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Entitled

*BIOHYDROGEN PRODUCTION OF A CO-CULTURE CONSISTING OF HALOPHYTIC CYANOBACTERIUM
PHORMIDIUM KUETZIGIANUM AND ACTIVATED SLUDGE BACTERIA USING DIFFERENT EXOGENOUS
CARBON SUBSTRATES AND SALT CONCENTRATION*

BY

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Abstract

Various studies have proved the ability of different types of cyanobacteria and algae to produce H₂ by splitting water molecules into hydrogen (H₂) and oxygen (O₂) using specialized enzymes (hydrogenase and nitrogenase) through the biophotolysis process. However, the production of O₂ acts as the main process inhibitor. Several researchers studied this O₂ sensitivity and proposed effective solutions to regulate O₂ concentration. By co-culturing algae with aerobic bacteria, the consumption of the resulting oxygen could be attained and thus reducing the sensitivity of the enzyme to the evolved O₂. In this study, a microbial consortium (co-culture) consisting of cyanobacteria *Phormidium keutzianum* and activated sludge bacteria was established to regulate O₂ concentration and enhance H₂ production. Different co-culturing ratios (algae: bacteria) such as 2:1, 1:1 and 1:2 were tested to find the optimum ratio for H₂ production. The effects of different exogenous carbon substrates (simple sugars) such as glucose, sorbitol, and mannitol were analyzed by supplementing the co-cultures with 10 g/L of sugar. In addition to study the effect of salt (NaCl) on H₂ production, different salt concentrations of 0, 10 and 20 g/L were tested. Results indicated that the amount of cumulative H₂ produced changed significantly by varying the carbon substrate. Glucose-supplemented co-culture produced the lowest amount of H₂ (278 ml L⁻¹) as compared to sorbitol-supplemented co-culture which produced the maximum amount of H₂ (980 ml L⁻¹). On the other hand, mannitol-supplemented co-culture produced (562 ml L⁻¹) of H₂. The results also showed that the addition of salt (NaCl) negatively affected H₂ production. By increasing the salinity level from 0-2%, the amount of total gas produced by glucose-supplemented co-culture was reduced from 2275 ml L⁻¹ to 734 ml L⁻¹, whereas cumulative H₂ reduced from 980 ml L⁻¹ to 176.8 ml L⁻¹ and 562 ml L⁻¹ to 333 ml L⁻¹ in sorbitol and mannitol-supplemented co-culture, respectively. This study proved the possibility of biohydrogen production by utilizing simple sugars and it can cause significant variations in the amount of the produced H₂ due to the differences in the metabolic pathways of different sugars by the involved algal and bacterial cells. This study also shows that physical factors (such as the effect of salt) affected the H₂ production process due to variations in the tolerances of the involved cyanobacterial and bacterial cells toward different salt concentrations.

Keywords: Algae, Salt tolerance, Hydrogenase enzyme, Nitrogenase enzyme, Wastewater, Metabolism, Chlorophyll