Assis, September 14th, 2021

Dear Editor-in-Chief:

We are submitting the manuscript entitled: "***Chemical characterization, hydrolysis and bioethanol production from municipal solid waste***", by Fabíola Ribeiro de Oliveira, Bruna Escaramboni, Pedro de Oliva Neto for consideration in ***Energy Conversion and Management: X***. The aim of this work was the production of bioethanol from the organic portion of the municipal solid waste (MSW), using a technology in conforms to the concept of biorefinery through enzymatic hydrolysis by *R. oligosporus* production of glucoamylases in solid-state cultivation and alcoholic fermentation of the hydrolysate by *S. cerevisiae*. Chemical characterization was initially applied to achieve this goal. The bioprocess proposed was:

a) **efficient** in convert starch into reducing sugar (relative high levels of yields were obtained).

b) **useful** for bioethanol production.

c) **innovative** by use of a new technology patented by UNESP (BR 102014031591-8 A2) for the enzymatic hydrolysis reaction.

d) **sustainable** for reuse of the MSW's organic portion.

Finally, the organic fraction of MSW was able to produce a high quantity of reducing sugars through hydrolysis and of bioethanol by fermentation. This manuscript is an original work of authors and has not been published before in any form. Besides, it is not under consideration by another journal at the same time. All the authors agree with this submission as well as the instructions and recommendations of the Journal were met.
Best regards,

*Corresponding author: Fabíola Ribeiro de Oliveira
Bioenergy Research Institute (IPBEN), Bioprocess Unit, Department of Biotechnology, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus Assis, Avenida Dom Antônio, 2100, 19806-900, Assis, SP.
Brazil
Ph: +55-11-989158530
Email: fabiola.oliveira@unesp.br

Highlights

- Characterization evidenced 67.21% of carbohydrates with potential for reuse.
- Technology patented by UNESP effectively converted starch into reducing sugars.
- Concentrated hydrolyzed liquid resulted in 57.75% of bioethanol yield.

Chemical characterization, hydrolysis and bioethanol production from municipal solid waste

Fabíola Ribeiro de Oliveira[a,*], Bruna Escaramboni[a], Pedro de Oliva Neto[a]

[a] Bioenergy Research Institute (IPBEN), Bioprocess Unit, Department of Biotechnology, Universidade Estadual Paulista "Júlio de Mesquita Filho" (UNESP), Campus Assis, Avenida Dom Antônio, 2100, 19806-900, Assis, SP, Brazil.

* Corresponding author: Fabíola Ribeiro de Oliveira

ORCID: https://orcid.org/0000-0001-5152-3467

Tel.: +55 11 989158530

E-mail address: fabiola.oliveira@unesp.br

Abstract

Food waste is increasing in the world and this residue when inappropriately disposed, cause serious problems in the environment, due to its toxicity for soil and water. Then, new technologies for the use of municipal solid waste (MSW) can decrease its damage to nature and expenses for society, besides open up the opportunity to obtain value-added products. There is also a strong interest in the development of fuels from renewable sources, aiming at lower environmental impacts than the widely used fossil fuels. In view of this, biomass from MSW is known for its great potential as a source for biofuels and biomolecules production. Therefore, the present work aimed to produce ethanol from the organic portion of the MSW. The proposed technology conforms to the concept of biorefinery, and it consisted in the enzymatic hydrolysis using glucoamylases produced by *Rhizopus oligosporus* in solid-state cultivation, through technology patented by UNESP (BR 102014031591-8 A2), which showed a conversion capacity of starch to reducing sugars (RS) of 67.38%, followed by alcoholic fermentation by *Saccharomyces cerevisiae*, producing 0.25 g of ethanol per gram of reducing sugar offered. Therefore, it was found that use of this lower cost source of enzyme results in good hydrolysis yielding, and consequently, considerable bioethanol production from MSW.

Keywords: Biomass, biorefinery, fermentation, reducing sugars, enzymatic hydrolysis

1. Introduction

The significant increase in the production of municipal solid waste (MSW) is an issue that has been studied and analyzed worldwide due to difficulties involved in its disposal, which requires appropriate locations, and searches for alternative ways for its reuse. It is estimated each person discards approximately 170 kg of organic matter per year [1]. Annually, 2.01 billion tons of MSW are generated in the world [2]. Only in Brazil 79.6 million tons of MSW were discarded in 2019, of which 29 million were destined for places considered inadequate such as dumps or landfills [1].

Inappropriate disposal of huge amounts of waste promotes accentuated environmental impacts, as shown by studies carried out in China and the European Union, in which waste of food consumption was estimated between 62.8 and 88 million tons per year. Noting that environmental impacts, in terms of ecological footprint, are in the order of 186 million ton CO_2 equivalent, 1.7 million ton SO_2 equivalent, 0.7 million ton PO_4 equivalent, in addition to 16292 Mm^3 of grey water footprint [3,4]. Other studies also reveal that places close to landfills have high levels of organic pollutants in soils and water even after 20 years of inactivity [5].

In addition, the high need for energy, the use of non-renewable sources and growing environmental pollution have generated an urgency in the search for energy alternatives, resulting in what is known as the biofuels policy. This strategy aims to reduce the emission of carbon monoxide and hydrocarbons and dependence on fossil fuels. Since it uses biomass as a raw material, reduces the net production of acid rain by emitting nitrogen and a lower sulfur content in its burning, thus releasing a lesser amount of greenhouse gases. [6,7].

Based on this idea, use of solid waste as a raw material in the production of alcohol has better environmental benefits compared to conventional methods. Once there is no negative impact that can come from land use, growth, harvesting, and methods of rigorous pre-treatment of the traditional process [8]. The organic part contained in MSW such as

bagasse, husks, oleochemicals and food waste has the potential to generate products of renewable origin such as biofuels, biosurfactants and recyclable materials. This is due to its heterogeneous composition, considering that, as a rule, contain important sources of sugars, lipids, carbohydrates, mineral acids, inorganic compounds, dietary fibers, phenolic compounds, carotenoids, and tocopherols, which can be used through bioconversion processes [9].

Despite having sufficient amounts of nutrients with potential for fermentation must, it needs prior processing, as occurs with carbohydrates, once ethanol-producing yeasts, such as *Saccharomyces cerevisiae*, are not able to use all types of carbohydrates present in wastes. Therefore, polysaccharides as the starch must be enzymatically hydrolyzed into glucose monomers [8–10].

This saccharification process takes place through some enzymes, generally in two stages, with alpha and beta amylases and glucoamylases being responsible for the complete degradation of starch into glucose, by starch's α-1-4 and α-1-6 glycosidic bonds hydrolysis [11]. An alternative to the conventional starch hydrolysis system is a single enzymatic extract and temperature, with a shorter reaction time, making starch hydrolysis more advantageous. This can be done through the enzymatic complex produced from the *Rhizopus oligosporus* fungus by bringing together the enzymes in a single amylolytic extract, with high performance, and also obtaining this input from agro-industrial residues or food waste as substrate, highlighting the sustainability of the process [12,13].

Ethanol biofuel can be obtained by the carbohydrate fraction of residues or sugars directly. However, other biofuels, such as biodiesel, can be obtained from the lipid fraction of biomass. Thus, from the enzymatic hydrolysis of residues, it is possible to separate them, through centrifugation, into distinct fractions, such as soluble carbohydrates, lipids, and fibers.

Knowing that the hydrolysate of the organic part of the MSW tends to be a rich must for fermentation, the present work consisted of the production of bioethanol from MSW. Biochemical characterization, hydrolysis of the organic portion under different conditions to obtain fermentable sugars, and alcoholic fermentation was used to achieve this goal.

2. Materials and Methods

2.1. Collect, separation and quantification of Municipal Solid Waste - MSW

Municipal solid waste was collected from different residences in the municipality of Assis, SP, Brazil. This material was autoclaved at a temperature of 121 ºC and pressure of 1 atm, during 20 min. Then the recyclable portion was separated from the organic one and both were quantified by their respective weights in relation to the total.

2.2. Organic fraction chemical characterization

After separation, the organic material was submitted to drying in an oven at 45 ºC, mixed and grinded, aiming at its homogenization for greater precision in chemical characterization regarding the contents of moisture, crude protein, lipids, ashes, starch and fibers [14]. For ashes' characterization, a change in the protocol was necessary, since the sample was kept in a muffle furnace at 900 ºC for 48 h, until weight became constant.

2.3. Enzymatic hydrolysis of MSW by R. oligosporus glucoamylases

For the enzymatic hydrolysis of the organic fraction, was used an amylolytic extract from *R. oligosporus* produced through technology patented by UNESP (BR 102014031591-8 A2), which occurs by solid-state fermentation processes and enzymatic extraction, providing a competitive lower-cost bioprocess [12,15].

The organic residue was mixed with 0.05 M sodium acetate buffer (pH 4.5) to obtain 20% (w/v) of reactional medium and gelatinized at 90 ºC for 30 min in a thermostated water bath (Te 183 – Tecnal – Piracicaba, SP, Brazil). Then, temperature was reduced to 50 ºC and enzymatic extrat, from the *R. oligosporus* cultivation, was added at 15 U/g of the substrate on

a dry basis. One unit (1 U) was defined as the amount of enzyme capable of releasing 1 µmol of reducing sugar per minute. The enzymatic hydrolysis occurred by 24 h incubation and, at the ending time, enzyme was inactivated in a boiling bath for 5 min, followed by cooling and centrifugation at 4000 rpm for 15 min, obtaining three fractions: lipidic, hydrolysate and fibers.

This reaction was performed in two different scales, first in falcon-type flasks (50 mL) then in a 2 L benchtop bioreactor (Tecnal – Tec-Bio Plus model) with a 500 mL working volume. Mechanical agitation and temperature control by water bath. Both were tested without or with manual stirring at intervals of 1 h during the first 10 h and in the last 4 h of reaction, with a pause between the tenth and the twentieth hour of reaction.

The hydrolysis yield was calculated from the concentration of reducing sugars (RS, g/L), the volume of hydrolysate (H, L), and the starch in the pre-hydrolysis sample (SPH, g), according to the equation:

$$\text{Yield (\%)} = [(RS * H)/SPH] * 100$$

A kinetic study was also performed, evaluating yield of starch to reducing sugars conversion, through different hydrolysis conditions: with and without stirring, with 24 h and 30 h of reaction and work volumes of 30 mL and 500 mL.

The hydrolysate fraction was characterized reducing sugars' quantification by the method of Miller (1959) [16], ashes, moisture, proteins by Kjeldahl's method [17], and soluble fibers by difference [14].

2.4. Alcoholic fermentation

Saccharomyces cerevisiae M26, isolated and stored by the team of the Industrial Microbiology Laboratory of the Faculty of Sciences and Letters - UNESP Campus Assis, SP, Brazil was spread in supplemented medium with the following composition (%, w/v): 2% sucrose, 1% yeast extract, 0.1% ammonium sulfate, 0.0028% $ZnSO_4.7H_2O$, 0.114%

147 $K_2HPO_4.3H_2O$, 0.024% de $MgSO_4.7H_2O$, 0.00104% $MnSO_4.H_2O$ and pH 5.0 [18]. The
148 spread was carried out in 3 steps: in test tubes containing 5 mL of medium, in 250 mL
149 Erlenmeyers containing 100 mL of medium and, finally, in 1 L Erlenmeyers containing 500
150 mL of medium. Then, a centrifugation was performed at 4 ºC, 3500 x g for 25 minutes.

151 Fed-batch process was performed according to the parameters of successful
152 methodologies by Dorta et al. 2006. To prepare the must, the hydrolyzed liquid obtained was
153 concentrated by raising the temperature, to evaporate the water, and stir until reaching the
154 amount of soluble solids accounted for at 20º Brix. At the fermentation, 9 g of *S. cerevisiae*
155 dry biomass was suspended in 75 mL of distilled water and 15 mL of must was added during
156 the feedings at 0, 1, 2, 3, 4, 5, 6, 7, 8 and 9 hours of the process, which lasted 24 hours with
157 stirring at 80 rpm in a shaker at 31°C [18].

158 At the end of fermentation, there was centrifugation at 4 ºC, 3500 x g for 25 minutes,
159 aiming to separate yeast and wine, followed by both must and wine distillation and evaluation
160 with a densimeter (Anton Paar DMA 35 Basic, São Paulo, SP, Brazil). Yeast samples were
161 taken at the beginning and at the end of fermentation to carry out moisture and cell viability
162 analysis in a Neubauer Chamber and Optical Microscope using methylene blue as dye.

163 Calculations were made according to the formulas:

164 Theoretical ethanol (g) = $CS * 0.511$

165 Theoretical ethanol (mL) = $CS * 0.6475$

166 Yield (%) = $(OE/TE) * 100$

167 Ethanol productivity $\left(\frac{g}{L*h}\right)$ = $(OE - IE) \ / \ (RT * MV)$

168 Where:

169 CS = consumed sugar (g); OE = obtained ethanol (g); IE = initial ethanol (g); RT = reaction
170 time (h); MV = must volume (L).

171 *2.5. Statistical analyses*

172 For data analyses, the analysis of variance statistical test (ANOVA) and comparison of
173 means by Tukey test at a significance level of 0.05 were used. For this, the software used was
174 BioEstat 5.0 (Institute for Sustainable Development Mamirauá, Tefé, AM, Brazil).

175 **3. Results and Discussion**

176 *3.1. Collect, separation and quantification of MSW*

177 In this study, 19.41 Kg of MSW were collected, in which 16.65 Kg were organic
178 material with moisture of 67.03±7.22% or 5.48 Kg of total solids and 2.76 Kg of recyclable
179 material. Therefore, MSW was composed of 85.8% organic compounds and 14.3% recyclable
180 ones, in which there was 1.79 Kg of plastic, 0.73 Kg of degradable material and 0.24 Kg of
181 metal (Table 1). After partial drying and grinding, 3.91 Kg of the organic fraction with
182 18.59% moisture was obtained, which is equivalent to 76.53% of the initial organic portion
183 due to water loss (Figure 2).

184 **Table 1.** Composition of the recyclable fraction in percentage (wet basis)

Recyclable Materials	% (w/w)
Metal	8.91
Glass	-
Plastic	64.66
Degradable	26.43

185

186 According to Tyagi et al. 2018, crude MSW, on a wet basis, after collection typically
187 contains 46% organic waste, including food waste, garden waste, wood and process waste,
188 followed by 17% paper, 10% plastic, 5% glass, 4% metal and 18% other materials [19].
189 However, several factors contribute to changes in its composition, such as the organic

fraction, which varies according to the place of origin, with a percentage of 50-70% in low-income communities and 20-40% in high-income communities.

Factors such as local culture, climate and geographic conditions are also added as influencers on the amount and composition of urban solid waste (USW) [20]. Another example is found in the study carried out in the city of Addis Ababa, Ethiopia by Tassie et al. 2019, in which the total wet-based MSW generated, recyclable materials such as metal, glass, plastic, paper, wood, and rubber were estimated in 15% of the total composition and 70% of the mass represented the portion of organic waste [21], a value 18.4% lower than that obtained in the present work. Probably in places where there is greater industrialization and/or greater consumption habits of more industrialized products, the percentage of organic matter is lower as the recyclable materials increase.

3.2. Organic fraction characterization

Chemical characterization of the pre-treated and homogenized organic MSW fraction was performed aiming to determine the possible applications of this material, such as biofuels production, the final objective of this study.

Carbohydrates, including fibers and starch, accounted for 67.2% of the organic fraction on a dry basis, followed by proteins (21.3%) (Table 2). Acid detergent fiber (ADF) and neutral detergent fiber (NDF) totaled 40.74%, of which 29.02% were hemicellulose and 11.72% cellulose and lignin.

Table 2. Comparison of the chemical composition of organic waste

References	Moisture (%, w/w)	Ash (%, w/w)	Proteins (%, w/w)	Lipids (%, w/w)	Starch (%, w/w)	Raw Fiber (%, w/w)	Carbohydrates (%, w/w)
[22]	nd*	1.46 ± 0.10	7.89 ± 0.50	5.95 ± 0.09	56.51 ± 0.60	20.2 ± 0.7	68.2
[23]	nd	5.7 ± 0.2	16.3 ± 6.2	nd	40.2 ± 4.4	nd	nd
[19]	72.8 ± 7.6	nd	17.7 ± 5.5	17.5 ± 6.6	nd	29.2 ± 15.0	55.5 ± 10.1
[24]	nd	nd	6.8 – 25.8	5.6 – 24.7	11.7 – 56.5	3.5 – 51.7	32.2 – 68.2
This study	18.59 ± 0.73	4.85 ± 0.15	21.34 ± 1.83	6.60 ± 1.25	26.55 ± 4.35	40.74 ± 1.67	67.21

*nd: not determined

Since it is well established that the composition of a raw material in terms of fat, starch, hemicellulose and cellulose determines its biofuel potential, the characterization of the organic fraction is essential. Aiming at it, is interesting to carry out a nutritional analysis as the main component of urban waste is food. [24].

Characterization of the MSW properties changes according to the regional, seasonal and socioeconomic contexts. Campuzano; González-martínez 2016 compiled the physical, chemical, elementary and chemical characteristics of 43 cities in 22 countries, showing the variation in waste characteristics attributed to the different cultural lifestyles and waste management systems found among these countries [25]. This variation does not allow, therefore, a generalization of residues' characteristics [19].

Knowing this, the results obtained in the present work are within the compositions described in the literature (Table 2). The high carbohydrate content and the low amount of lipids are also verified in those studies, while the ash content remained close to that described by Nishimura et al. 2017 [23]. However, the percentage of starch was found below that of some studies, although still within the range described by Barampouti et al. 2019 [19,20,24]. To establish a form of universal application of the methodologies proposed in this work, taking into account the heterogeneity of MSW, it would be interesting to standardize a minimum amount of starch needed before proceeding with hydrolysis and fermentation.

3.3 Hydrolysis of MSW by R. oligosporus glucoamylases

As the organic fraction of MSW is a complex mixture of easily digestible compounds, mainly starch materials, the use of carbohydrate hydrolysis processes highlights the potential for the production of MSW biofuels [22].

First, the amylolytic activity of the enzymatic extract of *Rhizopus oligosporus*, previously produced according to Escaramboni et al (2018) had 24.9 U/mL [12]. Thus, for

there to be 15 U/g of glucoamylase enzyme in the hydrolysis of 30 mL of reaction volume, with 20% (w/v) of residue, it was necessary to add 3.62 mL of the enzymatic extract.

After centrifuging the hydrolyzed material, the fractions were separated and quantified in volume and mass (Table 3). For the quantification in volume, the 30 mL of reaction medium was taken into account, while for the quantification in mass, the mass added in the reaction on a dry basis (6 g) was taken into account. Figure 2 shows the result of the gelatinization process, used for better effectiveness of the hydrolysis reaction. Proportion of total solids present in each organic fraction after hydrolysis was 70.32% of fibers (non-hydrolyzed material), 24.79% of soluble solids present in the hydrolyzed liquid and 4.9% of fat fraction. Figure 1 shows the phase separation after centrifugation.

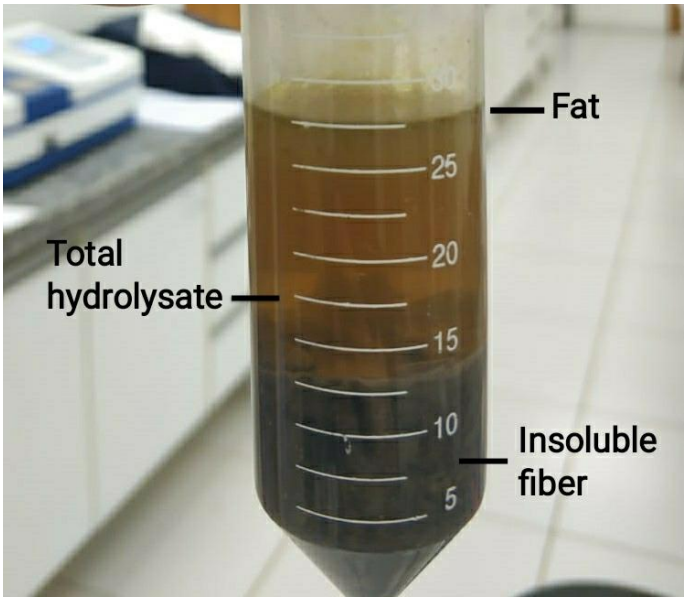
Table 3. Quantification in volume and mass of Municipal Solid Waste (MSW) fractions after hydrolysis by glucoamylases from *R. oligosporus*

Components	Reaction medium volume		Dry mass (20% reaction medium)	
	30 mL	% (v/v)	6.06 g	% (w/w)
Fat	3.25	10.83	0.3	4.89
Total hydrolysate	19.25	64.17	1.5	24.79
Insoluble fiber	7.5	25	4.26	70.32
Total	30	100	6.06	100

Reducing sugars (RS) quantification in the hydrolysate resulted in 33.78±0.49 g/L (3.38%). This result showed that only 40.42% of the starch was converted into RS. After scaling up the processes 16.7 times, with a work volume of 500 mL in a 1 L reactor, an increase on sugars yield was verified, once there was a 67.38% yield. The manual stirring process of one in one hour, used only in the hydrolysis scaling, can be responsible for the increase of the process efficiency. To evaluate this hypothesis, a kinetic study of the process

255 was carried out (Figure 3). This study found that stirring provides greater hydrolysis yield, as
256 it resulted in an increase in the concentration of reducing sugars reaching 5.4% after 24 h of
257 hydrolysis and 6.32% when extended to 30 h, being the maximum yield starch to RS
258 conversion rate of 75.62%.

259 **Fig. 1.** Phases' separation of MSW after hydrolysis and centrifugation.



260

261 Finally, the process was again carried out in 1 L flasks with 500 mL of working
262 volume, increasing the frequency of agitation and the reaction time to see if there would be an
263 improvement in the efficiency of the process, but the glucose concentration was similar to
264 previous test, with 5.6% RS and 67% (w/w) yield. In addition, the amount of soluble solids
265 present in the hydrolysate was measured through a refractometer, which resulted in 12.5
266 °Brix.

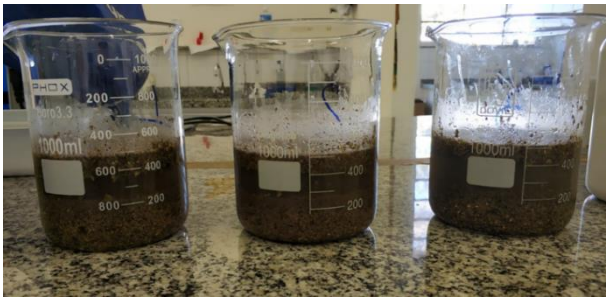
267 Table 4 presents RS concentrations obtained in the hydrolysis, considering statistical
268 analysis of the observed variations. Thus, it was noted that there was no statistical difference
269 in the results obtained at 24 and 30 h in a 500 mL reactor with manual stirring. However, on a
270 smaller scale there was a difference related to the presence or absence of stirring and related

to the time of hydrolysis. Even so, it is possible to affirm that the enzymatic hydrolysis yield is more related to the agitation than to the reaction time.

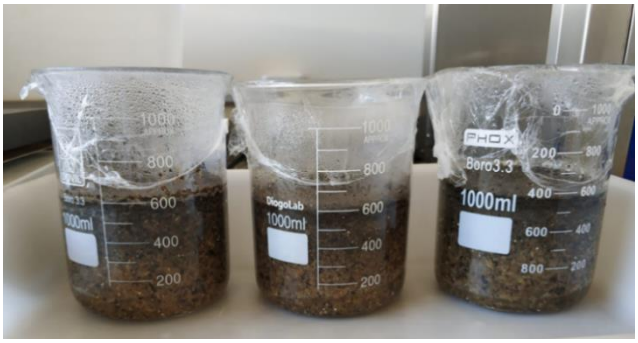
Fig. 2. (a) MSW's processed organic fraction (b) Appearance of the product before (c) and after 30 min of gelatinization of MSW at 90 ºC (d) Hydrolysate after 24 h fermentation.

(a)

(b)



(c)



(d)

Fig. 3. Kinetic study of starch hydrolysis in MSW at 50 ºC in a 50 mL Falcon tube, with the addition of 15 U/g of glucoamylase enzyme obtained from the cultivation of *R. oligosporus*. Bars represent the standard deviation of tests performed in triplicate.

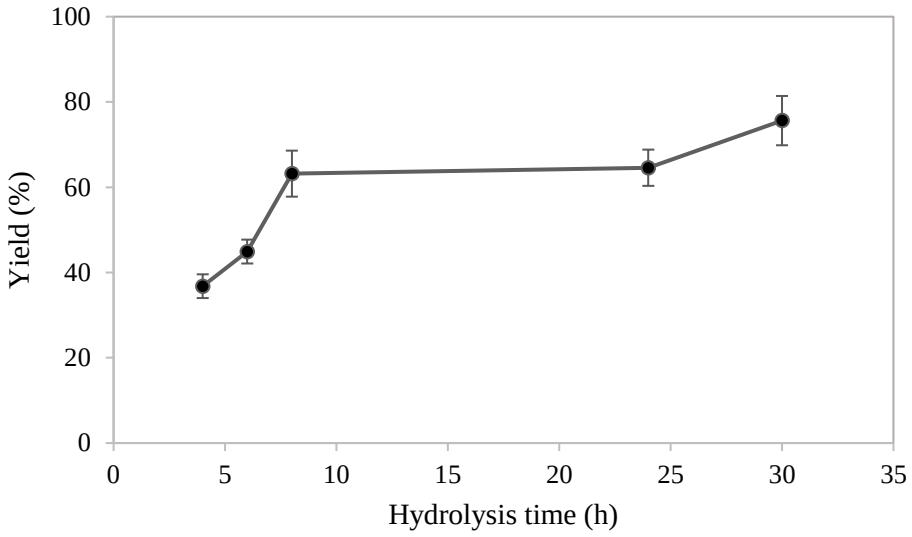


Table 4. Amount of reducing sugars (RS) and hydrolysis yield under different conditions of time and agitation.

Hydrolysis condition	Hydrolysis time (h)	Reducing sugars (g/L)	Hydrolysis yield (%)
Without stirring– Falcon 50 mL	24	33.79±0.49 a*	40.42
With stirring – Falcon 50 mL	24	53.97±4.25 b	64.60
With stirring – Falcon 50 mL	30	63.21±5.78 c	75.62
With stirring – Reactor 500 mL	24	56.32±9.95 b	67.38
With stirring – Reactor 500 mL	30	55.95±2.09 b	67.00

*Means followed by different letters indicate statistical differences ($p < 0.05$).

3.4. Chemical characterization of the fermentable fraction

This step evidenced the proportion of nutrients existing in the hydrolyzed liquid (with 78.69% moisture), which served as a must for fermentation. A large amount of soluble fiber (12.2%) was found, probably rich in hemicellulose, followed by reducing sugars (5.6%), 1.04% fat, 12.16% soluble fiber, 1.54% protein and 0.97% mineral residue.

3.5. Alcoholic Fermentation

After the propagation stage, 232.48 g of wet yeast were obtained, with 82.50% moisture. Thus, the amount of 9 g in dry basis required for fermentation was equivalent to 52 g in wet basis. Cell viability before (100%) and after (99.79%) fermentation was also verified, proving that there was minimal interference of the hydrolysate on *S. cerevisiae* growth and metabolism.

RS and total reducing sugars (TRS) were also determined after both concentration and autoclaving, aiming to verify the occurrence of a Maillard reaction, with a consequent reduction in the present RS (Table 5). Results show a concentration of 145.44 g/L in the must used for fermentation.

No statistical difference between the RS and TRS concentrations of the concentrated hydrolysate before and after autoclaving was found, proving that there was no significant loss of sugars by Maillard reaction during heating at 121ºC in the autoclave.

During the fermentation, 150 mL of must were added considering all the feeds, this amount resulted in the production of 177.3 mL of “wine” (fermented must), a volume quantified after centrifugation. The wine produced contained 1.91% of TRS, thus, it is known that the amount of total reducing sugar contained in the must was 14.54%. Therefore, it was possible to conclude that 18,42 g of sugar was consumed during fermentation.

Distillation process proved that there was no alcohol in the must and about 3.93% (% v/v) of alcohol content in the wine. Therefore, 177.3 mL of wine with 3.067% produced 5.44

320 g of ethanol or 57.75% yield. From these results, it was also possible to perform the
321 calculations of ethanol productivity, which resulted in 1.51 g/L.h.

322 **Table 5.** Concentration of reducing sugars and soluble solids after hydrolysis, concentration,
323 autoclaving and fermentation.

	Reducing sugars (g/L)	Total reducing sugars (g/L)	Soluble solids (°Brix)
Hydrolysate liquid	56.32±9.95 a*	80.19±1.80 d	12.5
Concentrated hydrolysate (must)	136.52±1.93 b	155.54±8.13 e	20
Autoclaved must	132.63±2.36 b	145.44±1.11 e	20
Wine	14.36±0.75 c	19.12±0.42 f	10

324 *Means followed by different letters indicate statistical differences ($p < 0.05$).

325 Table 6 shows the comparison of the results of this study with the literature,
326 highlighting the concentration of 30.68 g/L of ethanol produced in this study, since 5.44 g of
327 ethanol were obtained from 177.3 mL of wine. Thus, the results of this study are within the
328 standard obtained by the referenced articles, which also used urban solid waste as
329 fermentation must and *Saccharomyces cerevisiae* as inoculum [22,26–28].

330 Although some studies use different chemical pre-treatments to improve ethanol
331 production and different enzymes for MSW hydrolysis, it can be seen that there is a certain
332 constancy in the amount of ethanol produced from this source, which varies only according to
333 the amount of must used in the fermentation process [22,28].

334 If we consider 100% of the fermentable organic matter (ie, excluding recyclable
335 material) of the MSW we have 26.55% starch with hydrolysable potential. After enzymatic

hydrolysis of the starch present, we have 67.38% of the capacity to convert in reducing sugars (RS) therefore 17.66%. If 21.81 g of sugar offered in alcoholic fermentation produced 5.44 g of ethanol, we have a Yp/So (yield of ethanol produced by sugar offered) of 0.25 g/g. Therefore, of the 17.66% of RS, 4.42% (w/w) of ethanol were produced, that is, for every 100 Kg of MSW (considering only the fermentable fraction) we will have 4.42 Kg of ethanol produced. According to ABRELPE (2020), resources invested by Brazil in the collection of municipal solid waste (MSW) in 2019 were R$ 25 billion [1]. This is equivalent to approximately R$ 0.31/Kg (about 0.058 US $/Kg) of collected MSW, considering 79.6 billion Kg of MSW collected. Besides, considering that this amount of MSW would be capable of generating 3,52 billion Kg of bioethanol or 4,46 billion L and knowing that the average price of ethanol in Brazil is R$5.31, it would be possible to acquire a revenue of 23.68 billion reais.

Table 6. Comparison of fermentation results with literature.

References	Must Concentration* (g/L)	Yield (%)	Ethanol Concentration (g/L)	Ethanol Productivity (g/L.h)
This study	145.44	57.75	30.68	1.51
[26]	135.00	81.00	44.00	1.83
[22]	12.00	21.80	5.81	nd
[27]	120.00	nd**	39.00	nd
[28]	24.00	nd	9.50	nd

* Total reducing sugars

**nd: not determined

Thus, according to these values, if the carbohydrate and hydrolysis contents obtained in this work are similar to those processed in Brazil, we could produce around 3.16 billion Kg of ethanol per year. The Sugarcane Agroindustry Union (UNICA), based on data published by the National Agency for Petroleum, Natural Gas and Biofuels (ANP) and by the Brazilian Association of Pipeline Gas Distribution Companies (ABEGAS) compiles the quantity of ethanol consumed by Brazilians per year. Through this source it is known that in 2017, 25.56 billion liters of ethanol were consumed, which when converted into Kg by multiplying this volume in m^3 by the density of 789 Kg/m^3, is equivalent to 20.17 billion kg of ethanol. Thus, the production of 3.16 billion Kg of ethanol from MSW would be enough to supply 15.67% of the national demand.

According to FAO (2011) 1.3 billion tons of food waste are produced in the world [29], if the numbers of this work are close to the global average, it is possible to produce something close to 57.46 billion Kg of ethanol.

From the results obtained in this study, it was possible to verify several aspects related to the composition of municipal solid waste and its possibility of application, showing its viability in contributing to the reduction of the amount of incorrectly discarded MSW and its potential application in ethanol production. It is also important to stand out that RS concentration could be substantially increased if we consider the use of fibrolytic enzymes as xylanases and cellulases, although this would increase the cost of the process.

4. Conclusion

With regard to the organic portion of municipal solid waste from Assis-SP, Brazil, chemical characterization evidenced the presence of fibers and starches with potential for reuse, mainly through hydrolysis and subsequent fermentation. Use of the enzymatic extract produced from *Rhizopus oligosporus* fungus, through technology patented by UNESP, proved

to be effective in converting starch into reducing sugars. Finally, the concentrated hydrolyzed liquid obtained in this process resulted in good bioethanol yield, proving its potential for the production of this biofuel. Therefore, the work highlights the importance of separating recyclable and organic waste in the collected MSW, in view of the savings made both in the treatment of these wastes and in their use as raw material for obtaining liquid fuels (bioethanol).

Funding

This work was supported by Fundação de Amparo à Pesquisa do Estado de São Paulo (FAPESP, SP, Brazil, grant number 2019/24970-5).

Acknowledgments

The authors are thankful to the Studentship and São Paulo Research Foundation (FAPESP, SP, Brazil, grant number 2019/24970-5) for the funding. The first author acknowledges her parents, Marlene and Jucelino for the encouragement to research.

Author Contributions.

PON and BE conceived and designed the research. FRO conducted experiments as well as organized and analyzed the data with the help of PON and BE. FRO and BE wrote the manuscript. BE contributed with data statistical processing and edited the paper. All authors reviewed and approved the manuscript.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Ethical Approval

This article does not contain any studies with human participants or animals performed by any of the authors.

References

[1] ABRELPE - Associação Brasileira de Empresas de Limpeza Pública e Resíduos Especiais. Panorama dos resíduos sólidos no Brasil 2020.

[2] Kaza S, Yao L, Bhata-Tata P, Woerden FW. What a Waste 2.0. A Global Snapshot of Solid Waste Management to 2050. 2018.

[3] Scherhaufer S, Moates G, Hartikainen H, Waldron K, Obersteiner G. Environmental impacts of food waste in Europe. Waste Manag 2018;77:98–113. https://doi.org/10.1016/j.wasman.2018.04.038.

[4] Sun SK, Lu YJ, Gao H, Jiang TT, Du XY, Shen TX, et al. Impacts of food wastage on water resources and environment in China. J Clean Prod 2018. https://doi.org/10.1016/j.jclepro.2018.03.029.

[5] Pastor J, Hernández AJ. Heavy metals , salts and organic residues in old solid urban waste land fi lls and surface waters in their discharge areas : Determinants for restoring their impact. J Environ Manage 2012;95:S42–9. https://doi.org/10.1016/j.jenvman.2011.06.048.

[6] Demirbas A. Political, economic and environmental impacts of biofuels: A review. Appl Energy 2009;86:S108–17. https://doi.org/10.1016/j.apenergy.2009.04.036.

[7] Rasool U, Hemalatha S. A review on bioenergy and biofuels: sources and their production. Brazilian J Biol Sci 2016;3:3. https://doi.org/10.21472/bjbs.030501.

[8] Hegde S, Lodge JS, Trabold TA. Characteristics of food processing wastes and their use in sustainable alcohol production. Renew Sustain Energy Rev 2018;81:510–23. https://doi.org/10.1016/j.rser.2017.07.012.

[9] Lin CSK, Pfaltzgraff LA, Herrero-Davila L, Mubofu EB, Abderrahim S, Clark JH, et al. Food waste as a valuable resource for the production of chemicals, materials and fuels. Current situation and global perspective. Energy Environ Sci 2013;6:426–64. https://doi.org/10.1039/c2ee23440h.

[10] Dahiya S, Kumar AN, Shanthi Sravan J, Chatterjee S, Sarkar O, Mohan SV. Food waste biorefinery: Sustainable strategy for circular bioeconomy. Bioresour Technol 2018;248:2–12. https://doi.org/10.1016/j.biortech.2017.07.176.

[11] Mobini-Dehkordi M, Afzal Javan F. Application of alpha-amylase in biotechnology. J Biol Today's World 2015;1. https://doi.org/10.15412/j.jbtw.01010104.

[12] Escaramboni B, Fernández Núñez EG, Carvalho AFA, de Oliva Neto P. Ethanol biosynthesis by fast hydrolysis of cassava bagasse using fungal amylases produced in optimized conditions. Ind Crops Prod 2018;112:368–77. https://doi.org/10.1016/j.indcrop.2017.12.004.

[13] Escaramboni B, Castelli B, Mateus G, Abe M, Abel DP, Oliva Neto P de. Food Waste as a Feedstock for Fungal Biosynthesis of Amylases and Proteases. Waste and Biomass Valorization 2021. https://doi.org/10.1007/s12649-021-01511-0.

[14] Instituto Adolfo Lutz. Métodos físico-químicos para análise de alimentos. IV. São Paulo: 2008.

[15] Industrial P. Br 102014031591-8 a2 2016.

[16] Miller GL. Use of DinitrosaIicyIic Acid Reagent for Determination of Reducing Sugar 1959.

[17] Galvani F, Gaertner E. Adequação da Metodologia Kjeldahl para determinação de Nitrogênio Total e Proteína Bruta. Embrapa 2006;4.

[18] Dorta C. Sinergismo entre sulfito , ácido lático , pH e etanol na fermentação alcoólica de Saccharomyces cerevisiae PE-2 e M-26 Sinergismo entre sulfito , ácido lático , pH e etanol na fermentação alcoólica de Saccharomyces cerevisiae PE-2 e M-26 2006.

[19] Tyagi V, Fdez-güelfo LA, Zhou Y, Álvarez-gallego CJ, Garcia LIR, Ng WJ. Anaerobic co-digestion of organic fraction of municipal solid waste (OFMSW): Progress and challenges 2018;93:380–99. https://doi.org/10.1016/j.rser.2018.05.051.

[20] Mahmoodi P, Karimi K, Taherzadeh MJ. E ffi cient conversion of municipal solid waste to biofuel by simultaneous dilute-acid hydrolysis of starch and pretreatment of lignocelluloses. Energy Convers Manag 2018;166:569–78. https://doi.org/10.1016/j.enconman.2018.04.067.

[21] Tassie K, Endalew B, Mulugeta A. Composition , Generation and Management Method of Municipal Solid Waste in Addis Ababa City , Central Ethiopia : A Review 2019;9:1–19. https://doi.org/10.9734/AJEE/2019/v9i230088.

[22] Mahmoodi P, Karimi K, Taherzadeh MJ. Hydrothermal processing as pretreatment for efficient production of ethanol and biogas from municipal solid waste Corresponding author : Elsevier Ltd; 2018. https://doi.org/10.1016/j.biortech.2018.03.115.

[23] Nishimura H, Tan L, Kira N, Tomiyama S, Yamada K, Sun Z, et al. Production of ethanol from a mixture of waste paper and kitchen waste via a process of successive liquefaction , presaccharification , and simultaneous saccharification and fermentation. Waste Manag 2017. https://doi.org/10.1016/j.wasman.2017.04.030.

[24] Barampouti EM, Mai S, Malamis D, Moustakas K, Loizidou M. Liquid biofuels from the organic fraction of municipal solid waste : A review. Renew Sustain Energy Rev 2019;110:298–314. https://doi.org/10.1016/j.rser.2019.04.005.

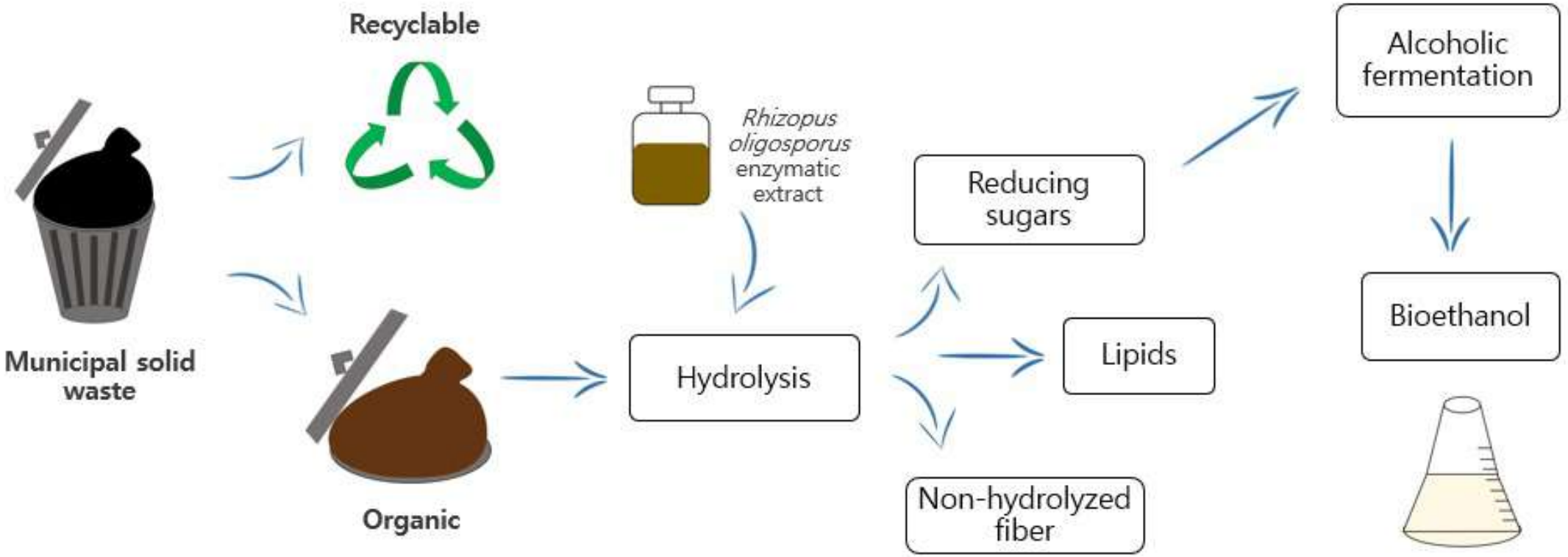
[25] Campuzano R, González-martínez S. Characteristics of the organic fraction of municipal solid waste and methane production : A review. Waste Manag 2016;54:3–12. https://doi.org/10.1016/j.wasman.2016.05.016.

[26] Kim JH, Lee JC, Pak D. Feasibility of producing ethanol from food waste 2011;31:2121–5. https://doi.org/10.1016/j.wasman.2011.04.011.

[27] Hong YS, Yoon HH. Ethanol production from food residues. Biomass and Bioenergy 2011;35:3271–5. https://doi.org/10.1016/j.biombioe.2011.04.030.

[28] Mahmoodi P, Karimi K, Taherzadeh MJ. E ffi cient conversion of municipal solid waste to biofuel by simultaneous dilute-acid hydrolysis of starch and pretreatment of lignocelluloses. Energy Convers Manag 2018;166:569–78. https://doi.org/10.1016/j.enconman.2018.04.067.

[29] FAO. Global losses and food waste. vol. 365. 2011. https://doi.org/10.1098/rstb.2010.0126.


Recyclable
Municipal solid waste
Organic
Rhizopus oligosporus enzymatic extract
Hydrolysis
Reducing sugars
Lipids
Non-hydrolyzed fiber
Alcoholic fermentation
Bioethanol

Declaration of interests

☒ The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

☐The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: