Tertiary Treatment of Coke-oven Wastewater through Microbial Route as a Polishing Step

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ABSTRACT

Coke-oven wastewater is generally characterized by an array of pollutants such as cyanide, phenol, ammoniacal-N, etc. The conventional primary (physico-chemical) and secondary (biological) treatment methods are not found sufficient to remove the pollutants below the permissible limits as set by the statutory bodies. Therefore, a tertiary treatment is the need of hour. Several advanced treatment methods are being used for the treatment of coke-oven wastewater such as electro-coagulation, electro-chemical oxidation, adsorption, membrane-based treatment, use of HOCl for oxidation of pollutants, hybrid methods: chemical pre-treatment followed by bacterial treatment, sequential bioreactors in different stages, etc. All these methods are associated with some limitations such as generation of secondary pollutants, high cost of chemicals, sludge generation, technically sophisticated nature, etc. Therefore, an alternative economic and eco-friendly tertiary treatment method is in search for the removal of pollutants from secondary treated coke-oven wastewater. Biological treatment is an environmentally friendly and cost-effective method. The studies on the bio-removal of multipollutant from the secondary treated coke-oven wastewater are quite terse. Therefore, the present research work entirely focuses on the simultaneous removal of multipollutant from synthetic and real secondary treated coke-oven wastewater using cyanobacteria/microalgae/bacteria/consortium in eco-friendly and economical manner. The phycoremediation of pollutants from synthetic secondary treated coke-oven wastewater using cyanobacterial consortium of *Leptolyngbya* sp. and *Planktothrix* sp., and biomass production were studied first. Lethal dose analysis was performed for test strain with varying concentrations of pollutants and maximum biomass obtained were 322.7±22.54 mg/L, 322.3±12.06 mg/L and 352±12.53 mg/L at 2 mg/L of phenol, 175 mg/L of ammoniacal-N and 30 mg/L of nitrate, respectively. One factor at a time (OFAT) approach was followed for finding out suitable operating condition for maximum removal by varying pH (8-10), inoculum size (IS) (5-10%) and initial concentrations (IC) of phenol (2-3 mg/L), ammoniacal-N (150-200 mg/L) and nitrate (30-40 mg/L). The most preferred condition as analysed by OFAT is as follows: pH 8, IS: 10% and initial concentrations of phenol: 2.5 mg/L, ammoniacal-N: 175 mg/L and nitrate: 30 mg/L. Response Surface Methodology (RSM) was used for obtaining optimum condition for removal of the said pollutants and biomass production. Experiments were further performed at the optimum conditions

as analysed by OFAT and RSM with synthetic secondary treated coke-oven wastewater containing mixed pollutants and results were compared. Both OFAT and RSM analysis results were observed effective for real wastewater treatment while from economic point of view, OFAT analysis results were found more suitable for the same. Biomolecules namely carbohydrate, protein and lipid molecules were extracted from treated cyanobacterial biomass to assess the prospect of value-added product formation. Mechanistic pathways for metabolism of pollutants were also discussed.

In the second stage of study, the effectiveness of phycoremediation technique as tertiary treatment for simultaneous removal of three model pollutants such as phenol, ammoniacal-N, and cyanide from real secondary treated coke-oven wastewater was assessed. A green route was employed for the treatment of coke-oven wastewater via the use of *Tetraspora* sp. NITD 18, collected from a contaminated site. Strain susceptibility was tested for the growth in the simulated solution of phenol (10-300 mg/L), ammoniacal-N (100-800 mg/L), and cyanide (1-10 mg/L), and subsequently, optimum concentration was assessed as 100 mg/L, 400 mg/L, and 2 mg/L, respectively. pH 7 and inoculum concentration 10% was found suitable for the removal of phenol (79.02±6.93%), ammoniacal-N (74.7±5.07%), and cyanide (80.4±0.015%) from their individual solution at optimal initial concentrations after 14 days. Biomass production with respect to time in presence of individual pollutants were examined, and the following order was found: ammoniacal-N>phenol>cyanide. The solution of mixed pollutants was prepared by mixing the said pollutants at their optimal level of initial concentrations at suitable pH 7. Such a solution was termed as simulated coke-oven wastewater (SCOW). Simultaneous removal of pollutants from SCOW and real secondary treated coke-oven wastewater was studied next.

In the third stage of study, a microalgal sample was collected and identified as a consortium of *Chlorella* sp. and *Synechococcus* sp. The culture cost was reduced by using poultry litter extract as supplementary material to BG-11 medium. Since major pollutants as present in real secondary treated coke-oven wastewater are phenol, ammoniacal-N (NH_4^+) and cyanide, several matrices were designed with these three major pollutants by varying their initial concentrations such as phenol (2-10 mg/L), cyanide (0.3-1 mg/L) and NH_4^+ (100-200 mg/L), termed as synthetic secondary treated coke-oven wastewater. Maximum removal was observed with individual solutions of phenol (4 mg/L), cyanide (0.6 mg/L), and NH_4^+ (175 mg/L) while maximum removal

in synthetic secondary treated coke-oven wastewater was observed at higher concentrations of phenol (8 mg/L) and cyanide (0.8 mg/L) and the same concentration of NH_4^+ (175 mg/L). Consortium was found effective to reduce the pollutants bellow statutory limits. Kinetic model was developed for predicting growth of consortium and observed that the poultry litter extract enriched BG-11 medium showed higher values of maximum specific growth rate (0.56 day^{-1}) and carrying capacity (1330 mg/L) than that in BG-11 medium only.

The next phase of study focuses on the simultaneous removal of multipollutant from synthetic coke-oven wastewater using a bacterial isolate, *Bacillus* sp. NITD 19, collected from the contaminated site. Growth of bacterial sample was studied in Luria-Bertani (LB) medium. The tolerance limit of each pollutant was studied with the bacterial strain. One variable at a time approach was followed to see the individual effect of operating factor on the removal of each pollutant. Effective conditions like pH 8, inoculum size (IS): 4% and initial concentrations of phenol: 100 mg/L, cyanide: 40 mg/L and ammoniacal-N: 400 mg/L were ascertained after OVAT analysis. Synthetic coke-oven wastewater was prepared by mixing these three pollutants at their most effective initial concentrations. The time variation for removal of pollutants from individual aqueous solutions and from synthetic coke-oven wastewater was studied at the most effective condition with the bacterial strain. Maximum removal of phenol (79.88±1.3%, 72.84± 1.21%), ammoniacal-N (82.83±1.23%, 80.25±1.49%), and cyanide (89.51±1.44%, 79.78±0.95%) were obtained from individual aqueous solution and synthetic coke-oven wastewater, respectively. Fourier-Transform Infrared spectroscopic (FTIR) study and Sodium Dodecyl Sulfate-Polyacrylamide Gel Electrophoresis (SDS-PAGE) analysis were performed for native and treated bacterial strain.

Finally, a bacterial-microalgal consortium was prepared and used for treatment of synthetic and real secondary treated coke-oven wastewater in an ecofriendly and economically feasible manner. Both suspended and immobilized cultures were used in the present study. Two microbial cultures - microalgal (Culture A: a consortium of *Chlorella* sp. (green algae) and *Synechococcus* sp.' (cyanobacteria)) and bacterial (Culture B: *Bacillus* sp. NITD 19) were used for preparation of bacterial-microalgal consortium (Culture C). To assess the function of microalgal consortium and bacterial strain in degradation of pollutants as present in coke-oven wastewater, several bacterial-

microalgal consortia (C1-C7) were constructed by mixing of bacterial culture (Culture B) and microalgal culture (Culture A) at different ratios such as 1:1 (C1), 1:2 (C2), 1:3(C3), 1:4(C4), 4:1(C5), 3:1 (C6), 2:1 (C7). All constructed consortia were cultured in different growth media such as modified minimal basal medium (MBM) (GM1), BG-11 (GM2), MBM+BG-11 (1:1 v/v) (GM3), poultry litter extract (PLE) enriched BG-11 (1:10 v/v) (GM4). The suitable culture ratio and medium were selected on the basis of growth of biomass. The maximum growth (3.08±0.57 g/L) was obtained with C4 culture when grown in GM4 medium. In the next phase of study, C4 was applied for the treatment of synthetic secondary treated coke-oven effluents (SSTCEs). The SSTCEs (SSTCE1-SSTCE4) were prepared by mixing phenol (10 mg/L), ammonium ions (200 mg/L), cyanide (0.8 mg/L), fluoride (10 mg/L), and nitrate (50 mg/L) in different media MBM, BG-11, MBM+BG-11, PLE enriched BG-11 and are termed as SSTCE1, SSTCE2, SSTCE3, and SSTCE4, respectively. The maximum removal of pollutants such as phenol (95.77±2.34%), ammonium ion (90.76±3.4%), cyanide (76.65±2.31%), fluoride (92.08±2.98%), and nitrate (88.71±3.37%) were obtained when Culture C4 was used for treatment of SSTCE4 for 10 days. Next, the whole live cultures, Culture A, Culture B, and constructed consortium Culture C4, were immobilized on to 'Earthen tea pot (ETP)' a commercial waste material. Different sizes of earthen tea pot (1 mm-3 mm) were taken as matrix for immobilization. Immobilized cultures were characterized using scanning electron microscopy (SEM) study and estimation of extra polysaccharide secretion (EPS). Immobilized cultures were then used for the treatment of SSTCE4, in batch mode and immobilized C4 Culture was found efficient to reduce all pollutants, such as phenol (0.23 mg/L, 0.35 mg/L, and 0.388 mg/L), ammonium ions (20.67 mg/L, 36.17 mg/L, and 47.03 mg/L), cyanide (0.021 mg/L, 0.035 mg/L, and 0.041 mg/L), fluoride (0.21 mg/L, 0.43 mg/L, and 0.46 mg/L), and nitrate (5.6 mg/L, 7.7 mg/L, and 9.28 mg/L), below the permissible limits within 5 days for 1 mm, 2 mm, and 3 mm sizes, respectively. Finally, real secondary treated coke-oven effluent was treated in a packed bed bioreactor containing immobilized C4 culture as bioagent under continuous mode.