

Adsorption of Radioactive Materials by Green Microalgae *Dunaliella Salina* from Aqueous Solution

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ABSTRACT

Introduction: Nuclear accidents release large quantities of radioactive materials into the environment. Iodine-131 and cesium-137 are two radionuclides released during nuclear accident, which can pose the greatest cancer risks. These radionuclides can be moved to other areas through rain and wind. The aim of this study was to develop efficient and economical biological methods for the absorption of water-soluble radionuclides released after a nuclear accident.

Material and Methods: The exposure of the algae to an aqueous solution of I-131 radionuclide was performed for 1, 2, and 3 h. The concentration activities of the samples were 27 $\mu\text{Ci/ml}$ and 270 $\mu\text{Ci/ml}$. After the removal of the alga by centrifuging, the activities of the sample solutions were measured using a calibrated dose calibrator. The measured activities at the mentioned periods of time were statistically significant for both groups ($P < 0.05$).

Results: The obtained results of the current study revealed that the activity of radioiodine-131 decreased 1, 2, and 3 h after adding algae, compared to the control group at the same time (21.8, 32.33, 39.84 for 27 $\mu\text{Ci/ml}$ and 15.38, 21.53, and 30% for 270 $\mu\text{Ci/ml}$, respectively). Furthermore, radioactive iodine is absorbed very well with this type of algae.

Conclusion: It can be concluded that *Dunaliella salina* can be used for the decontamination of radioiodine. This method can play a significant role in the decontamination of hazardous radioiodine after nuclear accidents.

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Introduction

Nuclear power plants generate more than 11% of the world's electricity [1]. When a nuclear power plant accident occurs similar to what happened in Fukushima, it causes the largest uncontrolled radioactive release into the environment [2, 3], which can lead to serious social and economic disruption for a large number of populations [3-5]. In other words, a nuclear accident can release many of radioactive materials, including iodine-131, cesium-137, and strontium-90 [6, 7]. The leakage and release of radioactive materials after a nuclear accident are considered as a major threat to public health [3, 8]. For instance, after the Great East Japan Earthquake, the release of radioactive materials from the Fukushima nuclear power plant posed health risks [9]. Radioactive materials can be released from a variety of ways, such as water, soil, and air [10, 11]. In case of accident, it is suggested to take potassium iodide tablets after accidental exposure to radioactive iodine as an effective protection against the irradiation of the thyroid [12]. Thyroid cancer is a rare and uncommon

type of cancer that occurs when abnormal cells begin to grow in thyroid gland and obviously related to external ionizing radiation exposure. Therefore, thyroid cancer is prevalent in populations exposed to ionizing radiation. The risk of thyroid cancer related to radiation exposure in studies of Japanese atomic-bomb survivors shows a decreasing trend during the post-exposure time or the increase of age-at-exposure [13, 14]. There are different ways to avoid spreading the contamination to other areas, such as certain bacteria and algae, that can absorb radioactive materials. Green micro-algae, as an absorbent of radioactive materials, can be very effective and helpful in removing contaminants [3]. Short-lived iodine-131, long-lived caesium-137, and strontium-90 are considered as the most important water-soluble radionuclides that contaminate aquatic ecosystems and food products, a major public health hazard, particularly form radiation dose [15]. Initial radiation exposure in the contaminated areas is normally due to short-lived iodine-131, followed by caesium-137. This

is known as the second main hazards, which are most commonly encountered after nuclear reactor accidents [8]. Large quantities of heavy metals can be accumulated by a variety of biomaterials including bacteria and fungi. Algae and yeast are believed to have a good performance in adsorption [16-19]. Zeolite, an aminosilicate, has been used effectively to absorb radionuclide materials as water decontaminating system in Fukushima [20]. The biosorption of radioactive metals can be an effective process for the decontamination aqueous solutions and algae as a new biosorbent material, which has been focused in recent research and investigations due to its high adsorption capacity [21]. It seems that sea water radioactive contamination is spreading more easily and contamination removal is difficult. Therefore, it is essential to use materials that can absorb these contaminants and prevent its spread to other areas. The current study was conducted to evaluate the absorption of radioiodine using a novel algal strain named *Dunaliella salina*. This halophile green microalga is known for its antioxidant activity, and can survive in highly saline conditions, such as sea salt fields.

Materials and Methods

Sampling and Cultures of Algal Strains

Dunaliella salina (Figure 1) was used to evaluate the absorption of radionuclides. It was previously isolated from Maharlu Salt Lake located in Shiraz (latitude 29.26 N, longitude 52.48 E), Iran, and identified based on rDNA ITS sequences (NCBI accession no. KC477401) to evaluate the absorption of radionuclides [22]. Algal suspensions were added to 250 mL glass flasks containing 100 mL of culture medium to give an initial cell density of 10^5 cells mL⁻¹ as described by Ben-Amotz et al. [23]. *Dunaliella salina* algae were identified based on morphological characteristics and identification keys. Moreover, molecular diagnostic methods were used to ensure the species identification. The purified algae were cultured in liquid medium in a large flask. The cultures were kept in a growth chamber under continuous and constant light intensity at $21 \pm 2^\circ\text{C}$. There were 3 samples in each group and in each sample contained about 10,000 algae. Algal cells at mid-logarithmic growth phase were exposed to an aqueous solution of I-131 radionuclide for 1, 2, and 3 h and experiments were repeated three times for each group.

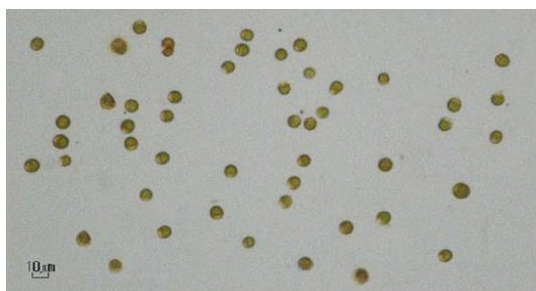


Figure 1. Identification of a novel strain of green algae, *Dunaliella salina*, microscopic image of *Dunaliella salina*

Radionuclide Uptake Assay

Radioiodine-131 was prepared from the Department of Nuclear Medicine, Namazi Hospital, Shiraz, Iran, with two activities of 27 $\mu\text{Ci/ml}$ and 270 $\mu\text{Ci/ml}$. In the next step, 5 ml of each of these activities was diluted with 30 ml of distilled water. Thereafter, 5 ml of the samples was separated for the control group and the rest (30 ml) were divided into 10 ml groups. Each one was added to a 40 ml solution containing algae. The control sample was mixed with 20 ml of distilled water to reach the same dilution ratio similar to other samples (1-5). In addition, there was a control group containing algae with no radioiodine. All the test groups were centrifuged for 10 min at 1500 rpm (ROTOFIX 32A, HETTICH, GERMANY) in 3 target time durations of 1, 2, and 3 h after the initiation of the examination. At the end of the experiment, solutions underwent dosimetry with calibrated dose calibrator (ISOMED 1000, Germany) of Department of nuclear medicine, Namazi Hospital, Iran.

Statistical analysis

Statistical analysis was performed in SPSS software (version 19) using the Mann-Whitney U and Kruskal Wallis tests.

Results

The current study evaluated adsorption radioactive iodine-131 in water solution containing *Dunaliella salina* microalgae, a type of halophile green microalgae found in the sea salt ground. *Dunaliella salina* has antioxidant activity since it can create a large number of carotenoids. In the present study, the samples were analyzed at different times of exposure. As shown in Figure 2 and 3, there were two control groups in the current study, one containing a tube of radioactive material without algae, and the other containing a tube with the algae. After adding the radioactive material to the control group containing algae, the activity at zero time was compared with the control group without algae. At this time, the difference between the two groups was not statistically significant ($P > 0.05$). In addition, at 1, 2, and 3 hours after the addition of radioactive Iodine-131, the activity was measured in a tube containing algae and measured with the control group without algae at the same time.

The findings of the present study showed that there was a statistically significant difference among all four groups in the two activities. Figures 2 and 3 display the activity of radioiodine-131 in the control group (without algae), and the experimental groups (1, 2 and 3 h after adding radioiodine-131 to tubes containing the algal solution). This means that the activity of radioiodine-131 decreases 1, 2, and 3 h after the addition of algae in comparison to the control group at the same time. The obtained results indicated a statistically significant difference in all groups ($P < 0.05$, Table 1). According to figures and Table 1, the decreasing trend in radioactivity in both groups (A and B) was 21.8, 32.33, and 39.84% for 27 $\mu\text{Ci/ml}$ and 15.38, 21.53, and 30% for 270 $\mu\text{Ci/ml}$, respectively.

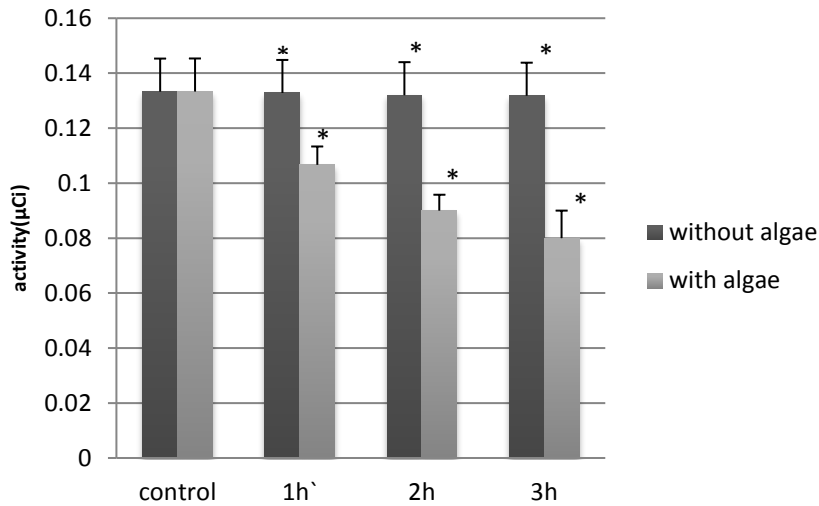


Figure 2. Comparison of activity concentration in 4 subgroup controls, 1, 2, and 3 hour after the initiation of the experiment and in activity of 27 µCi/ml (the initial activity before dilution), the results showed the mean±S.D of at least three independent experiments. *P<0.05 was compared with the control group; each of the groups in controls 1, 2, and 3 h were compared with a control group at the same time

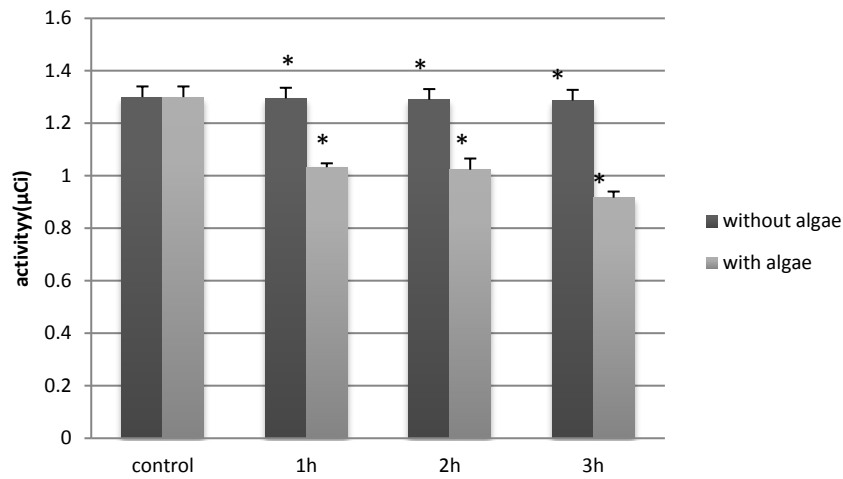


Figure.3. Comparison of activity concentration in 4 subgroup controls 1, 2 and 3 h after the initiation of experiment and in activity of 270 µCi/ml (the initial activity before dilution), the results showed the mean ± S.D of at least three independent experiments. *P<0.05 was compared with the control group; each of the groups in control 1, 2, and 3 h were compared with a control group at the same time.

Table 1. Activity concentrations in 2 groups of A and B and 4 subgroup controls 1, 2, and 3 h after the initiation of experiment (mean±SD)

		Control	1 hour	2 hour	3hour
		Mean±SD	Mean±SD	Mean±SD	Mean± SD
		(µCi/ml)	(µCi/ml)	(µCi/ml)	(µCi/ml)
Group A (activity: 27µCi/ml)	Without algae	0.133±0.012	0.104±0.0064	0.101±0.0057	0.982±0.0042
	With algae	0.133±0.012	0.106±0.0067	0.09 ±0.0057	0.08±0.001
Group B (activity: 270µCi/ml)	Without algae	1.3 ±0.040	1.29 ±0.037	1.271 ±0.056	1.256 ±0.012
	With algae	1.3 ±0.040	1.1±0.014	1.02±0.042	0.91±.023

Discussion

The current study was a report of the new species of microalgae *Dunaliella salina*. The occurrence of nuclear accident leads to a large spectrum of serious environmental problems. Cooling the reactor needs a large amount of water and decontamination systems to neutralize the radionuclide material, which is extremely expensive [24, 25]. Previous studies aimed to investigate

biomaterials, such as bacteria, fungi, yeast, and algae, as a cost-effective biotechnology for the treatment of wastewaters [16, 17]. However, the present study was a report of the characteristics of newly discovered green microalga *Dunaliella salina*, which have been recognized based on morphological characteristics and show accumulating radioactive nuclides by *Dunaliella salina* algae from water samples contaminated by radioiodine-131. Furthermore, we described the

accumulation of water-soluble radionuclides that can be released by nuclear reactors through a novel strain of alga.

Biosorption is known as a potential cost-effective biotechnology for the removal and recovery of heavy metal ions from aqueous solutions and high volume low-concentration containing heavy metal(s) [26, 27]. Another study introduced various mechanisms for binding metal materials to microalgae. The high capacity of these algae walls to bind with metals is their important feature [28]. In another study, it was shown that brown algal species, such as kelp, *Laminaria digitata*, were the most effective living accumulators of iodine and tissue concentrations [29]. Furthermore, brown algae have also been studied to absorb radioactive material and is effectively useful [30]. In a study conducted by Shimura et al., the ability of *Parachlorella sp. binos* (Binos) to accumulate strontium and cesium from water and soil samples collected from a heavily contaminated area in Fukushima was studied; however, iodine-131 in water and in laboratory conditions were addressed in the current study [3]. As the obtained results of the current study indicated the accumulation of radioactive materials, including iodine-131, is released in a nuclear accident by *Dunaliella Salina* algae from water samples. Moreover, the findings confirmed the effectiveness of the selected algae as biosorbent material.

As shown in figures 2 and 3, the activity differed in the three groups and control group. In the current study, a nuclear accident was simulated and radioactive materials, such as I-131, were released into seawater. Therefore, this species of algae was used to remove contamination. The obtained results showed that *Dunaliella salina* algae were capable of accumulating radioiodine-131 in water and could be used as an efficient sorbent for the removal of radioactive material in wastewater streams. In addition, the advantage of using these microalgae is that it can grow and spread very easily and in a very high volume rather than other strain. Nuclear power plant accidents similar to what happened in Fukushima need the development of decontamination systems that are both effective and affordable, which can be quickly used in the contaminated areas and minimize health risk. One of the benefits of this type of algae is its massive proliferation and its easy use in the path of nuclear contamination.

Conclusion

In this report, it was demonstrated that this novel alga strain simultaneously contaminate reduction iodine-131 released from nuclear power reactors accident. This method can be very cost effective and easy to carry out in the decontamination of radioactive materials. In practice, this method can yield to the removal of heavy materials from the environment using *Dunaliella salina* algae with two main mechanisms, the binding of metals to cellular surfaces (biosorption) and intracellular accumulation (bioaccumulation). Radionuclide materials will not be destroyed in this process; however, the

prevention of its spread can help humans to be protected from the exposure of dangerous radiation of iodine, strontium, and cesium radioisotopes. The current study aimed to introduce a new species of microalgae absorbing radioactive material, which can be easily cultivated and grow in seawater in different salinity.

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