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# Biosynthesis of titanium dioxide nanoparticles using *Bacillus amyloliquefaciens* culture and enhancement of its photocatalytic activity for the degradation of a sulfonated textile dye Reactive Red 31

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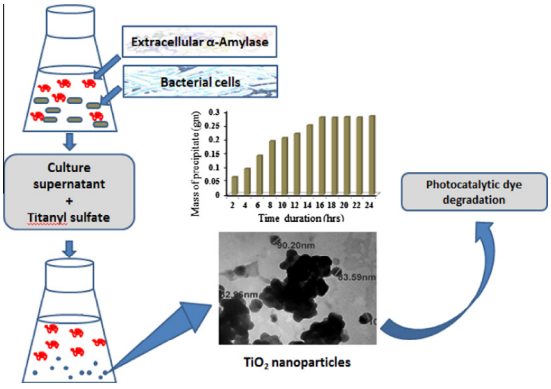
## HIGHLIGHTS

- *Bacillus* sp. was exploited for $TiO_2$ nanoparticle biosynthesis.
- Role of amylase in nanoparticle synthesis was determined.
- Synthesized nanoparticles were applied for the degradation of Reactive Red 31.
- Photocatalytic activity of synthesized nanoparticle was enhanced using metal doping.

## GRAPHICAL ABSTRACT

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## ABSTRACT

The present study aims at exploiting *Bacillus amyloliquefaciens* for the biosynthesis of titanium dioxide nanoparticles and also investigates role of bacterial enzymes in the biosynthesis of titanium dioxide nanoparticles. Bacterial synthesized as well as metal doped titanium dioxide nanoparticles were characterized by X-ray diffractometer (XRD), Fourier transform infrared spectroscopy (FTIR), Transmission electron microscopy (TEM), Energy dispersive X-ray spectroscopy (EDAX). Amylase activity (43.37 IU) in culture supernatant evinced a potential involvement of extracellular enzyme in $TiO_2$ nanoparticle biosynthesis. Crystallite size of bio-synthesized nanoparticles was found to be in the range of 15.23–87.6 nm. FTIR spectroscopy and native-PAGE (Polyacrylamide Gel Electrophoresis) clearly indicated involvement of alpha amylase in biosynthesis of $TiO_2$ nanoparticles and in their stabilization. TEM micrographs of the synthesized titanium dioxide nanoparticles revealed the formation of spherical nanoparticles with a size range of 22.11–97.28 nm. Photocatalytic degradation of Reactive Red 31 (RR31) dye was carried out using bio-synthesized $TiO_2$ nanoparticles under UV radiation. Photocatalytic activity of synthesized nanoparticles was enhanced by Ag, La, Zn and Pt doping. Platinum doped $TiO_2$ showed highest potential (90.98%) in RR31 degradation as compared to undoped (75.83%).



## 1. Introduction

In recent years, environmental pollution has become a major concern threating human life, ecosystem and biodiversity, resulting

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in ecological imbalance. Water pollution due to the discharge of colored effluents from dye industries is among them and considered to be most polluting in all industrial sectors [1]. Application of synthetic dyes in various industries such as paper, plastic, pharmaceuticals, dyeing, leather, and printing has increased significantly in recent years. Over 1000 different dyes and pigments are used worldwide in different industries with $\sim 7 \times 10^5$ tons of synthetic dyes produced annually [2]. A significant loss of dyes occurs during dyeing and finishing operations in textile and dye manufacturing industries, resulting in release of colored effluents into soil and water bodies. Dyestuff compounds are regarded as priority hazardous compounds by several governments owning to their high toxicity, carcinogenicity and environmental persistence [3]. Therefore, there is an urgent need for the development of efficient treatment methods for the remediation of waste effluents from textile and dye manufacturing industries.

Methods like chemical oxidation, ultrafiltration, adsorption, coagulation, microbial degradation, electrochemical and photocatalytic degradation were commonly employed for the decolorization and degradation of dyes present in colored effluents. Suspended $TiO_2$ nanoparticles have been largely used as an efficient catalyst for the degradation of organic contaminants present in water and aqueous wastes [4].

However, a critical drawback of $TiO_2$ nanoparticles is the relatively wide band gap ($\sim$3.2 eV), which is too large to allow efficient absorption of most sunlight and the recombination of the photogenerated electron–hole pairs takes place quickly on a time scale of $10^{-9}$–$10^{-12}$ s. A lot of research has been carried out to enhance the photocatalytic activity of $TiO_2$ including doping with metal ions such as Ag, Fe, and Pt [5–7]. The metal ion doping can modify the surface properties of $TiO_2$, hinder the recombination of photogenerated electron–hole pairs and increase the amount of the active sites [8,9].

The extensive use of toxic and hazardous agents applied in chemical procedures of nanoparticles synthesis has augmented the necessity in view of cheap and green chemistry approach [10]. The formation of nano-sized materials by microbial cells has come out as a promising tool for the synthesis of metal nanoparticles. The ability of the microbiological system to utilize, grow and survive in toxic, high metal concentration environment is already well established. Bacteria are organisms of choice due to easy handling, fast growth rate, high efficiency and lowest cost. A wide number of bacterial species specially those belonging to *Bacillus* sp. and *Lactobacillus* sp. have been reported for the production of titanium dioxide nanoparticles [11–13]. The mechanism of extracellular synthesis of metal nanoparticles using microbes is basically found to be enzyme mediated synthesis. A number of researchers supported enzymes for extracellular synthesis of nanoparticles [14].

In present study, for the first time *B. amyloliquefaciens*, isolated from dairy industry wastewater is exploited for titanium dioxide nanoparticle synthesis. Biosynthesized titanium dioxide nanoparticles were analysed for their photocatalytic efficiency towards degradation of a textile dye. Photocatalytic efficiency of synthesized nanoparticles was further enhanced by doping of titanium dioxide nanoparticles with other metals.

## 2. Materials and method

### 2.1. Materials

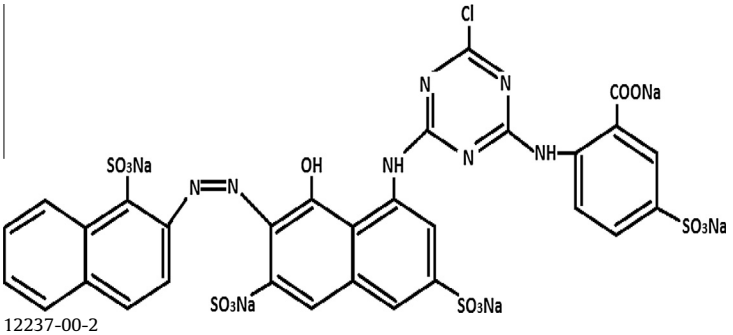
$TiOSO_4$ (Titanyl sulfate) was purchased from Himedia Laboratories Pvt. Ltd, Mumbai, India. Reactive Red 31 dye (Table 1) was obtained from a dye manufacturing industry in Vatva G.I.D.C., Ahmedabad, Gujarat. All the aqueous solutions were prepared in Milli-Q water. All other chemicals and reagents were from standard commercial sources and of highest quality available.

### 2.2. Screening, isolation and identification of bacteria for titanium dioxide nanoparticle synthesis

Bacterial strains were isolated from the effluent samples collected from a dairy industry, Mehsana, India. The spread plate method was used to isolate individual bacterial cultures from the dairy effluent. Each isolated bacterial cultures were allowed to grow as suspension culture in sterile distilled water containing suitable carbon and nitrogen source for 48 h and this was treated as a source culture. A significant amount of source culture is inoculated in the diluted medium. All the isolated strains were grown on nutrient agar medium (10 g peptone, 10 g meat extract, 0.5 g NaCl, 15 g agar, in 1000 mL distilled water) plates at 37 °C for 24 h. Each isolated strain was inoculated in 250 mL Erlenmeyer flasks containing 100 mL of medium and screened for nanoparticle synthesis.

A bacterial culture V7 was selected based on its ability to synthesize titanium dioxide nanoparticles. The potent culture V7 was identified based on 16S rRNA sequencing. Its isolated genome was subjected to PCR amplification of 16S rRNA using universal primers (Supplementary Fig. 1). A PCR mixture of 20 ng of extracted genomic DNA, 1 μL (100 ng each) of the primers, and 50 μL reaction buffer containing 1.0 μL dNTP mix (2.5 mM each), 1 X Taq buffer A (10 X) and 3 units Taq polymerase was prepared. PCR was performed using a thermocycler. Conditions set for

**Table 1**
General characteristics of Reactive Red 31.

| | |
|---|---|
| Chemical structure |  |
| CAS No. | 12237-00-2 |
| Molecular mass | 992.14 |
| $\lambda_{max}$ (nm) | 536 |
| Molecular formula | $C_{30}H_{15}ClN_7Na_5O_{15}S_4$ |