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RESEARCH ARTICLE

TRANSPORTATION OF *SPOROSARCINA PASTEURII* IN POROUS MEDIA WITH DIFFERENT PARTICLE SIZES

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ABSTRACT

The current study aimed to determine the transportation distance of *Sporosarcina pasteurii* (ATCC 11859) and the number of cells present in porous media. The experiments were carried out in continuous-flow columns, which were plastic columns with an inner diameter of 2.4 cm and a height of 50 cm, and which contained glass beads with average diameters of 0.25 mm, 0.50 mm and 1 mm to mimic porous media. To investigate cell transport through columns, suspension of *Sporosarcina pasteurii* was introduced into columns at a flow rate of 2 mL/min and the cell densities of OD_{600} 0.15, 0.75, 2.25. To count the bacteria in each section, the column was divided into five equal parts. The results showed that the most cells, which were counted as $1.72*10^{10}$ cells, were deposited in the columns packed with 0.25 mm glass beads for the experiments with OD_{600} 2.25, while the deposited cell number decreased at the bottom of the column. The cell deposition was greater at the bottom of the column in the case of columns packed with 1 mm glass beads. According to the findings, while using smaller glass beads resulted in more cell deposition in the porous media, using larger glass beads resulted in more cell transport through the porous media. It can be concluded that larger particle sizes may result in easier transportation conditions for cells transporting deep into porous media.

Keywords: *Cells, column, glass beads, porous media, Sporosarcina pasteurii.*

1. INTRODUCTION

Nowadays, transportation of bacterial cells gains importance due to the importance of developing environmentally friendly technologies, which can be listed in biotechnology methods including bioaugmentation and biotreatment, for many fields. Bacterial precipitation, which is related for producing insoluble organic and inorganic compounds using bacteria, is one of the environmentally friendly technology [1-2]. Bacterial precipitation has been used by many researchers to improve the soil properties [3-5], seal cracks [6], reduce hydraulic conductivity of porous media [7-8]. *Sporosarcina pasteurii* was used by many researchers for bacterial precipitation [9-12] because *Sporosarcina pasteurii* is a nonpathogenic bacterium with high urea activity [7]. The process of

bacterial precipitation carried out by *Sporosarcina pasteurii* relies on the metabolic activity of the bacteria. *Sporosarcina pasteurii* utilizes urease to break down urea into ammonia and carbon dioxide, leading to an increase in pH levels. This rise in pH triggers the precipitation of calcite in the presence of calcium ions [13]. In the most studies, the researchers used urea hydrolysis in a solution containing calcium chloride to introduce Ca^{2+} ions [14-15].

Various factors, including pH, temperature, nutrient presence, nutrient concentration, precipitation reagent concentration, and oxygen availability, influence the effectiveness of the bacterial precipitation process [16-17]. Another of these influencing factors is cell density, which correlates with the urease enzyme [9]. According to Eryürük, the amount of bacterial precipitation can be expressed as a function of the bacterial biomass deposited in the column [18]. Therefore, it is critical to understand the transportation distance of cells through the column to achieve bacterial precipitation. Glass beads of varying diameters were used to pack the columns in the current study, and the transportation distance was evaluated by introducing *Sporosarcina pasteurii* cell suspensions of varying densities.

2. MATERIAL and METHOD

2.1. Preparation of cell culture and measurement of OD

In this study, the transportation of cells was examined using *Sporosarcina pasteurii* (ATCC 11859) as the experimental bacterium. To create the culture medium (Tris-YE), a combination of Tris buffer (130 mM, pH 9.0), ammonium sulfate (10 g/L), and yeast extract (20 g/L) was utilized. To obtain a solid medium for stock culture, a mixture of 2% agar was added to 1000 milliliters of liquid medium. Prior to being mixed together, all of the components were subjected to separate autoclaving at a temperature of 121 °C for a duration of 15 minutes [7]. *Sporosarcina pasteurii* cells were acquired by introducing cells into Tris-YE medium and allowing them to incubate overnight at a temperature of 30 °C. The incubation process involved continuous shaking at a rate of 120 rpm. The collected cells were separated by centrifugation at 10,000 times the force of gravity (10,000 * g) for a duration of 10 minutes [7]. Afterward, the cells were washed twice with distilled water [7]. Finally, 200 mL of distilled water was used to obtain the cell suspension with the optical densities (OD) at 600 nm for 0.15, 0.75, and 2.25 (abbreviated as OD_{600} 0.15, 0.75, and 2.25) [7]. To measure the optical density (OD) values, a Hitachi U-1900 Spectrophotometer from Tokyo, Japan, was employed [7].

2.2. Experimental Setup and Conditions

The columns in the experiments were made of a plastic pipe with an inner diameter of 2.4 cm and a height of 50 cm (Figure 1).

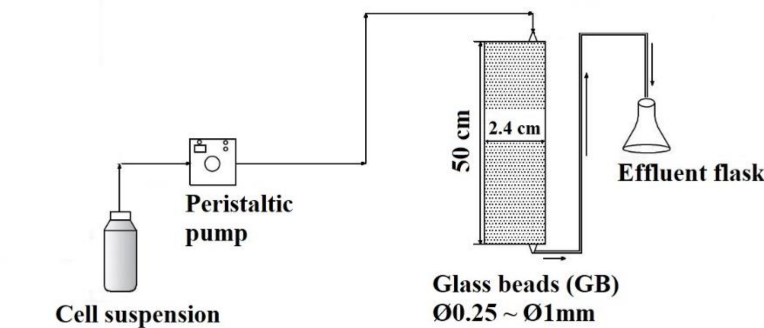

Figure 1. Experimental Setup.

In the experiments, glass beads of varying average diameters (0.25 mm, 0.50 mm, and 1.0 mm) were utilized as the porous media. Prior to each experiment, the glass beads were subjected to a cleansing process in which they were first washed with an acidic solution of 0.1 N HCl. Following that, the cells were subjected to multiple rinses with deionized water until pH was attained as 7.0. Saturated conditions were ensured for each experiment. A consistent temperature of 22 °C was maintained throughout all experiments, and the influent rate in the downward direction was regulated using a peristaltic pump. A suspension of *Sporosarcina pasteurii* cells, equivalent to four pore volumes of glass beads placed in columns, was introduced into the column at a flow rate of 2 mL/min. To ensure a uniform cell suspension during the introduction process, a magnetic stirrer was employed. Figure 2 indicates that the measured optical densities for 2.25, 0.75, and 0.15 at 600 nm were reflective of the following cell concentrations: $2.15 * 10^9$ cells/mL, $8.10 * 10^8$ cells/mL, and $5.89 * 10^8$ cells/mL, respectively.

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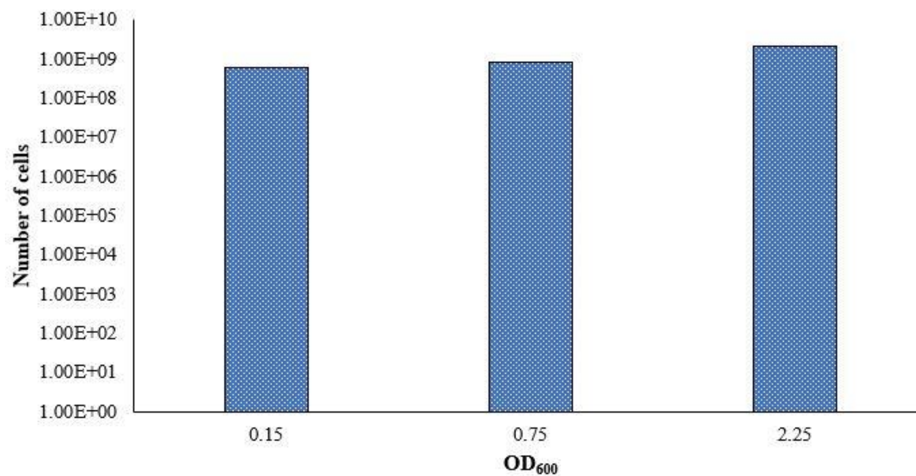

Figure 2. Number of cells for OD_{600} 0.15, 0.75, and 2.25.

The conditions for all the experiments were summarized in Table 1.

Table 1. Conditions for the experiments.

Glass beads size (mm)	OD_{600}
0.25	0.15
0.50	0.75
1	2.25

The column was divided into five equal parts. To determine the number of cells in each part, the glass beads from the corresponding part were transferred to a beaker and distilled water was added to the beaker. The quantity of cells deposited in the respective section was determined by sampling from the beaker. To quantify the cell population, a microscope model Olympus BX50WI from Tokyo, Japan, was employed.

3. RESULTS and DISCUSSION

This study was carried out to investigate the transportation of cells through porous media. As shown in Figure 3, in the columns consisting of small glass beads, cell accumulation was more in the upper parts of the column ($1.72*10^{10}$ cells in the columns filled with glass beads measuring 0.25 mm in diameter and introduction of OD_{600} 2.25 for 0-10 cm) comparing to lower parts ($5.50*10^{9}$ cells in the columns filled with glass beads measuring 0.25 mm in diameter and introduction of OD_{600} 2.25 for 0-10 cm 40-50 cm , while the cell accumulation increased in the lower parts of the column as the glass bead size increased ($2.05*10^{9}$ cells in the columns packed with 1 mm and introduction of OD_{600} 2.25 for 40-50 cm) comparing to upper parts ($7.00*10^{8}$ cells in the columns packed with 1 mm and introduction of OD_{600} 2.25 for 0-10 cm) . The accumulation of cells in the lower sections of the columns, which were packed with larger-sized glass beads, can be attributed to the enlargement of

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pore size in those beads. The smaller glass beads have smaller pore size which led more cell deposition in the columns packed with smaller glass beads size. The quantity of accumulated cells was additionally influenced by the cell densities of the introduced cell suspensions. Lower densities of the cell suspension led to a reduced number of cells accumulating in the column.

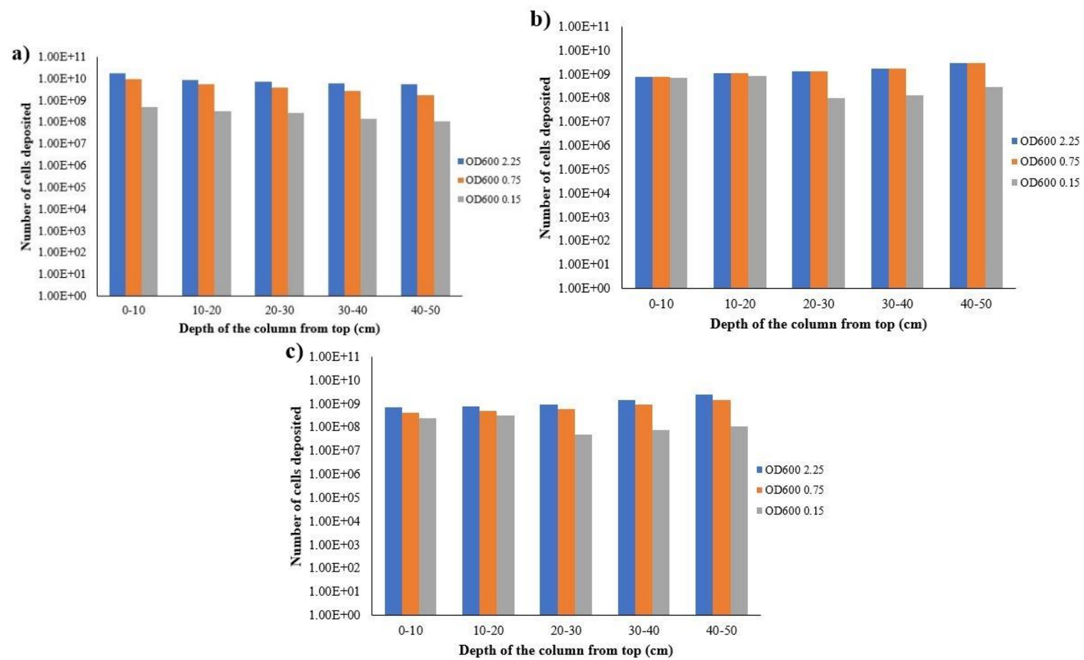

Figure 3. The total quantity of cells deposited in the packed columns with a) 0.25 mm GB b) 0.50 mm GB c)1 mm GB introducing cells suspensions with OD_{600} 0.15, 0.75, 2.25.

Figure 4 indicates that the total number of cells deposited in the columns. While the most cells accumulated in the column packed with 0.25 mm glass beads, the least cells accumulated in the column packed with 1 mm. This could be explained by the small pore size of porous media. In the column consisting of 0.25 mm glass beads, the cells deposited more because the pore size was smaller. Since the pore size was larger in the column consisting of 1 mm glass beads, the cells could not adhere to the glass beads and flowed away.

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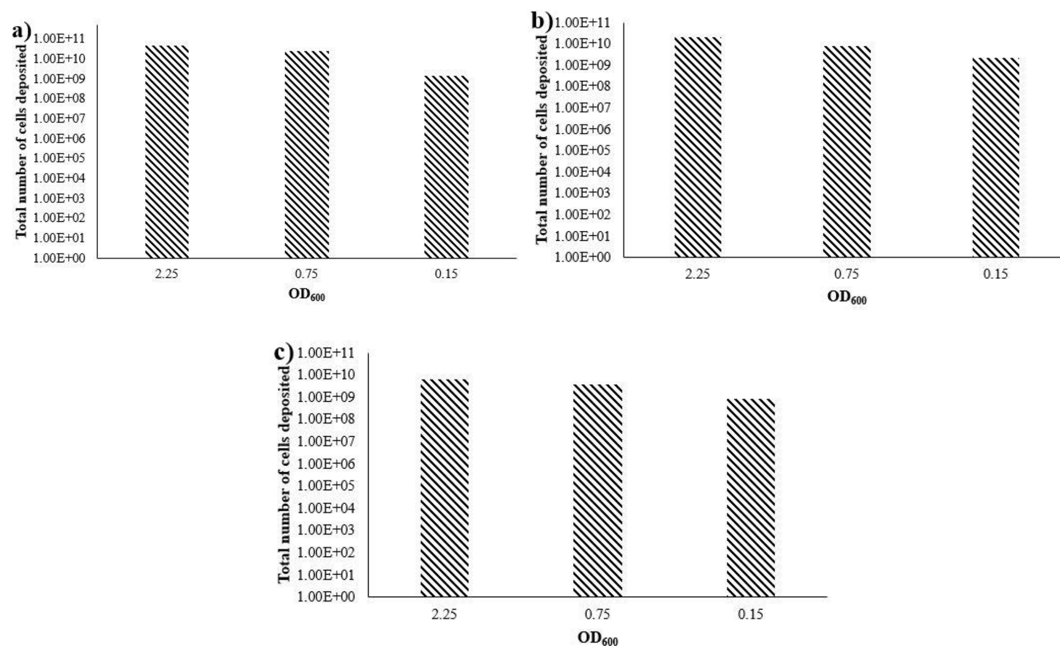

Figure 4. The number of total cells deposited in the columns packed with a) 0.25 mm GB b) 0.50 mm GB c)1 mm GB.

It can be said that the suggested method would be used in bioremediation methods by creating an impermeable layer by transporting bacteria to the deeper parts of the ground.

4. CONCLUSION

In this study, transportation of cells, which is crucial for environmentally friendly biotechnology methods, was investigated. It can be seen from the obtained results that the transportation of cells was effective when the porous media have larger size of particles. However, the total number of cells deposited indicated that more cell accumulation was achieved with smaller particle sizes. The smaller average pore size and correspondingly the pore volume could be reasoning more cell deposition in smaller glass beads. There was more accumulation in the upper parts of the columns containing small glass beads indicated that the biotechnology method to be developed will be effective at the surface level. On the contrary, there was more accumulation in the lower parts of the columns containing large glass beads indicated that the biotechnology method to be developed will also be effective at the lower levels.

This study indicated that density of cell introduced and the particle size of porous media would be crucial factors for improving biotechnology methods. This study can serve as an important motivation for future academic studies on bacterial transportation especially the transportation of *Sporosarcina*

pasteurii, a commonly utilized bacterium among researchers, has contributed to the advancement of biotechnology methods.

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