Waste management is one of the most serious concerns faced worldwide today. The management of wastewater released from the various industries contains hazardous organic pollutants (as classified by USEPA). It causes various adverse effects on the human and the environment. Therefore, it is imperative to develop efficient, economical and sustainable technique for the degradation of these hazardous organic pollutants. In this regard, bioremediation and photo-catalytic degradation have been proposed as the most favorable and sustainable techniques. Bibliometric analysis has shown that research communities are increasingly focusing on these two techniques of organic pollutant degradation from wastewater. Bioremediation practice has proved to be a huge success in the laboratory and also in many cases, in the natural environment. Most of the studies done on bioremediation of petrochemical waste employed bacteria for the degradation purpose owing to its very fast growth and easy adaptation in changing environmental conditions. Therefore, environmental microbiologists and biotechnologists have the challenging objective of solving these problems using microorganisms in bioremediation technologies. Biological methods have low operating costs and involve direct degradation of organic pollutants, without release of the toxic intermediates. However, the selection of microorganisms is critical factors in efficient degradation of petrochemical waste. To overcome the drawbacks of bioremediation, photo-catalysis can be considered as one of the most promising techniques. It is a viable alternative for efficient degradation of monocyclic and polycyclic petrochemical wastes. Literature survey revealed that the emerging trends in the photodegradation of organic pollutants among all used semiconductors TiO_2 is widely reported in the degradation processes along with other semiconductors/ nano-materials in visible and UV light irradiation. This advance oxidation processes

(AOPs) is evolving techniques for efficient sequestration of chemically stable and less biodegradable organic pollutants. However, there is need to develop more effective method, which consumes less energy and more efficient in pilot scale for the mineralization of pollutant. Photocatalytic processes are a viable alternative for efficient degradation of monocyclic and polycyclic petrochemical wastes and solve the problem of transformation from one form to another. The uses of alternative light sources such as LEDs are promising for reduction in power consumption. There is need to develop more efficient techniques in which solar energy are used for photocatalysis process. Presently only 5% of solar radiation are used for catalysis processes. However, there are various drawbacks associated with photocatalytic degradation such as high energy consumption and capital cost associated with photodegradation process. Toxic intermediates produced during photo-catalytic degradation are more toxic than the pollutants to various environments. It limits the wider adaptability of photo-degradation pathways.

The shortcomings in the present condition of the abovementioned techniques lead to their limited application. It includes low efficiency, high running cost, skilled man-power requirement, microbial specificity to pollutants and sensitivity to various environmental factor, which have limited their wider adaptability.

Therefore, efforts are being done for the technological development and in adopting various improved biological and photo-catalytic methods in the wastewater management. Therefore, in the present study, we evaluated the and photo-catalytic degradation processes for the efficient wastewater management technique. For biodegradation processes, various microbes were isolated from the contaminated sites

and used in the batch reactor for the degradation study of benzene and toluene. However, photo-catalytic degradation study was done by synthesis and characterization of Activated carbon based TiO_2 composite for degradation two dye compounds.

In the present study deals with biodegradation aspect of organic pollutants, in which various microbes has been isolated for the degradation of toluene and benzene from sites contaminated from petrochemical waste. These compounds are the priority pollutants, which adversely affects the human health and the environment. Among the isolated microbes, potential microbes were selected for biodegradation of toluene and benzene. The culture labeled as CH005 was very much close to *Acinetobacter junii* isolate OTU-a6 (GenBank Accession Number: KJ147060.1) based on nucleotide homology and phylogenetic analysis. Culture labelled as CH007 was identified as *Serratia marcescens* strain 35 dr (GenBank Accession Number: KJ729606.1) while CH010 was identified as *Klebsiella pneumoniae* strain GX120222 (GenBank Accession Number: KP091888.1). List of some other organisms along with their accession numbers, that have been used for degradation of toluene and benzene are included in chapter 4.

The parameters were optimized at which these microbial strains were showing maximum biodegradation of benzene and toluene. The pH is one of the most important factor controlling the growth and enzymatic activities of the microorganisms. In our study, pH 4.5 to 9.5 was selected to observe the toluene degradation efficiency of selected bacterial strains. We observed an enhancement in the percent degradation by mixed culture with an increase in pH from 6.5 to 7.5.

However, a reduction in degradation activity was observed beyond pH 7.5 due to lowered enzymatic activity. At pH 4.0, 6.5, 7.5, 8.5 and 9.5 toluene degradation by mixed culture was 12%, 48%, 52%, 46% and 30%, respectively. In the present study, the bacterium demonstrated the highest biodegradation at pH 7.5. Thus, optimum pH 7.5 was selected for further degradation studies.

Majority of the studies on toluene biodegradation have been performed with anaerobic microorganisms. In the present study, we evaluated the toluene degradation ability under aerobic conditions. With an increase in time gap, concomitant increase in percent degradation by both single and mixed culture was observed. Pure culture of *Acinetobacter junii* was able to degrade 69, 73 and 80% of 150, 100, and 50 ppm of toluene concentrations, respectively within 72 hours. The pure culture of *Serratia marcescens* was able to degrade 74, 77, and 82% of 150, 100, and 50 ppm of toluene concentration within 72 hours. However, *Klebsiella pneuminae* degraded 65, 79, and 89% of 150, 100, and 50 ppm of toluene concentration, respectively within 72 hours. It indicates that *Serratia marcescens* was the most effective amongst all the tested pure cultures, as they were able to degrade 74% of 150 ppm toluene. Moreover, we observed that 80% of toluene was degraded within 72 hours by the mixed bacterial culture. This might be attributed to the broad enzymatic capability of the consortium, which enhances the rate of degradation. Toluene degradation with time in the present study shows that the degradation occurs due to the enzymatic activities resulting from bacterial growth. The percent removal was very low up to 72 hours, showing saturation of enzymes involved in degradation. However, we selected 150 ppm benzene concentration for biodegradation and evaluated the degradation performance of mixed bacterial culture under optimum conditions. Benzene degradation by mixed

culture in mineral salt media (MSM) was determined to be 63% within 72 hours of incubation. It reached to saturation stage after 60 hours of incubation period. No further degradation was observed up to 72 hours, probably due to cellular enzyme saturation. Under optimum laboratory conditions, mixed culture of *Acinetobacter junii*, *Serratia marcescens* and *Klebsiella pneumoniae* has proved to be the most efficient bacterial strins in biodegradation in the present study. The present study will be beneficial in the industrial level treatment using biological treatment technologies

We observed the degradation intermediates produced by use of these bacterial strains. Toluene degradation by *Acinetobacter junii* isolate CH005 resulted in the formation of intermediate compounds such as 1-Isopropenyl-4-methyl-1,3-cyclohexadiene,1,3-Cyclohexadiene, 2-Methyl-5-(1-Methylethyl), 4-Methoxycarbonyl-4-butanolide Vinyl (2E,4E)-2,4-hexadienoate. However, benzene degradation by mixed bacterial culture resulted into formation of different compounds. The compounds produced were Phenol, 2,4, bis (1,1 dimethyl ethyl). Benzene acetaldehyde, alpha methyl (Trigueros, 2010) and Gamma butyrolactone These compounds are of general occurrence after aerobic bacterial degradation.

We also performed the morphological studies in our study to observe the effect of organic pollutant and their degradation intermediates on the bacterial strains. Surface morphology as revealed by SEM of the untreated and toluene treated *Acinetobacter junii*, *Klebsiella pneumonia*, *Serratia marcescens* and mixed culture showed that, all bacterial cells are cylindrical. However, some long cylindrical cells were transformed into ovoid and spherical structure after treatment, probably to escape themselves from toxicity. Bacterial systems have developed several types of

changes at the biochemical and physiological level which makes them suitable to survive under stressed conditions as observed in presence of organic compounds including benzene, toluene, ethylbenzene and xylene. The protective mechanisms also include changes in characteristics of cell membrane, alteration in cell shape, size, formation of vesicular structure on membrane and protein associated with stress tolerance, efflux pumps and energy pool maintenance.

In the present studies, the toluene and benzene biodegradation by bacteria *Acinetobacter junii*, *Serratia marcescens* and *Klebsiella pneumoniae* was best at pH 7.5 and at a temperature of 37°C. Temperature ranging from 30 to 40 °C has been used demonstrate the rate of hydrocarbon degradation. The bacterial species showed varying degree of ability in toluene degradation. Furthermore, mixed bacterial culture was found most suited for the degradation purpose with highest efficiency of degradation. Moreover, it is necessary to support the activities of these indigenous microorganisms by bioaugmentation and biostimulation in the polluted biotopes to enhance their degradation abilities.

The present study seeks to offer a scientific and technical overview of the current trend in the use of the photocatalyst for remediation and degradation of petrochemical waste as reported in the recent studies. The effect of various heterogeneous catalysts and their ecotoxicity has been briefly outlined. Also, the use of various photocatalysts for the degradation of petrochemical waste other than TiO_2 has been also azalysized. We performed the photocatalytic degradation of organic dye in self fabricated photo rector. Three reactors were used during this experiment. A photochemical reactor which consisted of a cylindrical reactor fitted in with 8 Phillips

UV lamps of intensity 960 LUX along with a magnetic stirrer shows schematic view of the photochemical reactor. For carrying out sonication reaction, a reactor fitted with a Sonicator (Hielsher Ultrasound Technology) was used. Figure 3.1 shows a schematic representation of a reactor consisting of a combined photo- and Sono-catalytic reaction. All these reactors were provided with cooling jackets with water flowing inside them so as to avoid overheating.

Various solutions consisting of different concentrations of dye, along TiO_2/AC catalyst was used for the photo-catalytic degradation experiment. The experiments were conducted in the three batch reactors as shown earlier. The samples were taken out from the batch reactor at fixed time interval and their concentration was measured. This data was then used for further calculations. In present study, degradation analysis of Direct Blue-199 and Acid Red-131 dyes was done following various photo-catalytic pathways. A catalyst, TiO_2 loaded on Activated charcoal, was prepared in the process and its characterizations was done, which showed an even distribution of TiO_2 (rutile phase) on the catalyst surface. The catalyst calcinations temperature was also optimized, which was found to be $\approx$ 350 ^{o}C. A first order reaction kinetics was then developed for the catalytic oxidation of dye using L-H model. The degradation of DB-199, via photocatalytic, sonocatalytic and sono-photocatalytic reactions, under optimized working conditions followed L-H model satisfactorily. These three reactors used for the degradation of dye were tested on the basis of degradation rates and energy consumption. Photo-catalytic reactor was found to be the best option amongst all the reactors used. The contribution of intermediates formed during the reaction is not considered in the kinetics study; however, there have been reports about the competitive involvement of intermediate products during the photochemical process.

The role of intermediates can be involved further for the development of reaction kinetics model. After optimization of the reactor conditions and efficacy, degradation reactions of dye (AR-131) were done in the photochemical reactor. The degradation was found to follow a first order kinetics mechanism (vis. Langmuir-Hinshelwood Model).

The release of these nano-materials into the environment has been reported to affect the plant growth mechanisms and development from the seeds germination to pollination. The understanding of the degree at which spent nano-particles affects seed germination and plant development is also an important issue. Therefore, we observed the effect of unused catalysts on environment after the photochemical degradation of dye. It was observed through the impact of these unspent catalysts on the seed germination of various plant species. The response of species to various composite nano-materials depends on the concentration, nature, and size of nanoparticles, which could have economic significance for agriculture. Various studies reported that the intermediates or end products formed in the photo-catalytic degradation process are sometimes more toxic than the original compounds. However, various studies have also supported that it increases the production of various crops, and improves the essential element content in plant tissue by increasing peroxidase, catalyse and nitrate reductase activity in plant tissues and enhancing their chlorophyll. Therefore, besides the advancement in the materials as the catalysts for degradation of persistent organic pollutant from water and air, their eco-toxicity should also be considered. Therefore, understanding of the mechanisms of interaction of nano-materials with the plants species is required.

In the present study, AC/TiO_2 nano-composite had positive impacts on the germination of *V. radiata* and *Solanum lycopersicon* seeds. It can be attributed to the fact that TiO_2 was able to penetrate the seed husks of these seeds. It might be attributed to the penetration of AC/TiO_2 nano-composite into seeds, which might break the husks to facilitate the water uptake, which resulted in the rapid seed germination and higher percentage of germination rates. Moreover, at the stage of seedling growth, activated carbon may also be providing the moisture. Compared to the control, enhanced germination was found at increased concentration, however, the growth of root and shoot of seedling either decreased or remained stable. It was observed that stems and roots of the seedlings were longer than those of the control with higher concentration of treatment. Carbon nano-materials are known for their abilities to enhance the seedling growth and development. Effect of nanomaterials on the plant growth has been found to depend upon the type of nano-materials, size-specific area, functional groups, concentration, plant species, soil type and condition. AC/TiO_2 nano-composite enhanced the shoot and root ratio depending on concentration. It envisaged that the increase in root and shoot ratio may be related to the concentration of catalysts; however, elaborative physiological studies are needed for the better understanding of this mechanisms. These results would help in mechanistic understanding of the interaction of nano-materials with plants species in the environment.

With majority of treatment techniques having major drawback of generation of another type of wastes, biodegradation and photocatalytic mineralization looks a very promising techniques. Overall, I strongly believe that there is a need to scale up this degradation technique from lab to land scale. For industrial wastewater treatment, there is a need to develop hybrid processes of photo-degradation followed by biodegradation.

The hybrid technique may be sustainable in all respect (economic as well as environmental). There is a possibility to develop hybrid techniques based on these two existing processes for more efficient degradation of organic pollutants

Recommendation:

We can make following recommendation based on the present study.

1. The need of the hour is to improve our understanding of the molecular reactions which form the basis of bioremediation.
2. Development of hybrid pathways through genetic manipulation of microorganisms is one of the promising techniques which would drastically improve the process of bioremediation.
3. Photocatalytic degradation needs more research to make these processes more efficient. Particularly, There is need to develop more efficient techniques in which solar energy are used for photocatalysis process. Presently only 5% of solar radiation are used for catalysis processes.
4. Another unexplored areas of the degradation of organic pollutant is to devise and promote hybrid processes (based on both the bioremediations and photocatalytic degradation routes) of two above-mentioned techniques. Therefore, combined processes can be the most promising technology for the remediation of environmental pollutant in future.
5. There is need of more research to understand the effect of nanoparticles on soil microbiology and plant growth.

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