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Chlorella vulgaris as a biological matrix for dairy effluent remediation

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Abstract

Phycoremediation, which uses the purifying capacity of microalgae and macroalgae to eliminate or biotransform contaminants, has emerged as a technology for wastewater treatment. The objective of the present work is to evaluate the potential of *Chlorella vulgaris* to remove nutrients present in effluents generated in a dairy farm and to know the concentration of chlorophyll *a* and the percentage of crude protein in the algal biomass obtained.
Chorella vulgaris was grown in culture medium with 25% effluent from dairy production for seven days. At the beginning and end of the assay, the following variables were determined: ammonium, nitrites, nitrates, total phosphorus, biological oxygen demand (BOD), chemical oxygen demand (COD), and pH. In addition, the amount (%) of crude proteins and chlorophyll-*a* were quantified in the obtained algal biomass. At the end of the assay, the following parameters decreased: ammonium, BOD, and COD=97.1%, 81.7%, and 80.8% respectively. In the pellet, chlorophyll-*a* and the percentage of proteins reached values of 1.68 µg $L^{-1}$and 3.75 % respectively. The results demonstrate the potential of *C. vulgaris* for the reduction of pollutants. In addition, it was shown that effluents from dairy production may be a less expensive alternative for the growth of microalga, with environmental and economic benefits.

Keywords: Phycoremediation; *Chlorella vulgaris;* Effluent of dairy production

Introduction

Intensive livestock production in emerging countries that base their economy on non-value-added raw materials generates large volumes of effluents that have a high content of solids, nutrients, organic matter, and microorganisms. These effluents can negatively modify the natural environments receiving them by superficial runoff or infiltration through the soil, contaminating the groundwater with possible damage to the health of the populations–especially the rural ones–that obtain the water from those aquifers (Herrero and Gil 2008).
In Argentina, dairy production has intensified in recent years. The most relevant indicator is the larger increase in the size of herds than in the surface or the number of dairy farms. This transformation of the production system increases the amount of effluents in milking facilities that do not have adequate infrastructure or planning on their final destination, causing environmental, economic and social problems (García 2013; De Grandis and Visintini, 2015).

The incorporation of nutrients to water bodies determines their chemical impairment by the excessive load of nitrates and phosphates that leads to the eutrophication of the system. This process can generate imbalances in the ecosystem due to changes in the structure and function of phytoplankton and zooplankton communities (Wu 1999). This process accounts for a third of the decline in biodiversity in rivers, lakes, and wetlands in the world (UN-Water 2015). In addition to the degradation of aquatic systems, these effluents are a source of proliferation of synanthropic pests (flies, rodents, among others) and the generation of undesirable odors when they are not adequately treated (FAO – INTA 2012).

Ecosystem damage includes natural hazards and negative impacts of water, soil and/or air pollution and is primarily considered as a risk to ecosystems and socioeconomic well-being (Sandifer et al. 2015). In the case of effluents from the dairy industry, the ecosystem services that are most affected by inefficient effluent management are the provisioning services (material benefits that people derive from ecosystems) and supporting services (necessary for the production of all other ecosystem services) (FAO 2016).

At the global level, several technologies have been developed for the treatment of dairy farm effluents. Among them, anaerobic lagoons and storage pits in the United States for later application as irrigation; through two stabilization ponds, the first being anaerobic and the second facultative (as in New Zealand); two lagoons as in Australia, or treatment through stabilization gaps in Uruguay. The efficiency of these treatments depends mainly on the quality and quantity of the discharge that is being generated in each facility, since the composition and concentration of the wastes vary in relation to the handling practices on each dairy farm (De Grandis and Visintini, 2015). On the other hand, the cleaning and purification achieved with the mentioned methods is not complete, so it is proposed that the process be improved using microalgae to remove the contaminating elements still present in the last phases of the effluent treatment that cannot be removed by other methods.

In order to respond to the growing problems of aquatic system contamination, in the last decade phycoremediation has been proposed as a new technology that takes advantage of the purifying capacity of microalgae and macroalgae (Park et al. 2011; Rawat et al. 2011; Prajapati et al. 2013) for the elimination or biotransformation of pollutants from wastewater (Dominicet al. 2009; Doušková et al. 2010; León and Chaves 2010; González-López et al. 2011; Abdel-Raouf et al. 2012; Infante et al. 2012; Prajapati et al. 2013; Maity et al. 2014). This technology was successfully used to remediate petroleum products, heavy metals, detergents, and industrial effluents of different organic and/or inorganic composition (Rachlin and Grosso 1991; Chong et al. 2000; Mehta and Gaur 2001; Salomon et al. 2003; Sáenz et al. 2004; Johnstone et al. 2006; Rodriguez et al. 2007; Vera et al. 2009). Kim et al. (2007) cultivated *Scenedesmus* sp. in culture medium with 3% of swine effluent and obtained a biomass rich in chlorophyll and carotenoids, besides reducing the concentration of carbon, nitrogen, and phosphorus by 12.9 %, 87 %, and 83.2% respectively. Morales-Amaral et al. (2015) reported that after cultivating the same genus of microalgae in open air reactors, the optimum percentage of the effluent in the culture medium was 30%. The reduction of nitrogen and phosphorus was proportional to the productivity of the biomass: 38.0 mg $_{N}$ L^{1} day ^{1} and 3.9 mg $_{P}$ L^{1} day^{1} respectively. Above this percentage, the yield decreased significantly, probably due to excess of ammonium. Moreover, the authors found that the algal biomass obtained had a high content of proteins, lipids, carbohydrates, among other value-added products. In this way, the efforts to cultivate microalgae in wastewater sought to obtain a double benefit, on the one hand, the production of biomass for different uses and on the other, a clean effluent using a relatively simple technology (Pulz and Gross 2004; Rawat et al. 2011).

The foregoing demonstrates the importance to develop tools for environmental management that consider the sustainability aspects of production systems. In this line, social and environmental health aspects should also be considered with a dual purpose: to optimize the possible uses and applications of the algal biomass obtained by technological processes and to improve the quality of diary effluents before their discharge into natural environments. This work aims to evaluate the potential of *Chlorella vulgaris* for nutrient removal from effluents generated in a dairy farm, and to know the concentration of chlorophyll-*a* and the amount of proteins in the algal biomass obtained.

Materials and Methods

Characterization of dairy effluents

The effluent was collected from a dairy farm in the locality La Penca and Caraguata, province of Santa Fe, Argentina. The dairy farm has 200 cows with an average production of 22 L day^{-}1milk per cow. The effluent treatment system includes the use of the effluent for irrigation. The effluent is previously treated in two stabilization ponds, the first anaerobic and the second facultative.

In order to know the characteristics of the effluent, the following physicochemical traits were recorded: ammonium (mg L^{-1}**)**, nitrites (mg L^{-1}), nitrates (mg L^{-1}), total phosphorus (mg L^{-1}), biological oxygen demand (BOD, mg L^{-1}), chemical oxygen demand (COD, mg L^{-1}) and pH. The determinations were made following the techniques proposed by the Standard Methods for the Examination of Water and Wastewater, 1998.

Preliminary tests

C. vulgaris was cultivated under increasing concentrations of effluent: 10%, 15%, 20%, 25%, and 30% effluent, diluted in BBM synthetic medium (Bischoff and Bold, 1963) in order to know the range of concentrations of effluent in which *C. vulgaris* could grow. The endpoint evaluated was the growth rate (μ) expressed in days $^{-1}$after5 days of culture. The culture conditions were: synthetic medium: BBM; volume of culture: 1000 mL; inoculum: 50000 cel.ml^{-1}; Tº: 25±1ºC; constant illumination: 120 μE m^{-2} s^{-1}; agitation: constant at 100 rpm; assay time: 5 days.

To evaluate the efficiency of *C. vulgaris* to remedy effluents from dairy production, the growth rate was determined using the following formula (1):

$\mu_{i\text{-}j} = (\ln X_j - \ln X_i) / t_j - t_i$ (days^{-1}) (1)

where μ i-j = rate of growth between times i and j; Xi = biomass over time i; Xj= biomass over time j (OECD

2011). After obtaining the maximum concentration of effluent in which *C. vulgaris* could grow, the respective crop growth kinetics was performed to determine the growth phases and the optimum time of harvesting in the definitive tests.

Definitive tests

The final assays with *C. vulgaris* at the previously defined highest concentration of effluent were performed in 2000 mL Erlenmeyer flasks, in quadruplicate, under the same culture conditions as the preliminary tests. Cultures were harvested in the exponential growth phase.

The microalgae were then centrifuged for 10 min at 3500 rpm. At the beginning and end of the test, the following variables were determined in the supernatant obtained: ammonium, nitrites, nitrates, total phosphorus, BOD, COD, pH, and conductivity, following the methodology proposed by the Standard Methods for the Examination of Water and Wastewater, 1998.

Later, in the algal biomass obtained, the amount of crude protein (Kjeldahl Method, Kjeldahl J. (1883)) and chlorophyll-*a* was measured. To quantify chlorophyll-*a*, in each replica, 250 mL of the medium with the microalgae was centrifuged (glass fiber filters S&S, diameter 55 mm), transferred to test tubes protected from light, with 10 mL of acetone at 90%. Subsequently, the filter was macerated, and the samples were kept refrigerated (4 °C) for 24 h. At the end of this time, the absorbance (Abs) of the supernatant was measured (wavelengths 665, 645 and 630 nm (model T60 spectrophotometer, PG Instruments) to calculate the chlorophyll-*a* content by applying the formula proposed by Strickland and Parsons (1972) according to equations 2 and 3:

$$\mathrm{C} = (11.6 \times \mathrm{Abs}\ (\mathrm{nm}665)) - \left(1.31 \times \mathrm{Abs}(645\ \mathrm{nm})\right) - (0.14 \times \mathrm{Abs}\ (630\ \mathrm{nm})) \quad (2)$$

Calculation of chlorophyll-*a* (µg L^{-1})

$$\mathrm{Cla} = \mathrm{C}/\mathrm{V} \times 10/\mathrm{L} \times \mathrm{S}/10 \quad (3)$$

where: **Cla**: chlorophyll-*a*; **C**: result of the equation of Strickland and Parsons; **V**: volume of the sample filtered (0,25 L); **L**: cuvette diameter (1 cm); **S**: volume of acetone used (0.01 L).

Results

Characterization of dairy effluents

The physicochemical characterization of the effluents is shown in table 1.

Table 1: Physicochemical characterization of effluent

pH (UpH)	Ammonium (mg L^{-}	Nitrates (mg L^{-1})	Nitrites (mg L^{-1})	Total Phosphorus (mg L^{-1})	BOD (mg L^{-1} – O_2)	COD (mg L^{-1} – O_2)
7.09	294	43.6	0.064	60.1	2981	6078

Remediation of effluents from dairy production using C. vulgaris

Preliminary tests

Theµ (days^{-1}) in effluent a concentration of 10%, 15%, 20%, 25% and 30% was: 0.10; 0.14; 0.16; 0.19 and 0.11 respectively. That is, the highest µ was reached at the effluent concentration of 25%, decreasing at higher concentrations. From these results, the definitive tests were carried out cultivating *C. vulgaris* with 25% effluent. On the other hand, to determine the different stages of growth and harvest time in the definitive cultures, growth kinetics was performed (Fig. 1). An optimum harvest time of 7 days was determined for the final assays (harvest at exponential growth phase).

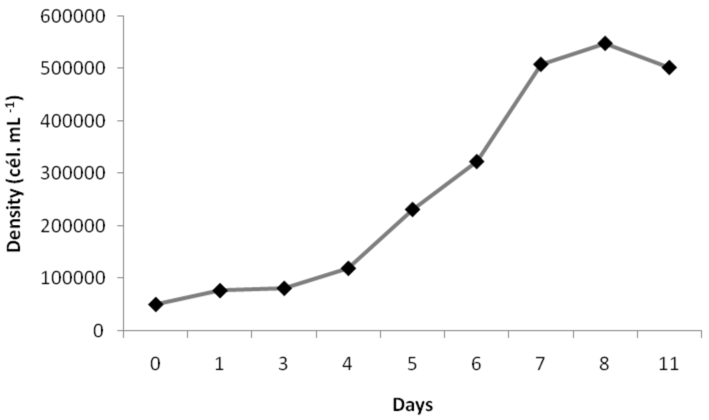

Fig. 1 Crop growth kinetics of *C. vulgaris* with 25% effluent from dairy production

Definitive tests

The results of the parameters analyzed at the beginning and at the end of the test in *C. vulgaris* cultures with 25% effluent are listed in table 2; in most cases there was a reduction of nutrients, with ammonium showing the most important reduction = 97.1%. In the pellet obtained at 7 days of culture, chlorophyll-*a* and the percentage of proteins reached values of 1.68 μg L^{-1} and 3.75%, respectively.

Table 2: Physicochemical characterization of the pure effluent and the supernatant obtained at the beginning and at the end of the final test, when cultivating *C. vulgaris* in culture medium containing 25% of dairy effluent.

	Pure Effluent	**25% Initial**	**25% Final**	**% reduction**
Ammonium (mg L^{-1})	294	67.2	1.9	97.1
Nitrates (mg L^{-1})	43.6	31.1	15.6	49.8
Nitrites (mg L^{-1})	0.064	0.067	0.107	-
Total Phosphorus (mg L^{-1})	60.1	48.4	19	60.7
COD (mgL^{-1} - O_2)	6078	1846	353	80.8
BOD (mg L^{-1} – O_2)	2981	947	173	81.7
pH (UpH)	7.09	7.58	8.79	-
Conductividad (μS cm^{-1})	4070	1665	1192	28.4

Discussion

In this work, high BOD reduction values were achieved (81.7%). In the studies that evaluated the potential of microalgae to remedy effluents, higher and lower values than those obtained in this work were reported. Colak and Kaya (1988) used microalgae to decontaminate urban wastewater and recorded BOD removal values of 68.4 %. In 2008, Hodaifa et al. reported similar results (67.4%) in cultures of *Scenedesmus obliquus*, using residual water from the production of olive oil as substrate. On the other hand, León and Chaves (2010) reported a reduction of 91.4% in treatments of stabilization ponds using microalgae.

Regarding the use of COD as a parameter of treatment efficiency, the results obtained (80.8%) are close to those reported in the literature. Hammouda et al. (1995) reported a COD removal of 89% and 91.7% for *Scenedesmus* sp. and *C. vulgaris* respectively. Hongyang et al. (2011) worked with *Chlorella pyrenoidosa* to bioremediate wastewater from soybean processing and obtained a COD removal between 80% and 84%, while Li et al. (2011) reached COD removal values of 90.3% and 90.8% using *Chlorella* sp. These authors concluded that microalgae rapidly used different organic compounds as a source of carbon, in addition to CO_2.

With regard to the removal of other compounds, Wang et al. (2010) reported the effectiveness of the use of digested effluent from dairy production as a nutrient supplement for the cultivation of *Chlorella* sp., obtaining removal values of several compounds similar to those found in this work: ammonia, total nitrogen, and total phosphorus =100%, 75.7%-82.5%,and 62.5%-74.7%, respectively. Moreover, the authors used 10%, 15%, 20%, and 25% dilutions of effluent, finding an inversely proportional linear regression (r^2= 0.982) between the growth rate and the initial turbidity during the first 7 days. These results could explain why in this study, *C. vulgaris* grew little or did not grow when it was cultured in more concentrated effluent (>25 %). On the other hand, Woertz et al. (2009) obtained ammonium and orthophosphate removal values of 96% and > 99% respectively, growing green algae supplemented with CO_2 and an effluent from dairy production. These values of ammonium removal are very close to those reported in the present work: 97.1%.

Another important aspect to be considered is the quality of the algal biomass obtained from the crops, i.e., the concentration of chlorophyll-*a* (1.68 µg L^{-1}) and the percentage of crude protein (3.75%). The results obtained here are lower than those reported in the literature for *C.vulgaris* cultures with and without effluents. Miao et al. (2016) cultivated *C.vulgaris* at concentrations greater than 80% of domestic wastewater, obtaining values of 40.9%-50.7% of proteins in the biomass. Ma et al. (2016) reported values of 55% protein in *C.vulgaris* cultures in wastewater with glycerol generated from biodiesel production. In cultures with synthetic medium (without effluents), Reno (2011) reported values of 46% of crude protein for *Chlorella* sp.; Morris Quevedo et al. (1999), 44%; Sansawa and Endo (2004), 12%; Andrade et al. (2006), 44%, and Zhengyun and Xianming (2006) obtained a production of 30% crude protein, while Sharma et al. (2012) reported between 54.2% and 52.6%, under different light regimes. Sankar and Ramasubramanian (2012) cultivated *C. vulgaris* in the same culture medium used in this work, reporting 36.16% of proteins after 20 days of culture, while Chia et al. (2013) cultivated *C. vulgaris* in CHU medium obtaining 50% of proteins.

As regards the concentration of chlorophyll-*a* for the same genus of microalga, Brito et al. (2016) reported values of 30.7µg L^{-1} chlorophyll-*a.* Otero-Paternina et al. (2012) reported the effects of concentrations of 1 and 1000 µg L^{-1} of phenanthrene on *Chlorella* sp., obtaining average values of chlorophyll-*a* of 1.05 ± 0.14 µg L^{-1} and 0.74 ± 0.15 µg L^{-1}, respectively. The differences found for the same species are likely to occur because, as is well known, in higher plants as well as in microalgae the composition of biomass and biochemistry may vary according to the surrounding conditions and the age of the culture (Lourenço et al., 1997; Renaud et al., 1999; Araújo and Garcia, 2005).

Another point to note is that *C.vulgaris* grown in medium with effluents from dairy production could be a source of raw material for the production of biopolymers such as cellulose, thus obtaining a double functionality: remediation of contaminated effluents and production of reusable biopolymers. Cellulose is a fibrous, tough, water-insoluble polymer that plays an essential role in maintaining the structure of cell walls. Moreover, cellulose is a biodegradable, biocompatible, and renewable natural polymer and hence, it is considered an alternate to non degradable fossil fuel-based polymers (George and Sabapathi, 2015)

Conclusion

The management of effluents involves planning their destination, minimizing negative impacts on the environment. To meet this goal, it is necessary to develop technologies and management recommendations to avoid the deterioration of the water quality, surface water courses and the environment in general, transforming this type of action into an investment for society.

In this sense, the results obtained demonstrate the potential of the microalga *C. vulgaris* for the reduction of pollutants using wastewater from the dairy industry as a culture medium. In addition, it was shown that effluents from dairy production may be a less costly alternative for microalga growth, allowing environmental and economic benefits to be generated through the purification of effluents and the production of biomass that can be used as feed products with high value added.

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