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Productivities and growth of the different algae on different Carbon sources from the candy industry

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TÍTULO:	Productivities and growing of the differences algae on different Carbon source from candy industry
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Abstract

The tests conducted with Chlorella and Scenedesmus have given various results with the different carbon resources given. Undoubtedly the best result is with glucose for two species 501.9 and 227.6, respectively. However, there are other alternatives such as galactine and sugar whose productivity is optimal for the species Scenedesmus. In turn, the optimal temperature for maximum Specific Growth Rate is 40.81ºC experimentally.

Keywords: Microalgae, Chlorella Vulgaris, Glucose, Scenedesmus, Peppermint Candy, Sugar, Black Candy, Galactine, Gumdrop

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Symbols and abbreviations

COD	Chemical Oxygen Demand
Abs	Absorbance
Chl	Chlorella sp
C. Sork	Chlorella sorokiniana
C. Vul	Chlorella Vulgaris
CS	Carbon source
Sce	Scenedesmus Obliquus
Glu	Glucose
PC	Peppermint candy
Sug	Sugar
Gal	Galactine
BC	Black candy
Gum	Gumdrop

Symbols And Units

Symbol	Description	Unit
Φ(T)	Function depends on the temperature	ºC
T	Temperature	ºC
μ	Specific growth rate	d^{-1}
x	Concentration of cells	g/L
t	Time	d
COD	Chemistry oxygen demand	mg/L

Aim of the thesis

The thesis aims to compare the experimental Specific Growth velocity with the theoretical values obtained in the different investigations with Chlorella algae and the Scenedesmus algae. At the same time, the comparisons of the experimental productivity obtained are determined to decide the best carbon source for the algae in a semi-batch system reactor. Then, in a batch system reactor, it is made the productivity experiment where it can see the different productivities with the different algae and the different candies.

1. Introduction

The industrial production of microalgal biomass is one of the most promising approaches to supplying next-generation foods, feeds, and biofuels. The high wide of molecules like carbohydrates, lipids, pigments, enzymes and vitamins, is made an object of the studies of this type of industry (Barros et al., 2019).

Mainly the studies are about the increase in large-scale microalgae, which means greater production of microalgae, and greater obtaining of products (Barros et al., 2019).

1.1. The microalgae

The group of microalgae is large and heterogeneous of microscopic algae, which are an almost untapped pool of metabolic versatility. Microalgae is a term used to describe a photoautotrophic organism, using CO2 and light to produce energy and biomolecules. All of these species are occurring in nature and, the potential exploration in the biotechnical manufacturing to the production of high-value biomolecules (Bumbak et al., 2011).

1.1.1. Autotrophic and Heterotrophic metabolisms

The knowledge of the metabolism is very extensive, from the simple reactions to the complete net of complex steps, including the enzymes, proteins, and the different cycles of the metabolism. Every microorganism shares some characteristics of the metabolism, and thus simplifies the studies about that. It found two types of metabolism more common the nature: Autotrophic metabolism and Heterotrophic metabolism. These types of metabolism share some characteristics and differences.

Autotrophic metabolism refers to the acquisition of energy and carbon from organic compounds. The autotrophic metabolism assimilated the inorganic carbon from the industrial greenhouse gas emission. (Lowrey et al., 2015). In contrast, heterotrophic metabolism acquires energy from organic carbon. One of the most attractive aspects of heterotrophic microalgae cultivation is the elimination of light requirements.

Under heterotrophic growth conditions, respiration rates equal or exceed the theoretical minimum cost of biomass synthesis. The ratio between CO_2 per carbon incorporated is valued at 0.4-1.4 for several Chlorella species. It means that the values for autotrophic growth are too much lower than the values for heterotrophic growth (Perez-Garcia et al., 2011). References to the energetic analysis of heterotrophic growth indicated a very favourable cell production per unit energy input (0.00924 g cells kJ−1 input) as compared to autotrophic growth (0.00177g cells kJ−1 input). These differences are made by the inefficiency of the photosynthetic process (Lowrey et al., 2015)

Mixotrophic cultivation is the growth mode where microalgae simultaneously use inorganic CO_2 and organic carbon sources in the presence of light (Perez-Garcia & Bashan, 2015)

1.1.2. Environmental factors affecting microalgal cultivation.

Light

The light is used by phototrophic microalgae containing chlorophyll a and chlorophyll b. The main function is the store of solar energy on carbohydrates or lipids. During this process, the microalgae can transform light energy into chemical energy with an efficiency of 50%. Just on a saturated medium of nutrients, the light of the critical factor for the activity. Microalgae require a specific light level to reach their maximum growth rate. Instead, If the light intensity is far above the saturation level, it will (Nur & Buma, 2019).

Temperature

The increases in metabolism in the major of the microalgae are by the increases in the temperature. The optimal temperature range for the growth of microalgae is between 25ºC and 30ºC but an increase in temperature affects the degree of dissemination of algal lipids in the form of saturated fatty acids content. The role of temperature as physical stress on lipid induction during microalgae cultivation in wastewater was studied. A mild increase in temperature can increase algae biomass production until a certain point after which protein synthesis gets affected. Low temperature induces the

generation of unsaturated fatty acids and leads to a decrease in membrane fluidity (Venkata Mohan et al., 2015).

In addition, the description of this variable with the growth is related to Equation 1. Relates the Specific growth Rate normalized and the temperature. This curve depends on three values: T_{min}, T_{opt} and T_{max}; where T_{min} is the minimum temperature at which the algae grow, T_{opt} is the optimal temperature where the growth is maximum, and the T_{opt} is the highest temperature where the algae work.

Equation 1

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

Carbon

There are many carbon resources from heterotrophic and mixotrophic microalgae such as arabinose, citrate, fructose, malate, lactic acid, lactose, peptone, urea, fulvic acids, ethanol, methanol, and sucrose. While glucose, glycerol, and acetate are used for heterotrophic cultivation for most commercial microalgae (Perez-Garcia & Bashan, 2015). The availability of carbon in the medium plays an important role in the synthesis of lipids of microalgae (Venkata Mohan et al., 2015).

Nutrients

The functional role of macronutrients and micronutrients, carbon, nitrogen, phosphorus, and potassium on heterotrophic cultivation of microalgae in domestic wastewater was studied on biomass growth and lipid productivity. In heterotrophic cultures, nitrogen availability plays an important role in lipid synthesis. Nitrogen limitation not only enhances lipid synthesis but is also responsible for the termination of other enzymes related to cell growth and propagation. Phosphorus plays an important role in the energy transfer of the algal cells as well as in the synthesis of phospholipids and nucleic acids. It was also reported that phosphorus deficiency promoted the accumulation of lipids in certain algae (Venkata Mohan et al., 2015).

Salinity

Growth and biomass productivity of Scenedesmus was also assessed in minimal media supplied with various concentrations of sodium bicarbonate, sodium chloride, seawater, glycerol, or sugarcane molasses. Among these growth conditions, high salinity, between 0.94 $g \cdot L^{-1}$ NaCl concentration or 25% seawater, resulted in the highest increase in growth and fatty acid content. The observed increase in lipid content in S. obliquus at higher salinity levels was confirmed in other reports (Msanne et al., 2020).

pH

The pH affects the biochemical reactions of microalgae. There is a range value for the increases and growth of the microalgae, which varies between 7 and 9. However, there is an optimal value between 8.2 and 8.7. in heterotrophic cultures the favourable values of pH fall between 6 and 7 (Venkata Mohan et al., 2015).

According to (Sakarika & Kornaros, 2016) Chlorella Vulgaris was grown heterotrophically at a pH range of 5.0–8.0. However, in extreme values (3.0, 4.0 and 11.0) it did not survive and at pH 9.5 aggregation of cells was observed. The best growth performance was observed at pH 7.5 and 8.0, with a Specific Growth Rate (μ_{max}) of 0.541 and 0.563 d^{-1}, respectively.

Table 1 compiled the different conditions and the other characteristics of the growth of the different experiments of the references.

Table 1. Theoretical experiments to compare

Microalgae	pH	T	μ_{max}	Carbon sources	Product	Ref
	-	ºC	d^{-1}	-	-	-
C. Vulgaris	6.0-7.5	36	0.18[1]	Glucose	-	(Nur & Buma, 2019)
C. Vulgaris	-	-	0.48	Glucose	-	(Abreu et al., 2022)
C. Vulgaris	6.5	28	1.28	Glucose	-	(Barros et al., 2019)
C. Vulgaris	-	-	0.91	Synthetic	-	(Lowrey et al., 2015)
C. Vulgaris	-	-	0.49	Acetate	Biomass	(Perez-Garcia & Bashan, 2015)
C. Vulgaris	-	-	0.34	Fulvic acid	Biomass	(Perez-Garcia & Bashan, 2015)
C. Vulgaris	-	-	0.15	Glucose	Biomass	(Perez-Garcia & Bashan, 2015)
C. Vulgaris	-	-	0.72	Glucose	Biomass	(Perez-Garcia & Bashan, 2015)
C. Vulgaris	-	-	0.42	Glucose	Biomass	(Perez-Garcia & Bashan, 2015)
C. Vulgaris	-	-	0.70	Glucose	Total carbohydrates	(Perez-Garcia & Bashan, 2015)
C. Vulgaris	-	-	0.08	Urea	Biomass	(Perez-Garcia & Bashan, 2015)
C. Vulgaris	7.5	-	0.541	-	Fatty Acids	(Sakarika & Kornaros, 2016)
C. Vulgaris	8.0	-	0.563	-	Fatty Acids	(Sakarika & Kornaros, 2016)
C. Pyrenoidosa	-	-	2.20	Glucose	Fatty Acids	(Perez-Garcia & Bashan, 2015)
C. Pyrenoidosa	-	-	1.10	Food waste hydrolysate	Fatty acids	(Perez-Garcia & Bashan, 2015)
S. Obliquus	-	-	1.08	Whey	Lipids	(Perez-Garcia & Bashan, 2015)

[1] μ_{max} units h^{-1}

1.2. Microalgae Fed-Batch cultivation mode

The fed-batch system is a semi-batch strategy used to avoid the limitations or inhibition of substances and the accumulation of toxic compounds. This is archived by replacing the medium periodically during the process (Nur & Buma, 2019). Fed-batch cultivation is the most effective technique for reaching high biomass concentrations in a short time and controlled manner. Perfusion technology is appropriate when inhibitory metabolites, which would otherwise affect biomass growth or product formation, need to be removed. High cell densities are attainable in fed-batch operation where the increasing biomass is retained and thus accumulates in the bioreactor. The pulsed addition of a carbon source is frequently employed in the high cell density cultivation of Chlorella. This is achieved in cultures grown heterotrophically by controlling the rate of addition of the organic carbon and energy source (Venkata Mohan et al., 2015).

1.3. Products from the microalgae

1.3.1. Pigments

The pigments are obtained from microalgae, they could be useful for food colourants, vitamins, cosmetics, and pharmaceutical applications. At the same time, the microalgae may contain a prominent level of Poly Unsaturated Fatty Acids (PUFA) which are suitable as a replacement for fish fatty acids. Documents refer to *C. Vulgaris*, which is more suitable for cultivation in a Palm Oil Mill Effluent (POME) medium compared to *S. Platensis* and *D. salina*(Nur & Buma, 2019).

1.3.2. Biodiesel

One option to produce biodiesel is to use microalgae o bacteria. The most common microalgae are *S. Platensis* could produce 100% of fatty acid methyl ester (FAME), the source of biodiesel. S. platensis is the best option compared to other microalgae like *D Salina* and *C. Vulgaris* with the production of FAME being 14,77 and 37,38%, respectively, using POME(Nur & Buma, 2019).

1.3.3. Other products

Apart from biofuel production, microalgae may serve as a potential renewable source for other commercial applications like environmental applications such as wastewater treatment and CO_2 mitigation; human nutrition; animal and aquatic feed; cosmetics production; high-value compounds such as fatty acids; stable biochemicals; biofertilizer; drug synthesis for antimicrobial, antiviral and anticancer treatments.

2. Materials and Methods

2.1. Materials

2.1.1. Microalgae

The two types of microalgae used during the experiments are: the Chlorella family: Chlorella Sorokoniana, Chlorella Vulgaris and Chlorella Sp; and Scenedesmus Obliquus

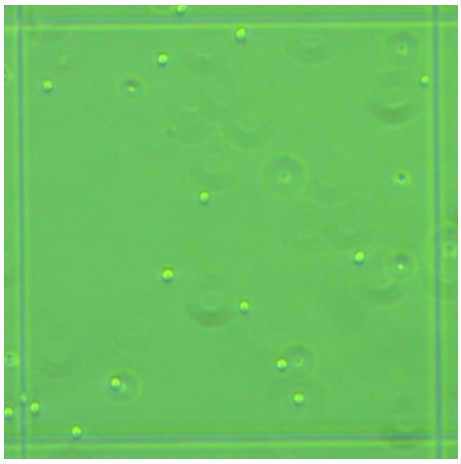

Figure 2-1. Photo by microscopy of the Chlorella Vulgaris

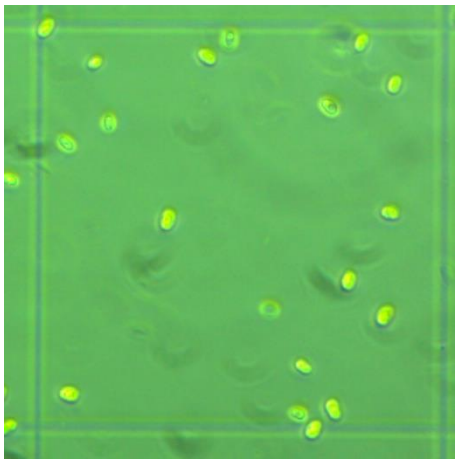

Figure 2-2. Photo by microscopy of the Scenedesmus Obliquus

2.1.2. MBBM Medium

It is mixed with nutrients to make optimal digestion for the microalgae. The MBBM medium is characterized by the concentration of the main nutrient like phosphate and nitrate, they are knowledge as macronutrients. Instead, they are another nutrient as Ca, Fe or Mg, in a low quantity of the mixed, they are important for digestion. They are known as micronutrients.

2.1.3. Glucose

Glucose is the main food of microalgae. It is destroyed the structure of the glucose to CO_2 and H_2O during heterotrophic digestion. There are different types of glucose like peppermint candy, galactine, sugar, black candy, gumdrop, and glucose. The

composition of these candies is described in Table 4 in section 0 compositions Table. It is assumed the value of the Cod is glucose in the carbon source.

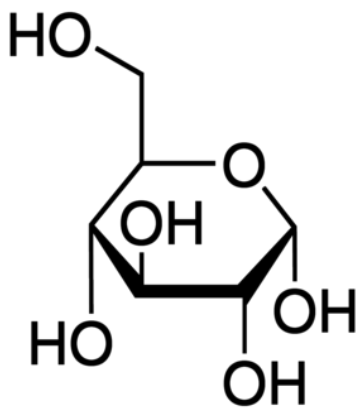

Figure 2-3. Molecule of D-Glucose

2.1.4. Reactor system

The piping and instrumentation diagram (P&ID) which describes the distribution and the controls of the reactors is drawn in the section of which name is A4-PI-101-1

2.2. Analytical methods

2.2.1. Spectroscopy instrument

This instrument can provide the measure of the COD, the concentration of the Nitrate and phosphate, the absorbance at 680 and 750 wavelengths, and the spectrometry of all the wavelengths spectrum.

The instrument is provided by Lange and with each test for the measure of the analyses of the main variables.

Figure 2-4. Spectroscopy instrument and the adaptations for the measures

2.2.2. Microscope and complements

The microscope is the instrument to see the algae in the dissolution sample. Using this technique, it is possible to count the number of cells of the square drawn in the Bürker camera to calculate the concentration of cells.

Figure 2-5. Microscope instrument

The complement of the microscope is the camera to take photos to count the number of cells. Another one is the Bürker camera, there used to count the cells in the square drawn in the glass. With this value, we can calculate the cell per unit of volume. Their measures are described in the picture below.

Figure 2-6. Bürker glass, tweezers, and glass

2.2.3. Oximeter, pHmeter and software controller

The oximeter is an instrument to know the concentration of O_2 dissolved in the reactor. It is controlled by software to maintain the constant value inside the reactor. it consisted of a compressor with works when the concentration of O_2 is low and stops when it is high.

The pHmeter is the instrument to measure the acids of the mix. It is controlled with the addition of HCl 0,1M to maintain a constant value for the optimal value of each reactor. Also, the NaOH 0,1M corrects the pH of the mixture.

The software controls each instrument to maintain the contact values consigned previously.

2.2.4. Python

The computational language using for the count of the cells and helping control the growth of the biomass. It makes the count of the picture taken with the microscope count in interesting.

2.3. Processing methods

2.3.1. Sampling

The quantity of the sampling there will be enough to do all the dissolution and measure of each reactor. The sampling is made by a pipette sterilised of 10mL. During the process, it is noted the date and the hour are to know the evolution of the values during the experiment. The minimal sampling it takes is 1mL to 5mL as a top because it is important not to variate the volume of each reactor.

2.3.2. Absorbance measure

The absorbance measurement is conducted by a spectroscopy instrument and the appropriate prismatic vessel. Before making the measure, it is necessary to dilute the sampling according to the data the day before. To make a correct measure the values must be lower than one value. The steps to make this measure are:

1. First, it will make a value of zero absorbance with deionized
2. Secondly, it will be measured with the sampling diluted using a vessel perfectly cleaned and dry. Repeat this step three times to certificate the stabilisation of the measure in each sampling

2.3.3. Spectroscopy measure

Spectroscopy is an important measure for determining the availability of the components in each reactor. in this case, it will find the two types of chlorophyll in the microalgae. The steps to follow to make the measure are:

1. First, it will make a value of zero absorbance with water deionized for each wavelength.
2. Secondly, it will be measured with the sampling diluted using a vessel perfectly cleaned and dry.
3. For each measure, it will save the data for their studies

2.3.4. Cells count

This procedure is used to know the number of cells concentrated in the medium. It is used the Bürker camera counts the number of cells in each square to determine the concentration of cells in each reactor.

1. The sampling is put between the Bürker camera and the glass until obtaining a homogeneous distribution of the mixture.
2. With the help of the camera installed in the microscope and the optimal software, photos are taken for analysis. For each reactor is taken eight squares of cells
3. For counting, the following rules are used: **Valid cells**, if is perfectly defined in the picture and it is inside as a whole in the square; **Invalid cells, if** it is not defined inside the square and on the picture taken.
4. According to the rules, it is the average of the counting for each square according to their reactor.

2.3.5. Chemical Oxygen Demand measure

The chemical Oxygen Demand (COD) is used to know the quantity of glucose dissolved in the middle. It serves to calculate the glucose consumed by microalgae in heterotrophic increases. The procedure to know the concentration is:

1. It dilutes the sampling to levels adequate for the measure of each component.
2. Filtrate the dilution to clean every solid present in the middle.
3. Follow the step that it indicates in the box of the equipment and made the reactions. For the measure of COD, is taken 2mL of sampling
4. The COD bottle is carried to the kiln for 15 minutes using the HT process and is waited 15 minutes later after finishing the digestion.
5. is made the measure of each bottle in the spectrum instrument.

Figure 2-7. COD box and kit

2.3.6. Nutrients measures

At the same time, the knowledge of the quantity of the nutrients like nitrate and phosphate is important. The procedure to know the concentration is:

1. It dilutes the sampling to levels adequate for the measure of each component.
2. Filtrate the dilution to clean every solid present in the middle.
3. Follow the step that it indicates in the box of the equipment and made the digestions, 1mL and 0,5mL of sampling for nitrate and phosphate measures respectively.
4. Let's stand the bottles for 15 minutes in the case of the nitrate and the phosphate.
5. To make the measure of each bottle in the spectrum instrument.

Figure 2-8. Phosphate box, kit and bottles A and C

Figure 2-9. Nitrate box, kit, and bottle B

2.3.7. Specific Growth Rates calculate method

It is calculated from t_0 to t_i, where it is the latest data of the experiment that there is exponential growth.

The representation of the logarithm of the concentration of cells versus time makes visualisation of the lineal zone which corresponds with the high growth rate. the slope of the linear zone will be the max growth rate (μ_{max}). The speed of the growth rate could be calculated in each interval evaluated. After that, the average is made. The mathematical method is explained in Equation 2 and Equation 3.

Equation 2

$$\mu_n = \frac{\ln\left(\frac{X_{i+1}}{X_i}\right)}{(t_{i+1} - t_i)}$$

Equation 3

$$\mu_{max} = \frac{\sum_0^n \mu_n}{n}$$

Mensurates the Specifics Growth Rates, it can determine the quantity of the cells in the exponential growth zone.

Equation 4

$$\begin{cases} x = x_0 \cdot e^{\mu \cdot (t_i - t_0)} \\ t_0 = 0 \end{cases} \rightarrow x = x_0 \cdot e^{\mu \cdot t}$$

2.3.8. Function Gompertz

It is a temporal function which describes the growth of the microorganism.

Equation 5

$$f(t) = a \cdot e^{-b \cdot e^{-c \cdot t}}$$

The process to calculate the constants is described below. From Equation 5, it is transformed into the equation:

Equation 6

$$\ln\left(\frac{N_i}{N}\right) = a \cdot e^{-e^{b - c \cdot t}}$$

The values of the constant it is made by Excel and specific software "Minitab"

1. It is defined as the cells of the constants in Excel.
2. It calculated the theoretical values for the same times in the experiment. It is calculated the differences for each point, and it is made the summary of the errors.
3. Helping by the "Solver" tool on Excel, it is made the curve minimizes the error between the experimental values and the theoretical values.
4. The "Minitab" software helps to certificate the values calculated by Excel

2.3.9. Exponential function calculus

Equation 4 is modified to compare the different curves.

Equation 7

$$x = x_0 \cdot e^{\mu \cdot t} \rightarrow \frac{x}{x_0} = e^{\mu \cdot t}$$

1. It is defined as the cells of the constant (μ from Equation 7) in Excel.
2. It is calculated the theoretical value for the same times in the experiment. It is calculated the differences for each point, and it is made the summary of the errors.
3. Helping by the "Solver" tool on Excel, it is made the curve minimizes the error between the experimental values and the theoretical values.

2.3.10. A theoretical model for the Specific growth rate with the temperature

1. It is defined as the cells of the constant T_{min}, T_{opt} and T_{max} on Excel's cells.
2. It is calculated the theoretical values for the same temperatures in the experiment applying Equation 1. It is calculated the differences for each point, and it is made the summary of the errors.
3. Helping by the "Solver" tool on Excel, it is made the curve minimizes the error between the experimental values and the theoretical values.
4. It is defined the restrictions: $T_{min}<T_{opt}$; $T_{opt}<T_{max}$

2.3.11. An experimental method to calculate kLa

Table 2. Summary of the probes for k_La calculus

Volume mL	Velocity rpm	Air -	Antifoam -
300	50	No	No
300	50	Yes	No
300	300	No	No
300	300	Yes	No
300	300	Yes	Yes
1000	300	No	No
1000	300	Yes	No
2000	300	No	No
2000	300	Yes	No

2.3.12. Experiments Conditions and compositions

Table 3 . Summary of the conditions in the experiments

Experiment	Algae	pH	T	Vol	Carbon source		Erlenmeyer					
			ºC	L	Reactor A	Reactor B	A	B	C	D	E	F
Test 1	C.Sork	7	25	0.3	Glu	-	-	-	-	-	-	-
Test 2	C.Vul	7	25	0.3	Glu	Glu	-	-	-	-	-	-
Test 3	Chl	7	25	0.3	Glu	Glu	-	-	-	-	-	-
Test 4	C.Vul	7	25	0.3	Glu	Glu	-	-	-	-	-	-
Test 5	C.Vul	7	25	0.3	PC	Glu	-	-	-	-	-	-
Test 6	Sce	7	25	0.3	PC	Glu	-	-	-	-	-	-
Test 7	Sce	-	25	0.15	-	-	Glu	Glu	PC	PC	Gal	Gal
Test 8	Sce	7	25	2.00	Glu	Glu	-	-	-	-	-	-
Test 9	Sce	-	25	0.15	-	-	Glu	PC	Gal	Sug	Gum	BC
Test 10	Sce	7	25	2.00	Glu	Glu	-	-	-	-	-	-
Test 11	Sce	-	25	0.15	-	-	Glu	PC	Gal	Sug	Gum	BC
Test 12	Sce	7	25	1.00	BC	Sug	-	-	-	-	-	-
Test 13	C.Vul	-	25	0.15	-	-	Glu	PC	Gal	Sug	Gum	BC

Table 4. Composition of the Carbon source used

Carbon Source	COD	NO3	NO2	PO4	P	NH4+
	mg/L	mg/L	mg/L	mg/L	mg/L	mg/L
Glu	204.00	-	-	-	-	-
PC	115.70	211	1.50	12.4	1.43	0
Gal	27.43	-	-	-	-	-
Sug	90.40	0	-	0	-	0
BC	96.30	0	-	0	-	0
Gum	7.91	0	-	0	-	0

3. Results and Discussion

3.1. Correlation between the absorbance and the concentration of cells

It is made the count of the cells to know the concentration of the algae cells in the reactor, described in section 2.3.4 Cells count. Then it is compared with the absorbance at 750 length-wave to compare the correlation between these parameters. It is made for Chlorella Vulgaris algae and Scenedesmus algae.

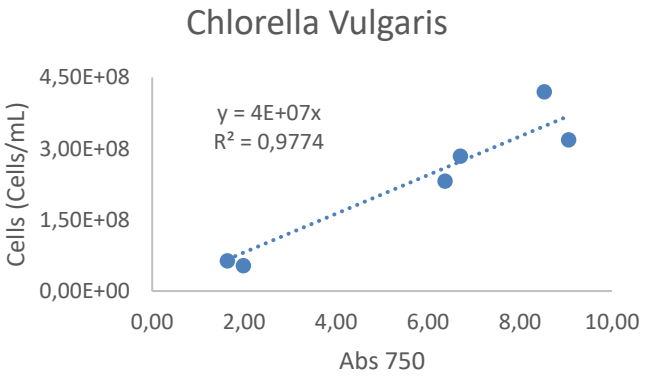

Figure 3-1. Correlation of the concentration of cells and the absorbance in Chlorella Vulgaris

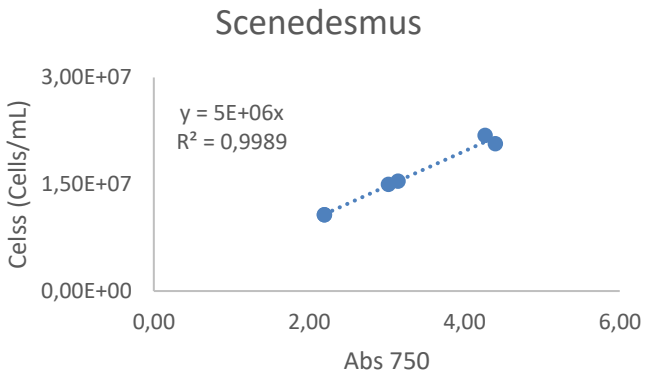

Figure 3-2 Correlation of the concentration of cells and the absorbance in Chlorella Vulgaris

This correlation permits it to work with the absorbance because it is worked with the cells at the same time.

3.2. Productivity comparison

The first experiment consists of determining the best Chlorella algae to grow in the same condition in every experiment. At the same time, the differences in the digestion in the reactors with antibiotics and the reactor without antibiotics.

These experiments used glucose at 200g/L and a dilution of phosphate (PO_4) and nitrate (NO_3) as a nutrient. The results of the different experiments are collected below in Figure 3-3, Figure 3-4, and Figure 3-5. It can use absorbance to know the growth of the microalgae because there is a relationship between the absorbance and the

concentration of the microalgae in the medium. According to that, it uses the formula to know the percentage of productivity.

Equation 8

$$\frac{(Abs680)|_{t=n} - (Abs680)|_{t=0}}{(Abs680)|_{t=0}} \cdot 100$$

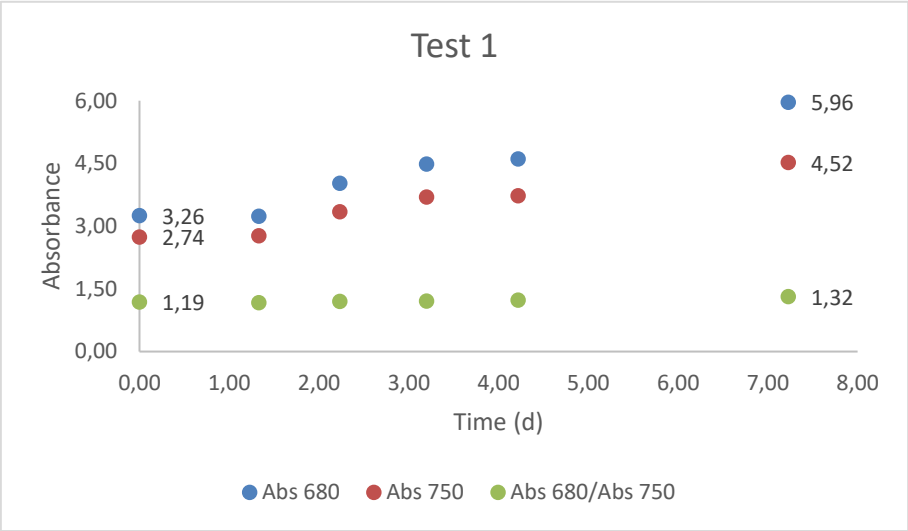

Figure 3-3. Evolution of the absorbance during the experiment Test 1

This experiment used chlorella and glucose as a carbon source, and it produced productivity of 82.8% applying Equation 8.

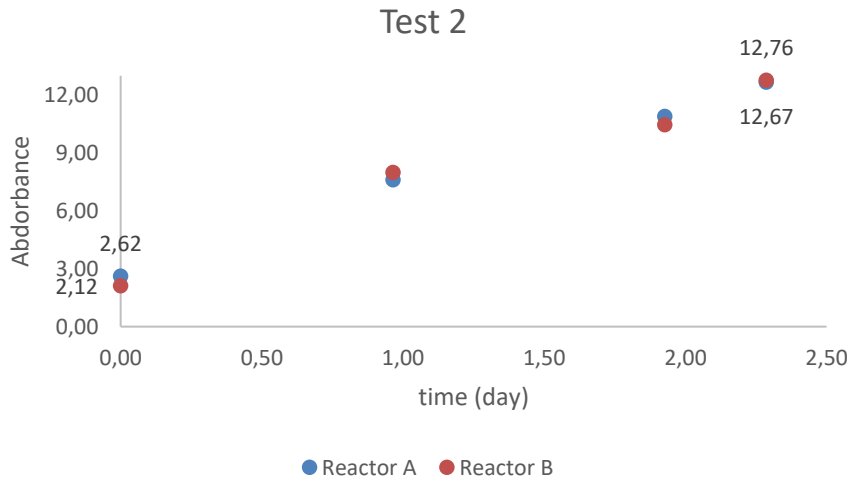

Figure 3-4. Evolution of the absorbance during the experiment Test 2

The experiment used the chlorella, on reactor B it introduced the antibiotic, and it can see, that there is not any difference in the productivity of the chlorella using the glucose as a carbon source. Applying Equation 8, the productivity of Reactor A and Reactor B is 383.6% and 501.9%

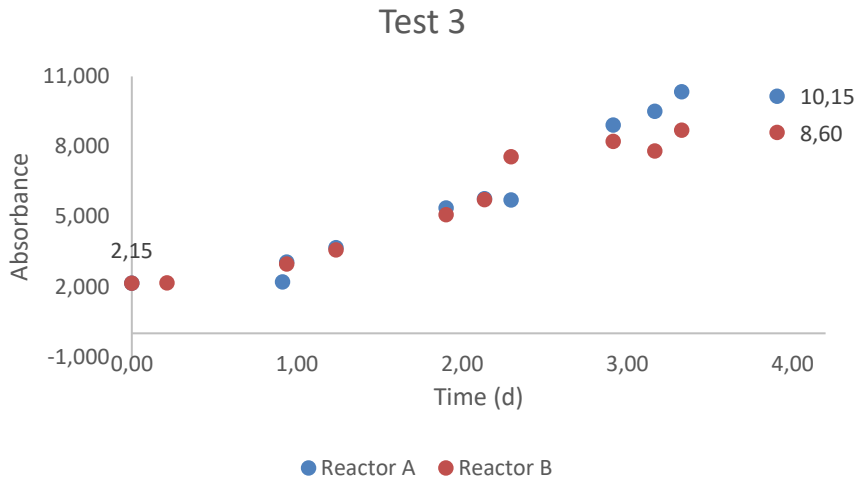

Figure 3-5. Evolution of the absorbance during the experiment Test 3

Applying Equation 8, it is getting productivity of 372.1% and 300.0% for Reactor A and Reactor B respectively.

It is made other experiments with differences are the use of the MBBM solution without NO_3 and the addition of the NH_4^+ as a nutrient. It is made this to compare the differences between Test 2 and this. The experiment is made with the second chlorella.

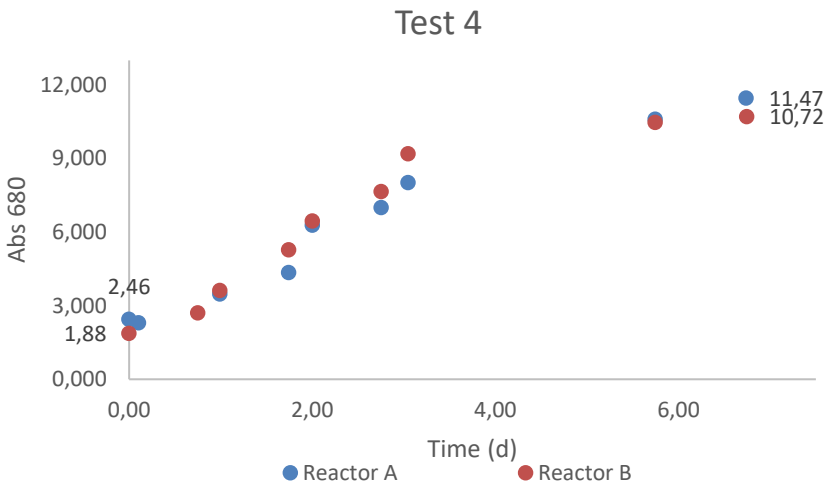

Figure 3-6. Evolution of the absorbance during the experiment Test 4

Watching the results, it is possible to compare the productivity in each other, using Equation 8. The productivity calculate is 424,3%, which is lower than the growth of Test 2.

To calculate the quantity of volume of the carbon source to add to each reactor, it is used Equation 9, using the glucose as a references COD

Equation 9

$$mL_{CS} = \frac{mL_{Glu} \cdot COD_{Glu}}{COD_{CS}}$$

Choosing the second chlorella is beginning the second part of the experiment. Now it uses one reactor with peppermint candy and the other with glucose, for the control of the experiment. During this experiment is introduced peppermint candy at the same portion of the COD of the glucose, which means it is put 5.2 mL of peppermint candy compared with the 3mL of glucose, calculated with Equation 9. In this case, it is used the MBBM without NO_3 and it is necessary to pour 1mL of NH_4^+.

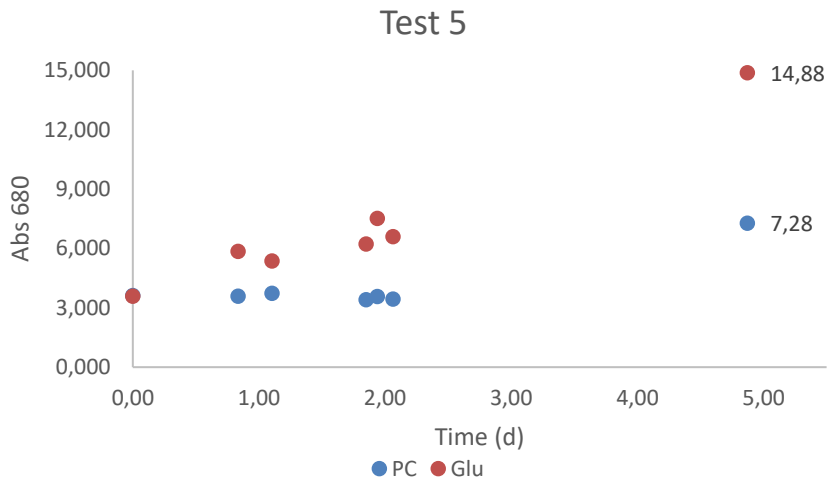

Figure 3-7. Comparison of the growth of the Chlorella Vulgaris between the peppermint candy and glucose

Watching Figure 3-7, the Chlorella Vulgaris has the best productivity with glucose in comparison with the peppermint candy. According to Equation 8, the Chlorella with glucose increases the mass by 522.1%, instead of the peppermint candy by 288.6%. This means there is a difference of 104.4% between these carbon sources.

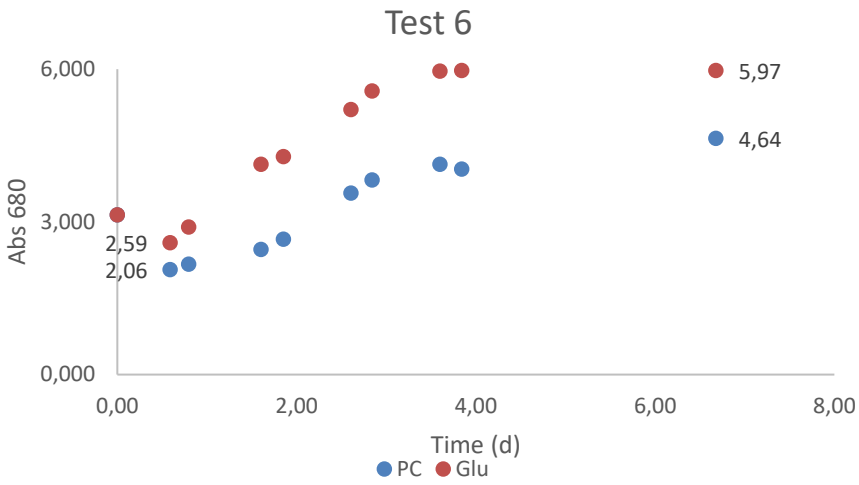

Figure 3-8. Evolution of the absorbance during the experiment Test 6

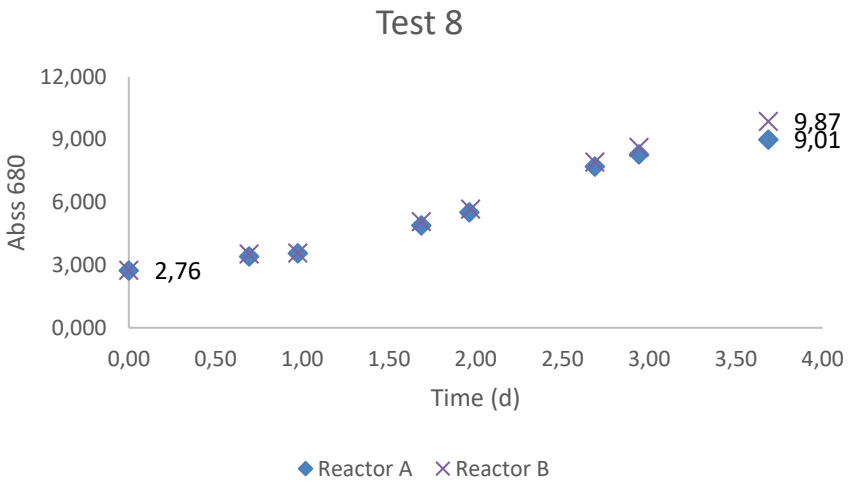

Figure 3-9. Evolution of the absorbance during the experiment Test 8 with Glucose

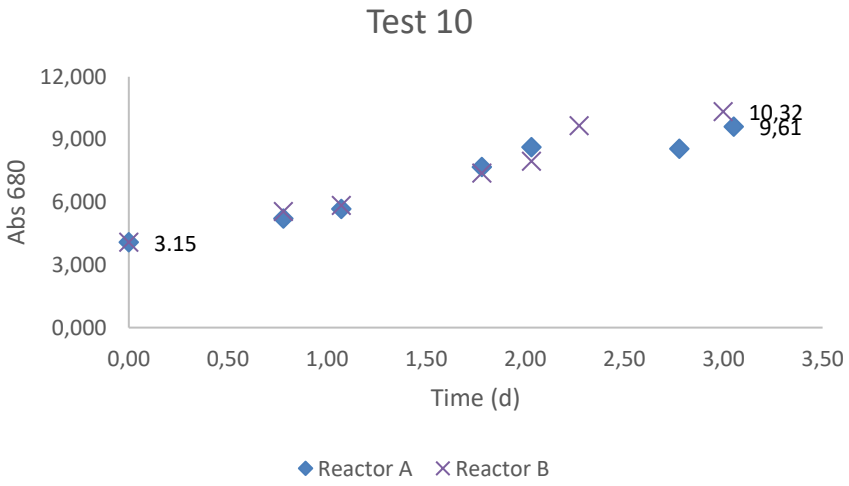

Figure 3-10. Evolution of the absorbance during the experiment Test 10 with Glucose

Figure 3-11. Growing of the Scenedesmus with BC (Reactor A) and Sugar (Reactor B)

Table 5. Summary of the results for the comparison of growth Rate

Experiment	Algae	Reactor A		Reactor B		Productivity (%)	
		Antibiotic	Carbon	Antibiotic	Carbon	Reactor A	Reactor B
Test 1	C.Sork	-	Glu	-	-	82.8	-
Test 2	C.Vul	-	Glu	X	Glu	383.6	501.9
Test 3	Chl	-	Glu	X	Glu	372.1	300.0
Test 4	C.Vul	X	Glu	-	Glu	366.3	470.2
Test 5	C.Vul	-	PC	-	Glu	102.8	314.5
Test 6	Sce	-	PC	-	Glu	125.2	130.5
Test 8	Sce	-	Glu	-	Glu	226.4	257.6
Test 10	Sce	-	Glu	-	Glu	227.6	205.1
Test 12	Sce	-	BC	-	Sug	58.2	51.9

3.3. Evolution of the growth in batch system reactor

The next test wants to demonstrate the removal of the different carbon sources by adding the same COD in each reactor. The six reactors used; are paired to check that they follow the same growth described in Table 3 with the different carbon sources.

Applying Equation 9, it is added 0.44 mL, 0.78 mL and 3.28 mL of glucose, peppermint candy and galactine to have 90 mg of COD in each Erlenmeyer. Previously, it prepared the medium with the algae. It is needed 15mg of Scenedesmus and a mix of 90 mL of MBBM and 810 mL of distilled water.

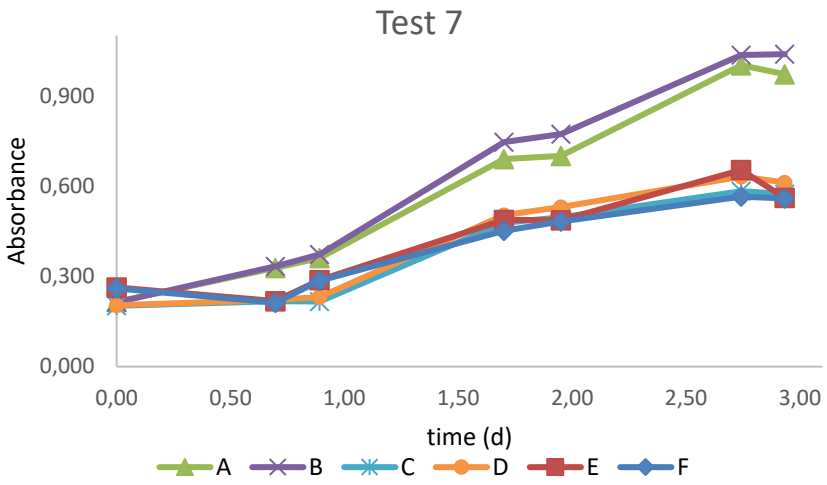

Figure 3-12. Comparison of the growth of the different carbon sources on Scenedesmus on Test 7

In Figure 3-12, the growth on each glucose medium is better than the peppermint candies and the galactine. As it can see, the Scenedesmus has the same growth as peppermint candies and galactine medium.

With the results before, it is made the same experiment with the rest of the carbon sources, to see the difference between theirs. Using Equation 9 and adding 90mg of COD, it is necessary to add 0.44mL, 0.78mL, 3.28mL, 1mL, 11.38mL and 0.93mL of glucose, peppermint candy, galactine, sugar, gumdrop, and black candy respectively on Test 9, Test 11, Test 11, and Test 13.

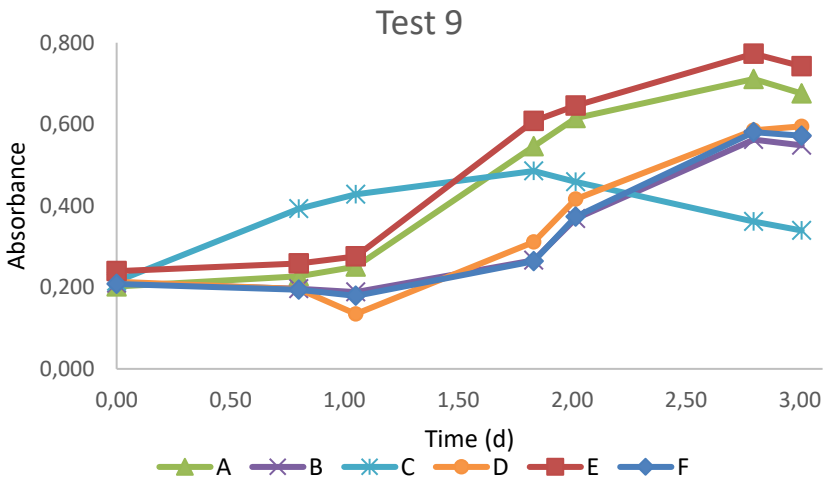

Figure 3-13. Comparison of the growth of the different carbon sources on Scenedesmus on Test 9

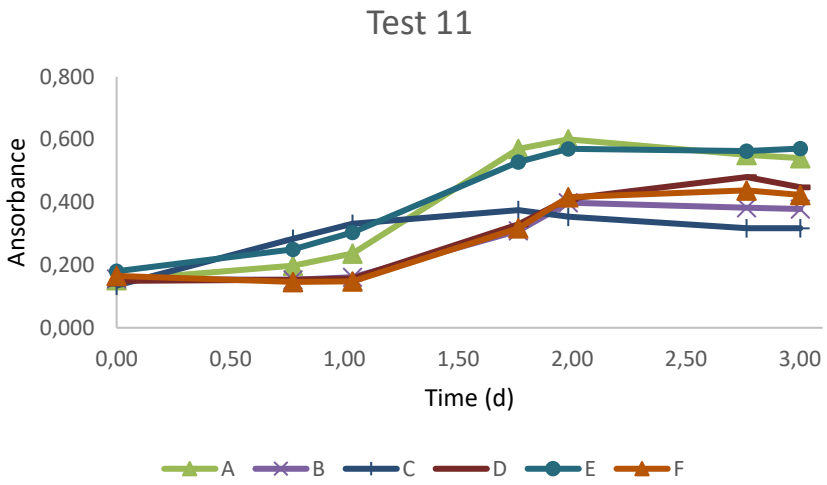

Figure 3-14. Comparison of the growth of the different carbon sources on Scenedesmus on Test 11

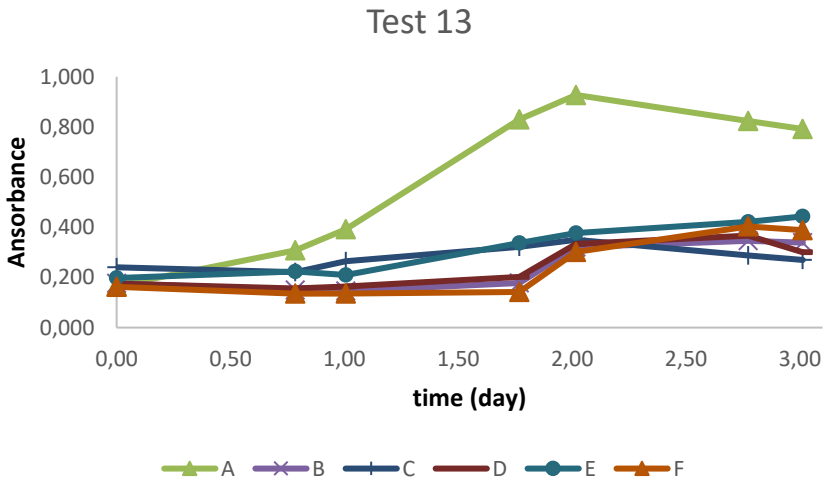

Figure 3-15. Comparison of the growth of the different carbon sources on Chlorella Vulgaris on Test 13

3.4. Specific growth rate comparison

Also, it is necessary to calculate the growth velocity. Presuming the absorbance at 750 length wave is correlated with the concentration of the cells, it is possible to calculate the velocity of growth with the absorbance.

It is necessary to compare the specific growth rate (μ). To calculate the value of the velocity it is necessary to know the concentration versus time. The method of the calculus is to obtain the slope of the straight, represented by the logarithm of the concentration of cells versus time.

Table 6. Summary of the Specifics Growth Rate for the different algae

Experiment	Algae	T	Vol	pH	Reactor A		Reactor B		Growth Rate (d^{-1})	
		ºC	L		Antibi	Carbon	Antibi	Carbon	Reactor A	Reactor B
Test 1	C.Sork	25	0.3	7	-	Glu	-	-	0.072	-
Test 2	C.Vul	25	0.3	7	-	Glu	X	Glu	0.761	0.844
Test 3	Chl	25	0.3	7	-	Glu	X	Glu	0.529	0.568
Test 4	C.Vul	25	0.3	7	X	Glu	-	Glu	0.540	0.603
Test 5	C.Vul	25	0.3	7	-	PC	-	Glu	0.259	0.227
Test 6	Sce	25	0.3	7	-	PC	-	Glu	0.435	0.336
Test 8	Sce	25	2.0	7	-	Glu	-	Glu	0.412	0.443
Test 10	Sce	25	2.0	7	-	Glu	-	Glu	0.293	0.294
Test 12	Sce	25	1.0	7	-	BC	-	Sug	0.136	0.158

3.5. Modelling of Gompertz Function

The curve is calculated by the process described in the section Processing methods. The result represented in graphics is the modelling calculated according to the data taken of N_i/N_0 versus time.

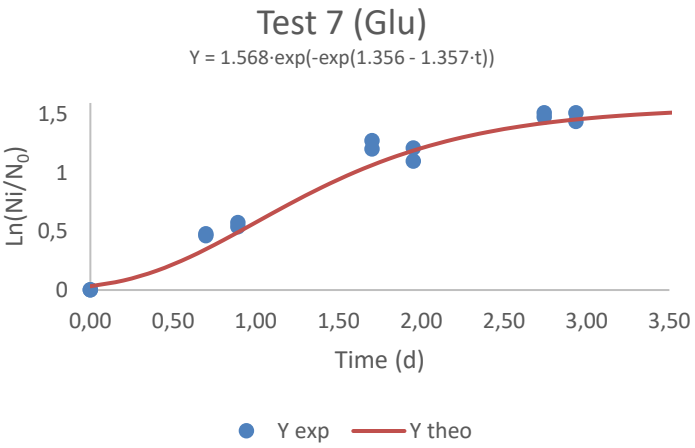

Figure 3-16. Scenedesmus algae in a batch system with Glucose

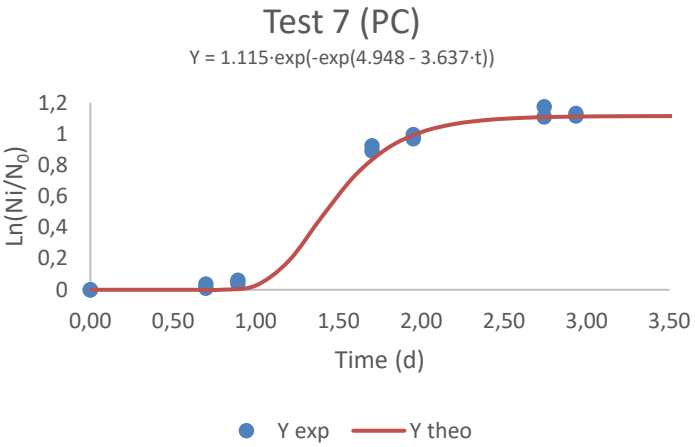

Figure 3-17. Scenedesmus algae in a batch system with Peppermint candies

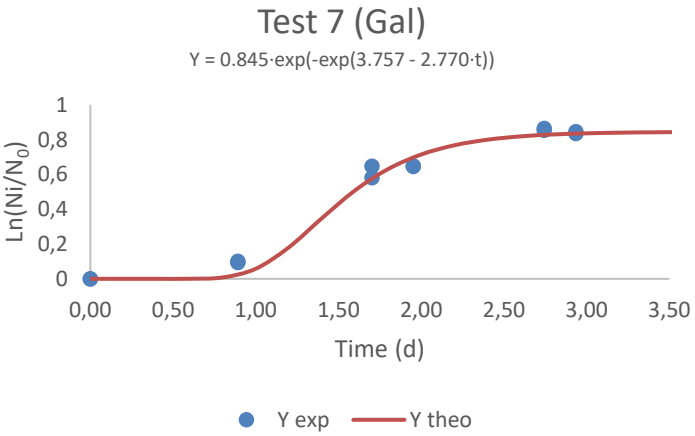

Figure 3-18. Scenedesmus algae in a batch system with Galactine

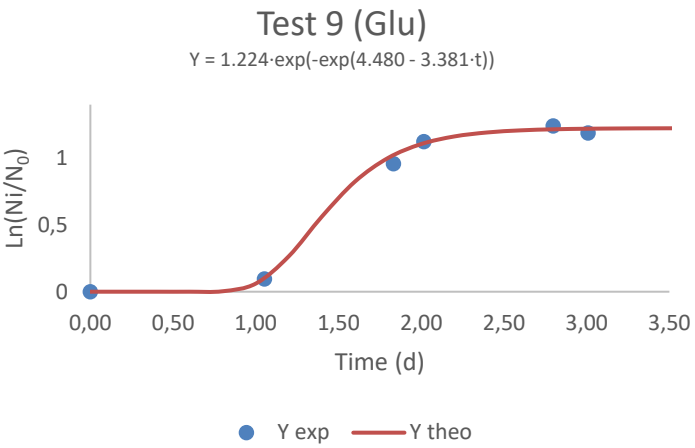

Figure 3-19. Scenedesmus algae in a batch system with Glu

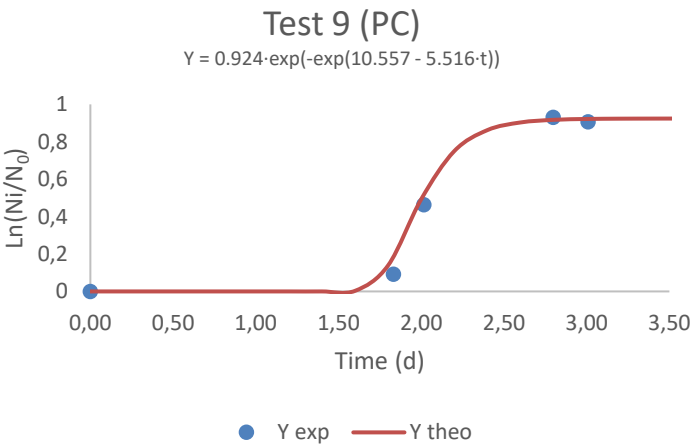

Figure 3-20. Scenedesmus algae in a batch system with PC

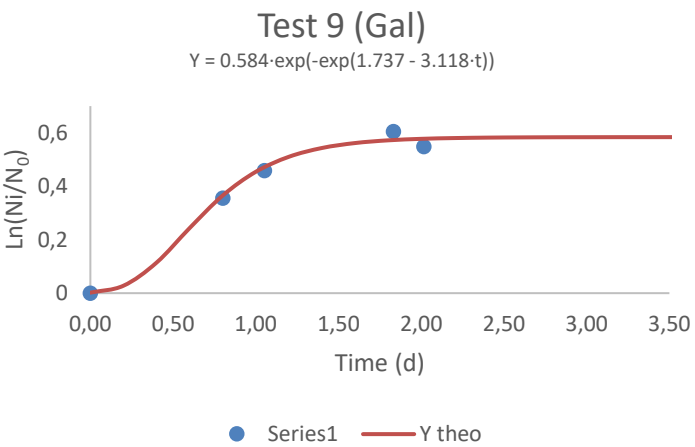

Figure 3-21. Scenedesmus algae in a batch system with Galactine

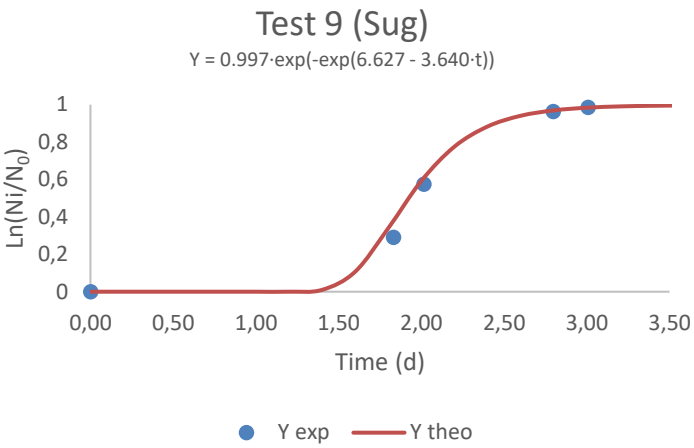

Figure 3-22. Scenedesmus algae in a batch system with Sugar

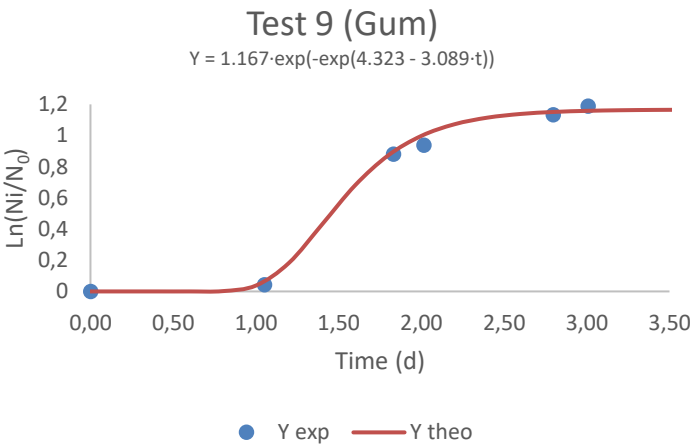

Figure 3-23. Scenedesmus algae in a batch system with Gum

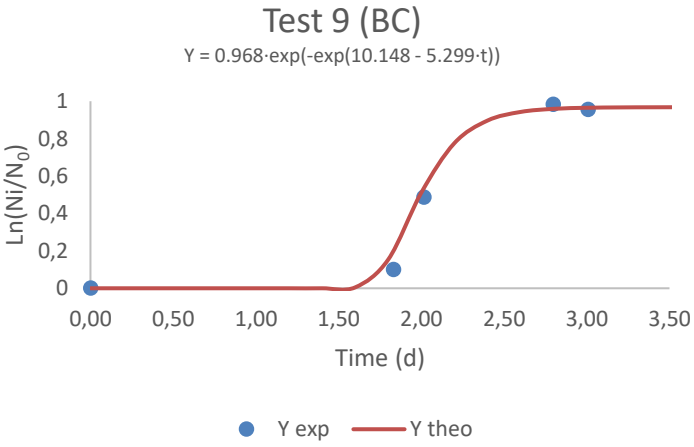

Figure 3-24. Scenedesmus algae in a batch system with BC

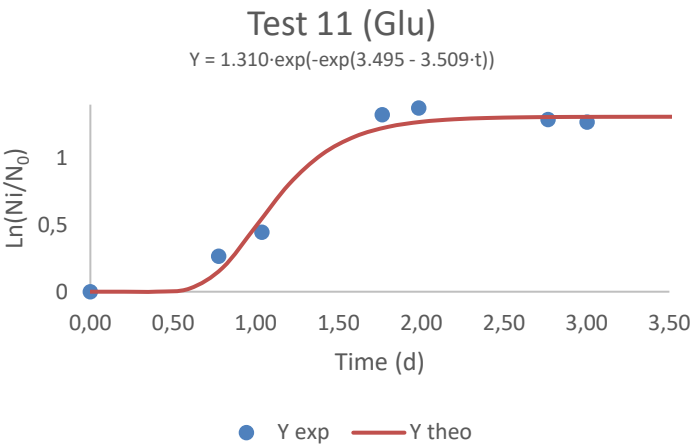

Figure 3-25. Scenedesmus algae in a batch system with Glucose

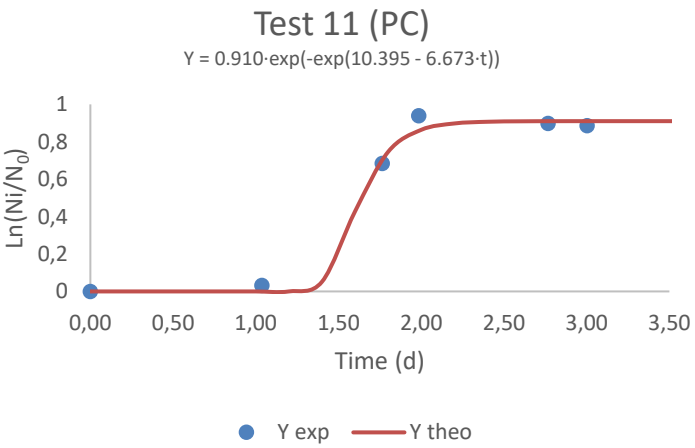

Figure 3-26. Scenedesmus algae in a batch system with PC

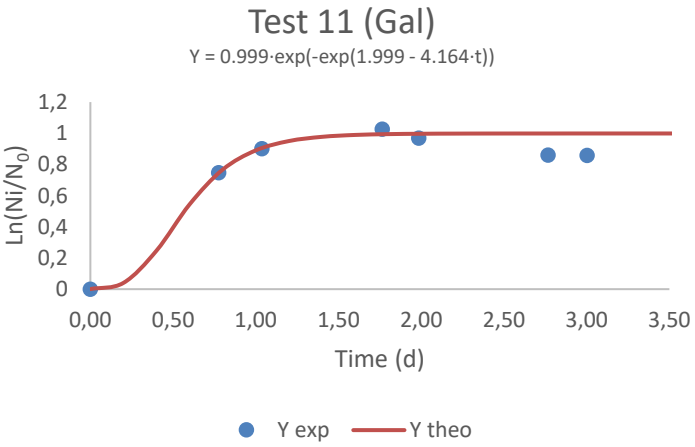

Figure 3-27. Scenedesmus algae in a batch system with Galactine

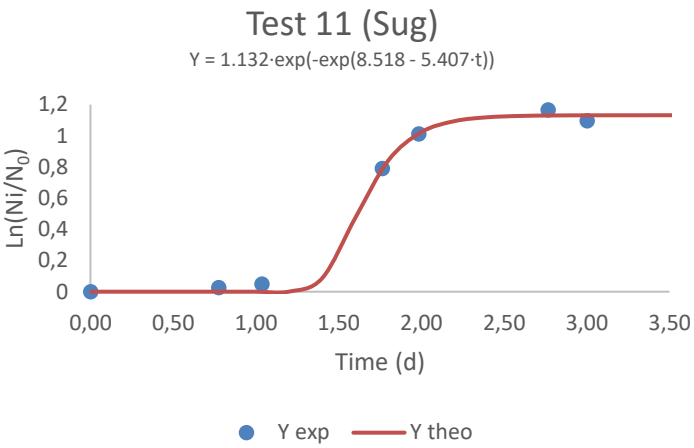

Figure 3-28. Scenedesmus algae in a batch system with Sugar

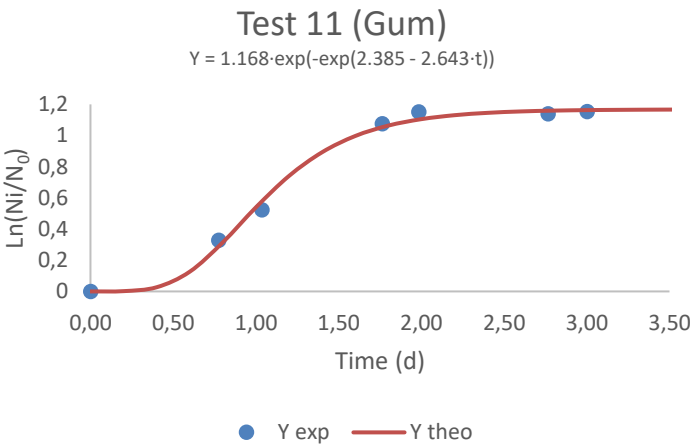

Figure 3-29. Scenedesmus algae in a batch system with Gum

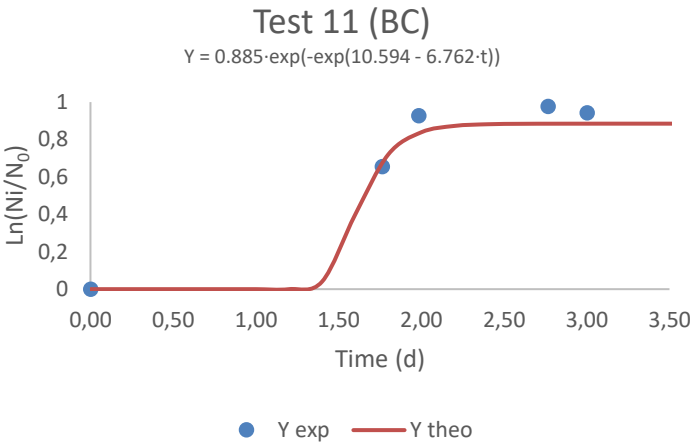

Figure 3-30 Scenedesmus algae in a batch system with BC

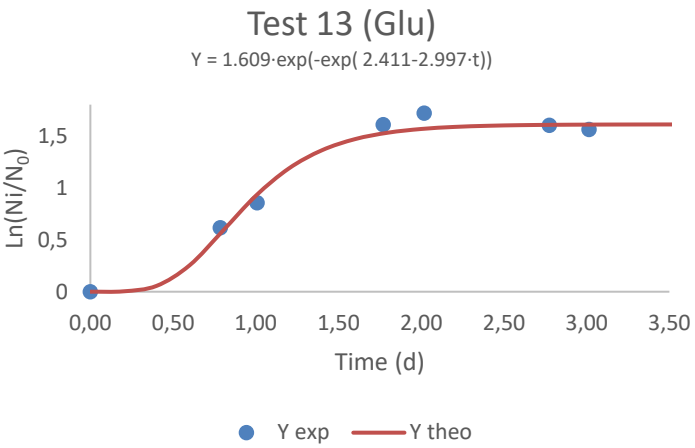

Figure 3-31. Scenedesmus algae in a batch system with Glucose

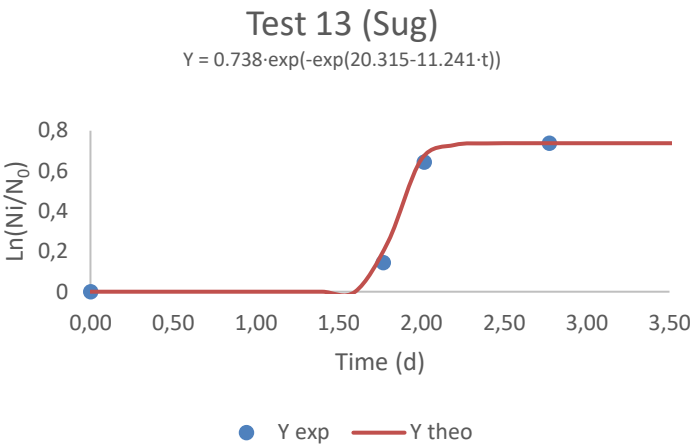

Figure 3-34. Scenedesmus algae in a batch system with Sugar

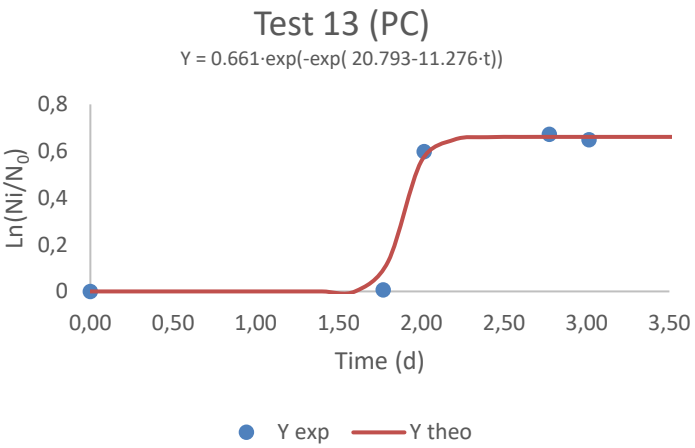

Figure 3-32. Scenedesmus algae in a batch system with PC

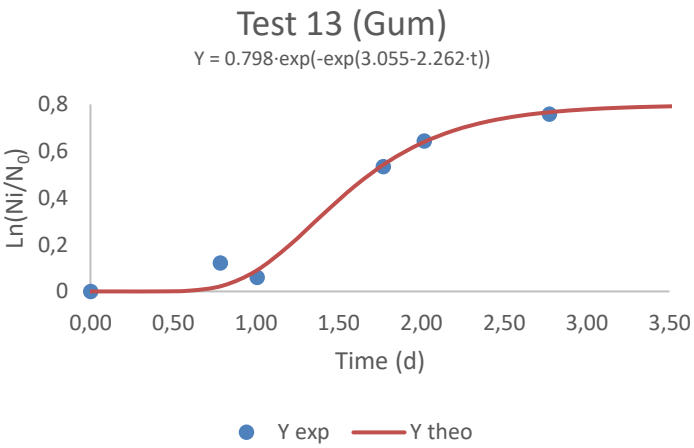

Figure 3-35. Scenedesmus algae in a batch system with Gum

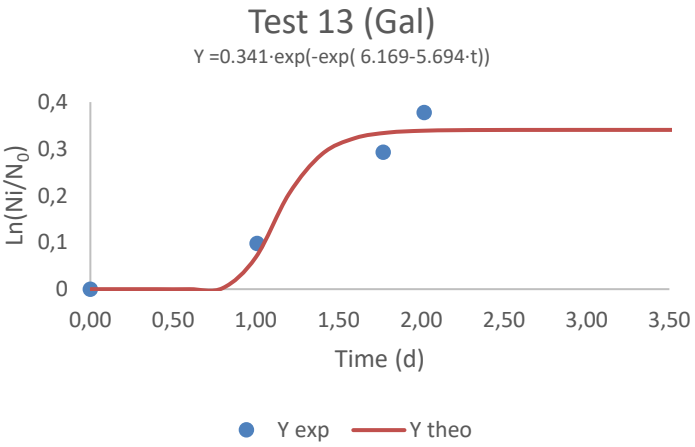

Figure 3-33. Scenedesmus algae in a batch system with Galactine

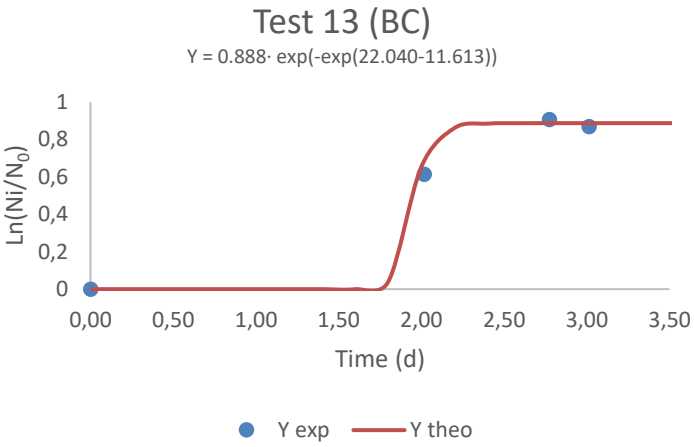

Figure 3-36. Scenedesmus algae in a batch system with BC

3.6. Evolution of the Growth Rate with the temperature

For this experiment, it is made some tests to calculate the constants which describe the exponential growth for different temperatures. The method to calculate the constant is described in the section Exponential function calculus. They are described like Equation 7. Figure 3-37, it can see the curves for the different temperatures.

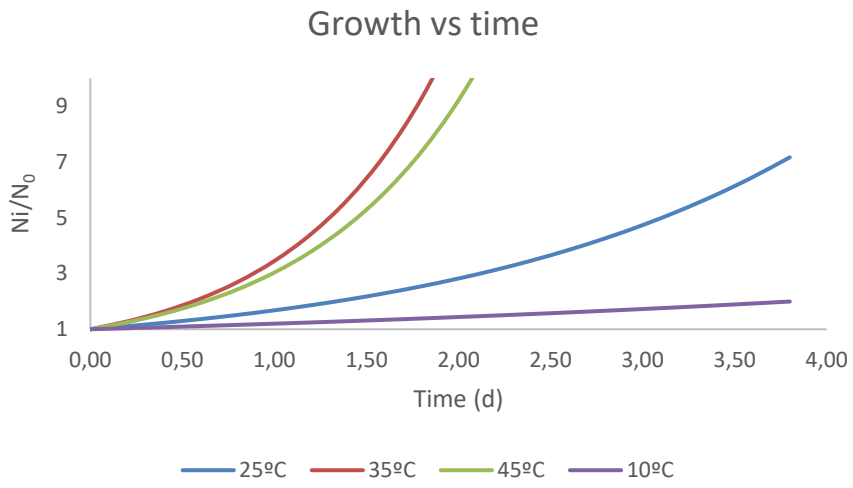

Figure 3-37. Theoretical exponential growth for different temperatures in Scenedesmus algae

Also, it can calculate the curve which relates the specific Growth Rate with the temperature.

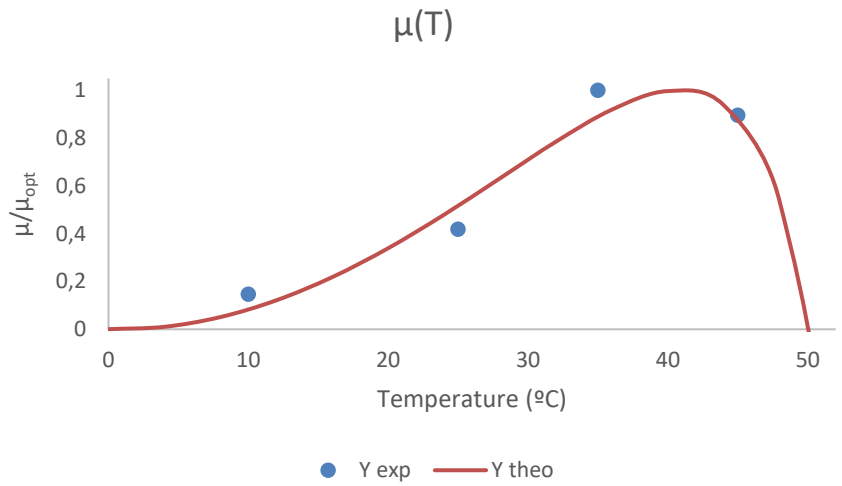

Figure 3-38. Relationship between the Specific Growth Rate with the temperature in Scenedesmus algae

The temperatures obtain (T_{min}, T_{opt} and T_{max}) with the process described in section 2.3.10 A theoretical model for the Specific growth rate with the temperature

T_{min} ºC	T_{opt} ºC	T_{max} ºC
0.75	40.81	50.06

To check these results is made the measure of the removal of oxygen at different temperatures, and the values of the slopes of the lines will be obtained and compared.

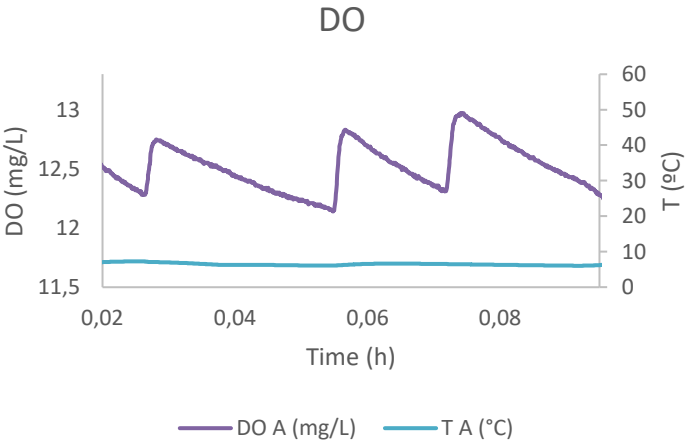

Figure 3-39. DO consumption at 5 ºC

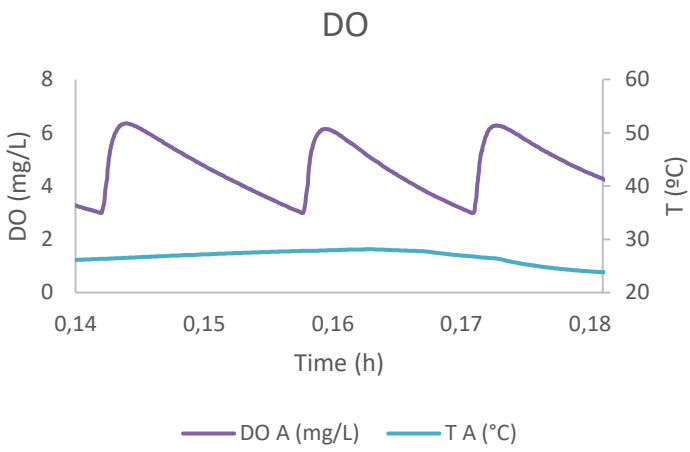

Figure 3-41. DO consumptions at 25ºC

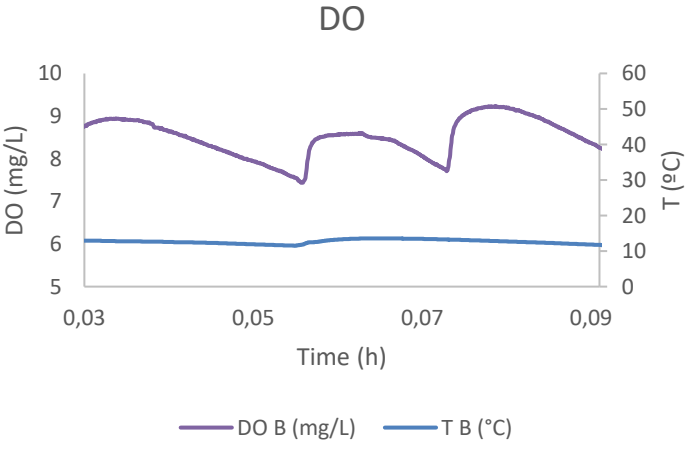

Figure 3-40. DO consumption at 10ºC

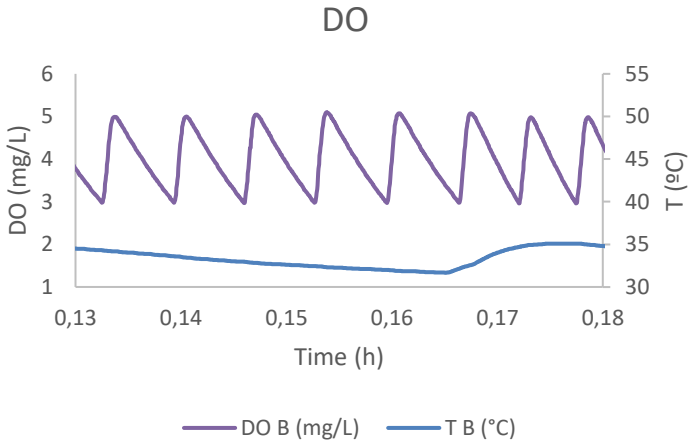

Figure 3-42. DO consumption at 30ºC

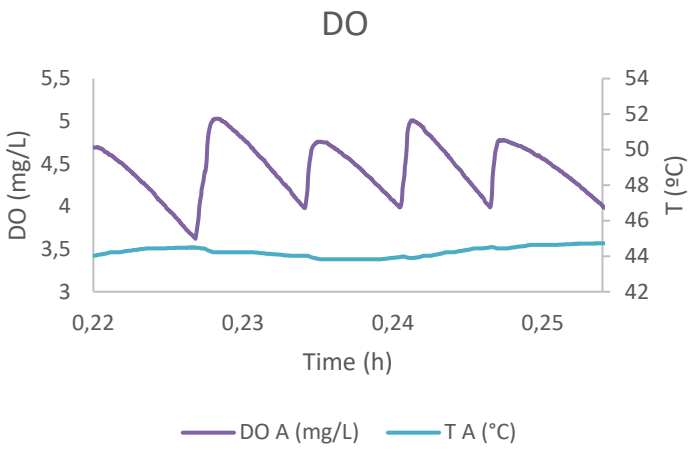

Figure 3-43. DO consumption at 45ºC

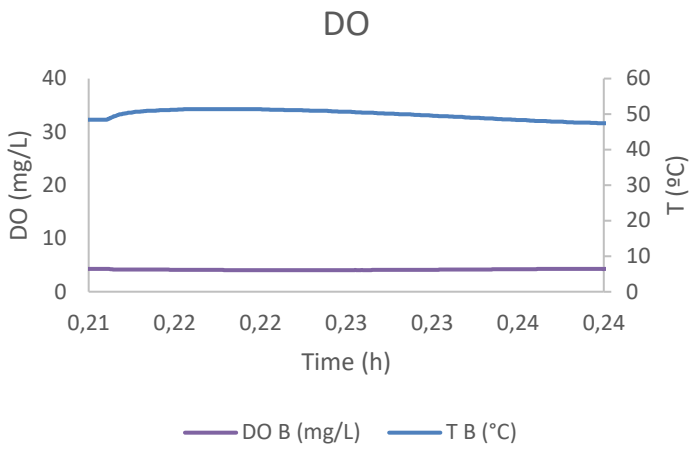

Figure 3-44. DO consumption over 50.05ºC

Table 7. Summary of the consumption of oxygen

$T_{average}$ ºC	OUR mg/L/h
6.4	31.07
12,7	121,34
27.7	309.24
32.7	391.21
43.8	161.19
50.1	0.01

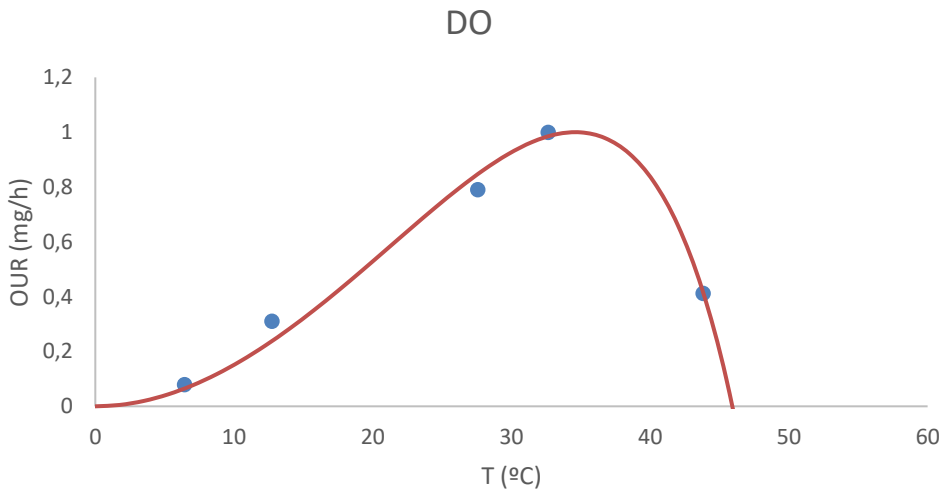

Figure 3-45. Consumption of oxygen at different temperatures

The temperatures are diverse in the experiment of the Specific Growth because the solution of the oxygen is a parameter which depends on the temperature.

T_{min} ºC	T_{opt} ºC	T_{max} ºC
0.00	34.61	45.92

These values are calculated with the process described in 2.3.10 A theoretical model for the Specific growth rate with the temperature.

3.7. kLa results at different rpm, volumes, and conditions

The representations of the results pretend to compare the differences between the air and agitation. The experiments are summarized in Table 2.

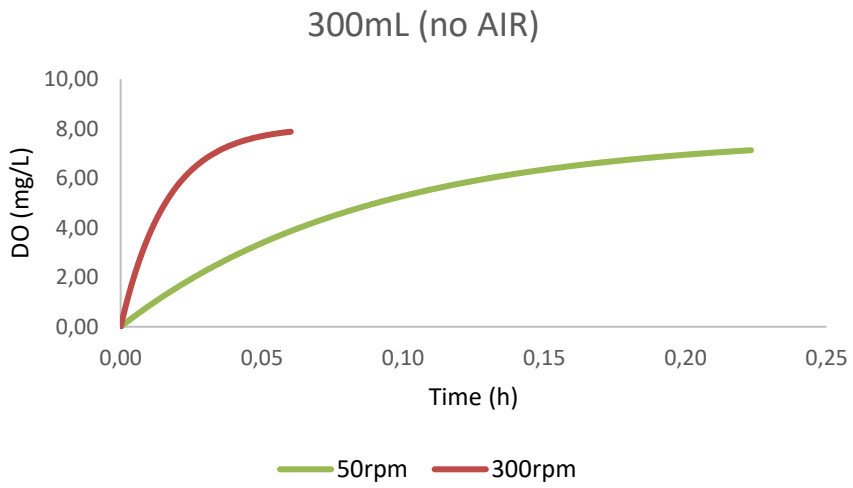

Figure 3-46. k_La differences between the 50rpm and 300rpm in 300mL with agitator

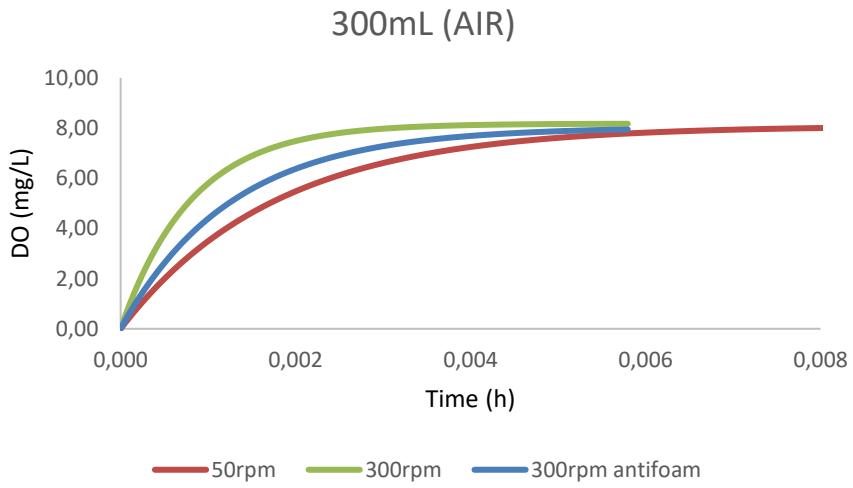

Figure 3-47. k_La differences between the 50rpm and 300rpm with and without antifoam in 300mL with agitator and air

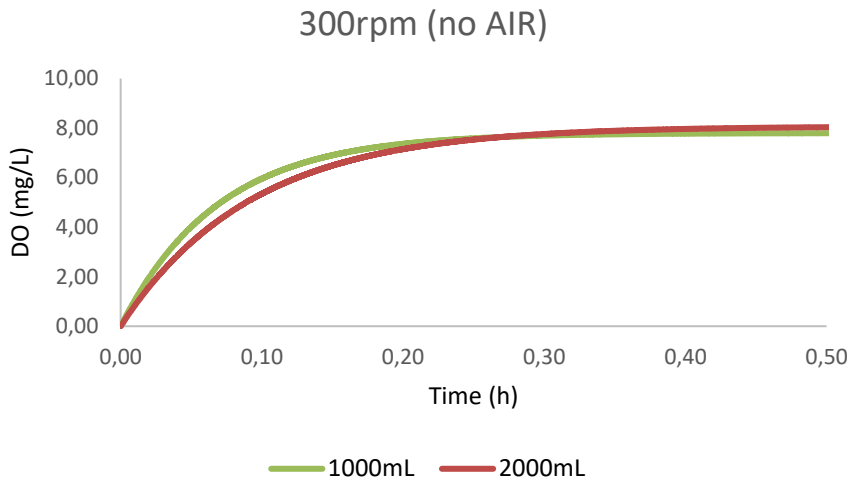

Figure 3-48. k_La differences between the 1000mL and 2000mL with 300rpm with agitator

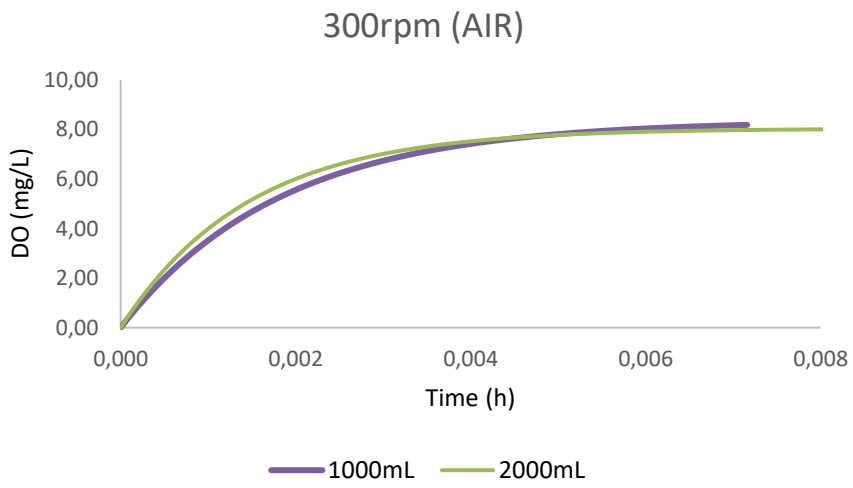

Figure 3-49. k_La differences between the 1000mL and 2000mL with 300rpm with agitator and air

Table 8. Summary of the results of k_La

Volume mL	Velocity rpm	Air -	Antifoam -	k_La $kgO_2/h/m^3$
300	50	No	No	11.51
300	50	Yes	No	565.21
300	300	No	No	60.80
300	300	Yes	No	1231.95
300	300	Yes	Yes	781.96
1000	300	No	No	14.44
1000	300	Yes	No	547.91
2000	300	No	No	10.85
2000	300	Yes	No	686.12

4. Conclusion

According to the first experiments, the best algae with glucose is the Chlorella Vulgaris because it has the best productivity and Specific Growth Rate of the other Chlorella algae. However, when it changed the Carbon Source with other candies like peppermint candy or Galactine the response is not an optimal value. Instead, the Scenedesmus algae have a better response with the other candies, but it is significative minor than the Chlorella Vulgaris as they don`t have a longer delay time in a Semi-Batch System in a Laboratory Scale.

Later, in the following experiments with Scenedesmus, the optimal values of Specific Growth Rate and OUR with temperature are studied, and the different results are obtained due to the incidence of temperature to the dissolution of oxygen in the medium. It is obtained and the maximum, minimum, and optimal value of the temperature in each case

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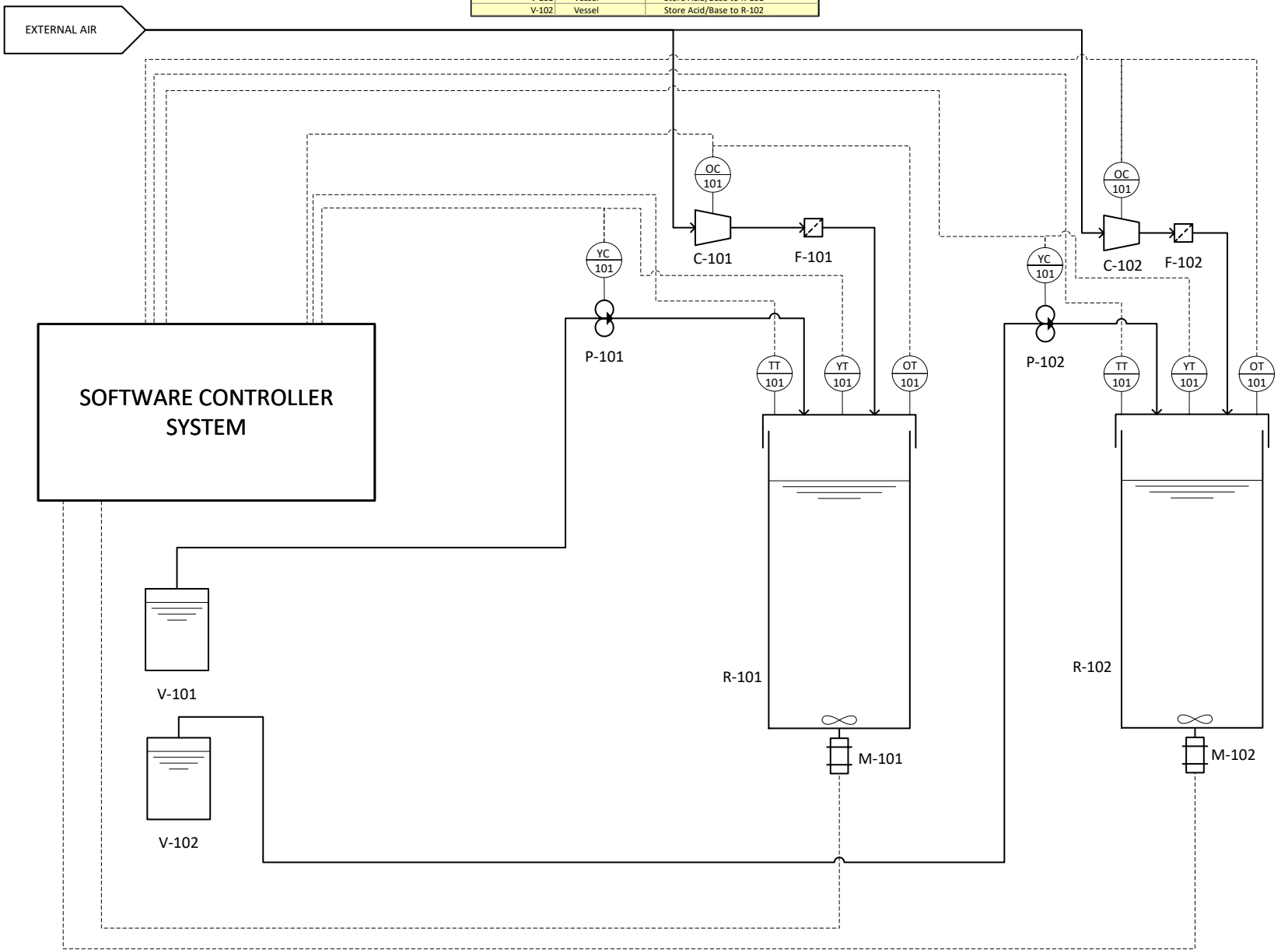
APPENDIX I

List Equipment		
Name	**Equipment**	**Descripción**
C-101	Compressor	Manage the air on R-101
C-102	Compressor	Manage the air on R-102
F-101	Filter	Filtre of the air to R-101
F-102	Filter	Filtre of the air to R-102
M-101	Magnetic Motor	Stirrer R-101
M-102	Magnetic Motor	Stirrer R-102
P-101	Pump	Dosage Acid/Base to R-101
P-102	Pump	Dosage Acid/Base to R-102
R-101	Reactor	Biological Reactor A laboratory scale
R-102	Reactor	Biological Reactor B laboratory scale
V-101	Vessel	Store Acid/Base to R-101
V-102	Vessel	Store Acid/Base to R-102



NOTES

OT- Oxigen Transmitter
OC- Oxygen Controller
YT- pH Transmitter
YC- pH Controller

REV	DESCRIPTION	BY	DATA
0	Reactor System	DAA	08/08/2022

LISENSED TO

LISENSOR

PLANT TITTLE
Distribution of the reactor System in the laboratory

DIAGRAM TITTLE
P&ID: Reactor System

GROUP

NAME
A4-PI-101-1

UNIT	PAGE	REV
1	1 of 1	0