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Bioconversion of Agro-industrial Wastes into Xanthan Gum

Fabiana P. dos Santos[a], Antonio M. Oliveira Jr[a], Tatiana P. Nunes[a], Carlos Eduardo de Farias Silva[b], Ana Karla de Souza Abud*[a]

[a]Food Technology Department, Federal University of Sergipe, São Cristóvão, Sergipe, Brazil
[b]Department of Industrial Engineering DII, University of Padova, Padova, Italy
ana.abud@gmail.com

Green coconut shell, passion fruit peel, straw and corn cobs, agro-industrial wastes, were evaluated as alternative substrates for xanthan gum production, which is one of the most important commercial biopolymers obtained by fermentation, widely used in the food industry and with applications in the oil, petrochemical, agrochemical and pharmaceutical industry. The residues were sanitized, dried at 50 °C and milled, being subjected to self-hydrolysis with water in a ratio of 9:100 (w:v) at 121 °C for 15 minutes with subsequent filtration. The extracted broth was placed in 250 mL Erlenmeyer flasks, supplemented with urea (0.01%) and potassium phosphate (0.1%), pH adjusted to 7.0, sterilized at 121 °C for 15 min and then fermented on an orbital shaker at 150 rpm for 100 hours by the bacterium *Xanthomonas campestris* pv. *campestris* (1078). The substrate consumption and the production of xanthan gum in green coconut shells (50% and 5.5 g/L) and passion fruit (54.7% and 6.7 g/L) were significantly higher than maize straw (straw - 72.6% (1 g/L) and cob - 46.6 (2.2 g/L)).

1. Introduction

Brazil is one of the countries that generate high volumes of agro-industrial residue, contributing to the organic waste production and causing environmental and public health problems, since they are subject to degradation, insects and rodents attraction, in addition to the stench and pollution of aquatic ecosystems.
In recent years, there is a growing interest in the efficient use of these residues, motivated by the increasing of world population, growing concern to the possible environmental impacts, high rate of losses and waste generated by the food industry. Being rich in macro (sugar and fiber) and micronutrients (minerals and vitamins), these residues can serve as a source of proteins, enzymes and essential oils likely to recovery (Coelho et al., 2001; Couto; Sanromán, 2006).
Various bioprocesses have been developed using organic residues as substrates for the production of various molecules with high added value, such as microbial proteins, organic acids, ethanol, enzymes and biologically active secondary metabolites. These processes can be economically viable and also help to solve environmental problems arising from its accumulation *in nature*.
In agribusiness food, the main use of waste is as a supplement for animal feed, being well accepted by cattle and goats. However, some limitations of this biomass make its use limited, including the large amount of water they contain, which causes problems of collection, transportation and storage.
Produced by strains of gram-negative bacterium *Xanthomonas campestris*, the xanthan gum is a water-soluble extracellular heteropolysaccharide with unique rheological properties, such as highly pseudoplastic behaviour, contributes to its wide-range applications as suspending, stabilizing, thickening and emulsifying agent for food, oil, petrochemical, agrochemical, cosmetic and pharmaceutical industry.
The global market of xanthan gum expects to reach USD 987.7 million by 2020 and, due to this, there is a number of studies to improve the strains, culture media, extraction and purification processes (Machado et al., 2012; Costa et al., 2014). Commercially, the xanthan gum production uses glucose or sucrose as carbon source, making the process limited by the processing costs. The optimization of biotechnological processes from low-cost substrates, as agro-industrial waste and by-products, is the most viable alternative for the xanthan gum production, providing a solution to solid waste disposal (Reis et al., 2010; Menezes et al., 2012; Gunasekar et al., 2014).

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Some alternative sources have been suggested, such as cheese whey (Silva et al., 2009), whey (Nery et al, 2008; Silva et al., 2009; Mesomo et al., 2009; Diniz et al., 2012), cassava serum (Brandão et al, 2010), apple juice residue (Druzian and Pagliarini, 2007), cocoa residue (Diniz et al., 2012), green coconut shell (Gomes, 2008), sugar cane broth (Faria et al., 2011), treated tapioca pulp (Gunasekar et al., 2014), waste sugar beet pulp (Yoo; Harcum, 1999), shrimp cell (Costa et al., 2014) and glycerin derived from the biodiesel production process (Reis et al., 2010). The highest yields depending on the presence of other carbohydrates such as lactose and pectin and others nutrient content, as soluble proteins, vitamins and minerals (Nitschke et al., 2001; Menezes et al, 2012).

This study evaluates the feasibility of using the waste hydrolyzate broth (green coconut shell, straw and corn cob and passion fruit peel) as a substrate for the production of xanthan gum. It is an attempt to add value to a waste while reduces the environmental impact, which currently does not suffer any kind of treatment and disposal.

2. Materials and Methods

The collected residues (green green coconut shell, passion fruit peel, straw and corn cobs) were cut into small pieces, rinsed with water, sanitized with 100 ppm of sodium hypochlorite for 15 minutes and then dried at 50 °C until constant weight, ground and then stored in plastic hermetic flasks for analysis.

The ground residues were submitted to self-hydrolysis with the distilled water in a ratio of 9:100 (grams of residue per 100 mL of water), autoclaved at 121 °C for 15 minutes. The suspension was filtered through TNT filter, generating the extract and the residual biomass.

The strain of *Xanthomonas campestris* pv. *campestris* (1078) was obtained from the Culture Collection of the Institute of Biology (Campinas-SP, Brazil), kindly provided by Professor Francine Padilha, from Tiradentes University (Aracaju-SE, Brazil). The strain was cultured on yeast malt (YM) medium containing (w/v) 0.3% yeast extract, 0.3% malt extract, 0.5% bacteriological peptone and 1.0% glucose. The maintaining culture was held in YMA, being added 2.0% agar to the standard YM. The mediums were sterilized at 121 °C for 15 minutes.

To obtain the inoculum, the strain was cultured in yeast malt (YM) medium (0.3% malt extract, 0.3% yeast extract, 0.5% bacteriological peptone, and 1.0% glucose) and incubated at ambient temperature for 24 hours with agitation at 150 rpm (orbits per minute).

The hydrolyzed extracts were supplemented with 0.01% urea and 0.1% potassium phosphate, adjusting the pH to 7 and transferred to 250 mL Erlenmeyer flasks, being sterilized at 121 ° C for 15 minutes.

After 24 h of culture, 25 mL inoculum was centrifuged and cells were washed with saline sterile solution (0.85% NaCl), centrifuged and resuspended in hydrolyzed broths. The fermentation was conducted on orbital shaker at 150 rpm for 100 hours. Samples were collected each 24 hours to observe cell growth and total reducing sugar consumption.

After fermentation, *Xanthomonas campestris* cells were removed by centrifugation at 5000 rpm for 15 minutes. The xanthan gums were precipitated from the supernatants by adding 96 °GL ethanol (3:1). The gum samples were separated and transferred to plates, dried at 50 °C for 24 h and stored in a sealed flask for analysis. The yield from each residue was calculated and the values were expressed in g/L (grams of gum per liter of culture medium) (Costa et al., 2014).

The pH of extracts, reducing sugar (RS) and total reducing sugars (TRS), in the *in nature* residue as in the pre-treated material (extract and residual biomass) were measured with the purpose of verify the hydrothermal efficiency. These results were expressed by the average ± standard deviation of triplicate measure. These sugars were determined by colorimetric method of the dinitrosalicylic acid (DNS) proposed by Miller (1959), where TRS has an initial hydrolysis step with 1.5 M H_2SO_4 in boiling bath for 20 min and subsequent neutralization with 2 N NaOH. The DNS analysis consists in after boiling the reagent and the sample for 5 min and read at 540 nm, correlating with a calibration glucose curve. Cell growth was determined by measuring optical density of cells at 560 nm in the fermentation broth and correlated with dry cell weight. The samples were centrifuged at 5000 rpm for 15 min before the measurements of sugars.

3. Results and Discussion

The sugar analysis, reducing sugar (RS) and total reducing sugar (TRS), before and after pre-treatment (self-hydrolysis) are presented on Table 1.

Apart from corn straw, which generate the lowest result on RS extract, green coconut shell, passion fruit peel and corn cob showed satisfactory pre-treatment condition, being of great importance for the production of xanthan gum. In the analysis of total reducing sugars (TRS) it was found that the process of self-hydrolysis was not satisfactory for the passion fruit peel and corn straw, being necessary studies of time and temperature conditions during this pre-treatment process, since most of the sugars remained in the waste. The higher

sugar concentration was obtained from green coconut shell, both for analysis of RS and TRS, followed by corn cob, passion fruit peel and finally, corn straw.

Table 1: Sugar analysis on in nature residue and in the pre-treated material (extract and residual biomass).

Residue	Reducing Sugar (RS) ($g_{glucose}/g_{residue}$)		Total Reducing Sugar (TRS) ($g_{glucose}/g_{residue}$)		
	In nature	Extract	*In nature*	Extract	Residual biomass
Green coconut shell	0.24 ± 0.09	0.37 ± 0.01	0.46 ± 0.02	0.41 ± 0.08	0.29 ± 0.07
Passion fruit peel	0.07 ± 0.03	0.12 ± 0.05	0.41 ± 0.01	0.25 ± 0.13	0.30 ± 0.15
Corn straw	0.09 ± 0.01	0.07 ± 0.03	0.40 ± 0.23	0.12 ± 0.09	0.18 ± 0.08
Corn cob	0.06 ± 0.03	0.12 ± 0.04	0.34 ± 0.18	0,35	0.26 ± 0.14

The pH and extraction yields are shown in Table 2. The extraction yield was calculated according to the volume of extract obtained after hydrolysis. The pH of green coconut shell, corn cob and corn straw are equal to 5.0, while passion fruit peel showed a more acid pH (4.0), not desirable for xanthan gum production. The volumes obtained in self-hydrolysis were satisfactory for green coconut shell, corn straw and corn cobs. The lower volume of extract found in passion fruit peel occurred in function of the gelling pectin, component present in large quantities in passion fruit peel, making difficult the extraction of self-hydrolysis.

Table 2: pH and extraction yield after pre-treatment

Residue	pH	Extract yield (%)
Green coconut shell	5.0	53
Passion fruit peel	4.0	40
Corn straw	5.0	57
Corn cob	5.0	55

The extracts derived from hydrolysis were fermented for the production of xanthan gum. In Figure 1 is presented the bacterial cell growth and in Figure 2 the consumption of sugars during the fermentation process.

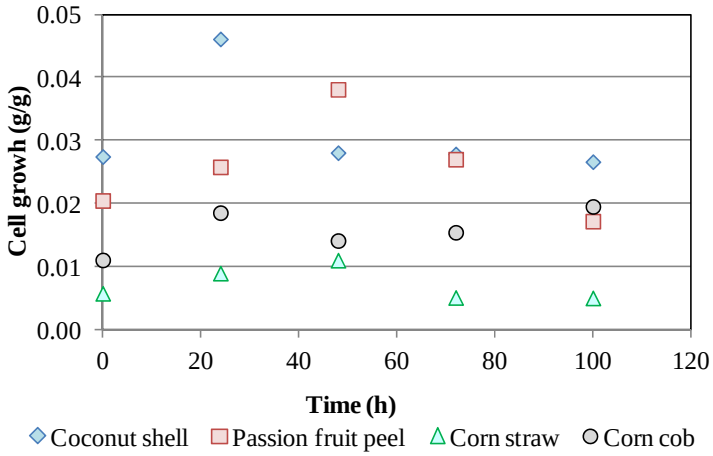
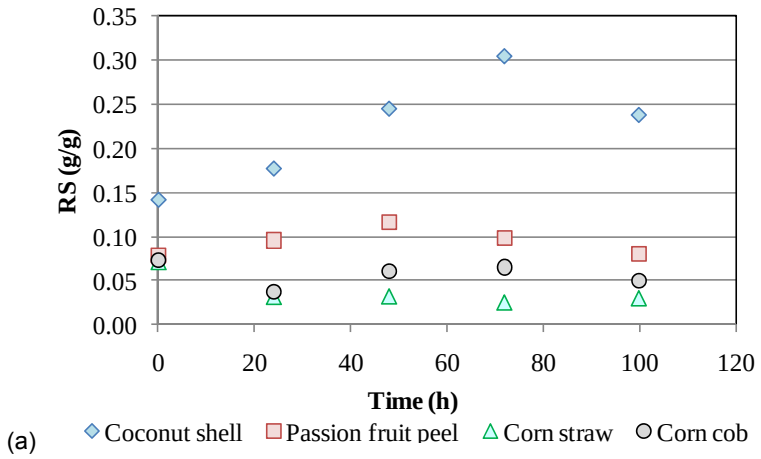


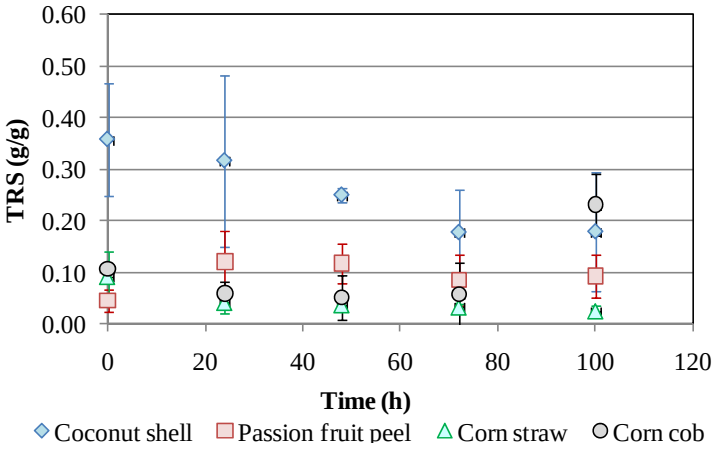
Figure 1: Cellular growth of Xanthomonas campestris from waste hydrolysis extract versus time.

It can be observed that after 24 hours of incubation, the growth of *Xanthomonas* in green coconut shell and corn cob were the best, while in the extracts corn straw and passion fruit peel the growing time occurred at 48 hours. The low growth of bacteria is possibly due to the lack of nutrients as well as to non-optimal conditions, such as the continuous agitation and temperature.

The analysis of reducing sugars (RS) from coconut husk showed a different result than expected, an increase in the glucose concentration present, that may be due to hydrolysis of substrate during the fermentation. Peel of passion fruit and corn cobs have an equivalent behavior. The corn straw, probably due to the low sugar, presented a slightly decreasing behavior.



(a)



(b)

Figure 2: Substrate consumption of Xanthomonas campestris from waste hydrolysis extract versus time: (a) reducing sugar (RS) and (b) total reducing sugar (TRS).

The TRS analyzes (Figure 2), indicate that the consumption of green coconut shell had the expected profile, i.e, hydrolysis of the sugar over the first 72 hours, justifying the growing profile of RS.
Passion fruit peel, probably in function of pectin hydrolysis, had an increase in the first 48 hours, followed by decay of total reducing sugars content.
Table 3 shows the quantity of sugars consumed in each extract fermented and the biopolymer yield after centrifugation, precipitation with ethanol and drying.

Table 3: TRS consumed and xanthan gum produced in the fermentation process

Residue	TRS Consumed (%)	Xanthan gum production (g/L)
Green coconut shell	50.0	5.5 ± 3.8
Passion fruit peel	54.7	6.7 ± 1.9
Corn straw	72.6	1.0 ± 0.04
Corn cob	46.6	2.7 ± 0.1

The extract of corn straw showed the largest consume and the lowest concentration of glucose present. As in the xanthan gum fermentation process it was added only urea and phosphate, this may have influenced the biopolymer production and, consequently, the low yield obtained.
Green coconut shell and passion fruit peels showed better performances, with moderate sugar consumption and higher xanthan gum productions. Passion fruit peels had a more consistent biopolymer because of the probable fractionating into pectin, which was converted to xantham gum. Bilanonovic et al. (1994) observed that xanthan gum from the pectin fraction was similar to that of the whole citrus waste, with the maximum of 9 g/L.

Nery et al. (2013), evaluating different scales of fermentation (shaker at 28 °C, 250 rpm for 60 h and bioreactor at 28 °C, 400 rpm, 0.5 vvm aeration and pH 7.0 for 60 h) in green coconut shells as the only carbon source, reach around 1.7 and 11 g/L of xanthan, respectively. Druzian et al. (2009), in the patent PI 0701765-0, also used green coconut shells and passion fruit as waste for xanthan gum productions of 5.2 to 13.3 g/L for green coconut shell and 3.5 to 8.6 g/L for passion fruit peel. These results are near to the obtained in this study, which differ by the use of self-hydrolysis as a pre-treatment, indicating that these residues are a good carbon source for the polysaccharide production.

The best results of xanthan gum from agro-industrial wastes were obtained from Druzian and Pagliarini (2007), using apple juice residue fermented at 28 °C and 250 rpm for 120 hours, reaching a biopolymer production of 45 g/L. Mesomo et al. (2009), in a bioreactor operating at 28 °C, initial pH 7.2, 390 rpm agitation and aeration of 1.5 vvm, obtained 36 g/L of xanthan gum employing cheese whey as carbon source.

These results shows the necessity of optimize the process conditions, especially in the carbon and nutrients sources, as also agitation and dissolved oxygen. However, it is also important evaluate the rheological properties and functional groups of the biopolymer.

4. Conclusions

Among the four residues used, the green coconut shell presented the best carbon source concentration and substrate consumption, with an average yield of xanthan gum of 5.5 g/L. Corn straw not generate large quantities of sugars in the pre-treatment causing less yield of gum (1.0 g/L), which may be due to lack of micronutrients. The passion fruit peel also showed significant amounts of xanthan gum (6.7 g/L). Apart from the product yield it is necessary evaluate their rheological properties and functional groups to identify the best carbohydrate source and use.

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