On the Utilization of Microalgae for Brewery Effluent Treatment and Possible Applications of the Produced Biomass

M. Filomena de J. Raposo¹, Susana E. Oliveira¹, Paula M. Castro¹, Narcisa M. Bandarra² and Rui M. Morais¹*

ABSTRACT


The effluent of a brewery, complete or diluted with deionised water or with normal culture medium, was used as the growth nutrient medium for Chlorella vulgaris and for a consortium obtained from the autochthonous flora of that effluent (microalgae, cyanobacteria and bacteria). The cultures were exposed to continuous light and aeration, at 25°C, and growth was evaluated by direct counting (C. vulgaris) or by chlorophyll determination (autochthonous flora). Total protein and lipid content, and amino acid and fatty acid profiles in the produced biomass were determined. The highest removal rate of nutrients present in the effluent was obtained when the complete effluent was used as the culture medium for the autochthonous flora – up to 5,855 g kg⁻¹ biomass d⁻¹ of nitrogen and up to 805 g kg⁻¹ biomass d⁻¹ of phosphate. A reduction of up to 27% in biological oxygen demand (BOD5, initial level of 2,172 mg O₂ L⁻¹) and up to 15% in chemical oxygen demand (COD, initial level of 1,340 mg O₂ L⁻¹) was observed in cultures of the autochthonous flora grown in different loads of effluent. A significant increase in aspartic acid, glutamic acid and valine content, and a higher level of the ramified fatty acids, of the 14:0:isobr, 18:4γδεζ, and the eicosapentaenoic acid, were found in the final biomass obtained from cultures grown with different loads of effluent, compared with the results obtained for the cultures grown in normal nutrient medium. The final microalgae biomass obtained, considering its protein and fatty acid content and the absence of heavy metals in significant amount, can be appropriate for use as animal feed or for biofuel production.

Key words: autochthonous flora, biological treatment, biomass valorisation, brewery wastewater, Chlorella vulgaris.

INTRODUCTION

The high organic load of effluents originating from different industries, namely those from the agro-food industries, is a major environmental problem. Among these, effluents derived from the dairy and brewery sectors are rich in organic compounds, such as proteins, phosphates, ammonia and/or nitrate, which must be removed from the residual waters before discharging into the environment. In addition, brewery effluent is usually composed of sugars, soluble starch, ethanol and volatile fatty acids, which are easily biodegradable.

There are several technologies associated with the treatment of effluents derived from the agro-food industries, such as physical, chemical, or biological, to remove the organic and inorganic nutrients, such as nitrogen (ammonia or nitrate) and phosphorus, as is the case of high rate ponds. Most of the biological treatment technologies involve the use of bacteria, but microalgae have already been applied for effluent treatment, either as single species, as is the case of Chlorella, Scenedesmus or Arthrospira, or as mixed cultures/consortia, to treat and remove nitrogen, phosphorus and chemical oxygen demand, from different types of effluents, such as dairy manures, or wastewaters from the wood-based pulp and paper industry, or olive-mill residues.

These organisms are also able to remove and incorporate heavy metals, such as lead, cadmium, nickel or mercury, present in effluents, and their use could be potentially more widespread. Microalgae also produce oxygen for bacterial decomposition of organic matter, and can play an important role in removing pathogenic bacteria through their bactericide action. Moreover, the biomass produced can be used as animal feed (fish and shellfish), as fertilizer to enrich soils with nutrients, or fixing particles, or even for extracting valuable compounds, such as pigments, enzymes or antibiotics.

Among the several microalgae used to treat effluents Chlorella is often found, as it is known for a long time that some species of this algae are able to grow in a mixotrophic environment; but scarce information is available on the use of autochthonous organisms, from the various types of wastewaters.

In this study, the potential use of a brewery effluent as a nutrient medium for microalgae growth was evaluated, resulting in the production of biomass, valuable for its composition, and amenable to be used as fertilizer or as feed for fish or other small animals. In addition, the capacity of C. vulgaris and the autochthonous flora of the effluent to remove some of the compounds present in the effluent was analysed.
MATERIALS AND METHODS

Biological material

*C. vulgaris* and a consortium (microalgae, cyanobacteria and bacteria) obtained from the autochthonous flora of a brewery effluent were used as described below.

Isolation and growth of the microalgae

*C. vulgaris* and *Arthrospira (Spirulina) platensis* were tested separately for growth in the referred effluent. Only the former was able to grow and thus was used as a control against the autochthonous consortium (microalgae, cyanobacteria and bacteria). The so-called autochthonous flora was isolated from the brewery effluent after filtration with glass filters GF/C Whatman, n°1822047; porosity: fine. The material retained in the filter was inoculated onto OHM (Optimal *Haematococcus* Medium) and BG (Blue-Green Medium – cyanobacteria) media, both with and without nitrate, to promote the growth of the microalgae possibly present, including some cyanobacteria that are able to fix nitrogen from the air, and thus are easily isolated in the absence of nitrate. The autochthonous flora obtained in BG medium was used as the inoculum for the experiments with the effluent. *C. vulgaris* was previously isolated in this laboratory from a waste discharge container and identified by the Department of Botany of the University of Coimbra. The microalgae was kept in OHM medium until required for use.

Cultures of *C. vulgaris* and the isolated consortium were established using the brewery effluent, and using OHM for *C. vulgaris* and BG for the consortium obtained from the effluent. All the experiments were carried out in a walk-in chamber, at constant temperature (25°C) and illumination (37 µE s⁻¹m⁻²) with cool white fluorescent lamps. The consortium was composed of green microalgae, cyanobacteria and bacteria. The main organisms present were identified by optical microscopy as *Chlorella, Scenedesmus, Oedogonium*, diatoms, and *Anabaena*. *Oedogonium* is not usually referred to as a microalgae.

Growth media

The OHM medium ingredients were as follows: KNO₃, 0.4 g L⁻¹; Na₂HPO₄, 0.03 g L⁻¹; MgSO₄, 0.12 g L⁻¹; CaCl₂, 0.11 g L⁻¹; EDTA, 0.006 g L⁻¹; CoCl₂·6H₂O, 0.012 mg L⁻¹; CuSO₄·5H₂O, 0.12 mg L⁻¹; CrO₃, 0.076 mg L⁻¹; MnCl₂·4H₂O, 1.12 mg L⁻¹; Na₂MoO₄·2H₂O, 0.121 mg L⁻¹; SeO₂, 0.006 mg L⁻¹; vitamins thiamine chloride hydrochloride, 0.175 mg L⁻¹; B₁₂, 0.15 mg L⁻¹ and H, 0.25 mg L⁻¹.

Two parts comprised the BG medium. Part I was prepared with NaCl, 35 g L⁻¹; tricine, 0.5 g L⁻¹; NaNO₃, 0.11 g L⁻¹; KH₂PO₄, 0.005 g L⁻¹; Na₂HPO₄, 0.045 g L⁻¹; H₂BO₃, 2.86 mg L⁻¹; MnCl₂·4H₂O, 1.81 mg L⁻¹; ZnSO₄·7H₂O, 0.222 mg L⁻¹; CuSO₄·5H₂O, 0.079 mg L⁻¹; Co(NO₃)₂·6H₂O, 0.049 mg L⁻¹ (pH 7.4). The two parts were mixed as equal parts. Both media were prepared with deionised water.

Growth was evaluated by direct counting with an Improved Neubauer chamber (*C. vulgaris*) or by chlorophyll determination (autochthonous flora). The extraction of chlorophyll was performed in 80% acetone and the chlorophyll concentration was calculated by the Jeffrey and Humphrey equations.

Algal biomass was harvested after sedimentation and centrifugation (10 min, 1,000 g). Dry weight was determined at 105°C until constant weight.

Influence of the effluent load on the microalgae growth

*C. vulgaris* was grown in the brewery effluent undiluted and diluted to 1:2 and 1:1 (v/v) with OHM culture medium. The inoculum was added at 6 mg chlorophyll L⁻¹ culture. The isolated consortium was grown using the same effluent dilutions used for *C. vulgaris*, but the effluent was diluted with deionised water. Nitrogen and phosphate concentrations in the effluent were brought to the levels present in the BG medium, which served as the control. The inoculum was added at 5 mg chlorophyll L⁻¹ culture. The pH of the effluent was corrected to 6.8–7.0. The scale-up process was from 1 L (glass culture flasks) to 15 L (plastic bags) and microalgae growth and removal of nitrogen and phosphates were followed. All experiments were performed in triplicate. Samples for analyses were taken in triplicate from each container.

Results from all the samples were combined and used to calculate averages, standard deviations and to perform the statistical analysis.

Influence of inoculum size on the growth of the isolated consortium

Growth of the isolated consortium in 1:1 (effluent: deionised water), with nitrogen and phosphate levels corrected to the BG medium levels, was tested using different concentrations of inoculum (1.5, 2.5 and 4.5 mg chlorophyll L⁻¹ culture). The pH was corrected to 6.8–7.0. Scale-up was from 1 L (glass culture flasks) to 15 L (plastic bags) and microalgae growth and removal of nitrogen and phosphates were followed. All experiments were conducted in triplicate.

Chemical analyses of the effluent

The BOD₅ and COD were determined using the methods described in Standard Methods for the Examination of Water and Wastewater. Quantification of ammonia was performed according to the Phenate Method and the nitrate content was determined by using the Nitrate Test Spectroquant kit, from Merck. Phosphates were determined according to the Vanadomolybdophosphoric Acid Colorimetric Method, described in Standard Methods for the Examination of Water and Wastewater.

Chemical analyses of the algal biomass

Analyses of the algal biomass produced by the end of each experiment were carried out. The amino acid content was determined according to the AOAC procedure and
the fatty acid profile was analysed according to the Lepage and Roy method\textsuperscript{19}, as modified by Cohen et al.\textsuperscript{7} Determination of the concentration of lead, cadmium, and nickel was based on the AOAC\textsuperscript{3} methodology. After incineration at 500°C, solubilisation and dilution in nitric acid, the sample was analysed in an atomic absorption spectrophotometer VARIAN mod. Spectr AA-20 at 217 nm, 228.8 nm and 232 nm, for lead, cadmium, and nickel, respectively. The mercury content was determined by the spectrophotometric method of atomic absorption, in cold phase, based on the European Standard\textsuperscript{11}.

**Statistical analysis**

Statistical analysis for the significant effect of the effluent on the growth ratios was performed using STATISTICA 6.0 (ANOVA/MANOVA) (StatSoft Inc., 1984–2001). Significance between means was assessed by the Multifactorial Tukey HSD post-Hoc test. Growth ratios were considered significantly different at $p < 0.05$. Significant effects of the biochemical parameters were statistically analysed by the means of the program STATISTICA 4.5, with ANOVA analyses, parametric and non-parametric (Kruskal-Wallis), for multiple groups and with the t-student test and Mann-Whitney (non-parametric) for groups of two.

**RESULTS**

**Effluent characterization**

Table I shows the characteristics of the effluent used during the experiments, in terms of organic matter content, expressed as BOD\textsubscript{5} and COD, and ammonia, nitrate and phosphate content.

**Influence of the effluent load on microalgae growth and nutrient removal**

The chlorophyll content for cultures grown in the effluent diluted 1:1 was significantly higher than for cultures grown in the control media, for either *Chlorella* (Fig. 1) or the autochthonous flora (Fig. 2). However, growth rates of autochthonous flora were twice as high as those obtained with the control medium (Table II), but were not statistically different from each other. By the end of each experiment, a slight decrease in the organic load of the effluent was noticed (Table III).

Concerning nutrient removal (Table IV), the highest nitrogen removal rate obtained with *Chlorella* was during growth in the control medium, with the lowest removal occurring for the complete effluent. Lower removal rates were observed for phosphate, again with control cultures presenting the highest rates. For the autochthonous flora, the highest removal rates of nitrogen and phosphate were obtained in complete effluent. An increase in the effluent load led to an increase in the removal rate. Removal rates of nitrogen in the control and 1:2 effluent cultures were not statistically different.

![Fig. 1. Growth curves of *Chlorella vulgaris* cultures, in complete effluent and effluent diluted to 1:1 and 1:2 (v/v) with growth medium (C. vulgaris) and deionised water (autochthonous flora), and in the control OHM and BG growth media.](image)
Table III. Removal of organic load from the brewery effluent by the autochthonous flora (values for BOD5 and COD were obtained from two different experiments with the correspondent percentages of removal shown in brackets).

<table>
<thead>
<tr>
<th>Experiment</th>
<th>Parameter</th>
<th>Initial values (mg O2 L⁻¹)</th>
<th>Final values (mg O2 L⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>BOD5</td>
<td>2,354</td>
<td>1,923 (18)</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>3,846</td>
<td>3,363 (13)</td>
</tr>
<tr>
<td>B</td>
<td>BOD5</td>
<td>1,340</td>
<td>977 (27)</td>
</tr>
<tr>
<td></td>
<td>COD</td>
<td>2,172</td>
<td>1,854 (15)</td>
</tr>
</tbody>
</table>

Table IV. Removal of nutrients from the brewery effluent by the microalga *Chlorella vulgaris* and by the autochthonous flora. Modal values for nitrogen and phosphate in g (nutrient removed) kg⁻¹ (produced biomass) d⁻¹. Standard deviation refers to all the replicate samples taken from the triplicate containers.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Rate of removal (g kg⁻¹ biomass d⁻¹)</th>
<th>Rate of removal (g kg⁻¹ biomass d⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen</td>
<td>Control 225 ± 11</td>
<td>Control 2,250 ± 197</td>
</tr>
<tr>
<td></td>
<td>1:2 effluent 139 ± 12</td>
<td>1:2 effluent 2,282 ± 113</td>
</tr>
<tr>
<td></td>
<td>1:1 effluent 103 ± 7</td>
<td>1:1 effluent 3,915 ± 172</td>
</tr>
<tr>
<td></td>
<td>Complete effluent 63 ± 0</td>
<td>Complete effluent 5,855 ± 212</td>
</tr>
<tr>
<td>Phosphate</td>
<td>Control 70 ± 0</td>
<td>Control 39 ± 5</td>
</tr>
<tr>
<td></td>
<td>1:2 effluent 32 ± 0</td>
<td>1:2 effluent 82 ± 3</td>
</tr>
<tr>
<td></td>
<td>1:1 effluent 23 ± 0</td>
<td>1:1 effluent 252 ± 9</td>
</tr>
<tr>
<td></td>
<td>Complete effluent 28 ± 0</td>
<td>Complete effluent 805 ± 15</td>
</tr>
</tbody>
</table>

Fatty acid content of the biomass produced

The fatty acid content of the *Chlorella* biomass (Table V) revealed differences concerning the polyunsaturated fraction, where the control biomass had higher levels of 18:2ω6, and lower levels of monounsaturated fatty acids, especially of the 16:1 and 17:1. Although the fatty acid content showed a general decrease with an increase in the effluent load, an increase in the ramified saturated fatty acid content and at higher extent in the 14:0isobr, 18:4ω3 and in the EPA (eicosapentaenoic acid) content occurred. The biomass produced from cultures of the autochthonous flora grown in the effluent presented a fatty acid content (49.5 g kg⁻¹ biomass) seven to eight times higher than the fatty acid content of the control biomass (6.0 g kg⁻¹ biomass) (data not shown).

Unlike what was observed with *Chlorella*, the saturated fraction of fatty acids of the biomass obtained from the isolated consortium was dominant when compared to the mono- and polyunsaturated fractions. However, the polyunsaturated fraction also showed a significant increase with an increase of the effluent load. The main components of the saturated fraction were palmitic acid (16:0) and myristic acid (14:0). The presence of the ramified saturated fatty acids, such as 14:0isobr and 17:1, can be related to the presence of bacterial contaminants. The most important fatty acids in the monounsaturated fraction were palmitoleic acid (16:1) and oleic acid (18:1). Linoleic acid (18:2ω6) and γ-linolenic acid (18:3ω6) appeared as the most important fatty acids in the polyunsaturated fraction (Table VI). Again, and unlike *Chlorella* biomass, the 06 family of the polyunsaturated group was the main component of this fraction.

Amino acid content

The protein content of the biomass obtained from cultures grown with effluent (both *C. vulgaris* and the
autochthonous flora) was always higher than in the biomass obtained from control cultures, the difference being statistically different. A significant increase in the amino acid content, both total and essential amino acids (Fig. 3), was observed, especially the aspartic and glutamic acids, and valine. Nevertheless, the ratio of essential/total amino acids was similar in all conditions and near 42.7.

**Incorporation of heavy metals**

The concentration of heavy metals in the algal biomass (Table VII) was higher than in the growth medium. Both in *C. vulgaris* and in the autochthonous flora biomass, the levels of Hg were between 0.05 and 0.22 mg kg\(^{-1}\) biomass, the maximum obtained when microalgae were grown in the complete effluent. The content of Cd was similar to Hg (maximum of 0.12 mg kg\(^{-1}\) biomass). The biomass of the autochthonous flora always presented higher concentrations of lead than the biomass of *Chlorella*, ranging from 2.9 to 6.25 mg Kg\(^{-1}\) biomass for the autochthonous flora, and between 1.0 and 2.0 in the case of *Chlorella*. Nevertheless, the results obtained were consistent, presenting higher values of lead concent-

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**Table V.** Concentration of the main fatty acids in the final *Chlorella vulgaris* biomass, grown under different effluent loads. Standard deviation refers to all the replicate samples taken from the triplicate containers.

<table>
<thead>
<tr>
<th>Fatty acids (g kg(^{-1}))</th>
<th>Control</th>
<th>1:2 effluent</th>
<th>1:1 effluent</th>
<th>Complete effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0 isobr</td>
<td>0.02 ± 0.002</td>
<td>1.57 ± 0.06</td>
<td>2.11 ± 0.18</td>
<td>2.03 ± 0.83</td>
</tr>
<tr>
<td>16:1</td>
<td>1.01 ± 0.13</td>
<td>5.95 ± 0.39</td>
<td>5.45 ± 0.42</td>
<td>4.70 ± 0.97</td>
</tr>
<tr>
<td>17:1</td>
<td>2.85 ± 0.24</td>
<td>9.31 ± 0.73</td>
<td>7.74 ± 1.04</td>
<td>3.08 ± 0.05</td>
</tr>
<tr>
<td>18:2 ω6</td>
<td>70.01 ± 7.47</td>
<td>14.81 ± 0.76</td>
<td>14.18 ± 1.13</td>
<td>13.75 ± 2.07</td>
</tr>
<tr>
<td>18:3 ω3</td>
<td>26.30 ± 2.06</td>
<td>13.04 ± 0.76</td>
<td>10.09 ± 0.88</td>
<td>41.18 ± 0.73</td>
</tr>
<tr>
<td>18:4 ω3</td>
<td>0.05 ± 0.0004</td>
<td>0.90 ± 0.07</td>
<td>0.10 ± 0.008</td>
<td>0.13 ± 0.07</td>
</tr>
<tr>
<td>20:5 ω3</td>
<td>0.04 ± 0.03</td>
<td>n.d.*</td>
<td>0.07 ± 0.01</td>
<td>n.d.</td>
</tr>
</tbody>
</table>

\(^{a}\) Not detectable.

**Table VI.** Concentration of the main fatty acids in the final autochthonous flora biomass grown under different effluent loads. Standard deviation refers to all the replicate samples taken from the triplicate containers.

<table>
<thead>
<tr>
<th>Fatty acids (g kg(^{-1}))</th>
<th>Control</th>
<th>1:2 effluent</th>
<th>1:1 effluent</th>
<th>Complete effluent</th>
</tr>
</thead>
<tbody>
<tr>
<td>14:0</td>
<td>0.14 ± 0.02</td>
<td>4.37 ± 0.29</td>
<td>1.19 ± 0.07</td>
<td>1.49 ± 0.02</td>
</tr>
<tr>
<td>14:0 isobr</td>
<td>0.06 ± 0.01</td>
<td>0.84 ± 0.10</td>
<td>0.45 ± 0.04</td>
<td>0.81 ± 0.06</td>
</tr>
<tr>
<td>16:0</td>
<td>2.47 ± 0.26</td>
<td>14.88 ± 0.55</td>
<td>22.99 ± 0.9</td>
<td>13.43 ± 0.1</td>
</tr>
<tr>
<td>16:1</td>
<td>0.39 ± 0.03</td>
<td>8.78 ± 0.68</td>
<td>2.85 ± 0.11</td>
<td>10.53 ± 0.12</td>
</tr>
<tr>
<td>17:1</td>
<td>0.04 ± 0.001</td>
<td>0.19 ± 0.01</td>
<td>0.20 ± 0.01</td>
<td>0.39 ± 0.01</td>
</tr>
<tr>
<td>18:1</td>
<td>0.40 ± 0.05</td>
<td>3.14 ± 0.07</td>
<td>3.78 ± 0.06</td>
<td>2.75 ± 0.02</td>
</tr>
<tr>
<td>18:2 ω6</td>
<td>1.01 ± 0.12</td>
<td>4.61 ± 0.16</td>
<td>9.09 ± 0.11</td>
<td>4.36 ± 0.12</td>
</tr>
<tr>
<td>18:3 ω6</td>
<td>0.55 ± 0.06</td>
<td>2.56 ± 0.06</td>
<td>5.94 ± 0.13</td>
<td>3.22 ± 0.12</td>
</tr>
</tbody>
</table>

Fig. 3. Essential amino acid profile of the *Chlorella vulgaris* and autochthonous flora biomass grown under different effluent loads.
Nickel levels were not quite different between the biomass of *Chlorella* and the biomass from the autochthonous flora, with the highest concentrations being registered in the biomass grown within the effluent. Moreover, concentrations in Ni increased when increasing the concentration of the effluent, all of these being significantly different from the ones of the control cultures.

**DisCUSSION AND CONCLUSIONS**

The variations in nutrient removal and growth of cultures observed in the brewery effluent were probably a result of the different compositions and concentrations of effluent for each batch of cultures. In the effluent, concentrations of phosphate were almost always higher than nitrogen, probably due to chemicals used in some of the brewery production units, as also reported by Driessen and Vereijken.

The removal rates of phosphorus were higher in the effluent, ranging from 54 and 66% (results of percentages not shown), similar to those reported by Gonzalez et al., and also higher than those observed in the control cultures. Nevertheless, phosphorus removal rates were always lower than those observed for N removal. The values for total N removal ranged between 85 and 90% for the effluent cultures. But here one has to consider that the organisms involved did not consume the entire N. In fact, despite being present at a higher concentration than nitrate, ammonia is easily removed because of the out-gassing to the atmosphere, due to the high pH, which shifts the equilibrium in favour of NH$_3$. Nevertheless, phosphorus uptake by algae was lower than nitrogen uptake because the nitrogen content of algae was approximately ten times higher than the phosphorus content.

The results obtained for nutrient removal (Table IV) are supported by previous reports, where high rates of N and P removal were observed during the growth of *Scenedesmus* sp. In reference to *C. vulgaris*, the highest nitrogen removal rate was obtained in the control cultures. Lower removal rates were obtained for phosphate, again with control cultures presenting the highest rates. For the autochthonous flora, the highest removal rates of nitrogen and phosphate were obtained in the complete effluent.

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<table>
<thead>
<tr>
<th>Heavy metals (mg kg$^{-1}$)</th>
<th><em>Chlorella vulgaris</em></th>
<th>Autochthonous flora</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mercury</td>
<td>0.04</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>Cadmium</td>
<td>0.01</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>Nickel</td>
<td>0.34</td>
<td>0.57</td>
</tr>
<tr>
<td>Lead</td>
<td>1.10</td>
<td>2.90</td>
</tr>
</tbody>
</table>

**Table VII.** Heavy metals content of the *Chlorella vulgaris* and the autochthonous flora biomass, grown under different effluent loads.

**Fig. 4.** Growth curves of the autochthonous flora cultures, in effluent 1:1 (v/v, in deionised water), for different concentrations of inoculum: I – inoculum of 1.5 mg chlorophyll L$^{-1}$ culture, II – inoculum of 2.5 mg chlorophyll L$^{-1}$ culture, III – inoculum of 4.5 mg chlorophyll L$^{-1}$ culture.
Algae also contribute to the treatment of wastewaters by supporting the growth and metabolism of epiphytes, such as bacteria, which may have contributed to the higher removal of nitrogen observed in the autochthonous cultures. An increase in the effluent load corresponded to an increase in the removal rate. The removal of nitrogen numbers in the control and in the 1:2 effluent cultures were not statistically different.

In general, the rate of N removal was much higher than the rate of P removal as also reported by Li et al. with algae grown in ponds. It should be noted that, as microalgae usually use inorganic phosphate rather than the organic compounds, only the inorganic phosphate was considered in the calculation of removal rates.

The highest protein content of biomass was observed when growth occurred with the effluent, for both cultures of Chlorella and the autochthonous flora. Similar results were verified by Sánchez et al. when treating an olive-mill wastewater.

In this work, the ratio of essential to total amino acids was similar in all culture conditions and was near 42.7, a value similar to that registered for egg protein. The results obtained for the quality of the proteins in the biomass are supported by Hammouda et al., who worked with Scenedesmus sp. and Chlorella vulgaris grown on wastewater as a nutrient medium, since similar protein profiles were obtained. However, new protein is not mainly due to ammonia uptake, since a significant part of it is lost to the atmosphere.

Relating to fatty acid composition, it seems that, in general, the best quality of lipids, in terms of the fatty acid profiles, was obtained from the biomass of the autochthonous flora grown in the effluent diluted 1:2, except for the 18:2ω6 and 18:3ω3 fatty acids.

In general, the content of heavy metals was similar in Chlorella and in the autochthonous flora cultures. In contrast, the lead content was much higher in the autochthonous flora. This could be related to the fact that for the autochthonous flora the effluent was not diluted with culture medium, but rather with deionised water, and thus there was no chelating agent in the medium. The presence of any chelating agent could reduce the adsorption ability of metals, for it is well known that metals in diluted chelated solutions are hard to remove. Hence, organisms from the autochthonous flora could adsorb lead in higher quantities instead of absorbing it. This high capacity of adsorbing heavy metal ions is mainly due to the charged functional groups on the cell walls of microalgae, which can act as binding sites for metals. In the case of Chlorella, since the effluent was diluted with culture medium, containing EDTA as a chelating agent, the algae would have absorbed metals until concentrations that were not toxic for the metabolic activities.

Concentrations of Hg were relatively high in biomass grown in the complete effluent. Nevertheless, for feeding animals with the biomass obtained, the values observed are within the range established by the EC for animal feed. Ni is the only metal whose maximum admissible limit is not yet legislated, but it is known that Ni presents a lower toxicity than the other heavy metals. In the US, for example, the US-EPA upper admissible limit, for humans, of Ni concentration in water is 0.04 ppm, whilst for Pb the upper acceptable concentration is 0.015 ppm. Moreover, the Dietary Reference Intake (DRI or Recommended Dietary Allowance, RDA) for nickel is 300–700 mcg, with a therapeutic range between 500 mcg and 50 mg.

Because of some discrepancy of nitrate and ammonia, and phosphate concentrations in the culture medium, and thus in the final biomass, simultaneous quantification of these compounds in the medium and protein concentration in the algal biomass, along with the total losses of volatile ammonium, would benefit clarification of the results obtained. In addition, removal rates could be improved if using a higher inoculum concentration or treating the effluent with a semi-continuous instead of a batch regime.

The study of the microbial composition, including the potential role of bacteria and proportions between different organisms of the autochthonous consortium, and variations during experiments, would also enrich future investigations. Consortia could be advantageous since bacteria are able to use ammonia, converting it to nitrate, which would be used by the algae. Furthermore, bacteria produce CO₂ during metabolism, this product being necessary for microalgae to carry out photosynthesis. Moreover, in consortia, different organisms could use different compounds present. Considering the protein and fatty acid content and the absence of heavy metals in significant amounts, it can be appropriate for use as animal feed or for biofuel production.

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