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Article

Nanocellulose and Bioethanol Production from Orange Waste using Isolated Microorganisms

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A biomassa procedente do processamento de laranjas (CPWO) foi utilizada na produção de nanocelulose e do etanol da segunda geração. Primeiramente, vinte micro-organismos foram isolados da CPWO e suas capacidades de fermentação foram testadas. Dois destes, identificados como *Candida parapsilosis* IFM 48375 e NRRL Y-12969 (ATCC 22019), foram selecionados para a posterior fermentação. A biomassa foi destilada a vapor para o isolamento do óleo essencial (1,5% g g^{-1} de CPWO seco) e convertida em uma mistura de açúcares fermentáveis (40% g g^{-1} de CPWO seco) usando hidrólise ácida ou enzimática. Os hidrolisados foram fermentados utilizando micro-organismos isolados e a *Saccharomyces cerevisiae*. A levedura *Candida parapsilosis* IFM 48375 foi a mais eficiente na fermentação dos açúcares obtidos desta biomassa e os maiores rendimentos de bioetanol (21% g g^{-1} de CPWO seco) foram alcançados. A nanocelulose (2,5% g g^{-1} de CPWO seco) e as nanofibras (0,5% g g^{-1} de CPWO seco) foram isoladas partindo de bioresíduos vindo da hidrólise enzimática e da fermentação alcoólica e este resultado é o primeiro do gênero.

Citrus processing waste from oranges (CPWO) was explored for the production of nanocellulose and bioethanol. After the isolation of 20 microorganisms from CPWO, their fermentation abilities were screened and two microorganisms identified as *Candida parapsilosis* strains IFM 48375 and NRRL Y-12969 (ATCC 22019) were selected for a further fermentation. The CPWO was steam distilled for the isolation of essential oil (1.5% g g^{-1} of dry CPWO) and converted into a mixture of fermentable sugars (40% g g^{-1} of dry CPWO) using acid or enzymes hydrolyses. Hydrolyzates were fermented with three different yeast strains, the two *Candida* sp. and *Saccharomyces cerevisiae*. *Candida parapsilosis* strain IFM 48375 accomplished excellent results in ethanol production (21% g g^{-1} of dry CPWO) from CPWO, higher when compared to other strains. Nanocellulose (2.5% g g^{-1} dry CPWO) and nanofibers (0.5% g g^{-1} dry CPWO) were isolated from solid residues obtained from enzymatically treated and fermented CPWO. To the best of our knowledge, this work reports for the first time the nanocellulose production from CPWO.

Keywords: citrus processing waste from oranges, nanocellulose, bioethanol, fermentation

Introduction

Recycling of agricultural and agro-industrial waste is growing in importance as a way to diminish the environmental impact caused by industrial and urban activities. Many recycling projects have been performed worldwide and also in Brazil. Among them, stand out the reuse of sugarcane bagasse and paper.[1,2] Orange juice is one of the most popular juices in the world and Brazil produces annually approximately 22 million tons of oranges, with the state of Sao Paulo generating alone more than 17 million tons.[3] After juice extraction, around half of the fruit weight becomes citrus processing waste from oranges (CPWO), a very interesting low cost material already used for producing 1G-bioethanol.[4-8] Botanically, CPWO is composed of peel, rag (segment membranes and cores), juice sacs, and seeds. Chemically, despite being a food industry residue, CPWO contains high value compounds like soluble sugars, pectin, proteins, appreciable amounts of fiber (cellulose and hemicelluloses), and lignin. CPWO can also be an important source of limonene that can be isolated as a by-product in relatively high yield.[9] CPWO

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is a good material for 2G-bioethanol production through either an acidic or an enzymatic hydrolysis followed by subsequent fermentation with *Saccharomyces cerevisiae*.[10,11] For example, in a fermentation of ground orange peels avoiding any pre-treatment it was found that the submerged fermentation provided superior ethanol yields compared to the solid-state fermentation.[4,7,10-12] The maximum ethanol yields from CPWO in a submerged fermentation method were 1.3% (v v^{-1}) and 1.4% (v v^{-1}) when *Saccharomyces cerevisiae* and *Candida albicans* were applied, respectively.[12]

Hydrolysis of CPWO with enzymes (cellulase, pectinase, and β-glucosidase) followed by fermentation with *Saccharomyces cerevisiae* and some bacteria or genetically modified microorganisms gave excellent quantities of ethanol.[13-16] Using orange peel waste in a novel lab-scale direct steam injection apparatus, depolymerization with a commercial cellulase, and fermentation with a *Saccharomyces cerevisiae* strain led to the high glucose and ethanol yields (50% m m^{-1}).[17] Also, a scale up experiment resulted in an excellent ethanol productivity (6 g L^{-1} h^{-1}). The final overall process yield (mass balance) at the bench-reactor scale of 140 L of bioethanol per metric ton of dry orange peel waste was reported.[17]

Although efficient processes starting from acid hydrolyzates of CPWO are already used for bioethanol production in the USA,[18-21] there are some serious fermentation problems still unsolved. Yeast cultures such as *Saccharomyces cerevisiae* can be inhibited in some cases.[11] For example, when very high sugar content, nutrient deficiency, very high or low temperature[22] and inhibitors[23,24] are present there is inefficient production of ethanol. Even if these limiting factors are absent, fermentation may cease prematurely due to substances produced by yeast during the normal course of fermentation,[25] like the ethanol obtained during fermentation.[22] Therefore, a search for the new microorganism strains and process improvement are still an open field of investigation.

Some interesting results, not directly linked to the CPWO, have also been published on nanocellulose[26] isolation from the solid residues of the cellulosic biomass remaining after processing for bioethanol production.[27,28] Also, bio-residues from wood fermentation to ethanol were used as raw material for industrial production of cellulose nanowhiskers,[29] and orange was used for obtaining microcrystalline cellulose.[30] Production of nanocellulose fibers and application of nanocellulose for composite materials have brought attention to valuable nanocellulose properties, such as high strength, light weight, unique morphology, biodegradability and renewability.[26] For example, nanocellulose has great applicability when used in composite materials because of its high stiffness as reinforcing material.[26] This biomaterial can be used for polymer composites and plastics, films, foams and gels, cosmetics, thickener and emulsion, implant material, biodegradable tissue scaffold, as a drug delivery vehicle, filter paper, concrete, for oil recovery, water treatment, transport, electronics devices, solar panels, paint pigments and ink, screens and coatings, among other applications.[31,32]

Our study had two aims: to evaluate the potential of CPWO for production of nanocellulose after acidic and enzymatic hydrolyses, and to investigate the feasibility of bioethanol production by fermentation of CPWO using selected microorganisms isolated from CPWO (*natural habitat*).

Experimental

Citrus processing waste (CPWO)

Citrus processing waste from oranges (*Citrus sinensis* (L) *osbeck*) as squeezed orange fruit was obtained from a local restaurant (Valinhos, SP, Brazil). Material was ground[33] with a food homogenizer to around 2 mm in diameter particles and stored at −20 ºC until use. From ground CPWO residues were determined moisture and ash contents using AOAC methods.[34] Pectins were extracted and analyzed according to Sudhakar and Maini method.[35] Acid detergent fiber (ADF), neutral detergent fiber (NDF) and acid detergent lignin (ADL) were determined with an Ankom 200 fiber analyzer. Acid detergent fiber (ADF) value refers to the cellulose and lignin contents. Neutral detergent fiber comprises ADF fraction plus hemicellulose. The difference between ADF and ADL was considered as cellulose, whereas difference between NDF and ADF was reported as hemicelluloses. All analyses were performed in triplicate.

Analytical Methods

Powdered CPWO residues were analyzed using X-ray diffractometer (XDR 6000, Shimadzu), at a scanning rate of 2 degree min^{-1}, Cu K_α radiation (λ = 1.54 Å) in the range of 2θ = 2º-60º with increments of 0.02º. Sugars and ethanol were quantified using a HPLC system (Waters, USA) with a refractive index detector (RID), photodiode array detector (DAD), a pre-column SH-G (6 × 50 mm) and a column (Shodex 1011, 300 mm × 8 mm) (Showa Denko, Japan). The column oven was maintained at 50 ºC. Degassed mobile phase containing 0.005 mol L^{-1} of sulphuric acid was used at a flow rate of 0.6 mL min^{-1}. Sugar peaks were detected based on the retention times of standards [glucose, fructose,

sucrose, xylose, galactose, 5-hydroxymethylfurfural (5-HMF), furfural] purchased from Synth and Sigma Aldrich. Ethanol fermentation broth contains mostly sugars and ethanol. Among these, the major components were identified separately by obtaining chromatograms of the individual components under the same conditions. The calibration curves were generated from the chromatograms of a series of standard mixtures at several concentrations. The relationship between peak area and the concentration was linear over the entire concentration range examined. Peak height vs. concentration was also linear over the same range. ^{1}H and ^{13}C NMR spectra were acquired on a Bruker Avance instrument operating at 250 MHz (^{1}H) and 62.5 MHz (^{13}C) using a 5 mm sample tube. These spectra were used to analyze the quality of the bioethanol. Scanning electron microscopy (SEM) analyses (JSM-6360LV, JEOL, Japan) were used to examine the bioresidues from the CPWO fermentation processes, as well to examine the yeast cells.

Isolation of microoganisms

Microorganisms were isolated from CPWO using solid media isolation procedure where a sample of CPWO was transferred to a Petri plate with a sterile solid medium containing yeast extract-peptone-dextrose (YPD) agar (20.0 g L^{-1} dextrose; 10.0 g L^{-1} yeast extract; 20.0 g L^{-1} peptone; 15.0 g L^{-1} agar and 30 mg mL^{-1} chloramphenicol) and incubated at 30 ºC for 24-48 h. After incubation, individual cells that have grown separately into discrete colonies were picked and transferred again to YPD agar and incubated at 30 ºC for 24-48 h. We have repeated this procedure for four times and obtained the colonies of a single species that were studied separately from all others.

Limonene extraction

Limonene was extracted from CPWO by steam distillation. Initially grounded CPWO was placed into 100 mL of water and kept under distillation temperature of 97-98 ºC. This way, water (steam) was introduced into the distillation apparatus, providing depression of the boiling points of limonene and other orange essential oil components. Then, after distillation, vapour was condensed yielding a two-phase system of water and limonene (approximately 80 mL). Quantity of total oil was measured applying Scott oil analysis by bromated titration.[36]

Acid hydrolysis

To elenmeyer flasks (250 mL) containing thawed CPWO residues (17.0 g), distilled water and sulphuric acid (98%) were added to reach an acid concentration of 0.5, 0.1 or 1.5% (m v^{-1}) to a final 100 mL volume. Next, samples were heated in an autoclave at 120 ºC (15 or 30 min). The hydrolyzates were analyzed for sugars, furfurals, acetic acid, and total phenols using HPLC. The treatment that enabled the highest sugar contents in hydrolyzates was selected and used for following fermentations. All acid hydrolysis experiments were carried out in triplicate.

Enzymatic treatment

To erlenmeyer flasks (250 mL) containing thawed CPWO (17.0 g) or autoclaved residues (17.0 g) and distilled water (total volume 100 mL) was added a cocktail of enzymes: pulpzyme HA (Novozyme), celluclast 1.5 L. (Novozyme) and β-galactosidase from *Aspergillus oryzae* (Sigma). Next, the slurries were incubated at 50 ºC during 48 h. The collected hydrolyzates were analyzed by HPLC. The enzymatic treatment that enabled the highest sugar yields from CPWO was selected as the best and was used for subsequent fermentations. Enzyme loadings were: 3.4 mg of pulpzyme HA, 3.4 mg of celluclast 1.5 L and 0.6 mg of β-galactosidase (*A. oryzae*) *per* g of CPWO. The enzymatic activities were measured as described by Ghose[37] for celluclast 1.5 L and pulpzyme HA. For β-galactosidase (*A. oryzae*), the modified method of Sigma from Kuby and Lardy[38] and Borooah *et al.*[39] was applied. The enzymatic treatment procedures were repeated three times.

After being hydrolyzed, the CPWO samples were heated to 105 ºC for 15 min to inactivate enzymes and subsequently fermented to ethanol using three different types of yeast.

Yeast cells and fermention

The commercial yeast (*Saccharomyces cerevisiae*, Sigma Aldrich) and two isolated microorganisms from CPWO, *Candida parapsilosis* IFM 48375 and *Candida parapsilosis* NRRL Y-12969, were aseptically inoculated into sterilized erlenmeyer flasks (150 mL) containing 50 mL of glucose yeast extract broth (GYE), and chloramphenicol (30 mg mL^{-1}). The flasks were incubated at 30 ºC for 24 h in a shaker with 100 × g. The inoculum was aseptically transferred to sterile erlenmeyer flasks (250 mL) containing 100 mL of GYE broth supplemented with 30 mg mL^{-1} of chloramphenicol, and flasks were incubated at the same conditions as cited above for 24 h. The cells were concentrated by centrifugation in sterilized centrifuge tubes at 10,000 × g and 15 ºC for 10 min. The cell count was determined using a Newbauer plate. Cells were concentrated to the level of 5.8×10^{8} cells mL^{-1} to

have 3.7 × 10^7 cells mL^{-1} in the final fermentation media at 10% (v v^{-1}).

Fermentations were executed in erlenmeyer flasks (250 mL) containing either acid or enzymatic hydrolyzed samples (100 mL), where after adjusting the pH to 5.0 (calcium carbonate), 10 mL of yeast inoculums were added. Each sample was incubated at 35 ºC for 72 h with agitation. Fermentation flasks were sampled at 0, 0.5, 1, 3 and 48 h to measure sugar and ethanol concentrations using HPLC. Individual experiments were carried out in triplicate.

Isolation of nanocellulose

Residue from the enzymatic hydrolysis of CPWO was filtered, washed with distilled water to remove the sugars and other soluble compounds and dried. Isolation of nanocellulose[40] started with bleaching of this solid residue using 4% m v^{-1} NaOH for 20 min at 120 ºC, that, after was washed with water, was treated with 1.7% m v^{-1} $NaClO_2$ (pH 4.5) for the next 20 min at 120 ºC and washed again. This way delignification and removing hemicelluloses, pectin and lignin was done.[31] The next procedure was defibrillation to nanocellulose using sonification (Ultrasonic processor, Sonics) operating at 750 Watt, 20 kHz and 4 J, using 20% of the pulse power for 10 min in an ice bath to avoid overheating. The fibrillated nanocellulose thus obtained was filtered through micro filter holder assembly (Merck, Millipore), washed with distilled water and dried. The bioresidue after fermentation was treated in the same way for nanocellulose production.

Results and Discussion

Our research started with successful isolation of 20 microorganisms from CPWO biomass that were screened for fermentation abilities. Two of the twenty isolated yeasts were selected due to their ability to ferment and produce ethanol. These two cultures were examined using scanning electron microscopy (SEM), and identified using sequencing tools and phylogenetic analysis of genes from ribosomal 18S *operon* as *Candida parapsilosis* IFM 48375 and *Candida parapsilosis* NRRL Y-12969 (ATCC 22019), at the Chemical, Biological and Agricultural Pluridisciplinary Research Center (CPQBA, Campinas, Brazil).

Second step in our research included thorough characterization of orange waste. CPWO contains significant amounts of water (73% m m^{-1}), proteins (7% m m^{-1}), lignin (3% m m^{-1}) and hydrolysable polysaccharides, pectin (9% m m^{-1}), cellulose (3% m m^{-1}) and hemicelluloses (5% m m^{-1}). CPWO samples were then treated for essential oil removal and oil yield was 2.5 g kg^{-1} of dry CPWO, a lower result when compared with other studies.[10,23,41] The CPWO compositional analysis has shown that several parameters, such as species of orange, climate (temperature, humidity), soil, infection and pests, planting methods and harvesting time, among others, influenced the quantities of pectin, cellulose, hemicellulose and lignin of a target biomass.[4,7,18] CPWO from Brazil has only half or less quantities of pectin when compared to data published on citrus wastes,[14] probably because in a tropical climate fruits needed less time to grow and become ripe.

Removal of essential oils from CPWO was an important step performed to avoid yeast inhibition during fermentation and to assure the best possible sugar conversion to alcohol.[10,14,21,42] Somewhat lower essential oil quantities (up to 1.5% g g^{-1} of dry CPWO) found in Brazilian CPWO were expected due to very high average temperatures and an easy limonene oxidation in moist air.

Then, sulphuric acid hydrolysis of CPWO was tested varying two process parameters: acid concentration and reaction time. In most trials, high concentrations of glucose (15.1 ± 0.5% g g^{-1} of dry CPWO), fructose (12.6 ± 0.3% g g^{-1} of dry CPWO), cellobiose (7.0 ± 0.3% g g^{-1} of dry CPWO) and arabinose (5.3 ± 0.2% g g^{-1} of dry CPWO) were produced. The most efficient were lower acid concentrations (0.5 and 1% m v^{-1}) and shorter hydrolysis time (15 min), as the sugar yields declined with increased acid levels in the reaction.

The enzymatic hydrolysis was carried out at 50 ºC and pH 4.0, similarly to previous reports,[18,19,43,44] that corresponded to activities of celluclast 1.5 L, β-galactosidase and pulpzyme HA of 68.5 FPU g^{-1}, 0.42 U mg^{-1} and 65.7 FPU g^{-1}, respectively (U is defined as a measure for enzyme activity and represents micro mol of product, D-glucose, formed *per* min.; mg^{-1} and g^{-1} are quantities of enzymes used for such activity, i.e., specific enzyme activity). Enzymatic saccharification of CPWO biomass also provided excellent yields of monomer sugars (up to 20.6% wt of dry CPWO).

The acid and enzyme hydrolyzed samples were inoculated with three yeast strains: *Saccharomyces cerevisiae* and two isolated *Candida parapsilosis* sp. strains, in independent experiments conducted at 35 ºC during 48 h.

High-ethanol yields (Table 1) from 7.4 to 10.7% m m^{-1} dry CPWO were observed on acid hydrolyzates with all yeasts. Only exception was when *Saccharomyces cerevisiae* with 1.0% (m v^{-1}) CPWO hydrolyzate was under trial. Ethanol productivity was lower when compared with other studies,[14] what could be explained due to the presence of 5-hydroxymethylfurfural in concentration of 0.6-0.8 g L^{-1}, a known inhibitor. Ethanol yields of fermented hydrolyzates were dependent on the type of the yeast applied (Table 1).

Table 1. Fermentation of CPWO acid hydrolyzates with yeasts

Yeast	*Saccharomyces cerevisiae*		*Candida parapsilosis* IFM 48375		*Candida parapsilosis* NRRL Y-12969	
Acid hydrolyzate	0.5% (m v^{-1})	1.0% (m v^{-1})	0.5% (m v^{-1})	1.0% (m v^{-1})	0.5% (m v^{-1})	1.0% (m v^{-1})
Quantity of ethanol produced in / g from 4.2 g of dry CPWO	0.45 ± 0.02	0.31 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	0.37 ± 0.01	0.35 ± 0.01
Ethanol productivity[a] / (g L^{-1} h^{-1})	0.10 ± 0.02	0.07 ± 0.02	0.07 ± 0.01	0.07 ± 0.01	0.08 ± 0.01	0.07 ± 0.01
Ethanol yield[b] / %	10.70 ± 0.5	7.40 ± 0.5	8.3 ± 0.3	8.3 ± 0.2	8.8 ± 0.4	8.3 ± 0.3

[a]Ethanol produced (g L^{-1}) divided by total fermentation time (h); [b]EtOH yield (%) = (ethanol produced in g from 4.2 g dry CPWO/4.2) × 100, where 4.2 = dry weight corresponding to 17 g of CPWO.

All studied yeast strains consumed primarily glucose and later fructose, but they did not consume cellobiose. The consumption of glucose from the acid (0.5% m v^{-1}) hydrolyzed sample using *Candida parapsilosis* IFM 48375 fermentation decreased dramatically compared to other sugars, and the yield of bioethanol in this case was 0.35 g starting from 4.2 g of dry CPWO (17 g of CPWO *in natura*) or around 42% of maximum expected yield considering that all sugars are glucose (1.7 g). The *Saccharomyces cerevisiae* fermentation from the acid (1.0% m v^{-1}) hydrolyzed sample resulted in lower ethanol yield (7.4% m m^{-1} dry CPWO or 0.07 g L^{-1} h^{-1}) compared with the *Candida parapsilosis* IFM 48375 fermentation (8.8% m m^{-1} dry CPWO or 0.08 g L^{-1} h^{-1}) and after 30 min of incubation, there was a slight decrease in carbohydrate consumption, which remained stable during the 48 h of fermentation.

Ethanol yields on substrate obtained in fermentations of enzymatic hydrolyzates are shown in Table 2 and varied from 17.9 to 20.2% (m m^{-1} dry CPWO). Isolated microorganism strains *Candida parapsilosis* IFM 48375 and NRRL Y-12969 accomplished excellent results in ethanol production from CPWO, higher or equal when compared to *Saccharomyces cerevisiae*, respectively. The ethanol productivity and yields, when compared to CPWO acid hydrolyzates (Table 1) were almost doubled. After the end of the fermentation processes, the fermented samples were distilled, and then analyzed by ^{1}H and ^{13}C NMR, and the spectra are shown in the Supplementary Information (SI) section (Figure S1).

Yeasts are excellent biotransformers, including for the fermentation of sugar to ethanol. The conventional yeast *Saccharomyces cerevisiae*, although commonly used in the fermentation industry,[2,13,14] is unable to ferment some unconventional sugars and could be inhibited by limonene traces still present in a treated CPWO biomass.[10] Therefore, screenings to isolate yeasts capable of fermenting different type of biomass is extremely important for biotechnological applications linked to production of second-generation biofuels. In this work, 20 microorganisms were isolated and two of these had great ability to ferment and to produce ethanol from CPWO during the fermentations. Excellent results were obtained using both species of *Candida parapsilosis* that could indicate an elevated tolerance to the fermentation process (bioethanol) of this strain. More detailed studies will be conducted to elucidate this hypothesis. The bioethanol generated in fermentations of citrus processing waste from oranges (CPWO) showed to be extremely pure and without presence of other compounds.

The X-ray diffractogram of the ground, dried and lyophilized CPWO is indicative for an amorphous crystalline material (data not shown).[33] In the cases of the residues obtained using acid and enzyme hydrolyses, higher crystallinity was observed, compared to the dried and lyophilized CPWO X-ray diffractogram. After enzymatic hydrolysis of CPWO, a brown residue was obtained that was washed with distilled water and dried. This crude residue was treated for lignin and hemicelluloses removal. The remaining biomaterial contained nanocellulose and nanofibers (ca. 200

Table 2. Fermentation of CPWO enzymatic hydrolyzates with yeasts

Yeast	*Saccharomyces cerevisiae*	*Candida parapsilosis* IFM 48375	*Candida parapsilosis* NRRL Y-12969
Quantity of ethanol produced / g from 4.2 g of dry CPWO	0.75 ± 0.04	0.85 ± 0.02	0.76 ± 0.03
Ethanol productivity[a] / (g L^{-1} h^{-1})	0.15 ± 0.03	0.18 ± 0.04	0.16 ± 0.02
Ethanol yield / %[b]	17.9 ± 0.2	20.2 ± 0.3	18.1 ± 0.2

[a]Ethanol produced (g L^{-1}) divided by total fermentation time (h); [b]EtOH yield (%) = (ethanol produced in g from 4.2 g of dry CPWO/ 4.2) × 100; where 4.2 = dry weight corresponding to 17 g of CPWO.

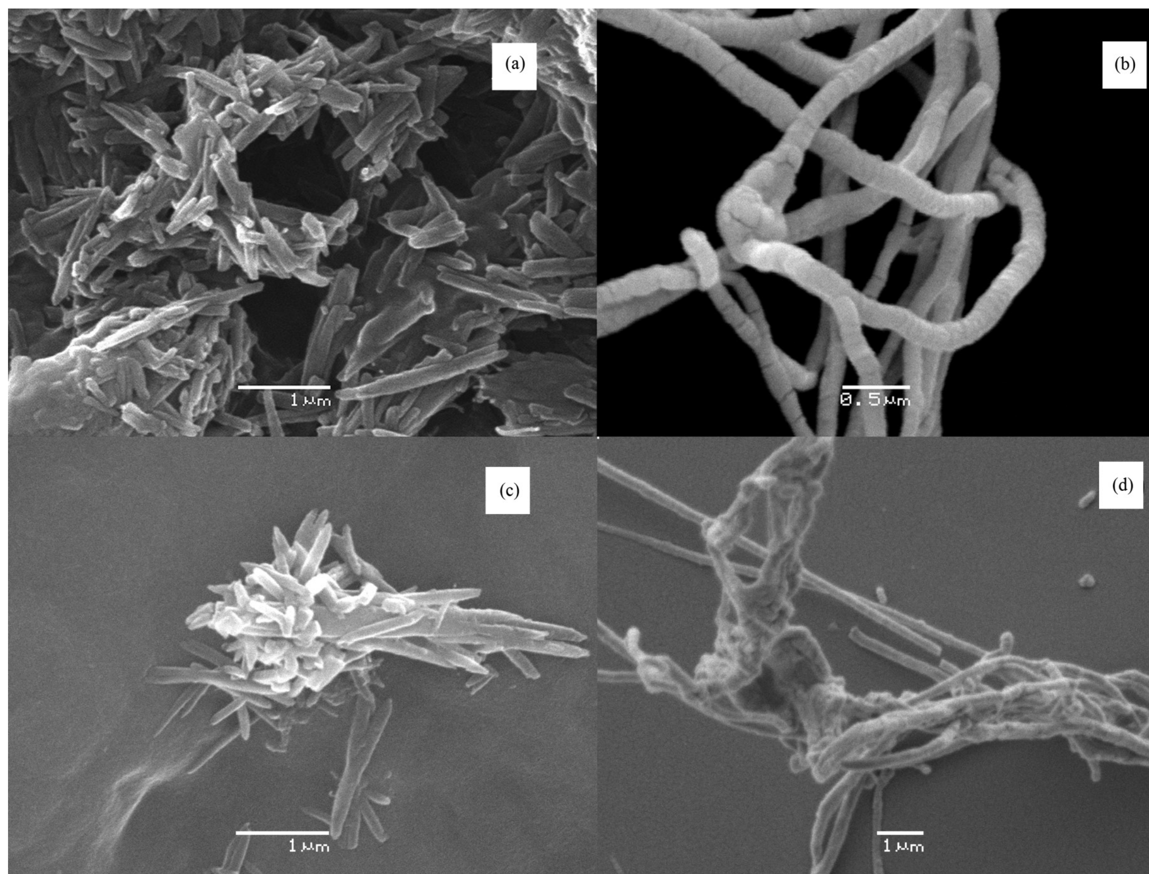

Figure 1. Scanning electron micrographs (SEM) of nanocellulose obtained from citrus processing waste from oranges (CPWO): (a) nanocellulose from enzymatic hydrolysis, (b) isolated nanofiber from enzymatic hydrolysis, (c) nanocellulose from fermented enzymatic hydrolyzate and (d) isolated nanofiber from fermented enzymatic hydrolyzate.

nm of widths) and the Figure 1 shows the SEM images of this biomaterial. The average nanocellulose sizes were around 180 nm in width and 1.3 mm in length (Figure 1a). The final residue after fermentation of enzymatic hydrolyzates also contained a nanocellulose of 150 nm width and 1.25 µm in length (Figure 1c) in 1.2% of dry CPWO and nanofiber after purification in a very low yield of around 263 nm width and ca. 13 µm in length (Figure 1d). This is the first time, to our knowledge, that biomass from CPWO is reported as a source of nanocellulose, potentially adding value to CPWO.

Although several researchers investigated shapes and size distributions of nanocrystals of cellulose obtained in hydrolysis of different type of fibers using acid and/or enzymes,[26,29-31,40] this is the first time that biomass from CPWO is reported as a source of nanocellulose, potentially adding value to CPWO. The quantity of this nanobiomaterial is approximately 3% of dry CPWO. Interestingly, nanocellulose crystals and fibers are obtained exclusively from enzymatically hydrolyzed CPWO residue and also from residue after fermenting enzymatically obtained CPWO hydrolyzates. Therefore, CPWO should be explored further as raw material for producing nanocellulose.

Conclusions

The citrus processing waste from oranges (CPWO) is presented herein as a valuable bioresource for obtaining: essential oil (D-limonene, 1.5% g g^{-1} of dry CPWO), bioethanol (20% g g^{-1} of dry CPWO) and nanocellulose (3% g g^{-1} of dry CPWO) expressed on dry mater basis. The viability of acid and/or enzymatic hydrolysis of this biomass was explored and the use of yeasts other than *Saccharomyces cerevisiae* in the fermentation of the hydrolyzates was compared. CPWO acid and enzymatic hydrolysis generated a mixture of glucose, fructose, arabinose and cellobiose. Enzymatic hydrolysis produced significant amounts of glucose and fructose, more than acid hydrolysis and no sub-products like furfurals. Fermentation processes indicated that the yeast *Candida parapsilosis* IFM 48375 was the most suitable to ferment sugars providing the highest bioethanol yields. A relevant feature of this work is that nanocellulose and nano-fibers were isolated from the solid residues from enzymatically treated CPWO in yields of 3% (g g^{-1} of dry CPWO).

Supplementary Information

Supplementary Information (NMR spectra of bioethanol obtained from citrus processing waste from oranges) is available free of charge at http://jbcs.sbq.org.br as a PDF file.

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