

## Bio-removal of Nickel ions by *Sporosarcina pasteurii* and *Bacillus megaterium*, A Comparative Study

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**Abstract.** The aim of this work was to study the potential of *Sporosarcina pasteurii* 586S and *Bacillus megaterium* 1295S isolated from sewage treatment plants (STPs) in removing of nickel ions from the aqueous solution. The bacterial cells were used as living and dead cell biomass. The efficiency of bio-removal process was investigated as a response for nickel and biomass concentrations, time, pH and temperature. The bio-removal capacity ( $Q_{max}$ ) of both strains were compared. The highest bio-removal percentage was recorded by dead cells in comparison to living cells. Dead cell biomass of *B. megaterium* 1295S exhibited higher efficiency for bio-removing of  $Ni^{2+}$  than *S. pasteurii* 586S at 196.4 and 200.2 mg  $Ni^{2+}$  g<sup>-1</sup>, respectively. It can be concluded that both bacterial strains have high potential to be applied in the biotechnology for removing of  $Ni^{2+}$  ions, however, dead cells of *B. megaterium* 1295S is the most potent.

### 1. Introduction

Bacterial cells have high affinity to removal of heavy metal ions, due to their novel structure in the cell wall and transport system in the cell membrane. Many of the transport systems are the administrator for transport of metal ions through the bacterial cell wall. Some of the transport system such as chemi-osmotic and proton gradient which called passive transport system has no need the energy, while others which called active transport system included ABC (ATP-binding cassette), MIT (metal inorganic transport, CHR (chromate transport), P-type and HoxN depend of the energy [1] The transport systems differ among the bacterial species and strains which lead to occurrence the differences in the efficiency of each bacteria in the removal of heavy metal ions. Bacterial cells have high potential to develop their mechanism to tolerate the toxicity of heavy metals by the accumulation it's in their cell walls, this properties is the key to select the most potent bacterial strains which might be used for the biotechnology and removal of metal ions from the contaminated wastewater (Al-Gheethi et al. 2014). The ability of the bacterial cells to develop their potential depend on the surrounding environment, bacterial strains which are living in high contaminated environment exhibited high potential to tolerate heavy metals and thus bio accumulate in their cell wall structure or



inside the cells as a non-toxic compounds. Sewage sludge are among several types of wastewater which are high contaminated with heavy metals and high bacterial diversity due to the availability of nutrients necessary for bacterial growth. Therefore, the sewage sludge represent a rich source for isolating bacterial strains with high efficiency for bio-removal of metal ions (Al-Gheethi et al. 2015). Nonetheless, as a results for the high bacterial diversity in these waste the potential to get a most potent bacterial strain to be applied in the biotechnology need to explore and compare between different bacterial strains in their effectiveness for metal tolerance and removal and this is the main focus of the current work. The present study is a continuous work for previous project in which the comparison between gram positive bacteria (*B. subtilis*) and Gram negative bacteria (*Burkholderia cepacia*) was conducted by Abdul-Monem et al. (2010). In the present study, the comparison between two bacterial strains belongs to Gram positive bacteria but it were isolated from biosolids generated from two sewage treatment plants (STPs). The aim of this work was to understand the role of isolation source on the ability of bacterial strains in removing of heavy metals.

The nickel ions are among the heavy metal ions available with the toxic concentrations in sludge due to the deficiency in the primary and secondary process to reduce it during the sewage treatment. Besides, nickel ions is one of the important element which is required as trace elements at as cofactors for several enzymes, while is toxic at high concentrations (Al-Gheethi 2015). Besides, the removal of this elements from the sludge is need for safe reuse of the sludge in the agriculture. Therefore, nickel ions was used in this study as a model to compare between the biosorptive capacity of *S. pasteurii* 586S and *B. megaterium* 1295S isolated from the sewage sludge, both strains exhibited high tolerance for the growth in the presence of nickel ions, but their ability to biosorb the metal ions from aqueous solution need more studies which were performed in this work.

## 2. Materials and Methods

### 2.1 Preparation of the bacterial cell biomass

*S. pasteurii* 586S and *B. megaterium* 1295S were isolated from sewage sludge and identified as described in previous work (Al-Gheethi 2015). The bacterial strains were selected among several bacterial isolates due to their ability to tolerate 15 mM of Ni<sup>2+</sup> ions. Pure culture of both bacterial were subculture separately on peptone glucose yeast extract (PGY) for 48 hrs at 37°C to produce of bacterial biomass. The bacterial cells biomass were harvested after 48 h where the maximum density of the bacterial cells are generated, the harvesting process were performed by centrifugation at 4020 rcf for 20 min and then washed three time with sterilized distilled water to remove the media residues. Thereafter suspended again with 10 mL distilled water. The bacterial suspended solution was divided into two parts, one part was used as a living cells (without treatment), while second part was subjected for the heating at 100°C for 15 min to prepare the dead cells (Abdul-Monem et al. 2010).

### 2.2 Bio-removal batch experiments

The efficiency of *S. pasteurii* 586S and *B. megaterium* 1295S for removal of Ni<sup>2+</sup> ions were compared based on their response for the effect different concentrations of Ni<sup>2+</sup> ions and biomass cells, pH, time and temperature. Nickel ions solutions were prepared with concentrations ranged from 0.1 to 0.8 mg L<sup>-1</sup>, biomass cell concentrations were investigated in the range between 0.1 to 3 mg L<sup>-1</sup>. The incubation time was conducted between 1 to 10 hrs, while pH ranged from 2 to 8 and temperature between 20 and 55°C. The biomass cells were separated from the nickel ion solutions by the centrifugation and the remaining Ni<sup>2+</sup> ions in the supernatant was determined spectrophotometrically (Snell and Snell, 1949) using UV-160 A spectrophotometer at 440 nm (Win. Aspect T20, 031-2004, Germany). The biosorptive capacity ( $Q_{\max}$ ) for both bacterial strains were determined by the inoculation of bacterial cells biomass in series nickel ions solutions as described by Gardea-Torresdey et al. (1998). The concentrations of remaining Ni<sup>2+</sup> ions in each solution was determined at the end of each experiment. The bio-removal efficiency and bio-removal capacity was calculated according to Equation 1 and 2 (Al-Gheethi et al. 2016).

$$E = \frac{C_i - C_f}{C_i} \times 100 \quad (1)$$

$$Q_{\max} = V \times \frac{C_i - C_f}{M} \quad (2)$$

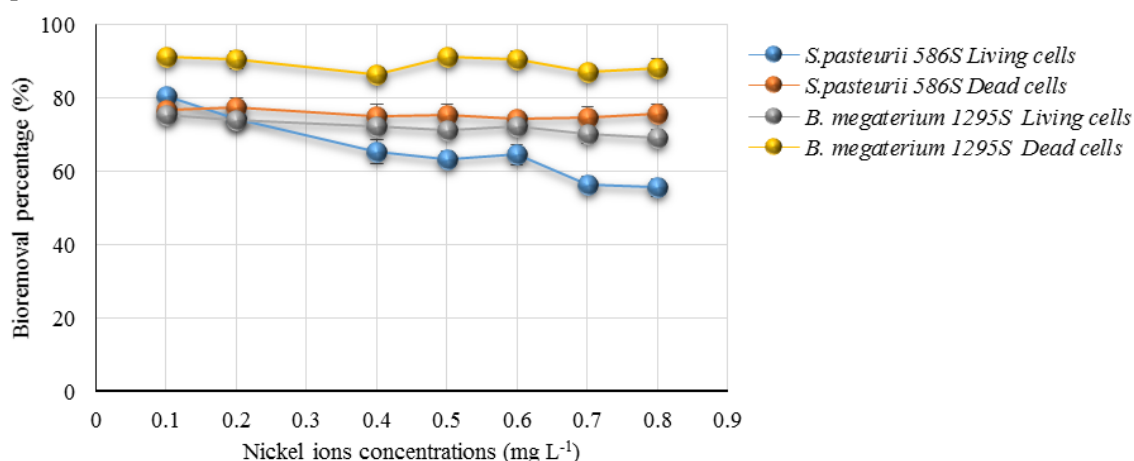
where;  $Q$  is amount of  $\text{Ni}^{2+}$  ions (mg) removed by gram of bacterial biomass;  $V$  is size of nickel solution (L);  $C_i$  represent  $\text{Ni}^{2+}$  ions (initial concentration, mg/L);  $C_f$  is  $\text{Ni}^{2+}$  ion concentrations in the supernatant determined at the end of the experiment;  $M$  is biosorbent added into the  $\text{Ni}^{2+}$  ion solution (g)

### 2.3 Statistical analysis

The comparison between the bio-removal efficiency of *S. pasteurii* 586S and *B. megaterium* 1295S were performed based on the analyses of the data using ANOVA tests by using SPSS software. Differences were considered significant at  $p < 0.05$ .

## 3. Results and Discussion

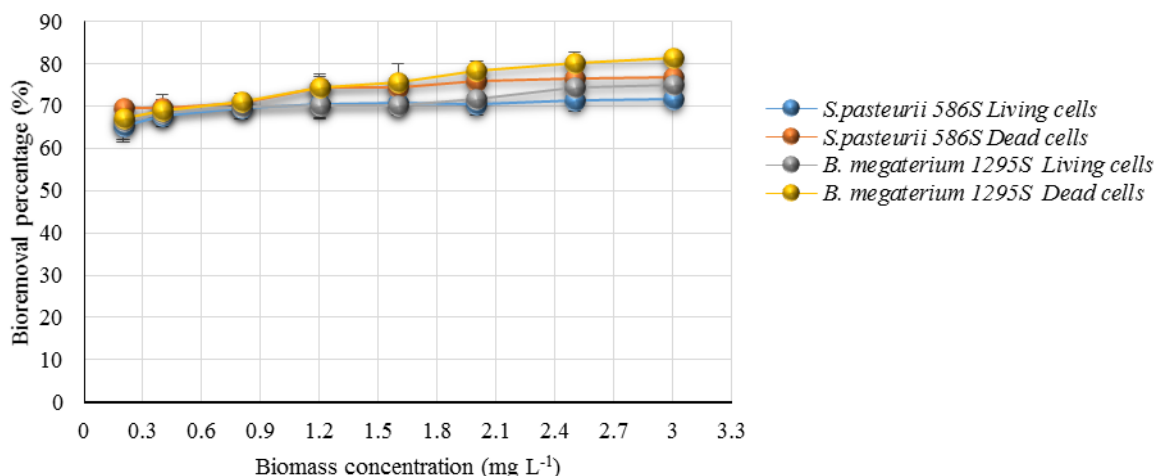
The comparison between bio-removal efficiency of  $\text{Ni}^{2+}$  ions by *S. pasteurii* 586S and *B. megaterium* 1295S is the main objective of the current work. In a response for the  $\text{Ni}^{2+}$  ions (Figure 1), it appeared that living cells of both bacterial strains exhibited high efficiency at low concentrations ( $0.1 \text{ mg L}^{-1}$ ) and reduce with the increasing of the metal concentrations to  $0.8 \text{ mg L}^{-1}$ . *S. pasteurii* 586S was more efficient than *B. megaterium* 1295S (80.46 vs. 75.4%, respectively). In contrast, the dead cells of both bacteria exhibited high efficient at high concentrations. However, *B. megaterium* 1295S occurred more efficiency than *S. pasteurii* 586S. The maximum removal of *B. megaterium* 1295S was recorded at  $0.5 \text{ mg L}^{-1}$  was 91.34 % while was 75.51% for *S. pasteurii* 586S. The authors in literature have indicated that the bio-removal efficiency by the bacterial wet cells gradually decreased with increasing initial concentrations (Jang et al. 2001; Padmavathy et al. 2003). Meanwhile, Ilhan et al. (2004) and Abdel-Monem et al. (2010) have mentioned that the nonviable cells (dead cells) frequently exhibit a higher affinity for nickel ions compared with viable biomass probably due to the absence of competing protons produced during metabolism. Indeed the bio-removal process by using living bacterial cells used in this study was conducted in the aqueous solutions, where no nutrients are available, therefore, these cells have no metabolic reactions. The reasonable explanation for increasing the bio-removal with the dead cells might be related to that the pre-treatment process for the cells by the heat lead to destruction of cell wall and thus increasing the diffusion of metal into the cell cytoplasm, where the cells was used as a store for the accumulation of metal ions.



**Fig. 1** Bio-removal process by *S. pasteurii* 586S and *B. megaterium* 1295S as a response for different concentrations of nickel ions

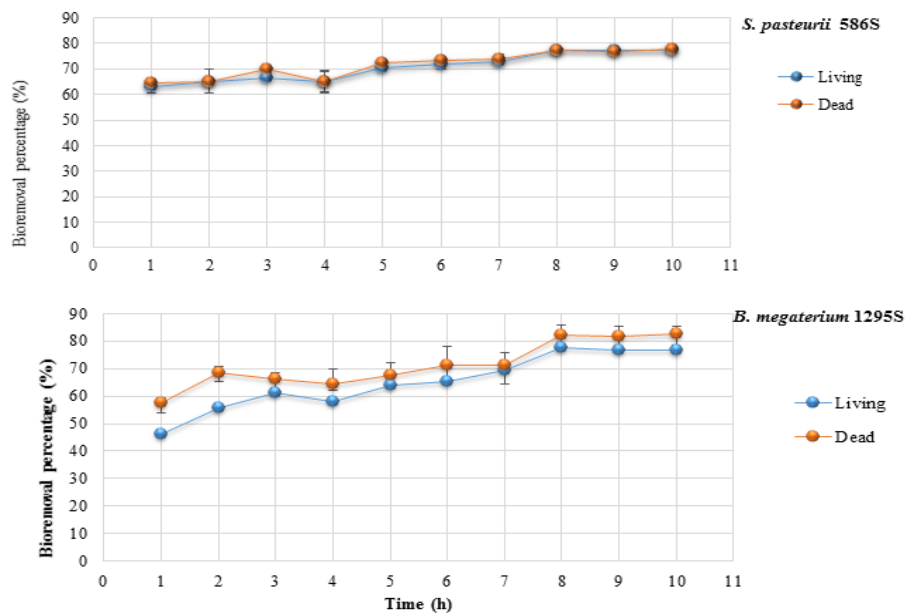
In the response for the bacterial cell concentrations, both bacterial strains increased significantly ( $p < 0.05$ ) in their removal percentage with the increasing of cell concentrations (Figure 2). The maximum removal was noted at  $3 \text{ mg L}^{-1}$  for both living and dead cells. However, the dead cell exhibited more efficiency than living cells in the cell concentrations of  $1.2 \text{ mg L}^{-1}$  and above. In a comparison between both investigated strains, it can be note that similar trends *B. megaterium* 1295S

was more efficient in the removal of  $\text{Ni}^{2+}$  ions than *S. pasteurii* 586S at the concentrations of  $2 \text{ mg L}^{-1}$  and above. The maximum differences was observed at  $3 \text{ mg L}^{-1}$  (81.5 vs. 76.61%, respectively). Moreover, it can be indicated that the removal percentage has a constant trends when the cell concentrations were  $2.5 \text{ mg L}^{-1}$  and above. These results agree with Al-Gheethi et al. (2014), who stated that the removal efficient of metal ions by the bacterial biomass increased with increasing of biomass doses to certain range due to the equilibrium limitations. In a study conducted by Selatnia et al. (2004) the results revealed that the biosorption rate of metals increased with increase in concentrations of biomass to limited values and then decrease due to the biomass granules, which are agglomerated.



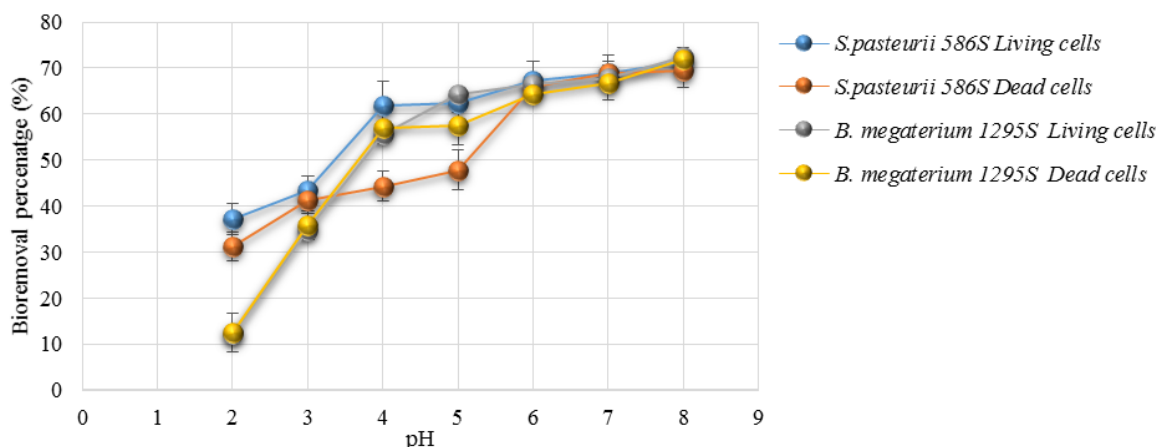
**Fig. 2** Bio-removal process by *S. pasteurii* 586S and *B. megaterium* 1295S as a response for biomass concentrations

The bio-removal efficiency of  $\text{Ni}^{2+}$  ions by bacterial strains in a response for the incubation time is depicted in Figure 3. There is no significant differences between living and dead cells of *S. pasteurii* 586S which might be related to the characteristics of bacterial cell wall, while *B. megaterium* 1295S has a significant differences in their removal efficiency between living and dead cells. In a comparison between both bacterial strains, it can be noted that *B. megaterium* 1295S has a wide range between 45 and 59% at the 1<sup>st</sup> h to 78 h and 85% at 10<sup>th</sup> h for living and dead cells, respectively. In contrast, the removal percentage of  $\text{Ni}^{2+}$  ions by *S. pasteurii* 586S ranged from 65 to 78% for living and dead cells, respectively. These findings indicated that *B. megaterium* 1295S has more flexible in a response for time. Moreover, based on the curve trends which appeared that has two stages. First stage started from 1 h to 7 h where the removal efficiency increasing with the time, while the second stage started from 8 to 10 h, where the removal percentage has not increased significantly. According to Ceribasi and Yetis (2001) the removal of metal by the biomass cells takes place in two stages: a rapid and a slow stage, the rapid stage is due to the surface biosorption on the bacterial cell wall, while slow stage is intracellular accumulation.



**Fig. 3** Bio-removal process by *S. pasteurii* 586S and *B. megaterium* 1295S as a response for time

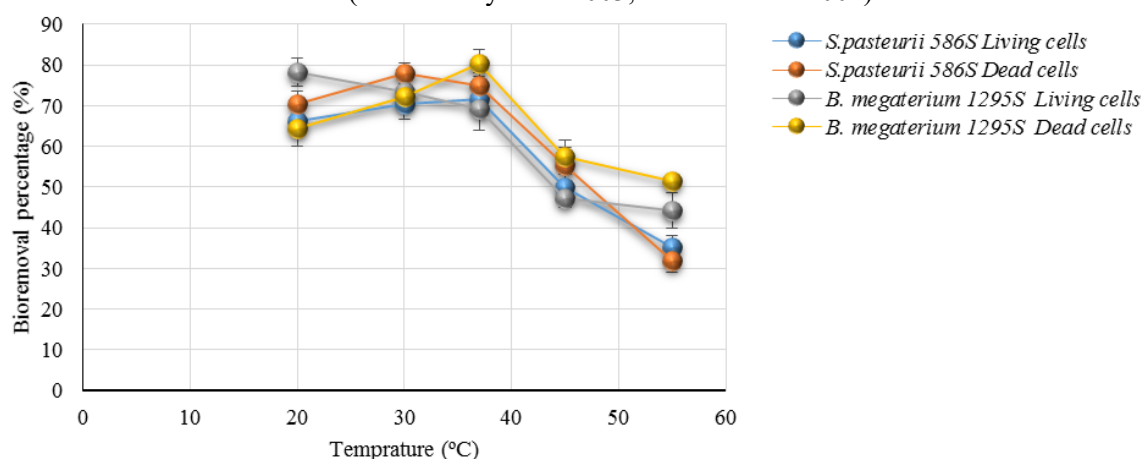
Bacterial strains investigated in the present work have different responses for pH values (Figure 4). *S. pasteurii* 586S (30 and 38% for living and dead cells, respectively) exhibited detectable removal at pH 2 and 3 compared to *B. megaterium* 1295S (only 10% for both living and dead cells). At pH 4, *B. megaterium* 1295S (56.94%) occurred more efficient than dead cells of *S. pasteurii* 586S (44.32%), but the living cells of this bacterium still the highest (61.82%). At pH 5 the living cells of both strains have similar removal percentage and more than the dead cells. In contrast, dead cells of *B. megaterium* 1295S (57.43%) was better than that of *S. pasteurii* 586S (47.83%). in pH range between 6 and 8, both bacterial strains (living and dead cells) have no significant differences in the removal percentage. the maximum removal for both strains were recorded at pH 8 (>70%). Many of authors have indicated that optimum removal efficiency for bacterial biomass is located between 6 and 8, while little removal is recoded at pH less than 3 due to the cation competition effects with oxonium (hydronium) ion  $H_3O^+$  (Kovaevi et al. 2000; Kaewchai and Prasertsan 2002; Al-Gheethi et al. 2014).



**Fig. 4** Bio-removal process by *S. pasteurii* 586S and *B. megaterium* 1295S as a response for pH values

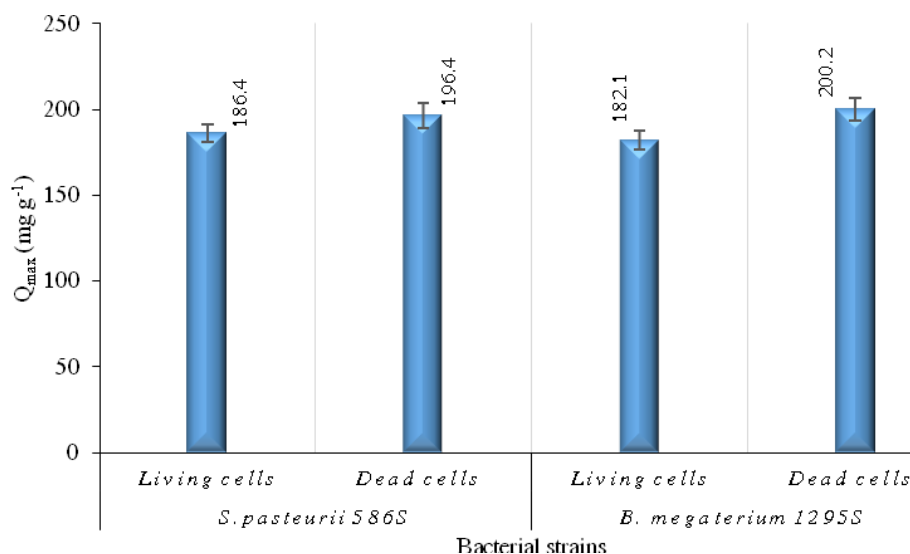
There are variations in the response of the bacterial strains as living and dead cells due to the change of temperature (Figure 5). The living cells of *S. pasteurii* 586S as well as the dead cells of both strains increased in their efficiency with the increasing in temperature from 20 to 30°C. Conversely,

living cells of *B. megaterium* 1295S reduced their effectiveness at 30°C compared to 20°C. The bio-removal efficiency for living and dead cells of *S. pasteurii* 586S decreased at 37 °C and above, while dead cells of *B. megaterium* 1295S appeared the maximum removal at 37 °C (80.34%). These findings might be explained based on the effect of temperature on the bacterial cells and in this case the bio-removal process appeared as endothermic process, where the bio-removal process increase with the increasing of temperature to limited values, or based on the effect of temperature on the metal ions where the removal process decreased with the increasing of temperature due to the releasing of metal ions form the active site to the solution (Padmavathy et al. 2003; Selatnia et al. 2004).



**Fig. 5** Bio-removal process by *S. pasteurii* 586S and *B. megaterium* 1295S as a response for temperature

The applicability of bacterial biomass for biotechnology depend on their bio-removal capacity ( $Q_{\max}$ ) which need be more than 150 mg g<sup>-1</sup> (Gadd 1990). In the present work,  $Q_{\max}$  for the living cells of *S. pasteurii* 586S was more than that for *B. megaterium* 1295S at 186.4 and 182.1 mg g<sup>-1</sup>, respectively) (Figure 6). Conversely, the dead cells of *B. megaterium* 1295S was better than that of *S. pasteurii* 586S (196.4 vs. 200.2 mg g<sup>-1</sup>). The bio-removal capacities of bacteria, algae and fungi is ranged range between 5-50 mg Ni<sup>2+</sup> g<sup>-1</sup> dry matter (Klimmek et al. 2001). However, the tolerant bacteria exhibited much more efficient than non-tolerant ones (Al-Gheethi et al. 2015). Therefore, the high  $Q_{\max}$  recorded here might be explained due to the ability of both strains to tolerate 15 mM of Ni<sup>2+</sup> ions. Further, the ability of tolerant bacteria to accumulate more metal ions in their cytoplasm is due to the mutation caused by the heavily exposure for the heavy metals which lead to make the uptake gate of the metal open without efflux process.



**Fig. 6** Bio-removal capacity for *S. pasteurii* 586S and *B. megaterium* 1295S



## Conclusions

It can be concluded that the bacterial strains, *S. pasteurii* 586S and *B. megaterium* 1295S isolated from the sewage sludge possess an important potential to remove nickel ions. The dead cells are more applicable than living cells, subsequently, *B. megaterium* 1295S is more efficient than *S. pasteurii* 586S.

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