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Research paper

# Enzymatic pre-hydrolysis of organic fraction of municipal solid waste to enhance anaerobic digestion

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ABSTRACT

Enzymatic pretreatment was proposed to enhance the anaerobic digestion of Organic Fraction of Municipal Solid Waste (OFMSW). Enzyme cocktails were produced at laboratory scale from *Aspergillus niger* fermentation on wheat bran (WB) and OFMSW. The effect of enzyme load, reaction time and agitation speed on OFMSW hydrolysis was determined. Results showed that enzymatic hydrolysis of OFMSW improved organic matter solubilisation. Indeed, pretreatment of OFMSW using WB and OFMSW cocktails resulted respectively in 37.1 and 34.6% change of the soluble chemical oxygen demand and 50 and 40.6% change of the reducing sugars.

Anaerobic digestion tests showed that enzyme-treated OFMSW yielded higher biomethane production than raw OFMSW. After hydrolysis by WB and OFMSW cocktails, methane potential of OFMSW increased from 189.2 mL $gVS^{-1}$ to around 607 and 672 mL $gVS^{-1}$ respectively. The result confirmed that the use of enzyme cocktails derived from raw waste bioconversion could be a valuable approach to improve biomethane potential of organic wastes.

## 1. Introduction

Organic fraction of municipal solid waste (OFMSW) is an organic waste originated from various sources, e.g., households, cafeterias, restaurants, etc. In Tunisia, more than 2.5 million tons per year of municipal solid waste (MSW) could be produced annually where OFMSW, represent 68% of total quantity [1].

Anaerobic digestion is considered the suitable method for the treatment of organic waste and for producing high-value products (biogas and biofertiliser) [2]. The low-level sludge production, low-level energy consumption and green energy recovery [3] makes this process advantageous. OFMSW is composed of various components such as proteins, starchy, fatty, and cellulosic materials which are difficult to be transformed into soluble fraction [4]. The hydrolysis of these components into simple soluble molecules is the rate limiting step of anaerobic digestion process [5,6]. Thus, different pretreatments methods of organic solid waste were applied to improve organic matter solubilization and thus enhancing methane yields. Among these processes, ultrasonication [7], microwave [8], thermochemical [9] or acid hydrolysis [10] and biological processes are highlighted. Brummer et al. [11] showed that enzymatic pretreatment is a suitable way to hydrolyze lignocellulosic materials of OFMSW because it requires less energy and mild environment conditions. This pretreatment can be regarded as an environment-friendly process [10], since it does not require the use of chemicals, reduce the energy input and minimize the production of inhibitors [12]. Moreover, commercial enzymes including carbohydrases, proteases and lipases and fungal enzymes can be used to improve the hydrolysis of organic waste [4]. Nevertheless, the high cost of commercial enzymes is inadvisable for their use. Therefore, the application of fungal enzymes, produced from the fermentation of organic waste, is an alternative method to make the enzymatic hydrolysis less costly.

Some fungal species preferentially degrade lignocellulosic materials [13]. *Aspergillus niger*, which is a generally recognised as a safe (GRAS) microorganism, is one of the most investigated fungi for lignocellulose degradation because of its production of several hydrolases. Therefore, in fungal cultures, enzymes including α-amylase, β-glucosidase, xylanases, pectinases, carboxymethyl cellulase can be produced extracellularly by the fungus itself so as to help the digestion of insoluble substrates such as cellulose [14,15]. Several agro-industrial by-products (wheat bran, wheat straw, rice bran, rice husk, rice straw and bagasse etc.) are available as substrates for the production of those enzymes, with WB being particularly reported to contain plenty of cello-oligosaccharides that increase several extracellular activities.

This study aimed to investigate the application of enzyme cocktails obtained from the *A. niger* fermentation on WB and OFMSW for the pre-

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treatment of OFMSW. Hydrolysis process variables, which are enzyme load, agitation speed and reaction time, were thoroughly optimised to maximize organic matter solubilisation. The effect of enzymatic pretreatment on anaerobic digestion of OFMSW was evaluated by the determination of biomethane potential.

## 2. Material and methods

### 2.1. Biological materials and substrates

#### 2.1.1. 1. A. niger

*A. niger* strain (CTM 1099) was obtained from the Tunisian Collection of Microorganisms present in Centre of Biotechnology of Sfax (CBS). For inoculation of fermentation, spore suspensions were prepared by growing *A. niger* for 5 days at 30 °C in Potato Dextrose Agar (PDA) medium. After that, spores were collected by washing plate surface by 50% glycerol solution. The collected spore suspensions were sorted at −80 °C until their use.

### 2.2. Anaerobic inoculum

Inoculum was obtained from an anaerobic digester treating activated sludge installed in a municipal wastewater treatment plant located in Chotrana, North of Tunisia. The collected sludge sample was incubated at 37 °C until exhaustion of biogas. The obtained biomass was used as inocula for batch anaerobic digestion tests.

#### 2.2.1. OFMSW sample

Municipal Solid Waste (MSW) used in this study was collected from a transfer station located in Sidi Mansour-Sfax (South of Tunisia). The OFMSW was obtained after manual sorting of MSW to remove foreign materials such as bones, plastics, hard ligaments, and other inorganic materials (metals, textile, glass, etc.). OFMSW samples were ground with a laboratory blender (Product Division USA, Torrington, CT) to reduce particle size in the range 1–3 mm as optimum size for anaerobic digestion previously demonstrated [16] and then stored at −18 °C until use as substrate for *A. niger* fermentation and anaerobic digestion. The characteristics of OFMSW are presented in Table 1. The OFMSW was characterized by high concentration of organic matter in term of COD and VS and moisture content (71.9 %). It was also rich on carbohydrates, cellulose and lignin which are a suitable components for proper enzymatic pretreatment method. Before experiments, the OFMSW sample was mixed for 2 h at 150 rpm in a horizontal shaker at room temperature in order to improve homogenisation. Then, the substrate was diluted with distilled water (40%, wet weight/water volume) to facilitate its solubilisation [17].

#### 2.2.2. Wheat bran

Wheat bran which was used as carbon source for *A. niger* fermentation was obtained locally and stored in a dark room at 4 °C.

**Table 1**
Characteristics of raw OFMSW sample

| Parameters | Content |
|---|---|
| pH | 4.5 |
| TS (%) | 29.13 ± 1.4 |
| Ash (%) | 1.2 ± 0.08 |
| VS (%) | 28 ± 1.3 |
| Moisture (%) | 71.9 ± 2.4 |
| Carbohydrates (%)* | 2.28 ± 0.2 |
| TKN (%)* | 1.67 ± 0.13 |
| Proteins (%)* | 8.56 ± 0.7 |
| Cellulose (%)* | 29.6 ± 2 |
| Lignin (%)* | 35.8 ± 2.7 |
| Strach (%)* | 4.52 ± 0.4 |
| Lipid (%)* | 6 ± 1.09 |

*based on dry weight

### 2.3. Enzymes production

Liquid submerged fermentations of *A. niger* were performed with a basal medium containing (g $L^{-1}$): wheat bran or OFMSW, 67; $KH_2PO_4$, 1; $K_2HPO_4$, 2.5; $MgSO_4$, 0.3; $CaCl_2$, 0.3 and peptone, 3.75. Urea, which is a nitrogen source, was added in a way to maintain C/N ratio equal to 10 in WB and OFMSW medium. Fermentations experiments were conducted as the protocol described by Hamza et al. [18]. Culture broths were filtered through muslin cloth to give enzyme cocktails representing the extracellular medium and it was directly used for OFMSW hydrolysis experiments. α-amylase, β-glucosidase and CMCase activities were measured in the filtrate in triplicate. The results were presented as mean ± SD (standard deviation).

### 2.4. Enzymatic hydrolysis assays

Two runs of experiments were conducted for enzymatic hydrolysis of OFMSW at 50 °C and pH of 4.5. In the first run, the optimisation of OFMSW hydrolysis with WB cocktail was carried out by testing different parameters: enzyme cocktail load, agitation speed and incubation time. Table 2 summarises the operational conditions for these experiments. The control reaction was carried out at the same conditions by adding water instead of enzyme. Samples were withdrawn periodically from the medium for further analyses. The hydrolysis efficiency was determined by monitoring the increase of reducing sugars and SCOD concentrations.

In the second run, OFMSW hydrolysis assays using WB and OFMSW cocktails were conducted under optimal conditions in order to prepare pretreated samples for anaerobic digestion tests.

### 2.5. Anaerobic digestion tests

Batch fermentations were performed using 250 mL glass bottles with working volume of 150 mL. Control containing only anaerobic inoculum, untreated and treated OFMSW with WB and OFMSW enzyme cocktails were added at a ratio of Volatile Solid inoculum/Volatile Solid substrate equal to 1. In order to preserve anaerobic conditions, all bottles were tightly closed with rubber stoppers and then flushed with $N_2$ for 3 min into the reactors to remove residual traces of oxygen from the bottles. Finally, all bottles were incubated at 37 ± 1 °C. BMP tests were carried out in duplicate. Daily methane production was monitored by measuring the volume of alkaline solution displaced from the measuring bottle and collected in graduated cylinder. The $CO_2$ contained in the biogas did not affect the volumetric methane measurement as it was dissolved in the alkaline solution (NaOH 5%). Methane yield was calculated by the Equation (1)

$$Methane\ \ yield\ \ (mL\ gVS^{-1}) = \frac{Cumulative\ \ methane\ \ production}{VS_{introduced}} \quad (1)$$

In order to evaluate the effect of enzyme preparations on the anaerobic digestion, volatile fatty acids and SCOD concentration were measured at the end of fermentation (50 days).

The maximum methane production rate ($R_{max}$) and lag phase (λ) were determined by fitting the modified Gompertz model (Eq. (2)) [7] to the experimental cumulative methane production curves.

$$M = P.\ exp\left\{-\exp\left[\frac{R_{max}.\ e}{P}(\lambda - t) + 1\right]\right\} \quad (2)$$

where M is the cumulative methane expressed in mL $CH_4$ $gVS^{-1}$; P is the maximum methane potential (mL $CH_4$ $gVS^{-1}$); t is the time (day); $R_{max}$ is the maximum methane production rate (mL $CH_4$ $gVS^{-1}$ $d^{-1}$) and λ the lag phase (day). P, $R_{max}$ and λ were determined using the software package Mathematica (Wolfram).

**Table 2**
Experimental conditions used for enzymatic hydrolysis of OFMSW

| Tested parameters | Varied conditions | Fixed conditions |
|---|---|---|
| Enzyme load (mL): | 0.7; 1.4; 2.1; 2.8; 3.5; 4.2; 4.9; 5.6; 6.3; 7 (β-glucosidase activity: 0; 1; 2; 3; 4; 5; 6;7; 8; 9; 10 IU $mL^{-1}$) | OFMSW concentration: 40 % pH: 4.5<br>Temperature: 50 °C<br>Agitation speed: 100 rpm<br>Reaction time: 2 hours<br>Working volume: 50 mL |
| Agitation speed (rpm) | 0; 100; 150; 200 | OFMSW concentration: 40 % pH: 4.5<br>Temperature: 50 °C<br>Enzyme load: 4.2 mL (β-glucosidase : 6 IU $mL^{-1}$)<br>Reaction time: 2 hours<br>Working volume: 50 mL |
| Reaction time (hours) | 0; 1; 2; 3; 4; 5; 6; 7; 8 | OFMSW concentration: 40 % pH: 4.5<br>Temperature: 50 °C<br>Enzyme load: 8.4 mL (β-glucosidase : 6 IU $mL^{-1}$)<br>Working volume: 100 mL |

### 2.6. Analytical methods

Total solids (TSs) and volatile solids (VSs) were determined according to the standard methods [19]. pH was measured by a pH-meter (Metrohm). SCOD was estimated as described elsewhere [20]. Reducing sugars were measured spectrophotometrically using the 3,5-dinitrosalisylic acid (DNS) method [21]. The change of SCOD and reducing sugars was calculated by Equation (3).

$$Change(\%) = \frac{Concentration\ in\ treated\ waste - Concentration\ in\ untreated\ waste}{Concentration\ in\ treated\ waste} \times 100 \quad (3)$$

Total Kjeldahl Nitrogen (TKN) was determined as described by Kjeldahl-N method. Total sugars were determined by the method of Dubois [22] with glucose as standard. The crude lipids content was measured by weight difference after extraction with hexane in a Soxhlet system. Total proteins were estimated multiplying organic nitrogen ($TKN - N–NH^{4+}$) by 6.25. The starch content was determined using the enzymatic method (α-amylase and amyloglucosidase). It corresponds to the difference between hydrolyzed and non-hydrolyzed reducing sugars [22].

The cellulose and hemi-cellulose content were determined according to the fiber analysis from the USDA Agriculture handbook [23] and the lignin content was determined by the method published by NREL [24].

The identification and quantification of volatile fatty acids (VFAs) as acetate, propionate, butyrate and isobutyrate, were carried out by HPLC. Samples were acidified with 1 M $H_2SO_4$, centrifuged for 15 min at 12 000 rpm, filtered through 0.4 µm filter (MILLIPORE) and finally injected (20 µl). The assays were performed on a Shimadzu CTO-10 AS apparatus composed of a Hiplex H column (300 mm × 7.7 mm) connected to UV detector (210 nm). The mobile phase was $H_2SO_4$ (0.017 M) at a flow rate of 0.6 mL $min^{-1}$, and the column temperature was maintained at 50 °C.

In addition, Fourier transform infrared (FTIR) spectroscopy was performed to qualify the chemical bonds between the functional groups of OFMSW raw waste and hydrolysate OFMSW. Spectra were recorded with Thermo scientific NICOLET 380 FTIR spectrometer. The spectra (400–4000 $cm^{-1}$) were measured at a spectral resolution of 4 $cm^{-1}$ and 32 scans per sample.

The β-glucosidase activity was determined using 1 mM *p*-nitrophenyl-β-D-glucoside (p-NPG) as the substrate (in 100 mM citrate buffer pH 5). An aliquot of 0.2 mL of 1 mM *p*NPG was incubated, with an appropriate diluted enzymatic preparation, at 50 °C for 15 min. The reaction was stopped by adding 0.5 mL $Na_2CO_3$ 1 M; the liberated p-nitrophenol (pNP) was measured at 400 nm [25].

The endoglucanase activity (CMCase activity) was determined using carboxymethylcellulose (CMC) according to the method of IUPAC [26]. One unit of CMCase activity corresponds to the release of 1 µmol of glucose equivalents per minute.

The α-amylase activity was determined using starch according to the method of Miller [27].

## 3. Results

### 3.1. Enzyme cocktails production

Fig. 1 shows the typical time courses of β-glucosidase, α-amylase and CMCase activities during *A. niger* fermentation on WB and OFMSW under optimal culture conditions. For the two substrates, a significant increase of fungal growth was visually inspected during the early 3 days from inoculation. Enzyme production was increased sharply after a lag phase of 3 days. Regardless of the medium, both β-glucosidase and CMCase reached their activity peaks in *A. niger* cultures after 7 days when the organism had established itself well in the deeper layers of WB and OFMSW. After 7 days of fermentation, the maximum level of β-glucosidase was of 71 and 63 IU $mL^{-1}$, whereas, the maximum level of CMCase reached 3.7 and 5.18 IU $mL^{-1}$ in WB and OFMSW cultures, respectively. Nevertheless, α-amylase was also obtained in the two broths culture reaching 131.3 and 50 IU $mL^{-1}$ after 6 days of culture in WB and OFMSW, respectively.

### 3.2. Optimisation of OFMSW hydrolysis using WB cocktail

#### 3.2.1. Effect of enzyme load

The effect of enzyme load on OFMSW hydrolysis was investigated. OFMSW concentration was fixed to 40% in medium reaction and the enzyme load varied from 0.7 mL to 7 mL of cocktail, which corresponds to varying β-glucosidase activities from 0 to 10 IU $mL^{-1}$. The hydrolysis reactions were carried out at pH 4.5 and 50 °C as optimum conditions determined in previous study [18]. The relationship between solubilisation of organic matter (reducing sugars and SCOD changes) and cocktail load was also examined (Fig. 2a). Result shows an increase of reducing sugars and SCOD concentration for all tested cocktail load. Nevertheless, the control (without addition of enzyme) did not show any change of reducing sugars and SCOD concentration over incubation time (Fig. 2a). The low tested volume (0.7 mL) resulted in 43 and 22% change of reducing sugars and SCOD concentration, respectively. It was also noted that the increase of enzyme load from 0.7 to 2.8 mL resulted in a slight variation of reducing sugars and SCOD changes. When the enzyme cocktail volume beyond 4.2 mL, the SCOD change remained stable (around 40%), but reducing sugars change increased with the enzyme load except with 7 mL. For economical issue an enzyme volume

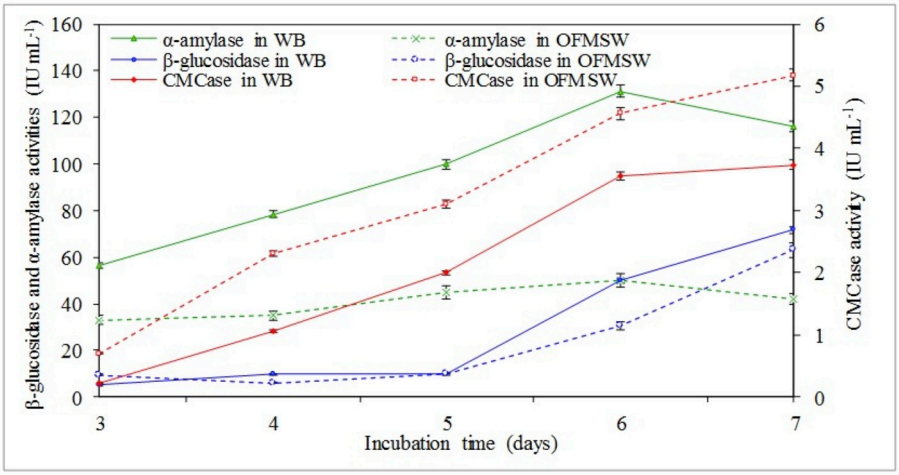



**Fig. 1.** Typical time courses of enzymes (α-amylase, β-glucosidase, CMCase) during *A. niger* fermentation in shake flask on wheat bran and OFMSW. (Values are means ± standard deviation of three determinations).

of 4.2 mL (6 IU $mL^{-1}$ of β-gulcosidase) was suggested as optimal load for maximizing the organic matter solubilisation. At this condition, the reducing sugars and SCOD changes were around 50% and 39%, respectively.

### 3.2.2. Effect of agitation condition

The effect of agitation on the reducing sugars and SCOD change was, also, studied (Fig. 2b). The biotransformation reactions were carried out at optimum β-glucosidase activity of 6 IU $mL^{-1}$ of reaction medium. Results showed the absence of enzymatic hydrolysis of OFMSW under static condition. However, the release of reducing sugars and SCOD was rapid and excellent at agitation condition. When increasing agitation from 100 to 200 rpm, reducing sugars and SCOD changes increases, respectively, from 52 to 58.5% and from 36 to 40.7% after 2 h of OFMSW hydrolysis (Fig. 2b).

### 3.2.3. Time course of enzymatic reaction

The time course of OFMSW hydrolysis by β-glucosidase enriched enzyme preparation was also evaluated by the determination of the reducing sugars and SCOD change (Fig. 2c). Experiments were carried out under the previously determined optimal conditions (6 IU $mL^{-1}$ of β-glucosidase, 100 rpm). In comparison to control, significant hydrolysis effect was shown by adding enzyme cocktail to OFMSW. In fact, 42% of reducing sugars and 26% of SCOD were released during the first hour of experiment (Fig. 2c). However, reducing sugars and SCOD change were improved with the increase of reaction time and reached 50% and 37.1% at 2 h of reaction, respectively. After that, the reducing sugars concentration was rising until 4 h, whereas no further changes in SCOD concentrations were found.

## 3.3. OFMSW hydrolysis at optimal conditions

To compare the effect of OFMSW enzyme cocktails to that of WB, hydrolysis experiments were conducted at optimal condition described above (6 IU $mL^{-1}$ of β-glucosidase, 100 rpm, 2 h).

Fig. 3 shows the reducing sugars and SCOD change after 2 h of OFMSW hydrolysis with enzyme preparation from *A. niger* fermentation on WB and OFMSW. The hydrolysis of OFMSW using WB enzyme cocktail gives reducing sugars and SCOD change of 50% and 37.1%, respectively, whereas, OFMSW enzyme cocktail led to reducing sugars and SCOD change of 40.6% and 34.6%, respectively (Fig. 3).

Raw and enzyme-treated OFMSW were analysed by FTIR spectroscopy (Fig. 4). All spectra have a strong peak at around 3200 $cm^{-1}$ The second main bands were identified at about 2900 and 2800 $cm^{-1}$. Besides, all samples presented a peaks at 1640 $cm^{-1}$and between 1740 and 1710 $cm^{-1}$. A small bands were observed around 1400 and 1200 $cm^{-1}$ region. A bands at 1050–1030 $cm^{-1}$ have a strong relative intensity in raw OFMSW compared to enzyme-treated OFMSW.

It is evident that most of the peaks are common in both untreated and treated OFMSW. Nevertheless, the IR spectrum of hydrolyzed samples using WB and OFMSW enzyme cocktails shows the decrease of some bands intensity.

## 3.4. Anaerobic biodegradability assays

Daily biomethane production and cumulative methane yield during anaerobic digestion of raw and pre-hydrolyzed OFMSW are shown in Fig. 5. It was noted from the results that the methane production from untreated and pretreated OFMSW starts from the first day (Fig. 5a). Nevertheless, the general trends of daily biomethane production for pretreated OFMSW with WB and OFMSW cocktails were similar. In these two batches, low biogas production from the 3rd to the 10th day was observed which then increased sharply during the fermentation period from 10th to 24th day. After that, a progressive decrease of the daily methane production was observed until the 50$^{th}$ day, where a low biogas production was detected due to the substrate consumption.

However, the methane production from untreated OFMSW dropped immediately after the peak reached at 3rd day, and a low methane volume was registered until the end of digestion. At the end of experiments, the biomethane yields were 607.3 and 672.6 mL $gVS^{-1}$ for OFMSW pretreated respectively with WB and OFMSW cocktails, (Fig. 5b), which were about 3.6 and 3.2-times higher than the untreated OFMSW (189.2 mL $gVS^{-1}$).

SCOD and TVFAs concentrations before and after anaerobic digestion are shown in Table 3. It can be seen that, SCOD removals reached 78.8% and 82.6% in OFMSW pretreated with WB and OFMSW enzyme cocktails, respectively, whereas no VFAs was detected in both batches at the end of anaerobic digestion.

In order to further assess the effectiveness of the enzymatic pretreatment, empirical non-linear regression models (Eq. (2)) were fitted to the data and the constants estimated are summarized in Table 4. The $R^2$ values presented in Table 4 and Fig. 5 showed that the regression curves fit well the experimental curves of raw and pretreated OFMSW. It can be noted, that the lag phase decreased after the application of enzymatic pretreatment. This can be also supported by the estimated maximum methane production rates ($R_m$) shown in Table 4. It increased from 11.2 mL $gVS^{-1}$ $d^{-1}$ for untreated OFMSW to 18.1 and 25.2 mL $gVS^{-1}$ $d^{-1}$ for treated OFMSW with WB and OFMSW cocktails, respectively.

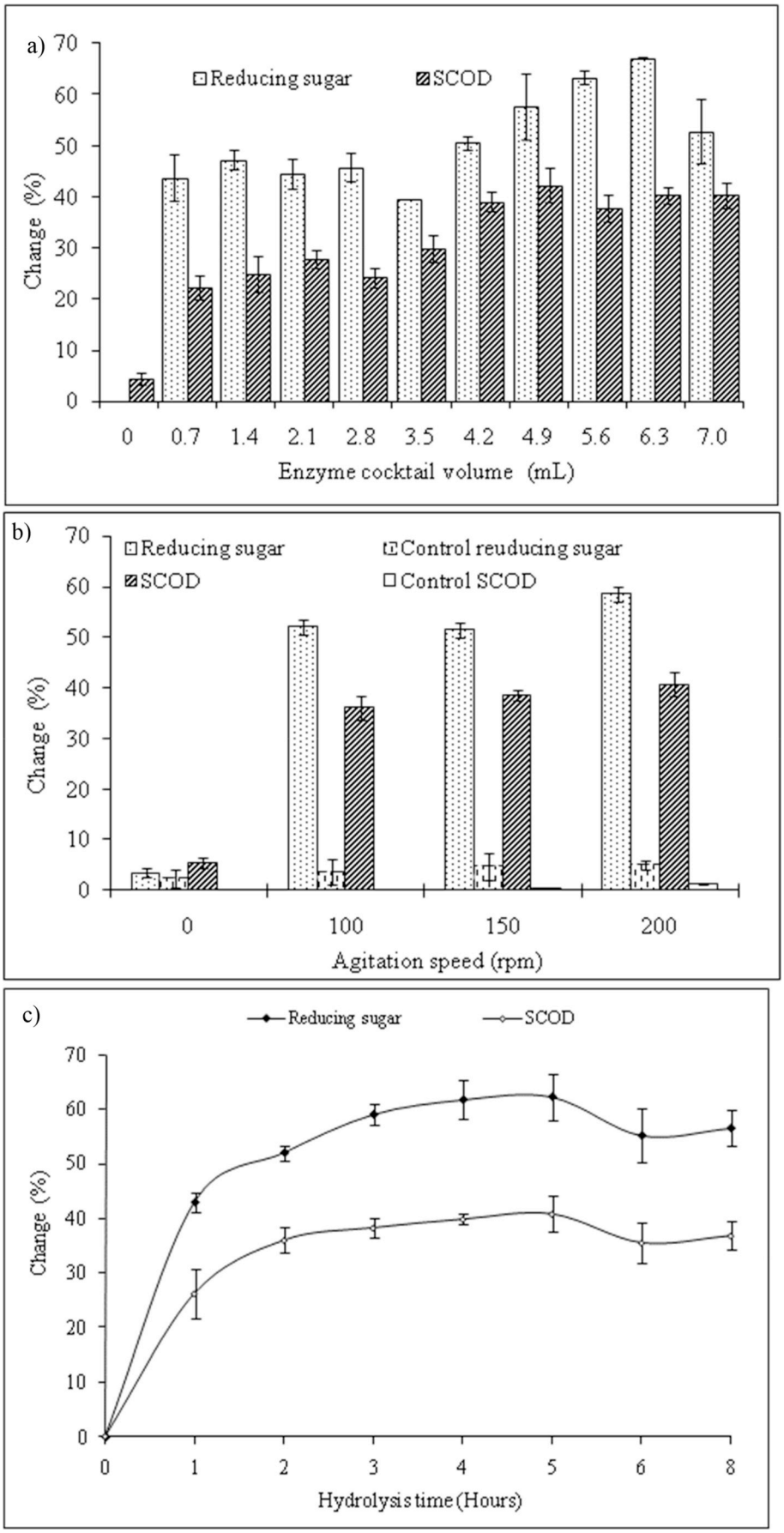



**Fig. 2.** Effect of β-glucosidase load (a), agitation speed and (b) time reaction on reducing sugars and SCOD change during OFMSW hydrolysis with wheat bran cocktail. Error bars represent standard deviation of mean (n = 3).

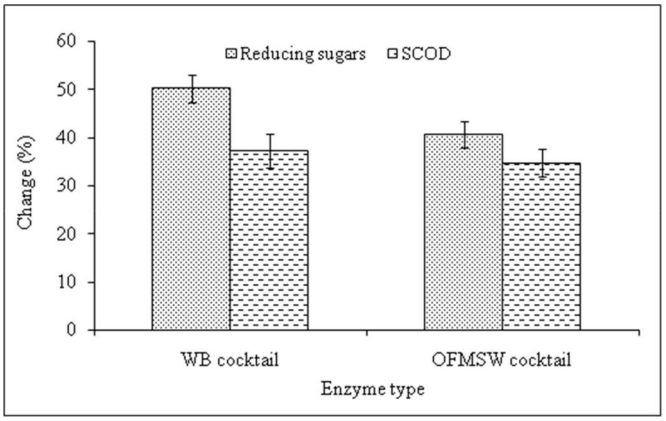



**Fig. 3.** Change of reducing sugars and SCOD after 2 h of OFMSW hydrolysis by wheat bran and OFMSW enzyme cocktails at optimal conditions (6 IU $mL^{-1}$ β-glucosidase, 100 rpm).

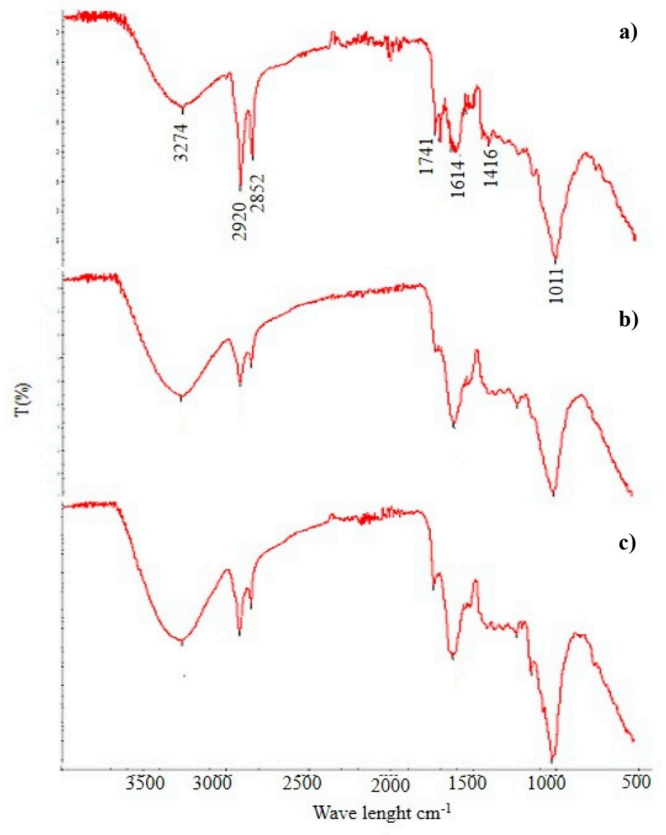



**Fig. 4.** FTIR spectra of untreated (a) and pretreated OFMSW using wheat bran (b) and OFMSW cocktail (c).

## 4. Discussion

In this study, two enzyme cocktails rich on β-glucosidase were produced from *A. niger* fermentation on a basal medium using WB and OFMSW as carbon sources. Results showed that WB is responsible of the induction of more enzymes compared to OFMSW substrate. The high enzyme production yield in WB is attributed to its particularly rich nutritional composition (vitamin B, proteins, carbohydrates, lipids, minerals and digestible nitrogen) [28]. In fact, the application of glycosyl hydrolase production process on an OFMSW-based growth medium is also very attractive, as it is a readily available carbon source.

To optimise enzymatic hydrolysis of OFMSW, different parameters such as β-glucosidase load, agitation speed and reaction time were tested by using WB cocktail. For control, the absence of organic matter solubilisation (SCOD and reducing sugar) confirmed that the enzyme addition is the essential factor explaining the hydrolysis of OFMSW carbohydrate polymers into reducing sugars. In addition, the stability of SCOD change noted beyond 4.2 mL could be explained by the fact that the enzyme overloading induce the inhibition of its stretching conformation that will causes enzyme inactivation [29]. Similar trends were observed by Kim et al. [30], who reported that a slight increase of SCOD was observed when the commercial enzymes dosage exceed 0.1% after food waste hydrolysis. At static condition, the OFMSW solubilisation with WB cocktail was not occurred. This indicated that agitation is an important parameter for enzymatic hydrolysis of OFMSW. It could accelerate the dynamic of mass transfer inside the medium and thus the enzyme effect [31]. However, the increase of agitation speed above 100 rpm does not affect significantly OFMSW solubilisation. On the basis of these results, the optimum agitation speed was fixed to be 100 rpm, which represents the lowest energy input rate.

The hydrolysis time is another parameter that has been demonstrated to have an effect on OFMSW hydrolysis. Generally, the hydrolysis process can be divided into two stages: In the first stage, macromolecules (cellulose and starch) are hydrolysed to soluble oligomers, and soluble oligosaccharides which are in second stage released to reducing sugars under the action of other enzymes present in the broth culture of *A. niger* such as carboxy methyl cellulase, xylanase, pectinase and esterase [32].

In the second part of experimentation, the OFMSW hydrolysis using WB and OFMSW under optimal condition was investigated. The multienzymatic composition of WB cocktail, which contains cellulases, arabinases, hemicellulases, glucanases and xylanases, capable of acting on a great number of components, in particular the soluble and insoluble fiber fraction [33]. These advantages causes modifications of several OFMSW components and thus more reducing sugars is released by WB cocktail than OFMSW cocktail. However, for the both cocktails, the synergism effect of varied enzymes on OFMSW hydrolysis resulted in SCOD and reducing sugar increases. Kiran et al. [34] observed an increase of SCOD and glucose by 94% and 95%, respectively, using fungal mash of *A. awamori* during hydrolysis of food waste. *A. niger* cultures conducted either WB or OFMSW yielded cocktails containing high activity of β-glucosidase, which is the key enzyme in hydrolysis of ligno-cellulosic materials. Therefore, cellulose, which is a relevant component of the polysaccharides in OFMSW, can be hydrolytically broken down into simple sugars by hemi-cellulolytic enzymes [6]. This degradation requires endoglucanases, exo-glucanases that can act from both the reducing (EC 3.2.1.176) and non-reducing ends (EC 3.2.1.91), forming cellobiose which is then cleaved into glucose monomers by β-glucosidases [35,36].

FTIR spectroscopy analysis was carried-out to evaluate the effect of enzymatic treatment on OFMSW (Fig. 4). The strong peak at around 3200 $cm^{-1}$ is assigned to the stretching vibration of hydrogen bond C–H and N–H groups for all samples. The bands identified at 2900 and 2800 $cm^{-1}$ correspond to the C–H stretching vibration in the aliphatic compound of untreated and treated OFMSW. The bands in the region 3200 and 2800 $cm^{-1}$ can be ascribed to carbohydrate and polysaccharide of rice, meat and vegetables characterizing OFMSW [37]. The peaks at 1740 and 1710 $cm^{-1}$, corresponds to stretching of the C = O bond of carboxylic acids of ester carbonyl group [38]. Besides, for all samples, the peak at 1640 $cm^{-1}$, which is assigned to the C = O group of amides, confirms the presence of proteins [39,40]. The small bands around 1400 and 1200 $cm^{-1}$ indicating the presence of lignin and cellulose [41]. An absorption in the 1050–1030 $cm^{-1}$ region, generally attributed to C–O stretching of polysaccharides or polysaccharide-like substances [42,43]. Therefore, a marked intensity decrease at about 2920 and 2850 $cm^{-1}$ (aliphatic C–H stretching) in pretreated OFMSW compared to untreated OFMSW may be explained by the enzymatic hydrolysis of carbohydrates and polysaccharides. The

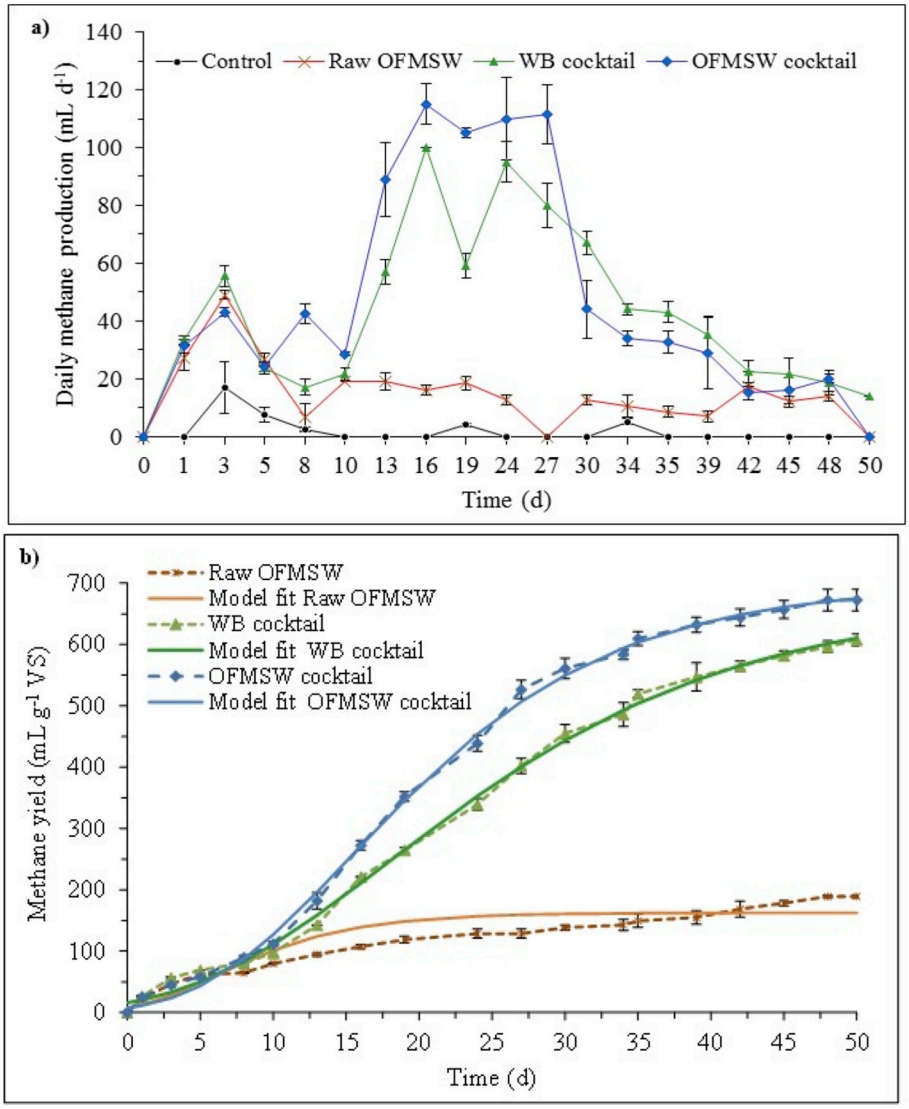



**Fig. 5.** Daily methane production (a) and cumulative methane yield (b) during anaerobic digestion of untreated and pretreated OFMSW with wheat bran and OFMSW enzyme cocktails. Solid lines indicate the simulation of the experimental data using the Gompertz equation.

**Table 3**
SCOD and TVFAs concentrations in the medium before and after batch anaerobic fermentation of raw and enzyme-treated OFMSW.

| | SCOD (g $L^{-1}$) | | TVFAs (g $L^{-1}$) | |
|---|---|---|---|---|
| | Initial | Final | Initial | Final |
| Raw OFMSW | 5.7 ± 0.28 | 10.3 ± 0.36 | 0.39 ± 0.04 | 4.45 ± 0.25 |
| WB cocktail | 5.9 ± 0.41 | 1.25 ± 0.08 | 0.57 ± 0.06 | 0 ± 0 |
| OFMSW cocktail | 6.27 ± 0.58 | 1.09 ± 0.09 | 0.6 ± 0.04 | 0 ± 0 |

**Table 4**
Kinetic parameters calculated from the theoretical model during anaerobic digestion of raw and treated OFMSW pretreated with different cocktails.

| Substrate | λ (d) | $R_{max}$ (mL $CH_4$ g $VS^{-1}$ $d^{-1}$) | P (mL $CH_4$ g $VS^{-1}$) | $R^2$ |
|---|---|---|---|---|
| Raw OFMSW | 6 | 11.19 | 162.52 | 0.932 |
| WB cocktail | 4.38 | 18.14 | 671.40 | 0.998 |
| OFMSW cocktail | 5.24 | 25.24 | 698.10 | 0.999 |

λ: Lag phase time, $R_{max}$: Maximum methane production rate, P: Estimated ultimate cumulative methane yield.

decrease of 1740 $cm^{-1}$ band confirms the hydrolysis of cellulose by β-glucosidase of the both cocktails (WB and OFMSW).

Anaerobic digestion performance was monitored by methane production profiles of untreated and enzymatic-treated OFMSW. The low methane produced from inoculum over digestion time demonstrated its stability and the absence of auto-digestion phenomena. The early methane production from untreated and enzymatic-treated OFMSW substrates can be explained by the presence of readily biodegradable fraction in both substrates such as VFA (Table 3) [44]. The lag phase observed in treated OFMSW batches is probably due to the conversion of organic matter into acetate. This result confirmed that the enzymatic pretreatment applied in this study led to a partial organic matter hydrolysis. At the end of fermentation, the low methane yield of untreated OFMSW is due to a severe inhibition of methanogens explained by VFA accumulation (4.0 g/L) [45,46]. However, enzymatic-treated OFMSW presents a high methane yield compared to the raw OFMSW. This indicated that enzymatic pretreatment improve the OFMSW hydrolysis step [47,48] and, thus, the methane production. According to Zieminski et al. [49] the pretreatment of sugar beet pulp and pent hops waste with a mix enzymatic preparation caused 19 and 13% increase in the biogas yield, respectively.

The stability of anaerobic digestion process was also assessed via

VFA and SCOD. The SCOD and VFAs reductions in enzymatic treated OFMSW were found to be highly correlated with important methane yields obtained after pretreatment by WB and OFMSW cocktails. Similar trends were observed by Yunqin et al. [50], who reported that VS and SCOD removal efficiency are consistent to methane yield of pretreated pulp and paper sludge with mushroom compost extract rich on cellulose.

The Gompertz model was used to compare methane production of untreated and enzymatic-treated OFMSW. It was observed that lag phase of treated OFMSW is shorter than raw OFMSW. In addition, OFMSW samples pretreated with OFMSW and WB cocktails gave an anaerobic digestion rate ($R_m$) faster about 1.6 and 2.25-times compared to untreated OFMSW, respectively. Similarly to kiran et al. [34] study, the pretreatment of food waste with fungal mash enhance the lag phase and the maximum methane production rate. Yin et al. [51] reported that the lag phase of enzymatic treated food waste, which was determined with Gompertz equation, is shortened to 2 time compared to the control.

## 5. Conclusions

The enhancement of anaerobic digestion of OFMSW by an enzymatic pretreatment was investigated. The used enzyme cocktails were produced from *A. niger* fermentation on WB and OFMSW as carbon sources. These substrates yielded enzyme cocktails rich in β-glucosidase, which have a crucial role in OFMSW hydrolysis as demonstrated in this study. The best enzymatic pretreatment conditions were 6 IU $mL^{-1}$ of β-glucosidase, 100 rpm and 2 h. At these conditions, SCOD rise by 37.1 and 34.6% in OFMSW pretreated with WB and OFMSW cocktails, respectively. Biomethane yields of OFMSW pretreated with OFMSW and WB cocktails were found to be, respectively, 3.6 and 3.2 times higher than raw OFMSW. The use of enzyme cocktails from organic wastes, which are a low-cost feedstock, is a recommended strategy for improvement of OFMSW hydrolysis and thus methane production. However, process scale-up and technico-economical analyses need to be conducted in future study.

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## Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.biombioe.2019.105286.

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