Original Research Article

Nanoparticles coupled with microorganisms mediated photocatalytic mineralization, detoxification and disinfection of phenolic waste water

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Abstract

Solarphotocatalysis is an important aspect of AOPs for the treatment of organic pollutants in industrial wastewater. The photocatalytic activity of Zinc Oxide nanoparticles also prove to be an efficient method for degradation of organic compounds and also have their lethal activity on microbial species. This study aimed at mineralization of phenol in wastewater, its disinfection and detoxification using the photocatalytic activity of ZnO andthe biodegrading ability of phenol degrading organisms. This had been successful in decreasing treatment time, cost effective, environment friendly and increased efficiency. The ZnO nanoparticles were synthesized chemically and characterized by UV-VIS spectroscopy and FTIR. Phenol degrading organisms were isolated and screened from soil sample. The coupled action of synthesized ZnO nanoparticles and phenol degrading microorganisms was studied under solar radiations for five consecutive days. The concentration of phenol analysed was degraded upto 93.4%. The COD and BOD were reduced upto 90.84% and 81.15% respectively. The reduction in TOC from 585 to 137mg/L represents mineralization of phenol and the by-products formed during its degradation. Pot culture and seed germination bioassays showed the reduction in toxicity of phenol after treatment by 60%. Reduction in TVC of coliforms during treatment confirmed lethal effect of ZnO and solar radiations.After treatment the photocatalyst was recovered, regenerated and reused effectively showing no significant reduction in photocatalytic activity and photodegradation of phenol.

Key Words: Phenolic wastewater, Photocatalysis, AOP, ZnO nanoparticles, Coupled Microbial degradation, Detoxification, Disinfection.

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INTRODUCTION

Phenolic compounds are released by many industries like petroleum, coal conversion, pesticide, paper and pulp industries. These compounds are toxic to microorganisms, animals and humans; and are classified as hazardous pollutants.[1] Phenol is a preceding pollutant included in the list of EPA (Environmental Protection Agency).[2,3] Chlorophenolic compounds are generated because of the chlorination of phenol containing water which leads to the protest and stench by consumers.[3] Therefore in the upcoming future Advanced Oxidation Processes (AOPs) may become the most necessary part of water treatment technologies for the pollutants which cannot be treated by conventional technologies because of their low biodegradability and /or high chemical stability.[4] Several reviews on treatment of wastewater by using AOPs are focusing on conventional methods like solar radiations (300nm wavelength), Heterogenous catalysis with UV/TiO_2 and Photo-Fenton. Fenton, which is H_2O_2 added to Fe^{2+} salts is combined with UV-VIS

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light to give Photo-Fenton reaction.[4] There are many oxidation processes using TiO_2, ZnO, Fe_2O_3, CdS, ZnS etc. like semiconductors. Under solar radiations ZnO has emerged to be more photocatalytically active than TiO_2 as it has high surface reactivity and large number of active sites[5] Inphotocatalytic process, the semiconductor photocatalyst has the ability to generate surface bound hydroxyl radicals that absorbs radiation according to its band gap when in contact with water and also generates pairs of valence-band holes.[6] Processes like UV-radiation, chlorination, ozone and membrane filtration are well known for disinfection, but have many advantages and disadvantages. Because of its high disinfection rate, residual effect and low cost chlorination is the most widely used disinfection process. However it may present a health risk due to NOMs (Natural Organic Matter) and DBPs (Disinfection by-products) generated. Ozonation and irradiation by using germicidal lamps (245nm) also pose limitations and problems. Therefore, to develop an alternative, the interest of researchers is growing for effective and economic disinfection techniques.[4] Solar Water Disinfection (SODIS) is an environmentally sustainable, simple and economically efficient solution for the treatment of drinking water which destroys pathogenic microorganisms causing water borne diseases and improves the quality of drinking water.[7] Photocataysis, is one of the disinfection technology in which the powder of photoexcited semiconductor is used which is lethal to microorganisms and this was first reported by Matsunaga and his colleagues in 1985.[4,8] The use of ZnOphotocatalysis for the inactivation of Gram positive *Bacillus subtilis* and Gram negative organisms *Escherichia coli* also has been reported.[9,10] The conventional methods of phenol degradation have certain limitations and therefore microbial catalysts in biodegradation of phenol is being preferred due to its potential to degrade phenol more efficiently and is more economical.[11] Complete mineralization of the organic pollutants by using hydroxyl radicals is always non-profitable. Therefore by coupling biotreatment and solar photcatalysis has emerged as a new technology. This approach is successful in reducing the plant size and increasing overall process efficiency.[12]

MATERIALS AND METHODS

Isolation and screening of phenol degrading organisms: Soil sample near the coke processing unit (Ambad, Nashik, India) was collected and suspended in minimal medium supplemented with phenol (50mg/l). The composition of minimal medium (gms/L) was KH_2PO_4-2.00, K_2HPO_4-7.00, $(NH_4)_2SO_4$-1.00, $MgSO_4,7H_2O$-0.10 and Sodium citrate-0.50. Eight bacterial strains (PD1, PD2, PD3, PD4, PD5, PD6, PD7, PD8) found to give good growth were then subcultured on minimal media slants containing 100mg/l of phenol concentration. The organisms which showed maximum growth in relatively short period of time were then characterized by studying morphological characters, Gram staining, motility and biochemical tests and further the species were confirmed by VITEC system and were used for further experiments.

Photocatalyst preparation and characterization: Zinc oxide nanoparticles were synthesized by chemical method using zinc chloride and sodium hydroxide and characterized by UV-VIS Spectroscopy and FTIR.[13]

Photocatalytic experiments: Experiment for phenol degradation was set up for comparative study of individual ZnOphotocatalysis, phenol degrading organisms and by coupledphotocatalysis and biodegradation, in waste water sample of 100mg/l phenol concentration. Photocatalysis was studied under solar radiations for 6 hours between 10am to 4pm and stirred continuously. After every 24 hours samples were analyzed for residual phenol concentration.

Analytical methods

Phenol Degradation and Mineralization: Phenol degradation in waste water was estimated by the decrease in organic carbon by measuring the COD (Chemical Oxygen Demand). Oxidant used was $K_2Cr_2O_7$ in combination with boiling H_2SO_4. Biodegradability of phenolic wastewater sample was evaluated by BOD (Biological Oxygen Demand) and TOC (Total Organic Carbon).[12,14] Phenol degradation was also estimated by Folin Lowry method. The maximum absorption was measured in the region of 660nm wavelength.[15]

Disinfection: Total Viable Count(TVC) was measured to estimate the number of coliforms present in the phenolic wastewater sample, to determine the extent of disinfection of sample after the treatment of individual ZnOphotocatalysis,[7,9] phenol degrading organisms and by coupledphotocatalysis and biodegradation. EMB agar was used as a selective and differential medium for the cultivation of coliform organisms.

Detoxification: Toxicity of treated phenolic waste water sample was measured by biological assays which determined the quality of waste water. Pot culture and seed germination of maize seeds were performed to measure the potential biological impact of treated waste water. The average root and shoot length of the crop plants were determined after a specific period of time.[16]

Reusability of photocatalyst: The reuse of photocatalyst was studied in order to establish the stability. Irradiation time, pH, phenol concentration and amount of photocatalyst like parameters were kept constant. The waste water sample was filtered by bacteriological membrane filter (pore size 0.4nm) in order to recover the photocatalyst from it. To regenerate, the filtered photocatalyst was washed thrice with distilled water, dried at 300^0 C and reused five times in the same process as the previous experiment.[6]

RESULTS AND DISCUSSION

Isolation and screening of phenol degrading organisms: Out of the initial eight isolates, two organisms were finally screened based on their maximum growth after subculturing on minimal media having phenol concentration 100mg/l. The biochemical tests of the given isolates where both of the isolates were found to be Gram negative, non capsulated, catalase positive and both utilize glucose as a carbon source. The VITEC identification for the isolates are shown in Table 1 and Table 2 and confirmed as *Bacillus pumilus*and *Pseudomonas aeroginosa.*

Table 1: Identification of PD3 organism by VITEC system

Identification information	Card: BCL	Lot Number: 239213510	Expires: Oct 18, 2017 12.00
Completed: Mar 24, 2017 03:01 IST	Status: Final	Analysis Time: 14.25 hours	
Selected Organism	94 % Probability ***Bacillus pumilus***	Bionumber: 1253105614566260	Confidence: Very good identification

Contraindicating Typical Biopattern (s)

Bacillus pumilus BNAG(92), LeuA(79), APPA(17), BMAN(22).

Biochemicaldetails:

1	**BXYL**	**+**	**3**	**LysA**	**-**	**4**	**AspA**	**(-)**	**5**	**LeuA**	**-**	**7**	**PheA**	**+**	**8**	**ProA**	**-**
9	BGAL	+	10	PyrA	-	11	AGAL	+	12	AlaA	+	13	TyrA	+	14	BNAG	-
15	APPA	+	18	CDEX	-	19	dGAL	-	21	GLYG	-	22	INO	-	24	MdG	(-)
25	ELLM	+	26	MdX	-	27	AMAN	+	29	MTE	-	30	GlyA	(+)	31	dMAN	+
32	dMNE	+	34	dMLZ	-	36	NAG	-	37	PLE	-	39	IRHA	-	41	BGLU	+
43	BMAN	+	44	PHC	-	45	PVATE	+	46	AGLU	-	47	dTAG	+	48	dTRE	+
50	INU	-	53	dGLU	+	54	dRIB	+	55	PSCNa	-	58	NaCl 6.5%	+	59	KAN	-
60	OLD	-	61	ESC	+	62	TTZ	+	63	POLYB_R	-						

Installed VITEK 2 System Version: 05:02

MIC Interpretation guideline: Therapeutic Interpretation guideline:

AES Parameter Set Name: AES Parameter Last Modified:

Table 2: Identification of PD7 organism by VITEC system.

Identification Information	**Card:** GN	**Lot Number:**241272040	**Expires:** June 8, 2016,12:00 IST
	Completed: Jun 8, 2016 18:53, IST	**Status:** Final	**Analysis Time:** 5.00 hours
Selected Organism	99% Probability ***Pseudomonas aeroginosa*** **Bionumber:** 0043053103500242 **Confidence:** Excellent identification		

Biochemical Details:

2	APPA	-	3	ADO	-	4	PyrA	-	5	IARL	-	7	dCEL	-	9	BGAL	-
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	-
17	BGLU	-	18	dMAL	-	19	dMAN	-	20	dMNE	+	21	BXYL	-	22	BAlap	+
23	ProA	+	26	LIP	+	27	PLE	-	29	TyrA	+	31	URE	-	32	dSOR	-
33	SAC	-	34	dTAG	-	35	dTRE	-	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	-	45	PHOS	-
46	GlyA	-	47	ODC	-	48	LDC	-	53	IHISa	-	56	CMT	+	57	BGUR	-
58	O129R	-	59	GGAA	-	61	IMLTa	-	62	ELLM	-	64	ILATa	+			

Installed VITEK 2 Systems Version: 06.01 Therapeutic Interpretation Guideline:

MIC Interpretation Guideline: AES Parameter Last Modified:

Photocatalyst preparation and characterization: ZnO nanoparticles were successfully synthesized and confirmed by UV-VIS spectroscopy and FTIR. In figure 1, the strong UV emission peak at about 380nm is the characteristic emission of ZnO.

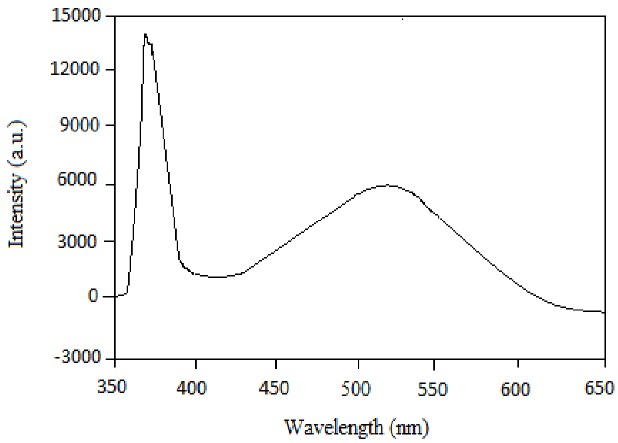

Figure 1: UV-VIS spectra of ZnO nanoparticles synthesized by $ZnCl_2$.

In the FTIR spectrum, the peak observed at ~370 cm^{-1} corresponds to the ZnO stretching vibrations. The water molecules present in the sample represent the peak in the range of 3000-3500cm^{-1}. The two peaks at 1369.46cm^{-1} and 1558.48cm^{-1} represent symmetric and asymmetric carboxylate bond vibrations respectively.

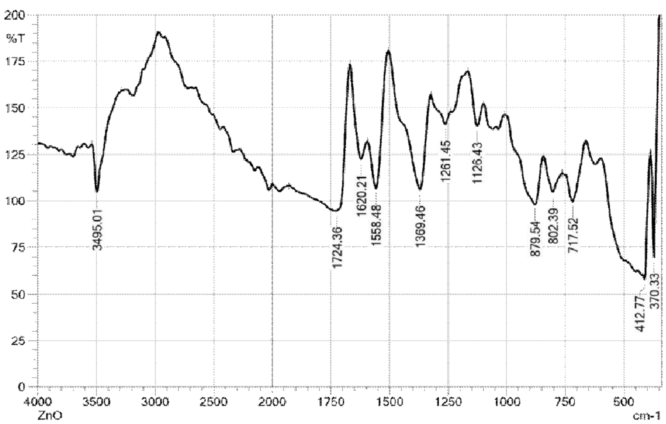

Figure 2: FTIR spectra of Zinc oxide nanoparticles.

Analytical methods Phenol Degradation and Mineralization: Wastewater after treatment can be discharged into water resource if it does not pose any harm to the water body or the ecosystem. The residual organic matter should be in the range that can be naturally biodegraded. Therefore, the standard of treatment prior to disposal of water is of great importance. In the present work, the amount of organic matter in phenolic waste water before and after stages of treatment was determined by BOD, COD and TOC. The phenol concentration in corresponding treatment stages was alsomeasured by Folin-Lowry method. The stages of bioconversion of phenol in waste water was studied by treating the sample by ZnOphotocatalysis, phenol degrading organisms and combined biotreatment. Table 3 shows that there was gradual phenol degradation in all three samples. There was considerable decrease in COD and TOC with increase in treatment time. The BOD had increased to a certain extent when sample was treated with ZnOphotocatalysis as the non-biodegradable matter is converted to biodegradable organic matter. In the coupled treatment photocatalysis was enough to achieve biodegradability of biologically recalcitrant phenolic compounds that could be further mineralized biologically. Thus, there was considerable decrease in TOC, eventually mineralization.

Table 3: Various parameter tests before and after treatment of phenolic wastewater.

	Before Initial sample	After ZnO	Treatment Organisms	Treatment Coupling
COD (mg/L)	980	440	313	89.7
COD % reduction	0	55.10	68.06	90.84
BOD (mg/L)	113	146	68.5	21.3
BOD % reduction	0	-29.2	39.38	81.15
Phenol Concentration	97	29	17.2	06.4
Phenol % reduction	0	70.10	82.26	93.4
TOC (mg/L)	585	468	315	137
TOC % reduction	0	20	46.15	76.58

Disinfection: The phenolic waste water sample was treated for 3 consecutive days with zinc oxide nanoparticles, isolated organisms and coupled treatment. The results showed a significant decrease in the number of coliforms in the sample treated by ZnOphotocatalysis. From the table 4, after 72hrs of treatment with ZnO, the percent reduction in coliform count was 99.73%, as ZnO and photolysis both are lethal to organisms. Treating wastewater with phenol degrading organisms, there was negligible reduction in coliform count. This may be due to the presence of toxic phenol and non-availability of nutrients to them. The treatment with coupled photocatalysis and microbial degradation showed a less reduction in TVC of 88%. This showed the efficiency of ZnOphotocatalysis in disinfection of wastewater.

Table 4: Total viable counts of coliforms after the treatment withZnO solar photocatalysis, Biodegradation and coupledZnO solar photocatalysis at various time intervals

	TVC Count of Coliforms		
Time	ZnO	Organisms	Coupled
0 hrs	5.2×10^4	5.8×10^4	5.6×10^4
24 hrs	2.5×10^3	5.2×10^4	4.2×10^4
48 hrs	3.8×10^2	4.4×10^4	8.9×10^3
72 hrs	140	3.6×10^4	6.7×10^2

Detoxification: Detoxification of phenol from wastewater sample after degradation and mineralization was analyzed by bioassays of seed germination and pot culture.

Seed germination: The germination test revealed that phenolic waste water sample was 100% toxic for seeds. When water samples treated with zinc oxide nanoparticles coupled with microbial degradation were provide to seeds, it showed 60% germinationi.e, 6 seeds out of 10 were germinated, as compared to control treated with distilled water.

Figure 3: Seed germination of pretreated Maize seeds treated with phenolic water (Test), Treated phenolic water (Treated) and Distilled water (Control)

Pot culture: From table 5, the average root and shoot length of crop plants in control was 14.61cm and 21.8cm respectively. The seeds sown supplied with phenolic waste water showed no germination and no growth. The treated water with Zinc oxide nanoparticles and microbial coupling, when supplied to crops, 5 seeds out of 8 germinated and the crops showed average root and shoot length of 8.2cm and 21.9cm respectively, conforming the partial detoxification of treated sample.

Control (Distilled water) Treated water sample

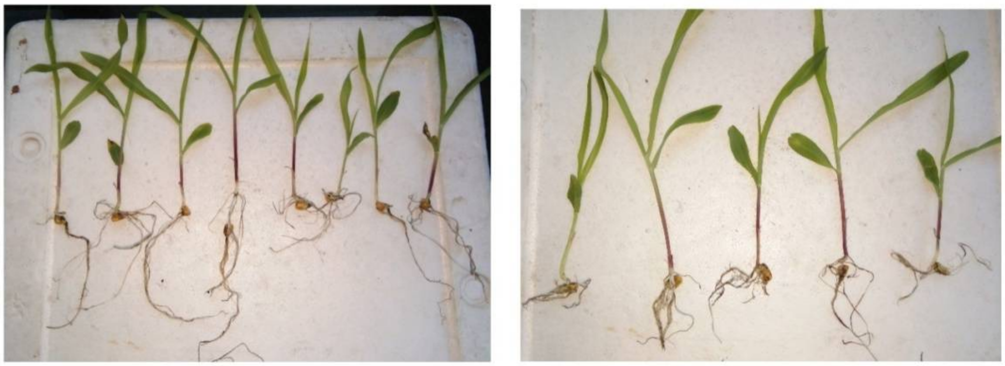

Figure 4: Observations for root-shoot length of plants treated with Distilled water (Control) and Treated phenolic wastewater

Table 5: Root-Shoot measurement of crop plants during pot culture

Root length (cm)	Shoot length (cm)	Root length (cm)	Shoot length (cm)
13.3± 0.2	23.2±0.3	8.5±0.5	25.2±0.1
14.4± 0.6	24.4±0.5	7.2±0.4	20.3±0.4
15.6± 0.4	21.1±0.4	9.3±0.5	19.5±0.5
13.8± 0.2	24.5±0.7	12.5±01	23.7±0.3
17.7±0.3	20.5±0.3	8.4±0.2	17.6±0.2
10.4±0.6	24.8±0.2	-	-
14.2±0.3	13.5±0.2	-	-
17.5±0.5	22.4±0.6	-	-

Recovery and reusability of photocatalyst

Photocalalystwas recovered by membrane filter technique followed by heat treatment andreused for five cycles. The results showed no significant reduction in photocatalytic performance in photodegradation of phenol, thus this indicated the stability of ZnOnanoparticles as a photocatalyst.

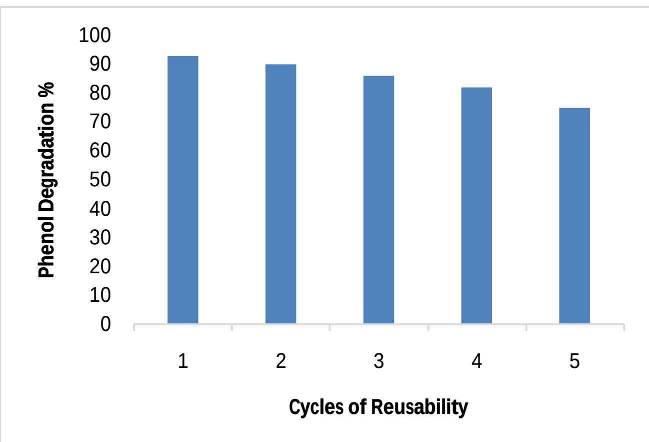

Figure 5: The efficiency of reused ZnOphotocatalysts for the degradation of phenol

CONCLUSIONS

The ZnO nanoparticles as photocatalystwere chemically synthesized and confirmed by UV-Vis Spectroscopy and FTIR. They were photocatalytically active in phenol degradation and when coupled phenol degrading organisms the treatment proved to be more efficient than individual ZnOphotocatalysis and biotreatment.Eight phenol degrading organisms were isolated from soil sample out of which two were more effective in degrading phenol. The decrease in COD and BOD represented degradation and bioconversion of phenolic compounds. Reduced TOC represented the further mineralization of phenol and by-products formed during its degradation. Detoxification of phenolic waste water due to coupling was most efficient than ZnOphotocatalysis or by using phenol degrading organisms alone. This was analyzed by pot culture and seed germination techniques. ZnOphotocatalysis proved to be more efficient in disinfection of phenolic waste water by reduction in coliform counts. Thus, under optimum conditions 100ppm of phenol was effectively photodegradedby 0.1gm of photocatalyst per 100ml of waste water. The photocatalyst after treatment was successfully recovered, regenerated and reused upto5 times without much loss in its efficiency. The photocatalytic degradation of various toxic organic compounds coupled with microbial degradation has been proposed as a viable process to treat the waste water as it has proved to increase the overall efficiency of the treatment.

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