ABSTRACT

The present study investigates the application of bacterial hyaluronic acid (HA) doped metal oxide nano-adsorbents for the degradation of benzene, toluene, ethyl benzene and xylene (BTEX) and polycyclic aromatic hydrocarbons (PAHs). Two bacteria, *Bacillus subtilis* and *Bacillus paralicheniformis* were utilized, and HA production was aseptically carried out using submerged fermentation by optimizing different cultural parameters i.e., medium (M4), initial pH, incubation temperature (72°C) and inoculum level. HA was extracted using two extraction methods, the physical and the ethanolic method. After optimization, *B. subtilis* produced more HA i.e., 472 µg/ml using the ethanolic method of extraction as compared to *B. paralicheniformis* (374.21 µg/ml), which is highly significant (*HS*, $p \leq 0.05$). Different metal oxide nano-adsorbents with absorption maxima at different wavelengths were synthesized like Ag2O (435 nm), Cu2O (300 nm), Al2O3 (300-320 nm) and ZnO (300 nm). The nano-adsorbents were subsequently coated with HA from both bacterial strains. The spectrophotometry of HA-doped nano-adsorbents revealed their absorbance peaks at different wavelengths as compared to free nano-adsorbents i.e., 300-380 nm for HA-Ag2O, 300-350 nm for HA-Cu2O, 280 nm for HA-Al2O3 and 350 nm for ZnO nano-adsorbents, which confirmed the formation of HA-doped nano-adsorbents. The FTIR analysis evaluated the specific stretching of each nano-adsorbent and functional groups of HA, proving the successful formation of HA-doped nano-adsorbents. Among all the nano-adsorbents, the HA-Ag2O nano-adsorbents were stable and demonstrated better performance, therefore, they were selected for optimization. Multiple parameters were optimized for the formation and constancy of HA-Ag2O nano-adsorbents, including AgNO3 concentration (15 mM), level of HA extract, procurement period, and incubation time (80°C). Further characterization of these optimized nano-adsorbents was then performed. XRD pattern confirmed the presence of Ag2O nano-adsorbents by showing peaks having hkl values of 110, 111, 200, 220, 311, 222 corresponding to 2θ angles that were different for free and HA-doped Ag2O nano-adsorbents. SEM was performed and it revealed the mean average size of individual nano-adsorbents (70.06±0.35 nm for free, 193.93±0.23 nm for *B. subtilis* HA-doped and 157.42±0.28 nm for *B. paralicheniformis* HA-doped Ag2O nano-adsorbents). EDX showed the elemental composition of HA-

Ag2O nano-adsorbents. Zetasizer revealed the 2 size distribution by intensity and zeta potential of prepared HA-Ag2O nano-adsorbents, with mean values of 171.6 nm and –13.6 mV for free, 821.1 nm and –23.5 mV for *B. subtilis* HA-doped and 556.5 nm and –13.7 mV for *B. paralicheniformis* HA-doped Ag2O nano-adsorbents, respectively. The optimized nano-adsorbents were subjected to various applications, in particular the antibacterial activity, antioxidant activity, photocatalytic dye degradation efficiency and degradation of BTEX and PAHs in refinery effluent. The antibacterial activity of HA-Ag2O nano-adsorbents demonstrated maximum potential against *S. aureus* in case of *B. subtilis* HA-Ag2O nano-adsorbents and *Lactobacillus spp.* in case of *B. paralicheniformis* HA-Ag2O nano-adsorbents with inhibition zones of 8±0.4 nm and 6±0.2 nm, respectively. Moreover, the antioxidant activity of HA-Ag2O nano-adsorbents was evaluated using the total antioxidant capacity (TAC) assay and the DPPH assay, showing a radical scavenging activity of 84.36±0.44% for *B. subtilis* HA-Ag2O and 83.6±0.44 for *B. paralicheniformis* HA-Ag2O nano-adsorbents. The photocatalytic dye degradation efficiency of HA-Ag2O nano-adsorbents was found to be 82.28 and 85.77% using *B. subtilis* and *B. paralicheniformis* HA-Ag2O nano-adsorbents, respectively, within 120 min. The maximum degradation efficiency of 76.2% and 68.5% was achieved in case of *B. subtilis* and *B. paralicheniformis* HA-Ag2O nano-adsorbents, respectively, for BTEX and PAHs. The overall findings suggested that HA-Ag2O nano-adsorbents are effective in degrading complex organic pollutants like BTEX and PAHs in refinery effluent, offering a promising solution for environmental remediation.