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Sugarcane bagasse as a potential bacterial carrier

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Immobilization of microorganisms into carrier materials is a promising tool in the formation of biofertilizers and biocontrol agents. Selection of an appropriate carrier material for cell immobilization is highly challenging since a carrier material should essentially facilitate cell immobilization, sufficient nutrition and a protective environment for the survival of immobilized cells. Further, there should not be a significant negative impact on ecological receptors of the receiving environment. Continuous supply of carrier materials at low cost is essential when scaling up laboratory made formulations into commercial products. Sugarcane bagasse (SCB) is a readily available industrial organic waste in Sri Lanka. However, it is underutilized as a carrier material in microorganism-based formations. Therefore, the objectives of the present study were to immobilize selected bacterial species in SCB and to estimate the viability of bacteria during storage in order to determine the potential of SCB as a bacterial carrier. The SCB matrix was prepared by grinding and sieving oven dried SCB to a fine powder. One portion of the fine powder was treated with 0.5 M NaOH while the other portion was kept untreated. *Bacillus cereus* at 1x 10^8 cell/mL optical density was used as the model bacterial inoculum. Four grams of both alkali-treated and untreated SCB matrices were inoculated with 50 mL of bacterial inoculum in 250 mL flasks. Immobilization of bacteria was facilitated by shaking at 150 rpm for 24 h. Bacteria-SCB matrices were collected by filtration and air dried for 1 h. Dried material was stored for 30 days at room temperature (approximately 30 °C) in sterilized screw-capped 250 mL flasks. Viability of bacteria in SCB matrices were compared with widely used sodium alginate bacterial carrier using the same model bacterial inoculum. Bacteria-sodium alginate homogenate was prepared at a final concentration of 2% (w/v) sodium alginate with 1x 10^8 cell/mL bacterial inoculum. Beads were prepared in 2.5% (w/v) $CaCl_2$ solution while stirring and washed with sterilized distilled water and air dried aseptically for 1 h. Beads were then stored for 30 days at room temperature in sterilized screw-capped 250 mL flasks. Viability of immobilized bacteria was determined by estimating colony forming units (CFU) per mL at different time intervals from 48 h to 30 days of storage. Results showed the presence of >3 x 10^8 CFU/mL at 10^{-6} which was the highest tested dilution until 14 days of storage for all three matrices. However, CFU of untreated SCB dropped up to 10 fold after 14 days at all dilutions whereas CFU of alkali-treated SCB and sodium alginate remained >3 x10^8 CFU/ mL at 10^{-6} for 30 days. Growth of immobilized bacteria in SCB carrier matrix with 3 x 10^8 CFU/mL at all dilutions confirms immobilization of tested bacteria in the SCB carrier matrix. Further, it confirms the viability of immobilized bacteria cells in the carrier material during storage at room temperature for 30 days. Scanning electron microscopic images showed attachment of bacteria on the surface of the SCB matrix. Therefore, we conclude the suitability of alkali treated SCB as a low-cost and locally available industrial waste as a carrier material for bacteria. Since *B. cereus* is a spore forming bacterium, during immobilization, cells may have survived as spores and germinated in the nutrient medium when cultured. Hence, further experiments with non-spore forming bacteria may support the evaluation of efficiency of cell multiplication in the alkali treated SCB matrix.

Keywords: Bacteria, Carrier material, Immobilization, SEM, Sugarcane bagasse

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