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Removal of Ranitidine Using Chlorella Sorokiniana MH923013

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Abstract

The frequent and widespread use of medicines and personal care products, particularly in the residential environment, tends to raise concerns about environmental and human health impacts. On the other hand, carbon dioxide accumulation in the atmosphere is a problem with numerous environmental consequences. Microalgae are being used to bioremediate toxins and capture CO2. The current study aimed to confirm the possibility of removing pharmaceutical contaminant (Ranitidine) at different concentrations by using the Chlorella Sorokiniana MH923013 microalgae strain during the growth time. As part of the experiment, carbon dioxide was added to the culture medium three times per week. Explanatory results revealed that gas doses directly affect microalgae growth and removal efficiency, as evidenced by faster and more productive cell adaptation compared to control cultures. The development profile of microalgae is significantly influenced by pure carbon dioxide bubbles. When compared to control flasks, carbon dioxide increased the specific growth rate and doubling time. During the 312 hours microalgae cultivation period, the Chlorella strain recorded the highest pollutant removal efficiency (58%), particularly at the pollutant concentration of 5 mg/l CO_2.

Keywords: Bioremediation, Carbon dioxide, Chlorella, Microalgae, Pharmaceuticals, Pollutants, Ranitidine.

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1- Introduction

The increased production of urban wastewater has been demonstrated to be one of humanity's major environmental concerns. The limited availability of clean water resources across many developing countries may indeed be caused by a failure to conduct appropriately treated wastewater or to empty liquid waste into nearby bodies of water at levels below the environmental level [1]. As a result of industrial, agricultural, and domestic activities, both organic and inorganic contaminants, as well as various pollutants tend to range from micropollutants to heavy metal ions and high nutrient loads, are released into the nearby bodies of water. Emerging contaminants include pharmaceuticals and personal care products (PPCPs), contrast media, plasticizers, food additives, wood preservatives, laundry detergents, surfactants, disinfectants, flame retardants, and pesticides [2].

These emerging contaminants (ECs) were discovered in low concentrations in conventional wastewater plants. On the other hand, these compounds can take place either naturally or artificially and go undetected in the environment [3]. Pharmaceuticals, which are contaminants, have garnered a great deal of attention recently. Although much is known about the toxicological and pharmacological effects of these potent, physiological substances at large concentrations, little has been known about their impact on the environment [4]. These pollutants are implemented in liquid facilities for waste treatment after being discharged into sewage systems, where there is a requirement to lower organic pregnancy, nitrogen, phosphorous, and pathogens [5]. Although these pollutants are hydrophobic, conventional methods of treatment cannot eliminate them. Because emerging pollutants are not removed and degraded, they cause serious problems. Even though low-dose exposure over time can cause harm to plants and fish by interfering with propagation and tissue accumulation [6, 7]. Chemical methods are widely used to treat wastewater from the pharmaceutical industry. However, significant drawbacks include harsh reaction conditions, high operating costs, and secondary pollutant production. Bioremediation is a biological process that transforms xenobiotic pollutants and non-conventional into less dangerous forms (the only end products are water and carbon dioxide) [8].

Pharmaceuticals and personal care products (PPCPs) are degraded by microorganisms (microalgae, bacteria, or fungi) through a variety of mechanisms, including bioadsorption, bioaccumulation, biodegradation, photolysis, and volatilization. Because PPCPs have various chemical and physical properties, microalgae select one or a combination of procedures to eliminate them from water [9]. The selection of microalgae for wastewater treatment is determined by their resistance to wastewater and their ability to grow in and absorb nutrients from wastewater [10]. Microalgae-based wastewater treatment is less expensive to operate and more environmentally sustainable than conventional

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wastewater treatment [11]. Some of the advantages include the portability of microalgae to climatic changes and also the varying nature of wastewater [12]. Microalgae treatment appears to be more productive than conventional wastewater treatment due to the fact it is capable of treating the wastewater in such a single motion that includes various strategies to stabilize nitrogen, carbon, and phosphorous ratios.

It can also be an environmentally friendly choice as it can transform carbon dioxide into chemical elements and fuel without causing environmental pollution, thereby lowering the emission of greenhouse gases [13, 14]. Plants and microorganisms normally absorb and consume CO_2 as part of their photosynthetic ability, trying to make this an attractive development opportunity. Microalgae and cyanobacteria, on the other hand, develop much faster than terrestrial plants and fix carbon dioxide 10 to 50 times more efficiently [15]. CO_2 fixation by microalgal species, in conjunction with the production of biofuel and treatment of wastewater, may represent an excellent alternative strategy for CO_2 mitigation [16, 17]. This study aims to know the activity of (Chlorella Sorokiniana MH923013) microalgae strain which is pure and isolated from the Iraqi soil, as well as registered at the Genetic Bank database in bioremediation Ranitidine through the growth rate and removal efficiency for this pollutant.

2- Materials and Methods

2.1. Preparation of Stock Solution

The emerging pollutant used was (Ranitidine HCl). Ranitidine Hydrochloride ($C_{13}\,H_{22}\,N_4\,O_3\,S$) Manufactured in India by Sun Pharmaceutical Industries Ltd (purity of 99.46% produced), degradation occurs with increasing temperature, ambient oxygen, and the presence of humidity. The medication (Ranitidine HCl) stock solution was prepared from sterile purified water, distilled that used filter paper, and kept in the dark until needed. The stock solution was prepared into four concentrations (5, 15, 25, and 35) mg/l.

2.2. Culture Media Sterilization

Microalgae sterilization is an essential step in the process. This procedure focuses on eliminating any undesirable germs in order to reduce contamination [18]. This was accomplished through autoclave sterilization of BG-11 media (BioReadyTM media – China), the culture media BG-11 used in this study was (15 L) which was prepared by dissolving 1.627 grams of BG-11 in 1000 ml of distilled water. The pH value was adjusted with 1M NaOH or HCl if it did not achieve the required value of 7.The autoclave took 15 minutes to reach its maximum temperature of 121 ℃ and pressure of approximately 2 bar.

2.3. Microalgae Species Cultivation

The pH of the BG-11 nutrient medium was corrected with (0.1 N) HCl and NaOH. A 5 ml stock of the *Chlorella Sorokiniana* MH923013 *microalgae* strain was grown in 500 ml BG-11 medium in a 500 ml conical flask with one repeater, yielding 2 copies of the error bar. All tests were carried out in an incubator with a continuous light intensity of 168 $\mu Em^{-2}s^{-1}$ and an ambient temperature of (24 $\pm$1 ℃).

2.4. Experimental Setup and Measurements

Laboratory experiments included applying a two-part system, the initial part of which consisted mainly of algal cultures placed in an incubator with continuous lighting and a temperature suitable for microalgae growth, and the second of which consisted of a sparing system that included the use of carbon dioxide gas with a flow rate 2 L/min and the presence of containers directly connected to the gas source as shown in Fig. 1 and Fig. 2. The strain used in this study was *Chlorella Sorokiniana MH923013*, which was divided into two groups: concentrations (5, 15, 25, 35) mg/l and a control group with one repeater for each concentration with the presence of a carbon dioxide as a first group, the second group contains the same concentrations and control as the first, but without the addition of the carbon dioxide for the second group. Each conical flask for concentrations contains 5 ml of culture media, which was then added to 500 ml of previously prepared and sterilized nutrition medium (BG-11). At the start of the experiments, the (Ranitidine HCl) was added in concentrations of (5, 15, 25, 35) mg/l. Aeration gas was pumped with a flow rate 2 L/min into the microalgae culture medium for (5 min) and for a volume of (50) ml of microalgal cultures for three times per week, to avoid a drop in pH. The flasks were manually shaken throughout growth to prevent clumping of microalgae cells and to maintain cellular proliferation. Microalgal culture samples have their growth kinetics measured using a UV spectrophotometer (GENESYS 10UV, USA) set to (680) nm [19]. The pollutant concentration was measured at (313) nm [20].

Fig. 1. Microalgae Farms after Added 50 ml of Carbon Dioxide to Culture Medium Concentrations (5, 15, 25, 35) mg/l and a Control Group with Light Intensity 168 $\mu Em^{-2}s^{-1}$ and an Ambient Temperature (24 $\pm$1 ℃)

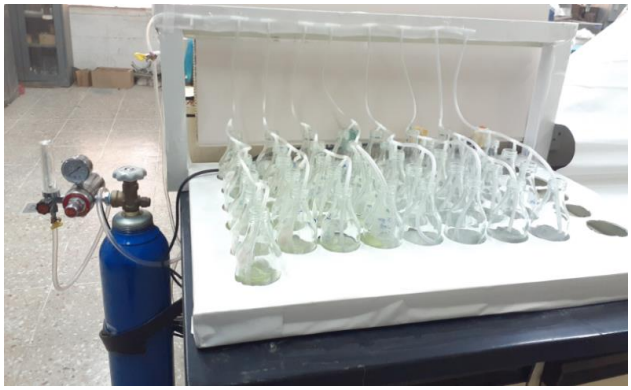

Fig. 2. Screenshot of the Experimental Set-Up Used in the Current Study (Sparging Carbon Dioxide Gas into 50 ml of Microalgae Culture Medium for 5 min with a Flow Rate 2 L/min at Room Temperature)

3- Growth Rate Kinetic

Growth curves related to algal biomass density over time were constructed using specific growth rate estimation. The specific growth was calculated using the straight line formula shown below [21].

$$S_g\ xn = \frac{dxn}{dt} \quad (1)$$

S_g is the specific growth rate (day^{-1}), xn is the biomass concentration of the cells (gL^{-1}).

By rearranging Eq. 1:

$$S_g = \frac{1}{xn}\frac{dxn}{dt} = \frac{d\ ln\ xn}{dt} \quad (2)$$

If S_g remains constant over time during the exponential growth rate, integrate Eq. 2 from (t_0 to t) as follows:

$$S_g = \frac{\ln(xn/xn_0)}{t - t_0} \quad (3)$$

xn is the final concentration of microalgal biomass at any time t, xn_0 is the initial cell concentration at the beginning of active logarithmic phase at time t_0.

The time required for doubling the cell mass is called as doubling time (t_d), the doubling time by setting the cell to be doubled ($xn = 2xn_0$) and ($t_0 = 0$) [22], can be calculated as follows:

$$t_d = \frac{\ln 2}{S_g} \quad (4)$$

In this study, the carbon source for metabolic activities was pure carbon dioxide gas. Nutrient availability, culture environment, and cultivation media all have an impact on microalgae growth profiles. *Chlorella Sorokiniana* MH923013 had the highest optical density (0.3795) at concentration of pollutant 35 mg/l with CO_2 after 312 hours of experimental cultivation.

4- Results and Discussions

Although the microalgae seem to be more sensitive to antibiotics and antimicrobials compared to all other types of PPCPs, no significant toxicity was observed in microalgae exposed to PPCPs from an antihistamine drug (Ranitidine). The presence of that kind of drug molecule had no negative impact on the rate of growth of microalgae. The current research demonstrated this fact by growing one type of microalgae (Chlorella Sorokiniana MH923013) that can grow in the Iraqi climate and was used in laboratory tests, as shown in Fig. 1. Light intensity, pH, temperature, carbon dioxide, and the nutritional content of the growing medium all have an impact on microalgae production systems, and some of them seem to be the most various environmental factors influencing algal advancement and biomass production. All of the above variables were carefully controlled in the current research to study the impact of Ranitidine on the growth of the microalgae used. The stable growth of all traditional cultures (control) showed a positive indication that such a type of pollutant could be dealt with in microalgae cultures in the future.

Sparging carbon dioxide into 50 ml of culture media has been observed to have a significant influence on the microalgae development profile, as it increases the number of microalgal cells. This improvement in microalgae strain was noticed after adding 2 L/min of CO_2 gas for five minute to 50 ml culture medium at various concentrations. Several experiments were performed during this time period to determine the volumetric flow rate of pure CO_2 in order to achieve complete CO_2 saturation while attempting to avoid a decrease in the pH of a main medium, if this occurs, may cause the death of microalgae cells.

The experimental results show that *Chlorella Sorokiniana* after 192 hours recorded optical density (0.148) for the concentration of pollutant (25 mg/l) with carbon dioxide, and (0.1205, 0.13) for pollutant concentrations with carbon dioxide (5 and 15) mg/l. *Chlorella* recorded optical density (0.102) in the existence of a carbon source at a pollutant concentration of 35 mg/l after 144 hours, indicating pollution consumption. The control group for *Chlorella* strain recorded optical density (0.342) with and without carbon source (0.1255) after 312 hours, sees Fig. 3.

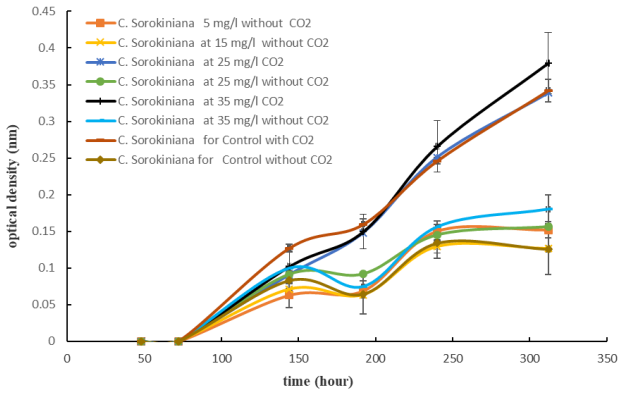

Fig. 3. Growth Rate of *Chlorella Sorokiniana* MH923013 Microalgae with and without Carbon Dioxide (Aeration Gas 2 L/min, Light Intensity 168 $\mu Em^{-2}s^{-1}$ and Temperature (24 ±1 ℃))

Table 1 displays the specific growth rate of a microalgae strain used in this research (*Chlorella Sorokiniana* MH923013) in addition to the strain's doubling time at various Ranitidine concentrations (0, 5, 15, 25, 35 mg/l). The results indicate that the concentration levels of the pollutant material in the culture media influence the responses of the doubling time and specific growth rate. When other process parameters (including the source of carbon dioxide) have been controlled, the specific growth rate and generation time (doubling time) of *Chlorella Sorokiniana* MH923013 significantly improved. The specific growth rates of this strain (0.0104, 0.0093, 0.0085, and 0.0096 hr^{-1}) and the doubling time (67, 75, 81, and 72) hr at various pollutant concentrations (5, 15, 25, 35) mg/l have indeed been registered, respectively. These results are slightly better than any of those gained in control bioreactors which registered a specific rate of growth ($0.0082 hr^{-1}$) and doubling time (84 hr). These findings indicate that the presence of such a type of pollutant in the culture medium has a positive effect on the growth rate kinetics. This impact, however, may be affected by the kind of microalgae used in cultivation.

For all Ranitidine concentrations, the specific growth rate and doubling time improved. Carbon metabolism enzymes such as ribulose-1,5-bisphosphate carboxylase/oxygenase (RuBisco) and carbonic anhydrase are affected by CO_2 abundance [23].

Table 1. The Specific Growth Rate and Doubling Time during 312 hours

Time (hr)	Microalgae strain	Concentrations mg/l	Specific growth rate (μ_r)		Doubling time (t_d)	
			CO_2	without CO_2	CO_2	without CO_2
312	*Chlorella Sorokiniana* MH923013	5	0.0144	0.0104	48	67
		15	0.014	0.0093	50	75
		25	0.0143	0.0085	49	81
		35	0.0142	0.0096	49	72
		Control	0.0114	0.0082	61	84

The volume of microalgae inoculation (*Chlorella Sorokiniana* MH923013) used in this investigation was (5 ml). This amount of inoculums has been approved by research regardless of the strain used [24].

Add 50 ml of culture medium with CO_2 as a dose resulted in a significant increase (2.02) in (X-factor) for 312 hours when compared to prior study [25] that resulted in (4.5) for *Chlorella Sorokiniana* MH923013.

The rate of growth was demonstrated in the current research (1.68, 1.76, 1.69, and 2.02) with concentrations (5, 15, 25, 35) mg/l of pollutant (drug) see Table 2.

Table 2. The Effect of Carbon Dioxide Addition on the Growth Rate in the Current Study after 312 hours

DIC Dosing	Microalgae strain	Concentrations mg/l	X factor based Control
50 ml	*Chlorella Sorokiniana* MH923013	5	1.68
		15	1.76
		25	1.69
		35	2.02

When pure carbon dioxide is used in cultivation systems, the pH rapidly drops, causing adverse environmental conditions for the majority of microorganisms, including microalgae. The inlet CO_2 concentration must not only be less than the critical value so that it will not satisfy the carbon requirements of microalgae. However, it should never exceed the maximum limit in order to prevent a large loss of CO_2 which cannot be utilized by the microalgae species and is released to the environment by the microalgae culture, as a result of which unnecessary environmental contamination occurs. As the concentration of pure CO_2 in the solution increases, the pH of the microalgae solution decreases. Nevertheless, as CO_2 levels start rising, photosynthetic capacity and microalgae growth keep increasing, causing pH to increase. Other several authors have already had comparable success.

According to [26] and [27], photosynthesis for carbon dioxide fixation tends to make a reasonable pH increase because of the accumulation of OH^- whereas CO_2 dissolution in water causes acidification resulting in the formation of carbonic acid. It has been observed that higher pH levels prevent cell growth through accumulating carbonates which microalgae cannot absorb. High concentrations also inhibited photosynthetic activity of microalgae through intracellular carbon anhydrase, resulting in a decrease of carbon dioxide residues in flue gases [28]. Carbon dioxide availability and solubility in the photobioreactor are directly proportionate to the pH of the microalgae culture medium [29]. The determining factor in the process of photosynthesis of microalgae is bicarbonate, and also the carbon compound proportions change as the pH changes (carbonate, carbon dioxide and bicarbonate) [28].

Regarding mass transfer, it ought to be that the presence of other gases can restrict the rate at which carbon dioxide transfer to the cultivation medium [30]. Prior studies used a gas mixed with CO_2 to regulate the amount of CO_2 dissolved inside in medium. Ying et al [31] used a mixture (95% nitrogen and 5 % carbon dioxide) as in cultivation of microalgae to regulate the quantity of carbon dioxide dissolved as well as keep the pH stable.

In the current study, the method of pumping gas used was pure carbon dioxide gas, which differed from previous studies. The gas was bubbled with (2 L/min) and for (5 min) into volume of fifty ml of microalgal cultures for three times per week, to avoid a drop in pH in main flask. Instead of all of the microalgae cultures, the spray system was used to direct the gas to a specific amount of culture medium. This method was effective and suitable because it attributed to an increase in production of

biomass while having no adverse influence on the pH value, as shown in Fig. 4.

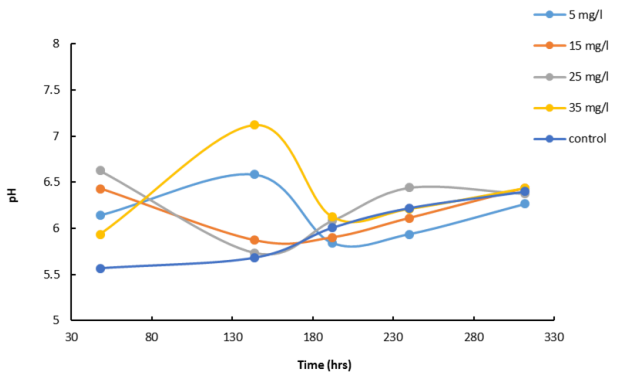

Fig. 4. pH Values after (50 ml) of Carbonic Culture Medium was Added to *Chlorella sorokiniana* MH923013 Culture Flasks

5- Pollutant Removal

Ranitidine is an environmentally significant medication because of its incomplete metabolism [32] since of poor discharge in wastewater treatment, microalgae will not be capable of completely eliminate it [33]. As shown in Table 3, the current research supports prior studies.

The *Chlorella* strain achieved the highest pollutant removal efficiency (58%) during the microalgae cultivation period of 312 hours, especially that produced at pollutant concentration 5 mg/l with CO_2, as shown in Table 3. Other carbon dioxide concentrations reported (30, 22.2, 10) % at (15, 25, 35) mg/l of Ranitidine. The maximum removal efficiency was observed at 5 mg/l including both cases with and without CO_2 , indicating that lower concentrations consume faster than higher concentrations. The lowest percentage removal for *Chlorella* was 10% with carbon dioxide and 14% without carbon dioxide at a concentration of pollutant 35 mg/l.

The increase in biological degradation could imply that enzymatic hydrolysis of microalgae is a viable drug resistance mechanism, several enzymatic pathways, including hydroxylation, methylation, nitrosation, and deamination, have been used to biodegrade the medication [34]. The pH and type of the extracellular polymeric particle sizes in the bioreactor may influence the capability of microalgae to absorb micropollutants [35].

Table 3. Efficiency of Removal after 312 hours of Cultivation

Microalgae	Concentrations mg/l	%Removal efficiency with CO_2	%Removal efficiency without CO_2
Chlorella Sorokiniana MH923013	5	58	53
	15	30	30
	25	22.2	21
	35	10	14

Because carbon and nitrogen are embedded in the suspended particles, microalgae find it difficult to use them. In co-cultivation, fungal extra - cellular enzymes can transform molecular organic compounds into soluble nutrients, in which enzyme-treated components could be absorbed [36]. As a result of self-reinforcing interactions between microalgae and fungi. In terms of nutrient removal (e.g. phosphorus, nitrogen), a co-cultivation process may be more effective than a monoculture system [8].

In comparison to a mono microalgae culture system, the combined culture of microalgae and fungi could absorb as well as digest more Ranitidine [37]. Fungi produce extracellular enzymes that degrade solid organic waste in to the soluble nutrients and CO_2, because microalgae cannot break down organic wastes, they find it difficult to metabolize and eliminate them from the environment [38].

The recent experimental results demonstrated that at a concentration of 5 mg/l, the removal percentages of the pharmacological pollutant in the monoculture system of microalgae ranged from 58% in the presence of a carbon source to 53% in the absence of a carbon source. This percentage may well be low when compared to the removal of ibuprofen and paracetamol by microalgae, as indicated in [39]. In this research, it was discovered that *Chlorella Sorokiniana* MH923013 microalgae consume some pharmaceutical contaminants more efficiently than some other types of microalgal species. Because Ranitidine was difficult to eliminate using a monoculture of microalgae, researchers needed to combine fungi and microalgae to create biopellets [40]. They combine fungi as well as micoalgae to produce biopellets, and they were capable of removing Ranitidine with such percentage of (50 ±19) % by biopellets and (30±12) % by fungus, but in this study, *Chlorella* was used and the highest removal percentage was (58%) during 312 hours.

The carbon dioxide gas was employed to enhance the rate of removal of pollutants by microalgae. When a carbon source such as CO_2 gas, was added, the removal rate increased by a factor of just one, as it appears to have done in some concentrations.

6- Conclusion

1. In a suitable environment, *Chlorella Sorokiniana* MH923013 microalgae was successfully grown.
2. Despite the availability of the contaminant at various concentrations, this study revealed that carbon dioxide has a direct impact on the growth of microalgae species, predicated on measured growth at doses of (50) ml, particularly in comparison to control groups of variable certified strains, which clearly showed slow and irregular growth.
3. At all concentrations tested (5, 15, 25, and 35) mg/l, the availability of a pharmaceutical pollutant Ranitidine appeared to have an impact on the specific rate of growth and doubling time at all concentrations tested (5, 15, 25, 35) mg/l; additionally, the growth process occurred on a regular basis in a carbon-rich environment.
4. The highest removal efficiency value was recorded for *Chlorella Sorokinana* MH923013 microalgae strain,

which was 58% in the presence of carbon dioxide and 53% without carbon dioxide at contaminant concentration of 5 mg/l.

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إزالة الرانيتيدين باستخدام Chlorella Sorokiniana MH923013

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الخلاصة

يشير الاستخدام المتكرر والواسع النطاق للأدوية ومنتجات العناية الشخصية ، لا سيما في البيئة السكنية، إلى إثارة المخاوف بشأن تأثيرها على البيئة وصحة الإنسان. يعد تراكم ثنائي اوكسيد الكاربون في الغلاف الجوي مشكلة لها عواقب بيئية عديدة. يتم استخدام الطحالب الدقيقة في المعالجة الحيوية للسموم والتقاط ثنائي اوكسيد الكاربون. هدفت الدراسة الحالية إلى تأكيد إمكانية إزالة الملوثات الصيدلانية (رانيتيدين) بتراكيز مختلفة باستخدام سلالة من الطحالب الدقيقة خلال فترة نموها (Chlorella Sorokiniana MH923013).

كجزء من التجربة، تمت إضافة ثنائي اوكسيد الكاربون ثلاث مرات في الأسبوع إلى مزارع الطحالب الدقيقة. كشفت النتائج التوضيحية أن جرعات الغاز لها تأثير مباشر على النمو الدقيق، وكفاءة الإزالة، ويتضح ذلك من تكيف الخلايا الأسرع والأكثر إنتاجية مقارنة بمجموعات التحكم. يتأثر معدل النمو للطحالب الدقيقة بشكل كبير بفقاعات ثنائي اوكسيد الكاربون النقي. عند المقارنة بقوارير التحكم، زاد ثنائي اوكسيد الكاربون من معدل النمو المحدد ووقت مضاعفة الخلايا. حققت سلالة الكلوريلا أعلى كفاءة في إزالة الملوثات (٥٨٪) خلال فترة زراعة الطحالب الدقيقة البالغة ٣١٢ ساعة ، خاصة تلك الناتجة عند تركيز الملوث ٥ ملغم / لتر بوجود غاز ثنائي اوكسيد الكاربون.

الكلمات الدالة: المعالجة الحيوية، ثنائي أوكسيد الكاربون، الكلوريلا، الطحالب الدقيقة، المستحضرات الصيدلانية، الملوثات، رانيتيدين.