%, pH at 10, and initial dye concentration of 4.427 g/l) which ensured the minimum SEC_{av} of 119.27 kWh/g.m^3. As the dye concentration in the regular textile wastewater is very low, it was recommended to have a dye enriching pre-treatment step for the regular textile effluent to achieve the desired high inlet dye concentration for ozonation. Under the optimized condition, the cost of electricity consumption in ozonation was found to be reduced significantly (by ~ 25-30 %). However, the nature, design and cost of the pre-treatment step (using adsorption, coagulation etc.) would determine the net gain in the overall treatment cost which may be focus of the future work.

7 Phyto-/Geno-Toxicity Assessments of the Dyeing Wastewater treated with Anaerobic-Aerobic Biodegradation (AnAB) *vs.* Ozonation-Aerobic Biodegradation (OAB) Processes

The effluents of textile and dyeing industries are some of the vast sources of wastewaters containing high loads of dyes, pigments, and various associated contaminants such as oil, detergents, dissolved and suspended solids, and other non-biodegradable toxic

matters (Kumar et al. 2007; Şen and Demirer 2003; Singh and Arora 2011). Since only 50 % of the applied dyes get utilized in the fabric, the rest is released with huge volumes of water into aquatic bodies, causing severe environmental hazards due to their high pH, temperature, COD (Chemical Oxygen Demand), BOD (Biochemical Oxygen Demand) and TOC (Total Organic Carbon) levels (Işik and Sponza 2007; Kousha et al. 2012; Roy, Biswas, et al. 2018; Roy, Sengupta, et al. 2018).

Over the last few decades, numerous treatment methods comprised physical, chemical, and biological pathways to degrade the recalcitrant dyes (Chaturvedi et al. 2021b; Paździor, Bilińska, and Ledakowicz 2019). Although physical methods such as adsorption and coagulation are good in reducing the dye loading in the wastewater, the disposal of adsorbents remains a concern. The chemical methods such as Fenton, photocatalysis, ozonation are fast and efficient in decolorizing the dyeing water; however, they suffer from high treatment costs and poor mineralization. The biological methods have been used as an economical and pro-environmental alternative that effectively treats dyeing wastewater. The anaerobic bio-reduction of the chromophore (-N=N-) in azo dyes is catalyzed by azoreductase enzyme in the presence of reducing agents (electron donor), nicotinamide adenine dinucleotide (NADH), and flavin adenine dinucleotide (FADH) (Jamee and Siddique 2019).

Further, the sequencing of anaerobic and aerobic processes with single and mixed bio-cultures have also been employed to yield enhanced decolorization and mineralization efficiencies for a wide range of industrial dyeing effluents. However, biological methods generally offer a slow rate of treatments compared to physical and chemical techniques. The investment costs of biological processes are 5–20 times lower than some chemical oxidation processes, and their operating costs are 3–10 times lower (Guieysse and Norvill 2014). However, biological processes are limited to treating biodegradable compounds and are not suitable for treating toxic or inhibiting molecules. In addition, their implementation is sometimes difficult due to the potential instability of bacterial populations that sometimes

need an acclimation process and growth control. It is helpful to oxidize pollutant macromolecules using a chemical reaction until the size of the macromolecules becomes small enough to be easily metabolized by biodegradation.

The incomplete detoxification achieved in chemical and biological methods has been one of the major concerns of the research activities performed in the last few years (Venkatesh, Venkatesh, and Quaff 2017). The toxicity of the treated effluents is attributed to the untreated dyes or the generated intermediates/ byproducts. The toxic byproducts of dyes may inhibit the biological culture; for instance, the anaerobic degradation of azo dyes produces complex aromatic amines, which pose a toxic effect to the bio-organisms. The chemical methods may also produce intermediates that are toxic or sometimes even more toxic than the parent pollutant. Although researchers have integrated physicochemical and biological methods to enhance detoxification (Chaturvedi et al. 2021b), complete detoxification of the dyeing water is rarely achieved. Next, various methods for evaluating the toxicity of the treated wastewater have been developed and used to determine the harsh impact of these toxicants on living beings (Blanco et al. 2018; Boehler et al. 2017; Carbajo et al. 2015; Chen et al. 2017; Libralato, Ghirardini Annamaria, and Francesco 2010; Rahimi et al. 2021). In this regard, several plants- and bacterial-bioassays have been adopted for assessing the physiological and behavioral changes shown by living organisms when their metabolisms are interrupted by the toxic compounds (Gu, Mitchell, and Kim 2004; Iqbal et al. 2017). However, no single toxicity assessment method can be labelled as the *gold standard* as each method is limited by the number/ kind of organisms used and the number of biological parameters assessed.

This study aimed at performing a comparative toxicity assessment of the treated dyeing wastewater using plant-and luminescent bacterial assays. Specifically, simulated dyeing wastewater (SDW) was treated using two systems, namely AnAB (Anaerobic Aerobic Biodegradation) and OAB (Ozonation-Aerobic Biodegradation) system and the

decolorization, mineralization, and detoxification in the treated wastewater were assessed and compared for both cases. The bacterial species immobilized in the bioreactor were obtained from the soil samples collected from a pond near a dyeing-waste discharge site of a carpet-dyeing unit. The decolorization and mineralization in the treated dyeing wastewater were evaluated using spectral absorbance, COD, and TOC determinations. The phytotoxicity assessment was carried out with seeds of *Vigna radiata*. The luminescent bacterial assay was prepared using a less-explored strain of luminescent bacteria *P. luminescens*. The toxicity of the treated dyeing wastewater was assessed based on plant growth and bioluminescence parameters for qualitative and quantitative comparisons.

7.1 Configuration of AnAB and OAB systems

A cylindrical borosilicate glass (internal diameter = 6 cm; height = 53 cm) based bioreactor was fabricated with a total volume of around 1500 mL and a working volume of 1200 mL for biodegradation of SDW. A total of 2000 mL of SDW was processed in each run with varying dye concentrations. Ports were provided at the top for sample collection and bottom for air supply, influent feeding, and drainage. For the immobilization process, washed and dried LDPE foam cubes were filled inside the reactor up to a height of 43 cm. The fixed bed of packing material was prepared using stainless steel sieves provided at the top and bottom of the packing. The ozonation reactor was also made of a cylindrical borosilicate glass column, and ozone was injected through a fine-bubble ceramic sparger from the bottom of the reactor. The ozone gas generation was done using Faraday's Ozonator A20G with a maximum ozone capacity of 20 g/h. Because of the physical and chemical constraints of ozone decomposition in wastewater, not all of the ozone produced was utilized for wastewater oxidation; part of it escaped from the reactor without mixing with wastewater. A 500 mL gas washing bottle containing a 2 percent KI solution was connected in sequence in the reactor's air effluent to trap the escaping ozone. The outlet from the semi-batch ozonation reactor was then treated in

the aerobic reactor. The schematic diagram of both the AnAB and OAB systems is shown in Fig. 7.1 (a and b).
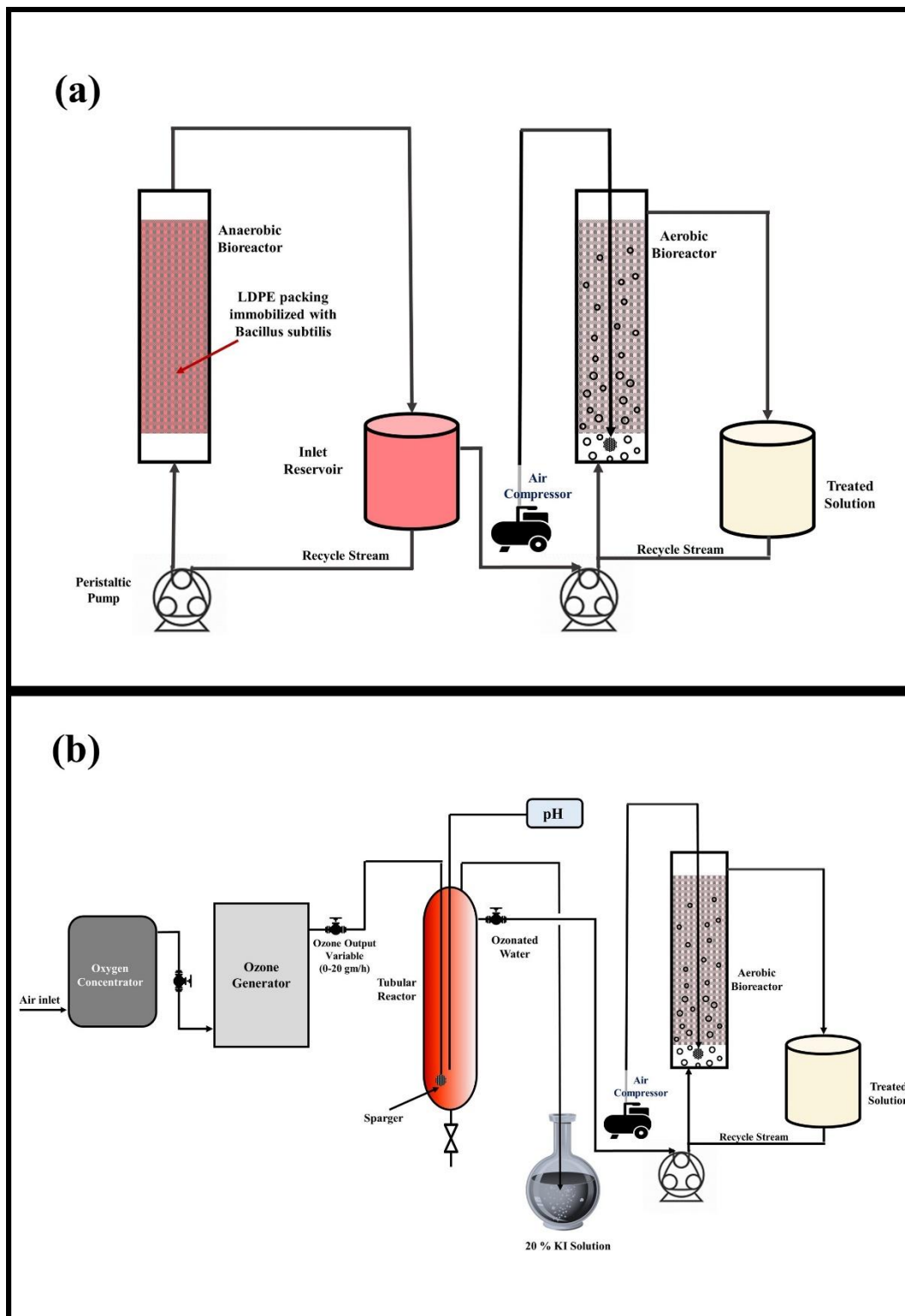
Figure 7.1 Schematic diagram of experiments: (a) anaerobic reactor followed by aerobic reactor (AnAB system); (b) ozonator reactor setup (with an oxygen concentrator and ozone generator) followed by aerobic reactor (OAB system)

7.2   Bacterial immobilization and their acclimatization to the SDW in the FBBR

The isolated bacterial species were immobilized on the LDPE packing medium in the FBBR. The process of immobilization took 20 days to complete. After a successful immobilization, the bacterial mass was acclimatized to the SDW made of Congo red dye in the FBBR. The acclimatization process makes the bacterial population adapt to the SDW in anaerobic conditions. In the initial run, the inlet concentration of Congo red dye to the FBBR was 50 mg/L, as shown in Fig. 7.2. After a complete decolorization, the cycle was repeated with the same dye loading in the subsequent run. The dye degradation rate was observed to be increasing from 5.45 mg/L.d to 11.21 mg/L.d in the second run. Next, the dye concentration in the inlet stream of FBBR was gradually increased in the range from 50 to 500 mg/L; a successive repetition of each cycle was also performed similarly as was done earlier. At every inlet dye concentration, the subsequent cycle showed an improved dye degradation rate than the previous cycle, suggesting bio-sludge's adaption to the dyeing water environment. The same has also been observed and reported in the literature that the bacterial sludge gets adapted to the degradation mechanisms and pathways when exposed to high pollutant concentrations (Khan, Bhawana, and Fulekar 2013). The process of adaptation yields alternation in the expression of genes that allow the secretion of enzymes responsible for the degradation of the pollutant. In this way, the microbes acquire genetic traits which improve their metabolic performance and survival over multiple generations. Finally, the FBBR acclimatized for dyeing water of concentration up to 500 mg/L was used in this study.
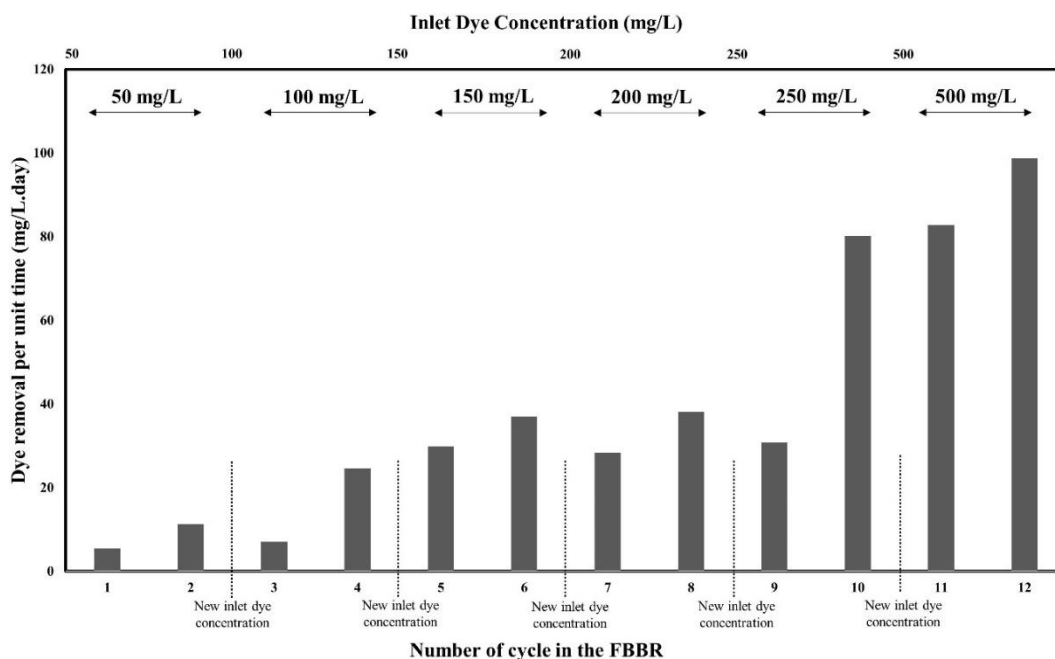
Figure 7.2 Bacterial acclimatization process in an FBBR. The FBBR system was exposed to varying inlet concentrations of Congo red dye (ranging from 50 - 500 mg/L) in different cycles

7.3 Performance evaluation of the hybrid AnAB and OAB systems

7.3.1 COD removal

Fig. 7.3 displays changes in the COD value during the treatment of simulated Congo red dye solution in the FBBR in anaerobic and aerobic phases of the AnAB system and ozonation followed by an aerobic phase of the OAB system for the initial dye concentration of 200 mg/L, which had the COD of 1370 mg/L (equivalent to the organic loading rate, OLR, of 1.37 kg COD/m^3. d). The faster removal of COD in the anaerobic phase (of the AnAB system) than the ozonation phase (of the OAB system) is apparent. It is attributed to the methanogenic activities of the microbial species during the anaerobic treatment (Zhang et al. 2011). A very fast COD removal of ~45 % was observed in the first 12 h of hydraulic retention time (HRT). However, the COD removal rate started decreasing afterward (as indicated by the inflection point in the COD curve in Fig. 7.3). It is attributed to the formation of complex aromatic amines that restrict further COD reduction during the anaerobic phase

(van der Zee and Villaverde 2005a). After 24 h of HRT, the COD removal efficiency reached about the near-maximum of ~53 %; and the system was then switched to the aerobic phase (phase II). The COD was observed to be reduced drastically with increasing the HRT. The reduction in COD level is attributed to the conversion of complex aromatic amines into simple amines via deamination and hydroxylation steps (Fernando, Keshavarz, and Kyazze 2014; Franca et al. 2019). At the end of 144 h of HRT, the maximum COD removal of 94 % was obtained. Other researchers have also used the anaerobic-aerobic integrated biological systems to increase overall COD removal and reduce operational costs (Punzi, Nilsson, et al. 2015b; Shoukat, Khan, and Jamal 2019).

The standalone ozonation process was lesser effective in the mineralization of SDW. In just 16 min of ozonation, the COD reduction reached ~ 30 %. However, the removal rate started decreasing and was almost saturated within 30 minutes of operation. After switching the system to the aerobic phase, the COD was further reduced. The final COD removal of the offered by OAB system was comparable with that of the AnAB system for the case of 200 mg/l of initial dye concentration.
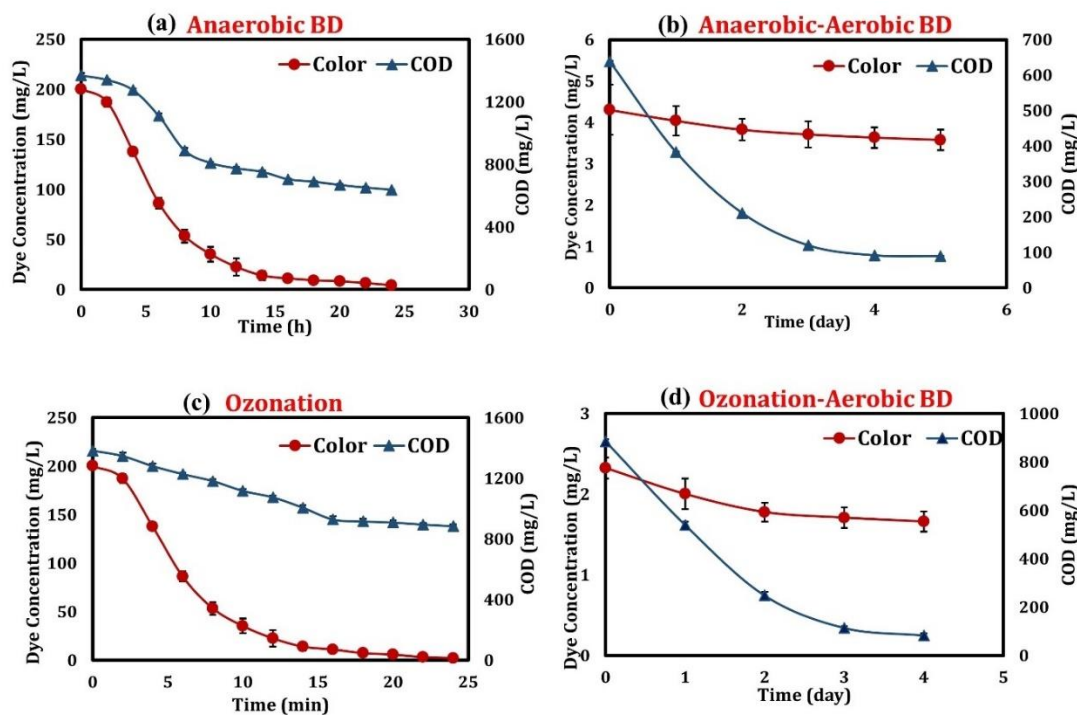
Figure 7.3 Color and COD removal with time for the (a) anaerobic biodegradation; (b) anaerobic-aerobic biodegradation; (c) ozonation process and (d) ozonation-aerobic biodegradation (at the initial dye concentration of 200 mg/L)

7.3.2   Color removal

The color removal of SDW with an initial dye concentration of 200 mg/L in the AnAB and OAB system is also presented in Fig. 7.3. The anaerobic phase played a vital role in the decolorization as a high color removal of ~97.28 % was achieved in the anaerobic phase itself at the end of 24 hrs. The anaerobic biodegradation aims at cleaving the chromophore azo bonds (-N=N-) *via* redox reaction using azo dye molecules as electron acceptors. Therefore, the presence of alternative electron acceptors such as nitrates, sulfates, oxygen, and ferric ions may inhibit the reduction of the azo dye molecules, thereby yielding insufficient color removal (van der Zee, Lettinga, and Field 2001; van der Zee and Villaverde 2005b). As the most color removal occurs in the anaerobic environment, it cuts down the aeration cost incurring in the successive aerobic step. Many studies have also reported that the anaerobic

biological systems offer maximum degradation of the color-causing compounds in the traditional systems (Amaral et al. 2014; Li et al. 2018; Yang et al. 2018).

Within minutes, the single ozonation process decolorized the SDW of 200 mg/l of initial dye concentration. The ozonation process generates many hydroxyl radicals ($\cdot$OH) in the alkaline medium. The radicals have a very high oxidizing potential, and they selectively attack the chromophore of dyes. As shown in Fig. 7.3(c), the process of ozonation almost completely decolorized the wastewater. Fig. 7.3(b) and 7.3(c) also display that the addition of a subsequent aerobic phase following the anaerobic phase offered a very slow rate of color removal in the case of azo dyes used in this study. However, the aerobic step reduced the COD level significantly, as discussed in section 7.3.1.

7.3.3   *Overall Performance AnAB and OAB System against varied organic loading rates*

The FBBR was first acclimatized up to the dye concentration of 500 mg/L. The OLR at the inlet of FBBR was varied from 1.37 to 2.23 kg COD/ m^3.d (equivalent to 200 to 800 mg/L of inlet dye concentrations) to evaluate the performance of the hybrid AnAB OAB system against shocks in the inlet dye-loading. The mineralization (TOC and COD removal) and decolorization were measured at various OLRs. Initially, the AnAB and OAB systems were fed with the influent dye concentration of 200 mg/L (equivalent to the OLR of 1.37 kg COD/ m^3.d). At this process condition, the degradation of color was found to be ~97.28 % in the first 24 h of anaerobic treatment (phase I of AnAB system), with the COD and TOC removal as 53.41 % and 41.57 %, respectively. Still, the standalone ozonation process (step I of the OAB system) decolorized the wastewater within 24 min completely. However, the mineralization via ozonation was lower. After 6 days of HRT, when the COD removal increased to a steady level of ~94 % and ~80 % for AnAB and OAB systems, respectively, the OLR was increased to 1.71 kg COD/ m^3.d by increasing dye concentration to 400 mg/L. At this condition, the degradation of color was still 96.85 % and 98.5 % for AnAB and OAB systems, respectively, but the COD removal decreased to 76.47 % and 71.15 % for both the

systems. After 15 days of the reactor operation, the dye concentration was increased to 600 mg/L for a corresponding OLR of 1.97 kg COD/ m^3.d. After reaching the steady stage, the COD, TOC, and color removal of 71.35 %, 51.41 %, and 97.84 % for the AnAB system; 62.57 %, 35.29 % 98.51 % for the OAB system, respectively were achieved. Finally, the OLR was increased to 2.23 kg COD/ m^3.d by increasing the inlet dye concentration to 800 mg/L. At this state, the FBBR took 15 days of AnAB hybrid operations (from day 28th – 43rd) to reach the steady state. After the 43rd day, the COD, TOC, and color reduction for the AnAB system reached steady values of 65.77 %, 46.26%, and 84.37 %, respectively. For the ozonation-aerobic biodegradation system, the final COD, TOC, and color removals were 55.16 %, 33.87 %, and 98.46 %, respectively, as shown in Fig. 7.4. In sum, a very high color reduction of near ~97% was obtained in just 24-56 min of ozonation operation in all cases of the inlet dye concentrations varied up to 800 mg/L (i. e. OLR of 2.23 kg COD/ m^3.d). However, the AnAB system was slightly better for wastewater mineralization (COD and TOC reductions) than the OAB system. So, the color reduction by the ozonation process and mineralization by the biological routes are recommended. The performance of the bioreactor declined when the inlet dye concentration was increased to 800 mg/L. It indicated that the FBBR, which was acclimatized at 500 mg/L of dye concentration, showed no inhibition in the performance until the inlet dye loading of 600 mg/L. Therefore, the prepared FBBR can remain stable against ~20% shock in the inlet dye loading rate.
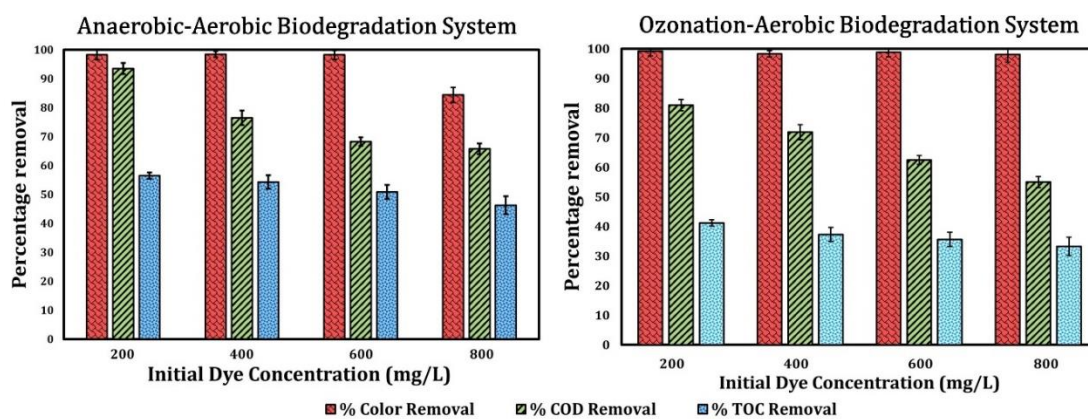
Figure 7.4 Overall performance of the AnAB and OAB system depicting the effect of an increase in the dye concentration from 200 mg/l to 800 mg/l on the percentage reduction in color, COD, and TOC. The data of color, COD, and TOC are the mean of three experiments ± standard error

7.4 Toxicity assessment

7.4.1 *Phytotoxicity analysis*

The release of toxic dyeing effluents into environments causes hazards to aquatic lives and impedes soil quality and fertility. The impact of untreated and treated SDW on the germination efficiency of *Vigna radiata* seeds was evaluated to assess their phytotoxicity. The *Vigna radiata* seeds exposed to 200 mg/L concentration of Congo red dye in the SDW sample showed a low percentage of germination (23.33 %), as shown in Fig. 7.5. In contrast, when the seeds were grown in the anaerobically and anaerobic-aerobically treated dyeing waters (i. e. AnTDW and AnATDW, respectively), they exhibited 86.67 %, and 90 % germination, respectively. The water samples treated using standalone ozonation showed slightly lower % germination of 70 %. It implies reduced phytotoxicity with wastewater treated using the AnAB and OAB systems.

Further, the plumule (shoot) and radicle (root) lengths were found to be significantly small (1.08 ± 0.1 and 0.98 ± 0.28 cm for AnAB system; 1.24 ± 0.16 and 1.11 ± 0.34 for OAB system, respectively) for the untreated SDW sample. However, the shoot and root lengths

improved significantly and were found close to the DiW (control) sample when the *Vigna radiata* seeds were germinated in the dyeing wastewaters treated in AnAB and OAB systems. They were seen as 11.33 ± 0.6 and 6.21 ± 0.53 cm respectively with the AnTDW, 7.84 ± 0.24 and 4.47 ± 0.28 cm respectively with the OTDW, 12.03 ± 0.72 and 4.75 ± 0.18 cm respectively with the hybrid AnATDW and 11.51 ± 0.22 and 5.27 ± 0.32 cm respectively with the hybrid OATDW. The standalone anaerobic biodegradation process showed a higher reduction in phytotoxicity than the standalone ozonation process. However, the toxicity levels of the OATDW samples improved and became comparable to AnTDW. Although the AnTDW and AnATDW samples were found to offer significantly lower phytotoxicity than the untreated SDW, the difference in the toxicity levels of AnTDW, AnATDW, and OATDW samples appeared to be shadowed by significant variations obtained in the phytotoxicity experiments. It indicates that the phytotoxicity analysis may not be used as a *precise* quantitative approach to assess the toxicity, mainly when the difference between the toxicity levels of the water samples is relatively small.

Sample	Germination (%)	Shoot (cm)	Root (cm)
DiW	100 ± 0	14.67 ± 0.84	7.78 ± 0.92
SDW	23.33 ± 4.71	1.08 ± 0.1	0.98 ± 0.28
AnTDW	86.67 ± 4.71	11.33 ± 0.6	6.21 ± 0.53
AnATDW	90 ± 8.16	12.03 ± 0.72	4.75 ± 0.18

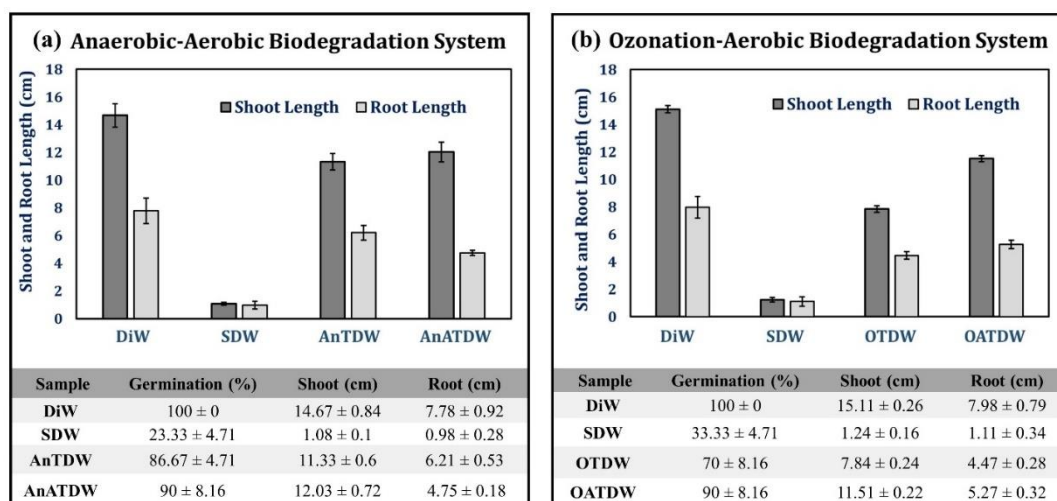| Sample	Germination (%)	Shoot (cm)	Root (cm)
DiW	100 ± 0	15.11 ± 0.26	7.98 ± 0.79
SDW	33.33 ± 4.71	1.24 ± 0.16	1.11 ± 0.34
OTDW	70 ± 8.16	7.84 ± 0.24	4.47 ± 0.28
OATDW	90 ± 8.16	11.51 ± 0.22	5.27 ± 0.32

Figure 7.5 Comparison of the germination %, root and shoot lengths after 5 days of exposure time in the treated and untreated samples obtained from anaerobic-aerobic biodegradation system (AnAB system) and ozonation-aerobic biodegradation system (OAB system), respectively

7.4.2 Bacterial toxicity analysis

The bacterial toxicity of the untreated- and treated-SDW was assessed by measuring the spectral intensities of luminescent *P. luminescens* bacteria in different wastewater samples. The *P. luminescens* bacterial culture with unity optical density was added in the samples for toxicity measurements. 1000 μL of *P. luminescens subsp akhurstii* was added to 5000 μL of every water sample comprised of DiW (control), SDW, AnTDW, AnATDW, OTDW, and OATDW to prepare assays for bioluminescence measurements. The bioluminescence intensity of bacteria in different water samples was measured after 30 min. and 24 h for the acute and chronic exposures, as presented in Fig. 7.6.
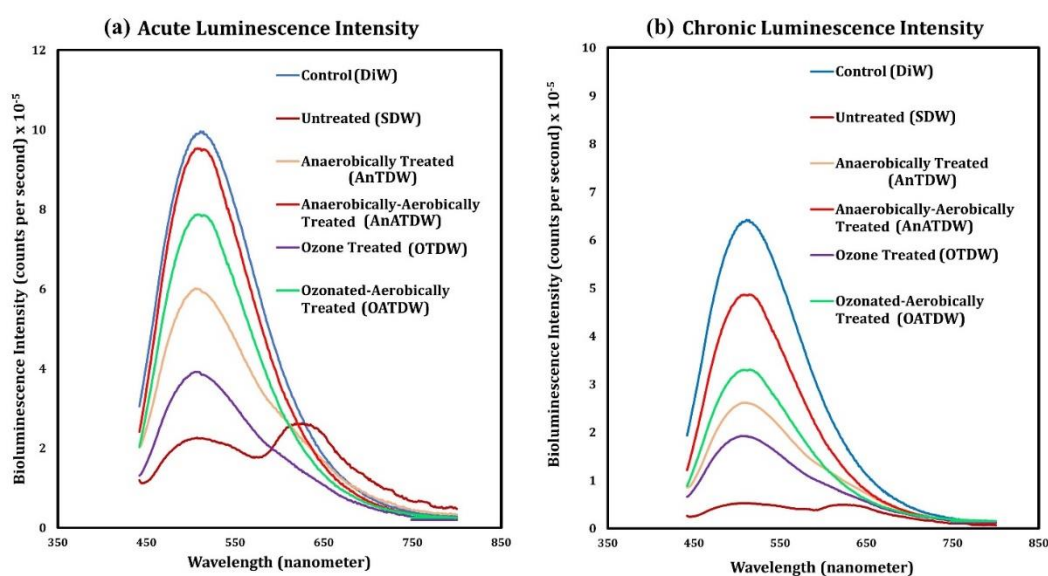

Figure 7.6 (a) Acute- and (b) chronic-bioluminescence intensities (counts per second) for the untreated (SDW), treated (AnTDW, AnATDW, OTDW and OATDW) and control (DiW) samples. The data points are a mean of three experiments ± SD based on the three independent determinations

It is apparent from Fig. 7.6 (a) that the bioluminescence intensity at 511 nm of the bacterial culture after acute exposure to untreated SDW came down to 2×10^5 counts per second compared to 10×10^5 counts per second for the control (DiW) sample. The bioluminescence intensity peak increased from $\sim 2 \times 10^5$ to 6×10^5 and $\sim 4 \times 10^5$ counts per

second for the AnTDW and OTDW samples, respectively. The subsequent aerobic treatment in the FBBR improved bioluminescence intensity for the sample AnATDW and OATDW to ~9.7×10^5 and ~8×10^5 count per second. It indicated that the acute toxicity level of the AnATDW reduced significantly and became almost comparable to that of the control distilled water and is much lower than the OATDW (as can be seen in Fig. 7.6 (a)). This is attributed to the enhanced degree of mineralization obtained in the subsequent aerobic treatment of toxic byproducts (such as aromatic amines) generated during anaerobic treatment of the SDW (Jayapal et al. 2018). However, the AnATDW offered chronic toxicity (24 h exposure) lower than the OATDW, as depicted in Fig. 7.6 (b).

The % bioluminescence inhibition, indicative of bacterial mortality, of *P. luminescens* during their acute and chronic exposure to the untreated and treated dyeing-water samples was determined at wavelength 511nm (using Eq. 3.2) are presented in Fig. 7.7. The untreated SDW offered the bioluminescence inhibition of *P. luminescens* as ~80% and ~90% during the acute and chronic toxicities. The dyeing water treated with the stand-alone anaerobic degradation in an FBBR offered an improved bioluminescence inhibition of 40.11 %, which was better than OTDW, i.e., single ozonation (69.32%) after acute exposure to P. luminescens. The bioluminescence inhibition was further reduced down to only 4.51 % when anaerobic-aerobically treated dyeing water, but with the OAB system, the inhibition was reduced to 21.64 %. After a prolonged toxic exposure of *P. luminescens* with the AnTDW, AnATDW, OTDW, and OATDW samples, the bioluminescence inhibitions were 59.03%, 24.43%, 67.75%, and 44.45%, respectively.
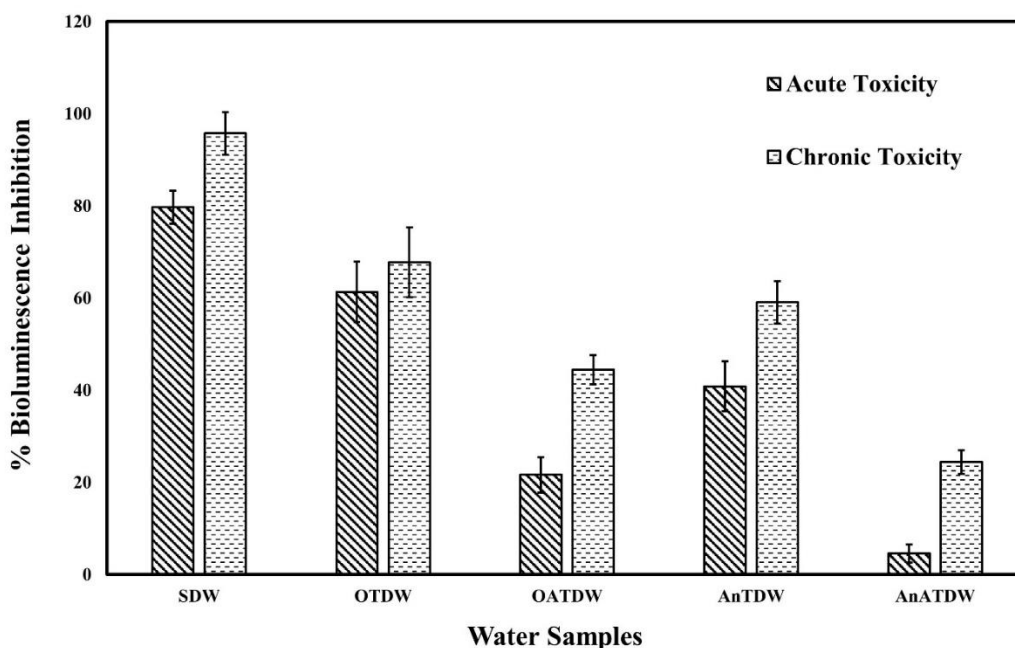
Figure 7.7 Bioluminescence inhibition of *P. luminescens* during their acute and chronic exposure to the treated (AnTDW, AnATDW, OTDW, and OATDW), untreated (SDW) dyeing water-samples with respect to the control distilled-water sample (DiW). Values are mean of three experiments for % bioluminescence inhibition ± standard error

The toxicity assessment study of the treated wastewaters against *P. luminescens* bacterial cultures and *Vigna Radiata* seeds showed significant improvement when the dyeing water was treated under the hybrid anaerobic-aerobic environments in an FBBR system. Both methods (bioluminescence and phytotoxicity) of the toxicity assessment showed that the anaerobic system standalone could only partially reduce the toxicity, further enhanced by a follow-up aerobic treatment. However, the bioluminescence analysis could reveal differences in the toxicity levels of the dyeing water samples even when the difference between their phytotoxicity levels was small or negligible. For instance, the toxicity of the AnTDW and AnATDW samples was better differentiated in the bioluminescence analysis than the phytotoxicity study. Therefore, the bioluminescence bacterial assay can be used for more quantitative and precise toxicity assessment than the plant-growth-based studies. Finally, this study recommended that the toxicity of the treated industrial wastewaters should not be

concluded based only on a single plant- or bio-assay; instead, both bio-markers should be assessed, and the bioluminescence inhibition can offer important information on the toxicity of the treated wastewater.

7.5 Conclusion

The FBBR was operated in the anaerobic and aerobic environments for the bioremediation of SDW, and the performance of the FBBR was evaluated at varied OLR ranging from 1.37-2.23 kg COD/m^3.d (equivalent to 200-800 ppm inlet dye concentration). The color removal efficiency of the anaerobic treatment was very high (from 92.37 to 97.84 %) within 24 hrs of treatment for the entire range of OLR used in this study. However, the stand-alone ozonation stage resulted in the removal of more than 98 % of the color (200 mg/l to 800 mg/l) in only 24-56 min. However, the COD and TOC removal in the anaerobic environment was limited to 23.85-53.41 % and 18.36-41.58 %, respectively, owing to the generation of aromatic amines. The anaerobic-aerobic hybrid treatment was used to enhance the COD and TOC removal to 65.77–93.50 % and 46.26–56.49 %, respectively, over the used range of OLRs, which is better than the ozonation-aerobic biodegradation stage. Based on Vigna radiata seeds, the phytotoxicity study exhibited 86.67 % and 70 % germination for AnTDW and OTDW, respectively, and 90 % germination for both AnATDW and OATDW in comparison to 23.33 % germination in the untreated SDW. It suggested that the toxicity of the treated water samples reduced significantly than the same to the untreated SDW; however, the AnTDW, AnATDW, and OATDW samples appeared to be offering comparable toxicity.

On the other hand, the bacterial toxicity study of the same dyeing wastewaters based on luminescent *P. luminescens* revealed a much higher bioluminescence inhibition of 40.11 % AnTDW sample and 21.64 % with the OATDW sample than that of only 4.51 % with the AnATDW after acute exposure. It implied that the toxicities of AnTDW, AnATDW, and OATDW samples were well-differentiated in the bioluminescence analysis even when the difference between their phytotoxicity levels was not significant. Therefore, the

bioluminescence bacterial inhibition analysis can offer an enhanced quantitative assessment of the toxicity in the treated water samples. After a satisfactory toxicity assessment, the treated wastewater can be reused for various purposes such as irrigation, sanitation, housekeeping, and other urban, industrial, environmental and recreational usages.