NATURAL MATERIALS AS CARRIERS OF MICROBIAL CONSORTIUM

Blanka Dadic[1], Tomas Corak Grdovic[1], Zorana Kovacevic[2], Jasna Hrenovic[1], Tomislav Ivankovic[1]
[1] University of Zagreb, Faculty of Science, Department of Biology, Horvatovac 102A, 10000 Zagreb, Croatia
[2] University of Zagreb, Faculty of Textile Technology, Savska cesta 16/9, 10000 Zagreb, Croatia
E-mail: blanka.dadic@biol.pmf.hr

ABSTRACT

Four different natural materials (natural zeolitized tuff, ZeoSand®, perlite, and crushed corncob) were tested as potential carriers of bacteria. A microbial consortium of different bacterial species was isolated and conditioned for bioaugmentation of biogas production reactors using *Miscanthus X giganteus* as substrate. The highest rate of immobilization of microbial consortium was obtained on perlite (2.52×10^9 CFU g^{-1}), followed by ZeoSand® (8.41×10^8 CFU g^{-1}), crushed corncob (7.41×10^8 CFU g^{-1}) and natural zeolitized tuff (3.96×10^8 CFU g^{-1}). Due to their porous structure, suitable surface for immobilization, and non-toxicity, all natural materials could be appropriate carriers of the microbial consortium.

Key words: natural zeolitized tuff, perlite, crushed corncob, microbial consortium, *Miscanthus X giganteus*

INTRODUCTION

In biotechnology, immobilization of the specific bacteria onto solid materials as carriers is frequently employed to achieve a higher density of bacterial cells and enhance metabolic activity in the bioreactors [1]. Natural zeolitized tuff (NZ) was extensively studied for the immobilization of bacteria *Acinetobacter junii* due to high availability and affordability, non-toxicity, and rough surface for immobilization [2] [4]. Furthermore, the immobilized bacteria have exhibited notable resilience to extreme environmental conditions [3]. NZ could be used as a biocarrier for the augmentation of biogas-producing reactors in which as substrate one could use olive waste or perennial grass *Miscanthus X giganteus*. Immobilized bacteria would enhance the process and carry out metabolic activities better than planktonic cells [4, 5].

This study aimed to evaluate the immobilization of a microbial consortium onto NZ and to compare its efficacy as a biocarrier with efficiencies of other materials such as perlite, corncob and ZeoSand®.

EXPERIMENTAL

The NZ from quarry located at Donje Jesenje, Croatia, and commercial ZeoSand® (Velebit Agro, Croatia) of the size fraction of 0.5–1 mm were used in this experiment. The NZ consisted of 50-55% clinoptilolite with the lower amounts of celadonite, plagioclase feldspars and opal-CT (10-15% each) and traces of quartz and analcime [4]. The mineral composition of the ZeoSand® was a minimum of 80% clinoptilolite, and other mineral components were not listed in the safety data sheet. Perlite was commercial product intended to be used for gardening (Special Mix B.V. manufactured by Gold Label, Aalsmeer, The Netherlands). Crushed corncob was domestic and not treated with pesticides and herbicides.

The NZ and ZeoSand® were washed with demineralized water prior to sterilization. Corncob was autoclaved at 121°C/20 min., and the rest of the carriers were dry sterilized for 1h at 105°C.

The microbial consortium was isolated and conditioned for bioaugmentation of biogas production reactors that use *Miscanthus X giganteus* as substrate [5]. The microbial consortium was suspended in 10 ml of sterile 0.3% NaCl. To immobilize the bacteria on each individual carrier, 50 ml of sterile LB medium, 0.5 g of carrier, and 0.5 ml of bacterial suspension were added to sterile plastic vials. The vials were incubated for 24 h at room temperature (24°C) on a rotatory shaker (5 rpm).

After incubation, number of bacteria immobilized onto each individual carrier was determined. A supernatant was decanted from vials, and the carriers were washed twice with sterile saline solution to eliminate bacteria not attached to the carriers. Then, 20 ml of sterile saline was added, and the vials were shaken on a vortex (45 Hz/3 min). The supernatant was serially diluted, inoculated on TGY agar (Biolife, Italy) and incubated at 37°C/24h. The grown colonies were counted, and the number of immobilized cells was expressed as CFU g^{-1} of carrier.

RESULTS AND DISCUSSION

During the incubation, the bacterial cells were successfully immobilized onto the surface of all the carriers (Figure 1.). Next, the goal was to determine the number of immobilized bacteria onto each carrier. Bacterial consortium showed a high affinity for immobilization onto perlite resulting in a value of 2.52×10^9 CFU g^{-1} (Figure 2.). The efficiency of perlite as a biocarrier of bacteria for augmentation of biogas-producing reactors using olive waste as substrate was reported by Ivankovic et al. [5]. They noticed that perlite was a suitable carrier due to the high number of immobilized bacteria that amounted to $2.1{\pm}0.9{\times}10^{11}$ and $3.4{\pm}0.6{\times}10^{10}$ CFU g^{-1} when the process was carried out in aerobic or anaerobic conditions [5].

ZeoSand® also demonstrated a significant affinity for immobilization, with a value of 8.41×10^8 CFU g^{-1}. The number of immobilized bacteria on NZ was 3.96×10^8 CFU g^{-1}. There was no statistical difference in number of immobilized bacteria between the NZ and ZeoSand®, and the most obvious difference is in the fraction of clinoptilolite, 50-55 % in NZ and >80% in ZeoSand®, suggesting that clinoptilolite content in the zeolitized tuff does not influence the immobilization of bacteria, already shown for some other zeolite samples [6]. Zeolite provides protection from harmful environmental conditions and allows bacteria to carry out metabolic activities [7]. On NZ a great rate of immobilization was reported for *Acinetobacter junii* (1.27×10^{10} CFU g^{-1}), *Escherichia coli* (4.53×10^8 CFU g^{-1}) and *Enterococcus faecalis* (0.13×10^8 CFU g^{-1}) [4].

The affinity of immobilization on crushed corncob resulted in number of immobilized bacteria of 7.41×10^8 CFU g^{-1}. Corncob is a type of material that is mostly made of lignin, cellulose and hemicellulose. It has a high bulk density, porous structure and provides a large amount of biodegradable carbon to immobilized bacteria [8]. Corncob is a suitable carrier for ureolytic bacteria due to its wrinkled and porous structure that allows bacteria to immobilize and to survive [8]. However, it was confirmed that corncob is not an appropriate carrier for plant growth-promoting rhizobacteria due to high carbon content and poor adherence [7]. Furthermore, corncob is not ideal for enriching anammox bacteria, but it could be used in research of denitrifying bacteria [9].

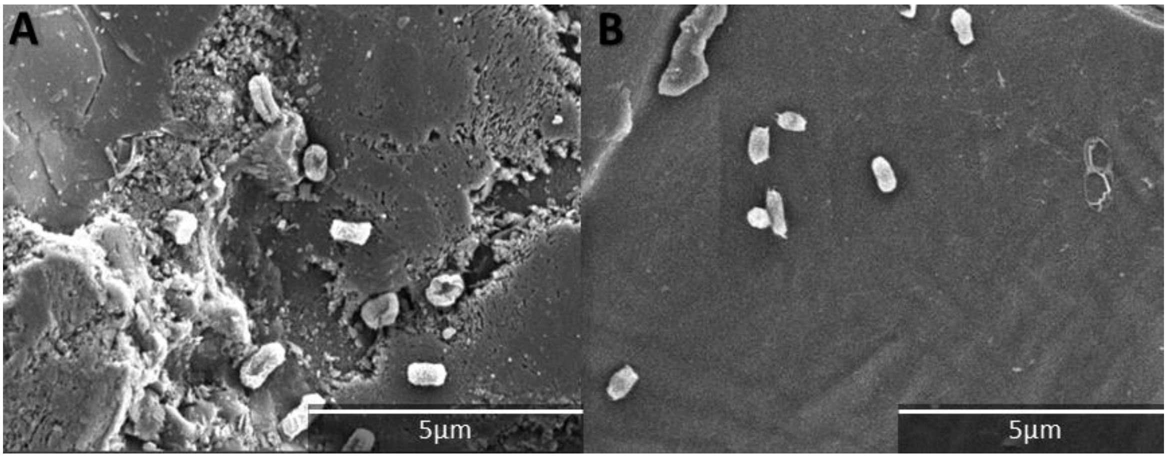

Figure 1. SEM images of bacterial cells immobilized on the surface on NZ (A, magnification 17 800 X) and crushed corncob (B, magnification 15 000 X)

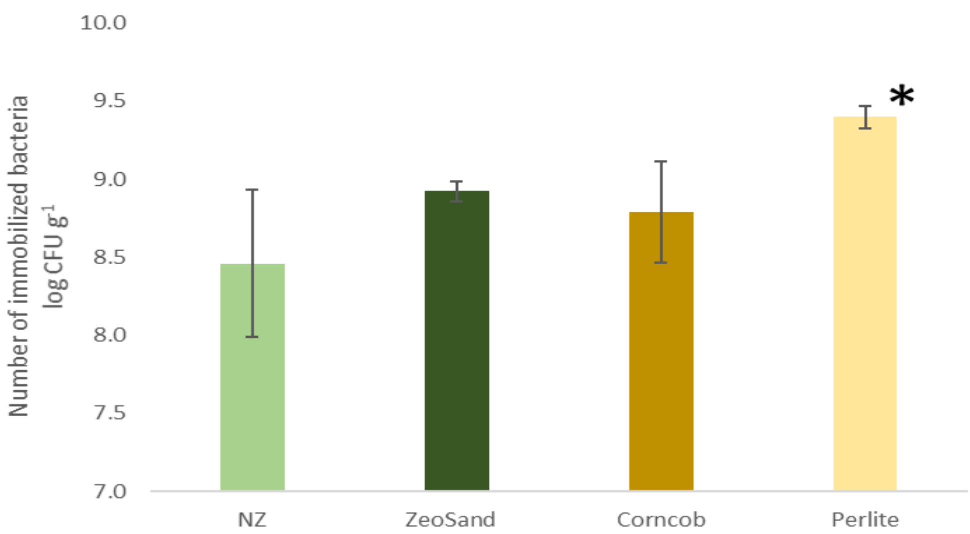

Figure 2. Number of bacteria immobilized on NZ, ZeoSand®, corncob and perlite. * Values that differ significantly ($p < 0.05$).

CONCLUSION

The highest rate of immobilization of microbial consortium was achieved on the perlite due to the large surface area of material that is available for bacterial immobilization. Number of immobilized bacteria did not differ significantly when NZ, ZeoSand® and crushed corncob were used as carriers. All tested materials could be used as potential biocarriers for the immobilization of microbial consortium and to be added to biogas producing bioreactors.

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