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INFLUENCE OF LOW TEMPERATURES ON *PSEUDOMONAS STUTZERI* BIOMASS STABILITY

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INTRODUCTION

As bacterial biotechnology progresses, there is a growing necessity to preserve cultures without the high costs and time-consuming processes, but with high genetic stability. Furthermore, it is vital to ensure viability and functionality are retained by stored stock cultures. Low-temperature storage, such as temperatures between -80 and 4 °C, is widely being used, but the implication to stability rarely investigated. Examine the stability of the produced biomass of *Pseudomonas stutzeri* strains during storage on low temperatures to maintain satisfactory vitality and viability of cells for further bioremediation processes.

MATERIALS AND METHODS

Pseudomonas stutzeri ATTC 17588 and *P. stutzeri* D1 (isolate from the Danube river water) were used. A precipitated biomass obtained after centrifugation of the cultivation fluid at the end of bioprocess (after 32 h at 37 °C) is divided into two equal parts. Biomass was resuspended in 8.5% NaCl solution. Further, the gained suspensions were distributed in sterile vials (2 ml) and were stored in the refrigerator at 4 °C and freezer at -20 ° C. The same procedure was applied to vials with biomass resuspended in sterile distilled water. At defined time intervals (0, 7, 14, 21, 28, 35 and 50 days), the number of viable cells was determined using the indirect method of streaking suspension on nutrient agar.

RESULTS

During storage period of 35 days, significant difference in cell concentrations at 4 °C did not observe for both tested strains, regardless of the used diluent. At the end of the incubation, bacterial concentration was decreased by approximately one log unit in both suspensions. Contrary, the effect of freezer temperature (-20 °C) had significantly different influence of bacterial stability. Briefly, biomass concentration decreased by approx. one log unit after only one week and remains constant until the 14th day. In the case of the reference strain, cell concentration in sterile saline decreased from approximately 9.7 to 6 log CFU/mL after 3 weeks, and maintained constant until one month, but at the end of the incubation period, growth of colonies did not observe.

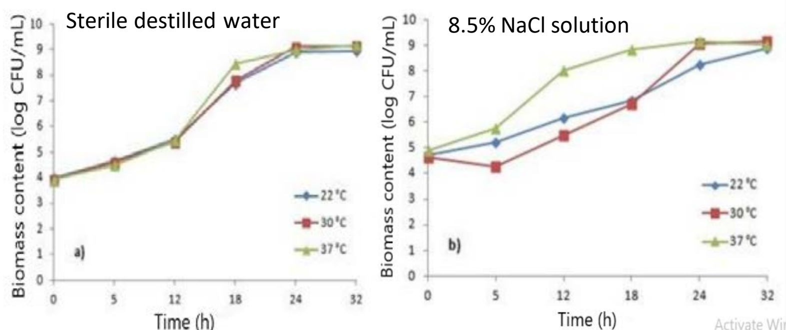

Figure 1. Influence of low temperatures on *P. stutzeri* biomass stability

In contrast, a temperature of -20 ° C has been shown to have a lethal effect on cells of the reference strain contained in sterile distilled leads after 3 weeks of storage. An effective way to solve this problem is to use cryoprotectant, such as glycerol, which are added in a certain volume to the cell suspension before storage. It can be concluded that the liquid for suspension preparation (saline and distilled water) has almost no effect on the vitality and viability of P. stutzeri D1 cells during storage at -20 ° C.

CONCLUSIONS

The presented results indicate the possibility of storing freshly prepared denitrifier suspensions at refrigerator temperature in sterile distilled water and sterile saline for a month while fully preserving cell viability and vitality. Additionally, the process of cell lyophilization can be avoided, which reduces the complexity of the preparation of the cell suspension.

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