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Biological neutralization and biosorption of dyes of alkaline textile industry wastewater

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ABSTRACT

The present work was aimed to secure biological neutralization and biosorption of dyes of an alkaline textile industrial effluent (ATIE) using an alkaliphilic bacterium, *Enterococcus faecalis* strain R-16 isolated from Gujarat coast. The isolate was capable and competent to bring down the pH of ATIE from 12.1 to 7.0 within 2 h in the presence of carbon and nitrogen sources. Carboxylic group concentration (CGC), NMR and FT-IR analysis revealed production of carboxylic acid as a result of neutralization. The unconventional carbon and nitrogen sources like *Madhuca indica* flowers or sugar cane bagasse supported the growth of bacterium with effective neutralization and biosorption of dyes from ATIE. The process proved to be efficient, inexpensive and eco-friendly as compared to conventional chemical neutralization process.

1. Introduction

India is facing enormous environmental challenges; particularly with respect to pollution of soil, air and water (Chakraborti et al., 2011). Rapid industrialization and growth of population have led to the problem of environmental pollution, especially of the aquatic environment with a multitude of contaminants. Most of the alkaline wastewaters are generated from soda ash, chloro-alkali, dye manufacturing, textile, pulp and paper, chemical, paint, food and beverage industries (Jain et al., 2011). The pH of wastewater directly affects the physiological function and nutrient utilization by microbes. The influx of effluent causes serious change in water chemistry which alters the pH of water bodies, and this consequently affects resources especially around the coastal belt (Vandevivere et al., 1998; Asamudo et al., 2005). The major issues with such effluents are (dyes) color, high pH and total dissolved solid (TDS), chemical oxygen demand (COD) and toxic wastes. Traditional treatment methods (Mohan et al., 2002) are laborious, expensive and hazardous which can further aggravate the environmental pollution (Babu et al., 2007). The biological treatment can be used as an alternative to these processes as this can be cost effective, environment-friendly and publicly accepted technology (Dafale et al., 2010). There are many reports on bioremediation of textile industrial effluent using microorganisms, specifically fungi (Asamudo et al., 2005; Verma et al., 2010); however, reports on biological neutralization using bacteria are relatively rare.

Gujarat is the second most industrialized state in India. It has a coastline of about 1666 km and most of the industries are located along the river basins, estuaries and coastal marine areas. Major problem encountered by alkaline effluent discharging industries is the treatment of their huge volume of wastewater of extremely high pH along with high salinity, TDS, COD and color. Common bioremediation processes use terrestrial microbes which are unsuitable for the effluents having high salinity, pH and color; hence, prior neutralization is essential as most of the microbial strains present in activated sludge process work well near neutral pH. Alternately, microbes, best suited to the extreme conditions of salinity and pH, can be used for neutralization of alkaline effluent. Generally, neutralization of alkaline effluent is being done purely by chemical means where a huge amount of acid is used which is neither economical nor safe as it poses serious health hazards. Conversely, neutralization of alkaline effluent by biological process using alkaliphilic bacteria is found to be quite safe. It has been reported that *Pseudomonas* sp. plays a very important role in the bioremediation of phenol containing waste effluent of dye industries (Sarnaik and Kanekar, 1995). Under extreme alkaline conditions, microbial utilization of benzoate, *m*-hydroxybenzoate and thiocyanate was reported delienating the probable use of alkaliphiles in effluent treatment rich in such compounds (Sorokin et al., 2001; Yumoto et al., 2003; Oie et al., 2007). Degradation of aromatic compounds and acetonitrile at either high salinity or alkalinity was reviewed (Peyton and Alva, 2003; Dimitry et al.,

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2000). Degradation of pyrene, a four ring compound, under alkaline condition, was reported by Habe et al. (2004). Most of the current effluent treatment processes are costly and produce large amount of solid wastes due to the use of chemicals for pH adjustment (Lim et al., 2010). It has been suggested that alkaliphilic bacteria could degrade pollutants under highly alkaline condition. However, reports on their application in effluent treatment are very rare. Nevertheless, few reports are available on neutralization of industrial effluent using alkaliphiles, where biological processes were developed for neutralizing highly alkaline beverage, chlor-alkali and textile industry wastewaters using alkaliphilic.

Dyes are natural or synthetic colorants used in various industries such as textiles, tanneries, paints, paper and pulp. Even if a small amount of dye is present in water (for example, even less than 1 ppm), it is highly visible and therefore undesirable (Sun and Yung, 2003). Effluents discharged from dyeing industries are highly colored and are toxic to aquatic life in the receiving water bodies (Brik et al., 2006; Tüfekci et al., 2007). Due to increasing environmental awareness and legal constraints imposed on discharge of effluents, the need for cost effective alternative technologies is essential for removal of dyes from industrial wastewater. Adsorption process is found to be the most suitable technique to eliminate pollutants from wastewater. Biosorption is an innovative technology using living or dead biomasses where the major advantages are its effectiveness and the use of inexpensive biosorbent material (Kumar and Bandyopodhyay, 2006; Volesky and Holan, 1995). *Enterococcus faecalis* is a normal flora in humans and animals. It is the second regular bacteria, after *Escherichia coli* as ecological agents (Yuan and Fu, 2003; Drahovska et al., 2004). Treatment of *E. faecalis* is difficult due to the possession of a large number of virulence factors and drug resistance. Enterococci have been traditionally considered as indicators of fecal contamination of drinking and recreational waters (Anonymous, 1999). However they are also present in high concentration in natural environments lacking exposure to heavy fecal contamination, such as rivers, seawater, and nonagricultural soils (Guardabassi and Dalsgaard, 2004). With increasing antibiotic resistance, enterococci are recognized as feared nosocomial pathogens that can be challenging to treat (Kayse, 2003). In a recent report, multidrug resistant pathogenic *Enterococcus* was isolated from environment air, sewage water and hospital wastewater (Park, 2003). In recent years, various natural adsorbents have been tried to achieve effective removal of various heavy metals and dyes. The present study focuses on isolation and identification of an alkaliphilic bacterium, *E. faecalis* R-16, having the potential to neutralize and biosorption of dyes an alkaline textile industrial effluent with a view to develop a cost effective and eco-friendly technology. Evaluation of efficiency of *Madhuca Indica* flower, sugarcane bagasse, wheat straw and rice straw powder as an adsorbent for the removal of dye from aqueous solutions indicated that *M. Indica* flower and sugarcane bagasse could be the best biomaterials as they could adsorb dye along with neutralization of alkaline textile effluent.

2. Materials and methods

2.1. Isolation, pH and salt tolerance and screening of alkaliphilic bacteria for neutralization study

Sample collection and isolation of alkaliphilic bacteria as well as screening of isolates for their neutralization potential were conducted as described earlier (Jain et al., 2011). pH tolerance of the seventy isolates was studied by growing each bacterial culture on the Horikoshi Medium (HM) agar plates and in broth with varying pH (7, 8, 9, 10, 11 and 12). Growth was recorded spectrophotometrically by measuring optical density (OD) at 600 nm against uninoculated HM broth after 24 h incubation at 30 °C. The cell death was also checked by streaking each isolate on HM agar plates having different pH (7–12). A salt tolerance study of potential isolates having capacity to neutralize alkaline HM broth was conducted by growing them at their optimum pH in presence of varying concentrations of NaCl (3.5%, 5%, 7.5%, 10% and 12.5%) in HM broth. Growth was determined by measuring absorbance at 600 nm after 24 h of incubation at 30 °C using spectrophotometer. Viability was checked by streaking each isolate on HM agar plates having different salt concentration (3.5–10%) and optimum pH. Screening of alkaliphilic bacteria for neutralization was performed using alkaline HM broth having pH 12.0, which was adjusted using saturated solution of sodium hydroxide (Gopalsamy et al., 2009). The pH was measured every 6 h upto 48 h by using a pH meter. The potential isolate which could reduce the pH in minimum time was extensively studied for bioremediation of alkaline textile industrial effluent (ATIE).

2.2. Identification and antibiotic susceptibility assay of R-16 alkaliphilic bacteria

Taxonomic characterization of strain R-16 was done on the basis of morphological, cultural and biochemical analysis according to Bergey's Manual of Systematic Bacteriology (Schleifer, 1984). This strain was also identified by Fatty acid methyl ester (FAME) analysis (MIDI Sherlock® Microbial Identification System) (Ibekwe and Kennedy, 1999) and 16S rRNA gene sequencing. For molecular identification using partial 16S rRNA sequencing technique, the isolate R-16 was grown in 5 ml of HM broth having pH 10 at 30 °C for 24 h. The biomass was harvested by centrifugation at 10,000 rpm for 10 min and washed twice in sterile tris–EDTA buffer (10:1 mol ratio, pH 8.0) and the wet biomass obtained was used for DNA extraction. The extraction and purification of DNA was carried out by the phenol–chloroform extraction (Marmur, 1961). PCR amplification of partial 16S rRNA gene sequences was performed using the forward and reverse primers as fD1 5′-AGA GTT TGA TCC TGG CTC AG-3′ and rP2 5′-ACG GCT ACC TTG TTA CGA CTT-3′ respectively (Weisburg et al., 1991). The reaction mixture for PCR amplification contained 10× PCR buffer 5 μl, 200 mM dNTPs 5 μl, 2.5 U Taq DNA polymerase, 20 pM of each primers (Sigma, India) and 50 ng of genomic DNA. Amplification was performed in a thermal cycler (Bio-Rad MyCycler, Thermal cycler, California, USA) for an initial denaturation at 94 °C for 4 min followed by 35 cycles of 94 °C for 1 min, 58 °C for 1 min and 72 °C for 2 min and a final extension at 72 °C for 5 min. The purified PCR product was sequenced and the 16S rRNA gene sequence was compared with those in the GenBank using the BLASTn program (Altschul et al., 1990). R-16 bacterium was tested for antibiotic sensitivity test using disc diffusion method (Jorgensen and Turnidge, 2007). R-16 bacterial inoculum ($\sim 8.65 \times 10^6$ CFU/ml) was applied to the surface of Mueller–Hinton agar on a large (150 mm diameter) plate. Antibiotic susceptibility tests were performed against 20 common antibiotics with recommended doses such as; Amikacin (30 μg), Ampicillin (10 μg), Amoxycillin (10 μg), Cefadroxil (30 μg), Cefoperazone (75 μg), Ceftazidime (30 μg), Ceftriaxone (30 μg), Chloramphenicol (30 μg), Ciprofloxacin (5 μg), Cloxacillin (1 μg), Co-Trimoxazole (25 μg), Erythromycin (15 μg), Gentamycin (10 μg), Nalidixic Acid (10 μg), Netilmycin (10 μg), Nitrofurantoin (300 μg), Norfloxacin (10 μg), Penicillin (10 μg), Tobramycin (10 μg) and Vancomycin (30 μg).These antibiotic dodeca disks are placed on the surface of inoculated agar plate and incubated for 24 h at 30 °C. The zones of inhibition around each of the antibiotic disks are measured recommended by the Clinical and Laboratory Standards Institute (CLSI, formerly the National Committee for Clinical Laboratory Standards or NCCLS) (Finegold and Martin, 1982).

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