## Bacteriophages for Sustainable Wastewater Treatment: Application in Black Water Decontamination with an Emphasis to DRDO Biotoilet

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Abstract : Bacteriophages are viruses that parasitize specific bacteria and multiply in metabolising host bacteria. Bacteriophages hunt for a single or a subset of bacterial species, making them potential antibacterial agents. Utilizing the ability of phages to control bacterial populations has several applications from medical to the fields of agriculture, aquaculture and the food industry. However, harnessing phage based techniques in wastewater treatments to improve quality of effluent and sludge release into the environment is a potential area for R&D application. Phage mediated bactericidal effect in any wastewater treatment process has many controlling factors that lead to treatment performance. In laboratory conditions, titer of bacteriophages (coliphages) isolated from effluent water of a specially designed anaerobic digester of human night soil (DRDO Biotoilet) was successfully increased with a modified protocol of the classical double layer agar technique. Enrichment of the same was carried out and efficacy of the phage enriched medium was evaluated at different conditions (specific media, temperature, storage conditions). Growth optimization study was carried out on different media like soybean casein digest medium (Tryptone soya medium), Luria-Bertani medium, phage deca broth medium and MNA medium (Modified nutrient medium). Further, temperature-phage yield relationship was also observed at three different temperatures 27°C, 37°C and 44°C at laboratory condition. Results showed the higher activity of coliphage 27°C and at 37°C. Further, addition of divalent ions (10mM MgCl2, 5mM CaCl2) and 5% glycerol resulted in a significant increase in phage titer. Besides this, effect of antibiotics addition like ampicillin and kanamycin at different concentration on plaque formation was analysed and reported that ampicillin at a concentration of 1mg/ml ampicillin stimulates phage infection and results in more number of plaques. Experiments to test viability of phage showed that it can remain active for 6 months at 4°C in fresh tryptone soya broth supplemented with fresh culture of coliforms (early log phase). The application of bacteriophages (especially coliphages) for treatment of effluent of human faecal matter contaminated effluent water is unique. This environment-friendly treatment system not only reduces the pathogenic coliforms, but also decreases the competition between nuisance bacteria and functionally important microbial populations. Therefore, the phage based cocktail to treat fecal pathogenic bacteria present in black water has many implication in wastewater treatment processes including 'DRDO Biotoilet', which is an ecofriendly appropriate and affordable human faecal matter treatment technology for different climates and situations.

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