



19th Brazilian Congress of Thermal Sciences and Engineering
November 06th-10th, 2022, Bento Gonçalves - RS - Brazil

ENC-2022-0557

**MATHEMATICAL MODELING AND SIMULATION OF BATCH REGIME
FOR IMMOBILIZED MICROALGAE PHOTOBIOREACTOR FOR
EFFLUENT TREATMENT**

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Abstract. *The increase of wastewater follows the expansion of the world population generating a deficit in basic sanitation and in the sewage collection provided. It is widely known that the United Nations (UN) instituted the 2030 Agenda, a plan for the sustainability of the planet, improvement of people's lives and world prosperity. There are 17 Sustainable Development Goals (SDGs) in the 2030 Agenda. We highlight the SDG 6: "Clean water and sanitation", which is aimed at basic sanitation and access to drinking water. Currently, the treatment system is divided into three stages: primary, secondary and tertiary. In the secondary stage, one makes use of microorganisms to remove organic matter from the medium, such as microalgae or bacteria. Preference has been given to the use of microalgae, classified as microorganisms of rapid cell growth with photoautotrophic capacity. However, the free state physical dimension of a microalgae makes the treatment process more expensive and potentially, impacts the treatment time, thus burdening the treatment. With that in mind, a method of immobilization of microalgae and the elaboration of a photobioreactor for the treatment of effluents was developed. Immobilization is a practice that consists of fixing algae within small spheres, which simplifies the separation methodology of microorganisms from the treated effluent. The immobilizing medium provides mechanical resistance and protects the culture from possible contamination. In order to demonstrate the functionality of the system, as a means of effluent treatment, a mathematical modeling of the effluent treatment was conceived. Fortran was the programming language used to solve nonlinear differential equations through temporal discretization. Runge-Kutta was the numerical method chosen to solve the equations of the model that are based on Monod's model. Monod's model predicts the growth parameters during the life cycle determining the amount of substrates and the number of microalgae along the lag phase, log phase and stabilization level. It also expresses the consumption of the substrates. Thus, the model allows the visualization of the biomass growth, consumption of inorganic substances and the treatment time under study.*

Keywords: *Bioremediation; Cell immobilization; Discontinuous mode; mathematical model; 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. Ambientalism has become an important factor as a safeguard to development of human life. That process can be shown by an action of the United Nations (UN) in the elaboration of the 2030 agenda. It is composed of 17 sustainable development goals (SDG) in which we focus on item 6 "clean water and sanitation" with a goal on the availability of fresh water to the population and urban sanitation.

Water treatment can be split in three processes, being primary, secondary and tertiary treatment. Primary treatment is based on removal of solid components, the secondary process is based on the removal of fine solids and removal of inorganic salts, and the tertiary process is based on chemical processes. Actually the water treatment process demands large scale industrial processes and technological knowledge hence why the actual process does not produce satisfactory results (Tom, 2021).

Considering the secondary treatment we plan to use microalgae due the organism capability to remove nitrogen and phosphorus compounds by means of photosynthesis, therefore using such compounds in its own development (Melo, 2014). Also according to Aslan and Kapdan (2006) it is possible to remove up to $21 \text{ mg} \cdot \text{L}^{-1}$ of ammonium and $7 \text{ mg} \cdot \text{L}^{-1}$ of phosphate, which demonstrates the viability of the use of microalgae as a means of water treatment.

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. 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 $0,1 \text{ KWH} \cdot \text{m}^{-3}$ to $15 \text{ KWH} \cdot \text{m}^{-3}$ (Lavriničs e Juhna, 2017).

To augment the efficiency of the treatment and control of the process a prototype of a photobioreactor was built. The piping was designed as a transparent PVC tube in order to easier allow the illumination of the reactor, also it used a couple of valves in order to control the passage of microalgae and water. To allow the construction a model was made in order to ease the construction and a mathematical model was made to determine the growth rate of the immobilized microalgae in the reactor.

2. BIBLIOGRAPHIC REVISION

2.1 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. According to the 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. 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).

The cultivation process can be made by two means, raceway ponds or tubular photobioreactor. Raceway ponds is the most used process due to the low cost to build and easier to do maintenance, the downside is that the biomass production is lower when compared to tubular reactor, also the carbon dioxide conversion rate is lower than in the tubular case, it can only be compared if you do not consider the amount of gas that goes through the system and is not absorbed (Chisti, 2007). Tubular photobioreactors are a closed system of cultivation, it is made of vertical or horizontal pipes in which can be determined the best light exposure. The advantages are that the contamination levels are lower and productivity is higher, although the maintenance costs are also higher (Silva et al., 2022). One more characteristic, according to Silva et al., (2022) is that microalgae grow freely in the medium, with a tendency to stay closer to the top of the pond as to do the aerobic process of water treatment.

During growth microalgae needs to gather every kind of nutrient needed through photosynthesis. 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).

2.2 Microalgae Immobilization

An improvement to the actual system of wastewater treatment with microalgae is to immobilize them. The process consists of using the medium of growth and mixing it with an sodium alginate medium, and slowly drip it on a calcium chloride medium, which forms a gelatinous material that has a specific porosity responsible to immobilize the algae. It is vital in the process to control pH of the alginate solution so as to avoid polimeric degradation. The whole process occurs on top of a magnetic stirrer and it should be kept in that state for six hours after the dripping (Giese, 2015).

The immobilization process eases the usage of microalgae according to Giese (2015) because it simplifies the process to recover the microorganism, due to the avoidance of time costing process such as filtration and the cost of the biochemical process demanded. Besides, it allows a higher durability due to the stability between the substrate and the organism, therefore resulting in high resistance to toxic compounds due to the gel immobilization (Abe et al. 2007).

The immobilization process can be achieved by artificial or natural means. Natural means is a spontaneous process that happens through electrostatic interactions, examples are: biofilm formation, microbial adsorption or absorption on synthetic supports. And the artificial means are represented by covalent reactions, such as encapsulation with calcium alginate (Araujo, 2018). Among the different processes to immobilize, it was chosen the artificial process due to the ease to execute and manipulate the microalgae cultivation and process of immobilization, the low toxicity of the compound and the high celular retention capability. The types of polimer most used are: agar, alginate, chitosan among other types (Giese, 2015).

2.3 Mathematical model

The equations were determined by the growth kinetics of fermentation processes, being chosen the monod model because it allows 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 substracts. Eq. (1) represents the specific growth rate in each iteration while Eq. (2) represents the derivative of the cellular concentration as a function of time.

For the substrate consumption rate it was considered a monod model but with an additional term that represents the substrate consumption based on the stoichiometric reaction of the microalgae formation. Each substrate was represented on Eq. (3) and Eq. (4) to nitrogen and phosphorus respectively (Balmant et al., 2011).

As a parameter for comparison the initial data used in the model was assumed based on the experimental work of (Xin et al., 2010) where its goal was the construction of a growth model for *Scenedesmus sp.* microalgae considering only one type of substrate as the limiting factor. The data used was KS_1 , KS_2 , S_1 and S_2 , defined respectively as 12,7 mg * L⁻¹, 0,27 mg * L⁻¹, 25 mg * L⁻¹, 2mg * L⁻¹. According to Balmant et al., (2011) the substrate consumption can be defined according to the stoichiometric equation of microalgae growth, considering the necessary mass of substrate necessary to the formation of a gram of biomass. Therefore the values of y_N and y_P are 0,397 and 0,04 respectively.

$$\mu = \mu_{max} * (S_1 / (KS_1 + S_1)) * (S_2 / (KS_2 + S_2)) \quad (1)$$

$$dX/dt = X * \mu \quad (2)$$

$$dS_1/dt = -y_N * \mu * X \quad (3)$$

$$dS_2/dt = -y_P * \mu * X \quad (4)$$

Where μ is the specific growth rate of the microalgae, d^{-1} ; μ_{max} is the maximum growth rate for the determined microalgae, d^{-1} ; S_1 is the nitrate mass present on the effluent, $mg * L^{-1}$; KS_1 is the constant in which the specific growth rate equals half of the maximum value of nitrate, $mg * L^{-1}$; S_2 is the phosphate mass present on the effluent, $mg * L^{-1}$; KS_2 is the constant in which the specific growth rate equals half of the maximum value of phosphate, $mg * L^{-1}$; dX/dt is the biomass growth from the microalgae, $mg * L^{-1}$; dX/dt is the microalgae growth mass, $mg * L^{-1} * d^{-1}$; X is the initial mass of biomass, $mg * L^{-1}$; dS_1/dt is the mass consumption of nitrogen, $mg * L^{-1} * d^{-1}$; y_N is the necessary mass of nitrogen to obtain one mg of biomass, dimensionless; dS_2/dt is the phosphorus mass consumption, $mg * L^{-1} * d^{-1}$; y_P is the necessary phosphorus mass to obtain one mg of biomass, dimensionless;

According to Balmant, 2011 the process to determine the constants y_N , e y_P was performed according to the chemical composition for the microalgae, thus performing a chemical balancing and considering the consumed elements such as phosphate and nitrate, it was possible to obtain biomass and oxygen gas as a product.

The microalgae cultivation system can 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 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 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 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).

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 tab. 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 $mg * L^{-1} * N$

3. RESULTS

As the microalgae will be immobilized, they should be confined in a tube that is transparent so it eases the light passage so it does not affect the photosynthesis process. To withhold the spheres on its designated tube it will use a valve with “y” type strainer filter. This equipment has a net that allows the effluent to pass but not the spheres, as the open spaces of the net have a smaller diameter. The equipment has a screw cap that gives access to the spheres, in case it needs a fast measurement or demand. On this spot a sample collector can be placed as can be seen on Fig. 1. The collector can be threaded and through the lever it is possible to collect the samples to determine the treatment levels.

As it is imperative that the algae sphere does not pass the connector folded in 45° is equipped with a metallic sheet made by foundry, considering that it is not a commercial tube. The holes will be made by a drill with a diameter lower than 5 mm and a threading will be made to remove the burr so it does not damage the spheres.

To control the flow of effluent the feeding system consists of a five liter reservoir, in a closed system to avoid odors in the cultivation area. The closing system is made of a silicone folder as there is a low pressure and low mechanical resistance demand.

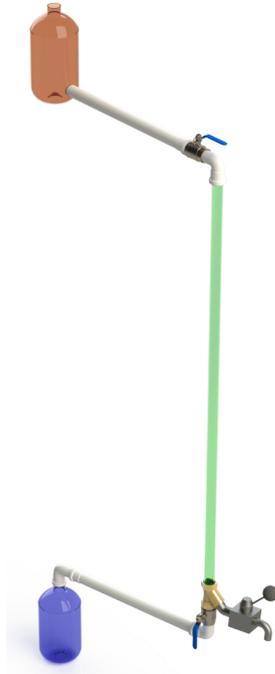


Figure 1. Model of photobioreactor for laboratory use

The pipe diameter was chosen as a DN40, considering the inside diameter is 40,94 mm the treatment volume is 2 liters being possible to determine based on the capacity of the tube how many spheres of immobilized microalgae fits in the tube.

As the immobilized microalgae has a diameter of approximately 5 mm, as can be seen in Fig. 2, it fits 30307 spheres in the piping, being able to estimate the productivity of the growth for this photobioreactor.



Figure 2. Immobilized sphere of microalgae.

The biomass growth shown in fig. 3 demonstrates that the treatment process takes six days long in a batch process. The initial concentration of microalgae was $172 \text{ mg} \cdot \text{L}^{-1}$ and after six days it reached $572 \text{ mg} \cdot \text{L}^{-1}$ which shows the secondary treatment with immobilized microalgae is efficient. The kinetic development of the growth is related with the model used, considering that the latency period is not represented and cell lysis is not interesting in this process, therefore the batch has to go through a recycle on the medium and spheres before that.

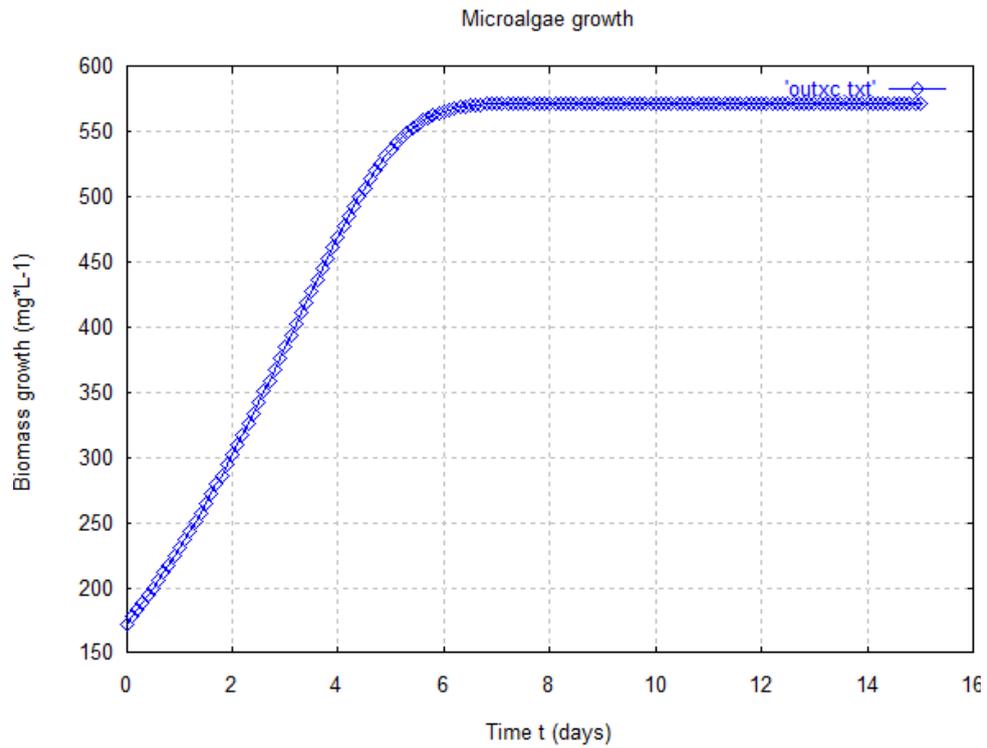


Figure 3. Biomass growth

Figure 4 shows nitrate consumption, used for cell growth, although the high consumption it is not the limiting substrate in the process as in the end there is still $9 \text{ mg} \cdot \text{L}^{-1}$, and according to Brazilian laws this amount of phosphate can be released in freshwater. If it is needed lower levels of nitrate the fluid has to go through another batch process so as to lower these levels.

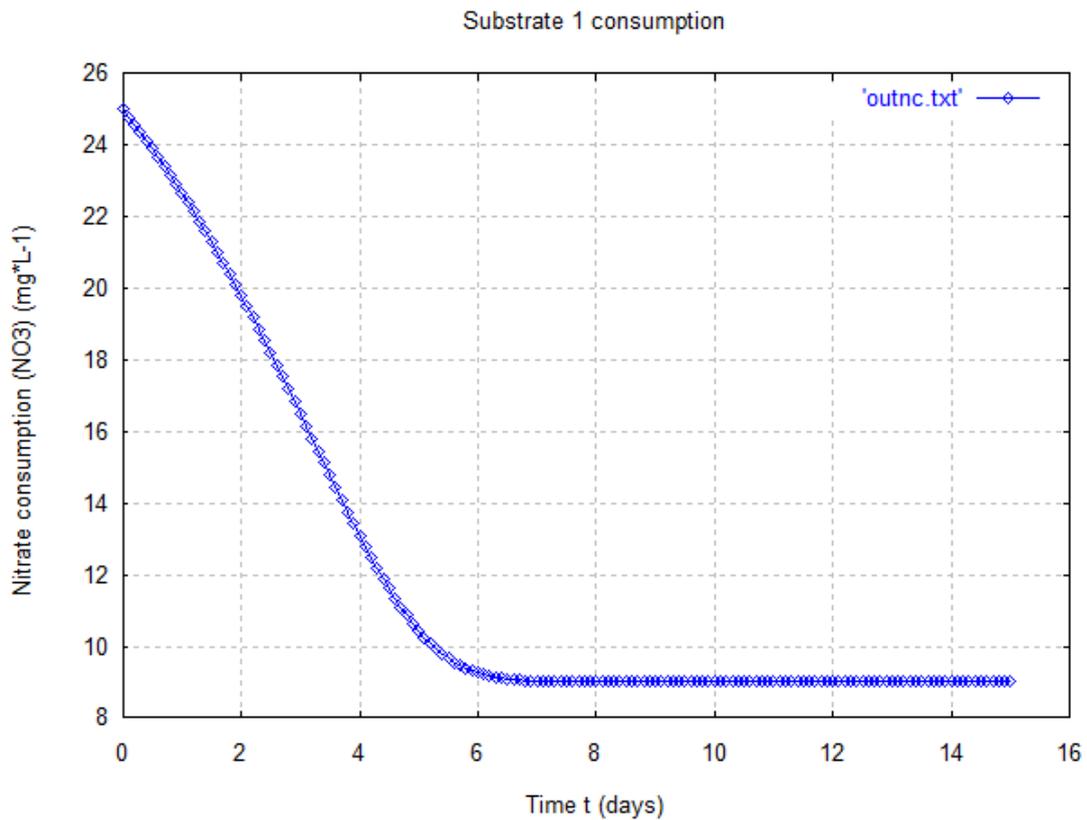


Figure 4. Nitrate consumption

Figure 5 shows phosphate consumption, used for cell growth, being this substrate the limiting factor during the process, as can be seen that in the 6th day it and the whole process comes to a halt. According to Brazilian laws this amount of phosphate can be released in freshwater.

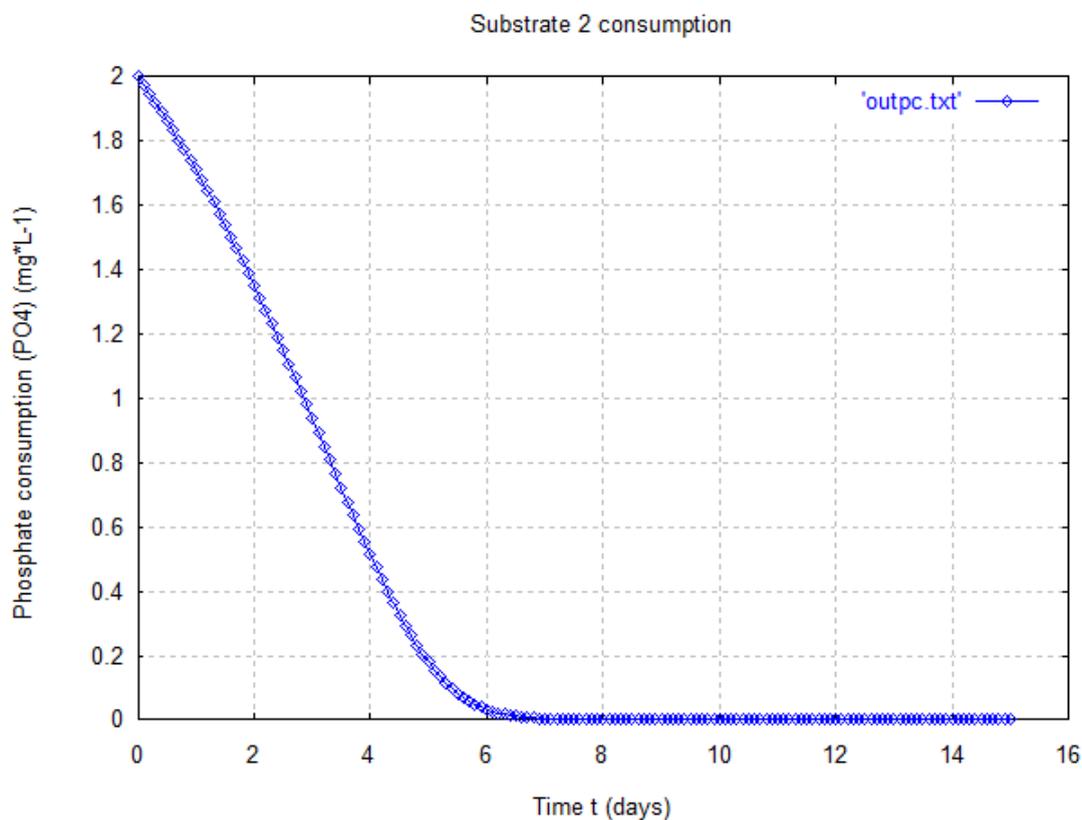


Figure 5. Phosphate consumption

4. CONCLUSION

The secondary effluent treatment process based on immobilized microalgae seems promising, based on the mathematical model, where it can be seen that the removal of nitrate and phosphate levels after treatment are compatible with CONAMA's legislation and the microalgae growth pattern is similar to the Monod's law as the model was defined. The photobioreactor is useful as it simplifies the filtration process due to the fact that all microalgae are stuck in the transparent tube thanks to the immobilization process and valves, saving energy and time on the process.

5. ACKNOWLEDGEMENTS

To the Brazilian National Council of Scientific and Technological Development - (CNPq) - [projects 407198/2013-0, 403560/2013-6, 407204/2013-0, 430986/2016-5, 443823/2018-9, 313646/2020-1, 310708/2017-6, 308460/2020-0, 446787/2020-5, no. 25/2021]; CAPES; Ministry of Education (MEC), Brazil - (projects 062/14 and CAPES-PRINT-UFPR-88881.311981/2018-01); Araucaria Foundation of Parana, Brazil (project 115/2018, no. 50.579 – PRONEX).

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