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Influence and optimization of growth conditions on phenol degradation by *Pseudomonas aeruginosa*

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With their relatively high toxicity, phenol and phenolics are water pollutants of major concern. The application of phenol biodegrading agents to treat phenol in wastewater has shown promising results. *Pseudomonas aeruginosa* is a well recorded phenol biodegrading bacteria. However, optimal growth parameters for efficient degradation of phenol may vary from one bacteria to another. Therefore, this study focused on testing the effect of growth conditions including strength and pH of the growth medium and inoculum size on phenol degradation by *P. aeruginosa* and to assess the phenol degradation efficiency by adding glucose as an additional carbon source. The bacterial strain of *P. aeruginosa* (MH031762) isolated from petroleum contaminated site in Sri Lanka was inoculated to Mineral Salt Media (MSM) with 1200 mgL^{-1} phenol under different growth conditions. Three-factor factorial design with three levels was used to determine the combination effect of strength (0.5x, 1x and 2x), pH (6.5, 7 and 7.5) of the medium and inoculum size (1%, 2% and 3%) on phenol degradation. Residual phenol in the medium inoculated with bacteria was measured using 4-aminoantipyrene spectrometric method under which the duration (in hours) it took for 50% phenol degradation was calculated. Under the growth conditions found to be as optimum, the biodegradation efficiency of phenol by *P. aeruginosa* was further tested by adding glucose as an additional carbon source at varying concentrations (0.1%, 0.25%, 0.50%, 0.75% and 1%). Pooled t-test was performed to test the phenol degradation efficiency in bio-stimulated media with glucose. According to the results, there was no combination effect by all the 3 factors together on phenol degradation by *P. aeruginosa* ($P = 0.186$). However, a significant impact was shown by the combination of the two factors, i.e. the strength and pH of the medium ($P = 0.002$). According to the Tukey's test, quickest phenol degradation resulted in the double strength (2x) MSM with pH 7.5 (71.82 h). Among the 3 levels of inoculum sizes assessed, 3% showed the highest phenol degradation rate by showing the lowest time (64±4.5 h) to achieve degradation of the amount of phenol by 50%. Further, glucose in the level of 0.1% enhanced the phenol degradation rate, by degrading the same amount of phenol within 58.9±0.2 h. In conclusion, optimal growth conditions of *P. aeruginosa* for fast degradation of phenol were determined as 2x MSM, pH 7.5 with 3% inoculum size. Further, addition of 0.1% glucose in the medium enhanced the phenol degradation. The findings of this study provide valuable information for designing bioremediation protocols to treat phenol in industrial wastewater.

Keywords: Bioremediation, Optimization, Phenol, *Pseudomonas aeruginosa*, Wastewater