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MATHEMATICAL MODELING OF IMMOBILIZED MICROALGAE CULTIVATION ASSOCIATED WITH EFFLUENT TREATMENT

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Abstract. *The indiscriminate disposal of household and industrial waste, coupled with deficiencies in sewage collection, has been increasing contamination and the formation of wastewater. Consequently, conventional effluent treatment carried out by treatment plants shows inefficiency rates, with difficulties in meeting minimum quality standards. On the other hand, the use of microalgae appears to be a promising alternative for wastewater treatment, as they are capable of removing pollutants and waste, such as phosphorus, nitrogen, and organic compounds, during their cultivation. From the biomass, various high-value bioproducts can be obtained, especially those related to biofuel production. However, microalgae cultivation currently presents many industrial challenges, especially those related to the cultivation system, such as cost and biomass recovery, thus immobilization technology may be a possible solution to these problems. The methodology consists of trapping cells inside a porous and semipermeable membrane in the form of a capsule through physical-chemical interactions. Thus, immobilization tends to improve the intrinsic characteristics of microorganisms and refine the process, as it reduces energy expenditure in downstream operations, allows for the reuse of cells, and increases the capacity for pollutant complexation. Therefore, considering the bottlenecks and the alternative approach, this work consists of developing a mathematical model for immobilized microalgae cultivation concomitant with wastewater treatment. In this sense, it is necessary to adapt the Monod model to describe the biomass accumulation process and the consumption of contaminants as substrates. Additionally, this article develops the first mathematical model for immobilized microalgae, demonstrating computational simulations capable of accurately describing experimental data obtained for biomass production. In summary, through the predictability that mathematical modeling is capable of providing, it is feasible to optimize the overall process, increasing productivity, reducing the time and final cost of biomass. It also allows for the adoption of cell immobilization as an economically and environmentally favorable option for pollutant removal from wastewater.*

Keywords: *Cell immobilization, Microalgae biomass, Modeling and Simulation, Waste to energy, Wastewater Treatment.*

1. INTRODUCTION

Along the development of the planet water has been the most important resource for the development of life. Lately the resource has been polluted thanks to the huge growth of the population, therefore occupying uninhabitable areas and the uncontrolled urban waste problem, not to mention the industrial waste, according to data from UNESCO, in 2017, the population rose from 314 million people to almost 500 million people in less than 20 years, being one of the motivations to the amount of residual water being generated. Another reason is the low sewage collection, especially considering that clean water distribution grows faster then sewage collection.

Data collected from the Agência Nacional de Água (ANA) in the same year has shown that only 43% of the brazilian national sewage is collected and treated, while 39% of organic matter is removed in the treatment plant, which is lower than what CONAMA establishes as acceptable to be sent to a course of water. CONAMA is the system responsible for drafting laws regarding the environment in Brazil.

To discharge treated water into freshwater bodies of water, it is necessary to comply with current CONAMA legislation, especially Resolution 357 of 2005, which cites the acceptable terms for discharge of water. Considering the main elements to be treated by microalgae, the acceptable limits are shown in Table 1.

Table 1. Acceptable levels of inorganic salts in freshwater.

Inorganic Parameters	Maximum Value
Total phosphorus (lentic environment)	0,020 mg * L-1 * P
Total phosphorus (intermediary systems with residency time between 2 and 40 days)	0,025 mg * L-1 * P
Total phosphorus (lotic environment and intermediary systems)	0,1 mg * L-1 * P
Nitrate	10 mg * L-1 * N

The amount of residual waters generated is one of the main concerns of humanity. The concern is based on the fact that the legislation determines an acceptable level so effluent can be discharged on water bodies. The actual process of conventional treatment of water consists of three phases, primary, secondary and tertiary treatment, which are based on physical, biological and chemical processes (Abdel-Raouf et al. 2012).

Primary treatment is responsible for removal of suspended solid material, through physicochemical processes, such as flocculation and decantation, being capable of withdrawing up to 40% of organic matter from effluent. Secondary treatment's main focus is based on biological processes to remove the leftover organic matter and also to remove inorganic salts, mainly nitrogen and phosphorus based matter. This process can be called phycoremediation depends on microalgae and bacteria to utilize organic and inorganic material in their development. Focusing on microalgae the process transforms the material into dioxide carbon and biomass that can be used in industrial processes. The problem of the actual water treatment process is that it demands large infrastructure and large scale industrial processes, allied to the technological knowledge hence why the actual process does not produce satisfactory results (Tom, 2021).

Even though it is possible to use microalgae as a means of treatment, the removal of the organisms from water demands either a long period of time, or high energy cost, due to the dimensions of the organism, sometimes of microscopic size, and that they grow freely in the medium. According to Molinuevo-Salces, (2019) the biomass harvesting process can cost up to 30% of the operational costs. And depending on the separation process between microalgae and water the energy consumption can fluctuate from 0,1 KWH/m^3 to 15 KWH/m^3 (Lavrinovičs e Juhna, 2017).

Therefore we propose a process to immobilize microalgae in a gelatinous polymer, thus favoring the algae fixation system, simplifying the filtering process of the medium and thus making the water treatment process cheaper. As a validation method of the water treatment process it is developed an mathematical model of the immobilized microalgae growth, showing biomass generation and consumption of nitrogen and phosphorus.

2. MICROALGAE

Microalgae are a kind of organism which doubles its own biomass in two hours. The process is realized by photosynthesis, which converts carbon dioxide into the subproducts needed by the algae. Biomass is composed mainly from carbon, nitrogen and phosphorus in the ratio of 50, 8 and 1% respectively. Therefore nutrient disposal is essential on the upkeep of the organism in bioreactors. Table 2 determines the relations between nutrients and the metabolic functions, and the nutritional deficiency can lead to low concentration of staches, lipids and chlorophyll and affects the microalgae development or the final product generation (Grobbelaar 2007; Borowitzka et al. 2016).

Table 2. Relation between nutrients and metabolic functions

Element	Functions
C	Structural component of macromolecule
N	Structural component of enzymes and protein
P	Components of nucleic acids, phospholipids and osmotic regulation

According to the chemical treatment process it goes through it can be produced biodiesel, methane, bio-hydrogen, nutritional supplements among other products, depending on the algae type, cultivation process and due harvest process (Yousuf, 2019; Neofotis, 2016; Chisti, 2007).

Microalgae cultivation has its advantages such as a high growth rate, the cultivation process can be realized on barren lands and it is not influenced by the different weather seasons. But it also has disadvantages, for the limiting factors temperature and light intensity affect the growth, in cases of too high temperatures or low light intensity the photosynthesis is affected and the growth rate is lowered (Yousuf, 2019)

During growth microalgae needs to gather every kind of nutrient needed through photosynthesis, if cultivated by photoautotroph or photoheterotroph means. These nutrients were added on the medium for the absorption (Chisti, 2007). Lately as a means of lowering the cost of microalgae production, residual waters are used as part of the medium of culture, due to the fact that it contains Nitrogen and Phosphorus. Therefore microalgae, in this situation, helps the water treatment process due to the withdrawal of these salts to reach acceptable levels (Strum, 2011).

2.1 **Immobilization process**

Waste treatment using microalgae still faces some challenges, such as the recovery of treated effluent. New technologies have been considered, such as cell immobilization. This technique involves trapping or fixing cells within a matrix through physical-chemical interactions. Furthermore, the main cell immobilization techniques can be classified into two main groups, such as immobilization with support material (adsorption, entrapment and encapsulation) and self-immobilization (cell self-aggression and self-flocculation), as exemplified in Figure 1 .

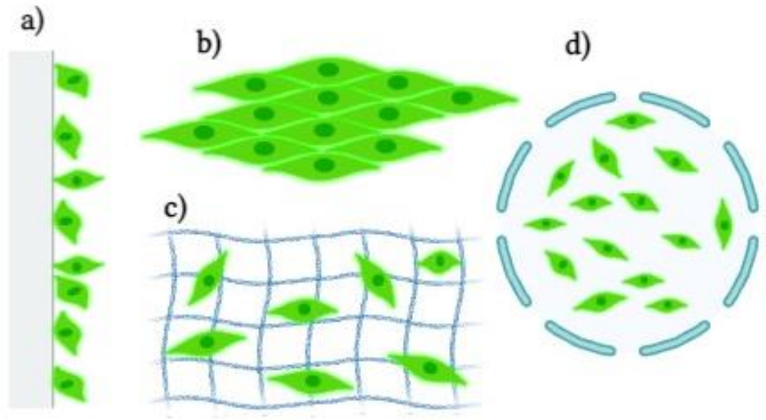

Figure 1. Microalgae cell immobilization system (da Silva et al., 2022)
(a) Microalgae attached to the support by adsorption; (b) Biofilm formation by self-immobilization; (c) Microalgae stuck in a matrix; (d)Encapsulated microalgae in a matrix.

The use of immobilized biomass for effluent treatment is increasingly being studied, as shown in Table 3. Immobilization tends to enhance the intrinsic characteristics of microorganisms, such as the removal of solids, as well as eliminating the sedimentation step. Therefore, the immobilized cell system can enhance efficiency and reduce costs in the biological process (Giese 2015).

Table 3. Treatment of residual matter through immobilized microalgae

Species	Residual waters source	Matrix	References
Scenedesmus intermedius	Pig manure	Sodium alginate	(Jiménez-Pérez et al. 2004)
Synechococcus elongatus	Artificial residual waters	Chitosan	(Aguilar-May e Sánchez-Saavedra 2009)
Chlorella vulgaris	Sewer of poultry slaughterhouse	Sodium alginate	(Hameed 2007)
Scenedesmus abundans; Chlorella vulgaris	Secondary effluent	Sodium alginate	(Kube et al. 2020)
Desmodesmus sp	Domestic residual waters	Sodium alginate	(Wang et al. 2020)
Lobosphaera sp.	Secondary effluent	Chitosan	(Vasilieva et al. 2021)
Pseudomonas putida	Artificial residual waters	polyvinyl alcohol	(Al-Zuhair e El-Naas 2011)
Chlorella pyrenoidosa	Secondary effluent of the activated slime	polyvinyl alcohol	(Huang e Wang 2003)

In general, immobilization offers several advantages, with the main ones being: cell reuse, resistance to contamination, and increased mechanical, chemical, and thermal resistance. The immobilization of microalgae cells enhances the cost-effectiveness of phytoremediation processes (Wittmann and Liao 2016).

Cell immobilization is a process that involves trapping or fixing cells within a matrix through physical-chemical interactions. The benefit of cell immobilization lies in increased productivity and reduced costs of bioprocesses. Immobilization has been employed to improve solid retention, eliminate sedimentation steps, and enhance the efficiency of treating highly diluted effluents (da Silva et al., 2022). Furthermore, it is possible to simultaneously treat wastewater and produce microalgae biomass. da Silva et al., 2022 using microalgae immobilized in an alginate and chitosan matrix, managed to achieve a reduction in phosphate concentration by approximately 80% and reached yields of up to 1.6 g/L of biomass, as shown in Figure 2 below.

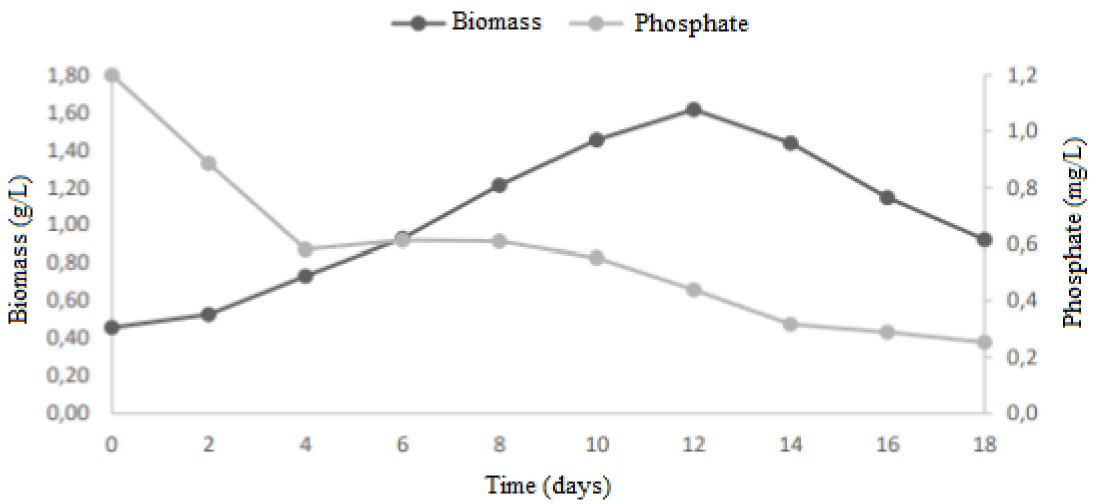

Figure 2. Cell growth curves and phosphate reduction in the medium according to the cultivation time **(**da Silva et al., 2022)

2.2 Mathematical model

The equations were determined by the growth kinetics of fermentation processes, we chose the Monod model because it allows us to foresee the microorganism growth rate as represented by graphs for control of the process (Som; Yahya, 2021).

The equations represent the concentration rate of microalgae based on two limiting substrates. Eq. (5) represents the specific growth rate in each iteration while Eq. (2) represents the derivative of the cellular concentration as a function of time.

The microalgae cultivation system used will be a batch process. The batch process consists of a closed process with input of organisms and medium, without changes in the medium. With population growth occurs substrate consumption and the formation of the desired product. The process can be controlled due to the following factors, being either the amount of cells and by-product, the concentration of nutrients, the control of the process, as pH, temperatures among other processes (Najafpour, 2007). The batch culture process can undergo constant stirring, which can be carried out mechanically or by gas blowing, also known as airlift. However, the demand for the agitation process depends on the desired by-product, as in certain cases the organism may respond better to a non-agitated medium (Blaby, 2011).

The batch culture process has disadvantages, firstly being the constant change of the culture medium, both by the decrease in nutrients and the presence of products of the organism, which can act as inhibitors, so if there is a point of increased production of products,it can be more complicated to control. In addition, at the end of cultivation the bioreactor needs to go through the emptying, cleaning and feeding processes, these processes taking up time when the reactor is not being productive (Blaby, 2011).

$$\frac{dx}{dt} = (x_{in} - x_{out}) * D + \mu * x - kd * x \tag{1}$$

$$\frac{dx}{dt} = (\mu - kd) * x \tag{2}$$

$$\frac{dS}{dt} = S_{in} - S_{out} + generated - consumed \tag{3}$$

$$\frac{dS}{dt} = (\frac{1}{y_s}) * \frac{dX}{dt} \tag{4}$$

$$\frac{dS}{dt} = (\frac{1}{m}) * \frac{dX}{dt} \tag{5}$$

$$\mu_x = \frac{\mu_{max} * S_1 * S_2}{(kS_1 + S1) * (kS_2 + S2)} \tag{6}$$

Where:

- $\frac{dX}{dt}$ is the microalgae growth mass, mg/L·d;
- X is the initial mass of biomass, mg/L;

- D is the dilution rate of the model d^{-1};
- kd is the rate of cell lysis d^{-1};
- $\frac{dS}{dt}$ is the mass consumption of nitrogen, mg/ L·d;
- S is the mass of inorganic matter present on the effluent, mg * L-1;
- μ is the specific growth rate of the microalgae d^{-1};
- μmax is the maximum growth rate for the determined microalgae, d^{-1};
- KS1 is the constant in which the specific growth rate equals half of the maximum value of nitrate, mg * L-1;
- KS2 is the constant in which the specific growth rate equals half of the maximum value of phosphate, mg * L-1;
- m is the angular coefficient of a line, obtained empirically.

3. RESULTS

The purpose of this modeling is to demonstrate the growth capacity of immobilized microalgae in an alginate and chitosan matrix. By means of graphs, the aim is to show the increase in microalgae biomass within the spheres, while simultaneously reducing the levels of nitrogen and phosphorus in the water systems to be treated. Furthermore, the analysis of organic matter consumption in the effluent to be treated is pursued.

To obtain a simulation comparable to the results obtained by da Silva et al., 2022, it was necessary to make some considerations. Therefore, it was assumed that, during cell growth, only phosphate would be consumed as a substrate, and the omission of the cell death factor was due to a lack of complementary data. An initial situation of 1.2 mg/L of substrate and 0.4 g/L of biomass were also considered. The parameters used in modeling were μ_{max} equal to 0.055 h^{-1}, m equal to 0.1545, KS equal to 9 g/L and Ys equal to 2.97 g of biomass/g of substrate. Thus, according to the considerations, Figure 3 was obtained.

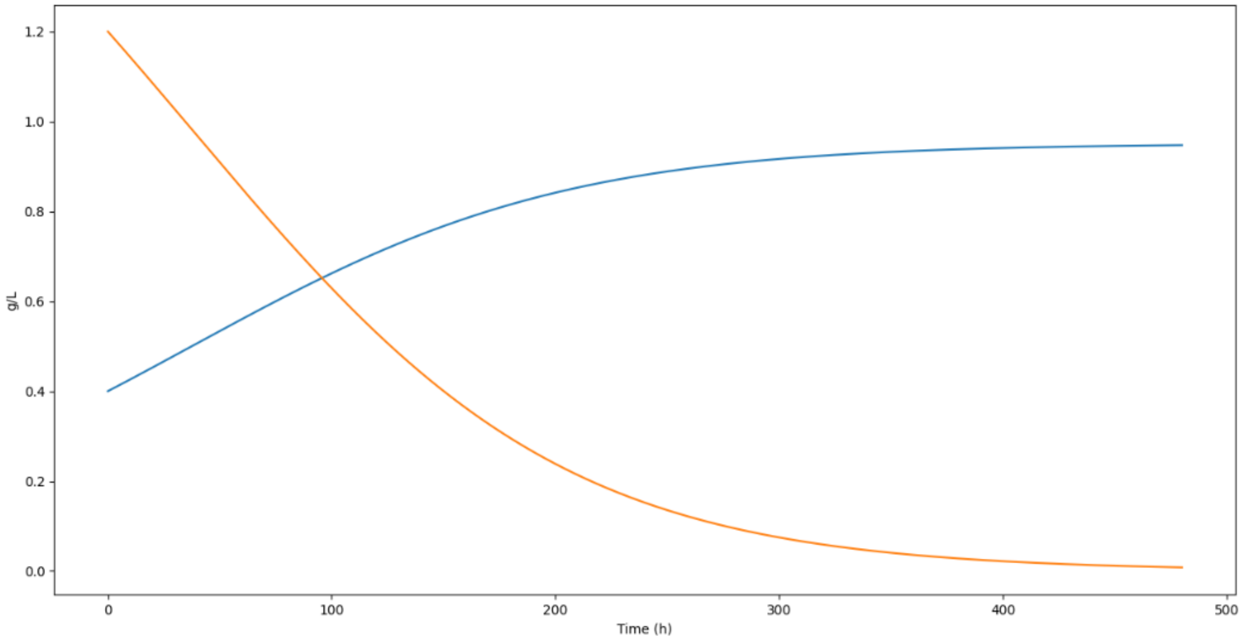

Figure 3. Mathematical results based on biomass growth

The curves of the mathematical model are similar to the empirical model, so we can state that this model partially allows the simulation of the growth of immobilized microalgae associated with effluent treatment. The simulation did not allow us to accurately predict the growth of immobilized microalgae, reaching a concentration value below the expected value after the simulated time. However, the consumption of substrate, which represents the treatment of the effluent, was satisfactory, reaching a phosphate reduction of almost 100%, close to that found experimentally, being greater than 80%.

The model's lack of precision is related to the disregard of the cell maintenance rate model, in addition to not considering the growth factor provided by the incidence of light, one of the essential sources of energy used by microalgae.

This approach offers a promising solution for wastewater treatment, using immobilized microalgae as a sustainable and efficient alternative for removing unwanted nutrients and organic matter from the effluent. Monitoring through graphs will enable tracking the progress of the treatment system, providing valuable insights into its performance and efficiency. Consequently, it is expected to contribute to environmental preservation and protection of water resources.

4. CONCLUSION

It is possible to infer that the immobilization of microalgae in an alginate and chitosan matrix is effective, guaranteeing cell retention in the spheres and preventing its dispersion in the culture medium. Simultaneously, the matrix exhibited adequate permeability to allow nutrient diffusion and allow microalgae growth within the spheres.

Although the process does not reach the level permitted by CONAMA for the disposal of effluents in water bodies, the treatment process using immobilized microalgae was carried out in a batch process. Thus, the process can be repeated by removing the contents of the reactor, cleaning it and placing the same effluent to be treated and a new set of immobilized spheres. This way the process can be repeated until the desired levels are reached.

The microalgae immobilization technology therefore demonstrates the potential to achieve high biomass yields as well as to carry out the process of phosphate bioremediation. The bioremediation potential of microalgae spheres is clearly evident, since nutrients likely to cause pollution are consumed during the bioremediation process.

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