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# Salinity on growth and chromium reduction abilities of *Magnetospirillum gryphiswaldense* MSR-1

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**Abstract** Use of bacteria in removal of heavy metals, including hexavalent chromium Cr(VI), has been proven to be as effective yet more eco-friendly compared to other existing technologies. However, polluted wastewater usually contains high amounts of salts in addition to the heavy metals and may interfere with the bioremediation process. Thus, the ideal bacterial strain for these scenarios must be resistant to the heavy metal and high salt concentration but still capable of removing the target pollutant. The current paper studies the effect of salinity on the growth and chromium reduction capabilities of the magnetotactic bacteria *Magnetospirillum gryphiswaldense* (MSR-1). MSR-1 was exposed to 20 mM, 50 mM, and 100 mM NaCl concentrations and monitored for 27 hours by measuring its optical density and chromium concentrations at different time periods. The results showed that the presence of NaCl does not greatly affect the growth of MSR-1 even at increased concentrations. On the other hand, the introduction of salt in the culture hampered the chromium reduction capacity of the strain with the addition 100 mM NaCl resulting in a decrease of 43% on the amount of chromium reduced.

**Keywords:** magnetotactic bacteria, chromium reduction, salinity, bioremediation, heavy metal

## 1. Introduction

Chromium pollution has been one of the most serious concerns in the past decades due to chromium's toxicity, prevalence, and poor disposal (Branco et al., 2008). In the environment, chromium predominantly exists in two forms: Cr(VI) and Cr(III). Investigations show that Cr(VI) is the more toxic form, attributing to its rapid transport across biological membranes and its ability to damage DNA (Arevalo-Rangel et al., 2013). Reduction of Cr(VI) to the less hazardous Cr(III) is thought to be an effective way to handle chromium pollution.

Use of biological methods are appealing options to address this due to their advantages such as low cost and high efficiency at lower metal concentrations (Ye et al., 2023). Magnetotactic bacteria (MTB) offers an additional benefit of being separable from the effluent due to the presence of magnetosomes (MS). MS are membrane-bound crystals that enable MTB to align along local magnetic field lines, a behavior known as magnetotaxis (Gareev et al., 2021). MTB has already been proven to be capable of bioremediating different metals. Results from Wu et al. (2023) recorded that *Magnetospirillum gryphiswaldense*, an MTB strain, can remove up to 40 mg/L Cr(VI).

There are few studies available on the performance of bacteria in reducing chromium in waters with high contents of salt ions, which are usually present in industrial effluents (Narayani and Shetty, 2013). Knowledge of the effects of factors such as salt concentration is necessary to design an effective treatment for polluted waters. Thus, the current work investigates the effect of salinity on growth and Cr(VI) reduction efficiency of a magnetotactic bacterium, *Magnetospirillum gryphiswaldense* MSR-1.

## 2. Materials and methods

### *2.1. Bacterial strains and medium preparation*

The bacteria *Magnetospirillum gryphiswaldense* MSR-1T (DSMZ 6361) was purchased from Leibniz-DSMZ (Deutsche Sammlung van Mikrooganismen und Zellkulturen GmbH, Germany). The pre-cultures were grown in flask standard medium (FSM) as formulated in a previous study (Heyen and Schuler, 2003). All samples contain sterilized ionic washing buffer (IWB) added with potassium lactate and ferric citrate as their base. IWB is composed of 0.1 g $KH_2PO_4$, 0.34 g $NaNO_3$, and 1 mL EDTA chelated trace mixture solution per liter. Potassium lactate was selected as the carbon source while addition of ferric citrate was set as it supplies iron for the development of magnetosomes by the bacterial cells. The added amounts of these two chemicals were also based on FSM.

### *2.2. Sample preparation and measurements*

MSR-1 was shaken cultured in microaerobic ($1\%O_2$:$99\%N_2$) serum bottles sealed with butyl-rubber stopper containing 5 mL FSM for 48 hours at culture

conditions of 30ºC and shaking at 120 rpm (Wu et al., 2023). The culture was mixed after with 35 mL FSM in 100 mL-serum bottles and shaken-cultured under the same conditions for another 16 hours. The optical density of the resulting culture was measured using UV-Vis spectrophotometer at 565 nm wavelength ($OD_{565}$). The cells were then washed twice with 40 mL IWB and centrifuged at 4ºC and 8500 rpm for 10 minutes.

The cells were resuspended in IWB with potassium lactate and ferric citrate, then added with their respective NaCl concentrations. All cultures were adjusted to the same concentration by setting their initial $OD_{565}$ to 0.5. Cr(VI) as potassium dichromate ($K_2Cr_2O_7$) was added to a final concentration of 10 mg/L.

The growth of the cells was monitored by measuring their $OD_{565}$ using UV-Vis spectrophotometer. The 1,5-diphenylcarbazide (DPC) method was used to determine the Cr(VI) concentration of the samples. Absorbance values were measured at 540 nm (Su et al., 2021).

## 3. Results and Discussions

Optical densities of MSR-1 in different salt concentrations at different time periods are shown in Figure 1.

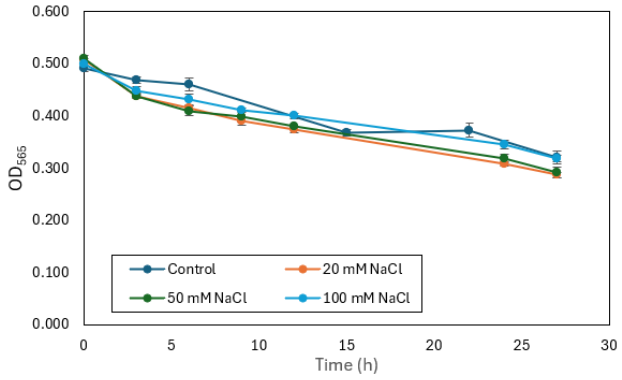


**Figure 1.** Optical density at 565 nm of MSR-1 whole cells exposed to different concentrations of NaCl.

Results in Figure 1 demonstrate that MSR-1 tolerated the presence of salt as it did not affect the bacteria's growth. In addition, no significant changes were observed as the concentration of the salt added increased. On the other hand, the summarized Cr(VI) reduction efficiencies in Table 1 shows a dramatic 34% drop from 91.35% efficiency was observed as 20 mM of NaCl was added to the MSR-1 cultures. Slight decrease to 53.31% and 48.33% Cr(VI) reduction efficiencies were noted as salt concentrations increased to 50 and 100 mM, respectively.

Many Cr(VI)-resistant bacteria also show tolerance in high salinity. However, it is also a common result that removal yield values decrease as salt concentration increases (Narayani and Shetty, 2013). This can be mostly attributed to the presence of salt's effect on enzyme stability (Braham et al., 2021). In this case, NaCl has more of a destabilizing effect on MSR-1's chromate reductase. This inhibition was also displayed in other studies (Ye et al., 2023; Arevalo-Rangel et al., 2013). Nevertheless, the substantial reduction efficiency of MSR-1 even after increasing the NaCl concentrations to 100 mM shows its great potential to be utilized for chromate reduction in highly saline wastewaters.

**Table 1.** Reduction efficiencies of MSR-1 whole cells exposed to different concentrations of NaCl.

| NaCl conc. (mM) | Cr(VI) reduction efficiency (%) | |
|---|---|---|
| 0 | 91.35 | |
| 20 | 57.96 | ±3.64 |
| 50 | 53.31 | ±2.03 |
| 100 | 48.33 | ±1.93 |

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