Biodegradation and ecotoxicological assessment of pectin production wastewater

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Abstract

The chemical composition of pectin production wastewater and its toxicity during biological treatment were investigated. Samples of wastewater from different steps of a pectin production wastewater biological treatment plant were investigated including the influent of the treatment (1), after denitrification tank (2), after anaerobic treatment (3) and final effluent (4). The conventional physico-chemical characteristics of samples did not indicate wastewater toxicity. However, toxicity assessments carried out on Vibrio fischeri and Scenedesmus subspicatus indicated low EC50 values. The fractionation of the samples using an XAD resin showed that the toxicity was associated with the organic matter. Wastewater apparent molecular mass distributions were 14.3, 25.0, 24.4 and 29.6 kDa for samples 1–4, respectively. Finally, characteristics of the sample by pyrolysis-gas chromatography-mass spectrometry (Py-CG-MS) demonstrated its polyphenolic nature and a 23% increase in the levels of such compounds after the first biological treatment step.

Key words: pectin wastewater; bioassays; ecotoxicity; pyrolysis-gas chromatography-mass spectrometry

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Introduction

The increasingly stringent legislation related to effluent pollution control requires a better characterization of effluents and its potential toxicity. Some effluents contain organic compounds that can not be able to be degraded by biological treatments and sometimes called residual or inert chemical oxygen demand (residual-COD) (Vidal et al., 2001; Hea et al., 2006; Figaro et al., 2006; Bilgili et al., 2008).

The appearance of these substances in effluent seems to be mainly related with the chemical composition of the influent, i.e., the nature of the raw material used in the process and the chemical and biological reactions occurring during the treatment. The influence of the chemical nature of the raw material used in the process can be clearly observed in pulp mill (Dahlman et al., 1995; Vidal et al., 2001; Kukkola et al., 2006), tannery (Horst, 2004; Schrank et al., 2004) and textile (Hüseyin et al., 2006; Sponza, 2006) processes. These use raw materials containing numerous types of phenolic compounds that are varied from simple monomers to high molecular weight polyphenolic polymers.

The second possibility is that the residual-COD is comprised of biopolymers, i.e., extracellular polymeric substances (EPS), due to cell metabolism and lysis. The chemical composition of EPS is reported to be very heterogeneous and these substances are sometimes referred to as humic-like substances (Frølund et al., 1996; Barker, 1999; Janga et al., 2007; Wilén et al., 2008).

In the literature there is no report of non biodegradable COD in pectin industry effluent, but in pectin production orange, lemon and apple peels are normally used as raw materials, in which the plant cell wall is composed roughly of 9%–25% cellulose, 20%–50% microfibrils, hemicelluloses, 10%–35% pectic substances, and 10% proteins (Fennema, 1996).

The chemical characteristics of organic matter in wastewater is usually carried out only through determination of the chemical oxygen demand (COD), biochemical oxygen demand (BOD) and total or dissolved organic carbon (TOC and DOC). The potential toxic effect of effluents on the environment can be highlighted by assessment through bioassays (Sponza, 2006; Barata et al., 2008), but their chemical nature remains unknown (Reemtsma, 2001).

In order to characterize and identify COD compounds in wastewaters, sophisticated and quite expensive analytical techniques are required, due to their complexity and heterogeneity. Pyrolysis and gas chromatography followed by mass spectrometry has been used for the investigation of chemical nature in industrial effluents (Calvo et al., 1995;
Ristolainen et al., 1999; Castillo and Barceló, 2001; Del Río et al., 2001; Hernández et al., 2001; Kukkola et al., 2006, Franke et al., 2008).

In this study, the chemical characterization and ecotoxicological assessment of wastewater from different steps of the biological treatment of a pectin production wastewater were carried out to correlate the chemical characteristics of wastewater with its toxic effects.

1 Materials and methods

1.1 Wastewater treatment plant and sampling

Industrial wastewater samples were collected from a pectin production plant located in northern Germany. The samples were collected during five different months of the year from different steps of the biological wastewater treatment, as shown in Fig. 1: sample 1 (influent); sample 2 (after denitrification tank); sample 3 (after anaerobic treatment) and sample 4 (final effluent). Samples were cooled at 7°C until the analysis. The ecotoxicological assays were performed immediately after samples arrival the laboratory.

![Diagram of the pectin production wastewater treatment](image)

**Fig. 1** Diagram of the pectin production wastewater treatment. (a) denitrification tank; (b) anaerobic treatment; (c): aerobic treatment (activated sludge); (d) clarifier; (e) sand filter. 1, 2, 3, and 4 represent samples.

1.2 Wastewater toxicity assessment

In order to evaluate the toxicity of samples, two tests were carried out: growth of the green alga *Scenedesmus subspicatus* and bioluminescence of the bacterium *Vibrio fischeri*. The assay with *S. subspicatus* was carried out as described by the International Organization for Standardization in Norm ISO 8692. The tests were performed in triplicates with different dilutions of wastewater by adding a nutrient solution and the algal growth rate was measured in percent inhibition of the growth for the algal test and bioluminescence for the bacterial test, i.e., EC50. The values were obtained by interpolation of the concentration-response curves and the estimation of EC50 and their respective confidence intervals calculated by the variance analysis of repetitions using the PROCGLM program of SAS®.

1.3 Physico-chemical analysis

COD was measured according to the internal reflux method using the LCK 314 kit (Dr. Lange Company, Germany). The determination BOD5 was carried out by the dilution method 5210 B (APHA, 1995). A modified Zahn-Welles test was performed to determine the biodegradability of sample 4 and to determine its residual-COD according to International Organization for Standardization in Norm 9888. The test was carried out for 7 d under aerobic conditions (continuous stirring at 70 r/min and aeration 3 mg O2/L) to determine the COD concentration of the sample periodically. Other chemical parameters were analyzed using NO3-N (LCK 339 method) and NO2-N (LCK 341 method) kit tests of Dr. Lange Company, and Merck Company (Germany) tests were used for the determination of NH4-N (Spectroquant 14752 method) and PO4-P (Phosphor-Test PMB 14848 method). Dissolved organic carbon was measured after the sample filtration through a 0.45-μm cellulose-acetate filter (Sartorius Company, Germany) in a total organic analyzer (Beckman Company, USA), according to ISO 8245. Sample colors were measured as absorbance at 436 nm in a spectrophotometer-DR2000 (Dr. Lange Company, Germany), according to ISO 7887 method.

1.4 Fractionation with XAD resin

Wastewater samples were fractionated with a non-ionic resin XAD-8 (Rohm and Haas Company, Germany), according to the method described by Sorouradin et al. (1993). The wastewater was previously acidified to pH 2 with 1 mol/L HCl. The resin volume was scaled down to a 500-mL water sample and the fractionation was run at a rate of 3 mL/min. Sample adsorptions were carried out with 100 mmol/L NaOH and the fractions were collected through a fraction collector apparatus and monitored by spectrophotometry absorbance at wavelength 254 nm to give DOC content (Kulovaara et al., 1995). As the control, a 100 mmol/L NaOH solution was used. The fractions were separated according to the maximum absorbance at 254 nm. Three different fractions were collected and the first did not show absorbance at 254 nm (no organic matter), the second had absorbance between 0.01 and 1.0, and the third fraction, representing the collection of sub-fractions, had absorbance values higher than 1.0 (high concentration of DOC). The toxicity assessment of fraction 3 of sample 4 (final effluent) was carried out on the green alga *S. subspicatus* and using the bacterial bioluminescence test with *V. fischeri*.

1.5 Apparent molecular mass distribution

The apparent molecular mass distributions of fraction 3 of the wastewater samples (obtained as described above) were determined by gel permeation chromat-
temperature was programmed to increase from 60 to 117°C, holding the temperature for 10 min. Helium was used as the carrier gas at rate of 1.1 mL/min. The mobile phase was 100 mmol/L NaOH at 1.0 mL/min. The molecular mass distributions of samples were compared with the calibration curves of molecules of known molecular mass provided by Sigma-Aldrich Company (Germany): blue dextran (2000 kDa), chymotrypsinogen (25 kDa), myoglobin (18 kDa), vitamin B12 (13.5 kDa) and cytochrome c (13.0 kDa). The molecular mass distributions of samples were calculated as described in Eq. (1).

$$K_{av} = \frac{V_e - V_o}{V_i - V_o}$$

where, $K_{av}$ is stationary phase fraction available for dispersion; $V_e$ is effluent elution volume (average value); $V_o$ is blue dextran elution volume; $V_i$ is column total volume.

### 1.6 Py-GC-MS analysis

The TOC contents for fraction 3 (with high organic carbon) of the samples were determined and the fractions were then freeze dried and analyzed on a pyrolysis-gas chromatography-mass spectrometry (Py-GC-MS) system. One milliliter of the freeze-dried sample was pyrolyzed at 550 and 700°C for 30 s using an infra-red pyrolyzer (Pyro 2a) interfaced to a gas-chromatograph (Shimadzu 17 A) coupled via a transfer line to a mass spectrometer (Shimadzu QP 5000). The pyrolysis products obtained were injected into the gas-chromatograph at 280°C and separated using a non-polar column containing silica with 5% phenyl (polysilphenylene siloxane) (column BPX-5, SGE, 48 m length × 0.32 mm i.d.). The gas-chromatograph temperature was programmed to increase from 60 to 320°C at 8 min⁻¹, holding the temperature for 10 min. Helium was used as the carrier gas at rate of 1.1 mL/min. The electron impact mass spectra (1 scan/s) were acquired at 1.4 kW and a mass range 40–400 of m/z was scanned. The identification of the pyrolysis products was based on mass spectral interpretation and comparison with the SNIST (Standard National Institute for Science and Technology, USA) which is available as software in the equipment. The peak size was expressed as the percentage area with respect to the total ion chromatogram.

### 2 Results and discussion

#### 2.1 Physico-chemical analysis

Chemical parameters of samples from different steps of the pectin production wastewater treatment were evaluated and are listed in Table 1.

All parameters used to measure organic carbon (BOD₅, COD and DOC) were reduced during the treatment. The low concentration of BOD₅ in the final effluent (sample 4) indicates its low biodegradability, which could be also confirmed by the high residual COD content and the low BOD₅/COD ratio. Reemtsma et al. (2001) affirmed that when BOD₅/COD was lower than 0.1, 90% of DOC can not be degraded by biological treatment. Around 30% of increment in color measured at 436 nm was observed up to sample 2. A high concentration of NO₃⁻-N in sample 4 could be explained by the use of nitric acid during the pectin production process. Physico-chemical analysis can indicate high concentrations of a pollutant which could lead to toxicity. However, the compounds analyzed in this study (Table 1), did not indicate potential toxicity of the samples.

Results for the sample toxicity toward *V. fischeri* and *S. subspicatus* are given in Table 2 as EC50 (effluent volume that inhibits 50% of the bacterial bioluminescence and algal growth).

The results for the wastewater toxicity were similar for both bioindicators. Sample 1 (influent) had the highest toxicity toward both the algae and bacteria. This sample contains all of the raw materials used in the pectin production. During the pectin extraction, organic solvents are used, mainly isopropanol, which is a known toxic

### Table 1

<table>
<thead>
<tr>
<th>Chemical parameter</th>
<th>Sample 1</th>
<th>Sample 2</th>
<th>Sample 3</th>
<th>Sample 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>2.1 ± 0.07</td>
<td>7.7 ± 0.04</td>
<td>8.2 ± 0.03</td>
<td>8.9 ± 0.07</td>
</tr>
<tr>
<td>COD (mg/L)</td>
<td>19855 ± 2866</td>
<td>7539 ± 1355</td>
<td>1100 ± 228</td>
<td>421 ± 87</td>
</tr>
<tr>
<td>Residual-COD (mg/L)</td>
<td>nd</td>
<td>nd</td>
<td>nd</td>
<td>394.6 ± 6.43</td>
</tr>
<tr>
<td>BOD₅ (mg/L)</td>
<td>13348 ± 2128</td>
<td>6288 ± 725</td>
<td>345 ± 108</td>
<td>4.6 ± 3.1</td>
</tr>
<tr>
<td>BOD₅/COD</td>
<td>0.7 ± 0.12</td>
<td>0.8 ± 0.20</td>
<td>0.3 ± 0.16</td>
<td>0.01 ± 0.01</td>
</tr>
<tr>
<td>DOC (mg/L)</td>
<td>8357 ± 231</td>
<td>2082 ± 23</td>
<td>202 ± 37</td>
<td>151 ± 41</td>
</tr>
<tr>
<td>Color</td>
<td>0.756 ± 0.121</td>
<td>1.086 ± 0.160</td>
<td>1.189 ± 0.107</td>
<td>1.115 ± 0.054</td>
</tr>
<tr>
<td>NH₄⁺-N (mg/L)</td>
<td>38 ± 5.4</td>
<td>71 ± 12.9</td>
<td>nd</td>
<td>26 ± 0.6</td>
</tr>
<tr>
<td>NO₃⁻-N (mg/L)</td>
<td>2780 ± 178</td>
<td>24 ± 1.9</td>
<td>nd</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>NO₂⁻-N (mg/L)</td>
<td>5 ± 0.4</td>
<td>&lt; 0.01</td>
<td>nd</td>
<td>&lt; 0.01</td>
</tr>
<tr>
<td>PO₄³⁻-P (mg/L)</td>
<td>17 ± 7.1</td>
<td>nd</td>
<td>nd</td>
<td>16 ± 4.3</td>
</tr>
</tbody>
</table>

Data are expressed as average values ± standard deviation (n = 5). nd: not determined. Samples 1–4 are referred to Fig. 1.
substance. Pedersen and Petersen (1996) investigated the toxicity of organic solvent mixtures on different bioindicators. A solution containing n-propanol (1000 mg/L) in a mixture of 20 organic solvents showed an EC50 of 8.3 mL/L for *V. fischeri* and 4.9 mL/L for the alga *S. capricornutum*. However, isopropanol has a high biodegradability, which could be observed in the reduction of the wastewater COD content and toxicity during the treatment.

It can be seen from Table 2 that there was a reduction in the toxicity from sample 1 to sample 3, and for sample 4 the toxicity slightly increased for both *V. fischeri* and *S. subspicatus*. The effects of chemical treatments like ozone, ultra violet (Hitchcock *et al.*, 1998; Melo *et al.*, 2006), and Fenton’s oxidation (Merie *et al.*, 2005) on the toxicity of wastewater are well described in the literature. The effect of these chemical treatments on the toxicity is greatly dependent on the nature of the wastewater and period of exposure. However, little attention has been paid to the effects that biological treatment may have on the wastewater toxicity. The results shown in Table 2 reveal an increase in the sample toxicity on comparing the wastewater after anaerobic and aerobic biological treatments. Such an effect could be attributed to the desorption of substances which were presented in anaerobic sludge when submitted to aerobic conditions. Klee *et al.* (2004) demonstrated changes in toxicity and genotoxicity of industrial sewage sludge samples following the treatment in bioreactors with different oxygen regimes.

The dark color of the effluent, mainly for sample 4 (Table 1), may influence the results of algal growth test, but this effect was overcome as discussed elsewhere (Reginatto *et al.*, 2000). The instructions of bioluminescence test for this type of correction are supplied by the manufacturer.

### 2.2 Fractionation of the samples

The absorbance values for the fractionation of sample 4 (effluent) are shown in Fig. 2. The results of the fractionation for the other samples are not given in this paper, but they differed from sample 4 only in terms of the eluted volumes. For sample 4 the fraction a collected (80 mL) did not show absorbance at 254 nm. The fraction b was composed of the sub-fractions collected between 80 and 305 mL, having absorbance values between 0.01 and 1.0. The fraction c represents the collection of sub-fractions with absorbance values higher than 1.0 (high concentration

![Fig. 2 Fractionation of sample 4 with XAD resin. Fraction a: no absorbance; fraction b: absorbance values between 0.01 and 1.0; fraction c: absorbance values higher than 1.0.](image)

### Table 3 EC50 values of fractions b and c of the final effluent (sample 4) assayed with *V. fischeri* and *S. subspicatus* and their respective confidence intervals (95%)

<table>
<thead>
<tr>
<th>Organism</th>
<th>EC50 (%, V/V)</th>
<th>Confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Whole effluent</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. fischeri</em></td>
<td>33.44</td>
<td>30.39–36.49</td>
</tr>
<tr>
<td><em>S. subspicatus</em></td>
<td>31.05</td>
<td>30.12–31.97</td>
</tr>
<tr>
<td>Fraction b</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. fischeri</em></td>
<td>55.41</td>
<td>53.21–67.62</td>
</tr>
<tr>
<td><em>S. subspicatus</em></td>
<td>S</td>
<td></td>
</tr>
<tr>
<td>Fraction c</td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>V. fischeri</em></td>
<td>22.28</td>
<td>19.31–25.25</td>
</tr>
<tr>
<td><em>S. subspicatus</em></td>
<td>38.08</td>
<td>36.13–40.05</td>
</tr>
</tbody>
</table>

S: stimulation of growth.

Toxicity assessment of fractions b and c toward the final effluent (sample 4) were carried out after adjusting the pH to 7.0 ± 0.2 to investigate whether the effluent toxicity is associated with the organic matter. Results for the toxicity of the final effluent fractions toward *S. subspicatus* and *V. fischeri* are shown in Table 3.

Results shown in Table 3 revealed that fraction b (with a low concentration of organic matter-absorbance values between 0.01 and 1.0) exhibited lower toxicity than fraction c and the whole effluent. The toxicity of fraction c (higher concentration of organic matter-absorbance values > 1.0) was similar to that of the whole effluent (especially in the case of the algae), indicating that the effluent toxicity was associated with the organic matter.

In addition, fraction b stimulated the algal growth and exhibited a lower toxicity toward the bacteria than the whole effluent. This effect is probably associated with the high concentration of nutrients, such as phosphorous and nitrogen, remaining in this fraction, as observed in the chemical characterization of the samples (Table 1).

The toxicity toward *S. subspicatus* and *V. fischeri* observed in Table 3 for the whole effluent should be the same as that presented in Table 2 for sample 4. However, while for *V. fischeri* the toxicity remains the same, for *S. subspicatus* the toxicity changed from 49.31 (46.81–51.81) in Table 2 to 38.08 (36.13–40.05) in Table 3. Such differences could be explained by the use of different samples of the effluent (sample 4) to perform the test. The test with algae could be influenced by the nutrient content of the effluent sample, which can change according to the industrial process.

### 2.3 Apparent molecular mass distribution

In order to obtain more detailed information on the chemical nature of the samples, the molecular mass distribution of fraction c of the wastewater samples was determined and the results are shown in Table 4.

According to Table 4, the sample molecular weight distribution increased by around 40% after sample 2, i.e., after the first biological treatment step, varying during the treatment process between 14.3 and 29.6 kDa. Barker *et al.* (1999) found that 89% of the material present in anaerobic effluents has a molecular weight of around 1 kDa. The values found in this study were closer to those found by...
Dahlman et al. (1995), who obtained fractions of effluents from the paper and cellulose industry with molecular mass values between 1 and 10 kDa by ultra filtration, which can be considered high.

2.4 Characterization by Py-GC-MS

In this study, the samples were initially analyzed by GC-MS without pyrolysis but very poor peaks were observed. Thus, a pyrolysis temperature of 550°C was applied and the fragments obtained were separated by gas-chromatography and characterized by mass spectroscopy. When Py-GC-MS is applied to high molecular weight compounds it provides data on their molecular composition in terms of different classes of compounds, e.g., sugars, phenolic derivatives, proteins, and etc. (Gray and McAuliffe 1993; Calvo et al., 1995; Franke et al., 2008). Around 20 compounds could be identified in fraction c of the samples from the different steps of the pectin wastewater treatment, through comparison with data given in the SNIST-spectral library. The number of components detected and their relative abundance as estimated via internal standards are given in Table 5.

The fragments shown in Table 5 were divided into three different classes of pyrolysis products, according to Gray and McAuliffe (1993), except the cyclohexadiene, the cyclooctatetraene and the azulene, which do not belong to any of the proposed compound classes. The classes of pyrolysis products are: (1) phenolic compounds and benzene derivatives (compound No.: 3, 6, 8, 9, 11, 12, 13, 14, 15, 16, 19), indicating mainly the appearance of polyphenols in the original structure of the wastewater; (2) furane and cyclopentenone derivatives of polysaccharides (compound No. 7); and (3) compounds containing nitrogen, like pyrrole derivatives (compound No.: 1, 4, 5, 18), suggesting the presence of proteins. The fragment classes of the pyrolysis products of the wastewater samples are summarized in Fig. 3 as the ratio of the relative abundance of the obtained fragments and the DOC content of each sample analyzed, to give an idea of the chemical nature of the samples.

The nature of the samples and the effect of biological treatment are clearly shown in Fig. 3. Sample 1 had the lowest content of phenolic compounds and benzene derivatives, around 50%. Up to sample 2, phenolic compounds and benzene derivatives are responsible for more than 70% of the compounds identified, indicating the polyaromatic nature of the samples. An increase of around 23% in the

![Fig. 3 Py-GC-MS fragment classes of the pectin production wastewater samples. Class 1: phenolic compounds and benzene derivatives; class 2: furanes and cyclopentenones, derivatives from polysaccharides; class 3: compounds containing nitrogen, like pyrrole derivatives; class 4: miscellaneous, cyclohexadiene, cyclo-octatetraene and azulene.](image-url)
content of phenolic compounds and benzene derivatives was observed after the first biological treatment step (sample 2), and the values were remained almost constant until the end of the treatment. The second product class of pyrolysis found is furans and the third most abundant product class consists of compounds containing nitrogen. The compounds containing nitrogen (class 3) decreased from samples 1 to 4, mainly between samples 1 and 2, probably due to the denitrification step in the wastewater treatment plant (Fig. 1).

The known low biodegradability of polyaromatics is in agreement with other physico-chemical characteristics of wastewater (Table 1), that is the low BOD5/COD and high residual-COD, mainly for sample 4. In addition, the increase in the polyaromatics after the first biological treatment step (denitrification) indicates that these compounds are partly formed during the biological treatment. Some researchers (Frølund et al., 1996; Houghton and Quaromy, 1999; Janga et al., 2007; Wilén et al., 2008) have reported that the chemical composition of extracellular polymers in sludge formed during biological treatment are very heterogeneous, containing proteins, carbohydrates, humic-like substances and uronic acids. Such compounds have a high molecular mass and can contribute to the polyaromatic nature of the pectin wastewater. Frølund et al. (1996) reported that the compounds of low biodegradability such as COD, sometimes referred to as humic-like substances and represent around 20%–50% of the COD content in effluent.

Figure 4 shows the relationship between the sample ecotoxicity expressed as 1/EC50 and the polyaromatics/DOC ratio. There is an exponential relationship between the polyaromatic compound contents and the toxicity, revealing that such compounds increase the toxicity. The correlation ratio was higher for V. fisheri ($r^2 = 0.9229$) than that for S. subspicatus ($r^2 = 0.8559$).

3 Conclusions

The results obtained using the classical chemical parameters to characterize wastewater, including BOD, COD and TOC, indicated a low biodegradability of the wastewater, particularly of the final effluent from the pectin production wastewater treatment system. However, this analysis was not able to indicate the observed toxicity of the samples toward V. fisheri and S. subspicatus. By the fractionation of sample 4, it could be observed that the toxicity is related to the organic material in the wastewater (fraction c). The Py-GC-MS analysis showed that there was an increase in the polyaromatic compounds during the wastewater treatment steps. This result is consistent with the low biodegradability of the samples indicated by a decrease in the BOD5/COD during the biological treatment. The first step of the biological treatment reduces the COD and BOD content but also changes the chemical structure of the effluent. Correlating the wastewater toxicity with the content of polyaromatic compounds, it could be observed that the sample toxicities are related to an increase in these compounds. The use of bioassays in combination with Py-GC-MS not only indicates the toxicity, but also reveals the formation of polyaromatic substances during the different steps of the wastewater treatment.

Acknowledgments

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References


effluent by free and immobilized lignin peroxidases and horseradish peroxidase. *Biotechnology Letters*, 11: 577–582.


