Cellulosic and Tannins Containing Wastewater Treatment Using MBBR Technology and Fungal Strain

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Abstract. Since the beginning, Mobile Bed Biofilm Reactor (MBBR) technology has been extensively used, both at the level of small on-site treatment units and at industrial scale. Moreover, this technology represents a starting point for many researches aimed at improving performance, such as the use of microorganisms, enrichment with anammox bacteria to accelerate nitrogen removal and more. Within the present paper, a new generation of carriers (consisting of a mix of high-density polyethylene + talcum + cellulose) was bio-augmented with a WRF (White Rot Fungi) strain, namely Cerioporus squamosus, in static conditions (data not shown in this paper). The wastewater, targeted for treatment, originated from National R&D Institute for Textile and Leather, INCDTP Bucharest, leather subsidiary, Leather and Footwear Research Institute, technological flux, characterized by high tannins concentration, and cellulosic content. Wastewater treatment aimed the reduction of COD value, as a water quality parameter, with satisfactory results, obtaining a percentage reduction rate of 48.53%. Also, GC-MS chromatography analysis was carried out on five vegetal tannins, used in leather treatment, highlighting main compounds for Mimosa, Chestnut, Gambier, Myrobalan and Quebracho natural tannins.

1. Introduction

Wastewaters containing cellulose fibers and various sources of tannins can lead to significant changes to the physicochemical properties of these sources of wastewater, rendering them more and more difficult to treat. Within National R&D Institute for Textile and Leather, along with its subsidiary, Leather and Footwear Research Institute, the generated wastewaters have high content of cellulose (due to the treatment and processing of textile materials) and tannins (due to tanning of leather-based materials).

Cellulose is a very abundant solid material as it is the main component in wood (40-45) % and other plant-based materials (till 90%) [1]. Cellulose is a raw material that is used in many industries like, pulp and paper, textile, tannery, wood, biotechnological industry, pharmaceutical. In 1839 this raw material was first segregated from plants by Anselme Payen [2]. Since then, the cellulose extraction has been developed, and now it can be extracted from different sources (plants, wood, tunicates, algae, bacteria etc. [3]). The global cellulose market size was 191.74 billion Euro in 2018 (Figure 1) and is projected to reach 266.47 billion Euro by 2026 [1].
Cellulose has a linear structure similar with a chain made from hundreds to thousands of linked glucose units. The polymerization degree varies for different sources such as 10000 for cellulose chains from nature or approx. 15000 for cellulose found in cotton [4]. In Figure 2 is presented the chemical structure of cellulose.

Vegetable tannins are widely used in the treatment of leather materials, representing an important stage in leather treatment, and for inducement of leather characteristic properties. Quebracho extracts are used to produce all types of leather (bags, saddles, belts, upper shoes, garment, car seats), but its qualities are highlighted when used to produce natural, full vegetable leather, like harnesses and vacchetta [5]. Chestnut extract results in: gross weight yield, excellent physical and chemical properties, even re-tanning and attractive and stable colour. In re-tanning of chrome leather, up to 30% of chestnut extract can be added to obtain smooth, compact and full leather with a good light fastness [6]. The application of Mimosa is very common worldwide and it can be modified by mixing with other types of tanning materials to adopt its characteristics to the specific leather type in re-tanning. Properties are natural soluble extracts, uniform in quality, pale in colour, low in salts and sludge formation [7]. Myrobalans are marketed in the form of whole nuts crushed nuts or solid and spray dried extracts which are used in tanning of hides. Powdered myrobalans extracted with cold water are used for dyeing red [8]. Gambier is widely used as an industrial raw material in the textile industry, cosmetic industry, pharmaceutical industry, and as an additive to food products. In traditional societies, gambier is used as a dye for textiles and rubber and for leather tanning. Gambier contains natural polyphenol compounds including tannins. Complex phenolic compounds such as tannins dissolve in polar solvents such as water (especially in hot water), methanol, ethanol, and acetone [9].

Treatment of tannins containing wastewaters is a difficult process, due to the high solubility of these chemicals in water, and to the fact that they inhibit growth of microorganisms in activated sludge [10-13].
2. Materials and Methods

2.1. GC-MS analysis

GC-MS analysis was carried out on pure solutions of each vegetal tannin, in order to assess the main compounds, on an Agilent Technologies 6890N spectrophotometer, with 7694E Headspace module (Agilent Technologies) and MS 5973N MS detector. The solutions were sonicated for 10 minutes, after which they were filtered and analyzed on gas chromatography with Mass Spectroscopy detection. The injection of the samples was done by the automatic liquid injection mode. The method and system parameters were as follows: Capillary column: DB-35 MS, length: 30 m, internal diameter 0,25 mm; layer thickness: 0,25 μm; injection system: splitless; injector temperature: 300°C; constant flow: 1,8 mL/min; carrier gas Helium; temperature program: 70 °C (2 min) at 310°C with 10°C/min, 310°C (5 min); injection volume: 1,0 μl; auxiliary: 300°C; MS detector: scan mode; scanning interval: 30-500 amu.

2.2. COD analysis

COD analysis was carried out according to SR ISO 6060, which implies the boiling with reflux for a certain duration, of the water samples mixed with mercury sulphate (III), with a known volume of potassium dichromate, in the presence of a silver catalyst in a strongly acidic environment (sulfuric acid), so that part of the potassium dichromate is reduced by the oxidizable materials present. The excess potassium dichromate is titrated with iron (III) sulphate and ammonium solution. The COD value is calculated from the reduced amount of potassium dichromate.

For the boiling stage with reflux, a C.O.D. thermoreactor was used, namely ECO6, Velp Scientifica type, with a temperature set to 200°C.

All reagents used to determine the chemical oxygen content were of a known analytical quality:

a) Sulfuric acid (p=1,84 g/mL). c(H2SO4)=4 mol/L;

b) Silver sulphate (Ag2SO4);

c) Potassium dichromate, reference standard solution. c(K2Cr2O7)=0.040 mol/L;

d) Iron (II) sulphate and ammonium, titrated solution. c[(NH4)2Fe(SO4)2 * 6H2O]=0.12 mol/L;

e) Feroin, indicator solution.

The chemical oxygen consumption (COD) expressed in milligrams oxygen per liter is calculated according to the formula (eq. 1):

\[
\text{COD (mg/L)} = \frac{8000 \cdot c \cdot (V_1 - V_2)}{V_0} \tag{1}
\]

Where: 
- \(c\) = concentration of the amount of substance of iron (II) sulphate and ammonium solution;
- \(V_0\) = the volume of the sample to be analyzed, before dilution (if performed), in milliliters;
- \(V_1\) = volume of iron (II) sulphate and ammonium solution, used for titration of the control sample, in milliliters;
- \(V_2\) = volume of iron (II) sulphate and ammonium solution, used for titration of the sample to be analyzed, in milliliters;
- 8000 = molar mass of \(\frac{1}{2} \text{O}_2\), in milligrams per liter.

2.3. Cerioporus squamosus functionalized HDPE carriers

HDPE carriers (with addition of talcum and cellulose) were functionalized, in static conditions, with a WRF representative, namely Cerioporus squamosus. First, the strain was grown in fresh culture, on Sabouraud nutritive broth, for 7 days, at 28°C. Afterwards, the broth, containing the fungal biomass, was poured over HDPE carriers (which were previously sterilized at 121°C, for 15 min.) enough for the liquid media to cover the carriers with just a few mm. The inoculated carriers were then incubated for 10 days, at 28°C. Treatment was carried out by extraction of bio-augmented carriers, and placement inside the testing aliquots, containing the wastewater.
3. Results and Discussions

Within FunCell project, textile and leather industry originated wastewater sample was subjected to COD reduction, with the help of Cerioporus squamosus functionalized HDPE carriers. Also, GC-MS analysis was conducted on the sample, in order to assess the main constituents of five tannins currently used in leather tanning: Mimosa, Quebracho, Chestnut, Gambier and Myrobalan.

Mimosa is a vegetable tannin, which is generally extracted from Acacia mollissima bark grown in Australia, Southeast Asia and South Africa. The bark of the tree is used for the tanning process, because it has a very high tannin content, up to 30 percent. The skin tanned with mimosa tannin has a reddish color. Mimosa extract is also used in natural medicine to treat headaches and diarrhea. The general structure of the main compound is presented in Figure 3.

Quebracho is a common name in Spanish for describing the species of very hard wood trees (wood density 0.9–1.3). The etymology of the name derives from quiebrahacha or quebrar hacha. Quebracho produces tannins that can be extracted from the heart of both red Quebracho (Schinopsis lorentzii) [17] and white (Aspidosperma quebracho-blanco). The general structure of the main compound is presented in Figure 4.

Chestnut extract is obtained from the heart and the sapwood (all the young layers located between the bark and the heart of a tree trunk, through which water and mineral salts pass) of the species of Castanea sativa and dentata. The general structure of the main compound is presented in Figure 5.

Gambier or gambir is an extract derived from the leaves of Uncaria gambir, a shrub native to Southeast Asia. Gambier is produced in Indonesia and Malaysia where it was an important commercial product at the end of the 19th century. It can be used as a tanning agent, food additive brown dye [16-17] and as a herbal medicine. Also known as pale catechu [17] or white catechu, it is often confused with other forms of catechu (which is an extract of Acacia species, especially Acacia catechu). The general structure of the main compound is presented in Figure 6.
Myrobalan is the dry extract of ripe fruits of Terminalia chebula (belonging to the Combretaceae family) and related species. These fruits are harvested from January to April and are dried by settling in thin layers, most of them in hue.

Characteristics:
- Color: yellow-brown to brown;
- Smell: very weak;
- Taste: astringent and slightly pungent;
- Form: mucilaginous. The general structure of the main compound is presented in Figure 7.

Myrobalan contains about 30% of the hydrolyzable tannins, which consist of chebulinic acid, chebulagic acid and D-galloyl glucose. Contains free tannic acid, gallic acid, ellagic acid and mirobalanine from the resin. Due to its antiseptic and healing properties, it is used in India as a medicine for different types of conditions such as: chronic ulcers or different types of wounds. Commercially, it is used in the dyeing and tanning industry.

GC-MS analysis results are shown in Tables 1-5, which highlight the presence of the five tannins used, together with their main compounds. This analysis will be the basis of future experiments on tannin degradation in aqueous solutions.

![Figure 7. Molecular structure of main compound found in Myrobalan tannin.](image)

### Table 1. Chestnut tannin main compounds.

| No. | Rt   | CAS     | Compound name                                | Area   |
|-----|------|---------|----------------------------------------------|--------|
| 1.  | 3.283| 98-01-1 | Furfural                                     | 51476  |
| 2.  | 5.258| 97-69-8 | N-Acetyl-L-alanine                           | 5344   |
| 3.  | 6.046| 6843-45-4| Methylimidazolidine-2,4-dione                | 22109  |
| 4.  | 9.180| 52485-92-4| Methyl α-D-ribofuranoside                    | 23898  |
| 5.  | 9.774| 4007-18-5| 3,4-dimethyl-sydnone                         | 12370  |
| 6.  | 9.871| 3201-20-5| 2,4-dihydro-4,4,5-trimethyl-3H-pyrazol-3-one| 4207   |
| 7.  | 10.027| 125425-35-6| Triethylene glycol monomethyl ether       | 8867   |
| 8.  | 10.427| 87-66-1| Pyrogallol                                   | 23157  |
| 9.  | 11.420| 2595-97-3| D-Allose                                      | 47297  |
| 10. | 13.837| 149-91-7| Gallic Acid                                  | 16914  |
Table 2. Gambier tannin main compounds.

| No. | Rt  | CAS     | Compound name     | Area  |
|-----|-----|---------|-------------------|-------|
| 1   | 8.571 | 120-80-9 | Catechol         | 52202 |
| 2   | 10.238 | 108-46-3 | Resorcinol       | 137335|
| 3   | 11.419 | 87-66-1  | Pyrogallol       | 29530 |
| 4   | 14.932 | 18979-60-7 | 4-Propylresorcinol | 64141 |

Table 3. Quebracho tannin main compounds.

| No. | Rt  | CAS     | Compound name     | Area  |
|-----|-----|---------|-------------------|-------|
| 1   | 8.565 | 120-80-9 | Catechol         | 189877|
| 2   | 10.243 | 108-46-3 | Resorcinol       | 704221|
| 3   | 11.414 | 87-66-1  | Pyrogallol       | 41921 |
| 4   | 14.932 | 18979-60-7 | 4-Propylresorcinol | 302142|

Table 4. Mimosa tannin main compounds.

| No. | Rt  | CAS     | Compound name     | Area  |
|-----|-----|---------|-------------------|-------|
| 1   | 10.243 | 108-46-3 | Resorcinol       | 639603|
| 2   | 11.419 | 87-66-1  | Pyrogallol       | 109404|
| 3   | 13.070 | 57-50-1  | Sucrose           | 44375 |
| 4   | 13.842 | 498-07-7 | Levoglucosan     | 20389 |
| 5   | 14.932 | 451-13-8 | Homogentisic Acid | 63595 |
| 6   | 15.655 | 146-72-5 | 3-O-methyl-D-glucose | 939664|

Table 5. Myrobalan tannin main compounds.

| No. | Rt  | CAS     | Compound name     | Area  |
|-----|-----|---------|-------------------|-------|
| 1   | 3.277 | 98-01-1  | Furfural          | 25302 |
| 2   | 10.017 | 67-47-0  | 5-hydroxymethyl furfural | 90084|
| 3   | 11.414 | 87-66-1  | Pyrogallol       | 251925|
| 4   | 13.076 | 57-50-1  | Sucrose           | 26676 |
| 5   | 13.837 | 498-07-7 | Levoglucosan     | 31507 |
| 6   | 15.040 | 33818-21-2 | 1,6-Anhydro-α-D-galactofuranose | 10391|
| 7   | 15.579 | 99-16-1  | Allantoic acid   | 11708 |
| 8   | 18.666 | 149-91-7 | Gallic Acid      | 564637|
COD analysis is a general indicator of the water quality, measuring the capacity of dissolved oxygen depletion, in the samples contaminated with organic matter. Specifically, the analysis determines the equivalent amount of oxygen required for chemical oxidation of organic compounds in water. Often, COD analysis is used to estimate BOD (Biological Oxygen Demand) values, between these 2 indicators existing strong correlations.

The most common method of determining chemical oxygen demand is the method that involves digestion of the sample for two hours at high temperature, under acidic conditions, in which potassium dichromate serves as an oxidant for the organic matter present in the water samples. Silver sulphate is present in the environment, as a catalyst, and mercuric sulfate is used to complex chlorinated compounds, possibly present in samples. Following digestion, the degree of oxidation is determined by indirectly measuring the oxygen requirement through the electrons consumed in reducing Cr$^{6+}$ to Cr$^{3+}$, by titration techniques or spectrophotometry. The level at which the determined experimental results are close as values to the theoretical values, first of all depends on the water load subjected to the analyzes and on the degree of oxidation, the COD analysis strongly depending on the composition of the analyzed water. According to the standard, a large number of organic compounds are oxidized in the proportion of 90% - 100%, and if the samples contain a large amount of such compounds (the case of municipal effluents), the COD value is a good approximation of the theoretical consumption of oxygen.

The result of the analysis revealed a COD value, for the tested wastewater, of 55,849 mg/L COD (Figure 8). Following biological treatment, with Cerioporus squamosus bio-augmented HDPE carriers (data not shown here), it was observed a percentage reduction of 48.53% of COD content.

4. Conclusion and perspectives

MBBR technology is not currently emerging as a novel treatment technology, for conventional wastewater, as the validates efficiency of this technology is already settled throughout the years. However, new demands, regarding treatment of industrial wastewater, also dictates necessity of novelty within the MBBR systems. FunCell project took a stab at obtaining novel generations of carriers, combining HDPE with talcum and cellulose.

Furthermore, bio-agumentation of these structures was possible with fungi strains, which indeed represents a novelty in this thematic area. Within this research paper, the authors carried out identification of main compounds from five main vegetal tannins widely used in the leather industry. Also, COD reduction was possible, with almost 50% reduction efficiency, from a source of natural wastewater sample, with bio-augmented HDPE carriers, thus making a step forward validation of this novel technology in the field of industrial wastewater treatment.
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5. References
[1] *** 2020, Cellulose market size, share & industry analysis, by derivate type (commodity cellulose pulp, cellulose fibers, cellulose ethers, cellulose esters, microcrystalline cellulose, nanocellulose, and others), by end-use industry (textile, food, chemical synthesis, pharmaceuticals, construction, pulp & paper, paint & coating and others) and regional forecast 2019-2026, Fortune Business Insights Publishing
[2] Castro R I and Morales-Quintana L 2019 Cellulose 26(5) 3009-3020
[3] Dong Y, Bi J, Zhu D, Meng D, Ming S, Guo W, Chen Z, Liu Q, Guo L and Li T 2019 Cellulose 26(12) 7355-7370
[4] Jiang Y, Zhang Y, Ding L, Joshua A., Wang B, Feng X, Chen Z, Mao Z and Sui X 2019 Carbohydrate polymers 223 115079
[5] Gurreiro O, Soldado D, Fialho L, Cachuco L, Garrido A, Francisco A and Jeronimo E 2019 Inclusion of rockrose and quebracho condensed tannins extracts in dairy goat diets– influence on the production, chemical composition and fatty acid profile of milk
[6] Quave C L, Lyles J and Horswill A R 2019 U.S. Patent Application no. 16/225,281
[7] El-Sayed A, El-Sakhaw M, Kamel S, El-Gendy A and Abouzeid R 2019 Egyptian Journal of Chemistry 62(5) 777-787
[8] Khan M A, Khan M, Srivastava P K and Mohammad F 2005 Colourage 52(12) 53-60
[9] Sy S and Kasman M 2019 IOP Conference Series: Materials Science and Engineering 546(2) 022032
[10] He Q, Yao K, Sun D and Shi B 2007 Biodegradation 18(4) 465-72
[11] Botezatu C, Duceac L D, Mastalier B, Stafie L, Jitareanu C M, Luca A C, Tarca E, Mitrea G, Iordache A C and Patrascu T 2018 International Journal Of Medical Dentistry 22(4) 346-350
[12] Tarnita D, Tarnita D.N, Popa D, Grecu D and Niculescu D 2010 Romanian Journal of Morphology and embryology 51(1) 145-150
[13] Berceanu C, Tarnita D and Filip D 2010 Journal of the Solid State Phenomena, Robotics and Automation Systems 166-167 45-50
[14] Hobbs C E 2019 Polymers 11(2) 224
[15] Gironi F and Piemonte V 2011 Chem. Eng. Res. Des. 89(7) 857–862
[16] Sakti A S, Saputri F C and Mun’im A 2019 Pharmacognosy Journal 11(1) 119-123
[17] Manalu D T and Armyanti T 2019 Agribusiness and Agricultural Economics Journal 2(1) 46-67