THE GROWTH OF MICROALGAE USING AN EFFLUENT FROM A BREWERY AS THE CULTURE MEDIUM

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ABSTRACT

When treating a effluent like this one from a brewery, one have to think that either the organic load, the nutrients, such as nitrogen, phosphorus, or metals, have to be removed. Biological treatment is the most useful process because the final residual water can be almost free from organic and inorganic matter, with biomass valorisation. Growth of microalgae was evaluated in effluent either as it is or diluted. Ammonium and nitrate nitrogen, phosphates, and some heavy metals, were measured in the initial effluent and also in the final wastewater. Besides, microalgae biomass was also analysed for the protein and fatty acid content.

INTRODUCTION

Treatment of effluents and wastewaters from the Agro-Food Industries has a relevant importance, both in Europe and in Portugal, either at the industrial or the economical level, and also from the environmental point of view.

Removing of the organic load, nutrients as (ammonium or nitrate) nitrogen and phosphorus, and also metals, such as iron and manganese (Mouchet, 1992), can be done using physical and chemical methods. However, biological methods for treatment of the residual waters has been being improved, either aerobic or anaerobic, depending on the organic charge (Droste, 1997) and the utilization of the final treated effluent.

One of the most profitable biological methods could be microalgae utilization, for these microorganisms are very able to remove and incorporate carbon, nitrogen, phosphorus (Hammouda *et al.*, 1995), or metals like iron and manganese, which can be dissolved in the wastewater. Moreover, they can even remove heavy metals like Pb, Cd, Ni and Hg (Chen *et al.*, 1998) and accumulate them in the biomass. Besides, after treatment, there can be some algal biomass valorization, in human (Jassby, 1988) or animal feed, as a protein source, or using some of their components like pigments, enzymes.

In this study, besides the evaluation of a brewery effluent utilization as a growing medium for microalgae, it was also shown that in the end, the algal biomass could be used as animal feed, after being analysed for protein, fatty acid content and heavy metals, eventually incorporated.

MATERIAL AND METHODOLOGIES

***Microalgae*: either *Chlorella vulgaris* or the autochthonous flora from the effluent were used to carry out this study.**

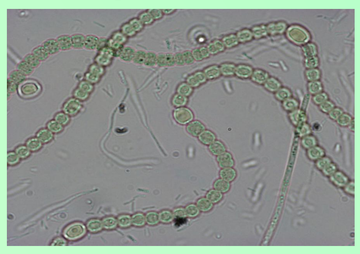
***Growth curves for biomass evaluation*: by direct counting, (*C. vulgaris*) and by chlorophyll quantification (autochthonous flora), in 80% acetone, and determined by Jeffrey and Humphrey (1975) equations.**

***Characteristics of the effluent* (table 1): BOD, COD, and phosphates (vanadomolibdophosphoric acid colorimetric method) analyses were made according to the *Standard Methods* (1998); ammonium quantification was done according to the Phenate Methodology (Weatherburn, 1967); for nitrates determination a kit from Merck (Spectroquant) was used and OD read on a UV spectrophoto-meter, under 338nm.**

***Analysis to the biomass*: Aminoacids content was determined according to the procedures of AOAC(1998). Analysis to the fatty acids respected Lepage and Roy (1986) methodologies, somewhat modified by Cohen *et al.*, 1988). Quantification of Pb, Cd, Ni was based on the AOAC(1990) procedures, but Hg followed the spectrophotometric method of atomic absorption, based on NP 2928 (1988).**

Table 1. Characteristics of a brewery effluent: range of values for indicated parameters.

Characteristics of the effluent	Range of values
CBO5	560-4778mg O2/L
CQO	565-7837mg O2/L
Ammonium	0.173-5.913mM
Nitrate	0.030-0.180mM
Phosphate	0.597-3.430mM



***Statistical analyses*: STATISTICA 4.5©, with ANOVA analysis was used to look for the significance of data, either parametric and non-parametric (Kruskal and Wallis) for multiple groups, and student's *t-test* and Mann-Whitney (non-parametric) for groups of two.**

Effluent was used as it is and diluted with distilled water; nitrogen and phosphorus were corrected to 9.5mM (as nitrate) and 0.124mM, respectively, when working with the autochthonous flora. These values are equivalent to the ones of BG medium.

Every experiment was carried out in triplicates, except for the cultures grown in plastic bag reactors, when only duplicates were made. All the analyses were made in triplicates.

RESULTS AND DISCUSSION

Biological treatment of a effluent can be done by anaerobic or aerobic processes. This case, some species of microalgae can be used, which sometimes are even able to degrade organic matter in the residual water and incorporate it in their own biomass. Besides they can also remove and incorporate with success nitrogen, phosphorus, and even some salts that dissolved in the effluent.

Actually, in this piece of work, not only *Chlorella vulgaris* but also the autochthonous flora from the residual water have shown a increase in their growth, more evident when a effluent from a brewery was used as the culture medium (Figure 1). These results are supported by previous works (Van-Coillie *et al.*, 1990; Picot *et al.*, 1991), when high rates of N and P removal were observed along with a more evident growth of the algae.

Besides, it was also observed that, in general, rate of N removal was more efficient that P removal (Table 2), as it was verified by Li *et al.* (1991) with algae grown in ponds.

Table 2. Effective capacity of treatment of the brewery effluent by the microalga *Chlorella vulgaris* and by the microalgae consortium obtained from the autochthonous flora of the same effluent.

Evaluated parameters		Initial values	Percentage de of removal		Initial values	Percentage of removal
		Chlorella vulgaris			Microalgae consortium	
CBO5		560-2730	—		1340-2354	18.3-27.1
CQO		800-3900	—		2172-3846	12.6-14.6
nitrogen (mM)	C	0.404-0.585	30.9-59.5	C	3.563-8.251	39.2-62.8
	Effluent	5.302-10.261	84.7-98.0	Effluent	7.935-10.502	31.3-98.0
phosphate (mM)	C	0.144- 0.429	66.4	C	0.116- 0.223	37.8-85.2
	Effluent	0.597-3.430	11.8-53.9	Effluent	0.109-0.993	10.1-93.0
Protein (mg/L)					0.036-1.908	21.8-71.8

In what concerns the quality of the protein, the results obtained are also supported by Hammouda *et al.* (1995). A significant increase in the aminoacids content was observed, namely in aspartic and glutamic acids and tyrosine. Besides, from the analysis to the *Chlorella vulgaris* biomass, it was verified that aminoacids concentration was always higher in the algae grown with effluent, difference being statistically significant both at essential and total aminoacids. Moreover, the highest results were obtained with the biomass that grew in the effluent diluted to 1:1 (Figure 1). However, the ratio essential aminoacids/total aminoacids is not different in all the conditions.

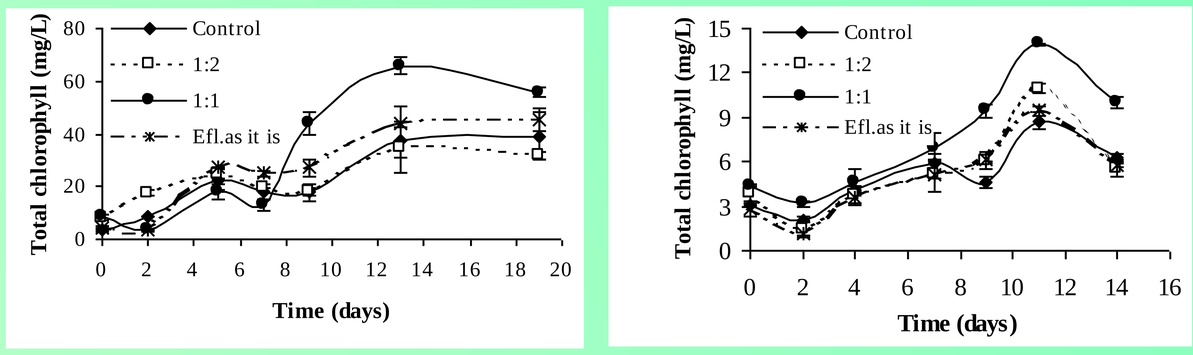


Figure 1. Growth curves of *C. vulgaris* (on the left) and the autochthonous flora (on the right) cultures, in effluent as it is (Effl) and diluted, against a control, in the usual growth medium.

Fatty acid contents (in percentage over the total fatty acids) showed the most evident differences in the polyunsaturated fraction, the control biomass having higher levels of 18:2ω6 and 18:3ω3, and lower percentages of monounsaturated, in special of 16:1 and 17:1. Although fatty acids content showed a general decrease with the increase of the effluent level, they also present an increase in the ramified chain fatty acids, and also a very significant increase in the 14:0isobr, 18:4ω3, and in the EPA (eicosapentaenoic acid).

In what concerns the incorporation of metals, concentration in the algal biomass is as high as the concentration in the growth medium (results not shown), as it was also observed by Chen *et al.* (1998) with *Pavlova lutheri* when exposed to higher and higher levels of different metals, as lead (Pb), copper (Cu), cadmium (Cd), nickel (Ni) or mercury (Hg). However, in the biomass samples, levels of Hg were between 0.04 and 0.21ppm in the control biomass and in the effluent (as it is) biomass, respectively, results being in the maximum range values established by the EC, in animal feed (Directiva 1999/29/EC). Lower than these limits were the contents in Cd (0.1ppm for the effluent as it is) and in Pb (1-2ppm). Ni is the only determined metal whose maximum admissible limits are not yet legislated; it presents, nevertheless, a lower toxicity than the other heavy metals.

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ACKNOWLEDGEMENTS

This work was supported by Project "Novos Sistemas de Reciclagem de Efluentes Agro-Industriais usando Biotecnologia de Microalgas com Valorização da Biomassa", from Programme Praxis XXI.

The authors would likle to thank UNICER-União Cervejeira AS for the effluente, but also Rui Mendes and Pedro Castro for their helpful work.