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Effect of dissolved oxygen concentration on biomass production in wastewater

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ABSTRACT

Biological wastewater treatment is a process that removes pollution caused by carbon, nitrogen and phosphorus. For this purpose, aerobic microorganisms must have an adequate amount of oxygen to avoid slowing down this process. This research therefore evaluated the influence of dissolved oxygen concentration and time on the rate of microbial growth in wastewater samples. To do so, a mixed culture of aerobic microorganisms was used with an equivalent concentration of SSV=150 mg/L, dissolved oxygen levels of 2, 3, 4 ppm, observation time of 5 days and concentration of pollutants equal to 800 ppm. It was determined that the growth of microorganisms responded to the cell synthesis phase, and it increased from 150 mg/L of SSV to 386.9, 412.07 and 423.7 mg/L, depending on the level of dissolved oxygen (2, 3 and 4 ppm). On the other hand, as the treatment time elapsed, the rate of microbial growth decreased, despite the fact that the significance of the effect of dissolved oxygen concentration was negligible. Finally, time and the interaction of both variables were relevant.

Keywords: batch reactor, bioremediation, microbial growth, oxygenation, wastewater.

Efeito da concentração de oxigênio dissolvido na produção de biomassa em águas residuais

RESUMO

O tratamento biológico de águas residuais é um processo que remove a poluição por carbono, nitrogênio e fósforo. Para isso, os microrganismos aeróbicos devem receber uma quantidade adequada de oxigênio para evitar a desaceleração desse processo. Nesse sentido, nesta pesquisa, foi avaliada a influência da concentração de oxigênio dissolvido e do tempo na velocidade de crescimento microbiano em amostras de águas residuais. Para isso, foi utilizada uma cultura mista de microrganismos aeróbicos com uma concentração equivalente de SSV=150 mg/L, níveis de oxigênio dissolvido de 2, 3, 4 ppm, tempo de observação de 5 dias e concentração de poluente igual a 800 ppm. Foi determinado que o crescimento dos microrganismos respondeu à fase de síntese celular e aumentou de 150 mg/L de SSV para 386,9, 412,07 e 423,7 mg/L, dependendo do nível de oxigênio dissolvido (2, 3 e 4 ppm). Por outro lado, com o passar do tempo de purificação, a taxa de crescimento microbiano diminuiu, embora a significância do efeito da concentração de oxigênio dissolvido tenha sido insignificante. Por fim, o tempo e a interação de ambas as variáveis foram relevantes.

Palavras-chave: biorremediação, crescimento microbial, esgoto, oxigenação, reator em lote.

1. INTRODUCTION

The limited availability of natural resources such as water has led to the construction of water treatment facilities to improve water management and safety (Caligan *et al.*, 2022). In this sense, conventional technologies such as artificial wetlands, bacterial beds, sequential biological reactors, among others have been discussed along with advanced treatment technologies such as reverse osmosis, microfiltration or ultrafiltration (Khatri *et al.*, 2020). The integrated methods of small-scale fixed-bed activated sludge in preconstructed reactors are highly efficient treatment for parameters such as BOD_5 , COD, TP, TKN, NH_4-N, TSS, fecal coliforms and total coliforms, even without a disinfection system, and can be optimized in two weeks (Naghipour *et al.*, 2022). Also, Advanced Oxidation Processes (AOP) such as catalytic ozonation, electrochemical oxidation, sonochemical and photocatalytic processes that, in combination with biological processes, including the use of enzymes and microorganisms, allow the degradation of emerging toxic pollutants in a sustainable way with hydroxyl, superoxide, hydroperoxyl and sulfate radicals (Babu Ponnusami *et al.*, 2023). Another modification of these treatments includes the use of bacteria associated with the phycosphere to control anthropogenic pollutants in wastewater treatment and whose interaction in a photobioreactor system allows us to understand the reduction of pollutants and improve the amount of biomass (Dang *et al.*, 2022).

In that framework, the biological treatments which coagulate the dissolved biodegradable organic matter and then separate it from the water are the most important (Meng *et al.*, 2019). This process can take place in the presence of oxygen (aerobic treatment) or in the absence of oxygen (anaerobic treatment) (Jung and Pauly, 2011). Being necessary to enhance the oxidation process so that aerobic microorganisms such as microalgae, cyanobacteria and photosynthetic live microorganisms (Janpum *et al.*, 2022) can convert organic wastes into inorganic by-products during wastewater bioremediation processes (Bahita and Belarbi, 2015).

Since biological treatment development can be limited by factors such as: temperature, pressure, oxygenation level, agitation, geometric shape, characteristics of the enclosures where the biological action takes place and the presence of suspended solids (Khatri *et al.*, 2020), which leads to high costs as part of the whole treatment process, with aeration being an essential step to reduce not only the energy used, but also the CO_2 emissions (Jung and Pauly, 2011).

For this reason, this study explored the relationship and influence of dissolved oxygen in wastewater on the rate of microbial growth during the treatment of contaminants in wastewater samples.

2. MATERIALS AND METHODS

Experimental tests were performed in a batch reactor implemented with two control systems, one for measuring the dissolved oxygen concentration and the other for temperature control, as shown in Figure 1.

This consisted of a fine bubble air diffuser, pressure regulator and solenoid valve that helped to guarantee the adequate DO concentration considered in the research design. The air entering the regulator came from a compressor at a pressure of 2 psi. The DO sensor had a range of 0 to 20 mA. The temperature control system consisted of a resistive temperature sensor (PT100. Range 0 to 800°C).

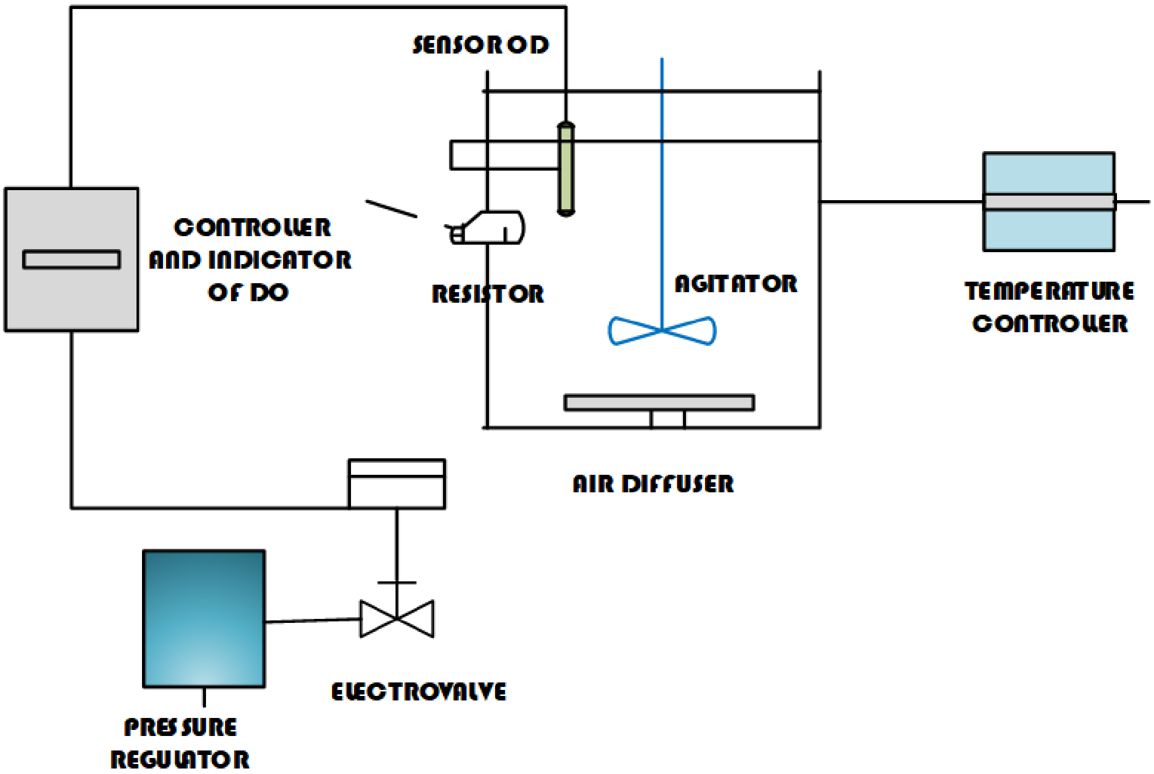

Figure 1. Schematic diagram of the experimental apparatus.

In addition, to guarantee the adequate amount of dissolved oxygen and meet the needs of the aerobic treatment, the selection of the type of aerator (fine bubble) that guarantees a better contact surface and degradation of the pollutants was taken into account, as well as the use of air flow control devices implemented in the aeration system. Likewise, the installation of the Data Logger allowed the continuous measurement of the DO concentration in the water to prevent it from falling below a critical level.

Then, 20L of synthetic wastewater were prepared according to the standardized protocol proposed by the (OECD, 2006), considering the quantities shown in Table 1 in order to ensure that the samples obtained have the same physicochemical characteristics of a wastewater sample treated in a conventional treatment system (Castellanos, 2019), avoiding the risk of exposure to pathogenic substances.

Table 1. Quantity of dissolved substances to prepare 20L of wastewater.

Substance	Amount
Peptone	8.36 g
Sucrose	1.88 g
Starch	4.40 g
Ammonium sulfate	1.44 g
Dibasic sodium phosphate	0.20 g

Subsequently, the decontamination tests were performed considering three levels of dissolved oxygen concentration (2, 3 and 4 ppm) at a temperature of 17°C. For this purpose, the wastewater samples were added and mixed in the Batch reactor with the aerobic microorganisms in an amount of 0.5 g/L. Finally, the dissolved oxygen and temperature control systems were activated, recording the time and determining the suspended solids from the volatile suspended solids (VSS) analysis.

2.1. Determination of volatile suspended solids

This procedure was based on the separation of suspended solids from a wastewater sample with a standard glass fiber filter. Then, with the retained solids, the following were determined gravimetrically: the solids dried at 105°C, the fixed solids and the volatile solids (VSS) incinerated at 550°C (OECD, 2006), as detailed in Figure 2:

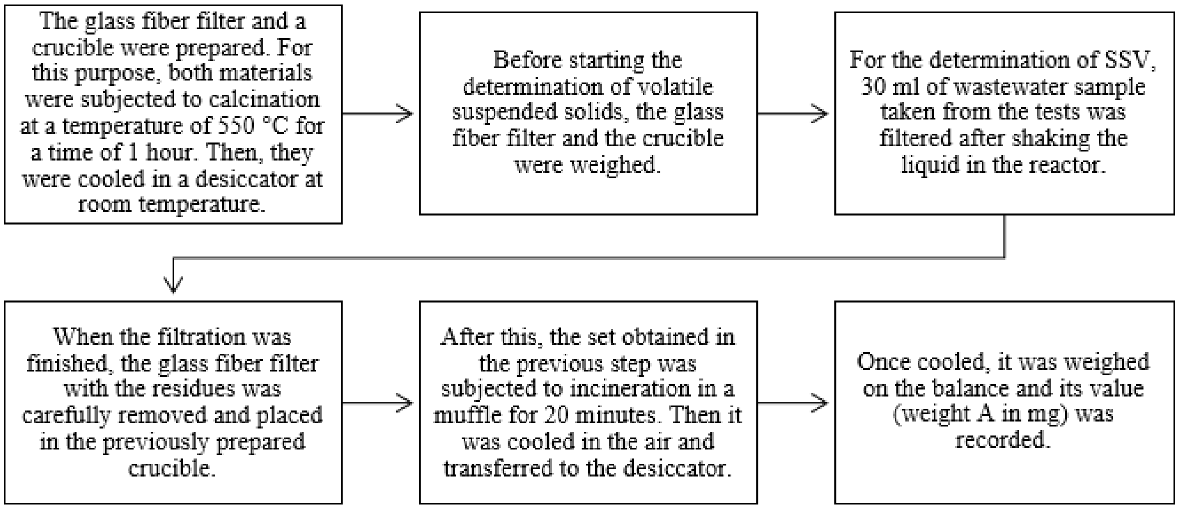

Figure 2. Procedure for obtaining SSV.

The recorded data were replaced in the following Equations 1, 2 and 3:

$$\frac{mg\ suspended\ solids\ (SS)}{L} = \frac{B-C}{v\ sample\ volume\ in\ L} \tag{1}$$

$$\frac{mg\ fixed\ suspended\ solids\ (SSF)}{L} = \frac{A-C}{sample\ volume\ in\ L} \tag{2}$$

$$\frac{mg\ volatile\ suspended\ solids\ (VSS)}{L} = SS - SSF \tag{3}$$

3. RESULTS

Table 2 shows the daily gravimetric measurements of the crucible plus glass fiber filter (C), of the crucible plus glass fiber filter with retained residues after drying at 105°C (B) and, finally, of the crucible plus glass fiber filter with residual solids after calcining at 550°C (A).

Figure 3 shows that the amount of volatile suspended solids increased on the first two days of biological reactor treatment and decreased on the fourth day. Meanwhile, on the fifth day, the cell synthesis phase came to an end in the three cases tested at 2, 3 and 4 ppm of dissolved oxygen concentration. After that time, the endogenous respiration phase began, where the concentration of VSS increased as a result of the metabolism of the microorganisms that favored the treatment process of the contaminated water.

Considering the average of the VSS of the five-day treatment, the total increase with respect to the initial amount was 257.5 mg/L. That is, a level equal to 1.7 times the initial value was reached as a result of the increased oxygen availability. In other words, dissolved biodegradable solids were more likely to become cellular material for microorganisms.

Table 2. Daily gravimetric measurements.

Dissolved oxygen concentration (ppm)	t (days)	C (mg)			B (mg)			A (mg)		
		I	II	III	I	II	III	I	II	III
2	0	18380.5	18199.1	17760.4	18385.5	18204.1	17765.4	18381.0	18199.6	17760.9
	1	18360.2	17780.3	18130.5	18367.4	17787.3	18137.4	18360.9	17781.1	18131.2
	2	18117.3	17721.4	17556.2	18126.8	17730.5	17565.0	18118.3	17722.4	17557.1
	3	17970.4	18235.5	17968.3	17981.2	18246.2	17979.2	17971.6	18236.7	17969.5
	4	17747.7	18327.7	17543.1	17759.4	18340.1	17555.2	17748.9	18329.0	17544.4
	5	18130	17675.6	18240.6	18142.6	17689.0	18253.7	18131.3	17677.1	18242.0
3	0	18460.3	18187.3	17755.2	18465.3	18192.3	17760.2	18460.8	18187.8	17755.7
	1	18119.5	17690.1	18147.2	18126.9	17697.5	18155.2	18120.2	17690.9	18148.1
	2	18123.2	17749.2	17767.2	18133.2	17759.6	17777.2	18124.2	17750.3	17768.2
	3	17989.2	18248.3	18335.1	18001.1	18260.6	18347.8	17990.5	18249.6	18336.5
	4	17732.1	18312.8	17868.1	17745.6	18326.1	17881.2	17733.6	18314.2	17869.5
	5	18102.3	17645.5	18324.6	18116.1	17659.4	18338.5	18103.8	17647.0	18326.1
4	0	18356.5	18284.1	18320.4	18361.6	18289.1	18325.4	18357.1	18284.6	18320.9
	1	17721.2	17825.7	18223.6	17729.5	17833.7	18231.8	17722.1	17826.6	18224.5
	2	18312.3	17861.1	17664.2	18323.3	17871.5	17675.3	18313.5	17862.2	17665.5
	3	18310.4	18340.9	18298.7	18323.2	18353.3	18311.1	18311.8	18342.3	18300.0
	4	17768.1	18351.6	17698.1	17782.2	18365.1	17711.9	17769.8	18353.1	17699.7
	5	18298.2	17851.4	18302.2	18312.6	17865.8	18316.0	18299.7	17853.0	18303.5

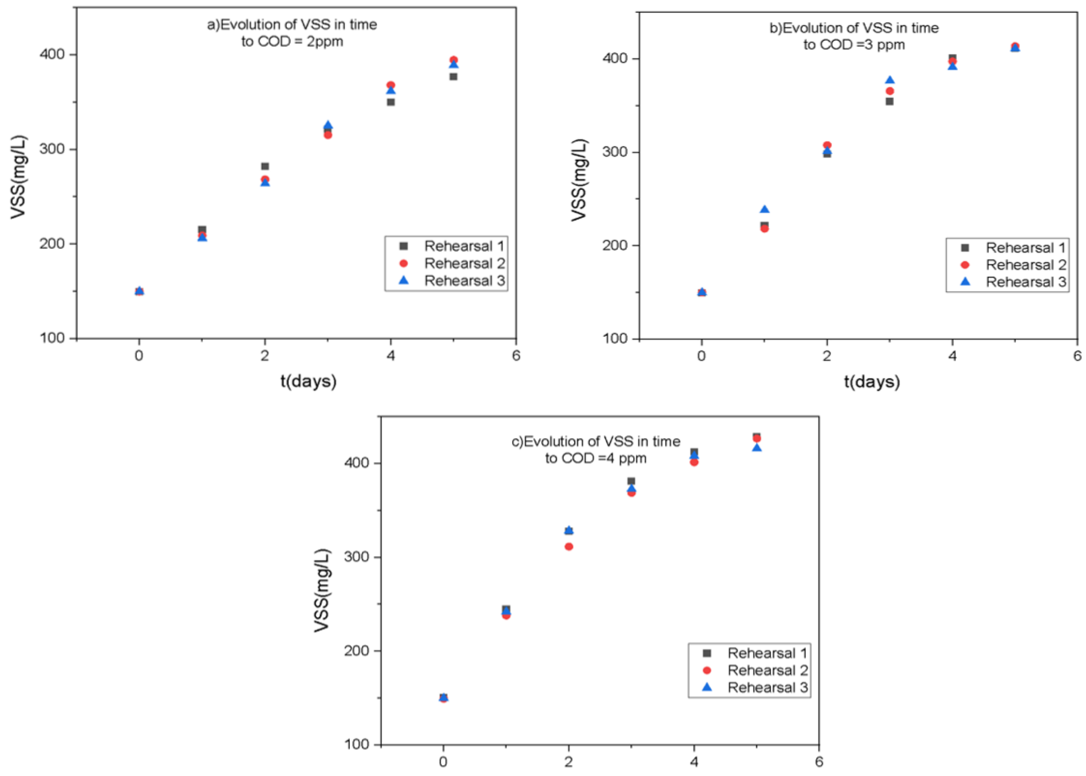

Figure 3. Evolution of the VSS as a function of time.

3.1. Growth rate of microorganisms

This parameter was obtained from the increase of the VSS during one day, establishing the following Figure 4:

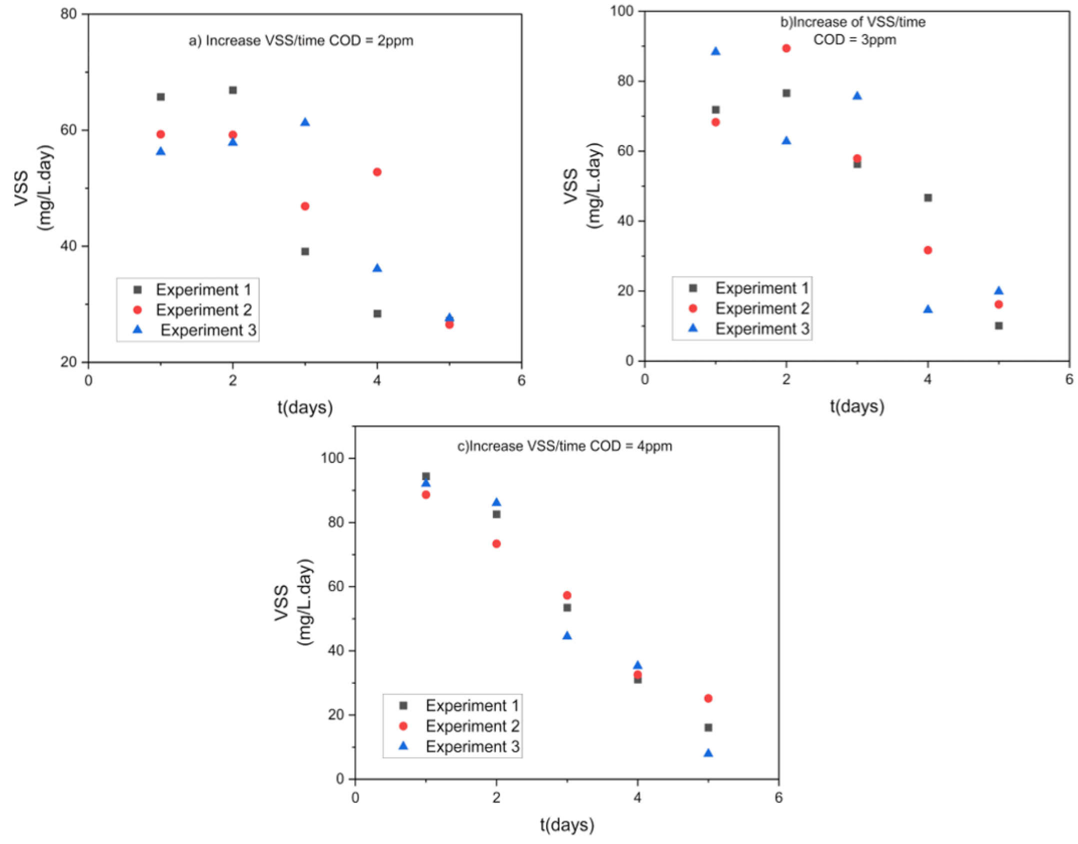

Figure 4. Daily bacterial growth rate.

In addition, a slower bacterial growth rate was observed when the dissolved oxygen concentration level was 2 ppm. Confirming that this parameter in initial moments or when the presence of dissolved substrate in the water is 800 ppm, the process of microorganism production and water decontamination is accelerated, but with low levels of chemical oxygen demand, the effect of substrate limitation is observed.

3.2. Relationship of the specific growth rate with the dissolved oxygen concentration

Calculating the specific growth rates for each dissolved oxygen level on a daily basis, and plotting these values graphically, as shown in Figure 5:

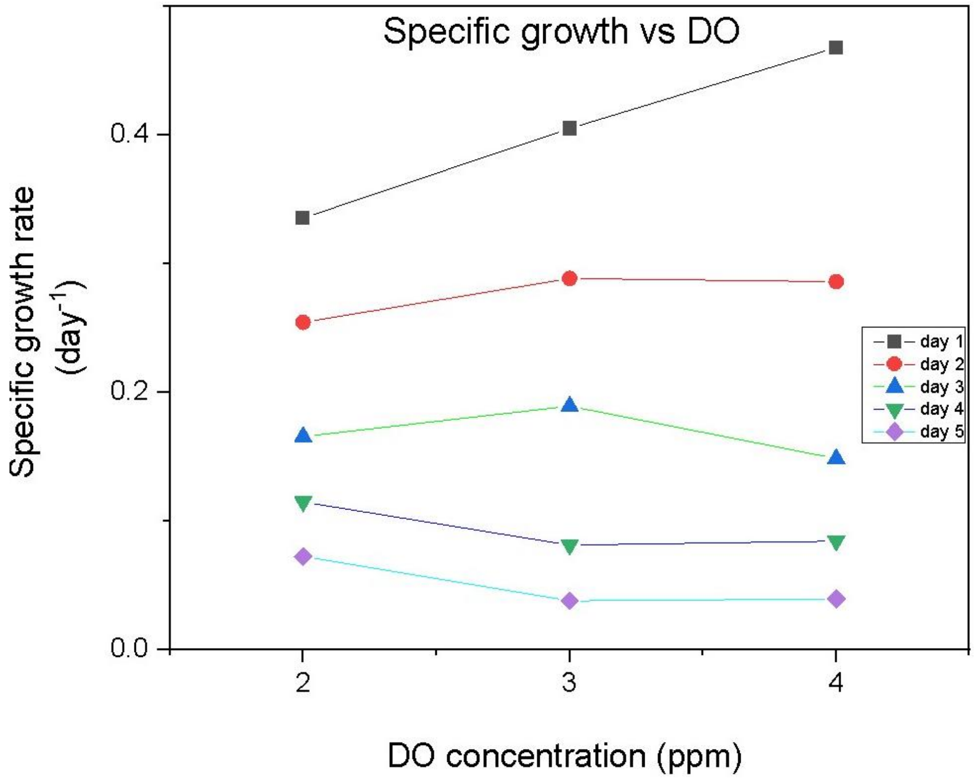

Figure 5. Specific growth rate obtained on a daily basis at each oxygen level.

The notable difference during the first and second day of treatment, as well as, the bacterial growth rate, when the level of dissolved oxygen concentration was 2 ppm, is lower than at 3 ppm, and this in turn is lower than at 4 ppm. The mathematical relationship obtained from the specific growth rate with the water oxygenation level is shown below (Table 3):

Table 3. Coefficients of determination.

Day	Mathematical Relation	R^2
1	$\mu = 0.0663C_{OD} + 0.2041$	0.9992
2	$\mu = 0.0158\ C_{OD} + 0.2291$	0.6882
3	$\mu = -0.0086\ C_{OD} + 0.1937$	0.1753
4	$\mu = -0.0152\ C_{OD} + 0.1393$	0.6741
5	$\mu = -0.0166\ C_{OD} + 0.0997$	0.7187

According to the coefficients of determination (R^2), after the second day, the relationship established between the two variables (dissolved oxygen and specific growth rate) is not defined or does not tend to be linear or quadratic.

4. DISCUSSION

In all cases, the concentration of volatile suspended solids (VSS) increased from the time of inoculation of the mixed culture that was previously adapted for a two-day period. This rate was indirectly associated with the daily growth rate of the microorganisms. This behavior was expected due to the fact that when working with discontinuous treatment systems, the concentration of substrate in the water decreases as a result of the metabolism and conversion of these substances in the cell wall of the microorganisms. These statements are supported by authors such as (Ekama *et al.*, 2006; Molina-Muñoz *et al.*, 2007) based on their experimental and theoretical research with bioreactors concerning enzymatic activities (acid phosphatases and dehydrogenase) and the biodiversity of the bacterial community present.

This phenomenon can have direct consequences on organoleptic parameters, such as the odor of treated water. Specifically, when the dissolved oxygen concentration is limited, especially under aerobic conditions, the formation of undesirable metabolites such as volatile sulfur compounds can occur. These compounds are commonly associated with unpleasant odors and they result from the incomplete degradation of organic matter and can negatively impact the sensory quality of treated water (Piotrowski *et al.*, 2023). Research underscores the importance of maintaining adequate levels of dissolved oxygen to prevent the generation of unwanted odors in treatment systems.

Beyond odor, the dissolved oxygen concentration also influences other parameters, including nutrient removal efficiency, sludge formation, and the activity of specific microorganisms (Quan *et al.*, 2012). An appropriate level of dissolved oxygen supports the activity of aerobic microorganisms responsible for nitrification, thereby contributing to the efficient removal of nitrogen compounds, as postulated by (Wang *et al.*, 2021), who also further emphasizes the significance of maintaining optimal dissolved oxygen balances in water treatment systems. This is not only critical for ensuring effective organic matter degradation and odor prevention but also for optimizing nutrient removal and other key biological functions of the system (Melo *et al.*, 2022).

In comparison with other biological processes, such as artificial wetlands, (Faulwetter *et al.*, 2009) emphasizes the critical relevance of dissolved oxygen concentration in microbial growth and, consequently, in the effectiveness of the pollutant reduction process. Although the influence of time and the interaction between variables underscore the complexity of the biological process undertaken, it is highly advisable to broaden the estimation of rates by considering multiple levels of oxygenation. This would facilitate a greater number of regression analyses with diverse equations, thereby contributing to a more comprehensive understanding of the process dynamics in various biological contexts, as discussed by (Copelli *et al.*, 2015; Mines *et al.*, 2017).

According to this, (Metcalf & Eddy, 1995) report that the growth of the bacterial population occurs with substrate limitation. Whereas (Couvert *et al.*, 2019) agree that, depending on the type of respiration of the microorganisms, the affectation given by oxygen concentration is not the same and that oxygen can be considered as an inhibitor of bacterial growth. Also Barreiro-Vescovo *et al.* (2020) and Mujtaba and Lee (2016) mention important aspects of the symbiosis found in their research, such as bacterial oxygen consumption that was limited to a few hours after the addition of wastewater; and the microalgae not only acted as oxygen producers, but manifested photorespiration events and endogenous respiration processes throughout the continuous culture treating the wastewater.

This confirms the importance of using a sequencing batch reactor operating with and without biomass recirculation, in addition to algae and bacterial aggregates to simplify aeration requirements and reduce high biomass harvesting costs (Papadopoulos *et al.*, 2023), as they provide the possibility to treat domestic wastewater without external air supply (Hakim *et al.*, 2022). Also, the results of (Du *et al.*, 2022) suggest that in the consortium of microalgae and bacteria, the biodegradation of aerobic bacteria produces CO_2 benefiting the photosynthetic oxygen evolution of the microalgae, which in turn favors the proliferation of aerobic microbes (Zhang *et al.*, 2018). Similarly, (Luan *et al.*, 2022) used an intermittently-aerated moving bed biofilm reactor and successfully treated synthetic rural wastewater without professional maintenance and with a lower amount of oxygen, ensuring high bacterial activity as well as autotrophic biomass ratio.

5. CONCLUSIONS

Regarding the evolution profile of the microbial concentration along the synthetic wastewater decontamination process, it was observed that it had a typical behavior of the cell wall synthesis phase and increased from 150 mg/L of VSS to 386.9, 412.07 and 423.7 mg/L, respectively, for the different levels of dissolved oxygen concentration (2, 3 and 4 ppm). This is explained by the fact that, as the level of dissolved oxygen concentration increases, so does the rate of microbial growth. Whereas the longer the purification time, the faster the growth rate decreases. On the other hand, the specific growth rate of microorganisms obtained on a daily basis and the dissolved oxygen concentration do not have a definite mathematical relationship. Therefore, the significance of DOC is negligible, while the time and the interaction of both variables were relevant.

Similarly, the level of dissolved oxygen is a critical factor influencing the efficiency of biological wastewater treatment. It affects parameters such as microbial growth rate and the activity of specific microorganisms. Therefore, maintaining an optimal balance of dissolved oxygen is essential, not only to ensure effective degradation of organic matter and odor prevention but also to optimize the removal of nutrients and other key biological functions of the system. Finally, it would be advisable to estimate the values of microbial growth rates as well as their activity, considering more levels of oxygenation to be able to perform a greater number of regression analyses with other forms of equations.

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