

Assessing Storage of Stability and Mercury Reduction of Freeze-Dried *Pseudomonas putida* within Different Types of Lyoprotectant

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Abstract : *Pseudomonas putida* is a potential strain in biological treatment to remove mercury contained in the effluent of petrochemical industry due to its mercury reductase enzyme that able to reduce ionic mercury to elementary mercury. Freeze-dried *P. putida* allows easy, inexpensive shipping, handling and high stability of the product. This study was aimed to freeze dry *P. putida* cells with addition of lyoprotectant. Lyoprotectant was added into the cells suspension prior to freezing. Dried *P. putida* obtained was then mixed with synthetic mercury. Viability of recovery *P. putida* after freeze dry was significantly influenced by the type of lyoprotectant. Among the lyoprotectants, tween 80/ sucrose was found to be the best lyoprotectant. Sucrose able to recover more than 78% ($6.2E+09$ CFU/ml) of the original cells ($7.90E+09$ CFU/ml) after freeze dry and able to retain $5.40E+05$ viable cells after 4 weeks storage in 4°C without vacuum. Polyethylene glycol (PEG) pre-treated freeze dry cells and broth pre-treated freeze dry cells after freeze-dry recovered more than 64% ($5.0 E+09$ CFU/ml) and $>0.1\%$ ($5.60E+07$ CFU/ml). Freeze-dried *P. putida* cells in PEG and broth cannot survive after 4 weeks storage. Freeze dry also does not really change the pattern of growth *P. putida* but extension of lag time was found 1 hour after 3 weeks of storage. Additional time was required for freeze-dried *P. putida* cells to recover before introduce freeze-dried cells to more complicated condition such as mercury solution. The maximum mercury reduction of PEG pre-treated freeze-dried cells after freeze dry and after storage 3 weeks was 56.78% and 17.91%. The maximum of mercury reduction of tween 80/sucrose pre-treated freeze-dried cells after freeze dry and after storage 3 weeks were 26.35% and 25.03%. Freeze dried *P. putida* was found to have lower mercury reduction compare to the fresh *P. putida* that has been growth in agar. Result from this study may be beneficial and useful as initial reference before commercialize freeze-dried *P. putida*.

Keywords : *Pseudomonas putida*, freeze-dry, PEG, tween80/Sucrose, mercury, cell viability

Conference Title : ICEWRE 2016 : International Conference on Environmental and Water Resources Engineering

Conference Location : Jeddah, Saudi Arabia

Conference Dates : January 26-27, 2016