Chapter V:

BIOREMEDIATION OF ARSENIC IN PACKED BED BIOREACTOR

5.1 General

Bacteria can be used either as free cells (suspension) or through immobilized cells (attached growth) for treatment of pollutants. The use of bacteria as free cells has several disadvantages, such as poor mechanical strength, low rigidity and direct exposure to toxic environments (Hasan et al., 2010). However, immobilization on packing supports greatly enhances efficiency and provides mechanical strength (Geed et al., 2017; Sonwani et al., 2019). In recent times, PBBR has been a preferred method of treatment for a wide range of pollutants. PBBR offers the advantage of a higher loading rate and increased removal efficiency as compared to free suspension systems (Sonwani et al., 2019). It is also simple to operate and has higher active biomass utilization (Geed et al., 2018). Since it is generally operated at ambient conditions, PBBRs are less energy-intensive than chemical processes. Various researchers have used different packing materials for PBBR such as sand (Altun et al., 2014), pyrex glass beads (Dastidar and Wang, 2012), etc. However, use of PUF as a carrier (also used as packing material) for bacteria immobilization to remove arsenic in PBBR is very limited. This study focused on the (i) immobilization of bacteria on PUF, (ii) evaluation of the performance of RPBBR under varying loading rates, and (iii) assessment of the reduction in toxicity using a luminescent toxicity test. The biotransformation ability of bacteria was also evaluated.

5.2 Working conditions of the reactor

The reactor was operated at room temperature, which varied between 25^{o}C to 35^{o}C (was mostly near 30^{o}C). The optimum temperature obtained from batch studies was 30^{o}C with As(III) and 35^{o}C with As(V) for TY6. For K7Pb, the optimum temperature was 30^{o}C in presence of As(V) and 35^{o}C in presence of As(III). TY6 showed maximum growth at pH 8 whereas K7Pb at pH 5. However, the difference in optical density (and thus bacterial growth) at pH 5 and pH 7 was very little. Hence, to keep the process simple and cost-effective, pH of the reactor was maintained near 7. Zeng et al. (2021) reported that PBBR performed well in the pH range of 5-8, with most optimum pH of 7 and temperature between 30-35^{o}C. *Pseudomonas aeruginosa* has been found to work best near pH 7 and pH 8 for the removal of heavy metal(loid)s in PBBRs (Al-Ansari et al., 2021; Singh et al., 2013). Extreme pH and temperature conditions affect bioaccumulation by bacteria and may also result in reduced biological activity possibly due to denaturation of protein. On daily basis, 25 mL of sample was collected for analysis of arsenic concentration in the effluent.

5.3 Effect of flow rate and initial arsenic concentration on removal efficiency

Flow rates and initial concentrations are among the most important parameters in the column study. The RPBBR was operated at flow rates of 5 mL/min and 10 mL/min. The removal efficiency was observed to go down with an increase in flow rate. Figure 5.1 represents the removal efficiency of As(III) and As(V) with TY6 at 5 mL/min and 10 mL/min, whereas Figure 5.2 represents the same for K7Pb. At a higher flow rate, the removal efficiency decreased for both As(III) and As(V) and the concentration of arsenic in the effluent increased. This could possibly be due to the fact that the residence and contact time decreased at increased flow rate and hence, resulted in lower sorption of arsenic (Podder and Majumder, 2018). At a lower flow rate, the biosorbent got more time for binding with metal(loid)s and thus biosorption/bioaccumulation was higher.

Also, at higher flow rates, resistance to external film mass transfer decreases, causing the adsorption sites to saturate more quickly. Conversely, at lower flow rates, the longer residence time enhances intra-particle diffusion, making it more effective (Abdolali et al., 2017). Similar studies for the removal of various pollutants have been reported that show comparable behavior in fixed bed columns (Abdolali et al., 2017; Acheampong et al., 2013; Jaiswal et al., 2023a; Podder and Majumder, 2016; Riazi et al., 2016). An identical trend was observed with an increase in the initial concentration of arsenic. At 250 µg/L, TY6 could remove 98.40% As(V) and 96.80% As(III) at 5 mL/min, whereas the removal efficiency was 92.38% for As(III) and 96.35% for As(V) at 10 mL/min. At 50 mg/L, the efficiency reduced to 69.53% for As(V) and 66.22% for As(III) at 5 mL/min, whereas TY6 could remove only 65.93% As(III) and 66.89% As(V) at 10 mL/min.

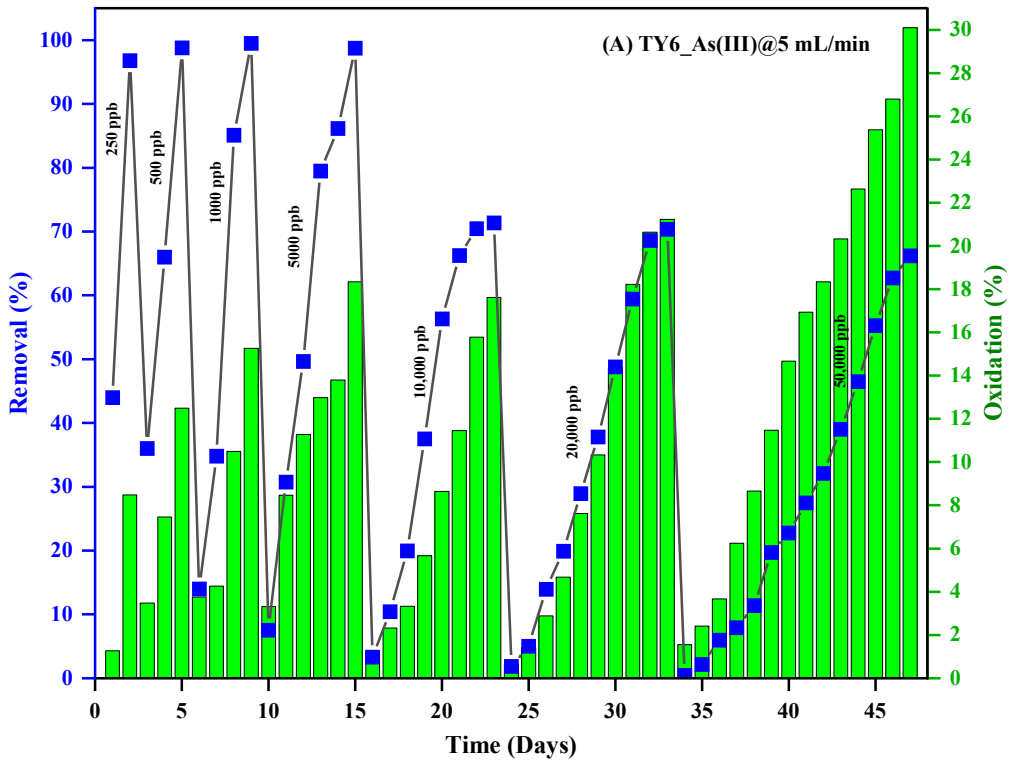
The trend was similar for K7Pb; however, the removal rate was higher as compared to TY6 (time involved was less). K7Pb, at 250 µg/L, removed 99.20% As(V) and 98% As(III) at 5 mL/min, whereas the removal efficiency was 95.20% for As(III) and 96.80% for As(V) at 10 mL/min. At 50 mg/L, the efficiency reduced to 71.55% for As(III) and 75.53% for As(V) at 5 mL/min, whereas K7Pb could remove only 89.97% for As(III) and 90.01% for As(V) at 10 mL/min. Higher concentration gradient and smaller mass transfer resistance may result in lower removal efficiency at higher concentrations (Abdolali et al., 2017). The low biotransformation capacity of the bacteria and higher toxicity may also be responsible for the slower removal rate of As(III) as compared to As(V). Katsoyiannis et al. (2002) reported that the removal efficiency of As(III) decreased as the concentration increased beyond 60 µg/L in a fixed bed up-flow bioreactor. Biosorbent prepared from the fermentation of biowaste showed saturation at 200 mg/L initially, but the breakthrough point decreased with an increase in the initial concentration of As(V) (Kim et al., 2020).

5.4 Biotransformation of arsenic by bacteria

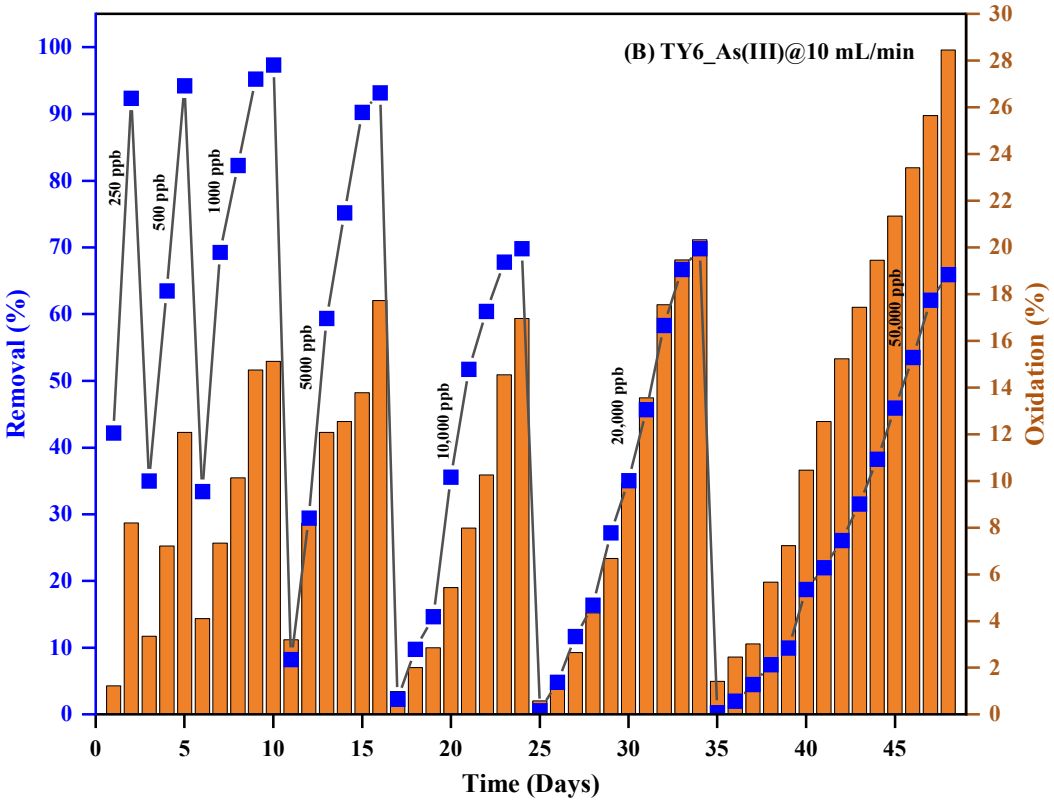
TY6 was able to oxidize As(III) and K7Pb could reduce As(V). Oxidation of As(III) varied between 0.67% to 30.11% at 5 mL/min and 0.58% to 28.46% at 10 mL/min (Figure 5.1). On the other hand, reduction of As(V) ranged from 1.32% to 21.22% at 5 mL/min and 1.03% to 20.46% at 10 mL/min (Figure 5.2). Both bacteria showed the presence of *arrA*, *arsC* [genes responsible for reduction of A(V) to As(III)] and *arxA*, *aioA* [genes responsible for oxidation of As(III) to As(V)]. However, TY6 could only oxidize As(III) to As(V) and K7Pb was only able to reduce As(V) to As(III) (refer to Section 4.6 for details). Zeng et al. (2021) reported that *Rhizobium* sp. A219 demonstrated the ability to completely oxidize 30 mg/L of As(III) within approximately 30 min in a fixed-bed bioreactor. pH and temperature, both factors were able to influence the oxidation efficiency. Dastidar and Wang (2012) reported that *Thiomonas arsenivorans* strain b6 could oxidize As(III) (with initial concentration varying between 500-4000 mg/L) between 48.2% to 99.3% in PBBR. The reactor was packed with spherical pyrex glass beads and had a hydraulic retention time of 0.2-1 d and was operated for 137 d. Reduction in efficiency of bacteria at higher arsenic concentration may be because higher arsenic concentration results in death of bacterial cells due to its toxicity and subsequently, it results in lower biotransformation and removal efficiency.

5.5 Characterization

The formation of biofilm on the carrier is influenced by operating conditions, including pH, temperature and nutrient availability. SEM images of packing material before and after immobilization have been shown in Figure B1 (refer to Appendix B). On day 0, the presence of micropores on the surface was observed and on day 20 (after immobilization), the formation of a thin film was observed, which confirmed the formation of biofilm on the PUF. The development of biofilm on the surface of the carrier requires both, metabolic activity of bacteria and presence of its extracellular compounds. The formation of biofilm on PUF demonstrates that the colonization of pores by bacteria was successful (Jaiswal et al., 2023a).



Operating conditions - Temperature: 25°C - 35°C; pH: 7.0



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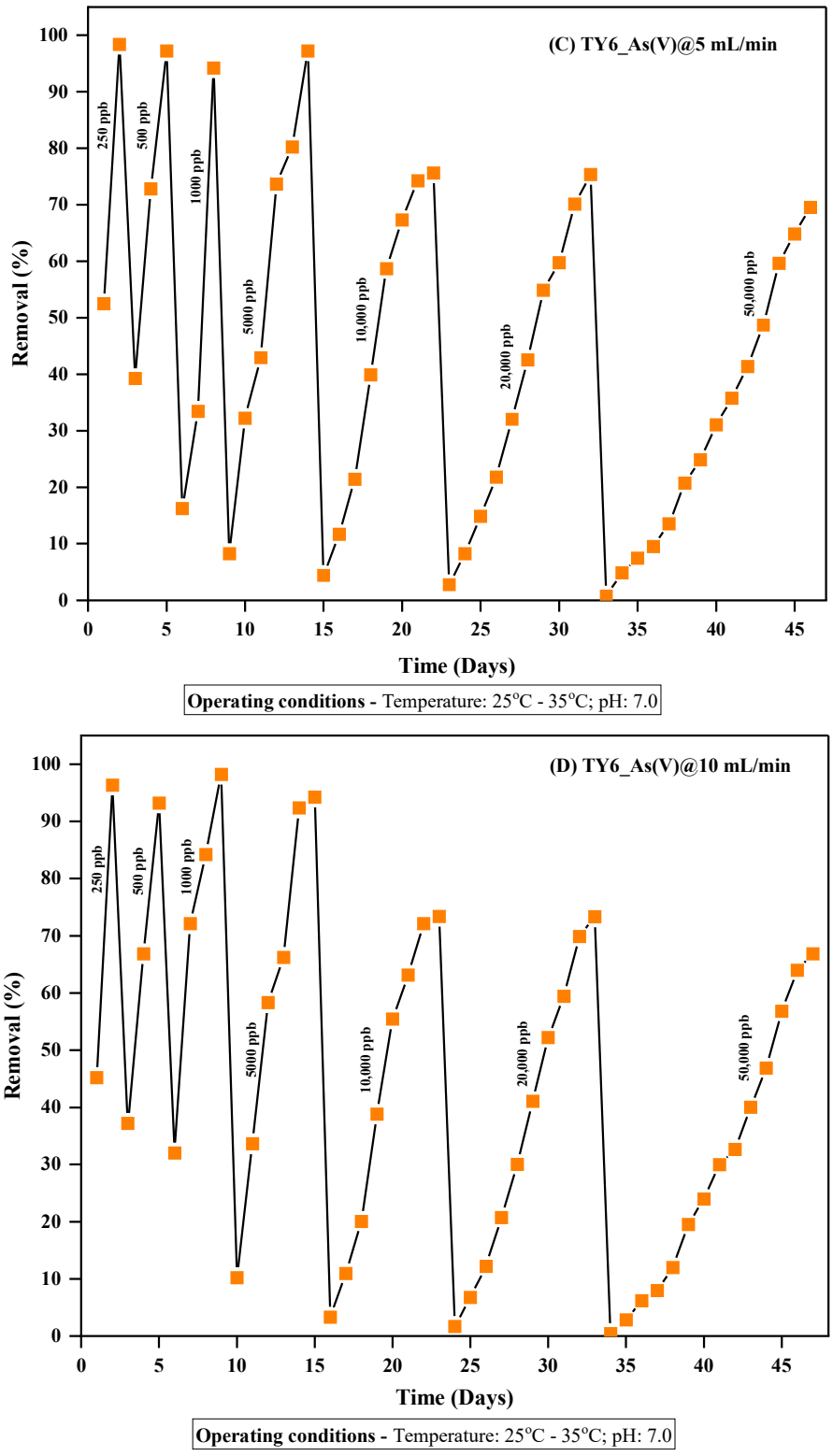
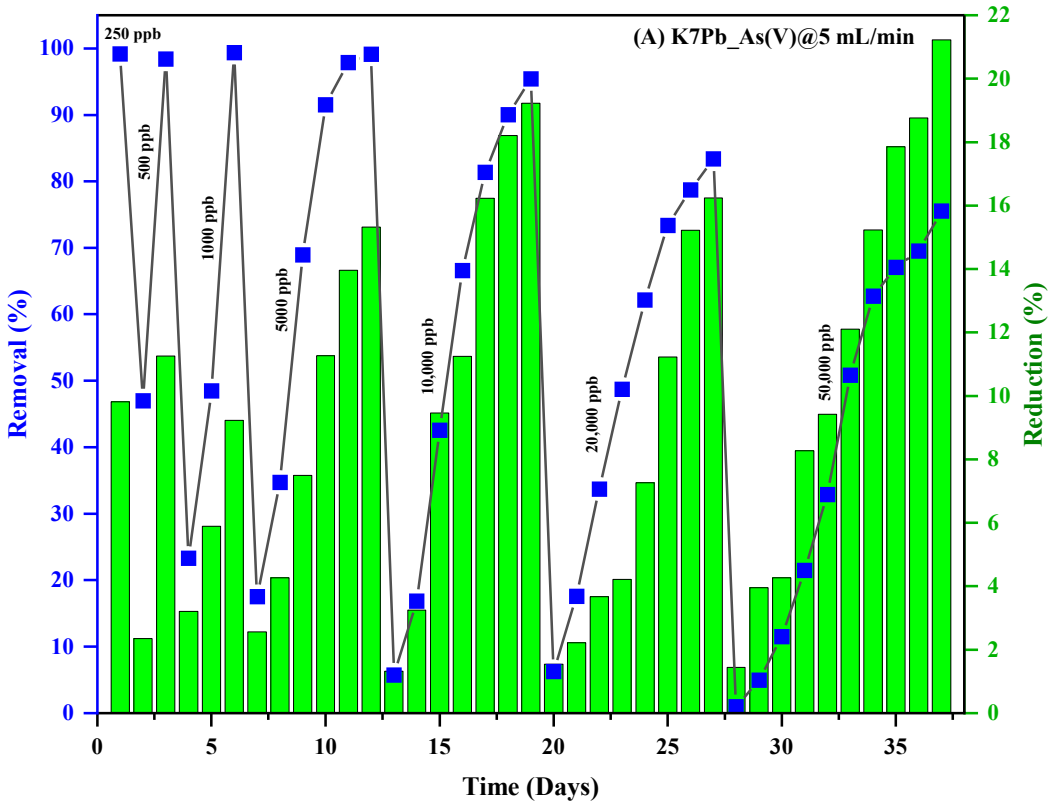
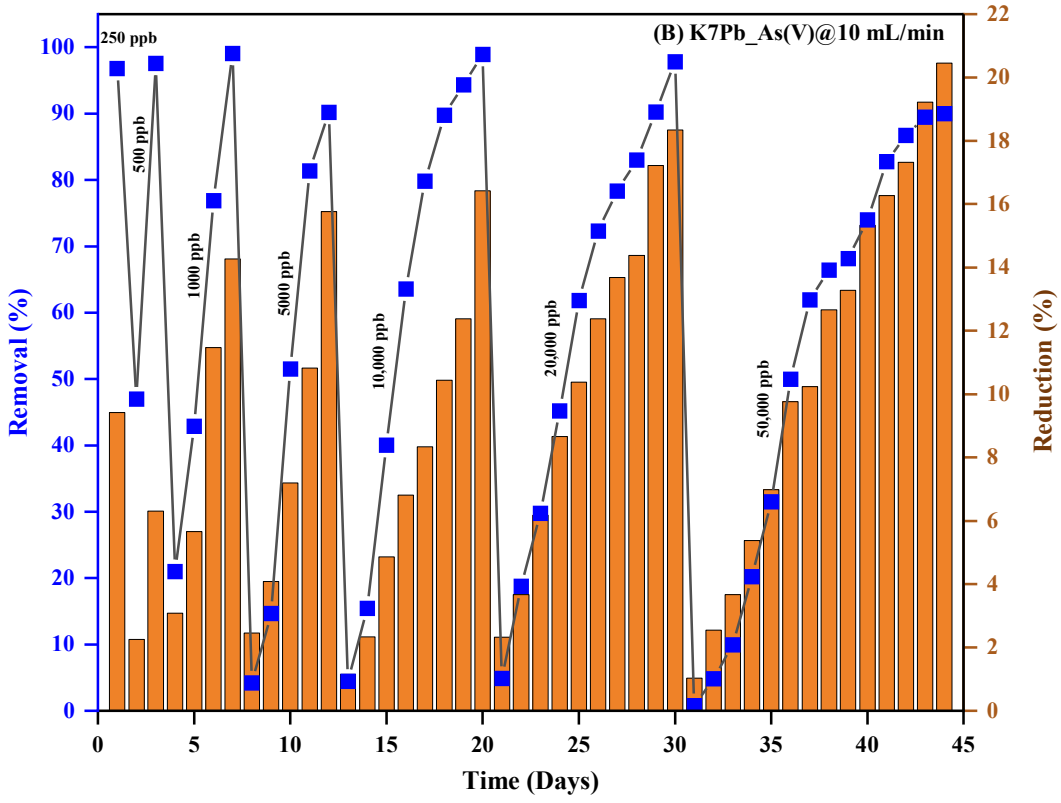


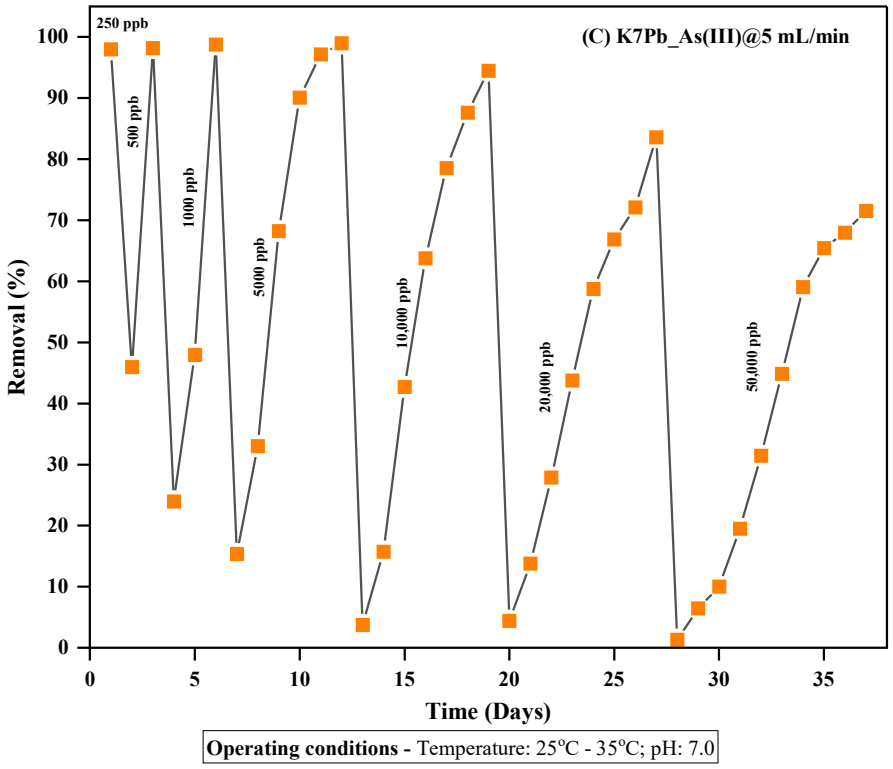
Figure 5.1: Removal of **(A)** As(III) @ 5 mL/min and its oxidation **(B)** As(III) @ 10 mL/min and its oxidation **(C)** As(V) @ 5 mL/min **(D)** As(V) @ 10 mL/min using TY6 in PBBR



Operating conditions - Temperature: 25ºC - 35ºC; pH: 7.0



Operating conditions - Temperature: 25ºC - 35ºC; pH: 7.0



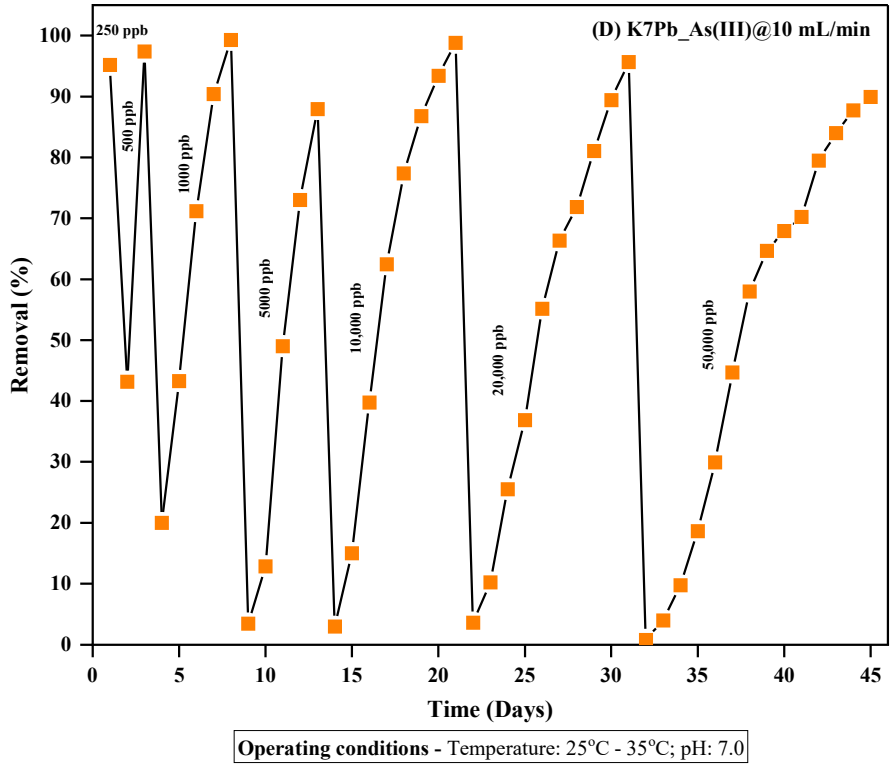


Figure 5.2: Removal of **(A)** As(V) @ 5 mL/min and its reduction **(B)** As(V) @ 10 mL/min and its reduction **(C)** As(III) @ 5 mL/min **(D)** As(III) @ 10 mL/min using K7Pb in PBBR

5.6 Batch and column study: Comparison of arsenic removal efficiency

A comparative study of arsenic removal using batch and column study with different initial concentrations has been shown in Figure 5.3. For batch study (free-cell), the removal was high for an initial concentration of up to 500 µg/L; however, the time required was very high (refer to Section 4.6 for details). Removal efficiency in case of batch study was very high at lower concentrations for both As(III) and As(V). TY6 could remove 99.97% and 98.12% As(III) at initial concentration of 50 µg/L and 100 µg/L, respectively. On the other hand, at the same initial concentrations, K7Pb could remove 98.02% and 96.42% As(V), respectively. As the concentration increased, the removal efficiency declined and the time required for removal also increased. The removal efficiency decreased significantly when the concentration was increased above 500 µg/L.

At further higher concentrations (10 mg/L), bacterial growth was greatly reduced and arsenic removal was only 14.26% for As(III) and 8.25% for As(V). The diminished efficacy of arsenic removal by free-cell bacteria indicates that higher arsenic concentrations are toxic to the cells. For immobilized cells, the removal efficiency was found to be higher. It was above 65% even at a concentration of 50 mg/L for both strains. Effective mixing due to recirculation may result in less mass transfer, leading to effective removal in PBBR (Talha et al., 2018). Compared to free cells, immobilized microorganisms have shown higher efficiency for pollutant removal at higher loading rates (Geed et al., 2017). Bag et al. (2010) reported an arsenic removal efficiency of 95% in a packed bed continuous flow reactor with bacteria *Rhodococcus equi* (JUBTAs02). An anaerobic bioreactor with microbial sulfate reduction could remove arsenic with an efficiency of 90% to >99% (initial concentration of arsenic was 5 mg/L) (Cohen and Ozawa, 2013). Along with arsenic, cadmium, chromium, copper, iron, lead and zinc were also removed.

Apart from biological removal, in an immobilized system, arsenic also gets adsorbed on the PUF surface, which may result in higher removal efficiency. However, the decrease in efficiency at higher concentrations may be attributed to non-adaptability of bacterial cells to such high toxicity and also due to reduction of active sites on the PUF surface. Higher biomass concentration availability per unit carrier volume in immobilized cells may also be responsible for higher efficiency of reactors.

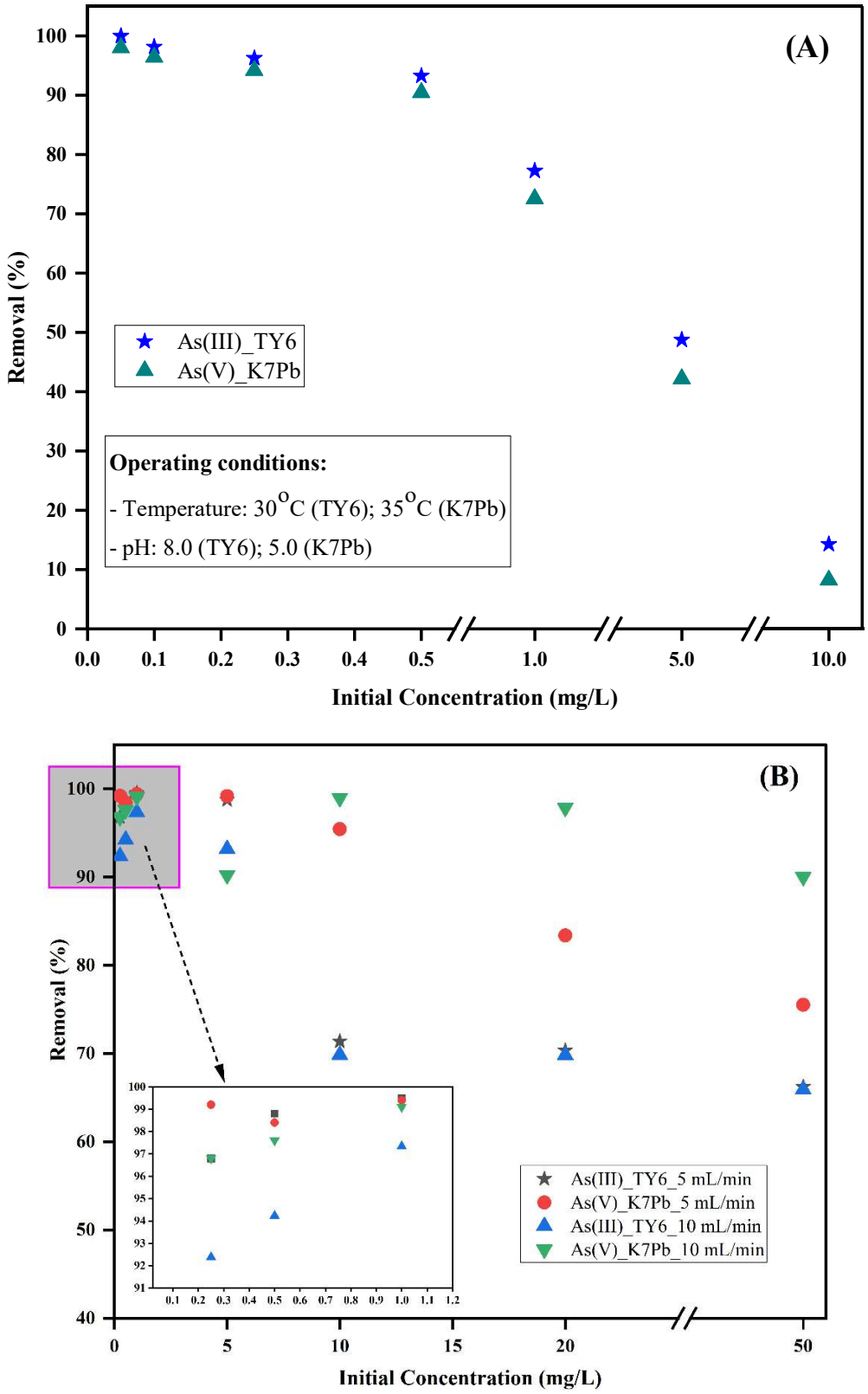


Figure 5.3: Removal of As(III) and As(V) using TY6 and K7Pb in **(A)** suspended free-cell study **(B)** RPBBR at different flow rates

5.7 Toxicity assessment

The acute and chronic toxicity of TY6 and K7Pb was assessed using *Photorhabdus luminescens*. The bacterial intensity of the sample treated with TY6 increased from $3.02x10^7$ (after 1 h) to $3.43x10^7$ (after 24 h), whereas the sample treated with K7Pb increased from $5.45x10^6$ (after 1 h) to $6.52x10^6$ (after 24 h) (Figure 5.4). The %BI for chronic toxicity was found to be higher than that of acute toxicity. The %BI decreased from 10.35% (after 1 h) to 3.51% (after 24 h) for TY6. However, in case of K7Pb, there was a slight increase in %BI from 5.06% (after 1 h) to 7.38% (after 24 h). This increase in toxicity may be because *Photorhabdus luminescens* was not able to grow properly in presence of As(V). In case of TY6, maximum intensity was observed between 340-360 nm, whereas, for K7Pb, it was between 520-525 nm. A significant decrease in bacterial intensity was observed in case of untreated samples as compared to control and treated samples for acute and chronic studies with TY6 and K7Pb. The comparable intensity of control and treated samples suggested a decreased toxicity of treated samples. Studies done for toxicity assessment in case of dye and pesticides have also reported similar conclusions (Tiwari et al., 2022; Tiwari et al., 2023).

5.8 Disposal of immobilized arsenic

Arsenic should be disposed off in ways that limit its risk of release back into the environment and, ideally, in those environments in which it was removed (Clancy et al., 2013). In developing nations such as India, the disposal of arsenic presents a significant challenge, resulting in hindrances in the implementation of various treatment technologies. Disposal practices currently employed include methods like blending with cow dung, stabilization, utilization of anaerobic digesters, soil disposal and use of landfills (Pathan and Bose, 2018). Arsenic should be encapsulated with a solidification process for safe disposal with long-term stability to minimize the leaching effect (Mandal et al., 2016). However, additional investigation is needed to find an alternate way of arsenic disposal so that there is no further risk of environmental pollution or contamination. Disposal in landfills can be an alternative but long-term tests need to be conducted to assess their leachability.

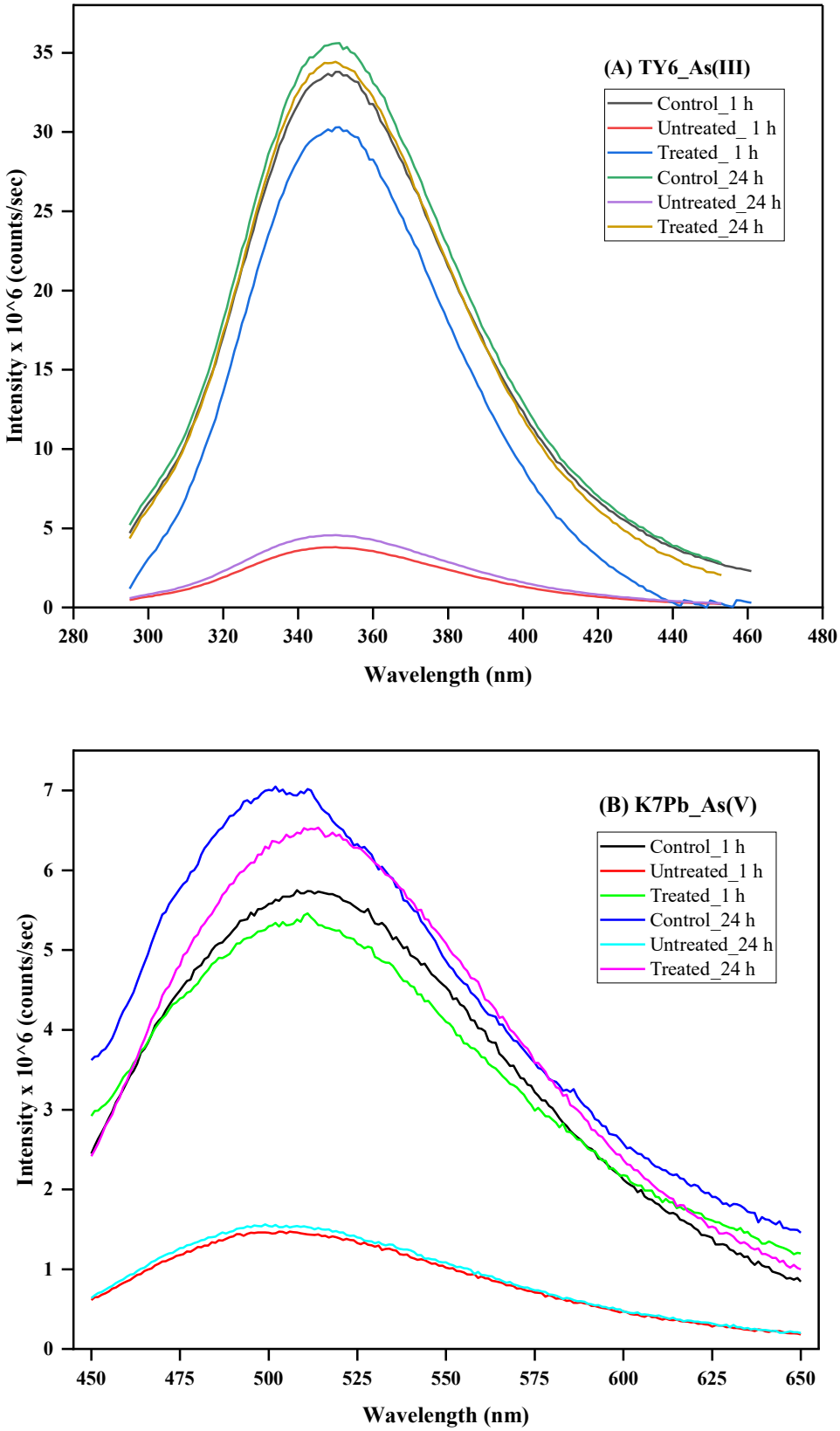


Figure 5.4: Bioluminescence intensities for acute and chronic toxicity in case of **(A)** TY6_As(III) **(B)** K7Pb_As(V)

5.9 Summary

This study investigated the use of bacteria-immobilized on PUF for arsenic remediation in a packed bed bioreactor. The effectiveness of *Proteus alimentorum* strain TY6 and *Pseudomonas aeruginosa* strain K7Pb for removal of As(III) and As(V) was evaluated. Initial arsenic concentrations ranged from 250 µg/L to 50 mg/L at varying flow rates. The removal efficiency of TY6 varied from 0.45% to 99.50% for As(III) and 0.75% to 98.40% for As(V). For K7Pb, the efficiency ranged from 0.79% to 99.30% for As(III) and 0.80% to 99.40% for As(V). Both bacterial strains demonstrated significant potential for arsenic biotransformation. TY6 was able to oxidize As(III) to As(V) and % oxidation ranged from 0.58 to 30.11%, whereas the reduction capacity of K7Pb from As(V) to As(III) ranged from 1.15 to 21.22%.