Porous cellulosics from leaves/bark of *Shorea robusta* G. & *Mangnifera indica* L., and husk of *Oryza sativa* L., of Indian origin, for bioprocess applications: textural characterization

M.N. Kumar[1], A. Bekatorou[1,]*, A.-I. Gialleli[1], K. Pissaridi[1], A.A. Koutinas[1], M. Kanellaki[1], C. Kordulis[1], J. Tirkey[2]

[1] Department of Chemistry, University of Patras, Patras 26500, Greece
[2] Central Tassar Research & Training Institute, Piska Nagri Ranchi India
Keywords: Indian cellulosic wastes, porous cellulose, delignification, carbonization, textural characterization
Presenting author email: abekatorou@upatras.gr

The need for innovative and sustainable technologies in food bioprocessing has brought upon a great interest on the use of cheap renewable resources such a waste lignocellulosics. Wood and husk are such materials that have been used for the production of porous cellulosics, after delignification, to design (a) natural filters for microbial load removal from liquid foods in "*cold pasteurization*" processes (Kumar *et al* 2016), and (b) immobilized cell biocatalysts to advantage bioprocesses such as alcoholic and lactic acid fermentations (Kumar *et al* 2014a) (Fig. 1). Further to these studies, this investigation aims to present a comparison of textural characteristics of the leaves, bark sawdust, and husk of three plant species of Indian origin, i.e. *Oryza sativa* L. (rice), *Mangifera indica* L. (mango tree), & *Shorea robusta* G. (sal tree), by chemical, porosimetry (BET), SEM, FTIR, and XRD analysis. Novel as well as previous data on the chemical/textural properties of the DCs are highlighted and reviewed (Kumar *et al* 2016, 2014a,b).

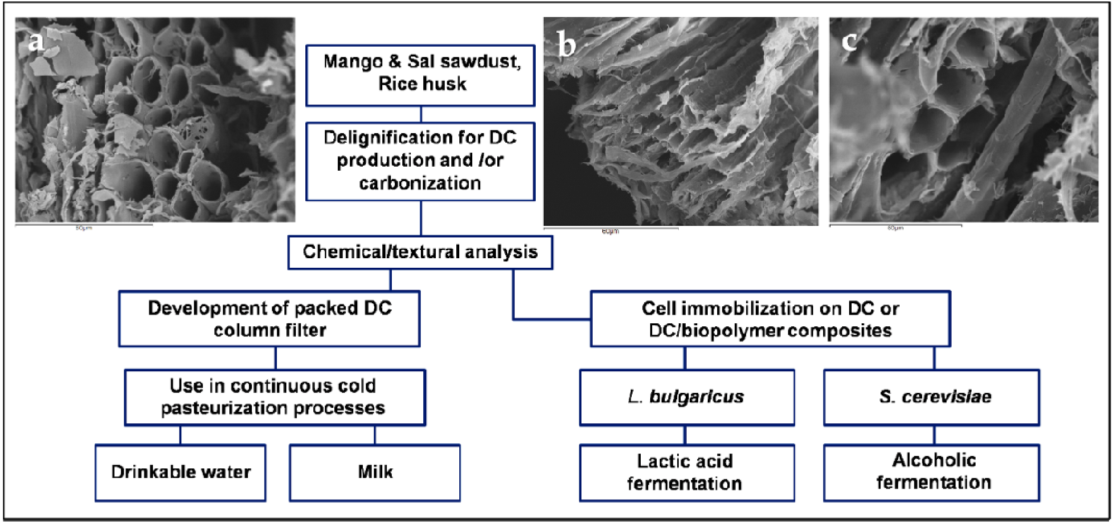

Figure 1. Bioprocess development based on porous DCs of Indian origin. SEM images of DCs: (a) Mango sawdust (b) Rice husk, and (c) Sal sawdust (Kumar *et al* 2016, 2014a,b).

Mango, sal wood sawdust and rice husk were delignified by boiling in 1% w/v NaOH (Koutinas *et al* 2012). The produced delignified cellulosics (DCs) were washed thoroughly and dried at 37°C prior to analysis. Carbonization of the DCs, to increase their porosity, was done at 350°C under nitrogen flow (0.6 L/min, 1.5 bar) for 6 and 12 h. Total nitrogen and crude protein determination was carried out using the Kjeldahl distillation method. For ash determination, amounts of each powdered DC were initially burned in a flame and then ashed at 550°C for 3-5 h. The acid soluble lignin content of the materials before and after the delignification, was assayed by National Renewable Energy Laboratory (NREL) methods. Scanning Electron Microscope (SEM) images of the DCs were taken on a model JSM-6300 (Japan) microscope after coating with gold in a Blazers SCD 004 Sputter Coater. The thermal stability of the DCs was carried out under nitrogen flow (200 mL/min) on a Perkin Elmer Diamond Thermogravimetric Analysis/Differential Thermal Analysis (TGA/DTA) system. Amounts of the DCs were dried at 100°C until constant weight for moisture removal, and then the temperature was increased to 1000°C (10°C/min). Porosimetry analysis was done by nitrogen adsorption-desorption experiments at −196°C on a Tristar 3000 porosimeter. The specific surface area was determined using the method of Brunauer, Emmet & Teller (BET), and the pore size and diameter distributions were quantified via the Barret Joyne & Halenda (BJH) method. FTIR analysis was carried out on both carbonized and non-carbonized DCs by dispersing the powdered samples in KBr pellets. IR spectra were recorded in the 4000-500 cm^{-1} region on a Perkin-Elmer spectrometer with a resolution of 4 cm^{-1} and 10 scans per sample. Background spectrum was obtained using a TC/KBr pellet. Finally, in order to determine the crystalline nature of the DCs, XRD patterns were obtained on an Enraf Nonius FR590 diffractometer with CuKa radiation generation. The intensity was measured between 2θ of 5-60°. The crystallinity index (CI) was calculated from the heights of the 200 peak (I002, 2θ=22.6°) and the intensity minimum between the 200 and 110 peaks (Iam, 2θ=18°). I002 represents both crystalline and amorphous material, whereas Iam represents the amorphous material.

Table 1. Porosimetry parameters of the carbonized DCs.

Heating Time (h)	Porosimetry parameter	Rice	Mango	Sal
	Porosimetry parameters of carbonized DCs at 250 °C.			
6	*BET surface (m^2/g)*	3.4	4.0	2.1
6	*Pore volume (cm^3/g)*	0.01	0.007	0.006
6	*Average pore diameter (Å)*	158	156	175
12	*BET surface (m^2/g)*	7.2	6.1	5.3
12	*Pore volume (cm^3/g)*	0.3	0.15	0.008
12	*Average pore diameter (Å)*	150	197	165
	Porosimetry parameters of carbonized DCs at 350 °C.			
6	*BET surface (m^2/g)*	10.0	8.4	8.7
6	*Pore volume (cm^3/g)*	0.06	0.05	0.03
6	*Average pore diameter (Å)*	231	199	200
12	*BET surface (m^2/g)*	12.2	11.3	16.2
12	*Pore volume (cm^3/g)*	0.07	0.04	0.2
12	*Average pore diameter (Å)*	262	166	265

DC extracted from sawdust and husk samples had higher cellulose content and lower hemicellulose and lignin contents than the original plant material. After delignification the rice husk had a 5-fold increase in surface area (6.6 m^2/g) and at least 3-fold higher pore volume compared to mango sawdust (1.05 m^2/g) and sal sawdust (0.6 m^2/g). Therefore, delignification caused an increase of 50-110% of the BET surface. The average pore diameter for rice husk was in the range of 100 Å indicating in relation to pore volume, that nanopores comprise more than 2% of the volume of DC as compared to the non-delignified materials. Carbonization of the DCs at 350°C for 6 and 12 h showed higher increase in the BET surface in the range 10-16 m^2/g; but with increase in BET surface there was a decrease in the mechanical strength of the material. The texture was similar to charcoal but more brittle in touch and more easily dissolved when in contact with liquid media. The results derived from the FTIR and NMR analyses confirmed that lignin and most of the hemicellulose were removed during the chemical process. The crystallinity of the extracted DCs was higher than that of the non-delignified samples.

The DCs were successfully used as biofilters for cold pasteurization of liquid foods at 4°C (Kumar *et al* 2016). The efficiency of the mango and sal DC was higher than the rice husk DC in all cases. The microbial removal load ranged from 80-100% for yeasts and 70-90 % for bacteria. Furthermore, novel biodegradable composite materials based on the DCs and microbial polymers (PLA, PHB), were prepared and successfully used as cell immobilization carriers in lactic acid and alcoholic fermentations (Kumar *et al* 2014a). In both cases, and both types of biocatalysts (plain or composite DCs), improvements were observed compared to free cells systems. The results of this study, show that the DCs have increased porosity characteristics after the delignification process, depending on their plant origin, and may be more suitable for bioprocessing such as cold pasteurization, enzyme storage carriers and immobilized cell biocatalysts. The delignification is necessary to remove lignin, which may be toxic to microorganisms, leave undesirable residues and cause discoloration in the target food products. Taking into account that the Greek wheat straw, used in a previous similar study (Koutinas *et al* 1981), contains 16% total lignin instead of 41.4% it is obvious that delignification of mango sawdust will lead to a material with increased porosity and therefore higher specific surface area as compared with straw. This work also indicates the potential of the DCs and carbonized DCs as nano/microporous adsorbent materials for water treatment, dyes, heavy metals and other toxic chemicals in industrial effluents.

Acknowledgements: The research work was sponsored by the National Overseas Scholarship, Ministry of Tribal Affairs New Delhi, India.

References

1. Koutinas *et al* 1981. A processing scheme for industrial ethanol-production from straw. *Energ. Conv. Manage., 21,* 131-135,.
2. Koutinas *et al* 2012. Nano-tubular cellulose for bioprocess technology development. *Plos One 7,* 4, e34350.
3. Kumar *et al* 2014a. Lactic acid fermentation by cells immobilised on various porous cellulosic materials and their alginate/poly-lactic acid composites. *Bioresour. Technol., 165*, 332-335.

4. Kumar *et al* 2014b. Preparation and design of porous biopolymer materials and their use for food bioprocess development. 28th EFFoST International Conference, Uppsala, Sweden, 25-28 November, 2014.
5. Kumar *et al* 2016. Application of nano/micro-tubular cellulose of Indian origin for alcoholic fermentation and cold pasteurization of contaminated water. *LWT-Food Sci. Technol., 69,* 273-279.