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Modeling the Behavior of *Chlorella Vulgaris* Microalgae in Water Treatment: A Kinetic Approach

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Abstract

In the modern era, there has been a notable surge in environmental pollution attributable to agricultural activities, urban expansion, industrialization, and various other contributing factors. This alarming trend has also taken a toll on our water resources, exacerbated further by the contamination stemming from human consumption-related wastewater discharges. To address these concerns, biological treatment approaches have gained widespread acceptance for wastewater treatment. The utilization of microalgae as a nutrient source, facilitating the removal of organic matter from wastewater, holds a pivotal role in bolstering the sustainability of wastewater treatment. The aim of this study, to mathematically model the removal of phosphorus and nitrogen from domestic wastewater using Chlorella Vulgaris algal culture. Experimental studies were conducted in a batch reactor, and removal efficiencies of nitrate nitrogen, ammonium nitrogen, and phosphate phosphorus were examined through measurements. The results indicate that microalgae efficiently perform the removal of pollutants process. As well as usage of microalgae in water treatment processes, a good microalgae kinetic model is highly important for nutrient removal, microalgae biomass accumulation, and enhancing operational settings in wastewater treatment. Kinetic modeling is a mathematical approach used to understand how a chemical reaction or process progresses or changes over time. Such models have various applications in all fields of science. Kinetic modeling can help us predict and optimize the behavior of reactions using computer simulations and mathematical analysis. Furthermore, specific growth rates of microalgae according to nitrogen and phosphorus nutrients were compared using the Michaelis-Menten equation for growth kinetics. According to the calculations, the nitrogen-based specific growth rate (NO_3^--N, NH_4^+-N) was determined as μ_{max}=0.053 day-1, and the phosphorus-based (PO_4^{3-}) specific growth rate was determined as μ_{max}=0.061 day-1.

Keywords: "Kinetic modeling, microalgaes, *Chlorella Vulgaris*, specific growth rate."

1. Introduction

Water plays a vital role as an essential resource, serving as a primary raw material in various industries, including pharmaceuticals, food and agriculture, petrochemicals, pesticides, and oil and gas, in addition to its household uses. The improper disposal of polluted water resulting from these industrial processes sustains significant environmental risks, which have become a growing cause for concern due to the wide array of pollutants involved. The excess release of phosphorus (P) and nitrogen (N) into aquatic ecosystems, resulting in eutrophication, raises environmental alarms due to issues such as solid waste production and the release of noxious substances into the atmosphere. This phenomenon also encourages the proliferation of harmful microorganisms, posing threats to aquatic ecosystems and degrading drinking water quality. This contributes to the widespread health issues observed in regions located close to the discharge areas [1].

Excessive use of fertilizers and pesticides in urban and agricultural areas leads to serious nitrate and phosphate pollution in surface and groundwater. Discharge of domestic wastewater, sewage, animal farms, processed food factories, and decomposition of organic matter release nitrogen into water environments. Therefore, studies related to nitrogen and phosphorus pollution and their treatment are of great importance [2].

As a result of the constrained capabilities of wastewater treatment facilities (WWTPs) in eliminating pollutants from wastewater, the discharged effluent, once treated, frequently retains minimal traces of contaminants. Despite the relatively low concentrations of some pollutants such as pharmaceuticals, endocrine disruptors (EDCs), microplastics (MPs), and persistent

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organic pollutants (POPs), they wield a substantial impact on the metabolism, development, and reproductive processes of organisms within the surrounding environment. Microalgaes offer a sustainable solution for tertiary and quaternary treatment processes due to their capacity to metabolize complex contaminants [3].

In situations where water is contaminated and purification becomes imperative, the selection of the optimal treatment approach is crucial to attain the desired purification goals. Traditional secondary treatments (such as activated sludge) and tertiary treatments (including processes like filtration and disinfection) often prove to be ineffective in adequately removing a majority of the emerging contaminants (ECs) that enter the wastewater treatment plant [1, 4].

Wastewater treatment using organisms such as bacteria, fungi, protozoa, and microalgae, which are capable of metabolizing parameters such as nitrogen, phosphorus, and carbon, has been extensively researched and studied. Numerous microalgae species, including "*Scenedesmus, Chlorella, Euglena, Oscillatoria, Chlamydomonas, and Ankistrodesmus"*, have demonstrated efficient growth in wastewater (WW) [1]. Among these organisms, microalgae have gained significant attention in wastewater treatment in recent years due to their fast growth rate and ability to thrive in challenging conditions [5].

Many researchers have proposed the use of microalgae as an innovative biological treatment method for nutrient removal in wastewater. Microalgae are also applicable in the tertiary treatment of wastewater due to their ability to absorb nutrients. Numerous studies have shown variations in the efficiency of nitrogen and phosphorus removal when using microalgae. These differences can be attributed to factors like the initial nutrient concentrations, which are influenced by various environmental conditions. Environmental factors, such as ambient conditions, light intensity, the nitrogen-to-phosphorus ratio, light and dark cycles, and the specific characteristics of the algal species, all play a crucial role in determining the effectiveness of nutrient removal through microalgae-based treatment processes [6].

Nitrogen and phosphorus in wastewater occurs in the tertiary treatment stage. The most common methods are biological processes such as anaerobic digestion and nitrification - denitrification. However, these methods are costly because they require multiple tanks and recycling of activated sludge. It is complex and wastes energy.

Efficiencies as high as 80-100% in nitrogen and phosphorus removal from wastewater have been reported for microalgae from different sources. Using microalgae to remove nutrients; it has various advantages, such as the nitrogen and phosphorus taken up by microalgae can be recycled through the production of fertilizer from microalgae biomass, or the resulting biomass can be used in the production of bioenergy, food, animal feed and medicine.

In the use of microalgae in wastewater treatment, the composition of the wastewater to be treated should be evaluated in advance. Because nitrogen/phosphorus molar ratios (N:P) seriously affect microalgae production and therefore nutrient uptake. Based on elemental composition for microalgal biomass, when the N:P ratios fall below 5:1, it results in nitrogen limitation, whereas N:P molar ratios exceeding 30:1 lead to phosphorus limitation [7].

In a study conducted by Şebnem A. and İlgi K. K., the effect of ammonia nitrogen and phosphorus concentration on the removal from wastewater by the algal species C. vulgaris was investigated, and kinetic coefficients were determined [8]. Experimental results showed that C. Vulgaris was able to completely remove ammonia nitrogen and achieve 78% efficiency in phosphate phosphorus removal. The discontinuous kinetic coefficients for the removal of ammonia nitrogen by C. Vulgaris were determined as $k = 1.5$ mg NH_4-N mg^{-1} chl-a d^{-1} and $K_m = 31.5$ mg/L. Similarly, for the removal of PO_4-P, the kinetic coefficients were found to be $k = 0.5$ mg PO_4-P mg^{-1} chl-a d^{-1} and $K_m = 10.5$ mg/L. Therefore, the removal rate of ammonia nitrogen is higher than that of phosphorus [8].

The binding mechanism of ions by algal biomass can vary depending on factors such as the type of algal species, the ionic charges involved, external environmental conditions like pH, ion concentration, biomass dosage, and temperature.

In a research study conducted by Melihe Amini et al., the capacity of unicellular green microalgae *D. salina* to uptake and remove NO_3^- and PO_4^{3-} from wastewater was investigated. The study examined several factors in batch systems, including the pH of the solution, the amount of microalgae biomass (0.05 g/L), and the initial concentrations of nitrate and phosphate (350 mg/L). FTIR experiments were conducted to understand how ions are absorbed by the algae. The most effective conditions for nitrate and phosphate adsorption and removal were found to be at pH 7, with 0.05 g/L of algae, and initial nitrate and phosphate concentrations of 350 mg/L. Under these conditions, *D. salina* exhibited a nitrate uptake capacity of 332 mg/g with a removal efficiency of 54%. Additionally, it demonstrated a phosphate uptake capacity of 544 mg/g with a removal efficiency of 82% [9].

A good microalgae kinetic model holds significant importance not only for the treatment processes involving microalgae but also for achieving nutrient removal, promoting biomass growth, and optimizing operating conditions in wastewater treatment. The kinetic modeling of *C. Vulgaris* microalgae can help optimize water treatment processes. This modeling can be used to evaluate factors such as nutrient input, growth rate of microalgae, biomass production, and efficiency of nutrient removal. Additionally, through this modeling, different scenarios can be simulated to examine the impact of various operating conditions and achieve the best performance.

Kinetic modeling involves some different mathematical equations that define the growth rate and nutrient taken of microalgae. These equations vary depending on factors such as nutrient concentration, temperature, pH, and some operational parameters. Understanding how *C. Vulgaris* microalgae behaves in water treatment, these equations are developed using laboratory experiments and data.

The Runge-Kutta method used in numerical analysis can be defined as an important type of open and closed iterative methods for solution approaches of ordinary differential equations. In a study conducted by Fatih Cantaş and his colleagues, the Runge Kutta algorithm was applied to fixed-size multimodal test functions, and a solution was sought to find the minimum point of the functions with different parameter values [10]. It is thought that this method can be used in cases where there are too many parameters in algae modeling.

The Monod (1) and Droop (2) models are frequently utilized mathematical models for studying microalgae growth. These models are employed to analyze the specific growth rates of microalgae. In Monod-type kinetics, the maximum specific growth rate is determined by the nutrient limitation, whereas in the Droop model, "the maximum specific growth rate" represents the growth rate of microalgae with an unlimited internal nutrient content [11].

The numbers of microalgaes has typically four phases over time; lag phase, exponantial growth phase, stationary phase, death phase. The following mass balance represents for modeling microalgaes;

$$\frac{dX}{dt} = \left(k_g - k_d\right)X \tag{1}$$

X is bacterial concentration, k_g is bacterial growth rate and k_d is bacterial death rate. k_g can be taken as μ.

The relationship between growth rate and concentration of substrate can be determined following emprical model;

$$\mu = \mu_{max}\frac{S}{K_s + S} \tag{2}$$

μ_{max} is maximum growth rate when food is abundant, S is substrate concentration (mg/L) and K_s is a half-saturation concentration. This model is sometimes called Michaelis-Menten model and also referred as Monod model [12].

$$\mu = \mu_{max}\frac{[A]}{K_A + [A]} \tag{3}$$

$$\mu_D = \mu_{D,max} * \left(1 - \frac{Q_0}{q}\right) \tag{4}$$

Monod and Droop models are often associated with equation 5.

$$\mu_{D,max} = \frac{\mu_m P_m}{P_m - \mu_m Q_0} \tag{5}$$

μ_m is Monod specific growth; μ_D is represented as Droop specific growth rate; $\mu_{m,D}$ is indicated as Droop maximum specific growth rate; [A] is denoted concentration of the nutrient A (mg/L); Q_0 is minimum cell quota; q is cell quota; ρ_m is represented as maximum nutrient uptake rate per cell [11].

While studies have indicated that the Droop model can faithfully replicate microalgae growth dynamics, researchers tend to favor the Monod model because of its simplicity in assessing the external nutrient concentration within the culture medium. While the Droop model takes into account the internal nutrient concentration, the Monod model is preferred due to its simplicity and ease of application in experimental studies, where the relationship between substrate concentration and the specific growth rate of microalgae is straightforward. However, in specific studies where the internal nutrient concentration is significant and can better explain growth dynamics, the Droop model is employed. There are also different models about growth rates [11].

The forthcoming global water scarcity issue is poised to become one of the most significant social and economic challenges of the 21st century. Microalgae-based wastewater treatment holds the potential to address this challenge by not only recovering nutrients but also generating clean water. Microalgae play a crucial role in the management and treatment of various types of wastewater, including industrial, agricultural, and municipal. These robust microalgae strains are adept at thriving in the harsh conditions presented by contemporary industrial and municipal waste environments [13].

The primary objective of this research was to create a kinetic model for the purification of contaminants from domestic wastewater, utilizing a culture of *C. Vulgaris* algae. Experimental data for this modeling were collected within a controlled laboratory setting, employing a batch reactor. To understand the growth kinetics of the microalgae, the specific growth rates based on nitrogen and phosphorus were evaluated and compared. The Monod equation was utilized as an analytical tool for this comparative analysis.

2. Material and Method

The primary objective of this research is to assess the effectiveness of *C. Vulgaris* algae culture in biological water treatment. Additionally, this study aims to quantify the specific growth rates concerning nitrogen and phosphorus nutrients.

The water sample used in this study was collected from the discharge point of the Eskişehir Municipal Wastewater Treatment Plant, where it is released into the Porsuk Stream. Fig. 1 illustrates the precise location of the sampling site.

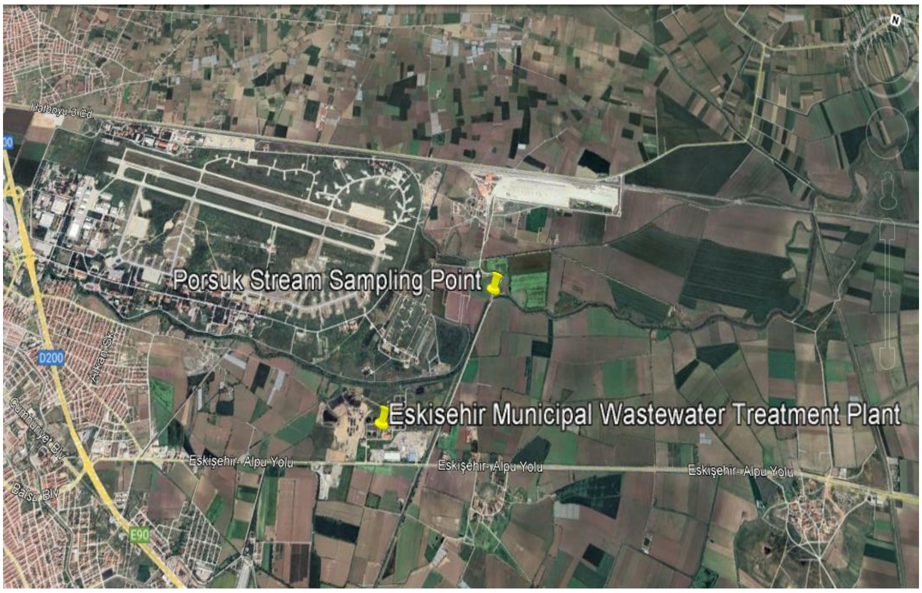

Fig. 1. Sampling location at Porsuk Stream

The experiment was set up using a batch reactor equipped with an Imhoff cone. Air was introduced into the system through a diffuser located at the lower part of the funnel. To provide illumination, a blue-red LED light source was utilized. A parabolic reflective surface covered with aluminum foil was placed around the cone to enhance the efficiency of the light source. In addition to monitoring NO_3^-N, NH_4^+-N, PO_4^{3-}, pH, and temperature values, chlorophyll-a measurements were conducted to observe the growth stages of the algae. pH adjustment during the experimental studies was carried out manually.

The experiments for NO_3^--N, NH_4^+-N, and PO_4^{3-} were conducted using the Hach DR2400 VIS spectrophotometer. Chlorophyll-a measurements, on the other hand, were performed using the Turner Design Aqua Fluor Fluorometer/Turbidimeter device to obtain in vivo Chl-a values [14].

After these experiments, calculations about kinetic modeling has been done. The microalgae growth kinetic model used the Monod model to account for nutrient limitations. Components of microalgae growth kinetic model include nitrogen types as NO_3^--N and NH_4^+-N and phosphorus type as PO_4^{3-}.

3. Results and Discussion

Phosphate, Chl_a and total ammonium nitrogen-nitrate nitrogen concentrations in experiments with *C.Vulgaris* in wastewater are shown in the graphs below (Fig. 2).

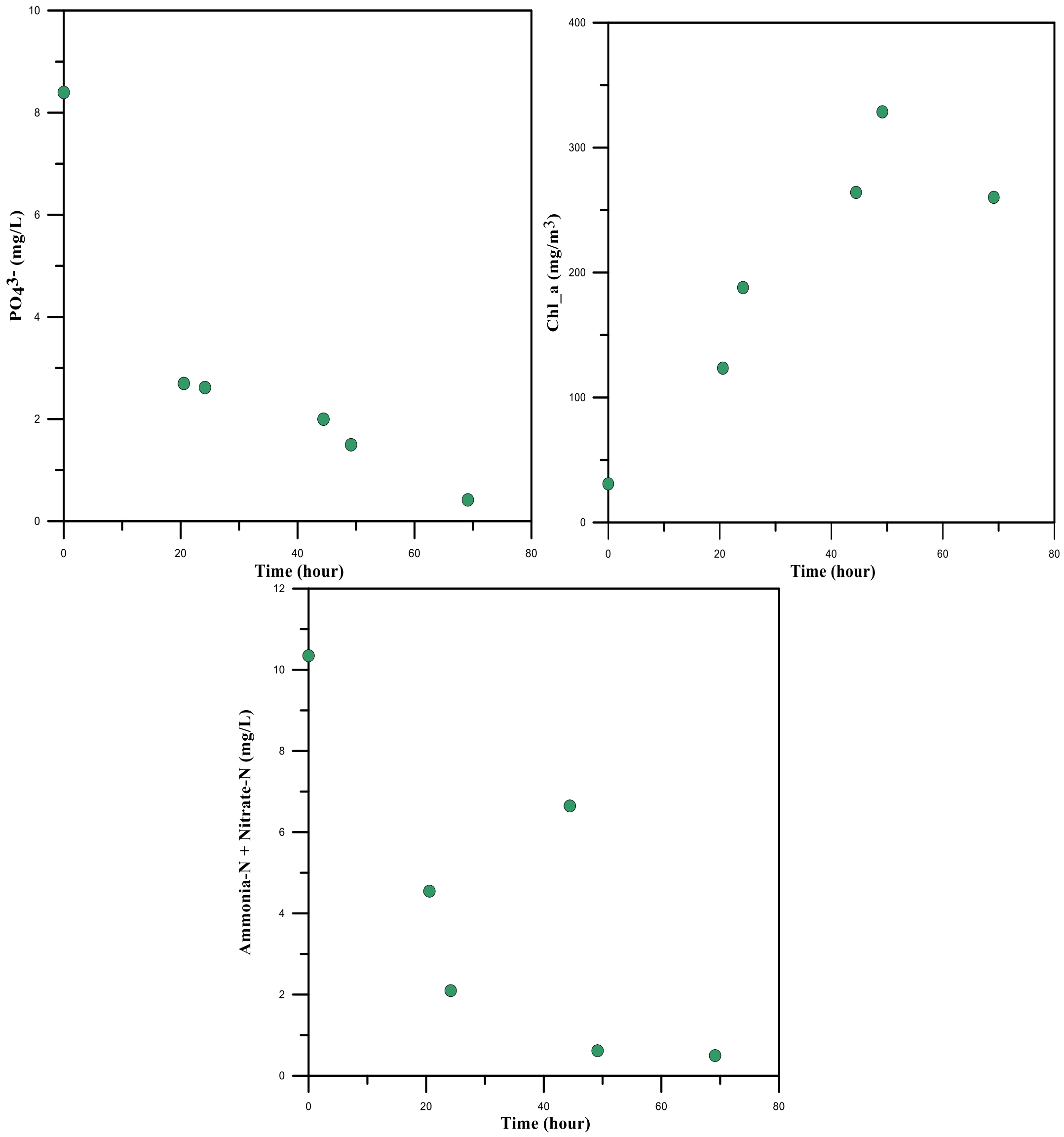

Fig. 2. PO_4^{3-} , Chl-a, total NO_3^- N and NH_4^+N concentration changes in Porsuk Stream samples

Equation 1 and 2 are integrated and resolved using linear techniques.

$$\frac{dX}{dt} = (k_g - k_d)X \qquad \mu = \mu_{max}\frac{S}{K_s + S} \tag{6}$$

In this study, the variable "X" represents the concentration values of chlorophyll-a (Chl-a), while "S" denotes the substrate concentration, encompassing total nitrogen in the form of NO_3^-N and NH_4^+-N, as well as PO_4^{3-}. Given the uncertainty regarding which specific type of nitrogen the algae primarily utilizes, the combined total of nitrate and ammonia nitrogen was employed as the nutrient source (Fig. 3 and Fig. 4). For substrate total NO_3^- -N and NH_4^+-N , μ_{max} is 0.053 day $^{-1}$, K_s is 0.028mg/L, and for substrate PO_4^{3-}, μ_{max} is 0.061 day $^{-1}$, K_s is 0.042 mg/L.

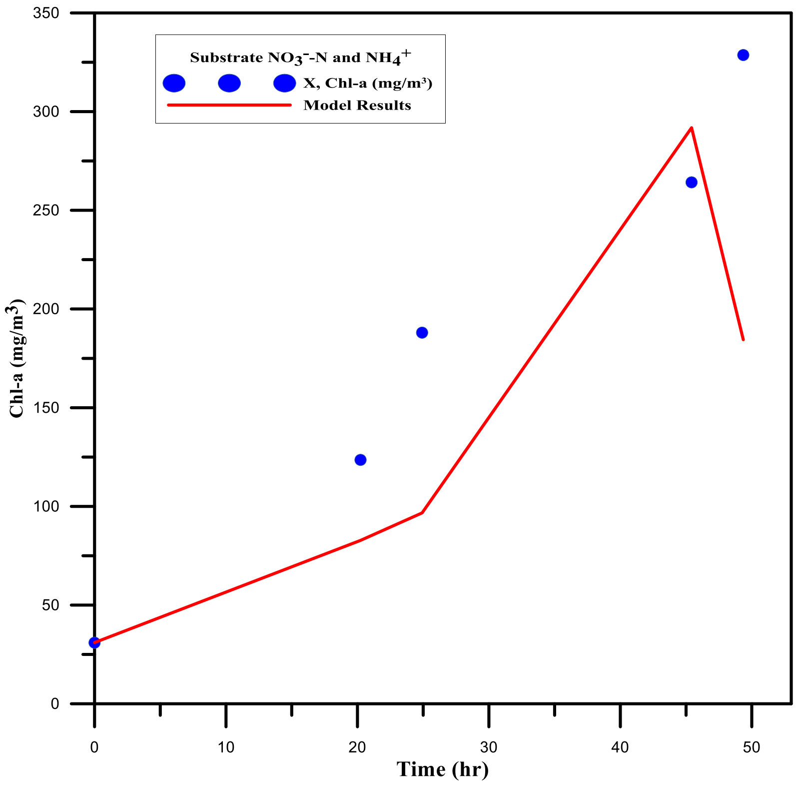

Fig. 3. Model and experimental findings for substrate concentration of NO_3^-–N and NH_4^+-N

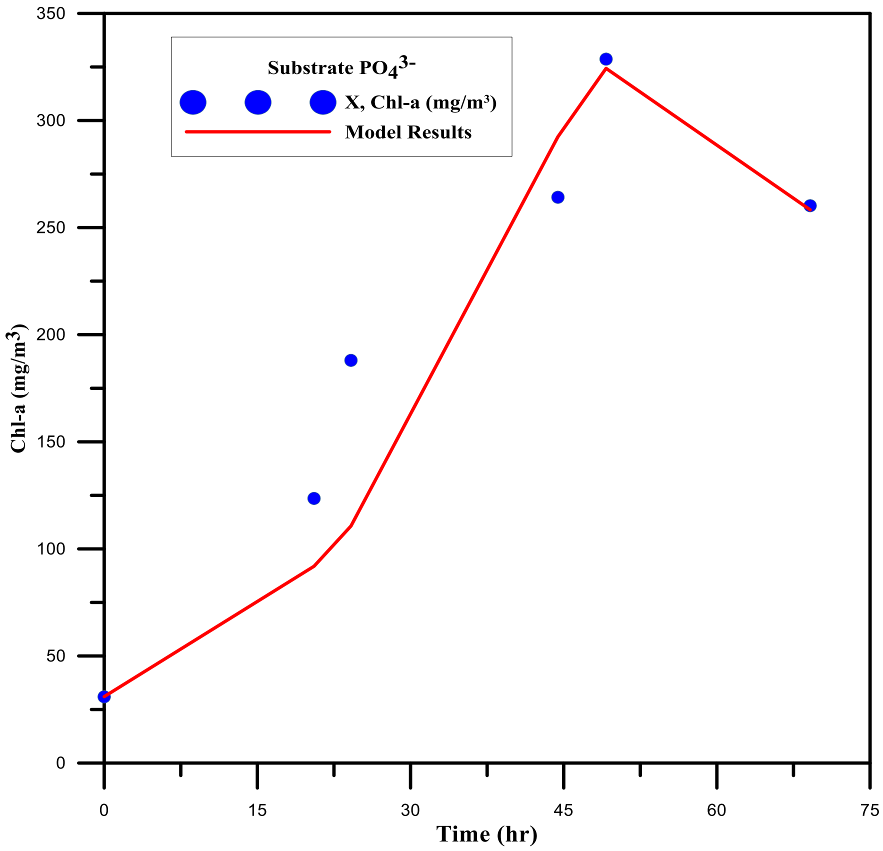

Fig. 4. Model and experimental findings for substrate concentration of PO_4^{3-}

The successful outcomes obtained from the application of microalgae in the elimination of nitrogen and phosphorus from domestic wastewater lead to the conclusion that their utilization is highly effective. Algae have been observed to actively contribute to the restoration of both wastewater and natural bodies of water. Moreover, their ability to rapidly and efficiently utilize the excess nutrients present in wastewater makes them a valuable asset in the treatment process. When employed in wastewater treatment, microalgae demonstrate their significant role, offering an economical approach with numerous advantages. Furthermore, microalgae have been suggested as a promising biological platform for mitigating carbon dioxide, a significant greenhouse gas, while simultaneously serving as a viable source for valuable compounds such as pharmaceuticals, cosmetics, nutritional products, animal feed, and biofuels. Besides, from an economic perspective, employing a microalgae-based system for the removal of nitrogen, phosphorus, and dissolved organic carbon from various wastewater sources proves to be a significantly more sustainable option compared to conventional systems. This is primarily attributed to the fact that microalgae systems can operate outdoors under natural sunlight conditions, leading to substantial cost reductions [3]. Moreover, bioremediation with using microalgae is a process that typically does not result in secondary pollution [1].

The maximum specific growth rate (μmax) of microalgae typically shows similarity between nitrogen and phosphorus nutrients across various substrates. Nevertheless, it's anticipated that the substrate affinity constant (Ks) would diverge due to differences in nutrient concentrations and utilization behaviors. Given that nitrogen and phosphorus exhibit distinct concentration levels and are assimilated by microalgae at varying rates, the Ks values are expected to mirror these disparities. Hence, the K_s values for nitrogen and phosphorus would be separate, capturing the specific nutrient demands and uptake kinetics of microalgae for each nutrient.

The incorporation of a reliable microalgae kinetic model holds great significance not only in treatment procedures but also in achieving efficient nutrient removal, promoting biomass growth, and optimizing operational parameters in wastewater treatment. A robust microalgae kinetic model serves as a valuable tool for accurately predicting and understanding the dynamics of nutrient uptake, biomass production, and the influence of various operating conditions. By utilizing such a model, wastewater treatment processes can be fine-tuned and optimized to enhance overall system performance and achieve desired treatment outcomes. In conclusion, the kinetic modeling of *C. Vulgaris* microalgae in water treatment can contribute to the development of more effective and efficient water treatment processes. This modeling provides a better foundation for understanding and optimizing the behavior of microalgae, enabling sustainable use of water resources and environmental protection.

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