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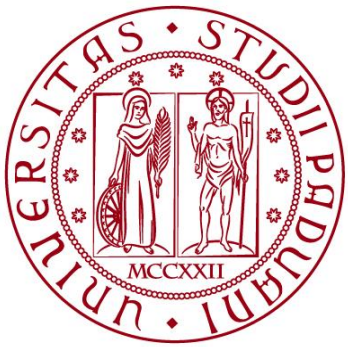

TESI DI LAUREA

A MODELING APPROACH TO DESCRIBE THE UPTAKE OF DIFFERENT NITROGEN SPECIES IN MICROALGAE BASED WASTEWATER TREATMENT

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A tutti coloro
Che mi hanno supportato durante questo percorso,
in particolare, ad Irene che mi è sempre stata vicino
con dolcezza, gentilezza e con il giusto spirito
nonostante il mio pessimo carattere.
Grazie

SUMMARY

ABSTRACT

Microalgae have a great potential in the removal of nutrients from wastewater, especially in the case of nitrogen species. Modeling can be helpful in the simulation of biological process. However, in the case of microalgae, many aspects still remain to be assessed.
The aim of this thesis is to evaluate two mathematical models developed in order to identify possible equations describing the removal of nitrogen forms from wastewater using microalgae.
In particular, the modeling approach tries to describe the competition between ammonium and nitrate that occur when the two nitrogen species are both present in the wastewater.
The methods considered in the thesis are the Solimeno model, and the Dixon model that account for the possible inhibition of ammonium on nitrate uptake by microalgae.
Matlab computational software was used to control the compatibility of the results of these methods with the data obtained in the laboratory for two different species of microalgae, which presented a different regulation of nitrogen metabolism.
The algal species used were *Chlorella protothecoides* and *Synechocystis*, which are widely used in the experimental setting for the removal of chemicals from wastewater. These two microorganisms are very important in this study because they can exploit both ammonium and nitrate, the two main forms of nitrogen in wastewater.
The models used were found not to be able to represent the experimental data, so they were modified based on Droop Model. In addition, a sensitivity analysis on kinetic parameter was carried out, to better ascertain which were the key parameters to represent the experimental data. Conclusions were drawn regarding some biological parameters of particular interest in the reactions involved.

CHAPTER 1: STATE OF ART

1.1 PRESENCE OF NITROGEN IN WASTEWATER

The constant increase of human activities due to the new necessities generated by the growth of the worldwide population produced a huge number of chemical compounds that are discharged in the water. Most of these compounds are based on Nitrogen (N) and/or Phosphorus (P). They affect the water basins because they are the main nutrients for algae growth, and their bloom can produce many problems such as eutrophication. Between the two elements the most spread one is nitrogen, which can be found in higher quantities and in different forms.
Among theme it is possible to identify the ammonium ions ($NH4^{+}$), nitrites (NO_3^{--}), nitrates (NO_2^{--}) and ammonia (NH_3). The last of the list is also toxic for the environment if it is present in the gaseous form. The explanation of this huge amount of Nitrogen is determined by the fact that it is one of the main elements that characterizes organic compounds, and most of the discards of the human body are based on it. For example, urea, peptides and amino acids are all present in households' water and represent a good percentage of the weight. After that their presence is also justified for the several reactions of oxidation that take place in the water, and compounds as nitrates are the common product of the biodegradation.
Moreover, there are some other industrial processes that can affect the water by adding nitrogen. These are the main reasons why it is important to develop an effective process to treat the water and reduce these concentrations before the releasing of the water in the environment.

1.2 WASTEWATER TREATMENT BASIC CONCEPTS

Treating wastewater is needed to reduce the number of components that can pollute the environment. Caused by the specific use of the water and also by the increase of the necessity of the use, the water that arrives at the wastewater treatment plants is rich in some chemical compounds that are removed by several steps.
Based on the origins of the wastewater, the concentrations of some pollutants such as BOD, COD, Nitrogen, Phosphorus and metals could change; for example, industrial wastewaters have statistically higher concentration of phosphorus or metals; on the other hands domestic wastewater has a higher concentration of BOD or COD.
BOD is the acronym to indicate the Biochemical Oxygen Demand, it is the amount of oxygen required to oxidize the organic fraction present in the water. It is a useful parameter that permits to estimate the amount of biodegradable matter (organic matter) presents in the wastewater and at the same time to estimate, through some experiment, the oxygen needed to complete the entire degradation.
The second parameter, COD has the meaning of Chemical Oxygen Demand, that describes the oxygen required to chemically degrade the substance organic and inorganic. Also this value, as the BOD, is important because it can define the quantity of oxygen that could be indispensable to remove (degrade) the organic and inorganic compounds from a specimen and the comparison with the value of BOD can help to understand which quantity of matter is higher presents inside.
Based on this definition it is obvious that the value of COD on a specimen for the test could be equal or higher than BOD.

The aim of the process is to improve the quality of the water based on some specific characteristics that are physical, chemical and biological. Physical parameters include: odor, color, temperature,

solid residues, turbidity and presence of oil. Chemical parameters are usually associated to the presence of solid and are identified by the use of BOD and COD. Biological parameters could be represented by a certain number of bacteria, so that the treatment has the aim to disinfect the water.

For all these parameters there is a limit value that must be respected to clean the water (Amit Sonume et al, 2004).

The target of the process is to remove the solids compounds and the toxic elements from the water and to do this are necessary some physical, chemical and biological stages. Due to some and different types of combinations it is possible to reduce the amount of BOD, Nitrogen, Phosphorus and all the other elements that are not necessary from the water.
The entire process is characterized by some fixed stages (figure 1): preliminary treatment, primary treatment, secondary treatment, tertiary treatment and disinfection.

The objective of preliminary treatment is the removal of coarse solids and other large materials often found in raw wastewater. Preliminary treatment helps to remove or to reduce in size. What is removed are heavy inorganic solids such as sand and gravel as well as metal or glass. These objects are called grit and excessive amounts of oils or greases (Amit Sonume, 2004).
The primary treatment has also the objective of removing a percentage of suspended solids, oils and BOD, in particular to reach this target it usually uses a primary settling. Through this technique it is possible to remove approximately 25-50% of the incoming biochemical oxygen demand (BODs), 50-70% of the total suspended solids (SS), 10% of Phosphorus and Nitrogen and 65% of the oil (Amit Sonume, 2004).

The secondary treatment process consists of the biological treatment of wastewater by utilizing many different types of microorganisms in a controlled environment. This process is the main core of the treatment, and it can be developed in different phases. In general, it is characterized by oxidation tanks where aerobic reactions based on the use of bacteria or algae are developed to reduce the concentration of SS and all the other chemical compounds that are used to the growth of the microorganisms. The aerobic tank is used to remove the great part of the Carbon and a percentage of P and N, however alone it is not enough to remove the rest of Nitrogen or Phosphorus to reach the legal target. For this reason, Nitrification and Denitrification sections are added to remove the remaining N and P. At the end of this stage there is a secondary settler that is used to remove the biomass that will exit from the previous tanks or, in case of abundant concentration of some molecules, some chemical compounds that increase the removal of some species could be added in the water. To increase the level of removal is used a recycle of the discharge flows that exit from the settler or/and from the tanks.

The next stage is called tertiary treatment. Tertiary treatment may be defined as any treatment process in which unit operations are added to the flow scheme following conventional secondary treatment. Additions to conventional secondary treatment could be as simple as the addition of a filter for suspended solids removal or as complex as the addition of many unit processes for organic, suspended solids, nitrogen and phosphorus removal (Amit Sonume, 2004).

Finally, the last step of the process is the disinfection, where the water is purified to reduce the number of bacteria that are present inside. The most common method to reach the target for the effluent is to use the UV radiation to kill the bacteria.

As we can see from the following figure (Fig. 1.8) this short description represents the main scheme of the flow of the wastewater, however it is to consider also the flow of the sludges that are produced

by the different steps of the treatment, and they have to be treated to reduce their size. This is the second reason that justified the presence of some recycling in the scheme for the wastewater.

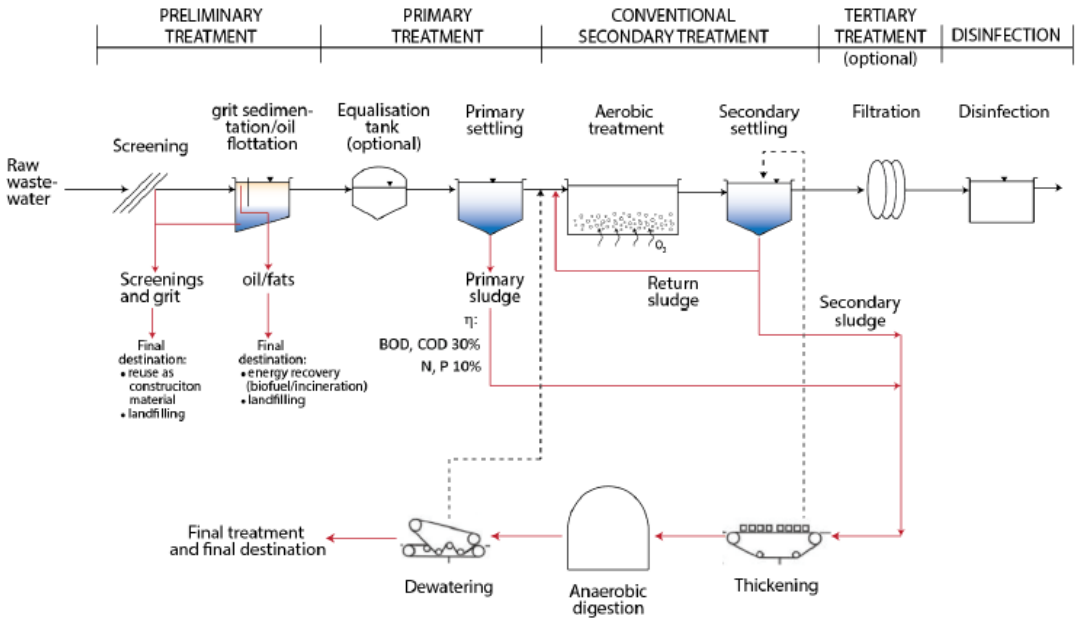

Figure 1.0: scheme of a wastewater treatment plant

1.3 ALGAE IN WASTEWATER TREATMENT

Considering the wastewater treatment, different processes exist to remove the chemicals from the water. A great part of them is based on the use of microorganisms in particular bacteria that are able to assimilate the nutrients (carbon, nitrogen and phosphorus) in aerobic or anaerobic conditions. However, in recent years there is also the possibility to apply algae or microalgae for wastewater treatment.

Microalgae that are used in the treatments, are living microorganisms that constitute the basis of aquatic food chains. They are a phylogenetically diverse group, encompassing a number of different phyla and classes of organisms. In some cases, cyanobacteria are also included (Camacho et al, 2019). The algae are organisms that carry out oxygenic photosynthesis by means of a cytoplasmic organelle, the chloroplast (E. Douglas et al, 2003).

Thanks to this process, they are able to convert inorganic matter into organic compounds. According to Classification of Algae (D. Sahoo, 2010) algae can be classified in two groups, namely prokaryotic and eukaryotic which were further divided into several divisions. Prokaryota has just one division i.e., Cyanophyta, whereas eukaryote was further divided on the basis of the nature of chloroplast membrane and genetic information. Summarizing, they can be classified into:

1. Group I: Prokaryota; in this class there are the Algae called *"Cyanophyceae*" that are characterized by Pigments with Chlorophyll a.

2. Group II: Eukaryota; characterized by Chloroplast surrounded by the two membranes of the chloroplast envelope. In this group there are *Glaucophyta, Rhodophyta and Chlorophyta*; some of them are defined by the presence of chlorophyll a and b.
3. Group III: Eukaryota; characterized by Chloroplast Surrounded by one Membrane of Chloroplast Endoplasmic Reticulum Envelope. They have chlorophyll a and b.
4. Group IV: Eukaryota characterized by Chloroplast Surrounded by two Membranes of Chloroplast Endoplasmic Reticulum Envelope.

One of the characteristics of this species, that permits to be very interesting for this point of view, is the fact that they can grow and live in hostile environments where the bacteria or other species cannot live. In fact, they can survive not only in the seawater or in freshwater but also in the soil, on the rock and hypersaline environments.
Analyzing the process, the final target of the system is to reduce the concentration and remove the pollutants, Microalgae-based bioremediation system offers various advantages over the common system because it works with the consumption of CO_2 (instead of the production), permit the removal of high percentage of Nitrogen and Phosphorus and produced green biomass and oxygen (T.Phan, et al.,2022). At the same time the cultivation of the Algae required a lot of time, water and nutrients.

1.4 VARIABLES THAT AFFECT ALGAE GROWTH

Microalgae cultivation requires specific environmental conditions including temperature ranges, light intensities, mixing conditions, nutrient composition, and gas exchange. It is also important to identify and specify all of them to permit correct growth of a specific culture of algae. They can be cultured using different metabolic pathways (photoautotrophic, heterotrophic, and mixotrophic) and by using different cultivation systems, commonly classified as open and closed systems (G.Zuccaro et al. 2017). Considering this last point, the growth can be affected not only by the common factors such as temperature or pH but also by the operational parameters like the hydrodynamics stress, mixing culture depth, dilution rate and harvest frequency.

1.4.1 TEMPERATURE

Most of the reactions; involved in the growth of algae are dependent on the temperature (S.V.Mohan et al.,2015). Based on this phenomenon algae can be subdivided in three main categories: psychrophiles
(<15 °C), mesophiles (<50 °C), and thermophiles (>50 °C). For each group the relative correct temperature is important, otherwise it is possible to notice inhibition phenomena that stop the growth or reduce the rate.
The main role of the temperature is related to obtaining a correct carbon fixation. Indeed, higher temperatures enhance CO_2 absorption and fixation but represent an inhibiting factor for the respiration metabolism and for the photosynthetic proteins, unbalancing energy transfer in cells (G.Zuccaro et al. 2017).
There are different models that can describe the possible effect and trend of the temperature, but the correct kinetic equation is complex to identify. Following the idea of Bechet (G.C. Okpokwasili, 2005), the value of the specific growth rate of photosynthesis μ (h^{-1}) is the product of two distinct functions of light intensity (Monod function) and temperature (Arrhenius equation) according to:

$$\mu = \mu_{mo} * \exp\left(-\frac{Ea}{KT}\right) * \frac{Iav}{K + Iav} \quad (1.1)$$

where μ is the specific growth rate (h^{-1}), μ_{m0} is the maximum specific growth rate (h^{-1}), E_a is the activation energy for photosynthesis (J), k is the Boltzmann constant (J K^{-1}), T is the temperature (K), I_{av} is the average light intensity in the culture broth (μmol m^{-2} s^{-1}), and K is a light half-saturation constant (μmol m^{-2} s^{-1}).

1.4.2 LIGHT

The light intensity is the most important variable because it can directly affect the photosynthesis process. The relationship between light intensity and photosynthetic rate is shown in the figure below (figure 1.1) where it is possible to observe the different light regimes.

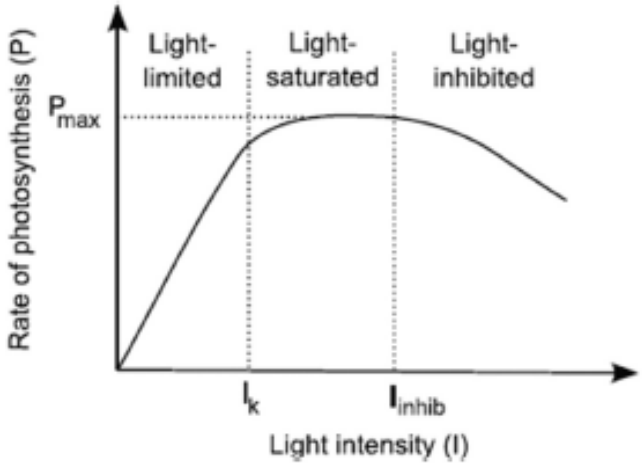

Figure 1.1: relationship between light intensity and photosynthesis (Microalgae Cultivation System, G. Zuccaro, 2020)

From the graph it is possible to observe three different areas that characterize the behavior of the algae based on the amount of light that they receive. The different zones are described in the following way:

1. $I < I_k$: the rate of photosynthesis is proportional to low light intensities and the photosynthesis is limited by the rate of photon capture;
2. $I_k < I < I_{inihib:}$ the rate of photosynthesis is usually maximal and independent from light intensity. This regime is light saturated, it means that the rate of photosynthesis is limited by rate of reactions following the photon capture
3. $I > I_{inihib}$: the rate of photosynthesis starts to decrease with the light intensity which, above a certain level can damage light receptors, such as key proteins, in the chloroplasts. This regime is known as photoinhibition.

Based on several analyses, the correct level of light intensity for the great part of the microalgae is about the range of 200-400 μmol m^{-2} s^{-1}.

Considering a generic case, for example a normal photobioreactor, the main variable that can influence the biomass growth is the light intensity. It is important to describe how it can change because the intensity along the reactor is not uniform and it depends from the reactor configuration. The function, that can describe the trend of the light in the reactors that are going to be used for the following reactions is the equation of Lambert-Beer.

The following equation describe the law that is used during the calculation inside the thesis:

$$I(z) = I_0 * e^{(-K_a * C_{X,out} * z)} \quad (1.\ 1)$$

where I_0 is the incident light intensity (μmol/m^2 *s) , ka is the biomass light absorption coefficient (m^2/g), Xout is the biomass concentration in the reactor (g/m^3), which is assumed to be uni-form along the reactor depth, and z is the axial coordinate of the culture depth (m) (E. Barbera et al.,2020). The importance of the light is determined by the fact that light reactions provide the conversion of light energy into chemical energy in the form of short-term energy storing molecules. This is possible because chlorophyll molecules absorb photons, which induce the excitation of a pair of electrons, leading indirectly to ATP and NADPH production (G.Zuccaro et al. 2017).

Another characteristic that can influence the growth and the chemical reactions is the penetration of the light through the surface or the reactors that contain the algae in the cultivation system.

1.4.3 NUTRIENTS

Microalgal cultures require some nutrients and micronutrients to grow. Of course, the amount and concentration must be enough to permit all the reaction of the metabolism of the cells. In literature it is possible to find an average ratio of different nutrients for the cultivation of photosynthetic microorganisms. The value was discovered by Redfield, and it compares the ratio between the three main elements that characterized the cells, namely Carbon, Nitrogen and Phosphorus, with the following molar ratio C:N:P = 106:16:1 (G.D. Price et al, 2005). However, the concept introduced by Redfield doesn't consider the great adaptability of microalgae, which can greatly change their composition depending on the surrounding environment.

Carbon

Carbon is the main element inside the microalgae; it is equal to 50%-65% of dry weight. It is taken up from inorganic compounds through the photosynthesis process which depends also on the following equation and the level of pH present in the solution.

$$CO_2\,(aq) + H_2O <\!\!-\!\!> H_2CO_3 <\!\!-\!\!> HCO_3^- + H^+ <\!\!-\!\!> CO_3^- + 2H^+$$

The equilibrium changes with respect to the value of pH:

- If the pH is lower than 6.5 (pH<6.5) H_2CO_3 is predominant
- when the pH is between 6.5 and 10 the dominant form is HCO_3^-
- at pH>10 more CO_3^{2-} becomes predominant

Carbon is fixed inside the microalgal cells through the Calvin cycle with the auxilium of the Rubisco enzymes. The pH can affect the uptake of inorganic carbon; in alkaline environments, carbon is metabolized mainly by active transportation than diffusion, excreting H^+ ions, able to react with HCO_3 to give CO_2. In the same environmental reaction, microalgal cells can regulate their intracellular pH by homeostasis. Photosynthetic activity, in particular the extracellular and intracellular conversion of bicarbonate to carbon dioxide is obtained according to: $HCO_3^- —> CO_2 + OH^-$.

Nitrogen

Nitrogen is the second most abundant element in the biomass of the algae with a concentration that is around 1-14% of dry weight; it is one of the main elements for the metabolism. It is necessary for proteins and the pigments; it is supplied both in inorganic and organic form as it is possible to understand from the following list:

- Nitrate (NO_3) is frequently supplied as $NaNO_3$ (J. Jeanfils et al, 1993).
- Nitrite (NO_2) is considered an intermediate of the nitrification processes due to bacteria, that is the oxidation of ammonia to nitrate, but it can also be an intracellular intermediate product by the nitrate reductase reaction (S. Yang et al., 2004).
- Nitrite oxide (NO) is a small and nonpolar molecule, able to diffuse directly in the cell (D.E. Santiago et al, 2010).
- Ammonium or Ammonia (NH_4^+) is the preferred nitrogen source of nitrogen for the microalgal culture because it has a less energy consumption when it is assimilated by the cells if we compare it with the other forms of nitrogen.

Ammonia can be toxic; when it is dissolved in the water with a wrong value of pH. If the ammonia is present as NH_3 form, is highly toxic for microorganisms.
The equilibrium of Ammonia depends on the pH, and it depends on the following equation and graph: $NH_4^+ + OH^- \rightarrow NH_3 + H_2O$. (O. Perez-Garcia et al, 2011).

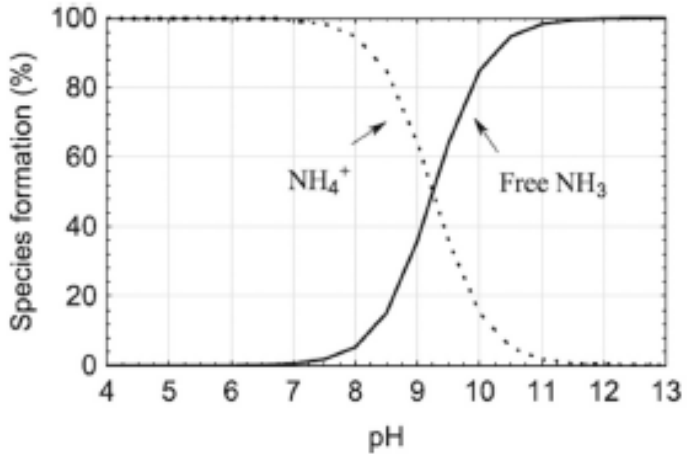

Figure 1.2: effect of pH on different form of Ammonia (Microalgae Cultivation System, G. Zuccaro, 2020)

Phosphorus

Phosphorus is the macronutrient representing the 0.05%-3.3% of the weight. Microalgal cells absorb phosphorus in orthophosphate form by the action of intracellular, extracellular transporters. The uptake rate of phosphorus is affected by available light, pH, temperature, ionic strength, and available ions (K+, Na+ and Mg2+). Microalgae are able to store large amount of phosphorus as intracellular reserve when it is present in excess in the cultivation medium, a phenomenon called luxury uptake.
Because of this property to store excess phosphorus, microalgae can be used to efficiently remove phosphorus from wastewater.

Other nutrients

Other micronutrients are required for microalgal growth, such as Magnesium that is present mainly in the ion form when the salts are dissolved in the water. It is important for the biochemical reactions because it is indispensable as an activator for several enzymes.
Presence of Sulfur (S) and of Calcium (Ca) are important for the metabolism of cells, however the value of pH can affect the assimilation.
Another important chemical element is Iron (Fe) that is involved in fundamental enzymatic processes such as oxygen metabolism, electron transfer, nitrogen assimilation, and chlorophyll synthesis and supplied as chelated complexes to increase its bioavailability (G. Markou et al, 2014).

1.4.4 IMPORTANCE OF PH

Metabolism is strict correlated with the value of pH of the solution; it has the capacity to influence the uptake of ions or the enzymatic reactions; in some other case can influence the the capacity to uptake carbon dioxide or the form in which will be present the ammonia in the water.
Based on the type of water where the microorganisms are growing there are two practical ranges of pH: 7.9-8.3 for marine water and 6.0-8.0 for the freshwater.

1.5 CULTIVATION SYSTEM

In general, microalgae grow by fixing inorganic carbons as CO_2 or as bicarbonate, and absorbing light as energy source; this process is called autotrophy.

Microalgae can be cultivated in reactors classified as open or closed. An open system is characterized by large surface areas, and it is widely exposed to the environment. The closed systems or photobioreactors (PBRs) can be classified in tubular, column, membrane or flat plate reactors. Concerning the operation mode, algae can be cultivated in either batch (discontinuous) or continuous mode.

1.5.1 OPEN PONDS

Open ponds are one example of an open system where there is possible to cultivate the algae. They are not expensive, and they represent the easiest way to grow the algae. This configuration has the capacity to provide enough light and an optimal hydrodynamic force that are good conditions to grow algae. The major problems are the fact that they require a lot of water (due to evaporation) that causes high costs and also the fact that the configuration requires a lot of space to build the structure.
The following pictures show a typical configuration of a raceway open pond.

Figure 1.3: open pond configuration (ENEA, sistemi per riproduzione delle microalghe, Report 2013)

The depth of the system cannot be too high because there is a direct connection between the concentration of algae during the growth and the light penetration coefficient. In fact, the depth is fixed around 20-30 cm otherwise the sunlight cannot reach the lower part and the cultivation is limited.

1.5.2 PHOTOBIOREACTORS

The photobioreactors (PBRs) are an example of closed systems; the choice to use them depends on the different advantages and disadvantages that this solution offers. In fact, this cultivation model requires more costs than the open system for the light illumination and for the feedings but on the other hand it is easier to control and monitor the problems of contamination, increasing the overall

productivity of the system. Different configurations exist that aim to obtain the maximum efficiency of the systems: column photobioreactors (bubble and airlift), flat-plate PBRs and tubular PBRs.

Column photobioreactors are simple cylinder devices with a radius that does not exceed 0.2 m to prevent possible problems with the light irradiation inside the culture and they are tall 2-3 meters. This method has some advantages such as the low costs and high mass transfer so consequently high efficiency on the use of CO_2. To avoid problems of sedimentation of microalgae cells it is needed a good ventilation from the bottom; and based on these details exist different configurations as it is possible to see in the figure 1.4.

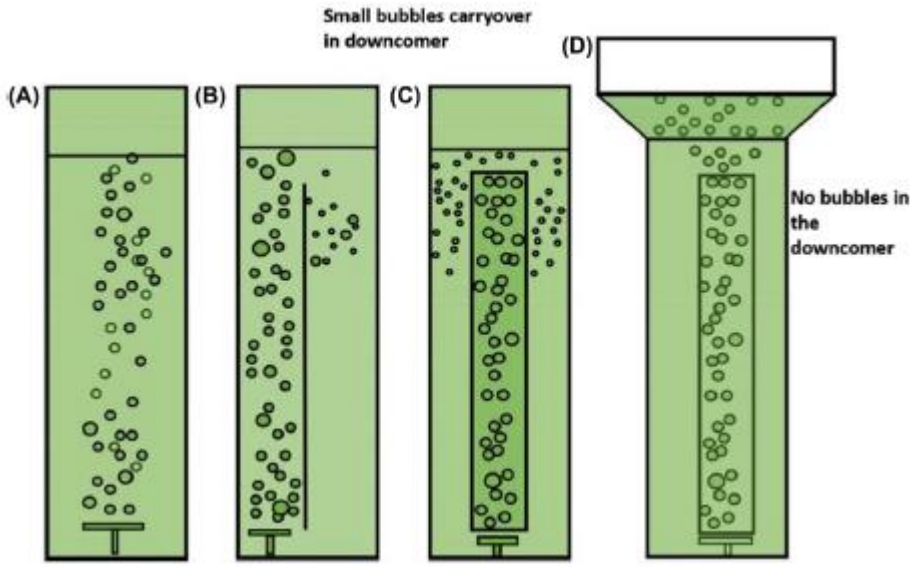

Figure 1.4: different configuration for the column reactors. a) bubble column, b) splitted column, c) internal loop airlift and d) internal loop airlift with gas separator

Flat-plates (Figure 1.5) are cuboidal-shaped reactors characterized by a high surface to volume ratio. These PBRs have many advantages, first of all the big, illuminated surface, and then the possibility to easily control the temperature of the cultivation and high gas-liquid mass transfer rate that is provided by the air bubbling.

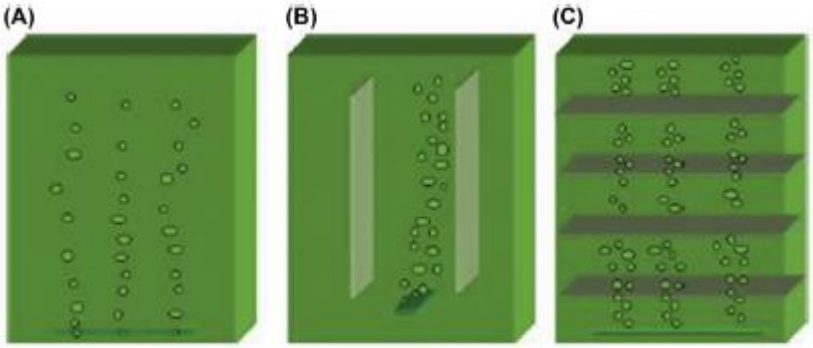

Figure 1. 5: flat-plate reactors. a) simple flat panel, b) flat panel with vertical baffles and c) flat panel with horizontal baffles

Finally, tubular PBRs (Figure 1.6) are types of reactors that are widely used in various applications; they can be arranged in different orientations, vertical, inclined or helical to maximize the sunlight capture. The diameter of the pipe is small, 10 mm, because the target to achieve is to produce cells with a high concentration.

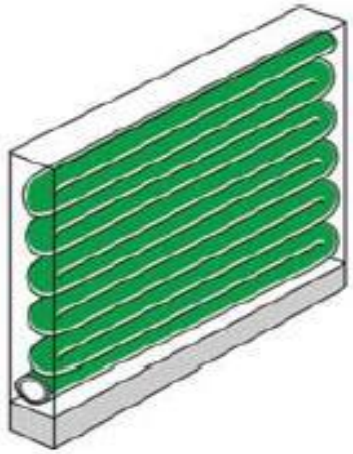

Figure 1.6: example of tubular reactor

1.5.3 BATCH SYSTEM

The cultivation of algae can be done according to different operation modes. The most common cultivation mode with the use of a batch reactor. Basically, this reactor is a closed and agitated vessel that works in a non-continuous way. The system is initially filled with a small concentrations of microalgae culture (inoculum), and with the cultivation medium with all the necessary concentration of nutrients (Ci).

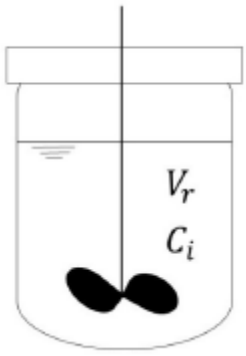

Figure 1.6: example of Batch reactor (lecture notes Bertucco, 2021)

However, this operating mode is characterized by a low productivity, high costs and a variable quality of the final product.

Algae growth in batch cultures experiences five different phases (described also in the figure 1.7). These are:

1- Lag: Initial period of slow growth;
2- 2- Exponential: Rapid growth and often cell division;
3- 3- Declining Relative Growth: Occurs when a growth requirement for cell division is limiting;
4- 4- Stationary: Cell
5- 5- Death/ Lysis: Cells begin to die due to lack of resources.

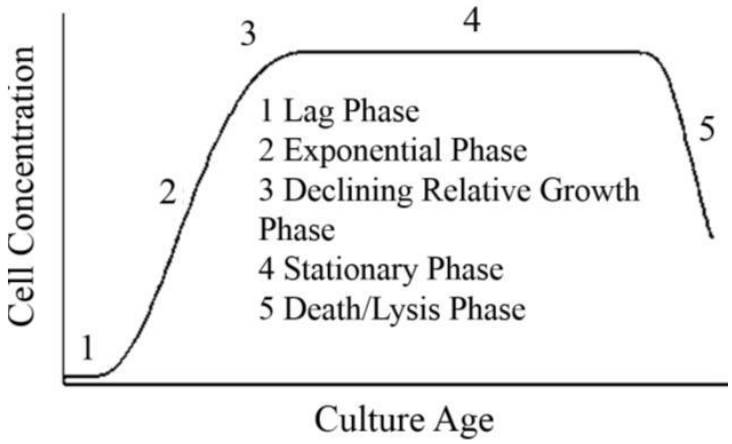

Figure 1.7: Five growth phases of algae cultures (Resource conservation in Microalgae, K. Price, 2013)

1.5.4 CONTINUOUS SYSTEM

As an alternative to a batch system, algae can be cultivated in an open system, in which a flow rate of fresh medium with the required nutrients continuously enters in the bioreactor, while the produced biomass is continuously withdrawn (Figure 1.6). In this case, the model that can better describe what happens is called a continuously perfectly stirred reactor (or CSTR).
The conditions in which this system works are the volume of the reactor must be the same during the time (Vr=constant), there is a volumetric flux that enter in the system (Ve) characterized by a concentration of pollutant (Cse), and a output flux (Vu) characterized by an outlet concentration (Csu).

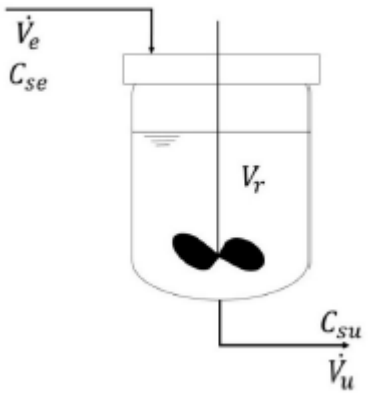

Figure 1.7: example of CSRT reactor with C_{xu} at the exit

To build the equation for the model of the figure 1.7 it is necessary to develop the following material balance:

$$\boldsymbol{A=E-U+R}$$

where the formula means that the accumulation (A) is given by the entrance (E) minus the output (U) and plus the reactions (R) that occur in the system that can produce or use some products or reagents.

1.6 TYPICAL MICROALGAE USED IN WASTEWATER TREATMENT

As it was explained before to treat the wastewaters there are different methods; some of them can be classified based on the type of biological reactions, the type of procedure that we use (aerobic step or anaerobic stages) and finally the type of microorganisms that are used to reduce the concentration of pollutants.
Considering this last point of the list the classification is splitted in two parts, the first one where the processes are based on the cultivation of bacteria (both in aerobic and anaerobic conditions) to reduce the concentration of the polluted compounds. The second case is based on the use of microalgae because some of them can use the major chemical compounds in the water as a source of nutrients. Moreover during their aerobic reaction for the uptake they do not release CO_2 but they use it to grow.
Based on this, the use of microalgae represents a potential good alternative for the wastewater treatment process. Different microalgal species can be used to this purpose, two of which are discussed in the following paragraphs.

The main advantage is the fact that the cultivation of microalgae in wastewater offers the unique opportunity to simultaneously achieve both nutrient removal and production of a high-value algal biomass even if the habitat is not favorable (A.Bertucco et al, 2019). In fact, the wastewater generally is rich of metal ions and chemical compounds that can inhibit the reaction for the growth of this algae. However, this species has a strong capacity of adaptation in this context, and it is possible to achieve strong results using them. In fact, their metabolism and the biomass cultivation need organic carbon, phosphorus and Nitrogen (As major nutrients) to survive and replicate (Y. Yu et al, 2013). All nutrients that are easy to find are abundant in the wastewater and based on some experiment it was possible to see a remarkable growth with a removal rate of 96% of phosphorus, 66% of nitrogen and 68% of COD (A.Bertucco et al, 2019).

1.6.1 *Chlorella sp.*

One genus that is frequently used to develop algal wastewater treatment is *Chlorella sp*.

Chlorella vulgaris and prothecoides are species that belongs to the family of *Chlorophyta.* This type of algae could be present in both marine and fluvial water so it can resist in salt and fresh water. Chlorella is a unicellular eukaryote green algae; its shape is spherical or elliptical characterized by a diameter between 2 and 10 nanometers (figure 1.9 gives an example of the dimensions).
The cell of this type of algae is characterized by a cell wall with a thickness, which improves with the growth conditions, that can change between 2 and 17 nm (Safi et al,2014). Inside the cell body is composed by nucleus, mitochondria, vacuoles, ribosomes, and a chloroplast parietal (figure 1.10) within which are chlorophyll a, b and carotenoids as accessory pigments (Clément-Lavoisiere, 2012).

Figure 1.9: cells of C. vulgaris (F. Scatolini, 2019, Utilizzo di C. Vulgaris)

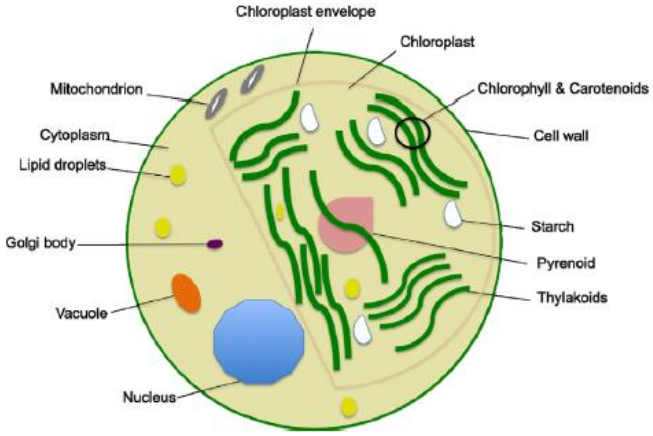

Figure 1.10: schematic structure of C. vulgaris (F. Scatolini, 2019, Utilizzo di C. vulgaris)

C. Vulgaris is a non-motile reproductive cell, that means it is asexual with rapid reproduction rate. In fact, in 24 hours the normal cell of this algae, under correct conditions can multiply by auto sporulation, which is the most common way for the reproduction of this species. In this manner, as it is possible to see in figure 1.11, it is possible to obtain 4 daughter cells, each of which has their own cell wall (C. Safi et al, 2014).

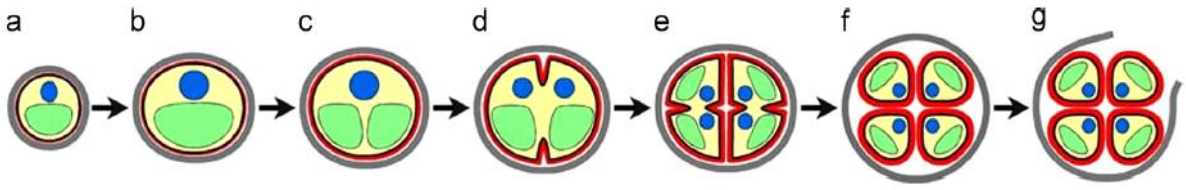

Figure 1.11: Drawings showing the different phases of reproduction: (a) cell-growth, (b) late cell-growth, (c) chloroplast diving phase, (d) protoplast diving phase, (e) late protoplast phase, (f) daughter cells maturation and (g) hatching phase

Chlorella vulgaris and protothecoides are species very versatile, so they can be used for different purposes, for example: production of biofuels, animal feeds, agrochemical applications and last but not least it can be used in the wastewater treatment during the secondary treatment.

Some recent studies discovered that *Chlorella vulgaris* is very useful to implement wastewater treatment. It has a potential to fix 75% of carbon dioxide when they are in the reactors, and they can absorb between 45-97% of nitrogen and 28-80% of phosphorus.
This level of removal is possible to reach because the algae have to satisfy their metabolism to grow, in fact all the previous substances represent nutrients (nitrogen, phosphorus, carbon dioxide, heavy metals) that are indispensable.
Thus, a faster growth rate accompanied by an elimination of water-contamination level is promising and advantageous process; another advantage of the us of *C.Prothecoides* is the fact that it has a great capacity to remove the ammonium nitrogen from the wastewater and for that reason it is one of the best microalgae to use to reach that result (C. Safi et al, 2014).

1.6.2 Synechocystis sp.

Synechocystis is a genus of cyanobacteria (formerly called blue algae) mainly represented by the strain *Synechocystis* sp.PCC6803. This microorganism is able to live in freshwater and it has the capacity and the ability to grow both phototrophically by oxygenic photosynthesis, and heterotrophically by glycolysis and oxidative phosphorylation.
The cells of this microalgae are very complex (see figure 1.12 and 1.13 below) and inside there are many structures that permit the growth and all the complex reactions for the metabolism. They can reproduce asexually way through the splitting of the main cell (L.A.Millis et al.,2020).

In recent studies, different useful applications for *Synechocystis* were proposed, for example as biotechnology platforms for the synthesis of pharmaceuticals. They potentially can contribute for the production of industrial compounds and biofuels, due to their highly efficient conversion of water and CO_2 to biomass using solar energy (L.A.Millis et al.,2020). But one of the areas of greatest interest for the use of this cyanobacterium is wastewater treatment. There are some characteristics that make this species like a very versatile organism to be used in this application. The advantages of this species are the fact that they can resist in different habitats, including those defined as extreme (with high or low temperature). Moreover, are promising candidates for environmental applications due to their ability to tolerate high levels of pollution, to degrade highly persistent organic contaminants and to remove heavy metals.

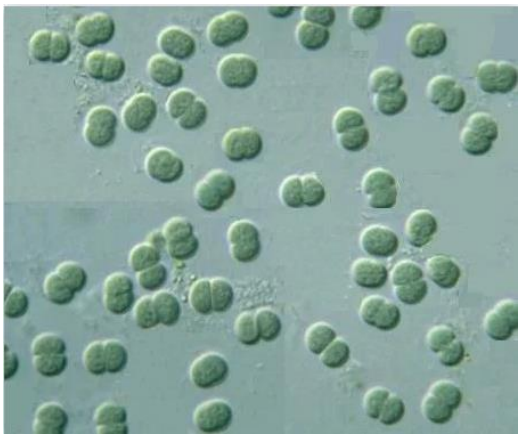

Figure 1.12: cells of Synechocystis observed in the laboratory

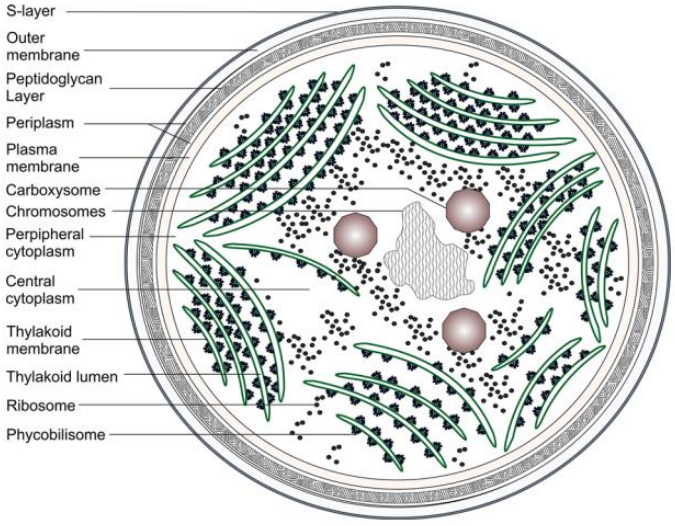

Figure 1.13: schematic view of a cell of synechocystis (Current knowledge and recent advances in understanding metabolism of the model cyanobacterium Synechocystis, Lauren A. Millis et al, 2020)

1.7 KINETICS OF MICRO-ALGAE GROWTH: MONOD AND DROOP MODELS

To describe the kinetic growth of the microalgae in this system it is possible to consider two different models that can describe the increase of the biomass during the time as a function of nutrients concentrations. The first model is the Monod model based on the amount of nutrients present outside the cells (i.e., dissolved in the medium), and the second model is called the Droop model that has a different approach and takes in consideration the amount of nutrients that are present inside the cells to estimate the growth. These two models are briefly described in the following sections.

1.7.1 Monod model

Monod model is generally used to describe the growth of microorganisms in a specific environment that is rich in all the nutrients that are important for their growth. The characteristic of this kinetic model is the fact that it mainly considers the concentrations of nutrients that are present in the environment to estimate the velocity and the final growth of the culture. The Monod model introduced the concept of a growth limiting substrate (G.C. Okpokwasili et al, 2005).
The growth rate is expressed by the following formula:

$$\mu = \mu_{max} * \frac{S}{Ks+S} \qquad (1.2)$$

Where μ = specific growth rate, μ_{max} = maximum specific growth rate, S = substrate concentration, K_s = substrate half-saturation constant (the figure 1.14 explains the behavior of the model). In Monod's model, the growth rate is related to the concentration of a single growth-limiting substrate through the parameters μmax and K_s.

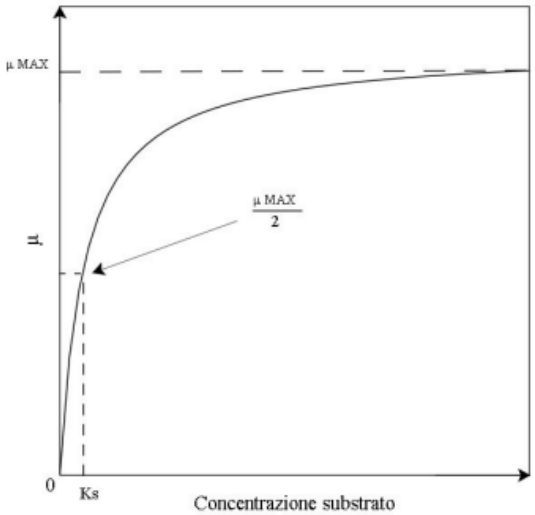

Figure 1.14: graph that shows the Monod relation regarding the variation of μ_{max}

1.7.2 Droop Model

However, for the aim of the research Monod's model is not precise enough so instead of it is preferred to use the Droop model. This second method can describe the growth rate considering the internal quota of nutrient presents in the cell of the microorganism. One of Droop's experimental results is

that the algae growth rate depends on the intra-cellular quota of nutrient q only. Moreover, he identified a threshold of intracellular quota, denoted $q\ m$ and referred to as the "subsistence quota",

under which algae do not grow. Droop proposed then the following model for the algae growth rate (V. Lemesle et al, 2008):

$$\mu(Q) = \mu_{max} * (1 - \frac{Q_{min}}{Q})$$

(1. 3)

It is possible to define μ_{max} the maximum growth rate, Q represents the cell quota and Qmin describes the minimum quantity needed to start the growth (figure 1.15).

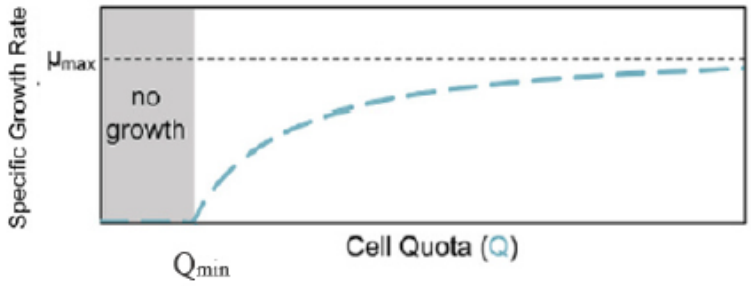

Figure 1.15: schematic representation of Droop model for the growth

Cell quota could be defined as the quantity of substrate within the biomass, in other words, a coefficient of demand and the reciprocal (in the absence of excretion) of the yield coefficient (Droop, 1983).
The evolution and the maintenance of the cell quota are due to the nutrient uptake on one hand and dissipated by cell multiplication at rate μ(Q) on the other hand, leading to the following equation:

$$q = \rho(S) - \mu(Q)$$

where $\rho(S)$ is the nutrient uptake rate that depends on the concentration of nutrient in the environment and his definition is based on another expression (1.4) where the value of Km explains the maximum uptake for the cells and Ks the half-saturation constant.

$$\rho(S) = K_m * \frac{S}{K_s + S}$$

(1. 4)

Considering the last equations and the figure above it is clear that there is a minimum level of growth and a maximum level, and this range depends on the amount of the quota cell.
Based on this process it is possible to give an explanation about the growth of the biomass of the microalgae.

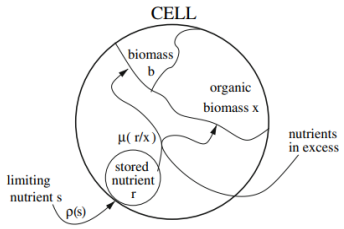

Figure 16: schematic view of the use of nutrient in the cell (based on Droop theory)

1.7.3 Co-occurrence of different nitrogen forms

As it was described before Nitrogen is one of the main nutrients that are necessary for the development of a cultivation of microalgae. This element is present in different forms in the water that reaches the wastewater treatment (see previous chapter).
Although microalgae are able to efficiently uptake both nitrates and ammonium when these are supplied as the only N source, when there is the co-occurrence of both these forms of nitrogen the level of uptake appears to be selective. Generally, the presence of ammonium seems to limit the uptake of nitrate. In particular the research has focused on understanding the effect of these two nitrogen forms on algal biomass growth.

For these reasons, different hypotheses are developed because there is the necessity to understand the reciprocal influence of the two forms on the respective uptake. Two models that could describe this behavior are present in the literature. Both models are based on the assumption that there is an additional parameter to be considered in the equations, accounting for the inhibition phenomena of nitrate uptake inhibition when ammonium is present.

The first model is called Dixon model; conventionally used for co-metabolism it is a development of the model of Monod in which it is also considered the presence of both the species of Nitrogen so in the equations there will be the presence of an element that underlines the inhibition effect during the uptake. The following formula explains how the growth rate can change with the presence of two substrates:

$$\mu_a = \frac{\mu_{max} * Ca}{Ka + Ca + \left(\frac{Ka}{Kb}\right) * Cb} \qquad (1.5)$$

Where C_a and C_b are the concentrations of the two nutrients (in this case the two forms of Nitrogen), and K_a and K_b are the half saturation constants of substrates A and B respectively. In the following chapter the formula will be explained with all the references and there will be a better context that will explain its function completely (S.K. Padhi et al, 2014).

The second type of model is the one developed by Solimeno et al. (cit.), obtained following the Monod equations. In fact, the formulation that will describe the inhibition effect is characterized by the normal uptake formula where a second part is added that shows the presence of a second substrate which can reduce the biomass growth.
By simplifying some members of the equation, the formula will have the following expression:

$$\mu_a = \frac{\mu_{max} * S_a}{Ka + S_a} * \frac{Kb}{Kb + S_b}$$

(1. 6)

In this formula there is the value of μ_{max} that represents the maximum growth rate, S the substrate concentration (S_a is the substrate of one of two forms of Nitrogen and S_b the other one), K_a the constant of half saturation for species "a" and K_b the constant for the half saturation for species "b" (A. Solimeno et al, 2015).

1.8 TRANSPORTERS FOR NITROGEN

Based on some studies the researchers have discovered the presence of some transporters that have the aim to help the cells, in this case the microalgae's cell, to import compounds into the cell. These transporters can be referred to as valves that allow the substance, in this case the target nitrogen species, to enter and pass through the cell membrane from outside to inside the cell. As a result of experiments conducted on the exchange of nutrients between the extern and the interior of the cell, it was also possible to identify that there are different types of transporters depending on the molecule that is to be absorbed. Consequently, to assimilate ammonium or nitrates, cells will use at least two different types of transporters.

In the case of ammonium, there are two transporters that allow the passage of that ion from one side of the membrane of the cell to the other; the systems in question are called HATS and LATS. The main difference between the two concerns the fact that the former is activated by low concentrations of ammonium while the latter works for high concentrations of the substance outside the cell wall. With regard to algae, few transporters have been identified, notably one only for *Chlorella* and 3 for *Synechocystis*.

Regarding nitrite and/or nitrate uptake, there are 3 different families of transporters that can be used by cells. However, in the case of algae such as *Synechocystis* the transporters used are of only one type: NrtABCD.

Consequently, depending on the type of algae, there are different transporters, and these also vary depending on the nutrient or molecule that the cell intends to assimilate; therefore, considering the co-presence of both species, based on some studies, it is possible to say that there is a possibility that the excess of either one may inhibit or alter the uptake by the cell.

AIM OF THE THESIS

Further developing research on the use of microalgae in wastewater treatment may lead to excellent results, especially being able to choose processes that are based on autotrophic algal species that have the ability to use carbon dioxide as carbon source and not as a waste product, and light as energy source. Certainly, this will be a huge advantage in both technical and economic terms for the development of new treatment methods.
However, among the aspects to be monitored and continued to be analyzed is the use of nitrogen as a source of nutrition for microalgae. Due to the presence of different nitrogen species, such as ammonium and nitrate, that can be both assimilated, the modelling of nitrogen removal has some challenges: nitrates and ammonium have also an antagonist interaction, which may lower the utilization of nitrate in the process. In fact, ammonium is a preferable source of nutrients than nitrates possibly reducing the overall efficiency of nitrogen removal.
Understanding and describing this phenomenon by a modelling approach could help in the design and operation of the system. However, the available models are not sufficient to describe this inhibition phenomenon.

Given this background, the following thesis will aim to consider and analyze the Solimeno and Dixon models, verify their applicability on the data obtained experimentally, in steady-state continuos cultures of *Chlorella protothecoides* and *Synechocystis sp.* and possibly improving the models to be able to capture this inhibition phenomenon.
Also, the uptake of nutrients by Droop will be included in the description to approximate the experimental results by understanding the influence of ammonium and nitrate in two species (*Chlorella protothecoides* and *Synechocystis spp PCC6803*).

CHAPTER 2: KINETIC MODELS

2.1 SYNECHOCYSTIS and CHLORELLA: EXPERIMENTAL CONDITIONS

This second paragraph is dedicated to the description of the mathematical equations that are to describe the reactions than take place in the reactor during the experiments. During this explanation two different method are presented. These two methods, Solimeno model and Dixon model, are the mathematical model that will be used in the Matlab software to compute the analytical results to compare with the experimental ones obtained in the laboratory. Both the model has the aim to develop a system of equation that permits to characterize the reactions that, in theory, happen in the system. After that it will present also the conditions in which the data of the experiment are obtained. The data that are used in this studies are taken by previous experiment in particular from the master thesis of Marta Carletti and Martina Stabile.

This chapter will briefly describe some theory than are used to analyze the development of cultivation of algal cultures. Some kinetic models are identified in the literature for describing the uptake pattern of these microorganisms. The two models considered in the following analysis are called: Solimeno's Model (from the researcher that studied the method) and the model of Dixon. The afore mentioned models will be described taking in consideration the functional parameters. After that based on some assumption, concerning the tests that are conducted, and boundary conditions of the system there is a short description of each passage that were applied to obtain the final system of equations. This result has the target to better describe the data obtained during the laboratory experiences.

The following paragraph gives an overview of the experimental conditions in which the two species of microalgae were cultivated in the laboratory.
Synechocystis and *Chlorella* samples, used for data collection, were grown in a continuous system.

Synechocystis was cultivated in a photobioreactor, shown in the following Figure 2.1 (as described in the introduction chapter) where temperature and incident light intensity were kept constant based on the description of the previous chapter that underlined their importance.
Temperature was set, for all experiment, at 30°C. The light, which was provided by LED panels outside the reactor, was 150 μmol photons/m^2/s.
Two types of stainless-steel polycarbonate flat panel reactors were used for the different experiments for the continuous system, the first one characterized by a thickness of 3 cm and a containing volume of 150 ml, and the second characterized by a thickness of 3.5 cm and a volume of 200 ml (Marta Carletti, 2021).

In the case of the cultivation of *Chlorella* the conditions regarding the temperature and the light intensity were the same than with *Synechocystis* (30° C and 150 μmol photos per second). Instead, the reactor volume and the thickness were equal to 3.5 cm of thickness and 200 ml of Volume (Martina Stabile, 2020).

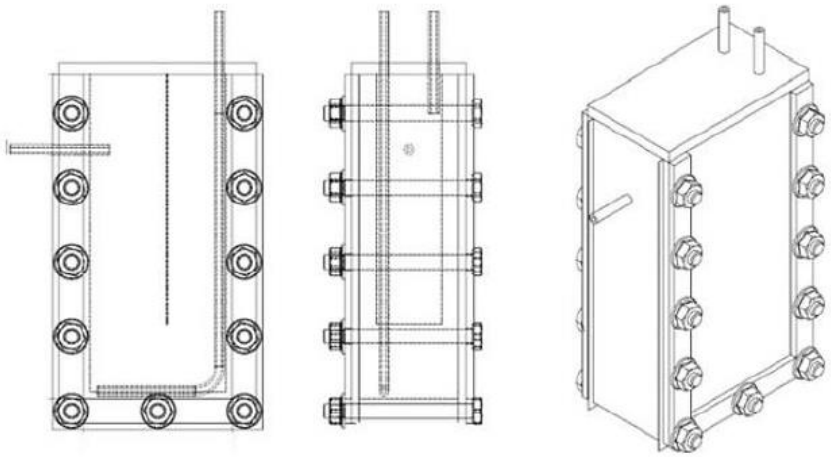

Figure 2.1: different side of the reactors used in the experiments (M.Carletti, 2021)

The purpose was to work with a reactor that approximated, in features and functionality, a CSTR reactor (described previously). To achieve this, it is important to work with a constant reactor volume (Vr) and a constant volumetric flow rate (V). Based on this concept it is possible to identify a fixed value of retention time (θ).

$$HRT = \theta = \frac{Vr}{V} \qquad (2.\ 1)$$

The HRT (hydraulic retention time) represents the value of time, express in d, that the cells are retained within the photobioreactor.
Having reached the steady-state condition, it was possible to proceed with measurements of concentrations of chemical compounds and the biomass that were present inside the reactor. Three were the variables that were measured: nitrate, ammonia, and the biomass. These results, together with the inlet composition (reported in the following tables 2.1 and 2.2 respectively) were used for a comparison with the model outcomes obtained with Matlab.
As it is possible to see from the values compared in the tables the samples had different compositions in terms of quantity and type of nitrogen compounds.
To better understand the behavior of the microalgae in presence of both the sources of nutrients, experiments were carried out with different concentrations of the two compounds. In particular, for *Synechocystis* there are experiments containing both nitrate and ammonium, as well as others characterized by the presence of only one of them.

The following tables (table 2.1 and table 2.2) contain the inlet concentrations of the two species of algae that were cultivated and the correspondent resident time and concentrations of Nitrogen and Phosphorus.

Data of *Chlorella*:

tau θ (d)	NO3 (g/m^3)	NH4 (g/m^3)	P (g/m^3)
0,65	60	30	10,8
0,71	30	80	10,8
0,72	100	100	10,8
0,7	40	10	10,8
0,7	10	40	10,8

Table 2.1: inlet concentrations of Ammonium, Nitrates and Phosphorus and the correspondence resident time for Chlorella experiments

Data of *Synechocystis*:

HRT= θ (d^-1)	NO3 (g/m^3)	NH4 (g/m^3)	P (g/m^3)
0,95	19,3	/	10,8
0,95	33,78	/	10,8
0,87	59,41	/	10,8
0,87	69,45	/	10,8
0,87	/	23,29	10,8
0,87	/	55,03	10,8
1,1	/	80,3	10,8
0,87	/	93,83	10,8
1,1	15,49	50,32	10,8
1,1	22,04	37,74	10,8
1,1	52,23	38,03	10,8
0,9	59,21	35,81	10,8

Table 2.1: inlet concentrations of Ammonium, Nitrates and Phosphorus and the correspondence resident time for Synechocystis experiments

2.2 KINETIC MODELS

As it is written in the short introduction, to describe the growth of algae inside the reactors and the uptake of the nutrients many equations are necessary.
Different purposes exist for the models that are considered in this thesis; the main one is the capacity to quantify the uptake of different forms of nitrogen for the two algal species and compare it with the laboratory results. The second target is to analyze and discover the values of some important parameters in relation to the biology that described the system of uptake of algae.
Both these aspects were investigated by the two models because it was important to compare them and understand which one could be more precise.

The following paragraphs will present all the equations used in the different models implemented in Matlab and describe all the steps performed to arrive at the final formulation that was found to be the most suitable.

2.3 DIXON MODEL

To be able to describe what happens inside the reactor requires the development of several equations where each has a distinct role.
In this paragraph the equations used and developed for Dixon's model are described, which were then written into Matlab to compare model results with experimental ones.

The system is characterized by 6 equations: the first describes the growth of the algae, the second, the third and the fourth consider the removal of the two forms of nitrogen (NO_3 and NH_4) and phosphorus, and the last two concern the total mass balance of the whole system for nitrogen and phosphorus.

2.3.1 Growth of algae

Algae growth rate (Rx) is a biological phenomenon that is influenced by multiple factors such as the amount of nutrients in the system, temperature, pH of the solution, and exposure to light that promotes or inhibits cellular reactions.
The equation describing this process can be summarized as follows:

$$Rx = (\mu g - \mu m) * Cx \qquad (2.\ 2)$$

where μg (d^{-1}) represents the growth rate of the microalgae, while μm (d^{-1}) describes the decay rate, (the mortality rate), and at the end there is Cx (g/m^3) that is the algae concentration at the reactor outlet.

Looking at the term related to algal growth, it can be said that it is influenced by a multitude of parameters; whereas about the mortality rate, the issue is relatively less complex since it can be simplified by saying that this value is influenced only by the specific decay rate K_d (d^{-1}) and the temperature.
Considering this concept, it is possible to write the equation as the following based on all the parameters that can influence:

$$Rx = [\mu_{max} * f(I) * f(N) * f(P) - K_d * f(T)] * \mathrm{Cx} \quad (2.3)$$

this equation can be written in this easier way:

$$Rx = \mu_{max} * [\mathrm{f(I)} * \mathrm{f(N)} * \mathrm{f(P)} - \mathrm{Kd} * \mathrm{f(T]} * \mathrm{Cx} \quad (2.4)$$

The equation (2.4) explains that the growth rate of the algae is a function of the maximum growth rate μmax (d-1), whose value changes based on the different species of microalgae (*Chlorella* and *Synechocystis* have different values), the nitrogen present in the system (f(N)), the amount of phosphorus (f(P)), the value of the temperature and the effect of the light (f(I)).

2.3.2 Effect of the light

Light has a fundamental effect on the growth of these microorganisms because photosynthesis, which is the basic process for algae development, is driven by the presence of it in the environment in which they live.

Following some studies, a relationship between the amount of light and the photosynthesis reaction was determined.
According to the studies done by Rèmond (Bernard & Rémond, 2012) there are three conditions of influence: the first is characterized by the fact that photosynthesis increases in a manner directly proportional to the amount of light received, the second case is characterized by reaching the maximum peak where photosystems are saturated, and finally the third phase is the one in which photosynthesis can be inhibited by the excess of light received. The picture below (figure 2.2) explains the three different phases that can occur.

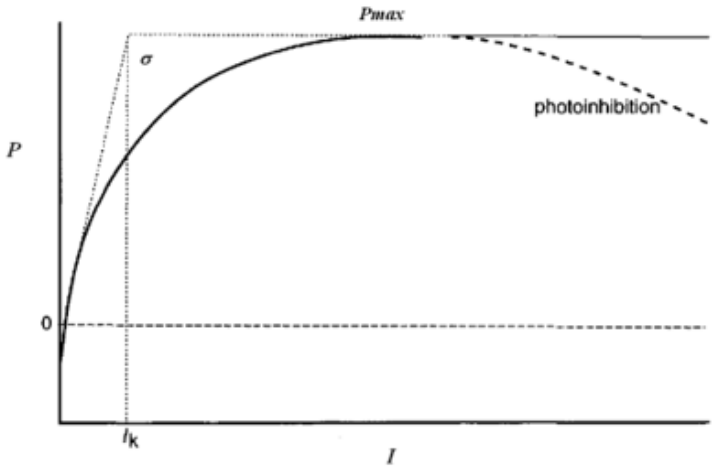

Figure 18: variation of photosynthetic rate as a function of light intensity (source: Bernard and Rèmond, 2012)

To describe this behavior and light dependence, there are several models in the literature but in the case of microalgae the model that better describes the relationship was developed by Bernard and Rémond and it was considered for these simulations.
The model takes in consideration the fact that the growth rate depends both from light intensity and temperature as the follows formulas describes:

$$\mu(T, I) = \mu_{opt}(I) * \phi(T) \quad (2.5)$$

where $\mu_{opt}(I)$ is the optimal growth rate which can be reached (at temperature T_{opt}), for a value of light intensity I (Bernard & Rémond, 2012).
The function that describes the influence of the light intensity is the following:

$$\mu_{opt}(I) = \frac{I(z)}{I(z) + K_I(\frac{I(z)}{I_{opt}} - 1)^2} \quad (2.6)$$

where the value I(z) represents the value of the light intensity that it is calculated through the equations of lambert – beer where the average speed is then calculated by making the integral mean of the values calculated punctually along the z coordinate.
Iopt represents the value of irradiance for which growth is maximal (with respect to light). It strictly related with the temperature, as it is possible to see from the following figure 2.3.
Finally, the last parameter Ki is the constant of half-saturation of the light.

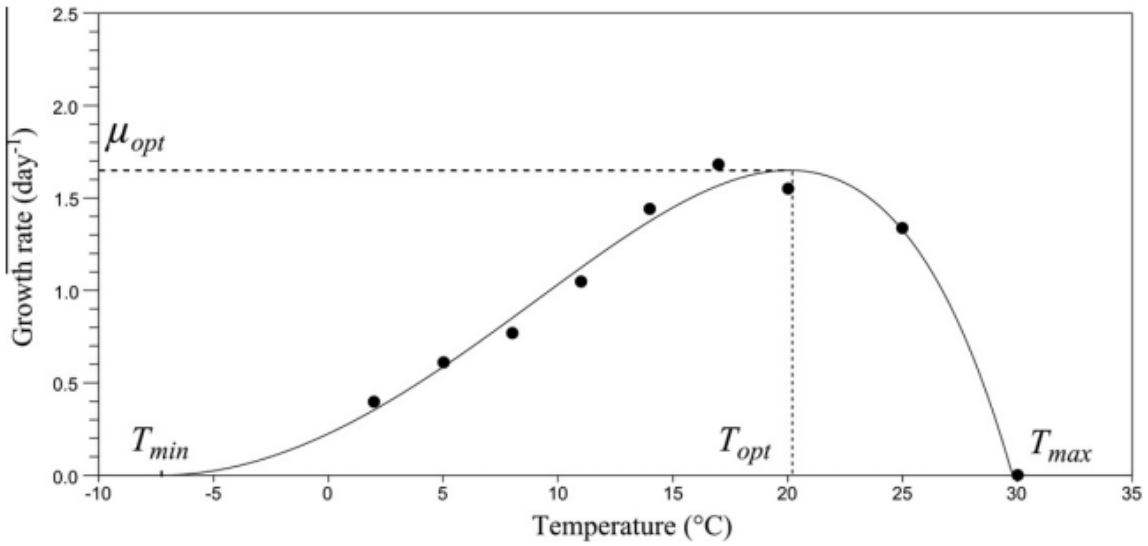

Figure 2.3: variation of μ_{opt} on the effect of the temperature

2.3.3 Effect of temperature

The other essential parameter for algae growth is temperature.
Each microorganism is characterized by a specific value of temperature that permits the optimal growth, according to Figure 2.3.
Considering the Bernard's model (Bernard & Rémond, 2012) is possible to explain how strong the influence of the temperature is considering the following groups of equations:

$$\mu_{max} = \begin{cases} 0 \text{ for } T < T_{min} \\ \mu_{opt} \cdot \phi(T) \text{ for } T_{min} < T < T_{max}, \\ 0 \text{ for } T > T_{max}, \end{cases}$$

where

$$\phi(T) = \frac{(T - T_{max})(T - T_{min})^2}{(T_{opt} - T_{min})[(T_{opt} - T_{min})(T - T_{opt}) - (T_{opt} - T_{max})(T_{opt} + T_{min} - 2T)]}$$

(2. 7)

The model is based on the fact that there may be three values of temperature that are important for the development and growth of algal culture, and in the overall view also the biology of the system. These three parameters have the role to fix three ranges, from which the biology of the system, particularly growth, will be different.

1. Considering temperatures below Tmin it is assumed that there is no growth because the environment is not favorable,
2. with values above Tmax the growth of microorganisms is reduced to a halt because conditions become extreme,
3. in the range of Tmax and Tmin algae can grow. Inside this range it is possible to define Topt that is the value at which there are the most favorable conditions to work. (Bernard & Rémond, 2012).

However, to develop these studies it was decided to conduct the experiment regarding the growth of microalgae and the removal of nutrients keeping constant the value of temperature for both the species *Chlorella* and *Synechocystis*. The value of temperature used in the photoreactors was 24°C.

2.3.4 Nutrient uptake

The heart of the model, that it is going to be described, is represented by the biological activities that allow to assume the nutrients and, consequently, to determine the growth of the microalgae. Before to start with the description it is necessary to take in consideration an assumption that is fundamental for the results of the research. For all the experiments there is not limiting element of factor that could stop the uptake or the growth of algae; that means that in the reactors, where there were *Chlorealla* and *Synechocystis*, carbon, nitrogen and phosphorus were abundant.

Following this assumption, it is possible to begin the description of Dixon's model being considering that the model of Droop was used to describe the uptake of nutrients that are needed for cellular metabolism.
In fact, the parameters called f(N) and f(P), in the equation 2.3, can be replaced by the following equations:

$$F(N) = \left(\frac{R_N}{Q_{min} + R_N}\right)$$

(2. 8)

$$F(P) = \left(\frac{R_P}{Q_{min} + R_P}\right)$$

(2. 9)

Based on the droop theory the capacity of growth of the biomass (microalgae) depends on the amount of share or "quota" of Nitrogen and phosphorus that can be assumed. In these cases, the value of Rn and Rp represent the internal "quota" of, respectively, nitrogen and phosphorus. While Qmin represents the minimum "quota" already presents in the cell.

2.3.5 Rate of growth of algae

Considering the previous formulas, the generic equation presented earlier can be replaced and completed with the following one:

$$R_x = (\mu_{max} * \frac{I(z)}{I(z) + K_I + \left(\frac{I(z)}{I_{opt}} - 1\right)^2} * \left(\frac{R_N}{Q_{min,N} + R}\right) * \left(\frac{R_p}{Q_{min,P} + R_p}\right) - K_d) \quad (2.\ 10)$$

As it is possible to see, the equation 2.3 was completely substituted by the equation that describes the influence of the light intensity and the two equations describing the "internal quota" of nitrogen and phosphorus.

After that must be calculated the average growth of algae inside the reactor, so the previous equation must be integrated respect the size of the reactor in which the reactions are taking place. In this way it is possible to obtain the average growth value along the depth of the reactor.
The average growth rate (Rx) is given by:

$$R_x = \frac{1}{W}\int_0^W \left(\mu_{max} * \frac{I(z)}{I(z) + K_I + \left(\frac{I(z)}{I_{opt}} - 1\right)^2} * \left(\frac{R_N}{Q_{min,N} + R}\right) * \left(\frac{R_p}{Q_{min,P} + R_p}\right) - K_d \right) * C_{xout} * dz \quad (2.\ 11)$$

Where "W" describes the thickness of the reactor (considered in meter "m") and $C_{x,out}$ is the algae concentrations at the outlet point of the reactor (g/m^3).

2.3.6 Rate of nutrient uptake

The growth of an organism is determined by the amount of nutrients that can be assimilated and used to replicate himself.
It is known that, considering Droop's method, the microalgae in question (*Chlorella and Synechocystis*) have in their cells a minimum amount of nitrogen and phosphorus (quota) that are needed for algal growth. There is also a maximum value of internal nitrogen and phosphorus that could be reached during the uptake and both the species has this limit that is different for the two algae.

There are two equations that describe the rate of nitrogen removal, one for nitrate (R_{NO_3}) and the second for ammonium (R_{NH_4}):

$$R_{NO_3} = \rho_{NO_3} * \frac{C_{NO3,out}}{C_{NO3,out} + K_{NO3}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2. 12)

$$R_{NH_4} = \rho_{NH_4} * \frac{C_{NH4,out}}{C_{NH4,out} + K_{NH4}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2. 13)

It is possible to understand that the removal rate of nutrients or the uptake of them from the wastewater is characterized by the interaction between three factors.

- The first one is the Droop factor that describes which can determines the amount of nitrogen absorbed by the cell.
- The second factor is the characterized by the presence of the concentration of nutrients, so the uptake might change based on the quantity of nutrients that are present in the medium.
- Finally, the third factor that interacts in this system is the velocity of maximum uptake rate (ρ_{NO_3} and ρ_{NH_4}). The values of ρ_{NO_3} and ρ_{NH_4} describes the velocity of uptake of the nutrients (gN/gX^{-1} d^{-1}).

However, the previous formulations described only the uptake of the single nitrogen nutrients, without accounting for the possible reciprocal inhibition effect; to consider this aspect, the removal rate equation was based on the Dixon's formula (S. Kumar Padhi et al. 2014).
However, the previous formulations described only the basic concept regarding the uptake of the nitrogen nutrients; to obtain one of the expressions that it was used to estimate the effectiveness of this process it is important to consider the removal rate based on the Dixon's formula (S. Kumar Padhi et al. 2014)
So, in the next passage the equations (2.12 and 2.13) will be modified using this formula (2.14) that resume the Dixon idea: and the following equation will be substituted within the previous two.

$$\mu_a = \frac{\mu_{max} * Ca}{Ka + Ca + \left(\frac{Ka}{Kb}\right) * Cb}$$

(2. 14)

As explained earlier this model was developed considering that there may be a possible inhibition between the two nitrogen species at the time when the plant cell must take them up. In particular it was thought that there could be a sort of competition between nitrate and ammonium; and although the first one (nitrate) is preferred over the latter this inhibition may cause a consistent reduction of removal for the species that is biologically defined as the preferred food of the algae.

This interaction between the two nitrogen species is considered in the formula when a ratio of the two K coefficients (K_a and K_b) appears and they represent the half-saturation constants of the two nutrients.
The definition of what is the species “a” or “b” depends on which side of the system is looking as it is possible to see in the two equations below (2.15 and 2.16).

As can be seen from the equation (2.14) depending on which one it is observed the ratio changes. Considering the system from the nitrate point of view the ratio will be described as the nitrate over

ammonia and this factor will be multiplied by with the ammonia concentration to complete the inhibition factor. The situation will be the opposite considering the ammonia point of view.

$$R_{NO_3} = \rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3} + \left(\frac{K_1^{NO3}}{K_2^{NH4}}\right) * C_{NH4}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2. 15)

$$R_{NH_4} = \rho_{NH_4} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4} + \left(\frac{K_1^{NH4}}{K_2^{NO3}}\right) * C_{NO3}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2. 16)

Where K_1 and K_2 are the half saturation constant for each species (gN/gm^3), C_{NO3} and C_{NH4} are the outlet concentrations (g/m^3) and the last part of the equations shows the Droop factor.

Finally, to simplify the equations, it was decided to name these two factors as α (in the Ammonium case, $\left(\frac{K_1^{NH4}}{K_2^{NO3}}\right)$) and β (in the Nitrate equation, $\left(\frac{K_1^{NO3}}{K_2^{NH4}}\right)$).

$$R_{NO_3} = \rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3} + \beta * C_{NH4}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2. 17)

$$R_{NH_4} = \rho_{NH_4} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4} + \alpha * C_{NO3}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2. 18)

However, as a result of the study conducted with Matlab scripts, it was observed that the best results were obtained considering the equations of removal rate of nitrogen without the Dixon formulation. For this reason, the latest calculations were made using the formulas without the inhibiting parameters and based only on the simple Droop theory (equations 2.12-2.13).

The complete equations for the nitrogen-based nutrient balance are described below ($B_{m,NO3}$ and $B_{m,NH4}$).

These equations consider the entire mass balance around the reactor and not just the removal rate, as the previous (2.19 and 2.20). To complete the equations with the aim to better describe what happens it is necessary to add another term in the balance of ammonium, $B_{m,NH4}$, accounting for its mass transfer with from liquid to gas phase, which is represented in the second part of the equation 2.22.

$$B_{m,NO3} = \left(C_{NO3}^{in} - C_{NO3}^{out}\right)/_{\theta} - \left(\rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)\right) * C_{x,alg}^{out}$$

(2. 199)

$$B_{m,NH4} = \left(\left(C_{NH4}^{in} - C_{NH4}^{out} \right) / \theta \right) - \left(\rho_{NO_3} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N \right)}{Q_{max,N}} \right) \right) * C_{x,alg}^{out} + K_{La} * \sqrt{\frac{D_{NH3}}{D_{O2}}} * Kh * p_{NH3} * \frac{C_{NH4}^{out}}{(1 + 10^{(pka - ph)})}$$

(2. 200)

As it has been anticipated before the second equation regarding the balance of ammonium is characterized by a new factor.
It was necessary to add another term to the overall balance of nitrogen in the system because the concentration of dissolved ammonium is affected by the chemical equilibrium with free ammonia (NH_3). This depends from a specific value of pH and temperature. Considering the molecules in the field it is dutiful to take in consideration that the phenomenon of mass transfer may occur. Specifically in this case, stripping of ammonia is considered.
This phenomenon is a physical chemical process that occurs under certain conditions and is characterized by the removal or in this case displacement of ammonia nitrogen from wastewater to the surrounding air and it must be considered in the calculation to identify the correct amount of ammonia nitrogen that will be removed during the process.

The principle behind this phenomenon is based on two basic concepts: ammonia in its pure form is an extremely volatile gas, and this shift follows the concentration gradient rule.
So, this means that the shift of ammonia (and nitrogen) from the wastewater, in which the algae are being grown, to the air outside the reactor is stimulated by the fact that the concentration of this substance is higher in the liquid than in the gas. Consequently, to try to reach a hypothetical equilibrium state and reduce this difference, this transfer occurs.
It occurs in relation to pH, as it regulates the equilibrium between NH_4^+ and NH_3, and thus is strongly influenced by this parameter. In all the experiment that have been studied of *Chlorella* and *Synechocystis* the pH used was 7 and, on this value, the resulting value of ammonia, that could be released, was calculated (Sperandio, 1997) with the following formula:

$$K_{La} * \sqrt{\frac{D_{NH3}}{D_{O2}}} * Kh * p_{NH3} * \frac{C_{NH4}^{out}}{(1 + 10^{(pka - ph)})}$$

(2.21)

The above formula makes it possible to calculate the amount of ammonia that might pass into the air, which is an almost negligible amount compared to the order of magnitude of wastewater nitrogen concentrations.

The equation (2.21) is characterized by certain parameters; the first of them is K_{La} which represents the overall mass transfer coefficient (d^{-1}), it identifies the ability of the compound to pass from water to air. Typically, it is referred to oxygen the value is directly proportional so higher is the value that characterizes the molecule higher is the ability of the molecule to move from one state to another.
Next, there are the mass diffusion values of ammonia (D_{NH3}, m^2/s) and oxygen (D_{O2}, m^2/s) representing the rate at which the two molecules diffuse.

Finally, the second part of the equation is characterized by the value of Henry's constant K_H (gN-NH_3/m^3 * s), which was calculated by a specific empirical formula (Sander, 2015) and depends on temperature:

$$H_{NH3}(T) = \left[4.63 * 10^5 * e^{2100*\left(\frac{1}{273.15+T}-\frac{1}{298.15}\right)}\right] * \frac{14}{17} \quad (2.22)$$

This number expresses how much a compound tends to volatilize, that is, to move from the liquid to the gas phase, and again the higher the corresponding number the higher the capacity (Sperandio, 1997).

Finally, we find the partial pressure values of ammonia p_{NH3}, the concentration of nitrogen in the reactor C_{NH4}^{out} and the influence of pH which is defined as the difference between the acid dissociation pka constant and the pH at which the reactor operates.

2.3.7 Phosphorus uptake

The second fundamental nutrient for the growth of plant organisms is phosphorus.
As was done for nitrogen, the Droop model was considered and based on that we obtained the following equation regarding the removal rate of this element ($R_{\_PO4}$):

$$R_{\_PO4} = \rho_P * \frac{C_P^{out}}{C_P^{out} + K_P} * \left(\frac{Q_{max,P} - \left(Q_{min,P} + R_{\_PO4}\right)}{Q_{max,P}}\right) \quad (2.23)$$

In this case there is not any type of inhibition, so it is not necessary to implement the formula with something else and it is possible to consider directly the overall balance.
If we take in consideration the whole mass balance of phosphorus presents in the reactor the equation is the following:

$$B_{m,PO4} = \left(\left(C_P^{in} - C_P^{out}\right)/_{\theta}\right) - \left(\rho_{PO4} * \frac{C_P^{out}}{C_P^{out} + K_P} * \left(\frac{Q_{max,P} - \left(Q_{min,P} + R_{\_PO4}\right)}{Q_{max,P}}\right)\right) * C_{x,alg}^{out} \quad (2.24)$$

2.3.8 Nitrogen and Phosphorus quota

The last two equations that will complete the Dixon model have the target to describe the overall balance of "quota" of Nitrogen and Phosphorus that is used in the system.
In fact, if we consider the definition of Droop the value of the quota is given by the uptake of the algae from the medium and its consumption for growth as it represents the main nourishment for the metabolism and reproduction of the algal culture.
As a result, the following equations (2.27 and 2.28) identify the sum of all the terms that contain the values of the "quota" or nitrogen or phosphorus.

$$B_n = \left(\rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3}} + \rho_{NH_4} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4}}\right) * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right) * \theta - R_N$$
$$* + \frac{1}{W}\int_0^W \left(\mu_{max} * \frac{I(z)}{I(z) + K_I\left(\frac{I(z)}{I_{opt}} - 1\right)^2} * \left(\frac{R_N}{Q_{min,N} + R_N}\right) * -K_d\right) * C_{xout} * dz$$

(2. 25)

$$B_{PO4} = \rho_{PO4} * \frac{C_P^{out}}{C_P^{out} + K_P} * \left(\frac{Q_{max,P} - \left(Q_{min,P} + R\_{PO4}\right)}{Q_{max,P}}\right) * \theta - R_{PO4}$$
$$+ \frac{1}{W}\int_0^W \left(\mu_{max} * \frac{I(z)}{I(z) + K_I\left(\frac{I(z)}{I_{opt}} - 1\right)^2} * \left(\frac{R_p}{Q_{min,P} + R_p}\right) - K_d\right) * C_{xout} * dz$$

(2.26)

2.4 SOLIMENO MODEL

Solimeno model is the second mathematical approach that it has been consider during analysis of the experimental data that had been obtained in the laboratory.
It has the same purpose of Dixon model, that is to describe the growth and the development of the two species of algae considered (*Chlorella* and *Synechocystis*) with hypotheses very similar to the previous model.
In fact, the presence of two forms of nitrogen that can cause hypothetical inhibition to the uptake system of the cells and the use of the Droop model have been considered as before.

The real difference with the model described above is that the inhibition term is expressed in a different form.

As mentioned above, the phenomenon that describes the growth of algae is extremely complex and depends on several factors (amount of nutrients, light, temperature and correct pH range). Consequently, although the model differs for some equations, the basic assumptions describing the development of algae are the same; in fact, the growth rate is always identified as the summation between the growth rate and the decay rate.

2.4.1 Growth of algae in Solimeno

Finally, since no other assumptions have been considered, the equation describing the growth rate of algal biomass is the same as that described in the Dixon model
The following formula describes the final result of the growth of the algae with Solimeno's method:

$$R_x = \frac{1}{W}\int_0^W \left(\mu_{max} * \frac{I(z)}{I(z) + K_I\left(\frac{I(z)}{I_{opt}} - 1\right)^2} * \left(\frac{R_N}{Q_{min,N} + R_N}\right) * \left(\frac{R_p}{Q_{min,P} + R_p}\right) - K_d \right) * C_{xout} * dz \qquad (2.28)$$

2.4.2 Solimeno nutrients uptake: nitrogen

The method considered by Solimeno for the removal of nitrogen from water and therefore the uptake of this nutrient within the plant cell derives from the Monod model.
The following equations are based on applying the standard Monod model, where the growth of the organism depends on the substrate present in the environment. After that the equation were implemented by the Droop model to specify the necessary presence of a minimum and maximum quota present in the cell.
The last step to complete the equations is to add a third term which represents the possible form of inhibition that will undergo the uptake process when the two forms of nitrogen will be present in the system.
In this way it is possible to write the complete formulas about the removal rate (R_{NO_3} and R_{NH_4}) of nitrogen:

$$R_{NO_3} = \rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3}} * \frac{K_{n1,alg}}{K_{n1,alg} + C_{NH4}^{out}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2.29)

$$R_{NH_4} = \rho_{NH_4} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4}} * \frac{K_{n2,alg}}{K_{n2,alg} + C_{NO3}^{out}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)$$

(2.30)

Also, in this case there are parameters such as the quota (Qmin, Qmax and R, [gN/gX]), the values of the velocity of uptake ρ_{NO_3} and ρ_{NH_4}, the half saturation constant for each component (K_{NH4} and K_{NO3}) and the values of half saturation described by Solimeno to explain the concept of inhibition: $K_{n2,alg}$ and $K_{n1,alg}$(Solimeno, 2017).

To simplify the reading, it was decided to assign the names of "Gamma" and "Delta" to the factors that according to this hypothesis will influence the uptake of nitrogen, thus obtaining the definitive expressions that were implemented in Matlab.
As in the case of the Dixon model, inhibition factors are also cross-referenced in the Solimeno model with respect to the equation of removal of one of the two nutrients. This means that in the ammonium removal equation the nitrate inhibiting factor will be present while in the second equation the terms will be reversed.
The following equations (2.31 and 2.32) describe not only the removal rate of the components but the whole balance of them:

$$B_{NO3} = \left(C_{NH4}^{in} - C_{NH4}^{out}/_{\theta}\right) - \left(\rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3}} * \frac{K_{n1,alg}}{K_{n1,alg} + C_{NH4}^{out}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)\right) * C_{x,alg}^{out}$$

(2.31)

$$B_{NH4} = \left(\left(C_{NH4}^{in} - C_{NH4}^{out}\right)/_{\theta}\right) - \left(\rho_{NH_4} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4}} * \frac{K_{n2,alg}}{K_{n2,alg} + C_{NO3}^{out}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right)\right) * C_{x,alg}^{out} + K_{La} * \sqrt{\frac{D_{NH3}}{D_{O2}}} * Kh * p_{NH3} * \frac{C_{NH4}^{out}}{\left(1 + 10^{(pka - ph)}\right)}$$

(2.32)

2.4.3 Solimeno nutrients uptake: phosphorus

The equation describing the uptake of phosphorus is like the previous ones with the only difference that in its case is not considered any inhibition factor because, according to the assumptions made this nutrient, does not interact or compete with the other two and above all it is not considered a growth limiting agent.
This equation describes the removal rate of Phosphorus:

$$R_{PO4} = \rho_P * \frac{C_P^{out}}{C_P^{out} + K_P} * (\frac{Q_{max,P} - (Q_{min,P} + R\_{PO4})}{Q_{max,P}})$$

(2. 33)

And finally, the following equation describes the complete mass balance for this nutrient:

$$B_{m,PO4} = ((C_P^{in} - C_P^{out})/_{\theta}) - \left(\rho_P * \frac{C_P^{out}}{C_P^{out} + K_P} * \left(\frac{Q_{max,P} - (Q_{min,P} + R\_{PO4})}{Q_{max,P}}\right)\right) * C_{x,alg}^{out}$$

(2.34)

2.4.4 Nitrogen and Phosphorus quota

To complete the system of equations it is also necessary to develop the two equations that consider the nitrogen quota present and the phosphorus quota, given by equations 2.35 and 2.36, respectively.

$$B_n = \left(\rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3}} * \frac{K_{n1,alg}}{K_{n1,alg} + C_{NH4}^{out}} + \rho_{NH_4} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4}} * \frac{K_{n2,alg}}{K_{n2,alg} + C_{NO3}^{out}}\right) * \left(\frac{Q_{max,N} - (Q_{min,N} + R_N)}{Q_{max,N}}\right) * \theta - + \frac{1}{W}\int_0^W \left(\mu_{max} * \frac{I(z)}{I(z) + K_I\left(\frac{I(z)}{I_{opt}} - 1\right)^2} * \left(\frac{R_N}{Q_{min,N} + R_N}\right) * -K_d\right) * C_{xout} * dz$$

(2.35)

$$B_{PO4} = \rho_{PO4} * \frac{C_P^{out}}{C_P^{out} + K_P} * \left(\frac{Q_{max,P} - (Q_{min,P} + R\_{PO4})}{Q_{max,P}}\right) * \theta - R_{PO4} + \frac{1}{W}\int_0^W \left(\mu_{max} * \frac{I(z)}{I(z) + K_I\left(\frac{I(z)}{I_{opt}} - 1\right)^2} * \left(\frac{R_{PO4}}{Q_{min,P} + R_{PO4}}\right) - K_d\right) * C_{xout} * dz$$

(2.36)

CHAPTER 3: RESULTS

In this chapter, the results that were obtained from the various interpolations conducted will be presented
It is necessary to anticipate that before to reach the final results, from which the relevant conclusions will be drawn, several attempts were carried out to select the best model to fit the data.

The interpolations carried out allowed, as anticipated in Chapter 2, had the aim to slightly modify the equations that were used inside the two mathematical models. The target of the entire study was to better understand which parameters are the most influent in the system that it was considered. And the interpolation is a good strategy to underline these parameters and to concentrate the study on them.

At the end, the entire approach was aimed to compare the two models that are described in the previous chapters. The comparison is necessary because it permits to analyze the different results from Dixon and Solimeno's methods and to understand which one is better than the other concerning the starting conditions. Through the results it was possible to reach a final result that permits to explain the behavior of the microalgae in these conditions.

3.2 Application of the models to experimental data

For the presentations of the results, it is possible to begin by describing the results of Solimeno's model.

As mentioned, in order to evaluate the effectiveness of a method, it was necessary to compare the results of the experimental outputs with the values obtained by MatLab simulation.
Below, Table 3.1 shows the results obtained in the laboratory, about the trials on *Chlorella protothecoides*, with reference to the inlet and outlet concentrations of the different compounds and all the operating variable and conditions of the sample such as the residence time.

I_0 (µmol/m^2 *s^1)	τ (d^-1)	L (cm)	T (°C)	c_x^{exp} (g/m^3)	Dev.St.	$N\text{-}NO_3^{in}$ (g/m^3)	$N\text{-}NO_3^{out}$ (g/m^3)	Dev.St.	$N\text{-}NH_4^{in}$ (g/m^3)	$N\text{-}NH_4^{out}$ (g/m^3)	Dev.St.	P^{in} (g/m^3)
100	0,65	3,5	24	173,05	0,51	60	49,88	1,30	30,00	9,42	0,89	10,8
100	0,71	3,5	24	147,23	0,28	30	22,80	1,20	80,00	49,73	0,49	10,8
100	0,72	3,5	24	311,28	4,13	100	42,09	2,63	100,00	73,42	3,73	10,8
100	0,7	3,5	24	176,44	4,97	40	28,44	1,92	10,00	1,13	0,51	10,8
100	0,7	3,5	24	256,67	2,05	10	9,32	0,24	40,00	17,13	2,56	10,8

Table 3.1: description of the main parameters of the specimen that was considered for Chlorella protothecoides

Analyzing the table above it is possible to observe, proceeding from left to right the following parameters that are used in all the studies:

- the specific light that is used to grow the algae (Io)
- the value of the residence time, that is, how long the whole remained inside the reactor before being extracted and analyzed,
- the length of the reactor that was used during the experiment,
- the temperature at steady state,

- Biomass concentration leaving the system after the residential time, basically it describes how much the microalgae grow,
- Standard deviation about the biomass concentration, the error on the measure of the algae concentration,
- the inlet concentration of nitrate (value measured prior to the preparation of the experiment)
- the outlet concentration of nitrate (value measured at the conclusion of the experiment),
- The standard deviation about the error on the measured nitrate concentration at the outlet,
- the inlet concentration of ammonium (value measured prior to the preparation of the experiment),
- the outlet concentration of ammonium (value measured at the conclusion of the experiment),
- The standard deviation about the error on the measured ammonium concentration at the outlet,
- The concentration of phosphorus for which there is not the correspondent outlet concentration.

The table 3.1 describes the data obtained in the laboratory for the growth and cultivation of *Chlorella protothecoides*. As can be observed the samples are characterized by being heterogeneous in the values of the concentrations. In fact, it can be observed that there are samples in which the concentrations of nitrate and ammonium are well above the average values of a classical wastewater discharge (e.g. the third sample). That it was done to understand during the process of calculation if the model can describe also strange and particular situations or for them it is necessary to implement another scheme.
In other conditions, the concentrations of nitrate and ammonium are reversed from each other (such as the fourth and fifth conditions), and in this case the purpose was to observe how the algal culture reacted and whether as hypothesized it removed, in percentage, more nitrate than ammonium.
Based on this observation the same attention to some these tests are reserved for Matlab's results.

3.3 Application of Solimeno's model to experimental results of *Chlorella protothecoides*

To obtain the results that were useful to analyze the efficiency of the method it was necessary to bring all the parameters of the table 3.1 and fill the script with them. After that it was decided the value of Lambda and Gamma, the two parameters that described the inhibition (see chapter 2). Finally through the use of the system of equations described in the Chapter 2 it was possible to obtain the fitting results.

After using the input concentrations into the Matlab script (see appendix for the complete script), it was realized that the gap between the values quantified in the laboratory and the concentrations estimated by the mathematical model were largely different.
Accordingly, a sensitivity analysis on Lamba and Gamma was carried out. The system has the aim to interpolate the value of gamma and lambda based on the expected result to produce some value that can reduce the difference with the experimental results. The target was to find a range of values for these parameters that could describe the uptake and the possible inhibition between the two species of Nitrogen.

Unfortunately, the results were not satisfactory, with considerable difference between the experimental concentrations and the simulated data. The results are anyway reported in the following table and graphs.
Table 3.2 summarizes the results of simulations concentrations of variables and the standard deviation

NO3 in (g/m^3)	NO3 exp (g/m^3)	NO3 out (g/m^3)	Dev.St.	NH4 in (g/m^3)	NH4 exp (g/m^3)	NH4 out (g/m^3)	Dev.St.	Cx exp (g/m^3)	Cx out (g/m^3)	Dev.St.
60	49,88	50,38	1,30	30,00	9,42	10,96	0,89	173,05	59,39	0,51
30	22,80	29,74	1,20	80,00	49,73	37,38	0,49	147,23	148,08	0,28
100	42,09	80,87	2,63	100,00	73,42	68,82	3,73	311,28	189,41	4,13
40	28,44	27,74	1,92	10,00	1,13	1,06	0,51	176,44	130,96	4,97
10	9,32	16,8	0,24	40,00	17,13	1,02	2,56	256,67	99,31	2,05

Table 3.2: results of Solimeno model, moving from left to right it is possible to read the nitrate concentrations (at inlet, the expected values and at the outlet) with the standard deviation, the concentrations for the ammonium and for the biomass

The first one described the outlet concentration for Nitrate after the implementation of Solimeno Method with a value of Gamma and Lambda equal to 2 and 3 (Figure 3.1). It is clear that the method, with these conditions, doesn't described well the reactions that took place inside the reactors. The orange line describes the place in which there is the perfect correspondence between the values. Higher is the distance higher will be the error and the method are wrong. Lower id the distance lower is the discharge and better is the model.

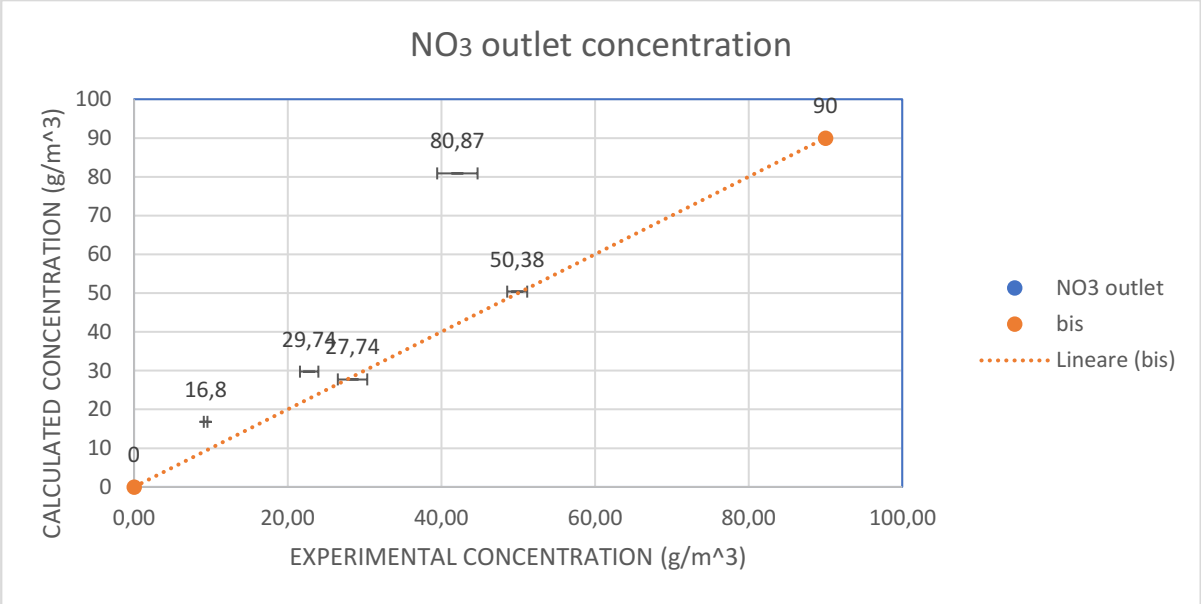

Figure 3.1: comparison between the experimental values and the calculated vales of outlet concentrations of Nitrate

To better underline the difference of some of the data here analyzed, it is preferable to develop the result using a histogram where it is possible to compare the experimental result and the mathematical results.
The histogram below (Figure 3.2) shows the gap between the experimental and simulated data.
In particular the case number 3 is the situation where all the combinations of Lambda and Gamma failed to fit the data.

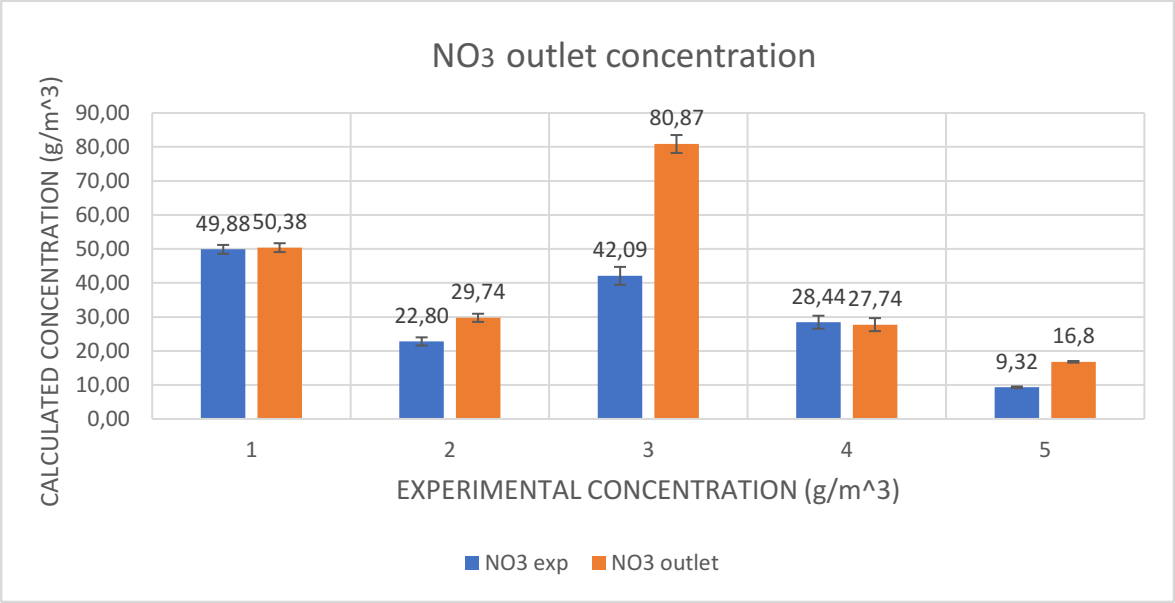

Figure 3.2: histogram that compared the difference between the concentration expected and the experimental one

Also, the concentrations related to the ammonium results were analyzed: in the following diagram there are the corresponding outlet values.
As in the previous diagram, also in this case with the standard deviation error it is possible to see the presence of a gap between the values. For some tests the error respect the experimental value is of 25% such as in the second case (see figure 3.3).

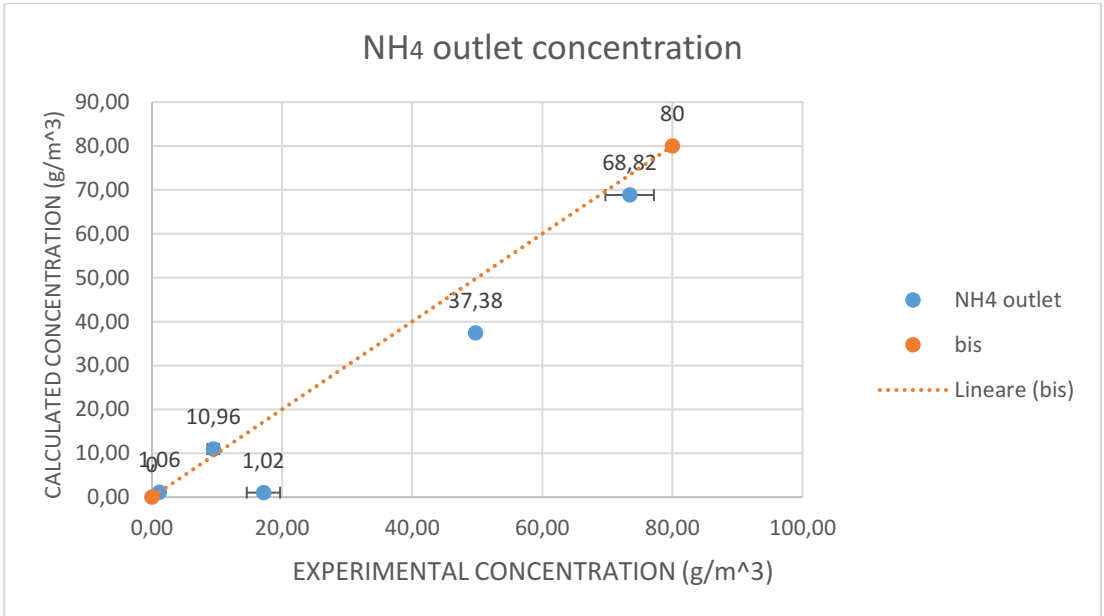

Figure 3.3: comparison between the experimental and calculated values of the outlet concentration of Ammonium

The histogram in Figure 3.4 better describes the limits of the approach used.

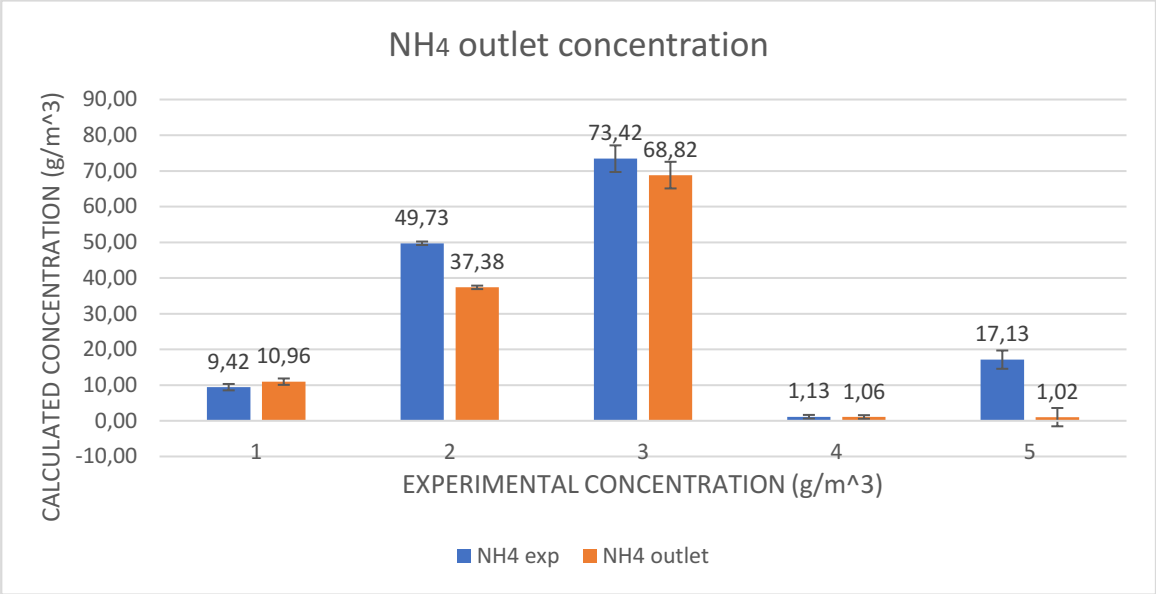

Figure 3.4*: histogram that compared the difference between the concentration expected and experimental*

Finally, results about biomass concentrations, as a results of the growth of the algae where analyzed, as a direct answer to the removal of nitrogen pollutants present in the wastewater. Figure 3.5 and Figure 3.6 clearly highlight the limit of the Solimeno's method in the case of *Chlorella protothecoides*.

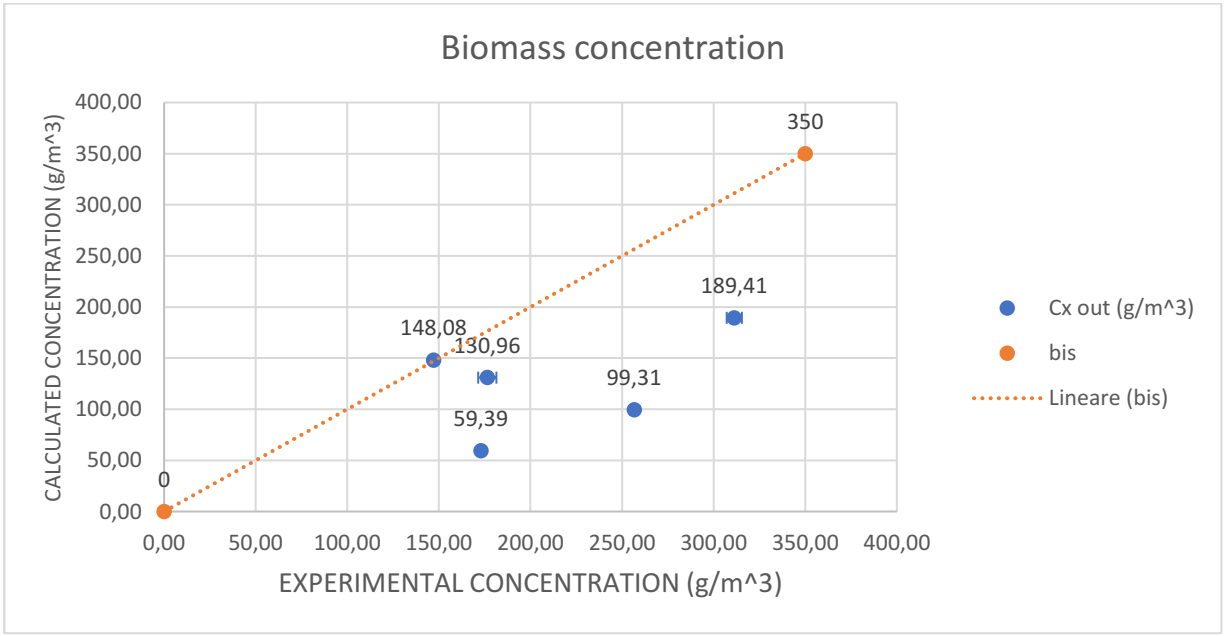

Figure 3.5: comparison between experimental and calculated values of outlet concentration of biomass

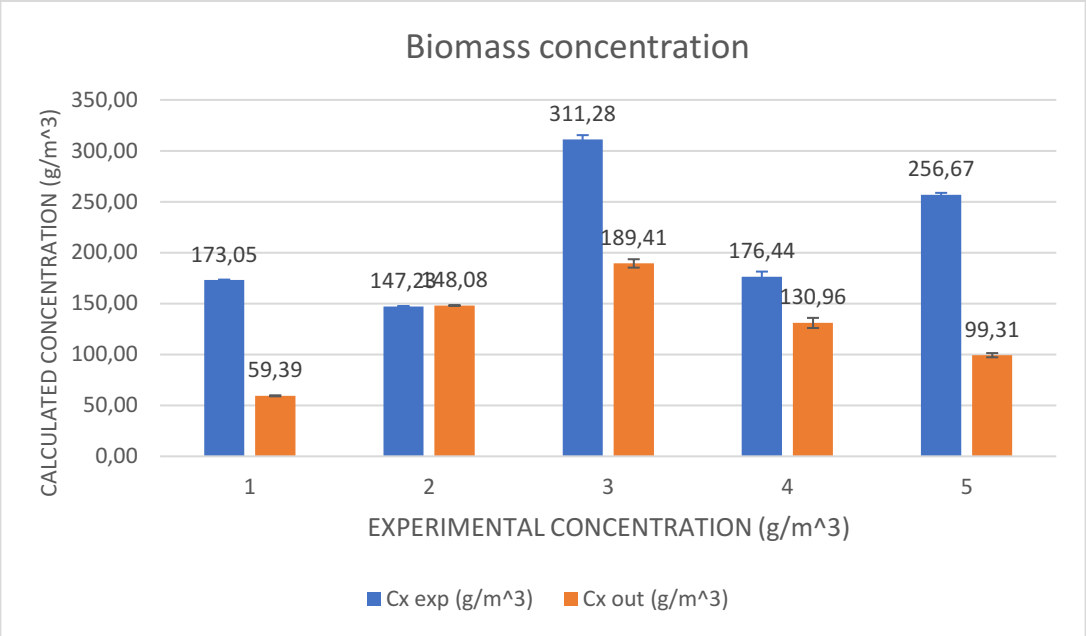

Figure 3.6: histogram of concentrations regarding the comparison between the different values of biomass

Even if the data obtained by the concentrations of the two forms are not so different respect the experimental data the situation for the Biomass is completely different. It appears clear that there is not any type of correspondence between calculated data from Matlab and lab data; moreover, the case described with all the histograms is the final result obtained after the interpolation analysis on the values of Gamma and Lambda to try to find a better correlation.
The model can be defined as inconclusive because in only one case the concentrations are the same; in the other cases the difference is about 40% -50%.

In summary, the Solimeno's model was not able to efficiently reproduce the trend of the experimental data. Indeed, it was very complex to find interpolations and values that could approximate the calculated results with the laboratory ones. As shown later, this model was found be less flexible than Dixon's method in which more similarities were found between the results.
Even with the introduction of two parameters (gamma and delta) it was not possible to reproduce the experimental results.

3.4 Application of Dixon model to the experimental results of *Chlorella prototheco*ides

Similar to what was done with the Solimeno method, the same approach was used by applying the Dixon model.

This model showed a lower error between the experimental results (laboratory) and those calculated (through Matlab), so it was used for further elaboration.

The data analyzed are the same as those used for the interpolation of values in the Solimeno method. Consequently, the following table (Table 3.1) is characterized by the same values present in the previous one.

The main purpose, also in this case, was to identify the final concentrations obtained using *Chlorella protothecoides* samples. In particular, more attention has been paid to the outlet values of ammonium and nitrates; finally, algal growth has also been considered to have a further data of feedback on the model.

3.4.1 First approach with Dixon model

As a result of this first approach where input data were simply replaced within the model, results were obtained (figure 3.7) that did not reflect perfectly the experiments. However, the margin of error could allow a further investigation to see if there was the possibility of modifying some parameter.

From the following graphs (Figures 3.7 and 3.9) that show the trend of nitrate and ammonium concentration, it is possible to see that the Dixon model can approximate the real results better than the Solimeno's one, as also reported in Table 3.2.

NO3 in (g/m^3)	NO3 exp (g/m^3)	NO3 dixon (g/m^3)	NO3 solimeno (g/m^3)	Dev.St.
60	49,88	46,09	50,38	1,30
30	22,80	15,31	29,74	1,20
100	42,09	67,04	80,87	2,63
40	28,44	23,18	27,74	1,92
10	9,32	7,749	16,8	0,24

*Table 3.2 :results of Dixon model compared with Solimeno model for C. prototheco*ides

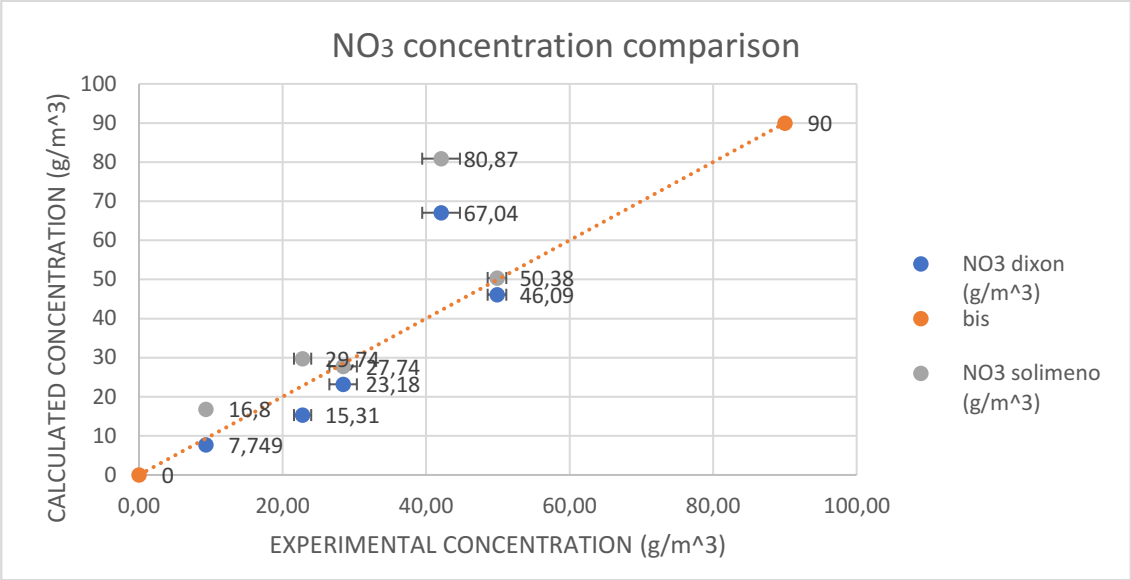

Figure 3.7: comparison between Dixon and Solimeno models on nitrate concentrations at outlet

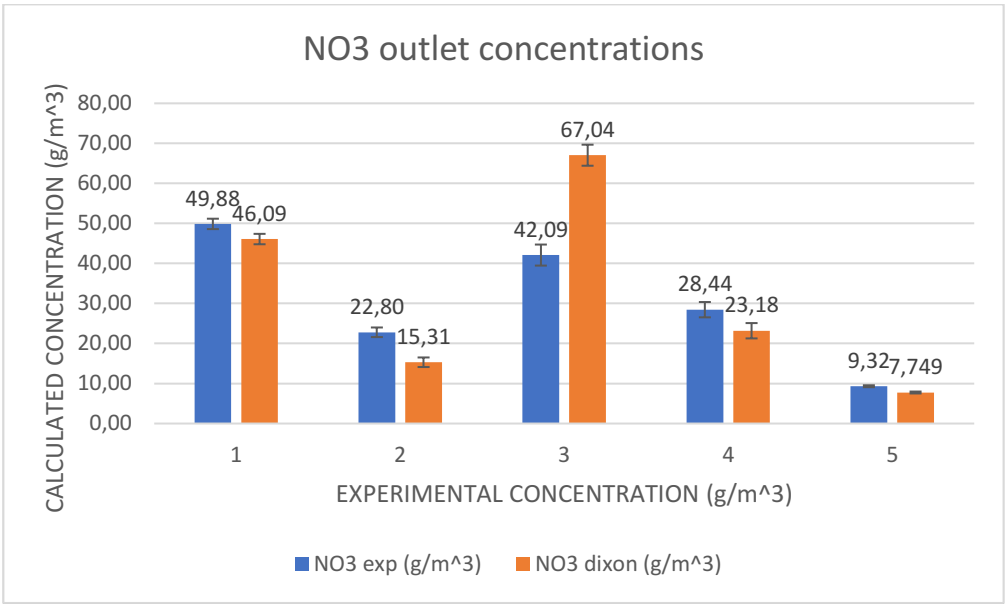

Figure 3.8: comparison between Dixon and Solimeno models regarding nitrate concentration at outlet in Dixon method

Considering the results obtained with the Dixon model on nitrate concentration is possible to see that the values obtained in the laboratory and the values calculated by the software display a similar trend. An important aspect to underline is the fact that this result is the first attempt obtained with this model, and in some cases it appears better than the results obtained after many trials with the Solimeno's one. One case in particular is important to observe: the calculated value of the third specimen is lower than that obtained with the Solimeno model, and it is closer to the real value (Table 3.2).

Looking at the ammonium concentration, the values obtained by Dixon are similar to the values obtained by Solimeno, even if these in some cases are better than the first one.The following table describe the results and it permits a possible comparison between the values of the two models.
The table 3.3 summarizes the results and allows a comparison between the values obtained with the two models with respect to the experimental ones.

NH4 in (g/m^3)	NH4 exp (g/m^3)	NH4 dixon (g/m^3)	NH4 solimeno (g/m^3)	Dev.St.
30,00	9,42	20,24	10,96	0,89
80,00	49,73	48,24	37,38	0,49
100,00	73,42	63,49	68,82	3,73
10,00	1,13	6,24	1,06	0,51
40,00	17,13314	25,61	1,02	2,56

Table 3.3: results of Dixon model compared with Solimeno model for C. protothecoides

The results are also reported in the two graphs that show a comparison with the Solimeno model (figure 3.8), and a comparison between the experimental data with the data that are calculated (figure 3.9).

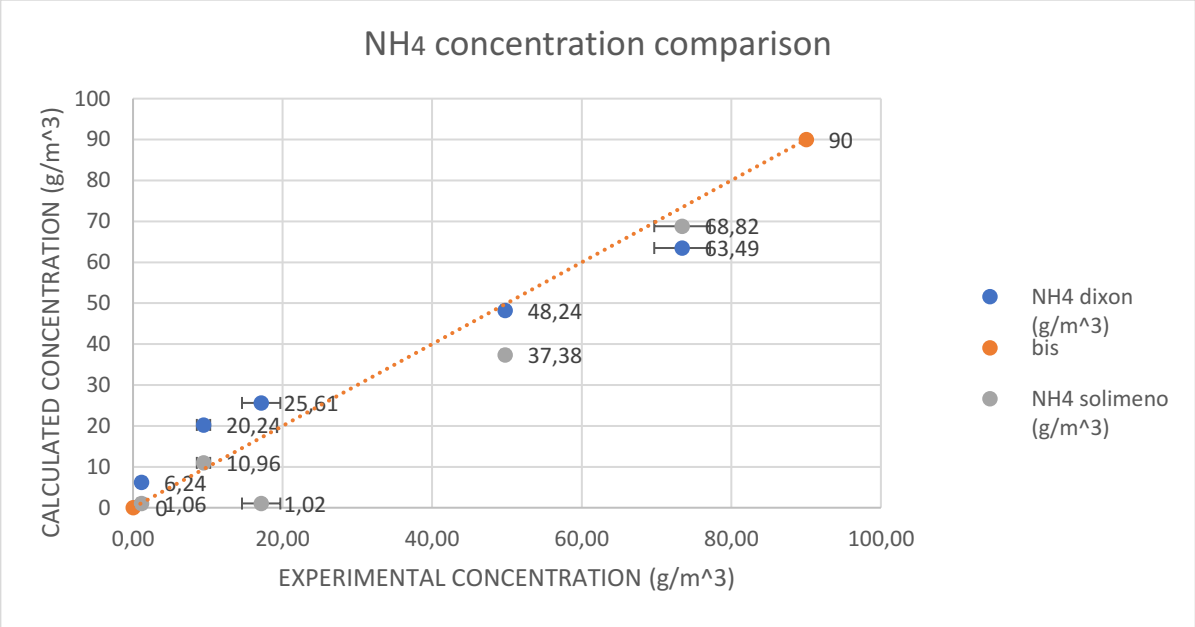

Figure 3.9: comparison between Dixon and Solimeno model on ammonium concentrations

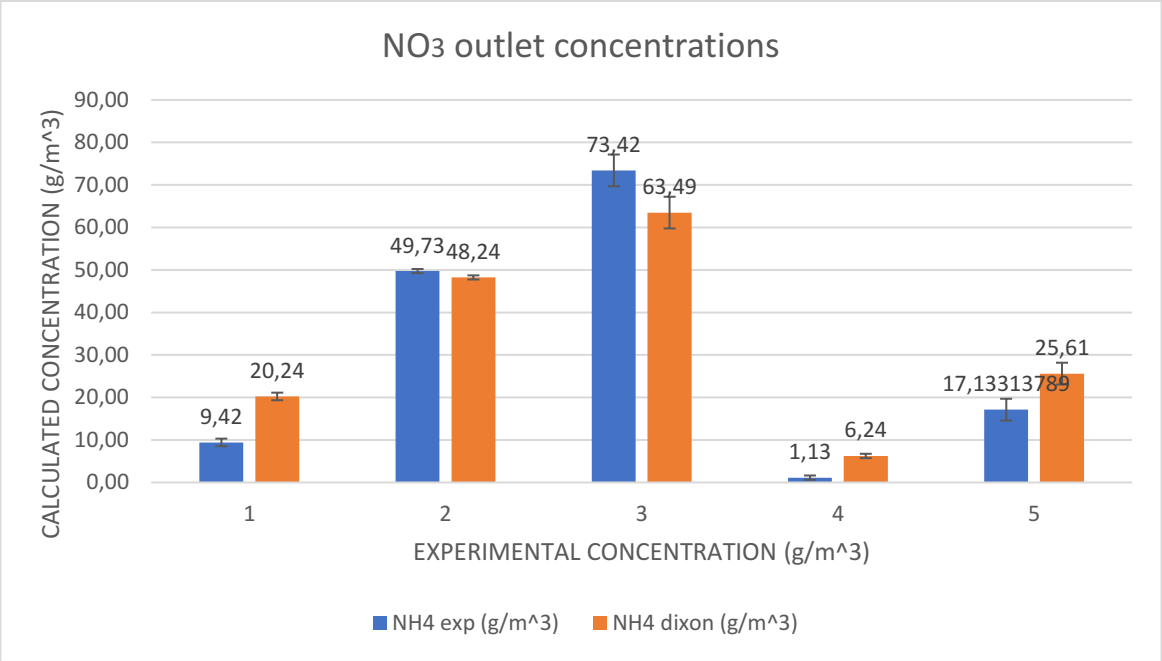

Figure 3.9: outlet concentration of Ammonium compared with the expected values

Figure 3.9 shows that there is a similar trend between the predictions of the Dixon model and the real results that are observed in the laboratory.
Moreover, it is possible to see that without any type of interpolation the data are closer to the experimental ones with respect to the values obtained with Solimeno. However, the real reason that allowed to choose which method was better to investigate is related to the prediction of biomass concentration (figure 3.10).
The table 3.3 resumes the data and values collected in the lab and from the mathematical model, which are also shown in the graphs (Figure 3.10 and 3.11).

Cx exp (g/m^3)	Cx out Dixon (g/m^3)	Solimeno (g/m^3)	Dev.St.
173,05	117,44	59,39	0,51
147,23	167,28	148,08	0,28
311,28	267,55	189,41	4,13
176,44	149,92	130,96	4,97
256,67	32,34	99,31	2,05

Table 3.3: results of Dixon model compared with Solimeno model for C. protothecoides regarding the microalgae concentrations

The graphs show the values of algal biomass concentration (*C. protothecoides*) at the reactor outlet. It is possible to see that there is a good feedback from this point of view, because 4 out of 5 conditions have a calculated value that is close to the real one. Moreover, these results are better than the results obtained from Solimeno. Nevertheless, in one case (case 5) the results of biomass were not acceptable because the difference between experimental and calculated values was very big.
However, these results are not perfect and in particular it is necessary to understand the reason why the predicted concentration of the last test is very small and far from the real one, and this is one of the reason of the future analysis conducted with this method.

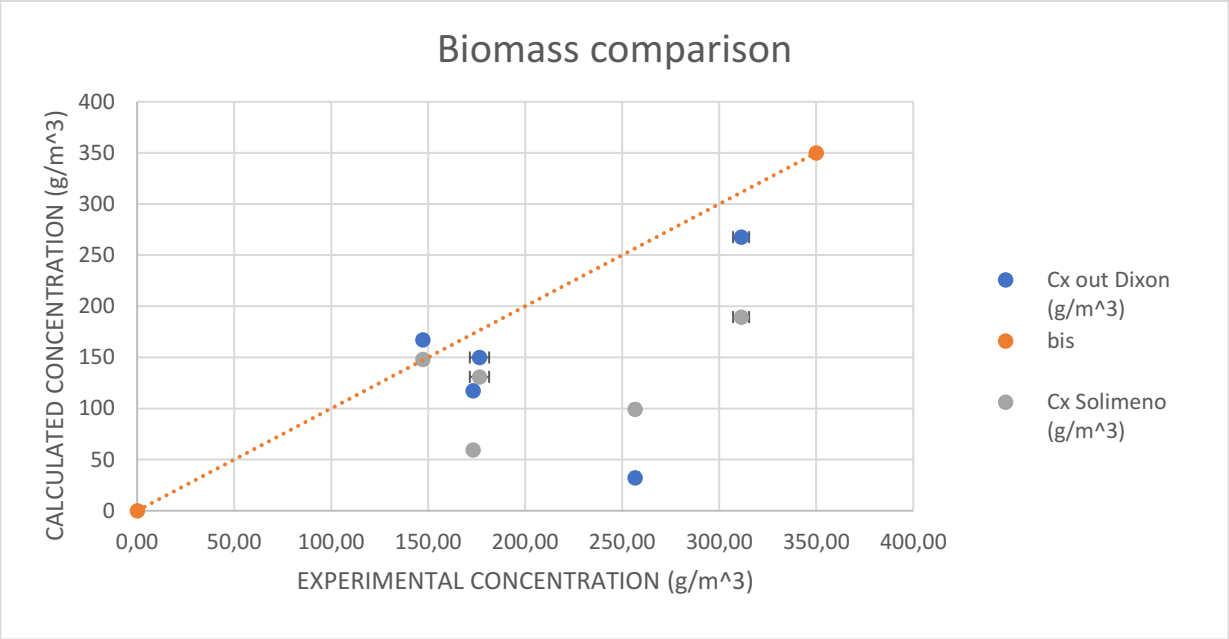

Figure 3.10: comparison between Dixon and Solimeno model on the biomass outlet concentrations

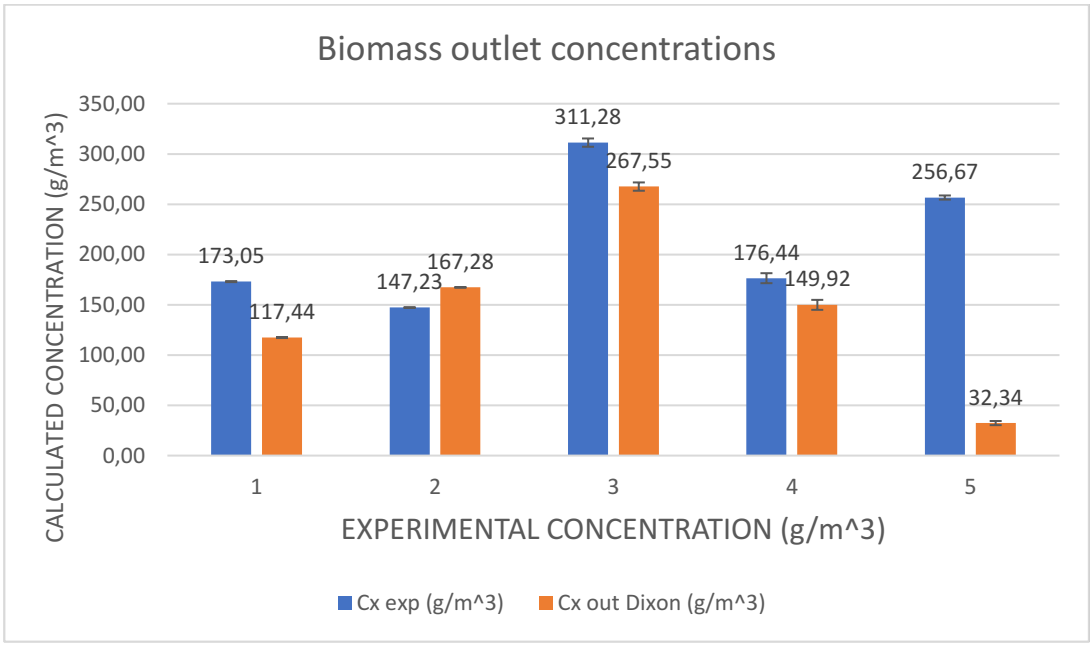

Figure 3.11: histogram showing the comparison between Dixon and Solimeno model on the biomass outlet concentrations

After this brief comparison some important points are discovered:

1. Dixon method is able to describe the reactions of uptake and the growth of the algae better than Solimeno theory;
2. The final results obtained with this method are not perfect and the error with respect the experimentally measured values is large for some conditions;
3. So, it is necessary to investigate the model with the final aim of understanding which parameters influence the result.

3.4.2 Analysis on Dixon model

Analyzing the previous results and trying to modify the values of the inhibition parameters (alpha and beta), to obtain better results, it was possible to notice that the results did not vary significantly. Accordingly, a sensitivity analysis was performed to understand which parameter influenced the model results the most.
During this study the attention was focused on some parameters that could affect the nitrogen uptake or the growth of microalgae. So, by means of interpolation of experimental data the values of the following parameters were modified to arrange a better result for the concentrations of the system.
.
During the interpolation, values like the maximum uptake rate (ρ_{NO_3} and ρ_{NH_4}), the half saturation constant (K_{NO3} and K_{NH4}), the overall mass transfer coefficient Kla, and the mortality rate Kd, are considered and changed because they can affect the growth of microalgae or the absorption of nutrients. For all these parameters a specific range of values was set because it is important that the solution obtained is characterized by realistic values from the biological point of view.
To perform the interpolation, a particular tool present in Matlab was used, which is described hereafter.

3.4.3 FMINSEARCH INTERPOLATION

In order to obtain better values and to evaluate which factor, at this point, most influenced the results of the equation system, an interpolation script was created, using the function called "fminsearch", which aims to minimize the “residues” (therefore the differences) between the results obtained in the laboratory and those calculated by the model. The corresponding Matlab script is reported in the Appendix.

At first it was decided to apply the ranges for each parameter were kept wide range because it was not clear which were the parameters that most affected the results. Afterwards, selected groups of parameters were investigated one or more at a time, and for each of them was defined a range of values in which they could move.
Consequently, the script was used to minimize the differences between the expected concentrations (lab results about biomass, ammonium, and nitrates) and the calculated values obtained, in relation to the parameters that were investigated in that moment.

The intervals were chosen to make the values realistic, or to keep an order of magnitude plausible because many high values would not have any kind of significance being impossible for the biology of the algae. They are reported hereafter for all the model parameters:

- $0.01 < \rho_{NO_3} < 5$; (interval that vas considered only in the first part of the study)
- $0.01 < \rho_{NH_4} < 5$; (interval that vas considered only in the first part of the study)
- 1.0*10^-9 < Kla < 1.0*10-^3
- $5 < K_{NO3} < 20$;
- $5 < K_{NH4} < 20$;
- 0.1 < Kd < 0.5
- 0.5 < alpha < 2;
- 0.5 < beta < 2.

Some of these intervals were chosen based on previous studies. In fact, they can vary depending on the species of algae, the pH and in particular for the concentrations of the substrate present. For these reasons, an important focus of this study is on the inhibition coefficients (alpha and beta) and also on the fact that the presence of two sources of nitrogen can manipulate or interact with the different transporters and change the velocity of uptake.

3.4.4 Effect of alpha and beta

What is possible to observe from the results presented below is a comparison between two situations that at the end of this first analysis justified a radical change in the equations that were used to calculate the mathematical concentrations.

The first case describes the results based on the change on the value of alpha and beta in the equations of the model.
The tool "fminsearch" was set to change the value of alpha during a first attempts and after the value of beta.
In the tables and in the diagrams all the data of the same species are together to show the similarities and the differences.

The first one describes the values of nitrates after the interpolation of alpha and beta. The table 3.3 below shows the best results possible with these values for the inhibition coefficient. After a quick observation it is possible to say that there isn't a huge difference between the columns that represents the model based on a value of alpha equal to 0.1 and a value of beta equal to 5.

DIXON NITRATE CONCENTRATIONS α =0,1 β= 5			
NO3 exp (g/m^3)	**NO3 (g/m^3)**	**NO3 (g/m^3)**	**Dev.St.**
49,88	47,84	46,17	1,30
22,80	16,86	13,84	1,20
42,09	69,95	68,68	2,63
28,44	23,95	23,11	1,92
9,32	8,25	4,79	0,24

Table 3.3: first changes in Dixon's model

Checking the results with a histogram (figure 3.12 below) it is possible to understand that in some cases, in particular the fifth and the third, the best value, that we can use for the coefficient beta, increases the difference between the calculated value and the experimental one
This is an important point because if there will be a similar correspondence also in the concentrations of ammonium and biomass it could be possible to consider in another way this factor.

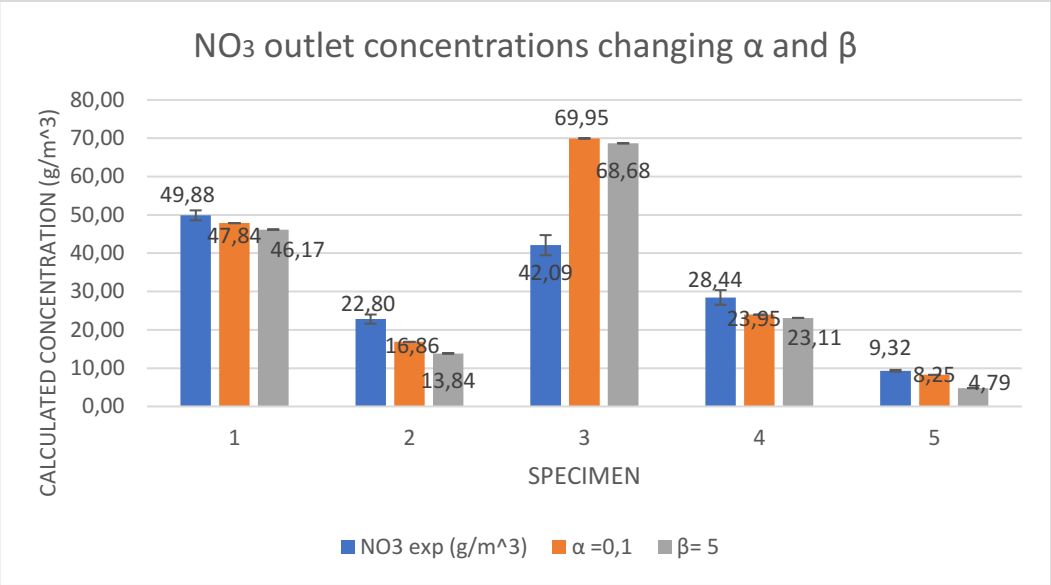

Figure 3.12: comparison between the expected value (blue column) and the calculated value of C.protethecoides based on the best possibility for alpha=0.1 and beta=5 (column orange and grey)

About the ammonium concentrations, the tables 3.4 resumes all the numbers related this nutrient from the expected values, described in the first column, to the different values found by changing alpha and beta. Iit is possible to observe the same trend as before. In fact, even if the results are not good compared to the laboratory's values, there isn't a huge difference between the numbers of the columns of alpha and beta.
After a comparison with the table above (table 3.3) it seems that the value of nitrate and ammonium with a modified beta parameters produced wrong results. Moreover, compared with the data obtained with the change of alpha there is an uptake of nutrients that is higher, and it is not related to the amount of starting concentration, but it seems a random behavior.

MODELLO DIXON α =0,1 β= 5			
NH4 exp (g/m^3)	**NH4 (g/m^3)**	**NH4 (g/m^3)**	**Dev.St.**
9,42	20,31	19,30	0,89
49,73	48,96	40,77	0,49
73,42	63,72	59,68	3,73
1,13	6,27	5,50	0,51
17,13	25,41	19,02	2,56

Table 3.4: comparison between the results obtained with the different values of alpha and beta and the expected (real) result

All these considerations appear clearer with the help of the following histogram that summarizes the final concentrations of ammonium in the two cases.

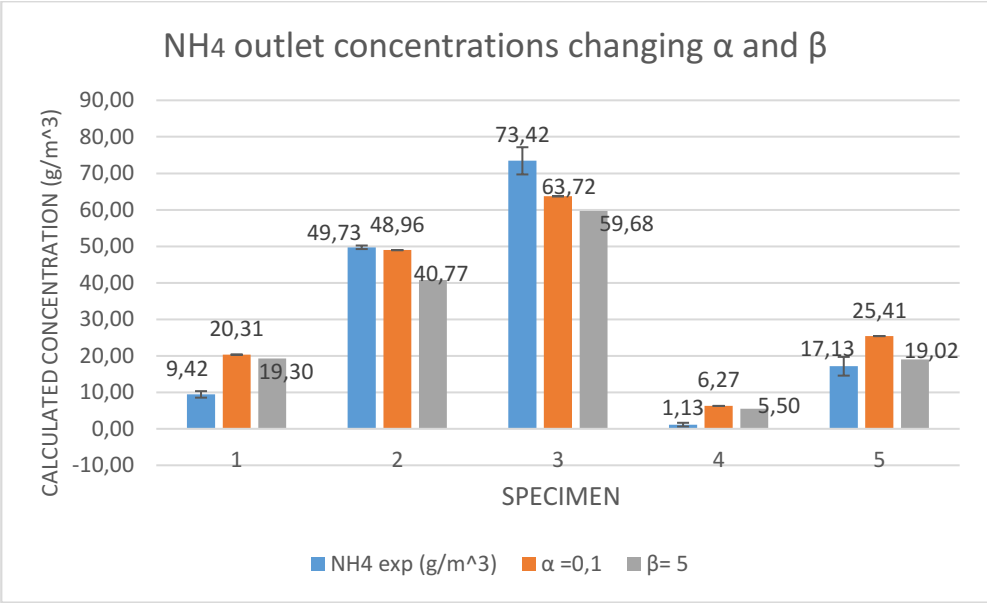

Figure 3.13: *comparison between the expected value (blue column) and the calculated value of C.protethecoides based on the best possibility for alpha=0.1 and beta=5 (column orange and grey)*

Table 3.5 and Figure 3.14 report the results for the biomass concentration, which showed a marked discrepancy between the experimental and simulated data.

MODELLO DIXON α =0,1 β= 5			
Cx exp (g/m^3)	**Cx (g/m^3)**	**Cx (g/m^3)**	**Dev.St.**
173,06	104,67	123,66	0,51
147,23	152,03	225,61	0,28
311,00	250,42	277,13	4,13
176,44	144,61	156,37	4,97
256,60	25,41	117,08	2,05

Table 3.5: comparison between the values of biomass calculated with two different coefficients for alpha and beta respect the real value obtained in the lab

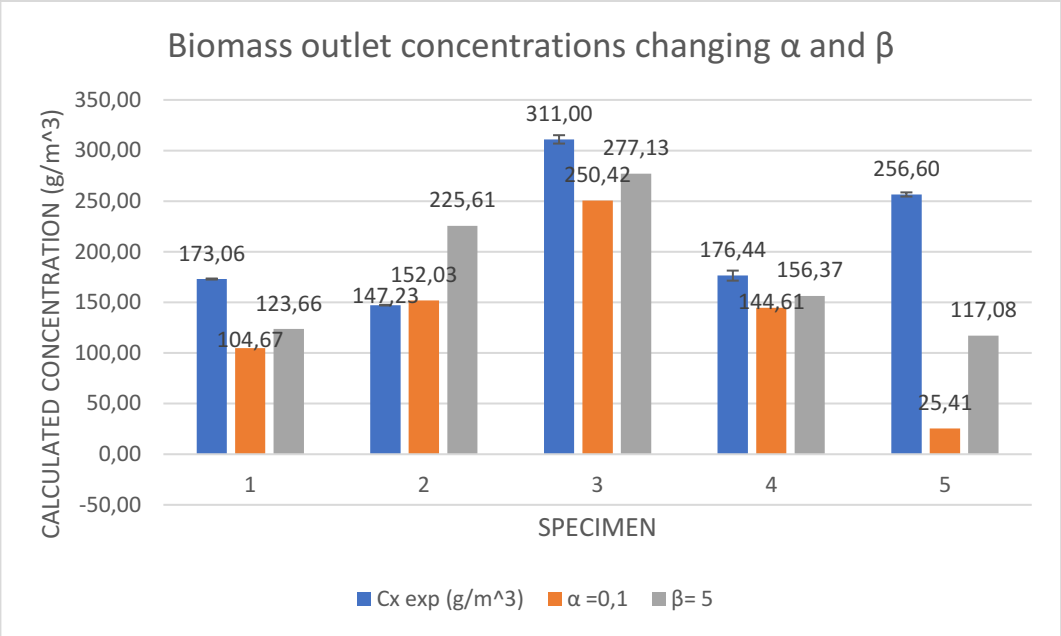

Figure 3.14: *histogram comparison between the values of biomass calculated with two different coefficients for alpha and beta respect the real value obtained in the lab*

The graph permits to understand that for 4 of 5 tests the concentrations calculated are underestimated and in no one of the cases there is a perfect match. Moreover, in this case the variations of alpha and beta produced results that are not similar to each other, if we compared the same test.

In conclusion from this part of the analysis was discovered that perhaps the value of alpha and beta are not the only one values that can affect the growth of algae and the uptake of nutrients. So, the inhibition factor, assumed for the presence of two different nutrients, is not fully determined. That means that more than one factor is implicated in the variation of the final concentrations.

To justify this affirmation, it probably necessary to compare these last results with the previous one in which there wasn't an interpolation of alpha and beta and for them it was used two values estimated (alpha=15 and beta= 17). The values present in the following tables are the outlet concentrations of biomass, nitrates and ammonium obtained during the comparison with the Solimeno model (see paragraph 3.4.1).

In this case the comparison is made with the use of only tables because the focus point is not the huge difference between the calculate values and the expected values but the little difference that exists between the 3 types of data that are produced with the Dixon's model based on the use of alpha and beta.

At first, we can discuss about the nitrate results, the table 3.6 shows how minimal are the differences between the 3 cases in which we use different values of alpha and beta.

	α =15	β= 17	standard
NO3 exp (g/m^3)	**NO3 (g/m^3)**	**NO3 (g/m^3)**	**NO3 dixon (g/m^3)**
49,88	47,84	46,17	46,09
22,80	16,86	13,84	15,31
42,09	69,95	68,68	67,04
28,44	23,95	23,11	23,18
9,32	8,25	4,79	7,749

Table 3.6: *comparison between the results obtained with the different values of alpha and beta (in this case 15 and 17), the generic result without interpolation and the expected (real) result regarding the nitrates compound*

It appears that even if there is a change of the coefficient that has the aim to describe a possible inhibition the final result doesn't change in a significant way. In fact, for most of the tests conducted in the lab the change is less than 1g/m^3 that is too small compared with the difference of the order of 10 g/m^3 that characterized the gap between the experimental values and the mathematical ones.

The same result can be observed for the concentrations of ammonium described in table 3.7. Also in this case, values are not sensible to the change of alpha and beta, confirming that the uptake of nutrients is not determined by these coefficients.

	α =15	β= 17	standard
NH4 exp (g/m^3)	**NH4 (g/m^3)**	**NH4 (g/m^3)**	**NH4 dixon (g/m^3)**
9,42	20,31	19,30	20,24
49,73	48,96	40,77	48,24
73,42	63,72	59,68	63,49
1,13	6,27	5,50	6,24
17,13	25,41	19,02	25,61

Table 3.7: *comparison between the results obtained with the different values of alpha and beta (in this case 15 and 17), the generic result without interpolation and the expected (real) result regarding the ammonium compound*

Similar conclusions can be drawn by considering the results of biomass (Table 3.8).

	α =15	β= 17	standard
Cx exp (g/m^3)	**Cx (g/m^3)**	**Cx (g/m^3)**	**Cx dixon (g/m^3)**
173,06	104,67	123,66	117,44
147,23	152,03	225,61	167,28
311,00	250,42	277,13	267,55
176,44	144,61	156,37	149,92
256,60	25,41	117,08	32,34

Table 3.8: *comparison between the results obtained with the different values of alpha and beta (in this case 15 and 17), the generic result without interpolation and the expected (real) result regarding the biomass*

For the reasons discussed so far, it was decided to change the approach and structure a new model able to represent the experimental data.

3.4.3 Analysis on the other parameters

At this point the other combinations among the different parameters listed above were considered. After a series of attempts that considered changing the values of Kn, kla, ρ, and kd, it was possible to obtain results that can be considered satisfactory and that allow the model to improve.

The best results were obtained through the interpolation of the values of the nitrate and ammonium uptake rates (rho). In fact, these parameters are related to the use of nutrients that the cell can eat. In particular, the values of the two rho were fitted on the experimental data case by case. The corresponding values of ρ_{NO_3} and ρ_{NH_4} are summarized in Table 3.9.
The reason why it was chosen to interpolate each case separately was the fact that the uptake velocity might change case by case because it is influenced by the boundary conditions such as the concentrations of nutrients at inlet. Based on this concept described in literature the different cases were developed one by one to see if this could be true.

ρ_{NO_3} (gN/gX*d)	ρ_{NH_4} (gN/gX*d)
0,212	0,946
4,96E-09	0,439
0,768	0,13
0,356	3,1
1,36E-09	0,918

Table 3.9: value of ρ used after the interpolation to obtain the result in the model of Dixon.

The table 3.10 below represents the outlet concentration for nitrate obtained after the consideration of different values of ρ.

NO3 in (g/m^3)	NO3 exp (g/m^3)	NO3 out (g/m^3)	Dev.St.
60	49,88	53,74	1,30
30	22,80	29,86	1,20
100	42,09	61,1	2,63
40	28,44	39,9	1,92
10	9,32	9,89	0,24

Table 3.10: result of nitrates for Dixon model with the use of different fitted values of ρ

As it is shown by the table there is a better correspondence in the values than the other context that are explained before. In fact, 4 of 5 tests produce a results that are quiet similar to the correspondent experimental ones. Also, the diagram below (figure 3.15) shows this trend.

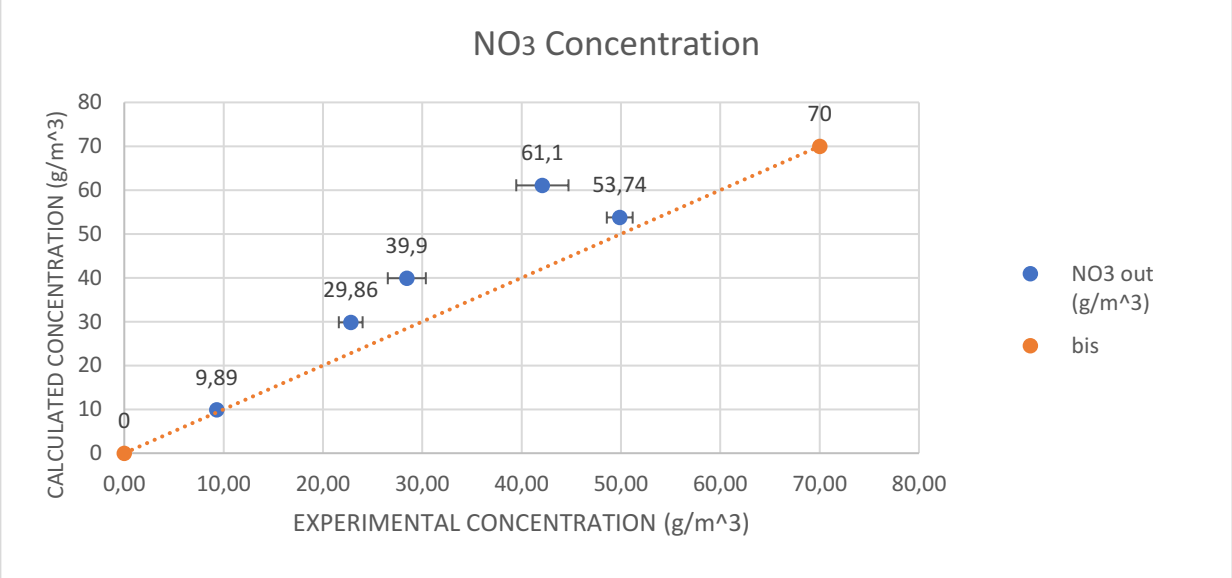

Figure 3.15: concentration of nitrates with the use of different ρ during the computational calculation

The results of ammonium concentration are presented both in table 3.11 and in the correspondent diagram figure 3.16.

NH4 in (g/m^3)	NH4 exp (g/m^3)	NH4 out (g/m^3)	Dev.St.
30	9,42	13,03	0,89
80	49,73	63,33	0,49
100	73,42	92,92	3,73
10	1,13	0,88	0,51
40	17,13	27,97	2,56

Table 3.11: results of ammonium concentrations after the use of different ρ

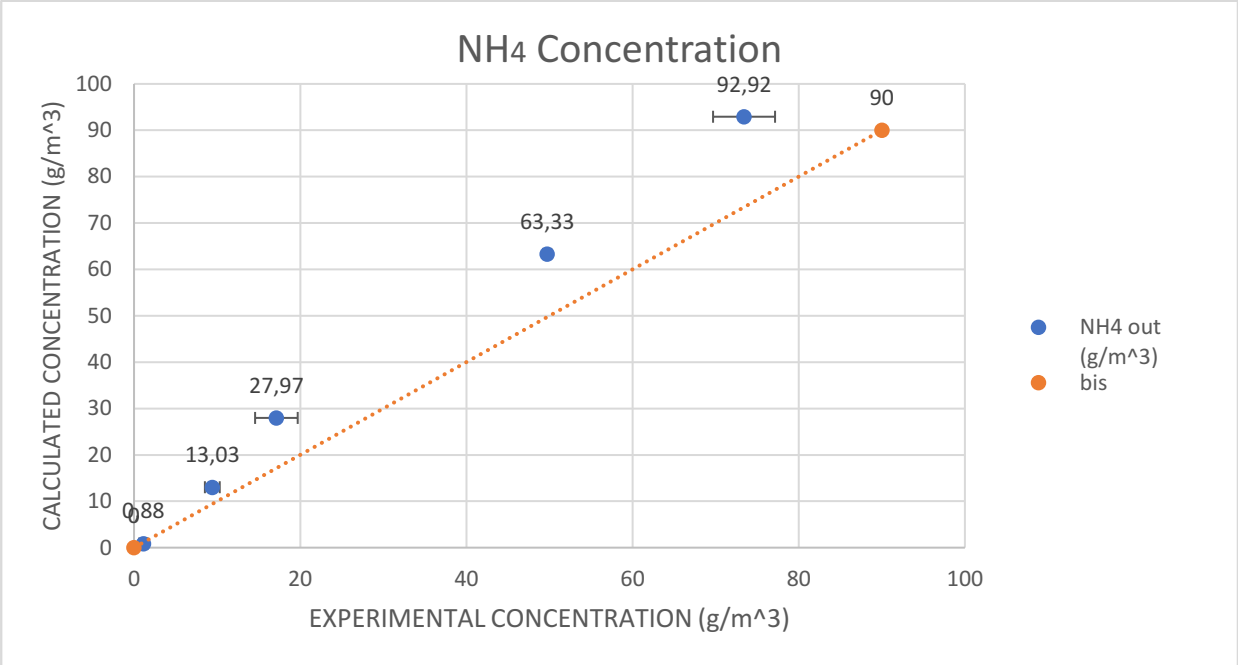

Figure 3.16: trend of the calculated concentrations of ammonium compared with the expected one after the change on the values of the uptake velocities

Also, in this case the trend of the results appears better that the other before because it seems that with the new values of ρ each concentrations started to move in direction of the expected values. The graph shows the presence of an improvement on this field, of course the results are not perfect, and it is necessary to investigate better to understand what can approximate the real values. In particular it is necessary that the gap of some test (number 2 and 3) could be reduced, since the error respect the expected result is almost 25% that is too large. It is necessary to implement something new to reduce it.

Finally, there are the results of the growth of microalgae. In this case the data are very good because for the first time the match is almost perfect. As it will be possible to see from the figure 3.17, the difference between the calculated values and the laboratory's values is less than 1%.

Cx exp (g/m^3)	Cx (g/m^3)	Dev.St.
173,05	173,68	0,51
147,23	148,87	0,28
311,28	316,88	4,13
176,44	175,96	4,97
256,67	258,08	2,05

Table 3.12: results for the biomass concentrations after the change of uptake velocities

Only in the second case there is an error that is a little bit higher than the other but, as it is possible to see from the graph, the value could be considered correct because the range created with the standard deviation is in the middle of the orange line.

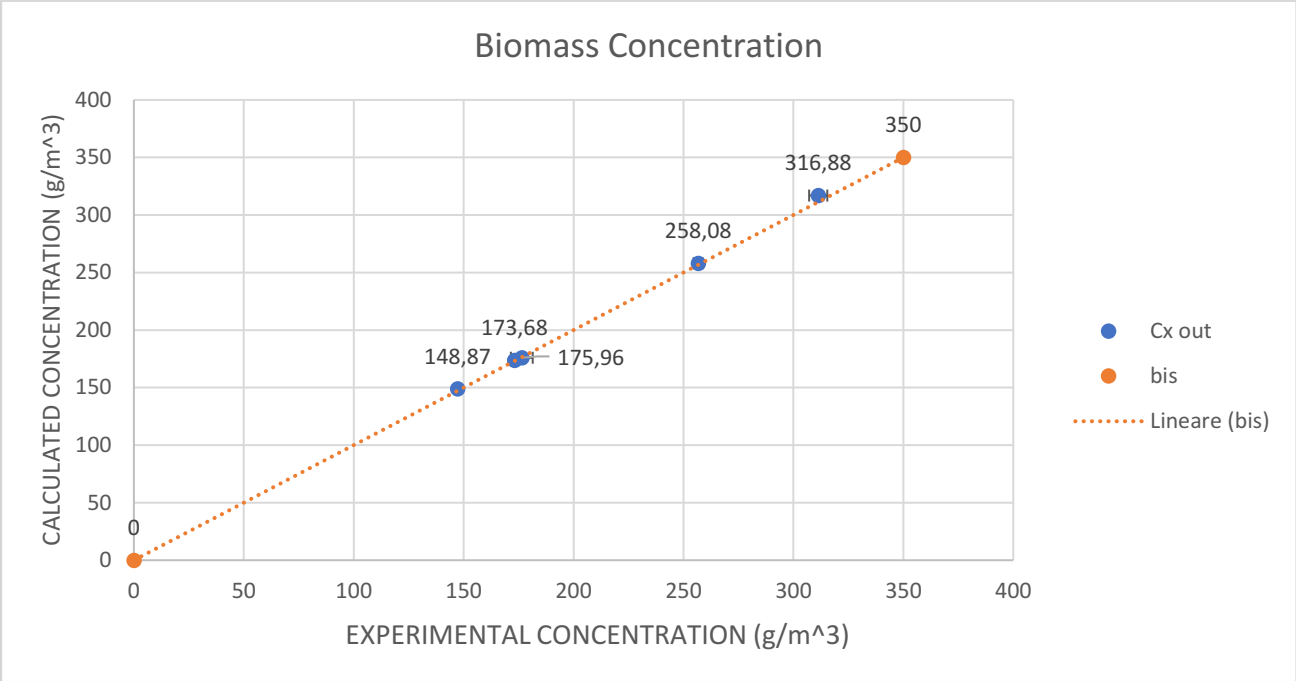

Figure 3.17: concentrations of biomass after the interpolation of uptake velocities

Considering the last results, it is necessary to reconsider the model of Dixon because after the change of the key parameters (alpha and beta) the results were not good and in a first look there was not a big change in terms of values of concentrations calculated. Otherwise, through the change of the other parameters, in this case the values of the uptake velocities, the numbers appear more clear and there is a good correspondence with the laboratory concentrations, especially for biomass.
Moreover, this result is obtained without changing the values of alpha and beta. This is an important point because it means that there was a radical change without considering two parameters the are at the base of the model.
Following this idea, it is possible that the system can depend on the action of other parameters such the ρ_{NO_3} and ρ_{NH_4}; for this reason the equations that describe the model are changed with the elimination of these two factors of inhibition as described in the following paragraph.

3.4.4 Return to Droop simple model

Based on the conclusions of the analysis discussed in the previous paragraph, it was clear that the inhibition parameters alpha and beta could be removed from the model, which means that the actual model equations could be simplified as the original Droop model.
The only two equations that are changed are those that describe the removal rate of the two species of nitrogen. The following equations are hence used (they are the same that were presented in chapter 2):

$$R_{NO_3} = \rho_{NO_3} * \frac{C_{NO3}^{out}}{C_{NO3}^{out} + K_{NO3}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right) \quad (2.\ 21)$$

$$R_{NH_4} = \rho_{NH_4} * \frac{C_{NH4}^{out}}{C_{NH4}^{out} + K_{NH4}} * \left(\frac{Q_{max,N} - \left(Q_{min,N} + R_N\right)}{Q_{max,N}}\right) \quad (2.\ 22)$$

Also in this case an interpolation was carried out (fminsearch) to evaluate if there are other parameters that affect the final concentrations.
The results produced form the interpolation based on the simplified model appeared to be always better than the previous ones. However, the great result of this approach was that it could identify more clearly which parameter was influencing algal growth and outlet concentrations.
In fact, one of the first results of the analysis on the simple Droop model is that it was possible to confirm that the values of the half-saturation constants (Kn) were not so influential on the outputs. Observing the results of different attempts carried out, it was seen that their value did not differ from the one taken from the literature. This allowed to conclude that in the observed system, despite the presence at the same time of different species of nutrients, the half-saturation constants did not affect the growth of algae and did not even modify the uptake. Following this result, they were cut-off from the parameters that are investigated.

Having obtained this information, it was decided to consider only the values of rho as modifiable parameters following each iterative cycle developed by the program. A partial consideration of their influence and importance on the final result and on the concentrations at the exit of the reactor had been found from the previous results. For this reason starting from the initial value of ρ (0.6 for nitrates and 0.62 for ammonium) it is developed the interpolation for each test to find two new values that can minimize the difference between experimental and calculated value.

3.4.5 Final results for Chlorella

After all interpolations were made for each sample and the new ρ values for each test were identified, the output concentrations were recalculated with the simplified Droop model.
It was noticed that this time the results were very prompting since, except for some cases, the concentrations were all similar.

Using the following graphs (3.18, 3.19 and 3.20) and table (3.12, 3.13 and3.14) it is possible to observe the definitive and final results for the system concerning the reactor with *Chlorella protothecoides* as the microalgal species.
For this last situation it was decided to reduce the range for the values of rhono3 and ρ_{NH_4}, namely:

- $0.01 < \rho_{NO_3} < 1$;
- $0.01 < \rho_{NH_4} < 1$.

Based on these new ranges we obtained this new values of uptake velocity (table 3.12):

ρ_{NO_3} (gN/gX*d)	ρ_{NH_4} (gN/gX*d)
0,292	0,861
0,1984	0,2972
0,8927	0,01006
0,433	1
1	1

Table 3.12: final values for uptake velocities after the analysis

The table 3.13 describes the final results about the concentrations of nitrates that are very close to the experimental ones.

NO3 in (g/m^3)	NO3 exp (g/m^3)	New range (g/m^3)	dev
60	49,88	51,48	1,30
30	22,80	24,28	1,20
100	42,09	55,32	2,63
40	28,44	26,11	1,92
10	9,32	2,26	0,24

Table 3.13: final concentrations of Nitrates after the use in the MatLab code of the new values for the uptake velocities for each sample

Looking the values in the table and comparing the second and the third column is possible to see that a real correlation exists between the change of the uptake velocity of nitrate and the final concentration of them. All the final values are very close to expected concentrations, except for the last specimen where the error is still relatively high. This results, compared with the previous one or

all the other explained in this study, underline the fact that the value of rho is fundamental for the removal rate of the system.

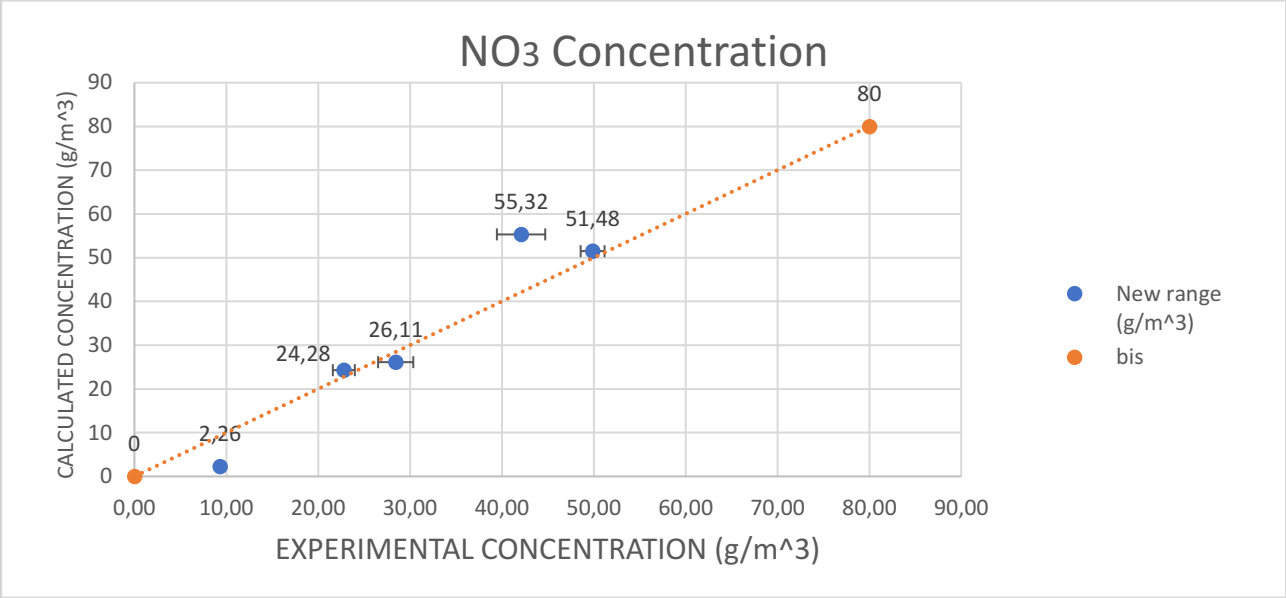

Figure 3.18: comparison between the expected concentrations and the calculated one after the introduction of the new values of ρ

Similar results that were seen for the nitrates appear also in the case of ammonium concentrations. In fact, the outlet concentrations calculated with the model are very similar to the data of the lab. The second and third columns show that there is a little difference from the two values at the same conditions, with a difference that is lower than 5% in most of the cases.

NH4 in (g/m^3)	NH4 exp (g/m^3)	new range (g/m^3)	dev
30	9,42	11,76	0,89
80	49,73	51,74	0,49
100	73,42	74,05	3,73
10	1,13	2,29	0,51
40	17,13	11,08	2,56

Table 3.14: final concentrations of ammonium after the use in the MatLab code of the new values for the uptake velocities for each sample

Figure 3.19 underlines how little is the gap between the values of the lab and the mathematical values obtained from the Matlab's script. In fact, great part of the results are located on the orange line, that means that exist a perfect correlation. Only one value, corresponding to the fifth specimen, is located a little further. It should be noted that also the correspondent result for the nitrate was a little bit far from the experimental result. A possible explanation is the fact that perhaps the conditions in that specimen were different with respect to the others.

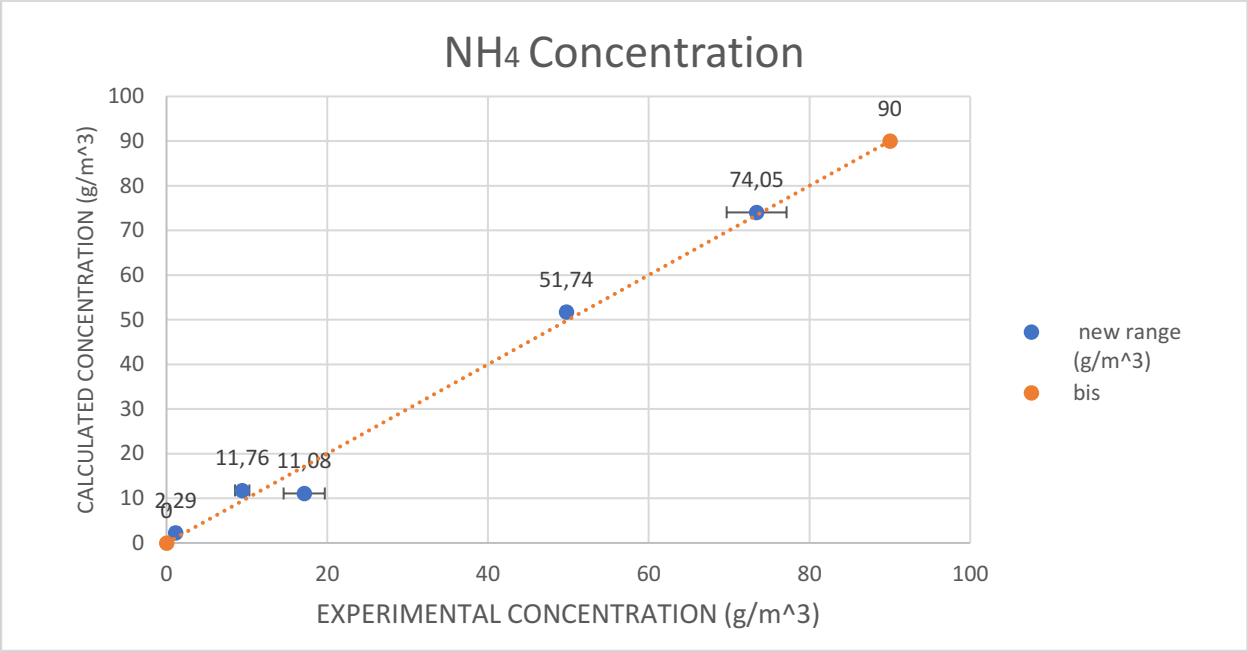

Figure 3.19: *comparison between the expected concentrations and the calculated one after the introduction of the new values of rho in the ammonium results*

The table 3.15 and the chart below (figure 3.20) illustrate the correspondence between many experimental and calculated values also for biomass concentration. In the first four samples there is a percentage error less than 1%. While in the last case, where the result is different, the percentage error that characterizes it is less than 5%. This is in line with what was observed in the nutrient data, in fact even in that case the fifth sample appeared to have deviations.

Cx exp (g/m^3)	Cx new range (g/m^3)	dev
173,05	173,04	0,51
147,23	147,14	0,28
311,28	313,50	4,13
176,44	175,54	4,97
256,67	247,09	2,05

Table 3.15: *final concentrations of biomass after the use in the MatLab code of the new values for the uptake velocities for each sample*

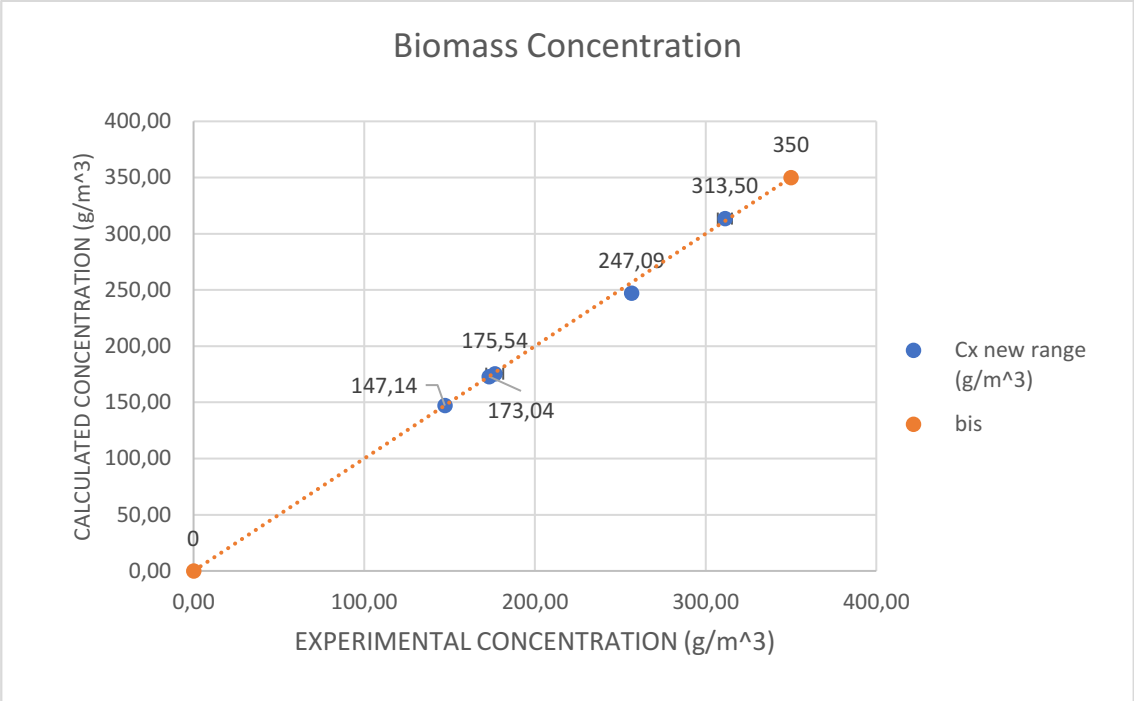

Figure 3.20: final graph about the final concentrations of microalgae

The latest results obtained show that there is a strong relationship between the outlet concentrations and the value of the uptake rates of the different algal species. Especially through the results obtained and observable in the tables of nitrates and ammonium it is possible to observe how this parameter can be considered as a regulatory parameter for uptake of nutrients by cells.
It can be noted, among the considerations that can be made, that the value of the uptake rate (ρ) appears to be increasing in samples where the concentration of ammonium is very low or in any case lower than nitrates. This can be a symptom that algae in the absence of ammonium try to assimilate as much as possible before their "quota" R runs out.

3.4.6 Parameters correlation

Based on the observation that a simple Droop model is able to represent the experimental data if the uptake rate are adjusted, a further analysis was made to ascertain possible parameters correlation between the ρ for nitrate and ammonium: it is necessary to confirm or disprove the fact that different combinations of rho that could lead to the same results exist.
Accordingly, of the values of ρ (for example ammonium) using the starting value 0.62 was fixed, while the other uptake velocity was fitted, to compare the data with the final results of Chlorella.
The following two tables compare the values of ρ, obtained before, with the value of this analysis. Parity plots are also reported in figure 3.21, 3.22 and 3.23.

ρ_{NO_3} (gN/gX*d)	ρ_{NH_4} (gN/gX*d)
0,4006	0,62
0,01	0,62
0,3469	0,62
0,479	0,62
1	0,62

Table 3.17: values of rhono3 when ammonium velocity is fixed

ρ_{NO_3} (gN/gX*d)	ρ_{NH_4} (gN/gX*d)
0,292	0,861
0,1984	0,2972
0,8927	0,01006
0,433	1
1	1

Table 3.18: interpolated values of uptake velocities

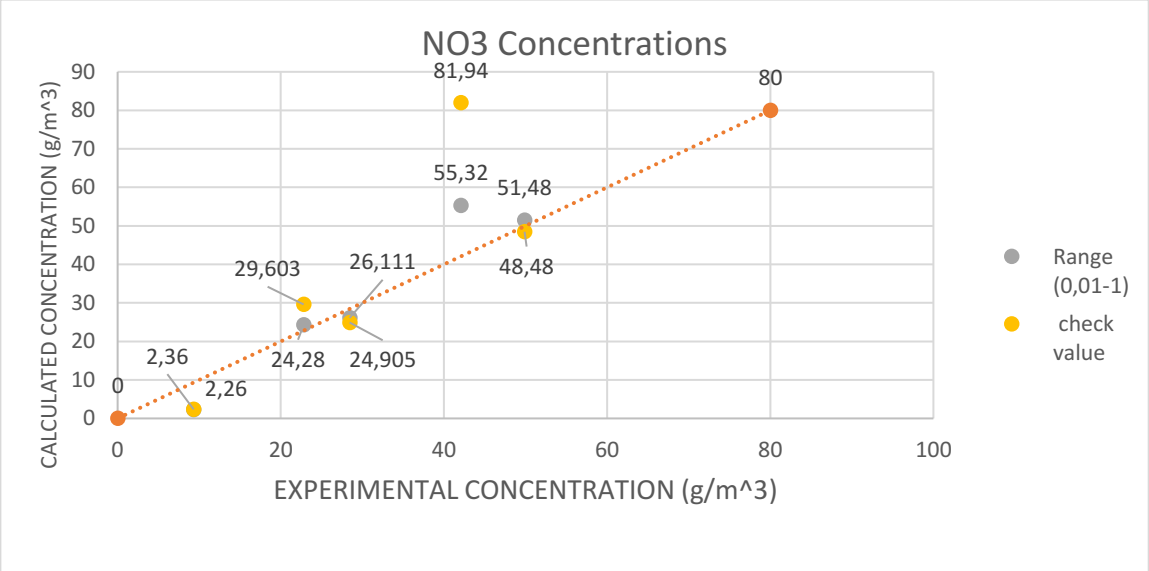

Figure 3.21: comparison between the nitrate concentration with the correct uptake velocities (grey) obtained during the previous study and the new one (yellow)

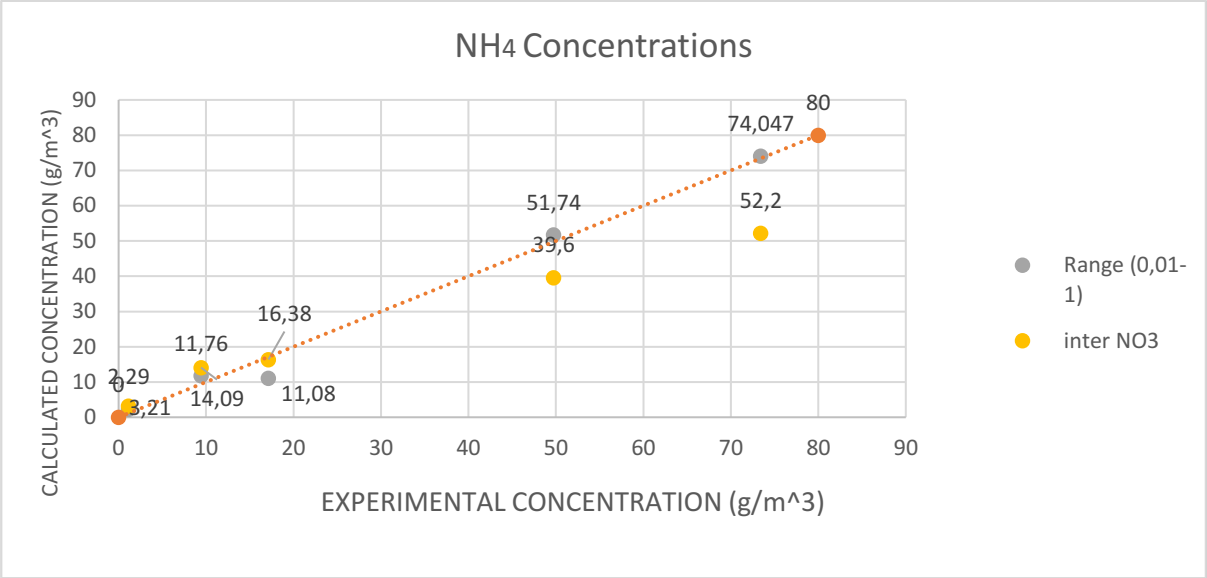

Figure 3.22: *comparison between the ammonium concentration with the correct uptake velocities (grey) obtained during the previous study and the new one (yellow)*

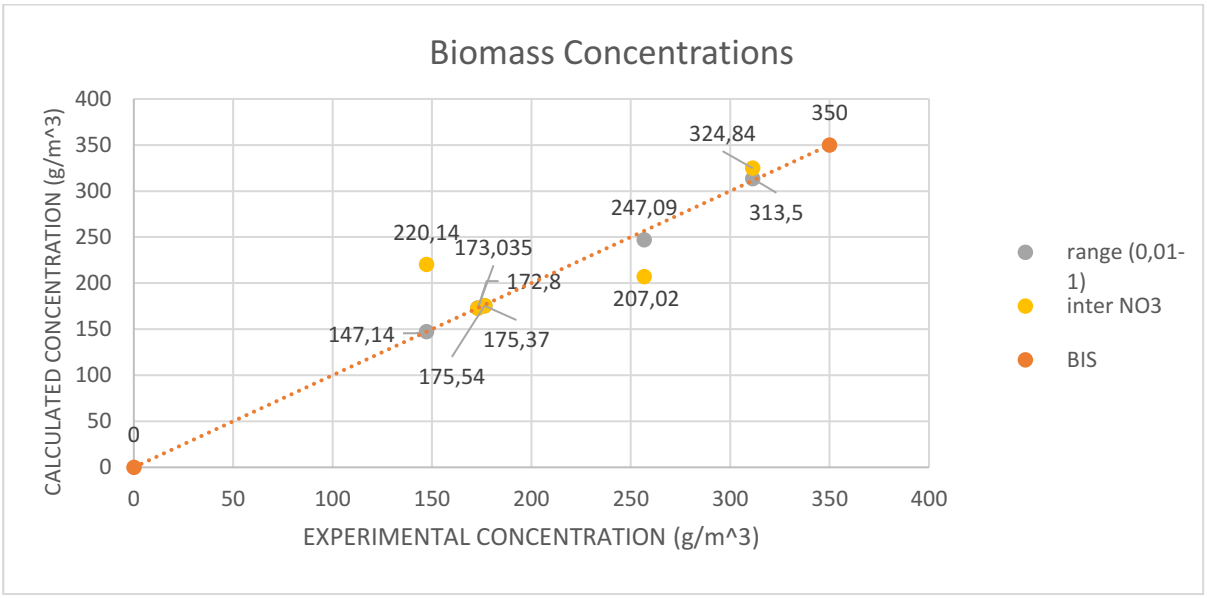

Figure 3.23: *comparison between the biomass concentration with the correct uptake velocities (grey) obtained during the previous study and the new one (yellow*

It is clear that fixing one of the rho is decreasing the freedom grade, resulting in an insufficient reproducibility of the experimental data. In fact, the error in these cases far exceeds 5% whereas in the previous context it was very close to 1%.
Thus, the parameters are not correlated, and they both need to be changed in order to reproduce the experimental data.

3.5 Model to represent the nutrient uptake by Synechocystisis spp. PCC6803

The same approach used to determine the parameters values in the case of *C. protothecoides* was used also for *Synechocystis,* that showed a different regulation on nitrate uptake when ammonium was present.
The process of achieving the results was comparable to the one developed and described above for *Chlorella*, with some differences that will be detailed in the following.

3.5.1 Calculation of qmin and qmax

In the case of Synechosysits, an additional step was needed, as the values of qmin and qmax (representing the minimum and maximum nitrogen levels present in the cell) were not available. As mentioned in the previous chapters, these two values are fundamental to determine the growth of the algae and the amount of nitrogen that can be assimilated. Consequently, in order to develop the same analysis carried out *on C. prothenoides* the values of the two parameters were retrieved by experimental data obtained under the supply of only nitrate and ammonium separately. Fminsearch function was the function used find the following values: qmin, qmax, ρ, kd and KN for both scripts. These values are put inside two scripts that had as boundary conditions (Table 3.17 and 3.18), the characteristic of the reactors, the concentrations of biomass and of the two nutrients and based on them the system should try to find the best value that could permit to find a correct final result

I_0 (µmol/m^2 *s^1)	**L (cm)**	**T (°C)**	τ (d) reali	**N_{NO3-} in ($g \cdot m^{-3}$)**	N_{NO3-} out ($g \cdot m^{-3}$)	dev std	Cx ($g \cdot m^{-3}$)	dev std
100	3,5	24	0,88	23,29	0,90	0,79	0,199	0,015
100	3,5	24	0,86	55,03	17,00	2,00	0,238	0,011
100	3,5	24	1,10	80,30	28,65	6,20	0,361	0,023
100	3,5	24	0,86	93,83	51,92	2,36	0,285	0,023

Table 3.19: boundaries conditions and all the data about the specimens with only nitrates

I_0 (µmol/m^2 *s^1)	**L (cm)**	**T (°C)**	**τ (d)**	NNH4+ in (mg·L-1)	dev std	NNH4+ out (mg·L-1)	dev std	Cx (g•L-1)	dev std
100	3,5	24	0,95	19,30	0,48	0,76	1,32	0,305	0,029
100	3,5	24	0,95	33,78	1,20	0,00	0,00	0,305	0,018
100	3,5	24	0,85	59,41	0,67	17,68	1,09	0,314	0,015
100	3,5	24	0,87	69,45	0,52	28,29	1,94	0,268	0,031

Table 3.20: boundaries conditions and concentrations for specimen with only ammonium

Values of qmin and qmax resulted similar between the two conditions, confirming that the internal quota in not dependent on the type of nitrogen source provided, while the other parameters where found largely different (table 3.20 and 3.21). The values of uptake rates are acceptable, being in the the range defined by literature and are also similar to the values found for *Chlorella*.

Qmin_N	0.00372
Qmax_N	0.1728
kd	0.05566
ρ_{NH_4}	0.5505
KNH4	16.73

Table 3.21: ammonium parameters

Qmin_N	0.00487
Qmax_N	0.175
kd	0.0977
ρ_{NO_3}	1.09
KNO3	1.58

Table 3.22: nitrate interpolated parameters

Accordingly, an average value for qmin and qmax was fixed, and the further analysis was focused on the other parameters only.

NH4 in	NH4 exp (g·m-3)	NH4 out (g·m-3)	dev
19,30	0,76	2,22	1,32
33,78	0,00	5,65	0,00
59,41	17,68	18,03	1,09
69,45	28,29	25,4	1,94

Table 3.23: results for samples with ammonium

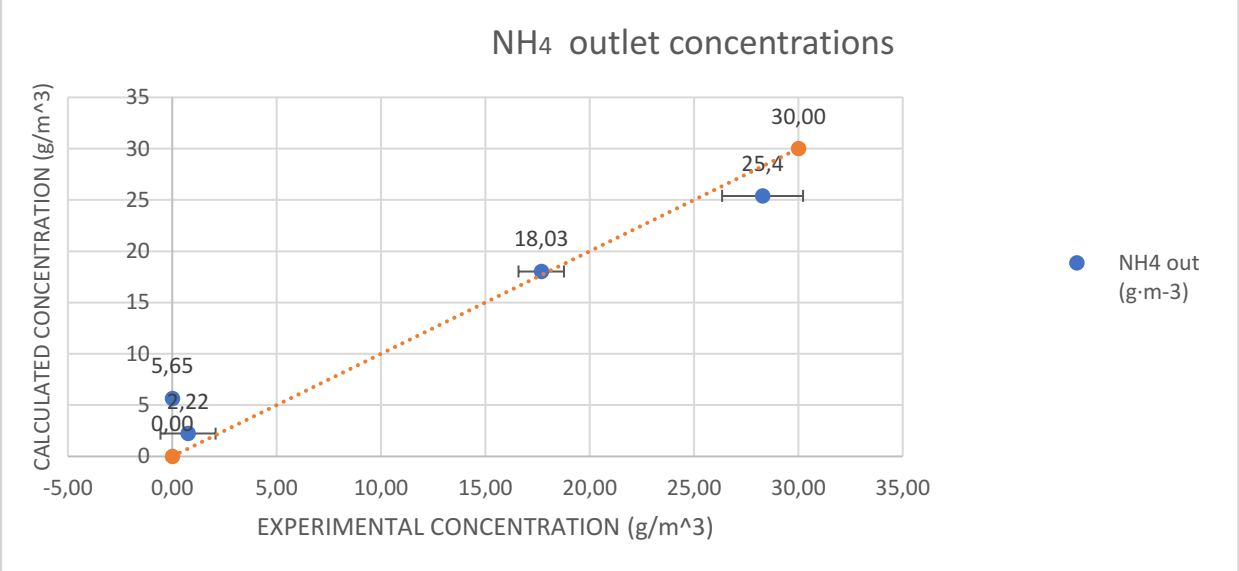

Figure 3.24: graphs that shows the ammonium concentration result respect the expected one

Since these tests are characterized by the presence of only ammonium the following table 3.24 will describe directly the results in terms of concentrations of biomass for each resident time.

CX exp (g·m-3)	CX out (g·m-3)	dev
305,000	256,84	2,900
305,000	273,71	1,753
314,400	265,48	1,476
268,167	242,17	3,112

Table 3.24: biomass concentrations for sample with ammonium as unique nutrient

The correlation was found poor, but it was considered acceptable to proceed with the next interpolation, where both nitrogen species were supplied to the reactor and differently exploited by the microalgal culture.

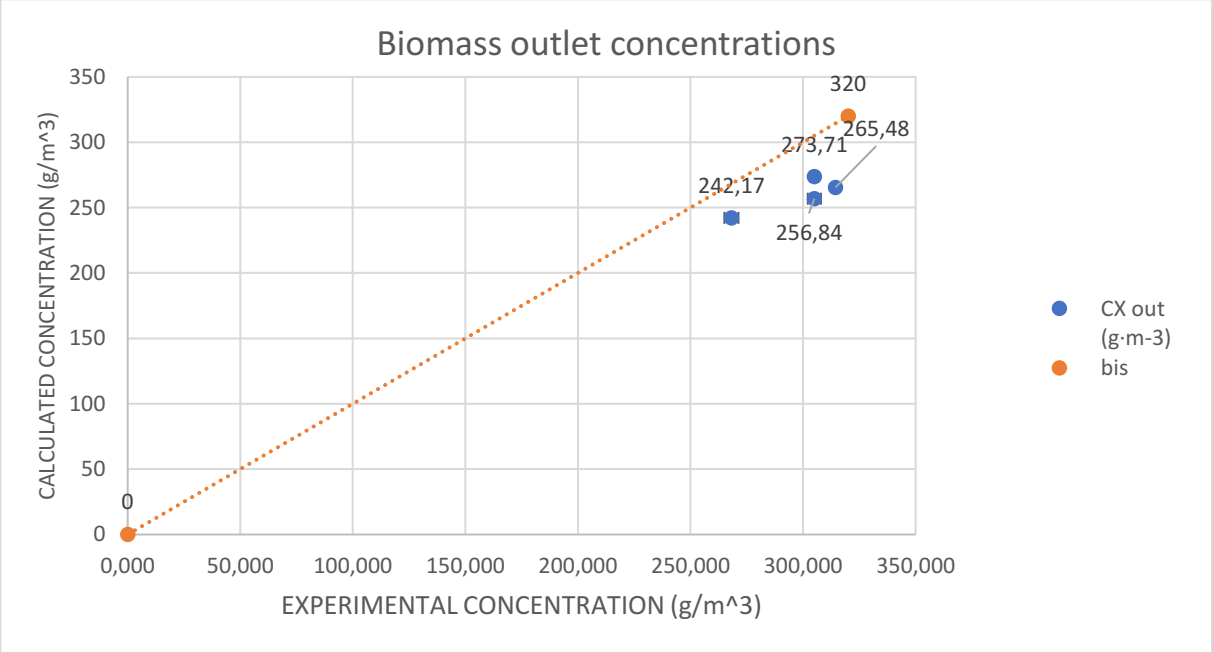

Figure 3.25: trend of biomass in the samples with only the ammonium

In conclusion, the values of "Qmin and Qmax" were obtained from the fitting of the experiments with only ammonium and only nitrate, and were used for further fitting.
For outgoing nitrate concentrations, the match is almost perfect with a margin of error of less than 5%. While for biomass there was a clear improvement compared to the previous case, however, the correspondences were optimal for two of the 4 tests conducted.

This allows us to assume that the values used for the nitrogen "quotas" are likely to be true to the actual values. Therefore, these values will be considered as fixed parameters in future interpolations and analyses.

3.5.2 Sensitivity analysis for uptake rate in Synechocystis

The analysis of *Synechocystis* growth un presence of two nitrogen species was finally considered. The method followed is the same as that done for *Chlorella* with the aim of comparing the final results of retrieved parameters between the two species.
the first analysis was carried out based on the data obtained under a mixed nutrients supply, including both ammonium and nitrate. All the parameters were initially retrieved in one step, with unsatisfactory results of correlation. In fact, the concentrations of nitrates was found to be underestimated, with opposite outcome in the case of ammonium.

Consequently, the following strategy was developed based on the erroneous results obtained.

- The reference values at the nitrogen "quotas" were to be kept constant because the microorganisms in the algal culture are the same. In fact, these samples are also determined by the presence of *Synechocystis*.
- Finally, considering the procedure carried out on the *Chlorella* tests, it was decided to apply the same procedure to identify the values of reaction rates using the fminsearch function.

Thus, a strategy similar to that one used in the case of *Chlorella* was applied: for each sample, the interpolation function fminsearch was applied in order to identify the uptake rate separately (ρ_{NO_3} and ρ_{NH_4}) that best describe the system considered, id order to possibly highlight a trend.
Also in this context the opportune assumptions have been made in order to limit the interpolation system. A specific range of values for uptake rate has been considered and to have a homogeneity of results the limits (lower and upper) are equal, so the final value could move between 0.01 and 1.5. the following table describes the numerical values of the uptake velocity for each sample.

ρ_{NO_3} (gN/gX*d)	ρ_{NH_4} (gN/gX*d)
0,536	1,5
0,086	1,45
0,604	1,3
0,73	1,2

Table 3.25: final values for the uptake velocities in presence of both the compounds

As a result of the single development of each interpolation, a significant improvement in the results was observed.
As can be seen from the following graphs, it is evident the trend changes and the better affinity between the experimental data and those calculated with the system of equations elaborated in Matlab.

NO3 in (g·m-3)	NO3 exp (g·m-3)	NO3 out (g·m-3)	dev stand
50,32	30,00	39,06	1,91
37,74	24,38	27,8	1,34
35,81	32,33	31,96	0,91
38,03	31,57	31,32	0,73

Table 3.26: final concentrations of nitrates

The table and the figure below are showing the possibility of a relationship between the values of rho and the concentrations that is assimilated or the concentrations that remains in the wastewater. In fact, 3 of 4 values are close to be perfectly like the expected results. This means that the value of ρ influenced the final results as expected.

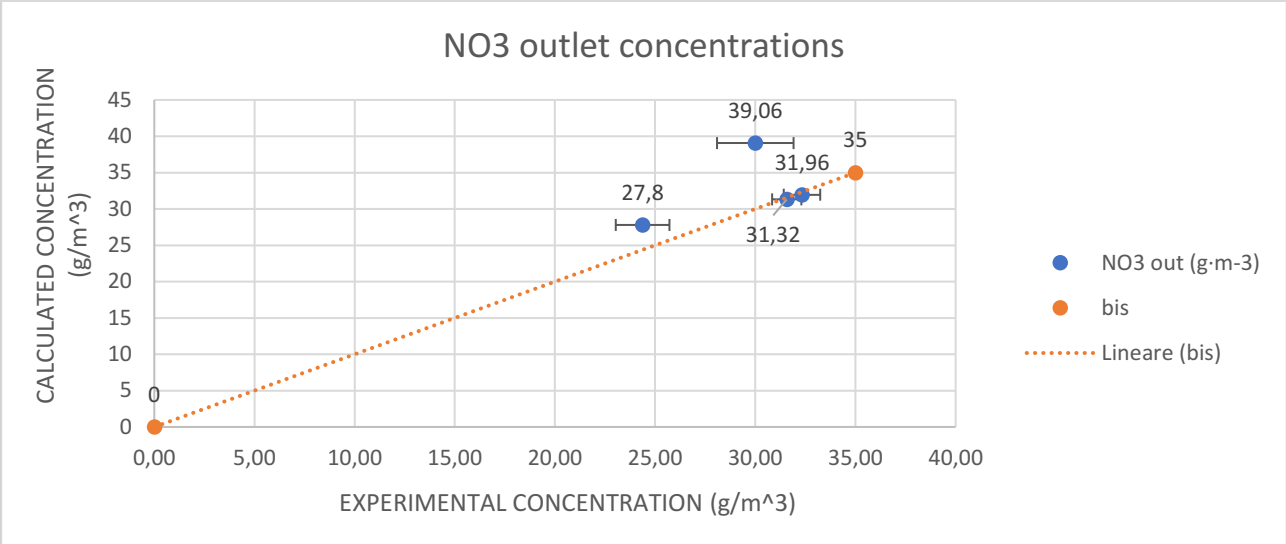

Figure 3.26: trend of nitrates in synechocystis samples

In the following, results of the concentrations of Ammonium are reported; in these cases the results are not perfect and in particular the error is a little bit higher. However, they are the best results that it was possible to produce for this compound. It could mean that the model that we are using is not correct for this species.

NH4 in (g·m-3)	NH4 exp (g·m-3)	NH4 out (g·m-3)	dev stand
15,49	0,00	1,55	0,00
22,04	0,26	2,48	0,34
59,21	19,69	21,67	3,70
52,23	3,87	10,94	1,05

Table 3.27: ammonium results

Also, the graph underlines that only in one case exists a consistent difference between the calculated results and the experimental ones. The others can be considered correct.

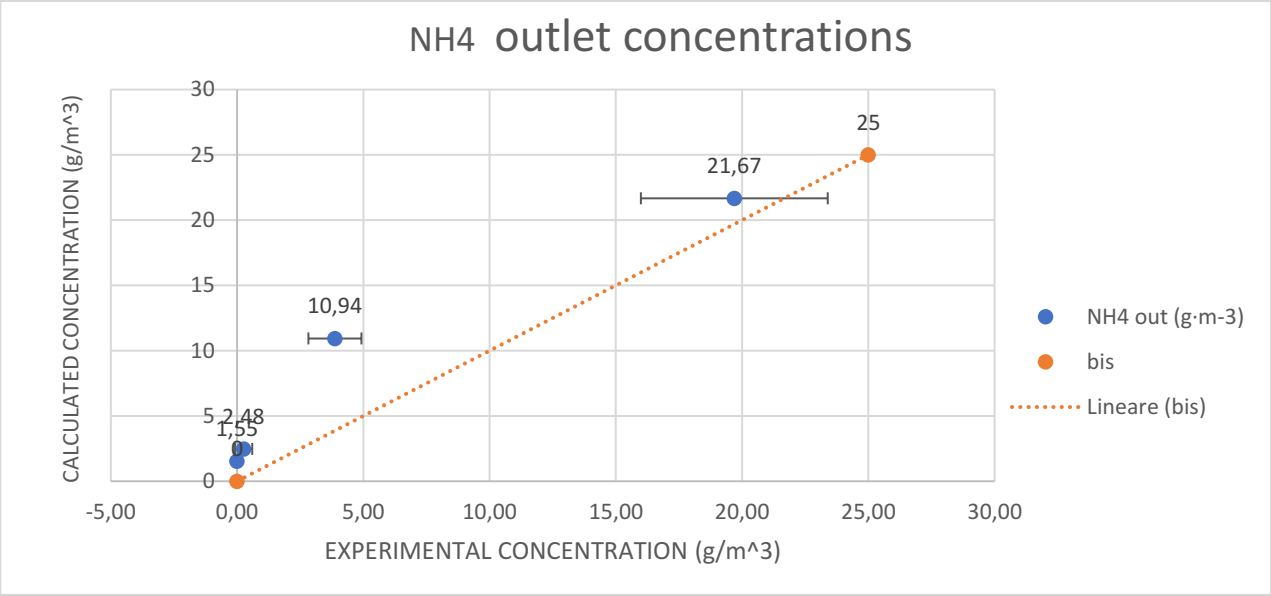

Figure 3.27: results for ammonium concentrations

The results of the biomass are reported in Table 3.28 and Figure 3.28.

.

Cx out (g·m-3)	Cx out (g·m-3)	dev stand
374,12	372,55	1,658
378,92	377,05	2,988
289,07	285,39	3,719
389,81	386,88	3,245

Table 3.28: biomass outlet concentration after the use of different velocities of uptake

Also, in this case there is a correlation between the values of rho and the final concentrations because great part of the samples is close to the real values, but the model is not perfect because one of them is outside the range.

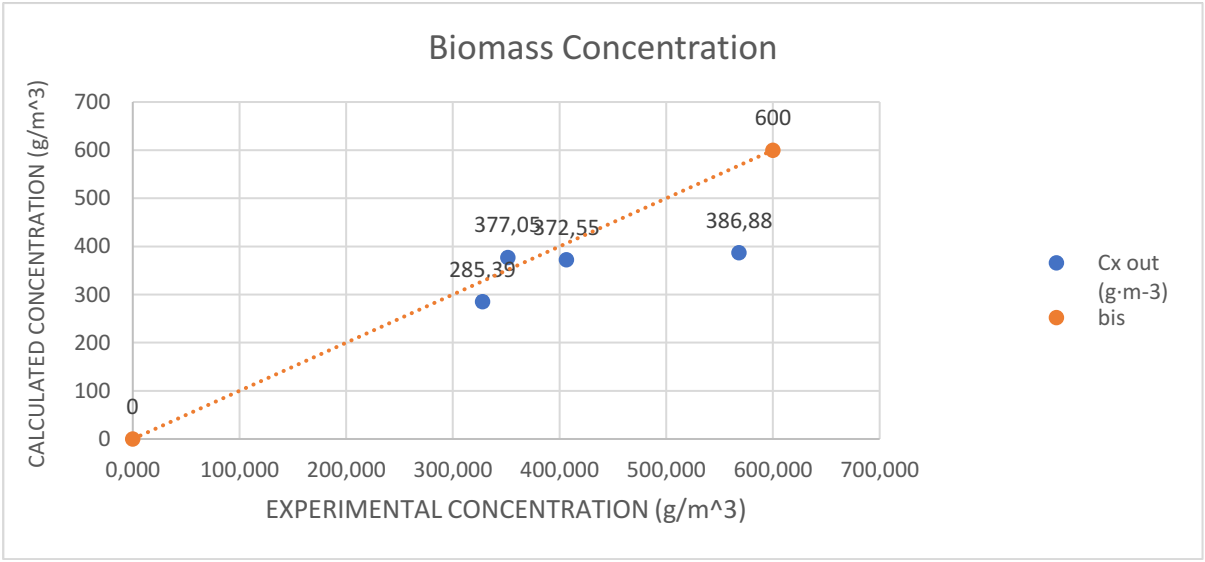

Figure 3.28: trend of biomass during the last analysis

The results obtained show that the values of uptake rates have a great influence on the estimation of the amount of nitrogen, ammoniacal or nitrate-based, which is absorbed. This effect is also reflected indirectly on the growth of biomass.
However, it is possible to observe that in this scenario, with *Synechocystis*, the correlation is not perfect so there is the possibility that the model is not perfect for these tests.

4. CONCLUSION

In this study, two experimental models were compared to possibly represent the competition between the uptake of ammonium and nitrate from wastewater by microalgae. Conventionally, the nutrient uptake in microalgae is described by the Monod model, which is not able to consider the possible variation of the internal quota of nitrogen in the algal biomass. For this reason, the models of Solimeno and Dixon were integrated with Droop Model and were developed based on previous studies. Method of Solimeno is strictly based on Monod theory and describes the nitrate uptake as possible when all the ammonium is completely used. Dixon model, instead, is meant to represent the co-metabolism of two coexistent substrates.

Two different species of microorganisms were selected in order to better represent the biological variability of microalgae: *Chlorella protothecoides* and *Synechocystis spp PCC6803*. The choice to use these two microorganisms is done due to their properties and in particular the fact that they can be used in wastewater treatment plants replacing traditional bacteria.

The thesis started with the application of the Solimeno and Dixon model on the experimental data obtained in continuous reactors, including the biomass concentration, the nitrogen consumption in their different chemical forms in reactors fed with known mixtures of nitrate and ammonium.
Both the models assessed were found unable to properly represent the variation of uptake metabolism of the two species when the ratio between nitrate and ammonium changed.

In fact, no one was correct for a precise description of the results and both of them were characterized by an elevated discrepancy between the values expected and calculated.
In fact, it was understood that the method developed by Solimeno and modified with the presence of Gamma and Lambda, the two inhibition coefficients developed during the study of the method based on the theory exposed in the previous chapters, to simulate the possible inhibition is not capable of describing what happens in the laboratory.
Even adjusting gamma and lambda no further improvements were obtained.

Dixon's model was found more promising to better describe the results, but after several tests, it was realized that the alpha and beta inhibition factors might be superfluous, affecting the reliability of the Dixon model as well. In fact, as reported in chapter III, the values of alpha and beta used did not help to find a good estimation of the final concentrations. After a sensitivity analysis on them and on the other important parameters (such as the half-saturation constant, the uptake velocity and the mortality rate) it was clear that the system, and in particular the removal rate, was mainly described by the uptake velocity. This is the main parameter that can be tuned to better describe the different uptake adaptation of the two microalgal species.

Therefore, the model was simplified to a Droop model for each nutrient that participate to the formation of internal nitrogen quota. This way, the presence of two equations considering separately the uptake of ammonium and nitrogen can simplify the description, given that the uptake rate of both nutrients is affected by the co-presence of the two nitrogen forms.
By focusing first on *Chlorella* and then with *Synechocystis* on the values of uptake rates, the desired findings were obtained.

To reach the conclusion that rho values played a key role, it was necessary to reminder the concept of transporters adaptation in the algal metabolism. In fact, to assimilate nutrients, cells need particular molecules, called transporters, that allow and facilitate the passage of the nutrient across the cell membrane. The greater these are in number or the better are conditions in which the cell lives better

will be the ability of assimilation. There are also some complex pathways of activity regulation that can affect the functionality of the transport.

As a result of the in-depth analysis on the two uptake parameters, it was possible to hypothesize and understand how these values directly influence the removal of nitrogen compounds in the effluent. In addition, different species show different characteristics of acclimation, possibly affecting the modeling description.

In the case of *Chlorella*, this value changed depending on the concentration of nutrients present in the reactor. Thus, it is possible to confirm that both the values (rhonh4 and ρ_{NO_3}) change based on the values of the concentrations present at the inlet of the reactor. However, it is possible to draw a correlation between the value of rho of ammonium and its inlet concentration. In particular, it appears that in case of a lower concentration of ammonium the value of ρ_{NH_4} is higher than the value of ρ_{NO_3}. It means that in the system, the microalgae are trying to assimilate as much as possible the ammonium present.

Different results were obtained in the case of *Synechocystis*; considering the point of view of the model it is possible to confirm that there is a correlation between the expected values and the calculated values. However, the correlation is not strictly like *in Chlorella*.
In this case it is possible to say that perhaps the simplified Droop model is not the correct or the perfect method that can approximate and interpolate this kind of specie of microalgae.

In conclusions, the available model present in literature were found not to be able to represent the microalgal uptake of different nitrogen species, because they were based on Monod model, and they apply an inhibition term which is not generally applicable for different algal species. A Droop model is able to simplify the description, charging all the acclimation phenomena on only one parameter, the uptake rate. However, due to the specificity of the uptake rate in microalgae, the model has to be adapted depending on the microalgal species.

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APPENDIX

MATLAB CODE

Script 1: Solimeno code

```
 clc
clear all
close all

%PARAMETERS

u_max = 4.5;      % d^-1,maximum specific growth rate of the microorganism
Ki = 73.4;        % umol m^-2 s^-1 light half saturation constant
Iopt = 413;       % umol m^-2 s^-1 optimal light intensity
ka = 0.09;        % m^2 g^-1 biomass light adsorbtion coeffiecient
KNO3 = 14.58;     % gN m^-3 Monod half saturation constant - NO3
KNH4 = 14.23;     % gN m^-3 Monod half saturation constant - NH4
kd = 0.10*u_max;  % d^-1 specific decay rate
u_d = kd *u_max;   % d^-1 mortality rate
%kd = 0.1;          % day^-1, decay rate
rhoNO3 = 0.6;     % gN gX^-1 * d^-1 maximum uptake constant NO3
rhoNH4 = 0.62;    % gN gX^-1 d^-1 maximum uptake rate constant NH4
Q_minN = 0.018;   % gN gX^-1 nitrogen mimimum internal quota of nitrogen
Q_maxN = 0.20;    % gN gX^-1 nitrogen maximum internal quota of nitrogen

GAMma = 1;          % HALF SATURATION OF GROWTH OF ALGAE THAT DECSRIBE THE INIBITION OF
AMMONIUM WITH THE UPTAKE OF NITRATE
DELta = 1;          % HALF SATURATION OF GROWTH OF ALGAE THAT DECSRIBE THE INIBITION OF
NITRATE WITH THE UPTAKE OF AMMONIUM

%STRIPPING PARAMETERS
Ph = 7;           % value of Ph in which the system works
Kla = 123;          % mass transfer coefficient between the water and the air for the
nitrogen (Ammonia)
pNH3 = 1.5*10-6;  % atm partial pressure of Ammonia
DO2 = 2.5;        % m^2*s^-1 mass diffusion coefficient for O2
DNH3 = 2.4;       % m^2*s^-1 mass diffusion coefficient for NH3
pKa = 9.25;       % - acid dissociation constant for ammonium-ammonia balance
T = 24;           % °C temperature in which works the system
T1 = T +273.15;   % °K temeparture of in Kelvin
Kh = ((4.63*10^5 * exp(2100*(1/273.15-1/T1)))* 14/17);    % gN-NH3 m^-3 atm^-1 value of
the Henry law constant


%LIGHT INTENSITY
I0 = 100;         % umol m^-2 s^-1 incidental light intensity
%DATA
W = 2.5;          % cm, reactor thickness
VR = 0.0002;      % m^3 reactor volume
z = 0:0.05:W;     % cm position vector along W
%RESIDENCE TIME
tau = 1.01;
%INLET
CqIN = [7.51 63.80 0 0];    % g/m^3 (NO3 NH4 X Qn)
%INITIAL STATE
C0 = [7.51 63.80 200 0.018];

%INTEGRATION
[t,Cq]=ode23(@BM,[0
100],C0,[],I0,u_max,ka,z,Ki,Iopt,W,tau,CqIN,Q_minN,kd,Q_maxN,rhoNH4,rhoNO3,KNH4,KNO3,GA
Mma,DELta,Ph,Kla,pNH3,DO2,DNH3,pKa,Kh);
```

```
%OUTLET VALUE
NO3out = Cq(:,1);          %outlet concentration NO3
NH4out = Cq(:,2);          %outlet concentration NH4
Xout = Cq(:,3);            % outlet concentration of algae
R = Cq(:,4);               % internal quota of N

%PLOT

%CONCENTRATION OF NITRATE
subplot(141)
plot(t,NO3out)
title('outlet concentration NO3out vs time'); %% Outlet concentration NO3 profile
xlabel('time (d)');
ylabel('Outlet concentration NO3 [g m^{-3}]');

%CONCENTRATION OF AMMONIUM
subplot(142)
plot(t,NH4out)
title('outlet concentration NH4out vs time'); %% Outlet concentration NH4 profile
xlabel('time (d)');
ylabel('Outlet concentration NH4 [g m^{-3}]');

%CONCENTRATION OF ALGAE/BIOMASS AT THE OUTLET
subplot(143)
plot(t,Xout)
title('outlet concentration Xout vs time'); %% Outlet concentration X profile
xlabel('time (d)');
ylabel('Outlet concentration Xout [g m^{-3}]');

%CONCENTRATION OF R
subplot(144)
plot(t,R)
title('R - Internal quota N vs time'); %% Internal quota  N ,profile
xlabel('time (d)');
ylabel('R - Internal quota N [g_N g_x^{-1}]');

%MATERIAL BALANCES IN A CSTR

function                        [balances]                        =                        BM
(t,Cq,I0,u_max,ka,z,Ki,Iopt,W,tau,CqIN,Q_minN,kd,Q_maxN,rhoNH4,rhoNO3,KNH4,KNO3,GAMma,D
ELta,Ph,Kla,pNH3,DO2,DNH3,pKa,Kh);

%BALANCE OF X (ALGAE)
Iz = I0.*exp(-ka.*Cq(3).*z);     %equation of Lambert_Beer
rx_z    =    Cq(3).*(u_max.*(Cq(4)./(Cq(4)+Q_minN))*(Iz./(Iz+Ki.*((Iz./Iopt)-1).^2))-kd);
%BIOMASS GROWTH RATE
rx_av = trapz(z, rx_z)./W;          %AVERAGE REMOVAL RATE
BMx = -Cq(3)./tau+ rx_av;            %MASS BALANCE OF BIOMASS

%BALANCE OF NITRATE

rNO3          =          rhoNO3.*(Cq(1)./(KNO3+Cq(1)).*(GAMma./(GAMma+Cq(2))).*((Q_maxN-
(Cq(4)+Q_minN))./Q_maxN));
BMno3 = (CqIN(1)-Cq(1))./tau-rNO3*Cq(3);

%BALANCE OF AMMONIUM
```

```
rNH4          =          rhoNH4.*(Cq(2)./(KNH4+Cq(2)).*(DELta./(DELta+Cq(1))).*((Q_maxN-
(Cq(4)+Q_minN))./Q_maxN));
BMnh4         =         (CqIN(2)-Cq(2))./tau-rNH4*Cq(3)-Kla*(sqrt(DNH3/DO2)).*(Kh.*pNH3-
(CqIN(2)/1+10^(pKa-Ph)));



%BALANCE OF R (QUOTA OF NITROGEN)

uqN = (u_max.*(Cq(4)./(Cq(4)+Q_minN)).*(Iz./(Iz+Ki.*((Iz./Iopt)-1).^2))-kd);
uqN_av = trapz(z, uqN)./W;
BMn = + rNO3.*tau + rNH4.*tau - (Cq(4)+Q_minN).*uqN_av;

%BALANCES
balances = [BMno3;BMnh4;BMx; BMn];


end
```

Script 2: Dixon code

```
clc
clear all
close all

%PARAMETERS

u_max = 4.5;      % d^-1,maximum specific growth rate of the microorganism
Ki = 73.4;        % umol m^-2 s^-1 light half saturation constant
Iopt = 413;       % umol m^-2 s^-1 optimal light intensity
ka = 0.09;        % m^2 g^-1 biomass light adsorbtion coeffiecient
KNO3 = 14.58;     % gN m^-3 Monod half saturation constant - NO3
KNH4 = 14.23;     % gN m^-3 Monod half saturation constant - NH4
kd = 0.10*u_max;  % d^-1 specific decay rate
u_d = kd *u_max;   % d^-1 mortality rate
%kd = 0.1;          % day^-1, decay rate
rhoNO3 = 0.6;     % gN gX^-1 * d^-1 maximum uptake constant NO3
rhoNH4 = 0.62;    % gN gX^-1 d^-1 maximum uptake rate constant NH4
Q_minN = 0.018;   % gN gX^-1 nitrogen mimimum internal quota of nitrogen
Q_maxN = 0.20;    % gN gX^-1 nitrogen maximum internal quota of nitrogen
alpha = 0.056;    % DIXON CONSTANT FOR AMMONIUM
beta = 17.7;      % DIXON CONSTANT FOR NITRATE

%STRIPPING PARAMETERS
Ph = 7;           % value of Ph in which the system works
Kla = 123;          % mass transfer coefficient between the water and the air for the
nitrogen (Ammonia)
pNH3 = 1.5*10-6;  % atm partial pressure of Ammonia
DO2 = 2.5;        % m^2*s^-1 mass diffusion coefficient for O2
DNH3 = 2.4;       % m^2*s^-1 mass diffusion coefficient for NH3
pKa = 9.25;       % - acid dissociation constant for ammonium-ammonia balance
T = 24;           % °C temperature in which works the system
T1 = T +273.15;   % °K temeparture of in Kelvin
Kh = ((4.63*10^5 * exp(2100*(1/273.15-1/T1)))* 14/17);    % gN-NH3 m^-3 atm^-1 value of
the Henry law constant


%LIGHT INTENSITY
I0 = 100;         % umol m^-2 s^-1 incidental light intensity
%DATA
```

```
W = 2.5;           % cm, reactor thickness
VR = 0.0002;       % m^3 reactor volume
z = 0:0.05:W;      % cm position vector along W
%RESIDENCE TIME
tau = 1.01;
%INLET
CqIN = [7.51 63.80 0 0];    % g/m^3 (NO3 NH4 X Qn)


%INITIAL STATE
C0 = [10 63.80 200 0.018];

%INTEGRATION
[t,Cq]=ode23(@BM,                                                                    [0
100],C0,[],I0,u_max,ka,z,Ki,Iopt,W,tau,CqIN,Q_minN,kd,Q_maxN,rhoNH4,rhoNO3,KNH4,KNO3,al
pha,beta,Ph,Kla,pNH3,DO2,DNH3,pKa,Kh);

%OUTLET VALUE
NO3out = Cq(:,1);          %outlet concentration NO3
NH4out = Cq(:,2);          %outlet concentration NH4
Xout = Cq(:,3);            % outlet concentration of algae
R = Cq(:,4);               % internal quota of N

%PLOT

%CONCENTRATION OF NITRATE
subplot(141)
plot(t,NO3out)
title('outlet concentration NO3out vs time'); %% Outlet concentration NO3 profile
xlabel('time (d)');
ylabel('Outlet concentration NO3 [g m^{-3}]');

%CONCENTRATION OF AMMONIUM
subplot(142)
plot(t,NH4out)
title('outlet concentration NH4out vs time'); %% Outlet concentration NH4 profile
xlabel('time (d)');
ylabel('Outlet concentration NH4 [g m^{-3}]');

%CONCENTRATION OF ALGAE/BIOMASS AT THE OUTLET
subplot(143)
plot(t,Xout)
title('outlet concentration Xout vs time'); %% Outlet concentration X profile
xlabel('time (d)');
ylabel('Outlet concentration Xout [g m^{-3}]');

%CONCENTRATION OF R
 subplot(144)
 plot(t,R)
 title('R - Internal quota N vs time'); %% Internal quota  N ,profile
 xlabel('time (d)');
 ylabel('R - Internal quota N [g_N g_x^{-1}]');

%MATERIAL BALANCES IN A CSTR


function                    [balances]                    =                    BM
(~,Cq,I0,u_max,ka,z,Ki,Iopt,W,tau,CqIN,Q_minN,kd,Q_maxN,rhoNH4,rhoNO3,KNH4,KNO3,alpha,b
eta,Ph,Kla,pNH3,DO2,DNH3,pKa,Kh);
```

```
%BALANCE OF X (ALGAE)
Iz = I0.*exp(-ka.*Cq(3).*z);     %equation of Lambert_Beer
rx_z    =    Cq(3).*(u_max.*(Cq(4)./(Cq(4)+Q_minN))*(Iz./(Iz+Ki.*((Iz./Iopt)-1).^2))-kd);
%BIOMASS GROWTH RATE
rx_av = trapz(z, rx_z)./W;          %AVERAGE REMOVAL RATE
BMx = -Cq(3)./tau+ rx_av;

%BALANCE OF NITRATE



rNO3    =    rhoNO3.*(Cq(1)./(KNO3+Cq(1)+alpha*Cq(2))).*((Q_maxN-(Cq(4)+Q_minN))./Q_maxN);
% REMOVAL RATE OF NO3
BMno3 = (CqIN(1)-Cq(1))./tau-rNO3*Cq(3);

%BALANCE OF AMMONIUM

rNH4    =    rhoNH4.*(Cq(2)./(KNH4+Cq(2)+beta*Cq(1))).*((Q_maxN-(Cq(4)+Q_minN))./Q_maxN);
%REMOVAL RATE OF AMMONIUM NH4
BMnh4      =      (CqIN(2)-Cq(2))./tau-rNH4*Cq(3)      -Kla*(sqrt(DNH3/DO2)).*(Kh.*pNH3-
(CqIN(2)/1+10^(pKa-Ph)));

%BALANCE OF R (QUOTA OF NITROGEN)

uqN = (u_max.*(Cq(4)./(Cq(4)+Q_minN)).*(Iz./(Iz+Ki.*((Iz./Iopt)-1).^2))-kd);
uqN_av = trapz(z, uqN)./W;
BMn = + rNO3.*tau + rNH4.*tau - (Cq(4)+Q_minN).*uqN_av;

%BALANCES
balances = [BMno3;BMnh4; BMx ;BMn];

end
```

Script 3: Simplified Droop model

```
clc
clear all
close all

%PARAMETERS

u_max = 4.5;      % d^-1,maximum specific growth rate of the microorganism
Ki = 73.4;        % umol m^-2 s^-1 light half saturation constant
Iopt = 413;       % umol m^-2 s^-1 optimal light intensity
ka = 0.09;        % m^2 g^-1 biomass light adsorbtion coeffiecient

%NITROGEN PARAMETERS

KNO3 = 14.58;     % gN m^-3 Monod half saturation constant - NO3
KNH4 = 14.23;     % gN m^-3 Monod half saturation constant - NH4
%kd = 0.10*u_max;  % d^-1 specific decay rate
%u_d = kd *u_max;   % d^-1 mortality rate
kd = 0.2;          % day^-1, decay rate
rhoNO3 =1;      % gN gX^-1 * d^-1 maximum uptake constant NO3
rhoNH4 =0.62;     % gN gX^-1 d^-1 maximum uptake rate constant NH4
```

```
Q_minN = 0.045;    % gN gX^-1 nitrogen mimimum internal quota of nitrogen
Q_maxN = 0.20;     % gN gX^-1 nitrogen maximum internal quota of nitrogen
%alpha = 5.028590e-02; %0.056;     % DIXON CONSTANT FOR AMMONIUM
%beta = 4.540995e+00; %25;       % DIXON CONSTANT FOR NITRATE

%PHOSPOROUS PARAMETERS

Q_minP = 0.0007;  % gP gX^-1 phosporous mimimum internal quota of phosporous
Q_maxP = 0.1;     % gP gX^-1 phosporous mimimum internal quota of phosporous
rhoP = 0.036;     % gP gX^-1 * d^-1 maximum uptake constant P
KP = 1.86;        % gP m^-3 Monod half saturation constant -P

%STRIPPING PARAMETERS


Ph = 7;            % value of Ph in which the system works
Kla =123; %123;          % d^-1 mass transfer coefficient between the water and the air
for the nitrogen (Ammonia)
pNH3 = 0;          % atm partial pressure of Ammonia
DO2 = 2.5;         % m^2*s^-1 mass diffusion coefficient for O2
DNH3 = 2.4;        % m^2*s^-1 mass diffusion coefficient for NH3
pKa = 9.401;       % - acid dissociation constant for ammonium-ammonia balance
T = 24;            % °C temperature in which works the system
T1 = T +273.15;    % °K temeparture of in Kelvin
Kh = ((4.63*10^5 * exp(2100*(1/T1-1/298.15)))* 14/17);    % gN-NH3 m^-3 atm^-1 value of
the Henry law constant


%LIGHT INTENSITY
I0 = 100;              % umol m^-2 s^-1 incidental light intensity
%DATA
W = 0.035;             % m, reactor thickness
VR = 0.0002;           % m^3 reactor volume
z = 0:0.0005:W;        % m position vector along W
%RESIDENCE TIME
tau = 0.7;
%INLET
CqIN = [10 40 0 0 10.8 0];    % g/m^3 (NO3 NH4 X Qn P Qp)
%INITIAL STATE
C0 = [10 40 200 0.1 10.8 0.1];  % g/m^3 (NO3 NH4 X Qn P Qp)

%INTEGRATION
[t,Cq]=ode23(@BM,                                                                [0
100],C0,[],I0,u_max,ka,z,Ki,Iopt,W,tau,CqIN,Q_minN,kd,Q_maxN,rhoNH4,rhoNO3,KNH4,KNO3,Ph
,Kla,pNH3,DO2,DNH3,pKa,Kh,Q_minP,Q_maxP,rhoP,KP);

%OUTLET VALUE
NO3out = Cq(:,1);          % outlet concentration NO3
NH4out = Cq(:,2);          % outlet concentration NH4
Xout = Cq(:,3);            % outlet concentration of algae
R = Cq(:,4);               % internal quota of N
Pout = Cq(:,5);            % outlet concentration of P
Rp = Cq(:,6);              % internal quota of P
%PLOT

%CONCENTRATION OF NITRATE
subplot(141)
plot(t,NO3out)
title('outlet concentration NO3out vs time'); %% Outlet concentration NO3 profile
xlabel('time (d)');
```

```
ylabel('Outlet concentration NO3 [g m^{-3}]');

%CONCENTRATION OF AMMONIUM
subplot(142)
plot(t,NH4out)
title('outlet concentration NH4out vs time'); %% Outlet concentration NH4 profile
xlabel('time (d)');
ylabel('Outlet concentration NH4 [g m^{-3}]');

%CONCENTRATION OF ALGAE/BIOMASS AT THE OUTLET
%subplot(143)
%plot(t,Xout)
%title('outlet concentration Xout vs time'); %% Outlet concentration X profile
%xlabel('time (d)');
%ylabel('Outlet concentration Xout [g m^{-3}]');



%CONCENTRATION OF R
 %subplot(144)
 %plot(t,R)
 %title('R - Internal quota N vs time'); %% Internal quota  N ,profile
 %xlabel('time (d)');
 %ylabel('R - Internal quota N [g_N g_x^{-1}]');

%CONCENTRATION OF PHOSPOROUS
subplot(143)
plot(t,Pout)
title('outlet concentration Pout vs time'); %% Outlet concentration P profile
xlabel('time (d)');
ylabel('Outlet concentration P [g m^{-3}]');

 %CONCENTRATION OF Rp
 subplot(144)
 plot(t,Rp)
 title('Rp - Internal quota P vs time'); %% Internal quota  P ,profile
 xlabel('time (d)');
 ylabel('Rp - Internal quota P [g_P g_x^{-1}]');



%MATERIAL BALANCES IN A CSTR


function                        [balances]                        =                        BM
(~,Cq,I0,u_max,ka,z,Ki,Iopt,W,tau,CqIN,Q_minN,kd,Q_maxN,rhoNH4,rhoNO3,KNH4,KNO3,Ph,Kla,
pNH3,DO2,DNH3,pKa,Kh,Q_minP,Q_maxP,rhoP,KP);

%BALANCE OF X (ALGAE)
Iz = I0.*exp(-ka.*Cq(3).*z);     %equation of Lambert_Beer
rx_z                                                                                    =
Cq(3).*(u_max.*(Cq(4)./(Cq(4)+Q_minN))*((Cq(6)./(Cq(6)+Q_minP)))*(Iz./(Iz+Ki.*((Iz./Iop
t)-1).^2))-kd);     %BIOMASS GROWTH RATE
rx_av = trapz(z, rx_z)./W;          %AVERAGE REMOVAL RATE
BMx = -Cq(3)./tau+ rx_av;

%BALANCE OF NITRATE

rNO3 = rhoNO3.*(Cq(1)./(KNO3+Cq(1))).*((Q_maxN-(Cq(4)+Q_minN))./Q_maxN);      % REMOVAL
RATE OF NO3
```

```
BMno3 = (CqIN(1)-Cq(1))./tau-rNO3*Cq(3);

%BALANCE OF AMMONIUM

rNH4 = rhoNH4.*(Cq(2)./(KNH4+Cq(2))).*((Q_maxN-(Cq(4)+Q_minN))./Q_maxN);        %REMOVAL
RATE OF AMMONIUM NH4
BMnh4      =      (CqIN(2)-Cq(2))./tau-rNH4*Cq(3)      +Kla*(sqrt(DNH3/DO2)).*(Kh.*pNH3-
(Cq(2)/(1+10^(pKa-Ph))));

%BALANCE OF R (QUOTA OF NITROGEN)

uqN                                                                                =
(u_max.*(Cq(4)./(Cq(4)+Q_minN)).*((Cq(6)./(Cq(6)+Q_minP))).*(Iz./(Iz+Ki.*((Iz./Iopt)-
1).^2))-kd);
uqN_av = trapz(z, uqN)./W;
BMn = + rNO3 + rNH4 - (Cq(4)+Q_minN).*uqN_av;

%BALANCE OF PHOSPOROUS



rP= rhoP.*(Cq(5)./(KP+Cq(5))).*((Q_maxP-(Q_minP+Cq(6)))./Q_maxP);
BMp = (CqIN(5)-Cq(5))./tau-rP*Cq(3);

%BALANCE OF Rp (QUOTA OF PHOSPOROUS)

uqP                                                                                =
(u_max.*(Cq(6)./(Cq(6)+Q_minP)).*(Cq(4)./(Cq(4)+Q_minN)).*(Iz./(Iz+Ki.*((Iz./Iopt)-
1).^2))-kd);
uqP_av = trapz(z, uqP)./W;
BMRp= +rP-(Cq(6)+Q_minP).*uqP_av;

%BALANCES
balances = [BMno3;BMnh4;BMx;BMn;BMp;BMRp];

end
```

Script 4: fminsearch interpolation

```
clc
clear

%ALL FIX PARAMETERS

u_max = 4.5;      % d^-1,maximum specific growth rate of the microorganism
Ki = 73.4;        % umol m^-2 s^-1 light half saturation constant
Iopt = 413;       % umol m^-2 s^-1 optimal light intensity
ka = 0.09;        % m^2 g^-1 biomass light adsorbtion coeffiecient

%NITROGEN PARAMETERS

KNO3 = 14.58;     % gN m^-3 Monod half saturation constant - NO3
KNH4 = 14.23;     % gN m^-3 Monod half saturation constant - NH4
kd = 0.2;          % day^-1, decay rate
rhoNO3 = 0.6;  %0.6;      % gN gX^-1 * d^-1 maximum uptake constant NO3
rhoNH4 = 0.62; %0.62;     % gN gX^-1 d^-1 maximum uptake rate constant NH4
Q_minN = 0.045;    % gN gX^-1 nitrogen mimimum internal quota of nitrogen
Q_maxN = 0.20;     % gN gX^-1 nitrogen maximum internal quota of nitrogen
```

```
%PHOSPOROUS PARAMETERS

Q_minP = 0.0007;  % gP gX^-1 phosporous mimimum internal quota of phosporous
Q_maxP = 0.1;     % gP gX^-1 phosporous mimimum internal quota of phosporous
rhoP = 0.036;     % gP gX^-1 * d^-1 maximum uptake constant P
KP = 1.86;        % gP m^-3 Monod half saturation constant -P

%STRIPPING PARAMETERS
Ph = 7;            % value of Ph in which the system works
pNH3 = 0;          % atm partial pressure of Ammonia
DO2 = 2.5;         % m^2*s^-1 mass diffusion coefficient for O2
DNH3 = 2.4;        % m^2*s^-1 mass diffusion coefficient for NH3
pKa = 9.401;       % - acid dissociation constant for ammonium-ammonia balance
T = 24;            % °C temperature in which works the system
T1 = T +273.15;    % °K temeparture of in Kelvin
Kh = ((4.63*10^5 * exp(2100*(1/T1-1/298.15)))* 14/17);    % gN-NH3 m^-3 atm^-1 value of
the Henry law constant

%DATA OF THE REACTOR
W = 0.035;             % m, reactor thickness
VR = 0.0002;           % m^3 reactor volume


z = 0:0.0005:W;        % m position vector along W

%PARAMETRI CHE SARANNO VARIATI CON LA FUNZIONE FMINSEARCH
%alpha=0.056;
%beta=17.7;
Kla =123;         % d^-1 mass transfer coefficient between the water and the air for the
nitrogen (Ammonia)

%PARAMETRI USATI NEL CICLO (numerati 1 2 3 4 5 in colonna)

I0vet= [100];  % 100 100 100 100 100 vettore con i 5 parametri luminosi
tauvet=[0.70]; % 0.65 0.71 0.72 0.70 0.70 vettore con i 5 tempi di residenza
CNo3INvet=[10]; %60 30 100 40 10
CNh4INvet=[40]; %30 80 100 10 40
CPinvet=[10.8]; %10.8 10.8 10.8 10.8 10.8
C0 = [10 40 200 0.1 10.8 0.1];  % g/m^3 (NO3 NH4 X Qn P Qp)

n=1;
par0=[rhoNO3];

LB =[0.01];
UB =[1];
options= optimoptions('fmincon');
%options = optimoptions('fmincon','Algorithm','interior-point');
par                                                                                =
fmincon(@MB,par0,[],[],[],[],LB,UB,[],options,C0,I0vet,n,u_max,ka,z,Ki,Iopt,W,tauvet,CN
o3INvet,CNh4INvet,CPinvet,Q_minN,kd,Q_maxN,KNO3,KNH4,Kla,Ph,pNH3,DO2,DNH3,rhoNH4,pKa,Kh
,Q_minP,Q_maxP,rhoP,KP);

function                                                                          err=
MB(par,C0,I0vet,n,u_max,ka,z,Ki,Iopt,W,tauvet,CNo3INvet,CNh4INvet,CPinvet,Q_minN,kd,Q_m
axN,KNO3,KNH4,Kla,Ph,pNH3,DO2,DNH3,rhoNH4,pKa,Kh,Q_minP,Q_maxP,rhoP,KP)


%CqIN=zeros(1,6);    %inizia il ciclo in posizione i
```

```
res=zeros(n,6);
for i=1:n
CNo3IN=CNo3INvet(i);
CNh4IN=CNh4INvet(i);
CPin=CPinvet(i);
I0=I0vet(i);
tau=tauvet(i);

%Kla=par(1);
rhoNO3=par(1);
%rhoNH4=par(2);
%KNO3=par(1);
%KNH4=par(2);
%PARAMETRI UTILI ALLA FINE (CONCENTRAZIONI TROVATE SPERIMENTALMENTE)

CXout = [257];   % 173 147 311 176 257 CONCENTRAZIONE SPERIMENTALE ALGHE USCITA
CNo3OUT=[9.31]'; % 49.87 22.79 42.08 28.44 9.31 CONCENTRAZIONE NO3 SPERIMENTALE
Cnh4OUT=[17.133]'; % 9.42 49.73 73.42 1.13 17.133 CONCNETRAZIONE NH4 SPERIMENTALE

%INTEGRATION
[~,Cq]=ode23(@BM,                                                                [0
100],C0,[],I0,u_max,ka,z,Ki,Iopt,W,tau,CNo3IN,CNh4IN,CPin,Q_minN,kd,Q_maxN,rhoNH4,rhoNO
3,KNH4,KNO3,Ph,Kla,pNH3,DO2,DNH3,pKa,Kh,Q_minP,Q_maxP,rhoP,KP);

%OUTLET VALUE


% NO3out = Cq(:,1); % outlet concentration NO3
% NH4out = Cq(:,2);          % outlet concentration NH4
% Xout = Cq(:,3);            % outlet concentration of algae
% R = Cq(:,4);               % internal quota of N
% Pout = Cq(:,5);            % outlet concentration of P
% Rp = Cq(:,6);              % internal quota of P


res(i,:)=Cq(end,:);

end
err= sum(CXout(i)-res(i,3)).^2+sum(CNo3OUT(i)-res(i,1)).^2+sum(Cnh4OUT(i)-res(i,2)).^2;
fprintf('%d,%d \n',err, rhoNO3)

%Devo aggiungere un comando per uscire dal cilco di minimi
end

%MATERIAL BALANCES IN A CSTR
function                        [balances]                        =                        BM
(~,Cq,I0,u_max,ka,z,Ki,Iopt,W,tau,CNo3IN,CNh4IN,CPin,Q_minN,kd,Q_maxN,rhoNH4,rhoNO3,KNH
4,KNO3,Ph,Kla,pNH3,DO2,DNH3,pKa,Kh,Q_minP,Q_maxP,rhoP,KP)

%BALANCE OF X (ALGAE)
Iz = I0.*exp(-ka.*Cq(3).*z);     %equation of Lambert_Beer
rx_z                                                                              =
Cq(3).*(u_max.*(Cq(4)./(Cq(4)+Q_minN))*((Cq(6)./(Cq(6)+Q_minP)))*(Iz./(Iz+Ki.*((Iz./Iop
t)-1).^2))-kd);     %BIOMASS GROWTH RATE
rx_av = trapz(z, rx_z)./W;          %AVERAGE REMOVAL RATE
BMx = -Cq(3)./tau+ rx_av;

%BALANCE OF NITRATE
```

```
rNO3 = rhoNO3.*(Cq(1)./(KNO3+Cq(1))).*((Q_maxN-(Cq(4)+Q_minN))./Q_maxN);      % REMOVAL
RATE OF NO3
BMno3 = (CNo3IN-Cq(1))./tau-rNO3*Cq(3);

%BALANCE OF AMMONIUM

rNH4 = rhoNH4.*(Cq(2)./(KNH4+Cq(2))).*((Q_maxN-(Cq(4)+Q_minN))./Q_maxN);       %REMOVAL
RATE OF AMMONIUM NH4
BMnh4      =      (CNh4IN-Cq(2))./tau-rNH4*Cq(3)      +Kla*(sqrt(DNH3/DO2)).*(Kh.*pNH3-
(Cq(2)/(1+10^(pKa-Ph))));

%BALANCE OF R (QUOTA OF NITROGEN)

uqN                                                                                =
(u_max.*(Cq(4)./(Cq(4)+Q_minN)).*((Cq(6)./(Cq(6)+Q_minP))).*(Iz./(Iz+Ki.*((Iz./Iopt)-
1).^2))-kd);
uqN_av = trapz(z, uqN)./W;
BMn = + rNO3 + rNH4 - (Cq(4)+Q_minN).*uqN_av;

%BALANCE OF PHOSPOROUS

rP= rhoP.*(Cq(5)./(KP+Cq(5))).*((Q_maxP-(Q_minP+Cq(6)))./Q_maxP);
BMp = (CPin-Cq(5))./tau-rP*Cq(3);

%BALANCE OF Rp (QUOTA OF PHOSPOROUS)




uqP=
(u_max.*(Cq(6)./(Cq(6)+Q_minP)).*(Cq(4)./(Cq(4)+Q_minN)).*(Iz./(Iz+Ki.*((Iz./Iopt)-
1).^2))-kd);
uqP_av = trapz(z, uqP)./W;
BMRp= +rP-(Cq(6)+Q_minP).*uqP_av;

%BALANCES
balances = [BMno3;BMnh4;BMx;BMn;BMp;BMRp];

end
```