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Removal of Sodium from Seawater Medium Using Photosynthetic Bacteria

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Abstract

The removal of sodium (Na) from seawater using two photosynthetic bacteria was investigated using *Rhodobacter sphaeroides* SSI (SSI) and *Rhodovulum* sp. which is a marine photosynthetic bacterium. Both *Rhodovulum* sp. and acclimated SSI were shown to grow well in a 3% NaCl supplemented glutamate-malate medium. The maximum rate of Na removal was 39.3% by SSI and 64.9% by *Rhodovulum* sp. after two days cultivation under static light conditions. However, Na was re-released back into the medium after two to three days. When a nutrient-supplemented seawater medium (3.3% NaCl, 13.10 gNa/l) was used, the maximum Na removal rates were 30.3% (9.05 gNa/l) by SSI and 48.9% (6.69 gNa/l) by *Rhodovulum* sp., under static light conditions. Similar growth and Na removal rates were found under aerobic dark cultivation. In this case, no re-release of Na was observed with either bacterium. Two stages culturing was conducted first, with *Rhodovulum* sp. and then with SSI replacement. The Na concentration was reduced to 0.79 gNa/l (94.0% removal) after cultivation for eight days under aerobic dark conditions. The supernatant was applied successfully as a liquid fertilizer in the cultivation of Japanese radish.

Keywords

Removal of Sodium, Marine Photosynthetic Bacteria, Desalinization, Replacement Culture, Liquid Fertilizer

1. Introduction

Photosynthetic bacteria that produce extracellular polymeric substances (EPS) on the cell surface adsorb cationic metals such as Cd, Pb, Cr, Hg, Cu and As at rates between 85% and 100% using the negative charge of the EPS [1] [2]. By the same process, *Rhodobacter sphaeroides* SSI (SSI) able to remove radionuclides at

rates of 95% for U, 82% for Sr and 58% for Co [2]. SSI has also been observed to adsorb Cs from waste water at rates approaching about 100% [3], but this seems to be closely related to the potassium transport system of photosynthetic bacteria [4]. In contrast, the marine photosynthetic bacterium, *Rhodovulum* sp. Accumulate abundant EPS on its cell surface allowing it to perform relatively strong self-immobilization [5].

The Great East Japan Earthquake occurred in March 2011. It released large amounts of radioactive materials, mainly Cs, from the Daiichi Nuclear Power Plant in Fukushima which then began to spread throughout the local and regional environment. In a previous study, we investigated the removal of radioactive Cs from the water and sediment mud of a swimming pool Fukushima City, demonstrating removal rates of almost 100% and 93% from the water and sediment mud of a swimming pool, respectively. This was achieved in four days using aerobic treatment with SSI immobilized alginate beads [6]. Removal of radioactive Cs from agricultural soil in Minami-Souma City and Namie Town, Fukushima was demonstrated using the same processes. In this case, the removal rate was between 60% and 75% [7]. Vegetables cultivated using the remediated soil were shown to have radioactivity of less than 100 Bq/kg which is within the recommended limit for human consumption in Japan [8]. These studies demonstrated the ability of the SSI strain to remove heavy metals, toxic metals and other cationic metals.

Panwichian *et al.* reported removal rates using newly isolated photosynthetic bacteria of the NW16 and the KMS 24 strains of 39% for Pb, 20% for Cu, 7% for Cd, 5% for Zn, and 31% for Na. These stains were cultured in a 3% NaCl seawater medium and exhibited Na reduction under microaerobic light (static light) and aerobic dark conditions. However, the precise function of Na reduction wasunclear [9]. Amezago *et al.* reported low-cost bio-desalinization of seawater using photosynthetic bacteria [10].

This study focused on the Na removal using two photosynthetic bacterial strains using a synthetic medium and a nutrient supplemented seawater medium (NSSW medium). Practical Na removal was demonstrated using both strains.

2. Materials and Methods

2.1. Photosynthetic Bacteria and Media

The marine photosynthetic bacterium used was *Rhodovulum* sp. [5] and a fresh water photosynthetic bacterium, *Rhodobacter sphaeroides* SSI [2] [3] was also used, both of which produce EPS on the cell surface. *Rhodovulum* sp. requires a medium in which the NaCl concentration is greater than 0.5%, whereas SSI will not normally grow when the NaCl concentration exceeds 0.5%. *Rhodovulum* sp. was isolated from the sea sediment mud of a shrimp cultivation pond in Thailand [5]. SSI strain was obtained as a spontaneous mutant which has self-flocculation ability from *Rhodobacter sphaeroides* S [2]. *R. sphaeroides* S has practically applications in wastewater treatment and has been used more than 40 years

in Japan [11].

Two NaCl supplemented glutamate-malate media (3% NaCl and 1% NaCl) were used for growth and Na removal. The composition of both GM media was as follows (g/l): sodium-L glutamate 3.8, DL-Malic acid 2.7, $(NH_4)_2HPO_4$ 0.8, Yeast extract 1.0, KH_2PO_4 0.5, K_2HPO_4 0.5, $MgSO_4 \cdot 6H_2O$ 0.2, $CaCl_2 \cdot 2H_2O$ 0.05, Thiamine-HCl 1×10^{-3}, Nicotinic acid 1×10^{-3}, Biotin 0.01×10^{-3}. This GM medium was modified from GM supplemented medium by Lascelles [12]. NaCl was then added to bring the concentrations to 3% and 1%.

An NSSW medium with 3.3% NaCl, was prepared by nutrients the addition of 5.0 g/l of glucose and 2.0 g/l of peptone. Seawater was taken from Hiroshima Bay, Japan. The initial pH of the medium was adjusted to 7.0 ± 0.1 using a HH_4OH solution or 6N-HCl. Media were sterilized by autoclave (at 121˚C, 20 min.).

To improve the growth of SSI in the 3% NaCl GM medium, acclimation was performed. First, the SSI was cultured in a 0.5% NaCl GM medium under static light conditions for two to three weeks. Slow growth was observed. The culture broth was then transferred to a 1% NaCl GM medium. Again, slow growth was observed. Cultivation was then performed three to five times in a 2% NaCl GM medium, and three to five times in 3% NaCl GM medium. The acclimated strain of SSI was then placed on agar plate of 3% NaCl GM medium. Cultivation of all strains was conducted at 30 ± 0.5˚C.

2.2. Cultivation

Pre-culturing was performed in a 300 ml conical flask (100 ml medium) under micro-aerobic condition with static light for three days. Illumination was supplied by a tungsten bubble at 5 klux (80 μE/m^2·sec) onto the surface of the flask. For the main culture, a 300 ml conical flask (200 ml medium) was held under the same conditions for five days. An aerobic dark culture was prepared in a 300 ml conical flask (medium 150 ml) placed on rotary shaker (120 rpm) for five days. Inoculation of the pre-cultured broth was conducted at 10% (v/v).

Culture replacement was performed in two stages. For *Rhodovulum* sp., culturing was first performed using the NSSW medium under both static light and aerobic dark conditions. After four days, the broth was centrifuged at 10,000 xg for 20 min to isolate the supernatant. Centrifugation was performed at room temperature (23˚C - 30˚C). This was sterilized using Millipore filter (0.45 μm pore size) and placed in a sterilized flask. In the second stage, SSI pre-cultured broth was inoculated at 10% (v/v) and cultivated for further four days under static light or aerobic dark conditions. On day eight, centrifugation at 10,000xg 20 min. was performed at room temperature (23˚C - 30˚C). In the next stage the experiment, the supernatant obtained was used as liquid fertilizer. Static light and aerobic dark culture were performed with 200 ml and 150 ml culture broth, respectively, in 300 ml flask. Cultivation temperature of *Rhodovulum* sp. and SSI was 30 ± 0.5˚C.

During the cultivation of both strains, 10 ml of culture broth was pulled out and measured OD_{660} for the growth evaluation every day. And then, culture broth was centrifuged (10,000 xg, 20 min.) at 4˚C. From the supernatant, Na concentration was measured.

2.3. Cultivation of Japanese Radish

Sprout of Japanese radish (*Raphanas sativas*) were cultured in the polyethylene container (10 × 20 × 5 cm) on 2 cm layer of cotton(put on the bottom) with a supply of deionized water to have wet condition. The container was placed near a window under indirect sunlight at roomtemperature (23˚C - 30˚C).

As a control, sprouts were sprayed with a commercial liquid fertilizer, diluted 1000 times HYPONeX stock solution (HYPONeX Japan Co. Ltd.) at one to three days intervals. A second container, supernatant as liquid fertilizer was sprayed in the same manner as the control. Growth and damages were observed for about one month.

2.4. Analysis

The growth of the photosynthetic bacteria was observed at OD_{660} using a spectrophotometer (Shimadzu, UV-1700). The Na concentration was measured using anatomic adsorption spectrophotometer (Shimadzu, AA-6200). Experimental errors of OD_{660} and Na were the mean value ± 5% and ± 10%, respectively. Data were the mean values from triplet experiments.

3. Results and Discussion

3.1. Na Removal from 3% NaClGM Medium

Figure 1(a) and **Figure 1(b)** show the growth and Na removal of photosynthetic bacteria for 3% NaCl GM medium (11.80 gNa/l), respectively. The SSI grew well even at this high salinity, and Na removal of 1.80 g/l (39.3% removal) was observed after three days culture under static light conditions (**Figure 1(a)**). Almost the same growth and Na removal pattern were observed under the aerobic dark conditions (**Figure 1(b)**). These results reflected the acclimation of the SSI, which is known as a fresh water photosynthetic bacterium and cannot normally grow when the NaCl concentration exceeds 0.5%.

In contrast, *Rhodovulum* sp. showed more rapid growth and greater Nareduction. The maximum Na removal rate of 4.25 gNa/l (64.9% removal) was recorded after one day under the static light conditions (**Figure 1(c)**), However, once growth was became steady, the Na concentration began to increased again, finally returning to almost the original level. A very similar pattern was observed under the aerobic dark conditions, although the rate of growth was slower.

It is well known that the energy status of the cells of photosynthetic bacteria affects the uptake of Na. Under high energy conditions, Na is incorporated, whereas, under low energy conditions Na is exported [10] [13]. In the final stage

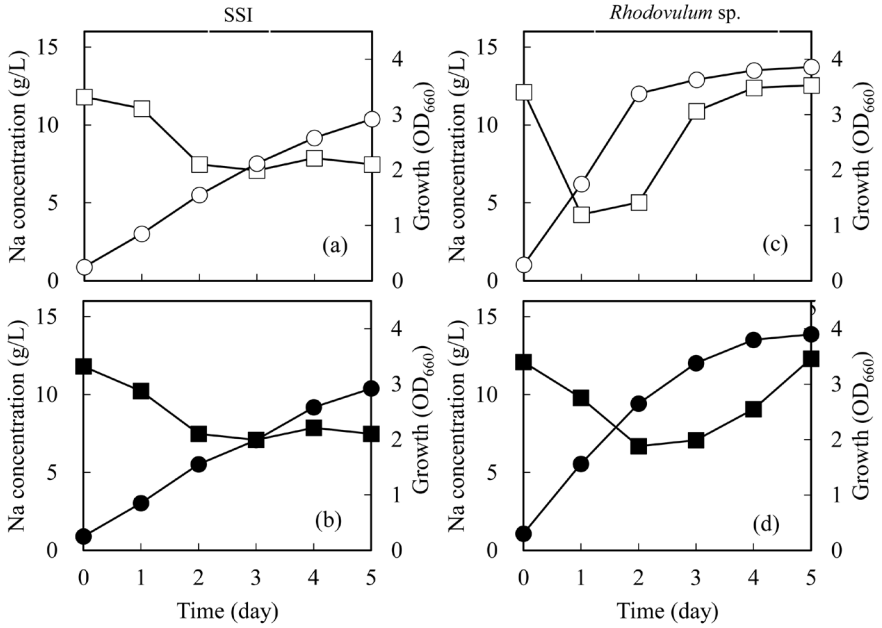

Figure 1. Growth and Na removal by *Rhodobacter sphaeroides* SSI and *Rhodovulum* sp. with 3%NaCl supplemented glutamate-malate medium. ○: growth under static light conditions; □: Na removal under static light conditions; ●: growth under aerobic dark conditions; ■: Na removal under aerobic dark conditions.

of growth, energy harvesting fell because of the limitations on carbon, light and oxygen, so that Na might be exported again into the medium.

3.2. Na Removal from 1% NaCl Supplemented GM Medium

As shown in **Figure 2(a)**, the SSI grew well in the 1% NaCl GM medium (3.93 gNa/l), and the Na concentration fell almost zero (about 100% removal) after one day under the static light conditions. However, after three days, Nabegan is to be exported into the medium. We observed almost the same growth and Na removal pattern under the aerobic dark conditions (**Figure 2(b)**).

The growth of *Rhodovulum* sp in the 1% NaCl GM medium (**Figure 2(c)**) was slower than that in the 3% NaCl GM medium (**Figure 1(c)**). Na concentration again fell to almost zero after one day both the static light and aerobic dark conditions.

In the removal of Na by SSI, the negative charge of the EPS on the cell surface appear to function in the same way the adsorption of cationic heavy metals and radionuclide [1] [2]. However, the process by *Rhodovulu*m sp. removes Na by its Na transport system of cells compared with the adsorption by EPS of negative charge [10] [13]. The functions of Na removal by *Rhodovulm* sp. needs further investigation.

3.3. Na Removal in the Seawater Medium

The SSI strain successfully grew in the NSSW medium removing up to 30.3 % of the Na after four days under both the static light conditions (**Figure 3(a)**) and

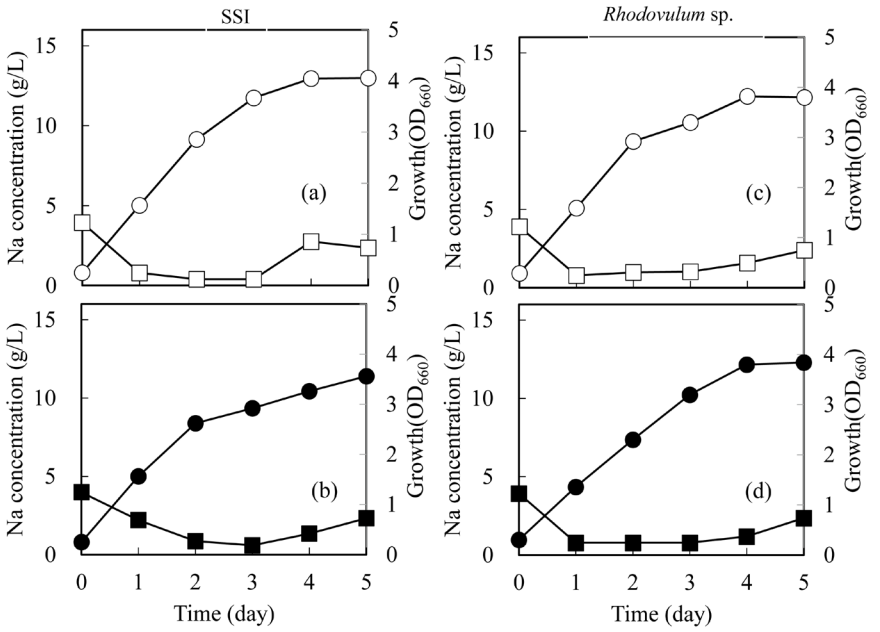

Figure 2. Growth and Na removal by *Rhodobacter sphaeroides* SSI and *Rhodovulum* sp. with 1% NaCl supplemented glutamate-malate medium. Symbols are the same as in **Figure 1**.

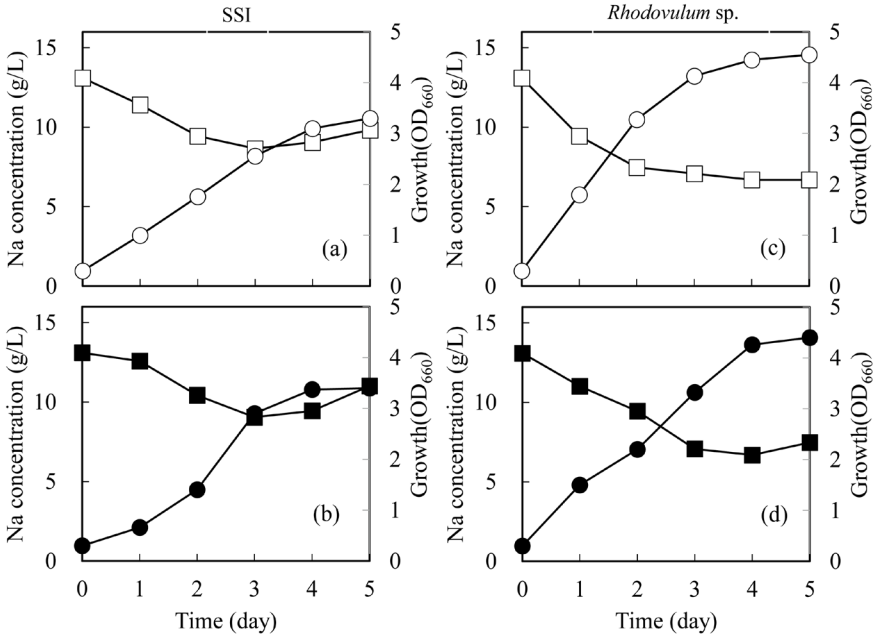

Figure 3. Growth and Na removal by *Rhodobacter sphaeroides* SSI and *Rhodovulum* sp. with a NSSW medium. Symbols are the same as in **Figure 1**.

the aerobic dark conditions (**Figure 3(b)**). This was almost the same as the results from the 3%NaCl GM medium (**Figure 1(a)**).

When *Rhodovulum* sp. was grown in the NSSW medium under the static light (**Figure 3(c)**) and aerobic dark conditions (**Figure 3(d)**), Na removal reached 48.9% after four days and did not subsequently reduce and no export of Na. The

addition of nutrients (containing peptone, high molecular weight organic materials) may have extended energy supply and suppressed Na export.

Both SSI and *Rhodovulum* sp. were able to remove Na from the NSSW medium under the aerobic dark conditions. This suggests that the use of photosynthetic bacteria offers a convenient, low-cost approach for the desalinization of seawater. In other desalinization processes that use photosynthetic bacteria, for example, cyanobacteria, large ponds are required to provide sunlight for growth of cells [10].

3.4. Two Stages Na Removal in the NSSW Medium by Culture Replacement

We observed relatively high Na reduction by *Rhodovulum* sp. and SSI in the NSSW medium and almost nore-export of Na into the broth. Na removal was performed in two stages. As can be seen from **Figure 4**, in the first stage, *Rhodovlum* sp. was cultured in the NSSW medium at 13.1 gNa/l. After four days, the Na concentration was reduced to 5.9 gNa/l (55.9% reduction) under static light conditions and to 5.51 gNa/l (57.9% reduction) under aerobic dark conditions. In the second stage, *Rhodovulum* sp. cells were separated using centrifugation, and the SSI culture was replaced using the supernatant after sterilization.

The minimum Na concentration of 0.79 gNa/l (0.2% NaCl concentration) was reached after four days of the second stage (total culture time of eight days) under both conditions. This represented a 94.0% removal of Na from the seawater. It must be emphasized that it was high level desalinization establishment using the two stages aerobic culture. For example, Panwichian *et al*. reported that reduction to 31% of Na removal was observed using photosynthetic bacteriacul-

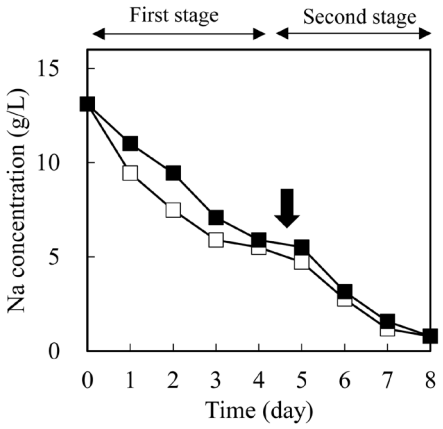

Figure 4. Na removal during *Rhodovulum* sp. culture with a NSSW medium in the first stage. Na removal during *Rhodobacter sphaeroides* SSI culture with nutrients supplemented supernatant of cultured broth in the second stage. Arrows indicate the *Rhodovulum* cells separation by centrifugation and supernatant obtained for replacement culture (see Materials and Methods). □: Na removal under static light conditions; ■: Na removal under aerobic dark conditions.

ture from the seawater [9]. The high level of Na removal from seawater found in our study has not been previously reported when using photosynthetic bacteria, including cyan bacteria.

3.5. Use of Culture Supernatant for Vegetable Culture

The supernatant obtained by centrifugation was used as a liquid fertilizer for the cultivation of Japanese radish. The growth and the appearance of sprouts were similar to the samples grown using the conventional liquid fertilizer of HYPO-NeX solution for one month (data not shown).

The supernatant contained photosynthetic bacterial metabolites such as amino acids, organic acids and extracellular products. These might support vegetable growth at a low Na concentration. For example, a photosynthetic bacterial supernatant usually contains 10 - 30 μmole/I of 5-aminolevulinic acid (ALA). This extracellular product supports the growth of rice seedling [14]. ALA has already been commercialized and is used as a liquid fertilizer in the cultivation of crops, vegetables and flowers [15].

This study suggests the possibility of producing liquid fertilizer from seawater after two stage treatment using photosynthetic bacteria. This would support agriculture in regions where fresh water is scarce, such as islands and coastal areas of Japan. However, further investigation and long term observation of this liquid fertilizer (to assess its safety) will be needed before applying it to the cultivation of vegetables, plants or flowers.

3.6. Proposals for Practical NaCl Removal from Seawater and Production of Liquid Fertilizer

Based on our results, we propose the practical process of Na reduction and bioconversion of seawater into liquid fertilizer as shown in **Figure 5**. After nutrients are added and the seawater issterilized with addition of Cl_2, aeration beginning. The *Rhodovulum* sp. seed culture broth is inoculated after the Cl_2 level fell below 0.1 mg/l. A dense inoculation of about 10% (v/v) is used to maintain the dominance of the *Rhodovulum* sp. A seed culture broth is obtained from the NSSW medium in a Jar ferment or culture. After sterilization in an autoclave, pure culture broth is obtained, to preserve the dominance of photosynthetic bacteria, anaerobic digestion liquor from food wastes, agricultural wastes or night soil are preferred as nutrients. Lower fatty acids such as acetic, propionic and butyric acids are suitable carbon source for photosynthetic bacteria but not preferable for other bacteria [11] [15].

Photosynthetic bacteria can grow without large contamination under low oxygen level with a dissolved oxygen level below 1.0 mg/l. In Japan, practical wastewater treatment plants handling food processing wastewater mainly use activated sludge treatment. Photosynthetic bacteria are usually dominant under the low oxygen level in this aerobic treatment [11] [15]. But, fresh photosynthetic bacterial seed cultures must sometimes be added to maintain this dominance. This is not practically challenging in practical applications [11] [16] [17].

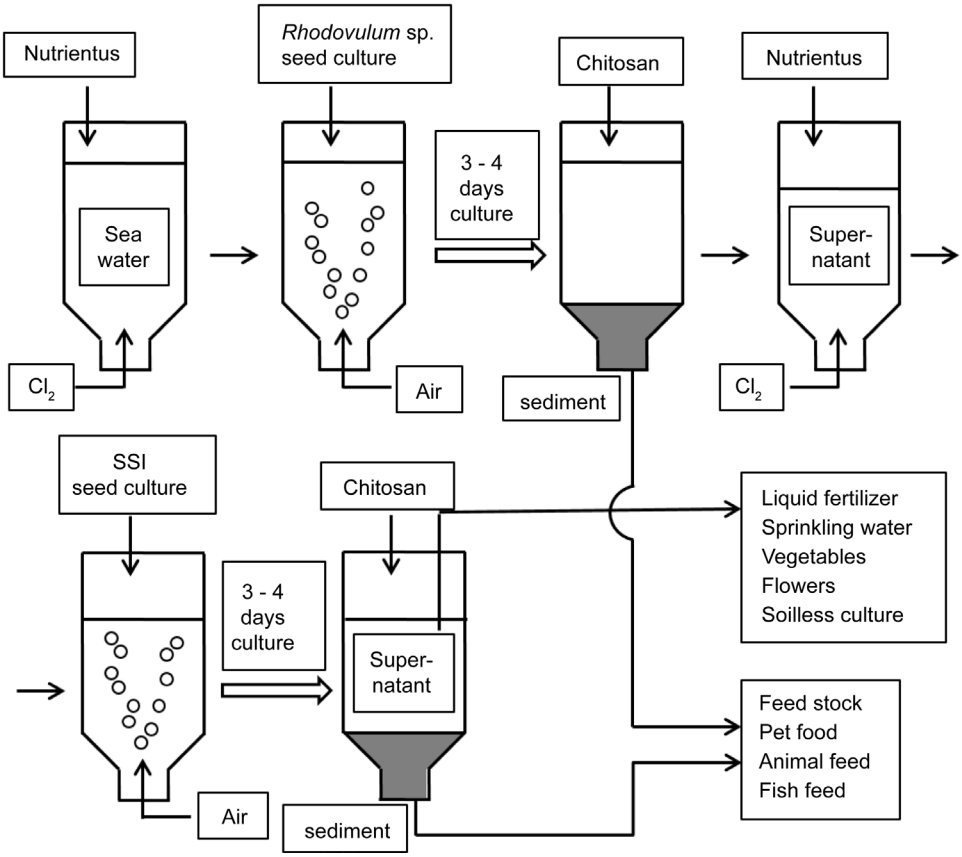

Figure 5. Schematic of the practical process of seawater bioconversion into liquid fertilizer or sprinkling water using photosynthetic bacteria. Steps: 1) Add nutrients and Cl_2; 2) Aeration and *Rhodovulum* sp. seed culture inoculum (<0.1 mgCl_2/l); 3) Three to four days culture; 4) Add chitosan and cells sediment recovery; 5) Add nutrients and Cl_2; 6) Aeration and SSI seed culture inoculation (<0.1 mgCl_2/l); 7) Three to four days culture; 8) Add chitosan and cells sediment recovery. Supernatant of SSI replacement culture is used as a liquid fertilizer or sprinkling water. Nutrients are the wastes from food processing or agriculture.

After cultivation of *Rhodovulum* sp., chitosan sedimentation is performed to separate the cells. Chitosan is a good coagulant and for photosynthetic bacteria and can easily sediment the bacteria. As chitosan is a natural material, the sedimented cells can also be used as a feedstock for production of pet food, animal feed and fish feed. After three to four days of SSI culture, the cells are again sedimented and separate using chitosan treatment. The supernatant obtained can be used as a liquid fertilizer or sprinkling water for vegetables, flowers and water source for soilless culture or hydroponic culture.

In this study, we demonstrated possibility of the bioconversion of seawater into liquid fertilized by removal of Na using two strains of photosynthetic bacteria. The comparative cost of this alternative approach is currently being investigated.

4. Conclusions

The removal of Na by photosynthetic bacteria was investigated, and the follow-

ing results were obtained.

1) Acclimated SSI was shown to grow well in a 3% NaClGM medium (11.8 gNa/l). The maximum Na removal was 39.3% (7.16 gNa/l) under static light conditions and 36.7% (7.47 gNa/l) under aerobic dark conditions.

2) *Rhodovulum* sp. also grew well in the 3% NaCl GM medium maximum Na removal of 64.9% (4.2 gNa/l) was observed after one day of culture under both conditions but Na began to be released back into the broth after two days.

3) In the NSSW medium (3.3% NaCl, 13.1 gNa/l), SSI was shown to remove up to 30.3% of the Na (9.05 gNa/l) after four days under the static light conditions, and *Rhodovulum* sp. up to 48.9% (6.69 gNa/l). In this case, almost no Na was later exported. Similar results were observed under the aerobic dark conditions.

4) Tow stage culturing first, with *Rhodovulu*m sp. then with an SSI replacement culture was performed under the aerobic dark conditions using the NSSW medium. Finally, the Na concentration was reduced to 0.79 gNa/l (94% removal) after eight days. This represented a high level of desalinization.

5) The supernatant (0.2% NaCl) was used in the cultivation of Japanese radish, demonstrating its use as a liquid fertilizer.

6) Our study demonstrated a practical approach to the bioconversion of seawater into liquid fertilizer using *Rhodovulum* sp. and SSI.

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