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M.M. Saleh[1,2], M.M. Gaballah [1], *B.K. Zaydan[2], A.K. Sadvakasova[2]

[1]Suez Canal University, Ismaillia, Egypt

[2] Al-Farabi Kazakh National University, Almaty, Kazakhstan

*e-mail: zbolatkhan@mail.ru

Bioremediation of polluted water by using wild and mutant strains of microalgae *Parachlorella kessleri*

Pollution of the aquatic ecosystems with different types of pollutants affect on water quality. In this study, wild and mutant strains of green microalgae, *Parachlorella kessleri* were used. The wild and mutant strains introduced into the polluted water of Al-Sayyadin lagoon which is polluted by sanitary and agricultural wastewater and heavy metals. The results showed that, the growth of algal strains was very poor in the samples which contained only water due to the competition between other microorganisms and bacteria, this poor growth affect on the remediation potential of the algal strains. In the samples that contained mixture of medium and water, the wild and mutant strains well grown and the remediation potential was powerful in removing Ammonia, Phosphate, Co, Zn and reducing the levels of BOD and COD in the polluted water.

Keywords: remediation, pollutants of wastewater, heavy metals, wild and mutant strains of green microalgae *Parachlorella kessleri.*

М.М. Салех, М.М. Габаллах, Б.К. Заядан, А.К. Садвакасова

Parachlorella kessleri микробалдырының табиғи және мутантты штамдарын ластанған судың биоремедиациясында пайдалану

Бұл жұмыста *Parachlorella kessleri* жасыл микробалдырлардын жабайы және табиғи штамдары қолданылды. Ауыл шаруашылық қалдық сулармен және ауыр металдармен ластанған Әл-Саядин суының жабайы және мутантты штамдарына әсері зерттелді. Коректік орта мен ластанған су араласқан үлгілерде, аммоний, фосфат, кобальт және цинк иондарын алып тастаған жағдайда, жабайы және мутантты штамдардың қарқынды өскені анықталды. Бұл үлгіде микробалдырлардың биоремедиациялық потенциалы жоғары болғаны бақыланды.

***Түйін сөздер*:** ремедиация, ластанған сулар поллютанттары, ауыр металдар, микробалдыр *Parachlorella kessleri*-ның табиғи және мутант штамдары.

М.М. Салех, М.М. Габаллах, Б.К. Заядан, Садвакасова А.К.

Биоремедиация загрязненной воды с использованием диких и мутантных штаммов микроводоросли *Parachlorella kessleri*

В исследовании были использованы дикие и мутантные штаммы зеленой микроводоросли *Parachlorella kessleri*. Изучено влияние воды водоема Ал-Саядин, загрязненной сельскохозяйственными стоками и тяжелыми металлами, на рост диких и мутантных штаммов зеленой микроводоросли. Установлен активный рост клеток диких и мутантных штаммом микроводорослей в вариантах, содержащих смесь питательной среды и загрязненную воду, при исключении из среды аммиака, фосфата, кобальта, цинка, что способствовало улучшению потенциала биоремедиации загрязненной воды.

***Ключевые слова*:** ремедиация, поллютанты сточных вод, тяжелые металлы, природные и мутантные штаммы микроводоросли *Parachlorella kessleri*.

Nowadays, there are many problems that may threaten waterbodies allover the world, such as the pollution of many aquatic ecosystems with different types of pollutants that affect on water quality. The rapid increase of the industrial activity has caused a progressive deterioration of the water quality over the past decades. Numerous natural and chemical substances have been used and released without knowledge of the possible impact on the structure and function of aquatic ecosystems [1]. Bioassays are based on the response of living organisms to putative toxic substances in the water [2]. This approach does not allow identifying a given chemical substance but it indicates the presence of a pollutant above a potentially harmful threshold. Different unicellular and multicellular organisms have been used for this purpose including bacteria, protozoa, algae, invertebrates and fishes. It well known that the biomass of algae (living or dead) have immense capacity to sorb metals and have been applied to treat wastewaters [3, 4]. Different physiological responses such as growth rate, biomass, photosynthesis, fluorescence, and movement behavior have been monitored as criteria for the toxicity of a given water sample [5].

One of the main interests for microalgae in biotechnology is focused on their use for heavy metals removal from effluents and wastewater [6,7] stated that due to the high toxicity of the salts of heavy metals, as well as their ability to accumulate in the organisms and to be transferred along the trophic chain, they occupy a special position among environmental pollutants [8].

In this study the bioremediation of the polluted water of the Al-Sayydin lagoon was done by using wild and mutant strains of green microalgae *Parachlorella kessleri* (*PC*). These strains were used to investigate the efficiency of both strains in improving the physical, chemical parameters of water and efficiency of these strains in absorbance of some heavy metals from water.

Materials and Methods

Study area Al-Sayyadin lagoon located at south of Ismaillia city at the western side of the lake Timsah. This lagoon is polluted by sanitary and agricultural wastewater. Indeed, the accumulated heavy metals result in metal loading of the recently deposited sediments and water. These toxic pollutants in suspended and bottom sediments may access food web for aquatic macro-invertebrates and fish (Figure1 & 2).

Microalgal strains. The microalgal strain of *Parachlorella kessleri* (*PC*) and *PCMut*2, *PCMut*3, *PCMut*4 obtained from Kazakh National university- Al-Farabi, Biotechnology department culture collection. The strains were grown in TAP medium in 250 ml Erlenmeyer flask at 28̊C and were exposed to continuous illumination at a light intensity of 120μE $m^{-2}s^{-1}$.

Figure 1 – Geographical location of Al-Sayyadin lagoon

Results and Discussion

Design of the Bioremediation experiment. Water samples were collected from Al-Sayyadin lagoon (Fig.1) for only one time during the study. Physical and chemical parameters and some heavy metals were analyzed in the water. For all algal strains, the samples were divided as: Sample No.1: Negative control was 1000 ml of the culturing medium. Sample No.2: Dilution 4:1 was 800 ml culturing medium + 200 ml of the polluted water to reach 1000ml. Sample No.3: Dilution 3:2 was 600 ml culturing medium + 400 ml of the polluted water to reach 1000ml. Sample No.4: Dilution 2:3 was 400 ml culturing medium + 600 ml of the polluted water to reach 1000 ml. Sample No.5: Dilution 1:4 was 200ml culturing medium + 800 ml of the polluted water to reach 1000 ml. Sample No.6: Positive control was 1000ml of the polluted water. All those samples were inoculated with the algal strains. These samples were incubated for 7 days at the same growth condition of light and temperature.

Investigation of the growth of algal strains in the samples. Examination of the algal cells was done by using light inverted microscope of the OLYMPUS series. The initial cell density was about $1x10^5$ cells/ ml. Counting the number of cells everyday for a period of 7days by using UTERMÖHL's technique (1958) under the light microscope [9].

Microalgal strains. Wild type of *Parachlorella kessleri* and mutant strains *PCMut2, PCMut3* and *PCMut4* are shown in (Figure 2 a, b, c, d).

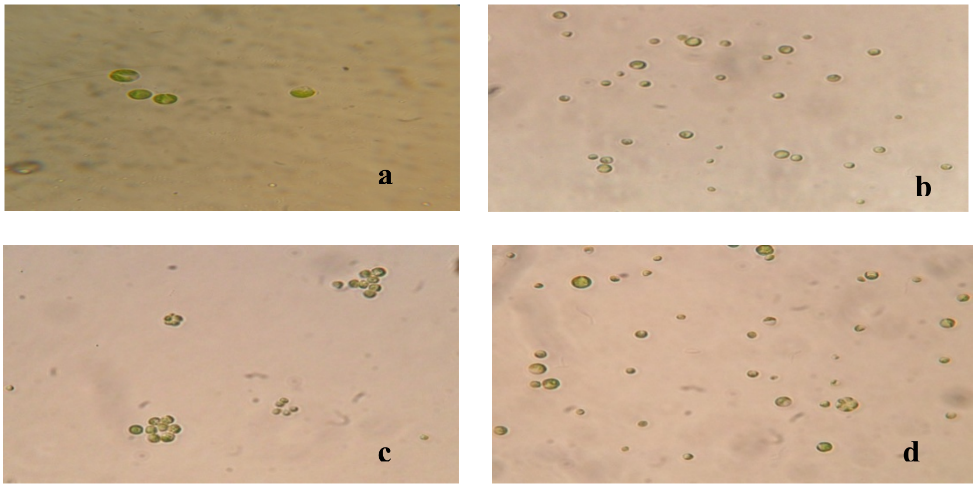

Figure 2 – *Parachlorella kessleri* (wildtype) (a), *PCMut2* strain (b), *PCMut3* strain (c), *PCMut4* strain (*d)*

Analysis of the physical and chemical parameters and some heavy metals of water samples. Water samples were filtered onto Whatman GF/C glass fiber filters to get rid of the algal cells before the analysis of the physical, chemical parameters and some of heavy metals. According to standard methods for examination of water and waste water (APHA), The temperature, pH were measured. Biological oxygen demand (BOD_5), chemical oxygen demand (COD), total dissolved solids (TDS), Nitrates, Nitrites, Ammonia, Phosphate, Cadmium, Cobalt and Zinc were analyzed in samples No.2, No.4 and No.6 for both algal strains after 7 days.

Table 1 – Parentages of cells numbers in different water samples

Strains	Water Samples					
	Neg. cont.	Dilution 4:1	Dilution 3:2	Dilution 2:3	Dilution 1:4	Pos. cont.
PC	100	92,4	66,2	72,2	58,7	14,5
PCMut2	100	216,3	204	218	93,6	29,3
PCMut3	100	81,3	145,4	174,9	67	13,8
PCMut4	100	125,7	152,2	143,3	141,9	27,3

Growth of algal strains in the water samples. Counting the number of cells of all algal strains in different water samples every day for a period of 7 days *PC, PCMut2, PCMut3* and *PCMut4* and the comparison between the best dilution that affect on the algal growth was shown in Table 1. The number of cells of both wild and mutant strains was the lowest in the positive controls which was contained the polluted water in comparison with all water samples. The algal strains in that case give us an indication that this water was highly polluted. On the other hand, the number of cells was the highest in negative controls which was contained the TAP medium for the wildtype. In *PCMut2* and *PCMut3* strains the best growth was in the dilution 2:3. The *PCMut4* strain was the only strain that showed the best growth in the dilution 3:2. The other factor that affect on growth of the strains was the presence of other microorganisms and bacteria that compete our strains and lowered their growth compared with the other samples that contained mixture of water and culturing medium, because in the case of presence of the culturing medium with water enhanced the algal strains to grow faster than the other microorganisms that not adapted with that culture medium like our strains. This result was confirmed by the results of Olumayowa *et..al.*, 2013 [10], microalgae increase in growth by 1.5 and 2.5 fold in autoclaved wastewater to get rid of other microorganisms and bacteria, so that, the microalgae growth in wastewater can be enhanced by autoclaving the water. Wastewater microorganisms including some anaerobic bacteria and viruses can be toxic or outcompete microalgae species. Absence of microorganisms could be one explanation for improved growth in autoclaved media [10].

Analysis of physical, chemical parameters and some heavy metals in water samples after bioremediation using Parachlorella kessleri and mutant strains. After a bioremediation using the algal strains for 7 days, the physical, chemical parameters and some heavy metals were analyzed in three water samples for each strain. The analysis of water in sample of dilution 4:1 showed a decrease in all parameters than the control in all wild and mutant strains. The remarkable decrease was occurred in the levels of Ammonia, Phosphate, Cobalt, Zinc.

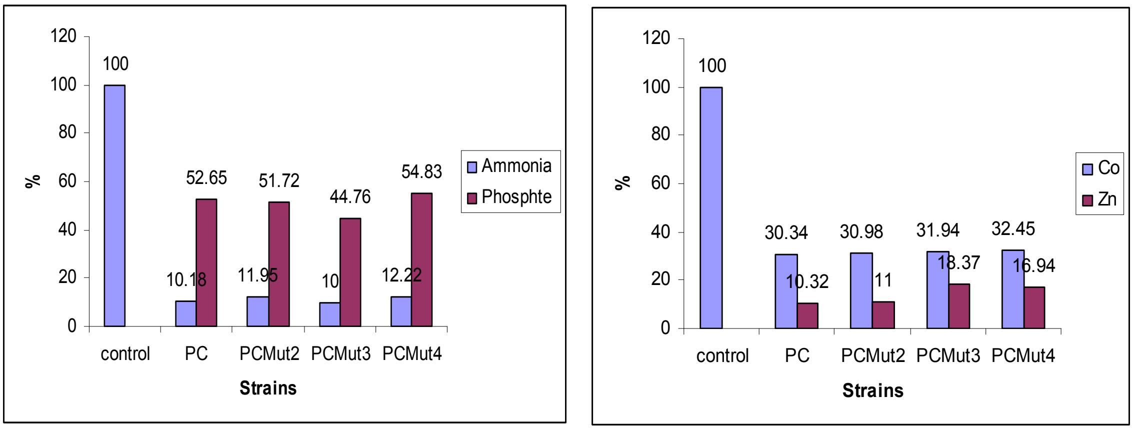

Figure 3 – Percent of Ammonia, Phosphate (a); Co and Zn (b) in water after bioremediation by using *Parachlorella kessleri* and mutant strains

The efficiency of the four strains in remediation potential was determined for Ammonia, Phosphate, Cobalt , Zinc removal and BOD, COD reduction which was clear in the analysis of water in both dilutions 4:1 and 2:3 samples. On the other hand, due to the poor growth in the samples of positive controls, which contained only polluted water, all the wild and mutant strains had no efficiency in treating the water but some parameters were increased than the control, and that result was explained previously by Cho, *et..al*, 2011. Due to the well growth of the wild and mutant strains in the samples of dilution 4:1, the results of water analysis showed remarkable decrease in Ammonia, Phosphate, Cobalt and Zinc (Figure 3 a, b).

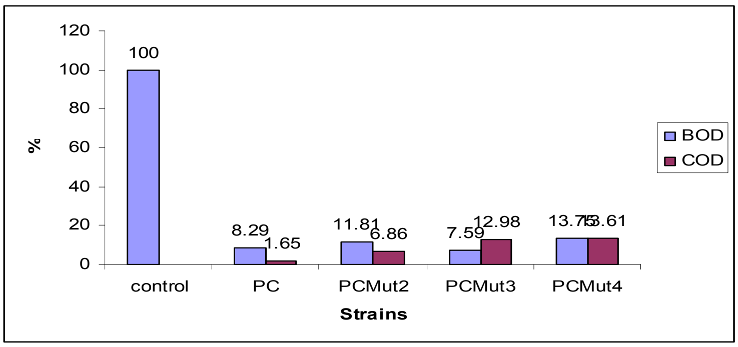

Figure 4 – Percent of BOD and COD in water after bioremediation by using *Parachlorella kessleri* and mutant strains

These N and P removal values were better or equivalent to those seen previously by algae from wastewaters, however, it is important to note that the N and P concentrations vary markedly in these different wastewater types [10]. The reduction in the levels of the BOD and COD, for the four strains in dilutions 4:1 showed in (Figure 4).

From the above results we proved that all the wild and mutant strains had approximately the same efficiency in water treatment because there was no significant difference ($P > 0.05$) between the wild and mutant strains in treating the polluted water because they had the approximate results. We concluded that the used doses of UV radiation didn't affect on the efficiency of remediation potentials in treating polluted water in the tested mutant strains.

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Ю.А. Синявский*, М.К. Кошимбеков, Ж.М. Сулейменова
Казахская академия питания, г. Алматы, Казахстан
Казахский Национальный медицинский университет им.С.Д.Асфендиярова, г. Алматы, Казахстан
e-mail: sinyavskiy@list.ru

Иммунный статус больных с ожоговой травмой на фоне приема специализированного продукта питания

В статье излагаются данные по оценке иммунного статуса больных с ожоговой травмой на фоне медикаментозной терапии, получавших специализированный продукт «Сергектік»

Ключевые слова: специализированный продукт, иммунитет, ожоговая травма.

Y.A.Sinyavsky, M.K.Koshimbekov, Zh.M. Suleimenova

The immune status of patients with burn injury in patients receiving specialized food

This paper presents data on the assessment of the immune status of patients with burn injury on a background of drug therapy, treated with a specialized product "Sergektik"

Keywords: custom product, immunity, burn injury.

Ю.А.Синявский, М.К.Кошимбеков, Ж.М.Сүлейменова

Арнайы тағам өнімін тұтыну кезіндегі күйік жарақаттары бар науқастардың иммундық мәртебесі

Мақалада «Сергектік» арнайы тағам өнімін тұтыну кезіндегі дәрілік терапия алып жатқан күйік жарақаттары бар науқастардың иммундық мәртебесі туралы деректер көрсетілген

Кілт сөздер: арнайы өнім, иммунитет, күйік жарақаты.

В патогенезеожоговой болезни, ключевую роль играет состояние иммунологической реактивности, с которой связаны риски развития инфекционно-воспалительных осложнений, очищение раны и интенсивность репаративных процессов, а также состояние микробиоценоза толстого кишечника[1-4].По данным литературы, абсолютно все авторы указывают на наличие у пациентов с термической травмой комбинированного вторичного иммунодефицита, связанного стравматическим стрессом, ожоговым шоком,выраженными нарушениями со стороны центральной и