ON THE UTILIZATION OF THE ALGAL BIOMASS GROWN IN A BREWERY EFFLUENT IN ANIMAL FEED

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INTRODUCTION

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

One of the most profitable biological methods could be microalgae utilization. Besides, after treatment, there can be some algal biomass valorization in 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:
- ***Chlorella vulgaris***
- **autochthonous flora from the effluent**

Growth curves for biomass evaluation:
- **direct counting (*C. vulgaris*)**
- **chlorophyll quantification (autochthonous flora)**
  - **in 80% acetone, and determined by Jeffrey and Humphrey equations (1).**

Characteristics of the effluent (table 1):
- **BOD (2)**
- **COD (2)**
- **Phosphates**
  - **Vanadomolibdophosphoric Acid Colorimetric Method (2)**
- **Ammonium**
  - **Phenate Methodology (3)**
- **Nitrates**
  - **Merck (Spectroquant) kit and OD read on a UV spectrophotometer, at 338nm.**

Analyses to the biomass:
- **Aminoacids (4)**
- **Fatty Acids (5) (6)**
- **Pb (7)**
- **Cd (7)**
- **Ni (7)**
- **Hg (8)**

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

Experiment:
- **Nitrogen (autochthonous flora)**
  - **corrected to 9.5mM (as nitrate)**
- **Phosphorus (autochthonous flora)**
  - **corrected to 0.124mM**
- **triplicates**
- **duplicates**
  - **cultures grown in plastic bag reactors**

Effluent:
- **as it is**
- **diluted with distilled water**

Analyses:
- **in triplicates**

Statistical analyses:
- **ANOVA**
- **Student's *t-test***
- **Non parametric tests**
  - **Kruskal and Wallis**
  - **Mann-Whitney**

RESULTS AND DISCUSSION

In this 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, 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).

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 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

In what concerns the quality of the protein, a significant increase in the aminoacids content was observed. 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.

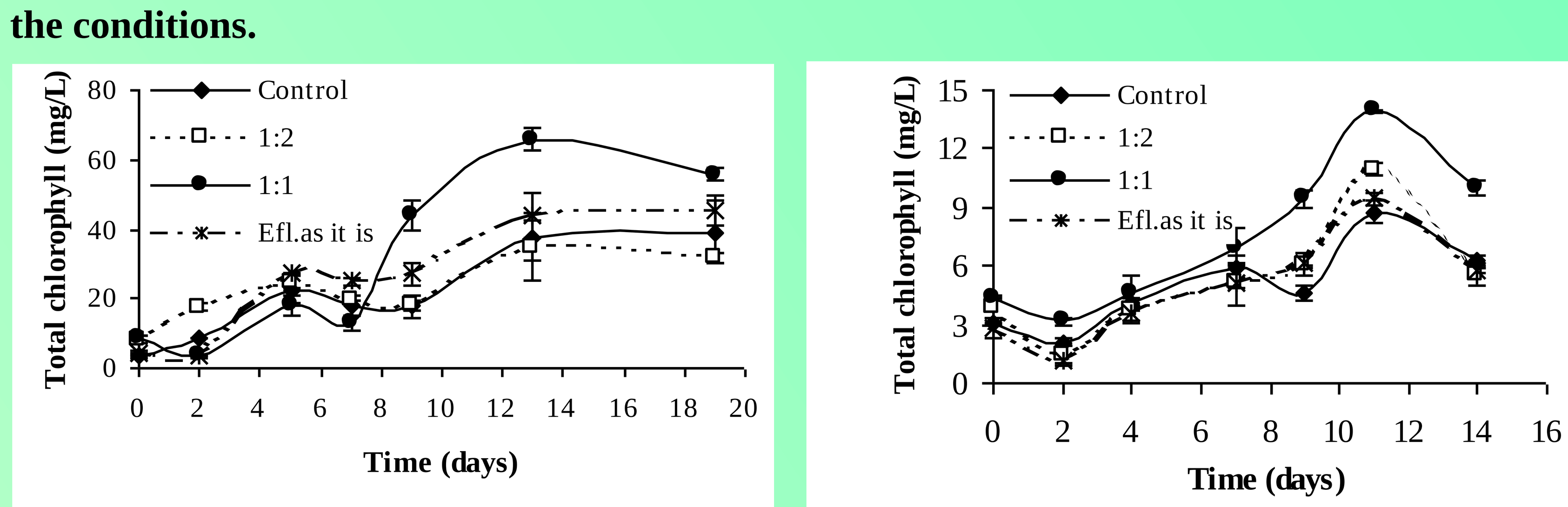

Figure 1. Growth curves of *C. vulgaris* (on the left) and the autochthonous flora (on the right) cultures, in effluent as it is (Effl) and diluted, against a control, in the usual growth medium.

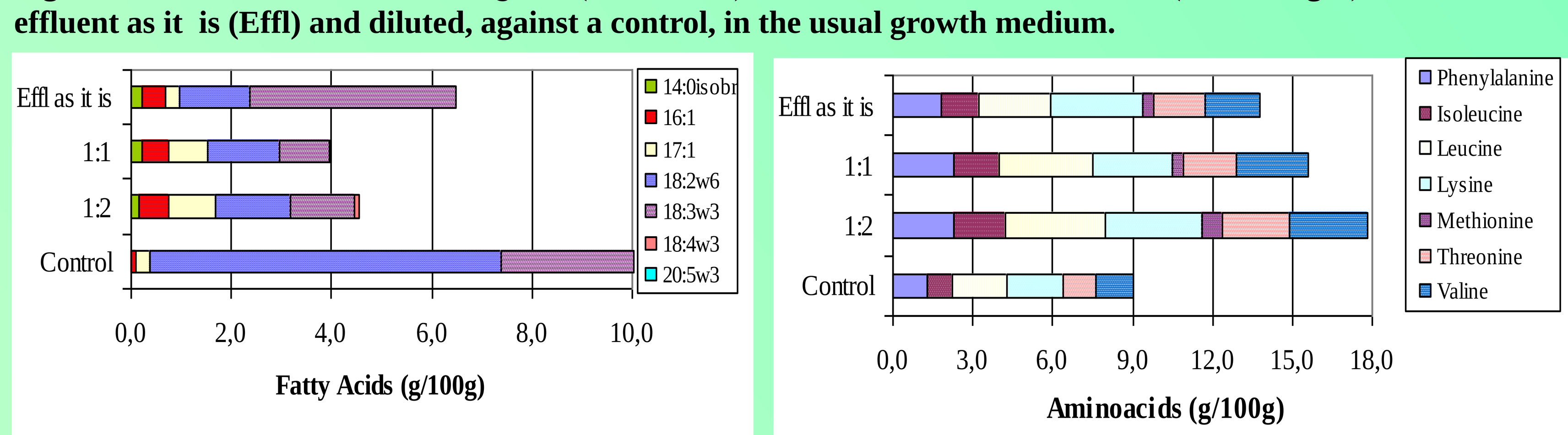

Figure 2. Different percentages obtained for fatty acid composition (on the left) and aminoacids content (on the right) with the biomass grown in the effluent as the growth medium, against the control (usual growth medium).

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 (figure 2).

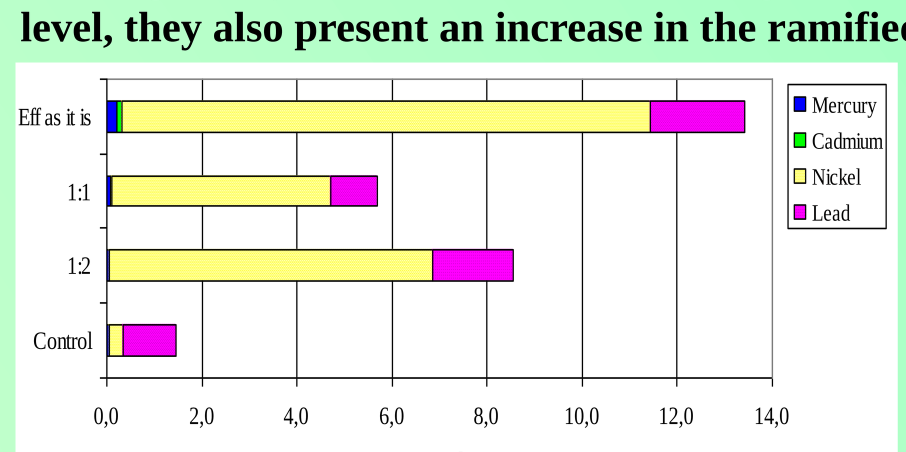

Figure 3. Contents in mercury, cadmium, nickel, and lead in the biomass samples from the effluent, either as it is or diluted, and the control (usual culture medium).

In what concerns the incorporation of metals, concentration in the algal biomass is as high as the concentration in the growth medium. The results (figure 3) are in the maximum range values established by the EC, in animal feed (9). 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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