21 days (i.e. for maize: 46 vs. 22 and 21 g Carbon/100g mat. and 5 vs. 39 and 33 g Ash/100g mat. and for charred maize: 62 vs. 31 and 32 g Carbon/100g mat. and 10 vs. 42 and 42 g Ash/100g mat.). This difference can be attributed to the mass of NaCl in the incubation solution since M** and CM** (Table 1) were analyzed for organic elemental composition and ash content before incubation treatment (i.e.: without contact with the incubation solution).

Table 1. Organic elemental composition and ash content of raw and charred corn residues feedstocks and samples at 21 days of incubation.

Material	C	H	O*	N	Ash
	(g/100g mat.)	(g/100g mat.)	(g/100g mat.)	(g/100g mat.)	(g/100g mat.)
M**	46.0 ± 0.1	5.6 ± 0.1	43.2 ± 0.0	0.60 ± 0.01	4.6 ± 0.1
M21	22.1 ± 2.0	2.9 ± 0.3	35.4 ± 4.3	0.31 ± 0.01	39.2 ± 2.9
iM21	21.6 ± 3.8	2.9 ± 0.5	41.9 ± 8.2	0.37 ± 0.01	33.2 ± 3.9
CM**	62.0 ± 0.4	0.9 ± 0.1	22.6 ± 0.3	4.51 ± 0.14	10.0 ± 0.4
CM21	31.3 ± 1.3	2.2 ± 0.1	24.5 ± 3.9	0.37 ± 0.01	41.7 ± 2.9
iCM21	32.4 ± 0.6	2.4 ± 0.0	22.9 ± 4.0	0.46 ± 0.05	41.9 ± 3.4

*Values calculated by difference. **Before treatment. Legend: mat.: material, iM21: inoculated maize (corn stover) at day "21" of the experiment, iCM21: inoculated char maize at day "21" of the experiment.

Biodegradation of biomass char in terms of soluble protein content The soluble protein content measured in non-inoculated (M and CM) and inoculated feedstock materials (iM and iCM) also provided results (shown in Fig. 4) which are in accordance with the results reported in the previous sections. First, concentration of soluble protein in M (606, 743 and 753 ug/mL) was higher than in CM (202, 150 and 177 ug/mL) for all the batches of 7, 14 and 21 days respectively of incubation at 25 °C. In addition, soluble protein content in M increased during 21-day incubation period, whereas in CM it was observed to be almost stable (considering their standard deviation), possibly meaning that the corn residues contained more soluble protein than the corn residues char. When the biomasses were inoculated with the microbial consortium, soluble protein contents kept increasing in inoculated samples (iM and iCM) as the residence time increased from 7 to 21 days. This increase of soluble protein could be attributed to extracellular enzymes produced by the microbial consortium. High content of cellulose and hemicelluloses in the corn residues feedstocks could induce *Trichoderma reesei* in the consortium to produce cellulase and hemicellulase enzymes (Juhász et al. 2005). In addition, the release of cellular debris from the microbial growth period during the whole experiment (up to 21 days) could also result in the increase in soluble proteins. On the other hand, more conditions and analysis would be required in order to confirm that protein contents comes from microbial activity and to discern whether one microorganism (*Trichoderma reesi* or *Candida*) developed more intensively from the results obtained.

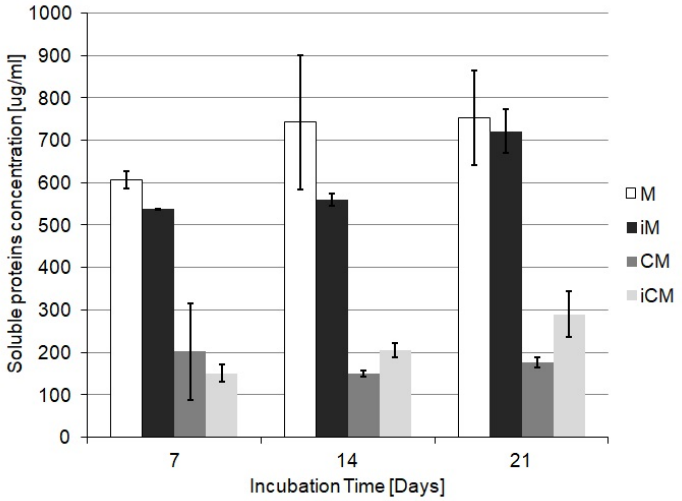

Figure 4. Soluble proteins concentrations for biodegradation experiment samples with respect to time. Legend: M: raw maize (corn stover) residues, CM: char maize residues and i: inoculated

It was observed that maximum degradation levels (in iM & iCM; related to highest soluble protein content) were obtained at 21 days (Fig. 4). In addition of supporting the observation of the degradation increasing with respect to time (Fig. 3), proteins analysis also supports the previous observation concerning iCM which degraded less than iM. As shown in Fig. 4, it is possible to notice that the soluble protein content in the inoculated char was lower than iM for every incubation time. In fact, soluble protein content was

obtained in every case; in non-inoculated samples as well as inoculated samples. In the case of corn stover residues, M samples soluble protein content appeared higher than in iM, which was significantly higher than iM protein content at 7 days (P = 0.005, Fig. 4). Higher soluble protein in M than iM could be due to soluble protein, which in iM, could be consumed by the microorganisms for their growth. While in the case of CM vs. iCM, it was somehow the other way around, especially at 21 days of incubation where soluble protein content was found at significantly higher levels than in CM (P = 0.025, Fig. 4). In the case of M, the presence of protein within the non-inoculated samples (Fig. 4) may be explained from the protein content of the plant biomass which solubilized. Following this previous observation, the protein content increase in the iM with respect to time (Fig. 4) does not necessarily mean an increase of protein resulting from microbial growth. Nevertheless, elemental nitrogen analyses (Table 1) obtained from 21 days incubation samples only, shows significantly higher levels for both inoculated feedstocks (iM vs. M and iCM vs. CM at P values of 0.002 and 0.04 respectively). Strain of *Trichoderma* in association with other microorganisms, such as *Trichoderma harzianum* and *Clostridium butyricum* (Veal and Lynch 1984) were observed to possibly fix N_2. As a result, the possibility that *Trichoderma reesei* and the *Candida* yeast strain would have fixed gaseous nitrogen could be considered as an hypothesis to study further. Nonetheless, this elemental organic nitrogen content difference between inoculated and non-inoculated could also be attributed partly to the inoculum itself, which could account in the mass to a certain extent.

The presence of proteins content observed in biomass char; inoculated or not could result from proteins in raw biomass remaining after thermal degradation (Fig. 4). Despite a different biomass matrix, Kebelmann et al. (2013) presented the TGA-DTG analyses performed on algae biomass protein where the mass loss by degradation and volatilization of proteins mostly occurred at temperatures from around 350 to 450 °C, therefore at temperatures higher than the actual char production at 300 °C used in the present study. However the fragmentation and denaturation of protein can be observed at lower temperatures since gaseous losses from proteins (eg.: CO_2, NH_3 and H_2O) at temperatures between 200-300 °C were reported by Kasarda and Black (1968). The bicinchoninic acid method used for assessment of soluble proteins (see Methodology and Method) is based on the formation of a Cu^{2+}-protein complex under alkaline conditions, followed by reduction of the Cu^{2+} to Cu^{1+}. The amount of reduction is proportional to the protein present, however it was shown that fragments of protein such as the peptide bond may also reduce Cu^{2+} to Cu^{1+}. As a result, there are proteins being known to be thermally degraded at temperatures from 200-300 °C (Kasarda and Black 1968), protein content observed in results could also be fragments of proteins (eg. peptides), remaining from the thermal degradation under mild pyrolysis conditions. This could explain the "observed presence of protein" in the non-inoculated char solution. Results for proteins in char (CM and iCM) should then be pondered with respect to the limitation of that method (the Sigma-Aldrich, Bicinchoninic Acid Protein method BCA1 AND B9643 used along these experiments). Despite this previous questioning concerning the applicability of the protein method used for dissolving char material, it is still possible to note that there was significantly more protein (P = 0.025) in iCM than in CM at 21 days of incubation. As a result microbial development could explain this significant

difference. This soluble protein content at 21 is however about 2.3 lesser in iCM than in iM (significantly lesser for $P = 0.006$, Fig. 4).

Biodegradation of biomass char in terms of microbial growth The colony forming units of the *Candida* yeast in the corn residues and the charred corn residues on the PDA and TSA medium are presented in Table 2. A greater growth of the *Candida* yeast in the corn residues than in the corn residues char was observed, which is consistent with the recalcitrance to biodegradation with biomass char results presented above in this text. Cellular concentrations of the yeast cultivated on PDA and TSA were always higher in iM than iCM and significantly more at 14 days on PDA medium (8.73E7 CFU/mL vs. 2.48E7 CFU/mL, iM and iCM respectively, $P = 0.003$). The growth of *Trichoderma reesei* in iM and iCM was observed along with the yeast growth by its formation of yellowish filamentous in PDA medium. As shown within Table 2, *Candida* yeast colony developed more into the PDA medium compared to the TSA medium; about twice (for the non-charred maize). The presence of the *Trichoderma reesei* within the consortium seems to affect yeast development since the yeast growth appeared to be affected on PDA agar where yeast shrank when *Trichoderma* was present compared to TSA; where the fungus did not appear. No bacteria development was observed on TSA medium.

Table 2. Colony forming units (CFU) obtained in function of Petri dishes growing media

Treatment	TSA (CFU/mL)	PDA (CFU/mL)
iM0	3.80.E+04 ± 2.55.E+04	9.34.E+04 ± 8.92.E+04
iM7	1.27.E+07 ± 9.20.E+06	3.18.E+07 ± 3.77.E+06
iM14	3.30.E+07 ± 1.06.E+07	8.73.E+07 ± 1.62.E+07
iM21	4.00.E+07 ± 4.53.E+07	7.20.E+07 ± 3.78.E+07
iCM0	3.80.E+04 ± 2.55.E+04	9.34.E+04 ± 8.92.E+04
iCM7	9.93.E+06 ± 7.02.E+05	1.22.E+07 ± 3.01.E+06
iCM14	2.25.E+07 ± 1.02.E+07	2.48.E+07 ± 3.27.E+06
iCM21	1.91.E+07 ± 9.72.E+06	1.59.E+07 ± 8.28.E+06

Legend: TSA: Tryptone Soya Agar, PDA: Potato Dextrose Agar, iM0: inoculated maize (corn stover) at day "0" of the experiment, iCM21: inoculated char maize residues at day "21" of the experiment.

FUTURE WORK In terms of future work, it could be of interest to run these experiments with many types of char materials. It would also be relevant to compare the biodegradation behaviour of mild pyrolysis bioenergy-biomaterial feedstock material during storage vs. during such experiment in order to assess whether or not such type of protocol for laboratory analysis conducted during only 21 days of incubation could be indicative of expected biodegradation behaviour. For future work, since mass balance analysis showed significant difference and due to the simplicity of such analysis, arriving to a simple laboratory treatment followed with a mass-differential based analysis could be useful for biomass char biodegradation resistance assessment. Conducting this study with separated strains would also be of interest in order to possibly associate observations with specific microorganism. On the other hand, the continuation research to target an optimal microorganisms consortium for such type of laboratory assessment method application could also be relevant.

CONCLUSION The development of laboratory analytical assessment of biomass char recalcitrance to microbial degradation is of interest with the increasing development of biomass char applications (eg.: bioenergy and soil amendment). Along these experiments, the inoculation of corn residues biomass char produced under mild pyrolysis (15 min. at 300 ± 10 °C) using consortium of *Trichoderma reesei* and a *Candida* yeast showed significant lesser mass losses compared to the raw inoculated biomass (corn residues) at 14 and 21 days of incubation. The colony counting of *Candida* yeast development was greater in samples with inoculated biomass than with inoculated biomass char, which was significant on potato dextrose agar at 14 days of incubation. Continuation research to develop an optimal microorganism consortium representative of biomass char storage as well as soil amendment for biodegradation recalcitrance laboratory assessment appears as a domain to explore further.

Acknowledgements. Authors would like to thank their colleagues Karine Saucier and Frédérique Bélanger-Lépine for discussions held with them or their technical assistance. Elena Nadezhina and Francine Bélanger-Gariépy from the Laboratoire d'Analyse Élémentaire de l'Université de Montréal are also acknowledged. This study was also possible from the financial support from the Fonds de recherche du Québec - Nature et technologies.

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