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Kinetic of the Sewage Treatment: The Consumption of Organic Carbon of The Microalga *Chlorella sp*.

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As well known, microalgae are eukaryotic or procaryotic microorganisms able to photosynthesize, namely transforming inorganic substrates and sun light into organic compounds and chemical energy. They result very promising in treating civil wastewaters thanks to their ability to employ nitrates and phosphates as nutrients (Lima et al., 2019). Autotrophic microalgae are, anyway, not useful in decreasing the organic carbon content of wastewaters, and for this reason, they cooperate with heterotrophic bacteria. The usefulness of microalgae-bacteria consortia in treating wastewaters and the ratio of their inoculum was investigated in a previous work (Lima, 2022a). Contrarily to autotrophic microalgae, mixotrophic microalgae are able to decrease the organic content of the matrix in which they are grown. In this work, we preliminarily investigated the capability of the autochthonous microalga *Chlorella sp*. CW2 to grow in mixotrophy and decrease the organic content of the artificial wastewater in which they are grown. Several batch cultivations were performed with glucose in different concentrations. Kinetic parameters were obtained and employed to determine the dilution rate (D) ideal for the abatement of glucose from the artificial wastewater.

1. Introduction

Microalgae are photosynthetic microorganisms which represent an alternative biomass to land-plants because of their high productivity and ability to grow in wastewaters. They are able to effectively removing nitrogen and phosphorous from wastewaters when cultivated in autotrophy; otherwise, when cultivated together with heterotrophic bacteria their ability to decrease the Chemical oxygen demand (COD) increases (Lima, Brucato, Caputo, Grisafi, et al., 2022). When cultivated in mixotrophy, microalgae may instead exploit the carbon source of the culture medium and simultaneously use the light as energy source. In this way algae may decrease the organic carbon of the sewage, and therefore abate COD and total organic carbon (TOC). It is well known that the use of microalgae in wastewater treatment has several advantages, such as the removal of pollutants such as ammonia (NH_4^+), nitrates (NO_3^-) and phosphates (PO_4^{3-}) and also of heavy metals, oxygen production that can be used by the heterotrophic bacteria to degrade compounds present in the matrix and CO_2 removal. There are several examples of lab-scale and pilot-scale outdoor cultivation of microalgae with the aim of treating wastewaters; the practice is anyway not widespread on higher scale because of the lack of reliable and reproducible parameters that strongly affect growth, nutrient abatement and, consequently, the industrial scale-up of the systems. For the industrial treatment of civil sewage, normally outdoor reactors called raceway ponds are employed. In order to design and appropriately size the raceway reactors, some kinetic parameters are needed, such as the specific grow factor μ and the substrate constant K_S (Doran, 2013). These are normally obtained from batch data, by measuring the concentrations of the microorganisms and that of the nutrient of interest. In this work, a synthetic wastewater was designed and characterized to be employed for the cultivation of an autochthonous strain of the microalga *Chlorella sp* CW2, previously isolated from wastewaters and molecularly characterized. The microalga was cultivated in several batches with growing glucose concentrations 2.2 mg L^{-1}, 6.7 mg L^{-1},12.6 mg L^{-1}, 22.2 mg L^{-1}, 40.0 mg L^{-1}, 89.3 mg L^{-1}, 210.2 mg L^{-1} and 250.0 mg L^{-1} with the aim of calculating the growth rate of each cultivation. The result showed that the growth rate increased together with glucose concentration.

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The kinetic parameters were estimated through a Langumuir Plot, as suggested by Doran (Doran, 2013). Furthermore the decrease of glucose was followed in each cultivation, with the result that the alga was able to employ each of the tested glucose concentration and that the rate of decrease is independent from the employed concentration.

2. Material and Methods

2.1 Microalgal growth

Microalgae *Chlorella sp. Pozzillo*, previously isolated, was grown in a synthetic wastewater with the following composition: glucose 221.7 mg L^{-1}, NH_4Cl 153 mg L^{-1}, $NaNO_3$ 160 mg L^{-1}, $CaCl_2{\cdot}(2H_2O)$ 165 mg L^{-1}, Na_2SO_4 63.37 mg L^{-1}, K_2HPO_4 56.37 mg L^{-1}, $NaHCO_3$ 200 mg L^{-1}, EDTA (Na_2 salt) 6.37 mg L^{-1}, $FeCl_3$ (6 H_2O) 4.85 mg L^{-1}, $CuSO_4$ (5 H_2O) 0.02 mg L^{-1}, $ZnSO_4$ (7 H_2O) 0.03 mg L^{-1}, $CoCl_2$ (6 H_2O) 0.02 mg L^{-1}, $MnCl_2$ (4 H_2O) 0.28 mg L^{-1}, Na_2MoO_4 (2 H_2O) 0.01 mg L^{-1}.
A pre-culture for each sample was set up by inoculating 10 mL of sample from a stock culture in 100 mL of synthetic sewage. When cells were in late exponential phase (after about 8 cultivation days), 10 mL of the cell suspension were used to inoculate the sewage. 150 mL of culture were grown in 250 mL Erlenmeyer flasks placed in an oscillating incubator (Corning Lse) under a 200 µmol m^{-2} s^{-1} photon flux at 27°C. Light intensity was measured with a Delta Ohm-HD 9021 quantometer equipped with a Photosynthetic Active Radiation (PAR) probe (Delta Ohm LP 9021 PAR). The algae were cultivated for 3 days. The concentration of the microalgal suspension was monitored several times a day by measuring the absorbance at 750 nm in a spectrophotometer (Cary 60 Uv-vis, Agilent technologies). An experimental correlation between the dry weight (DW) and the optical absorbance at 750 nm was obtained as follows:

$$X = 0.2315 * Abs_{750\ nm} + 0.0264 \quad (1)$$

Where X is the concentration of microalgae in g L^{-1} and Abs_{750nm} is the absorbance at 750 nm. The R^2 was 0.9897. In order to obtain the kinetic parameters, microalgae were grown into the synthetic growth medium with different glucose concentrations: 2.2 mg L^{-1}, 6.7 mg L^{-1},12.6 mg L^{-1}, 22.2 mg L^{-1}, 40.0 mg L^{-1}, 89.3 mg L^{-1}, 210.2 mg L^{-1} and 250.0 mg L^{-1}.

2.2 Analysis on the synthetic sewage

For the measurement of pH and conductivity, a pHmeter equipped with a conductivity probe was employed (CRISON MM 41).The content of carbon and in particular Inorganic Carbon (IC) and Non-Purgeable Organic Carbon (NPOC), was assessed by a TOCL CSH/CSN analyzer Shimadzu.
The content of glucose was analysed through HPLC. The suspension was filtered through 0.2 µm membranes (CA, Millipore) and sugars were analyzed by means of a HPLC Dionex UltiMate 3000 equipped with a column Rezex ROA – Organic acid H+ operating at 60°C and using 0.6 mL/min of a 5 mM H2SO4 aqueous solution as eluent.

2.3 Determination of the kinetic parameters

To obtain the kinetic parameters of carbon consumption, μ_{max} and K_s, of the species *Chlorella sp*. grown into shaking flasks under different glucose concentrations, an experimental approach for recovering kinetic parameters from batch data was applied. The specific growth rate µ may be expressed by the simple equation:

$$\mu = \frac{\mu_{max}\, S}{K_s + S} \quad (2)$$

where S is the concentration of glucose, and μ_{max} and K_s are the kinetic parameters. This equation is called Monod Equation and describes the relation between the concentration of limiting substrate and the specific growth rate µ.The specific growth rate µ was calculated while the cells were in exponential phase, by using the following expression:

$$\mu = \frac{\ln X - \ln X_0}{t - t_0} \quad (3)$$

where X_0 is the biomass concentration at time t_0 and X is the biomass concentration at time t.
The kinetic parameters were estimated through a Langmuir Plot (Lima et al., 2022b). To obtain the kinetic parameters, μ_{max} and K_s, Equation (2) can be rewritten according to Langmuir (Doran, 2013) as:

$$\frac{S}{\mu} = \frac{1}{\mu_{max}}\, S + \frac{K_s}{\mu_{max}} \quad (4)$$

A Langmuir plot of S/µ versus S should give a straight line, the kinetic parameters were obtained from its slope ($1/\mu_{max}$) and intercept (K_s/μ_{max}). In order to obtain the kinetic parameters of the Monod equation, batch cultivations were carried out in the synthetic sewage with different glucose concentration: 2.2 mg L^{-1}, 6.7 mg L^{-1},12.6 mg L^{-1}, 22.2 mg L^{-1}, 40.0 mg L^{-1}, 89.3 mg L^{-1}, 210.2 mg L^{-1} and 250.0 mg L^{-1}.

3. Results and discussion

3.1 Analysis of the synthetic sewage

The synthetic sewage was analysed for its content of organic and inorganic carbon, pH and conductivity. In Table 1 the characteristics of the synthetic wastewater are reported.

Table 1: Characteristics of the synthetic sewage

pH	conductivity	NPOC	IC	N:P	NH_4^+:NO_3^-
8.1	1157.58 mS cm^{-1}	87	33	14.64	1.53

The synthetic sewage was designed to simulate the composition of an average civil sewage after the primary treatment, in which there is the removal of suspended solids. Several studies were taken into account for its realization (Ji et al., 2018; Tianle Li et al., 2022; Rani & Ojha, 2021) and the sewage parameters are in the range of what previously suggested. There is a prevalence of nitrogen over phosphorous, that is in fact often the limiting nutrient in oxidation ponds. There is also a slight prevalence of ammonium nitrogen over nitrate nitrogen. The synthetic sewage is enriched with a mix of micronutrients normally present in microalgal growth media added for avoiding nutritional deprivations in their growth.

3.2 Kinetic parameters

In order to obtain the kinetic parameters of the Monod equation, batch cultivations were carried out in the synthetic sewage with different glucose concentration: 2.2 mg L^{-1}, 6.7 mg L^{-1},12.6 mg L^{-1}, 22.2 mg L^{-1}, 40.0 mg L^{-1}, 89.3 mg L^{-1}, 210.2 mg L^{-1} and 250.0 mg L^{-1}. The growth of microalga *Chlorella sp*. at 27°C and with different glucose concentration is shown in Figure 1.As it can be seen, the growth is related to the glucose concentration. The slowest growths are shown with the lowest glucose concentrations of 2.2 and 6.7 mg L^{-1} and it gradually increases with increasing glucose concentration. The highest reached concentration is observed with the concentration of 250 mg L^{-1} of glucose.

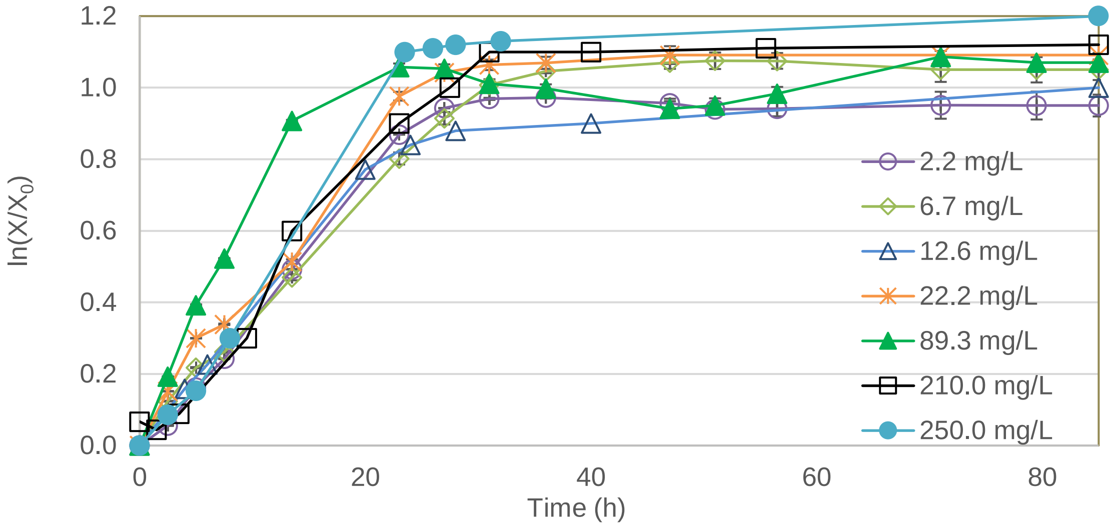

Figure 1: Growth of the microalga Chlorella sp. *at 27°C in shaking flasks with different glucose concentrations.*

By employing a linear regression from data in the exponential phase of Figure 1, the specific growth rates were calculated by using Equation 3 and reported in Table 2, together with the R^2 values and the relevant S/µ. It is possible to observe that the growth rates are growing together with the glucose concentration. The observed growth rate were similar to what was previously observed for *Chlorella sp* growing into sewage (Wang et al., 2010).

In Figure 2, a Langmuir Plot is shown, obtained by plotting the Equation (4). From the intercept and the slope of the linear regression, the kinetic parameters μ_{max} (0.047 h^{-1}) and K_s (2.06 mg L^{-1}) are obtained. The kinetic constants were applied in Equation 2 and plotted in Figure 3; the typical Monod curve can be therefore observed. The substrate constant K_s is the value at which the growth rate is the half of the maximal. This value is very small, in the order of mg L^{-1} as typical for carbohydrate substrates (Doran, 2013). This means that the microalga *Chlorella sp* CW2 is able to use even very small concentrations of glucose, and that the growth rate μ is influenced by glucose concentration even if it quickly approaches the maximum growth rate μ_{max} for glucose concentration larger than Ks.

The calculated maximum growth rate is similar to what found previously by other authors, for example in exponential phase for *Chlorella sp*. growing in raw centrate (Y. Li et al., 2011).

From the observed data, it can be concluded that in a continuous system employing the microalga *Chlorella sp.* CW2 and a similar wastewater than the one employed here, the dilution rate, defined as

$$D = \frac{F}{V} \tag{5}$$

Where F is the inlet flow inside the reactor and V the volume of the reactor, cannot exceed the value of μ_{max}, 0.047 h^{-1} in order to avoid the washing out (Doran, 2013). It is also evident that the ability to removing organic carbon is very high, as the K_s is very small.

Figure 4 shows the consume of glucose vs time when starting from different glucose concentrations. The results confirm that microalga *Chlorella sp. CW2* is able to employ the glucose at all the tested concentrations. The removal occurs already in the first hours after the inoculum. A similar trend was observed before (Tingting Li et al., 2022). Microalga *Chlorella sp.* is therefore able to decrease the organic content of the synthetic sewage when growing in mixotrophy whatever is the employed glucose concentration. It is worth noting that the real wastewaters have more complex carbon sources compared to simple glucose solutions and this may result in decreased ability to abate the COD/TOC, therefore further experiments should be done to assess the carbon consume in real wastewaters.

Table 2: Values of specific growth rates and R^2 calculated with different glucose concentrations and a fixed illumination at 27°C in Chlorella sp.

S (mg L^{-1})	μ (h^{-1})	S/μ	R^2
2.2	0.0333	66.6	0.99
6.7	0.0352	188.9	0.95
12.6	0.0381	331.1	1.00
22.2	0.046	482.0	0.95
40.0	0.046	869.6	0.99
89.3	0.046	1941.6	0.99
210.2	0.0463	4540.1	0.99
250.0	0.048	5208.3	0.99

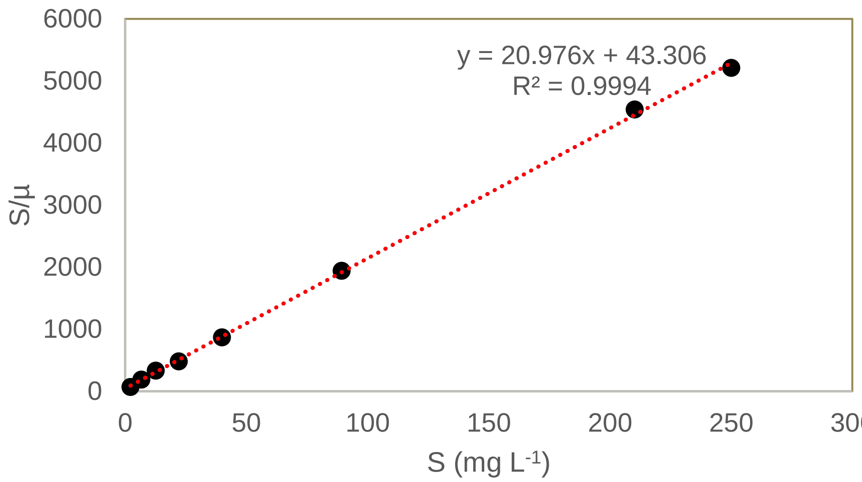

Figure 2: "Langmuir Plot" of experimental values obtained with different glucose concentrations.

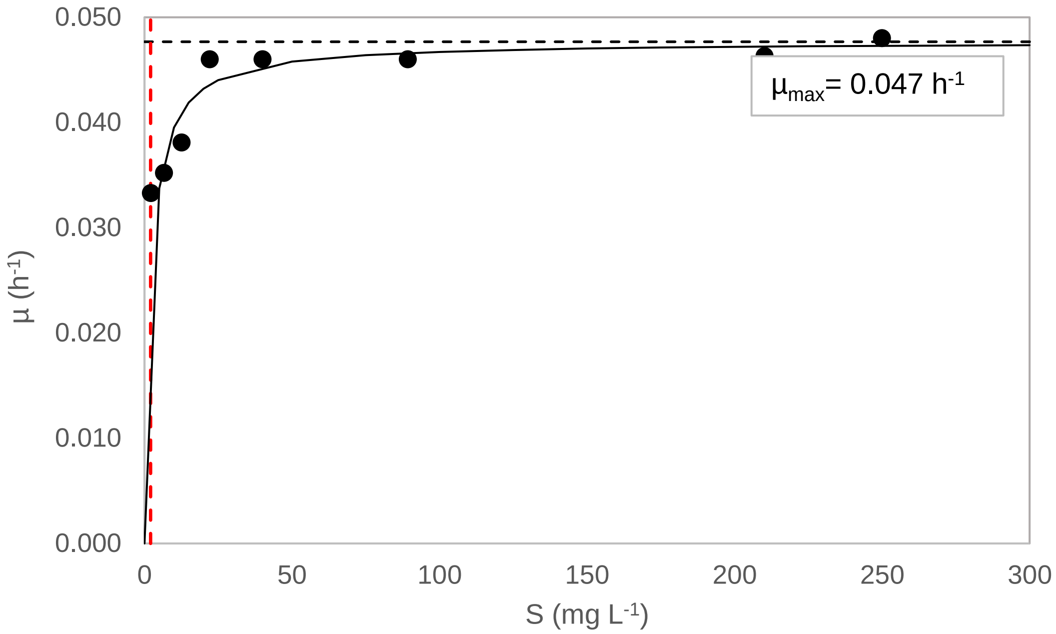

Figure 3: Specific growth rate versus different irradiation intensities of the microalga Chlorella sp. *Black circles, experimental data; line, Equation (2).*

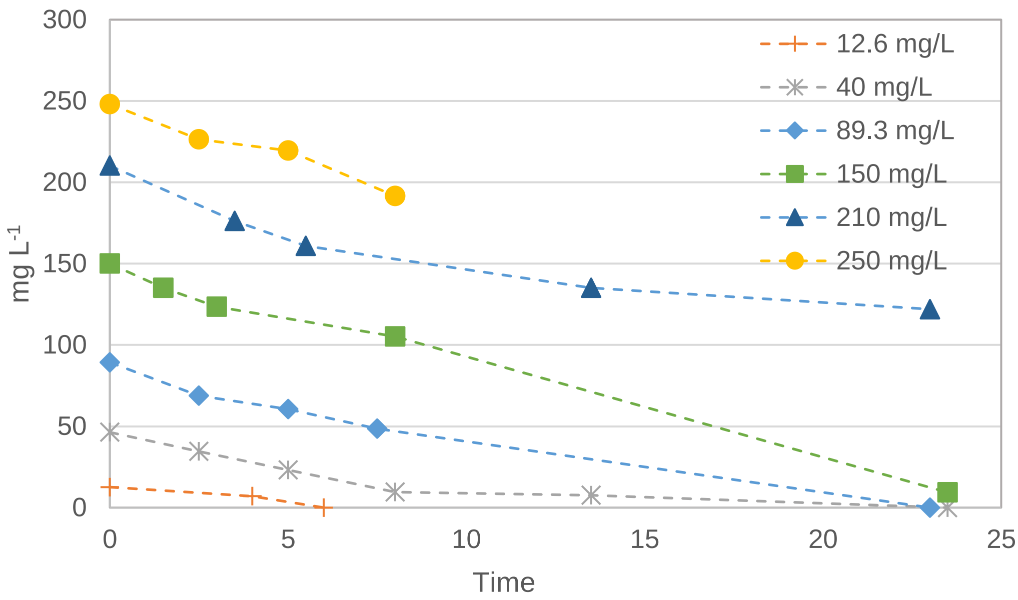

Figure 4: Kinetic of the removal of glucose from synthetic wastewater with different initial concentration of glucose.

4. Conclusions

In this work, a synthetic wastewater was designed and characterized, showing typical features of civil sewages. The wastewater was employed for the cultivation of an autochthonous strain of the microalgae *Chlorella sp* CW2. The microalga was cultivated in several batches with growing glucose concentrations with the aim of calculating the growth rate of each cultivation. The specific growth rate ranged from 0.0333 to 0.048 h^{-1}, with a similar range of other works in literature. The specific growth rate was observed to increase together with glucose concentration. The kinetic parameters were estimated through a Langumuir Plot and resulted 2.06 mg L^{-1} as K_s and 0.047 h^{-1} as μ_{max}. Also, the decrease of glucose was analysed for each cultivation. The results suggest that the alga was able to employ each of the tested glucose concentrations, indicating that it is a promising strain for being employed in mixotrophy for the abatement of organic matter in civil sewage.

Nomenclature

μ= specific growth rate, h^{-1}
S= substrate concentration, mg L^{-1}
K_s= substrate constant, mg L^{-1}
μ_{max}= maximum specific growth rate, h^{-1}
X= biomass concentration, g L^{-1}
D=dilution rate, h^{-1}

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