Effect of Biofilm in Waste Water Treatment by Using Bactericidal Activity of Nitrogen Doped Metal Oxide Nanoparticles

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Abstract

Background: Biofilm of different microorganisms can stabilize harmful components from waste water. It can be effectively use for the treatment of waste water.

Objective: Development of Rotating Drum Biological Reactor (RDBR) for the treatment of waste water and to study the effect of biofilm developed on RDBR for the reduction in BOD, COD and phenol concentration in waste water. Synthesis of TiO2 nanoparticles to study antimicrobial property against developed biofilm.

Results: Synthetic waste water with initial concentration of phenol as 50 mg/lit and with COD and BOD values 2080 mg/lit and 1600 mg/lit respectively was treated in RDBR. After 60 hrs of operation phenol concentration drops to 0.0028 mg/lit and COD, BOD values were reduced to 178 mg/lit and 71.2 mg/lit respectively. The reduction of phenol concentration was upto 99.7%.

Conclusion: Based on the experimental results we can conclude that rotating drum biological reactor (RDBR) technology is one of the promising technology for the treatment of waste water. After treatment of synthetic waste water in RDBR there was drastic reduction in the initial concentrations of COD, BOD and Phenol was observed. Results show the reduction in phenol concentration upto 99.7%. It has been also found that nitrogen doped TiO2 nanoparticles shows good antimicrobial activity over the biofilm developed.

INTRODUCTION

Water is the most important constituent for all the living things. Water contamination is now a major problem in the global context as a consequence of industrialization, globalization, population growth, urbanization and warfare combined with increased wealth and more extravagant lifestyles (UN- World Water Development Report, 2006). Eutrophication of lakes and the sea is caused by discharge of nutrients originating from of human activities, industries and agriculture, which threatens the maintenance of biodiversity and human health. Suspended, colloidal or dissolved degradable organic materials, quantities and ratios depend on the nature of the wastewater characteristics of wastewater are measured in terms of chemical oxygen demand (COD), Biological oxygen demand (BOD), and volatile suspended solid (VSS).

Biological wastewater treatment is therefore of utmost importance for the wellbeing of our water bodies. Biological treatment is one of the most widely used removal methods as well as for partial or complete stabilization of biologically degradable substances in wastewater and wastes. Most biological waste and wastewater treatment processes employ bacteria as primary micro-organisms; certain other micro-organisms may play an important role. Degradation of organic matter is effected by its use as food by micro-organisms to produce protoplasm for new cells during the growth process, population dynamics of bacteria is biological treatment depends on environmental factors which include pH, temperature, type and concentration of essential nutrients (e.g. Nitrogen, Phosphorous, Sulphur etc.), essential minerals, osmotic pressure, media toxicity, by-products and degree of mixing.A biofilm can be defined as a complex coherent structure of cells and cellular products, like extra-cellular polymers (Characklis, 1990), which either form spontaneously as large, dense granules (Lettinga et al., 1980), or grow attached on a static solid surface (static biofilms) or on suspended carriers (particle supported biofilms) (Heijnen, 1984). Biological wastewater treatment is mainly carried out by prokaryotes, even if fungi, protozoa, algae and rotifers may also be represented (Bitton G, 2005). Municipal wastewater is composed of organic material, i.e. proteins, carbohydrates, fats and oils; nutrients, mainly nitrogen and phosphorus; as well as trace amounts of recalcitrant organic compounds and Metals (Bitton G, 2005). The most frequently found prokaryotes in biological wastewater treatment systems belong to the classes Alpha-, Beta- and Gamma proteobacteria, Bacteroidetes and Actinobacteria (Wagner M and Loy A, 2002).These bacteria in aquatic environments are predominantly not in a free-floating planktonic stage, but have a tendency to attach to surfaces and form multi-species communities called biofilms (Metcalf & Eddy,
The biofilm form during water treatment process can be roughly divided into 1) the fixed-medium systems, and 2) moving-medium systems (Rodgers et al., 2003). In the fixed-medium systems (fixed bed bioreactor; FBBR) the biological reactions take place in the biofilm growing on a static medium. In the moving medium systems the biofilm media are kept in continuous movement by mechanical, hydraulic or air forces. Rotating bed bioreactors (RBBR) are an application of moving bed biofilm reactors with a higher filling rate of carrier elements.

Nanoparticles in waste water treatment: The waste water treatment plant in which trickling filter, RBC, packed bed reactor are used involve formation of biofilm, which reduces the COD of treated water (Nicholas P. Cheremisinoff, 2003). More recently, several natural and engineered nanomaterials have also been shown to have strong antimicrobial properties, including chitosan (Qi et al., 2004), silver nanoparticles (nAg) (Morones et al., 2005), photocatalytic TiO2 (Cho et al., 2005; Wei et al., 1994), fullerol (Badireddy et al., 2007), aqueous fullerene nanoparticles (nC60) (Lyon et al., 2006), and carbon nanotubes (CNT) (Kang et al., 2007).

The biofilm form during water treatment process can be stabilised/ degraded by using the metal oxide nanoparticles such as TiO2, the surface of TiO2 crystal is occupied by O2 atoms with high electron density. The bactericidal effect of TiO2 is attributed to the decomposition of bacterial outer membranes by reactive oxygen species [ROS], primarily hydroxyl radicals [*OH] which leads to phospholipids peroxidation and ultimately cell death.

Nitrogen Doping Of Metal Oxide Nanoparticles: TiO2 is the most commonly used semiconductor photocatalyst. Among the different nanomaterials, it is the most studied. Activated by UV-A irradiation, its photocatalytic properties have been utilized in various environmental applications to remove contaminants from both water and air (Gelover et al., 2006; Murray et al., 2007; Salthammer and Fuhrmann, 2007). A wealth of information on TiO2 photocatalytic inactivation of bacteria has been acquired over the last 20 years (Matsunaga et al., 1985; Wei et al., 1994). TiO2 can kill both Gram-negative and Gram-positive bacteria, although Gram-positive bacteria are less sensitive due to their ability to form spores (Wei et al., 1994). More recently, nano-sized TiO2 was also reported to kill viruses including poliovirus 1 (Watts et al., 1995), hepatitis B virus (Zan et al., 2007), Herpes simplex virus (Hajkova et al., 2007), and MS2 bacteriophage (Cho et al., 2005). The concentration of TiO2 usually required to kill bacteria varies between 100 and 1000 ppm, depending on the size of the particles and the intensity and wavelength of the light used (Wei et al., 1994). An attractive feature of TiO2 photocatalytic disinfection is its potential to be activated by visible light, e.g. sunlight. Metal doping has long been known to improve visible light absorbance of TiO2 (Anpo et al., 2001), and increase its photocatalytic activity under UV irradiation (Choi et al., 1994).

Materials and Methods

Materials:
Potassium Dichromate (K2Cr2O7), Sulphuric acid (H2SO4), Sodium thiosulfate, Starch, Phosphate buffer solution and allylthiourea solution (0.5%) were purchased from Merck Specialties Private Limited (Mumbai, India). Titanium isopropoxide (Ti (OCH(CH3))2)3 and ethanolamine were purchased from Sigma Aldrich. All the other chemicals used were of analytical grade with highest purity.

Organisms and culture maintenance: Bacillus subtilis (NCIM 2549), Pseudomonas putida (NCIM 2847), Nocardia hydrocarbonoxydance (NCIM 2386), Pseudomonas aeruginosa (NCIM 2074) and Rhodococcus terrae (NCIM 5126) were obtained from National Collection of Industrial Microorganisms (NCIM), Pune, India. All microorganisms are maintained on nutrient agar slant at 4°C and these are subcultured at regular interval in departmental laboratory.

Pure Culture and Mix Culture: Each microorganism were grown in a nutrient broth separately and incubated for 24 hrs at 37°C each in 100 ml conical flask. The inoculums were taken from each flask and mix culture was developed in 500 ml conical flask.

Biofilm development on different materials:
All the flasks from the above procedure were subjected to flask level study. In this strips of different material such as PVC, GI, Glass, and Nylon fiber was taken. The best optimum condition such as pH, temperature, dilution and material will be selected from above study and the selected material was used for fabrication of drums which was used in rotating drum biofilm reactor (RDBR).

**Determination of Chemical Oxygen Demand (COD):**
COD is a measure of the capacity of water to consume O\textsubscript{2} during the decomposition of organic matter and the oxidation of inorganic chemicals such as ammonia and nitrite. COD was calculated according to the method given in the book of S.S.Dara (1999).

**Phenol estimation:**
The standard brominating solution is a mixture of potassium bromide and potassium bromated (KBr+KBrO\textsubscript{3}). The bromine (Br\textsubscript{2}) liberated from these solution in the presence of concentrated HCl, phenol undergoes bromination to form 2, 4, 6 tribromophenol. The excess of bromine which is not used by phenol reacts with KI solution and liberate iodine. The liberated iodine was titrated against standard 0.1 N Sodium thiosulfate solution. Thus the phenol was estimated by calculating the amount of bromine consumed during the reaction in terms of 0.1 sodium thiosulphate solution.

**Determination of Biological Oxygen Demand (BOD):**
Biological oxygen demand or BOD represents the quantity of oxygen required by bacteria and other micro organisms during the biochemical degradation and transformation of organic matter present in waste water under aerobic conditions. BOD test is of great value in the analysis of sewage, highly polluted waters and industrial effluents. BOD was calculated according to the method given in the book by S.S.Dara (1999).

**Biofilm development:**
For the biofilm development different materials can be used as attachment surface but PVC (polyvinylchioride) gives good result for attachment of biofilm. The mix cultures of bacteria was taken and pour in rotating drum biological reactor (RDBR) which was made from one and half liter of PVC jar with provision of inlet and outlet and centrally located shaft carrying PVC drum with filter. A batch of 500 ml mix culture was run for 7 to 8 days at 2 rpm at room temperature.

<table>
<thead>
<tr>
<th>Constitute</th>
<th>Stock concentration (g/l)</th>
<th>Quantity used (ml/5l)</th>
<th>Final concentration (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bactopeptone</td>
<td>32.25</td>
<td>27.3</td>
<td>352.75</td>
</tr>
<tr>
<td>MgSO\textsubscript{4}·7H\textsubscript{2}O</td>
<td>10.00</td>
<td>25.0</td>
<td>50.00</td>
</tr>
<tr>
<td>MnSO\textsubscript{4}·H\textsubscript{2}O</td>
<td>1.60</td>
<td>25.0</td>
<td>5.00</td>
</tr>
<tr>
<td>FeCl\textsubscript{3}·6H\textsubscript{2}O</td>
<td>0.175</td>
<td>25.0</td>
<td>1.00</td>
</tr>
<tr>
<td>CaCl\textsubscript{2}</td>
<td>100</td>
<td>25.0</td>
<td>3.75</td>
</tr>
<tr>
<td>KH\textsubscript{2}PO\textsubscript{4}</td>
<td>52.25</td>
<td>33.4</td>
<td>349.4</td>
</tr>
<tr>
<td>K\textsubscript{2}HPO\textsubscript{4}</td>
<td>107.00</td>
<td>33.4</td>
<td>715.56</td>
</tr>
<tr>
<td>Phenol</td>
<td>-</td>
<td>-</td>
<td>50.00</td>
</tr>
</tbody>
</table>

Table-1. Concentration of different components in synthetic waste water

**Preparation of TiO\textsubscript{2} nanoparticles:**
Nanoparticles were synthesized according to the process shown in the Fig.1. Chemicals- Precursor solution of 4 ml Titanium isopropoxide (Ti (OCH (CH\textsubscript{3})\textsubscript{2})\textsubscript{4}) (Sigma Aldrich 97%), ethylene diamine (Strem, 99%) or ethanolamine (Sigma Aldrich, 99%) and 200 ml of anhydrous ethanol.

The precursor solution was refluxed for 24 hrs and hydrolyzed by adding drop wise 20 ml distilled water. The yellow precipitate then centrifuge and dry under vacuum. The resulting powder then sintered in the air at 200 °C for 1 hr.10ml of Ti (OCH (CH\textsubscript{3})\textsubscript{2})\textsubscript{4} and isopropyl alcohol (5:95 volume ratio) was slowly added into 100ml of distilled water at pH 2, adjusted by HNO\textsubscript{3}. The mixture was stirred for 12 hrs; and centrifuged. The precipitates then wash and dry under vacuum, and kept in plastic vials. Characterizations of these particles were done by using X-RD and particle size distribution.
Reflex 4 ml of precursor solution for 24 hrs

\[ \text{Hydrolyze by adding drop wise 20 ml distilled water} \]

\[ \text{Yellow precipitate} \]

\[ \text{Centrifuge and dry under vacuum} \]

\[ \text{Powder sinter in the air at 200 } \degree \text{C (1 hr)} \]

\[ \text{Kept in a plastic vials} \]

**Fig.1.** - Fabrication of TiO₂ Nanoparticles.

**Biofilm development:**
After running the batch of 500 ml mix culture of bacteria’s for 7 to 8 days at 2 rpm at room temperature, biofilm was observed after 8 days on the surface of drum and appears as transparent and sticky growth (Fig.2).

**Phospholipids peroxidation:**
Bacterial cell suspension were kept at 4°C prior to the photocatalytic experiments. 10 ml of bacterial cell suspension was taken and 1 ml of 0.01 mg/lit aqueous solution of nitrogen doped TiO₂ particle was added. Then this suspension was kept in Petri dish under the light radiation for 6 hrs to facilitate photocatalytic activity of TiO₂. Also simultaneously second Petri dish was prepared without N₂ doped TiO₂ solution. 1 ml of suspension from each Petri dish was taken and spread on two different agar plate and label as Petri dish 1 and 2 respectively. Then these agar plates were incubated for 24 hrs at 37 °C and observed after 24 hrs.

**Results and Discussions**

**Biofilm development:**
After running the batch of 500 ml mix culture of bacteria’s for 7 to 8 days at 2 rpm at room temperature, biofilm was observed after 8 days on the surface of drum and appears as transparent and sticky growth (Fig.2).

**Treatment of Synthetic Waste Water:**
Phenol and phenolic compounds found as major constituent in effluents of many chemical industries. 500 ml of synthetic waste water containing 50 mg/lit of phenol and other constituents such as K₂HPO₄, CaCl₂ etc. was treated in RDBR. Water was fed from inlet and batch was operated for 48 hrs, samples are taken from 12 hrs and concentration of COD, BOD and phenol was measured (Fig.3, 4 and 5). It was found that mix bacterial cultures were capable of degrading phenol by 99.70 % approximately in 60 hrs.

**Fig.3 Reduction in COD value of synthetic waste water after treatment in RDBR.** The initial concentration of COD which was 2080 mg/lit at 0 hrs was reduced to 178 mg/lit after 60 hrs.
Fig. 4. Reduction in BOD value of synthetic waste water after treatment in RDBR. The initial concentration of BOD which was 1600 mg/lit at 0 hrs was reduced to 71.2 mg/lit after 60 hrs.

Fig. 5. Reduction in phenol concentration of synthetic waste water after treatment in RDBR. The initial concentration of phenol which was 50 mg/lit at 0 hrs was reduced to 0.0028 mg/lit after 60 hrs.
**Phenol Degradation study at flask level:**

A flask level study was done for estimation of phenol concentration that can be treated by biofilm. For these four different samples 300 ml of synthetic waste water containing varying concentration of phenol like 0.1, 0.2, 0.3 and 0.4 ml/lit was taken in 500 ml conical flask and inoculated with mix culture of bacteria. Result shows that bacterial growth can occur satisfactory at about 0.4 ml phenol concentration and cannot survive concentration above 0.6 ml per liter phenol. (Table 2)

<table>
<thead>
<tr>
<th>Sr. No.</th>
<th>Concentration (ml/lit)</th>
<th>After treatment (ml/lit)</th>
<th>Phenol degraded (ml/lit)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.1</td>
<td>0.00150</td>
<td>0.0985</td>
</tr>
<tr>
<td>2</td>
<td>0.2</td>
<td>0.00125</td>
<td>0.1980</td>
</tr>
<tr>
<td>3</td>
<td>0.3</td>
<td>0.00183</td>
<td>0.2981</td>
</tr>
<tr>
<td>4</td>
<td>0.4</td>
<td>0.00470</td>
<td>0.3950</td>
</tr>
</tbody>
</table>

**Synthesis of nitrogen doped TiO<sub>2</sub> nanoparticles:**

TiO<sub>2</sub> has strong antimicrobial activity and it can act on wide range of microorganism. Doping of TiO<sub>2</sub> particles with nitrogen enhances the antimicrobial activity and facilitate use of TiO<sub>2</sub> under visible spectrum. Nitrogen doped TiO<sub>2</sub> particles were prepared by solgel method using titanium isopropoxide as TiO<sub>2</sub> precursor and ethanolamine as doping precursor. 24 hr reflux method in ethanol can be used but vigorous mixing by stirrer can give TiO<sub>2</sub> nanoparticles within a 4 hrs.

**Phospholipid peroxidation by Nitrogen doped TiO<sub>2</sub> nanoparticles:**

Results shows bacterial growth was observed on Petri plate which was not treated with TiO<sub>2</sub> solution (Fig.5). On other hand TiO<sub>2</sub> treated suspension does not give any growth on second Petri plate (Fig.6).

**Fig.5. Appearance of bacterial growth on agar plate which was not treated with TiO<sub>2</sub> nanoparticles.**

**Fig.6. Agar plates treated with TiO<sub>2</sub> suspension indicating the absence of bacterial growth.**

**Conclusion**

Based on the experimental results we can conclude that rotating drum biological reactor (RDBR) technology is one of the promising technology for the treatment of waste water. After treatment of synthetic waste water in RDBR there was drastic reduction in the initial concentrations of COD, BOD and Phenol was observed. Results show the reduction in phenol concentration upto 99.7%. It has been also found that nitrogen doped TiO<sub>2</sub> nanoparticles shows good antimicrobial activity over the biofilm developed.

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