Determination of bacterial counts in immobilized beads using strain BS19 isolated from Antarctic soil

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Environmental pollution caused by oil spills has become serious concerns in recent years. One heterocyclic compound found in petroleum is carbazole (CAR), which resides in the environment because it is difficult to decompose naturally. CAR is a useful substance that can be used in a variety of materials, but it is toxic and mutagenic. Bioremediation involves immobilization of the bacteria, which improves degradation efficiency and maintains high bacterial concentrations.[1)] However, for stable bioremediation, it is essential to monitor the bacteria in the beads. Conventionally, monitoring the number of bacteria inside fixed beads has been done by breaking the beads. However, breaking the beads may affect the bacteria inside the beads. Therefore, monitoring is performed using resazurin, which is non-toxic and does not affect the bacteria inside the beads. Resazurin is also known as alamar blue. It is a blue-violet non-fluorescent substance, that is reduced to the red fluorescent substance resorufin upon cellular uptake. This reaction is irreversible and is used for cell viability in bacteria, fungi, plants, and animals.[2)] However, long-term reactions with cells should be avoided because resorufin will eventually be reduced to dihydroresorufin, which is non-fluorescent.

In this study, bacterial counts of BS19, a bacterium isolated from Antarctica, were calibrated by resazurin assay and colony count method. 2.6 mL of MSM, 0.3 mL of 0.4 mM resazurin, and 0.1 mL of BS19 strain cultured at 0.1 % CAR concentration with adjusted bacterial concentration were prepared and mixed in test tubes. After incubation at 30 °C for 30 minutes, 100 µL was transferred to a 96-well plate, and the fluorescence intensity was measured using a plate reader to generate a calibration curve.

References

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