



Investigating the Urease Activity of *Sporosarcina pasteurii* for Potential Usage of Microbially Induced Calcium Carbonate Precipitation

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Abstract

The urease activity of *Sporosarcina pasteurii* was investigated using different number of bacterial cells. Resting *Sporosarcina pasteurii* cells and 500 mM urea solution were placed into the beakers to measure the change in electrical conductivity of the solution. The electrical conductivity values were varied between 0.382 mS/m and 6.224 mS/m for 10 min. The highest electrical conductivity was measured for addition of the largest number of cells (10×10^9) to the solution. Resting *Sporosarcina pasteurii* cells and 500 mM urea and 500 mM CaCl_2 solution were also placed into the beakers to observe and evaluate microbially induced CaCO_3 precipitation. The amount of microbially induced CaCO_3 precipitation was changed between 4 mg and 28 mg in the experiments for ten minutes. The highest amount of CaCO_3 precipitation was observed in beaker with the highest number of cells (28 mg) than in those with the smallest number of cells (4 mg). Hence, the amount of microbially induced CaCO_3 precipitation was increased in direct proportion to the cell number in the beakers. The experiments suggest that the urease activity *S. pasteurii* is depended on the number of cells and more cells of *S. pasteurii* leads more microbially induced CaCO_3 precipitation in a short time period.

Keywords: Electrical conductivity, Microbially induced calcium carbonate precipitation, Number of cells, *Sporosarcina pasteurii*, Urease activity.

Mikrobiyal Olarak İndüklenen Kalsiyum Karbonat Çökeltisinin Potansiyel Kullanımı için *Sporosarcina pasteurii*' nin Üreaz Aktivitesinin Araştırılması

Öz

Sporosarcina pasteurii'nin üreaz aktivitesi, farklı sayıda bakteri hücresi kullanılarak araştırıldı. Çözeltinin elektriksel iletkenliğindeki değişimi ölçmek için dinlenme halindeki *Sporosarcina pasteurii* hücreleri ve 500 mM üre çözeltisi beherlere eklendi. Elektriksel iletkenlik değerleri 10 dakika süreyle 0.382 mS/m ile 6.224 mS/m arasında değişiklik göstermiştir. En yüksek elektriksel iletkenlik, çözeltiliye en fazla sayıda hücrenin (10×10^9) eklenmesi için ölçülmüştür. Dinlenme halindeki *Sporosarcina pasteurii* hücreleri ve 500 mM üre ve 500 mM CaCl_2 çözeltisi de mikrobiyal olarak indüklenen CaCO_3 çökeltisini gözlemlemek ve değerlendirmek için beherlere yerleştirildi. On dakikalık deneylerde mikrobiyal olarak indüklenen CaCO_3 çökeltisi miktarı 4 mg ile 28 mg arasında ölçülmüştür. En yüksek CaCO_3 çökeltisi miktarı, en az hücre sayısına (4 mg) kıyasla en fazla hücre sayısına sahip beherde (28 mg) gözlemlendi. Bu nedenle, mikrobiyal olarak indüklenen CaCO_3 çökeltisi miktarının, beherlerdeki hücre sayısıyla doğru orantılı olarak arttığı söylenebilir. Deneyler, *S. pasteurii*'nin üreaz aktivitesinin hücre sayısına bağlı olduğunu ve daha fazla *S. pasteurii* hücresinin kısa bir süre içinde daha fazla mikrobiyal olarak indüklenen CaCO_3 çökeltisine yol açabileceğini göstermektedir.

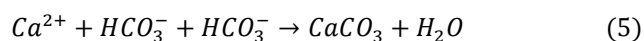
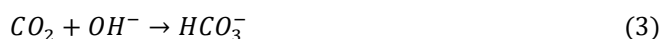
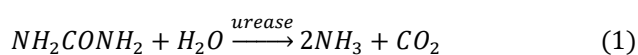
Anahtar Kelimeler: Elektriksel iletkenlik, Hücre sayısı, Mikrobiyal olarak indüklenen CaCO_3 çökeltisi, *Sporosarcina pasteurii*, Üreaz aktivitesi.

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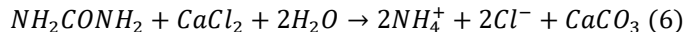
1. Introduction

Microbially induced calcium carbonate has been studied by many researchers to develop biotechnology methods such as improving construction materials [1], decreasing hydraulic conductivity of porous media [2], changing soil properties [3-5] increasing oil recovery [6] because of widespread presence of ureolytic bacteria in the environment. Although there are a number of species which produce CaCO_3 minerals, *Sporosarcina pasteurii* was used by many researchers widely and there are numerous reports on bacterial CaCO_3 precipitation by *S. pasteurii* [7-12] because *S. pasteurii* is not a pathogenic bacterium, it is not suppressed by ammonium, and it provides high urease [2].

S. pasteurii produces urease which result in urea hydrolysis and this hydrolysis causes ammonia and carbon dioxide [2]. The ammonia gives rise increasing pH of the ambience and, which in turn induces calcite precipitation in calcium rich environment. The hydrolysis of urea in a solution with calcium chloride was used in the researches mostly [13-14]. The reaction steps and overall reaction can be identified as [4];



Overall reaction:



To understand the potential usage of microbially induced calcium carbonate precipitation (MICCP), urease activity of *S. pasteurii* needs to be identified. Therefore, the amount of MICCP was measured and the relationship between cell concentration and change in electrical conductivity of urea solution was investigated using different number of cells of *S. pasteurii* in present study.

2. Material and Method

2.1. Microorganism used in the experiments and culturing

S. pasteurii [American Type Culture Collection (ATCC) 11859] was used as microorganism to evaluate urease activity of cells. The medium (Tris-YE) for cultures consisted of Tris buffer, 130 mM (pH 9.0); $(\text{NH}_4)_2\text{SO}_4$, 10 g; and yeast extract, 20 g; to which 2% agar per liter of pure water was used for solid medium which was used for culturing. Autoclave at 121°C for 15 min was used for sterilization of medium elements [2].

1 L Tris-YE medium was used to obtain culture of *S. pasteurii* [2]. 30°C and 120 rpm shaking were employed for overnight for the culture. The bacterial cells were harvested using centrifuge at 10000*g for 10 min and rinsed with pure water two times [2]. Eventually, *S. pasteurii* cells were dissolved again into 100 mL

pure water to obtain an optical density of 2.25 (abbreviated as OD_{600} 2.25) at 600 nm. OD_{600} 2.25 was corresponded 10^9 cells/mL. Resting *S. pasteurii* cells were used in the experiments.

2.2. Experimental setup and conditions

Batch experiments were carried out at 22°C as constant temperature. The electrical conductivity of the solution was measured because the amount of urea hydrolyzed can be related to the production of ammonium. Two moles of ammonium (NH_4^+) is released while the hydrolysis of 1 mole of urea occurs. The electrical conductivity of the solution was increased because of the hydrolysis reaction. The concentration of urea hydrolyzed and change in electrical conductivity of the solution was identified as [15]:

$$\text{Urea (mM)} = \Delta \text{Electrical conductivity} \left(\frac{\text{mS}}{\text{m}} \right) * 0.1111 \quad (7)$$

50 mL of urea solution (500 mM) was filtered using a 0.22 μm filter for sterilization and poured into 100 mL beakers. 1 mL, 2 mL, 4 mL, 8 mL, and 10 mL cell suspensions, which were corresponded to 10^9 cells, $2*10^9$ cells, $4*10^9$ cells, $8*10^9$ cells, and $10*10^9$ cells of *S. pasteurii*, respectively, were added to the beakers. Finally, the bacterial cells were added to the beakers.

A magnetic stirrer was employed to provide the homogen cell suspension in the beakers. The initial electrical conductivity of all solutions was measured using conductivity meter and noted. Then, the change in electrical conductivity of all solutions was measured for each minute for 10 minutes. The electrical conductivity value for each experiment was calculated by subtracting the initial value from the final value.

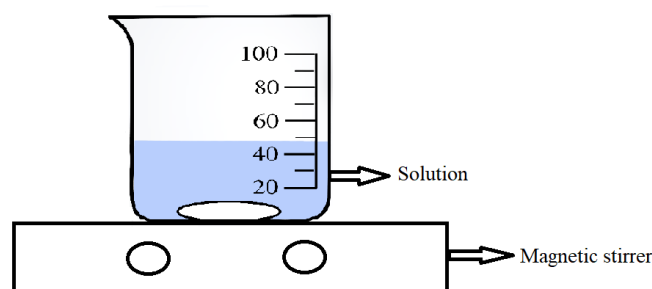


Figure 1. Experimental setup

To obtain MICCP, 50 mL of 500 mM urea and 500 mM CaCl_2 solution (precipitation solution) was also filtered using a 0.22 μm filter for sterilization and poured into 100 mL beaker. 1 mL, 2 mL, 4 mL, 8 mL, and 10 mL cell suspensions were added to the precipitation solution for 10 minutes. After 10 min, the reaction was stopped using N-(n-butyl) thiophosphoric triamide as inhibitor. After that, 0.1 N HNO_3 was used to dissolve CaCO_3 formed in the beakers. The specimens were prepared to measure calcium concentration using ICP-AES (inductively coupled plasma atomic emission spectroscopy). When the measurements were completed using ICP-AES, the amount of CaCO_3 which had formed into the beakers was computed.

3. Results and Discussion

The initial electrical conductivity value was measured as 0.253 mS/m. After adding different number of cells to the solutions, the urease activity was observed beginning from the first minute to the tenth minute.

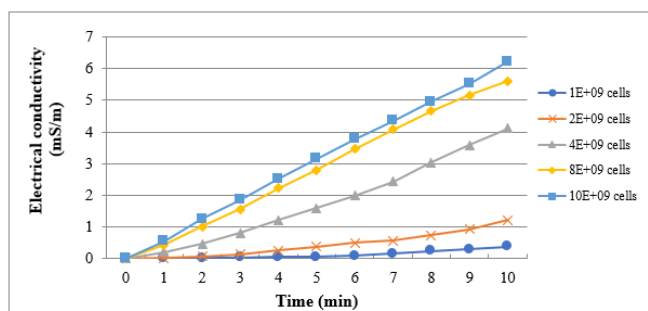


Figure 2. The change in electrical conductivity for different number of cells in time

The electrical conductivity values were changed between 0.07 mS/m and 0.547 mS/m for the range of 10^9 cells and 10×10^9 cells, respectively, in the first minute (Fig. 2). The electrical conductivity values were increased in time for all conditions. The highest electrical conductivity was measured as 6.224 mS/m for addition of 10×10^9 cells to the solution for 10 min. As indicated figure 2, the electrical conductivity had closer values for 10^9 cells and 2×10^9 cells, and for 8×10^9 cells and 10×10^9 cells for 10 min. The increase in number of cells in the solution was resulted in high rate of hydrolysis. Hence, it can be said that the electrical conductivity was increased depending on cell numbers added to the solution.

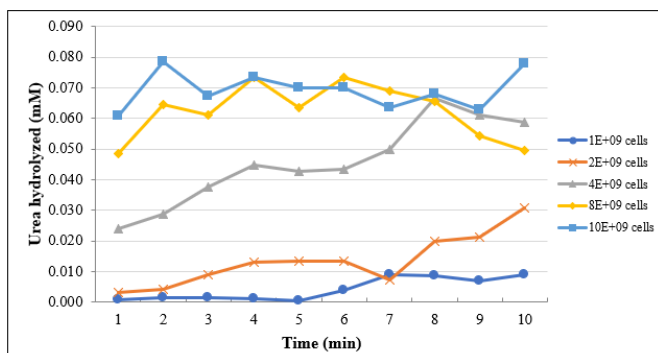


Figure 3. The change in concentration of urea hydrolyzed for different number of cells in time

In the presence of 10×10^9 cells, the concentration of urea hydrolyzed, which was the highest value, was 0.078 mM urea after ten minutes. The lowest concentration of urea hydrolyzed for ten minutes was measured as 0.009 mM for 10^9 cells added to the solution. As indicated in figure 3, all conditions have urease activity from the first minute of the experiment. However, the urease activity was reduced when 10^9 cells was added to the solution. As shown in figure 3, increase in number of cells was resulted in rise of the concentration of urea hydrolyzed. Thus, it can be said that when there is no nutrient to enhance the number

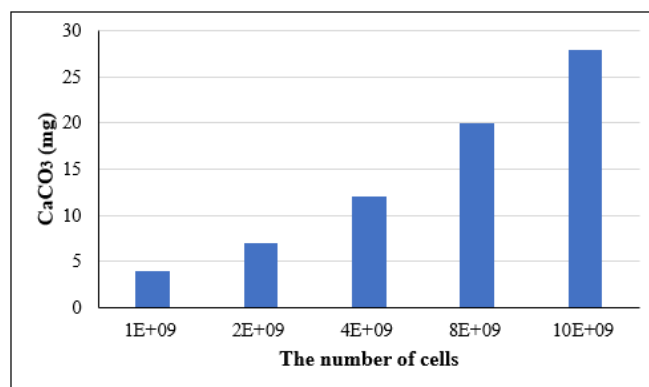


Figure 4. The amount of CaCO₃ precipitated

The range of the amount of CaCO₃ precipitation was calculated between 4 mg and 28 mg as shown in figure 4. The greatest amount of CaCO₃ for *S. pasteurii* was achieved (28 mg) when the bacteria concentration of 10×10^9 cells was added to the precipitation solution. Previous studies agreed producing large amounts of urease enzyme in the presence of urea [16-17] and, ultimately, the higher concentration of CO₃²⁻ were released and bound to the more concentration of Ca²⁺ to form MICCP.

4. Conclusions and Recommendations

In this study, urease activity of resting *S. pasteurii* cells was investigated. The results were indicated that highest urease activity was achieved when the number of cells was 10×10^9 , which was 10 times larger than the lowest number of cells used in the experiments. It should be noted that the urease activity *S. pasteurii* is depended on the number of cells.

The resting cells of *S. pasteurii* without nutrients to simulate the case of lack of nutrient in the environment. The effect of bacterial growth using nutrient sources such as yeast extract etc. should be examined to understand the potential usage of *S. pasteurii* for microbially induced calcium carbonate precipitation.

In summary, *S. pasteurii* has higher potential to precipitate amount of CaCO₃. More cells of *S. pasteurii* leads more MICCP in a short time period. Therefore, time should be taking in consideration to apply MICCP technology.

of cells in the environment, high number of *S. pasteurii* cells could be used to get higher urease activity.

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