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Biodegradation of reactive blue 19 with simultaneous electricity generation by the newly isolated electrogenic *Klebsiella* sp. C NCIM 5546 bacterium in a microbial fuel cell

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ABSTRACT

Reactive Blue-19 (RB19) is an anthraquinone based vinylsulphone dye, which is very difficult to degrade by chemical oxidation because its anthraquinone structure is stabilized by resonance. It has 75–80% fixation efficiency on the cellulose (cotton textiles) and the unfixed dye persists in the environment for long periods. Therefore, textile industry effluents containing reactive blue (RB) 19 dye in a significant concentration need to be treated. Microbial fuel cell (MFC) is the newest approach for generating energy (electricity-bioelectricity) from the textile wastewater using bacteria. In this study, synthetic textile wastewater containing Reactive Blue 19 (RB19) dye (50 mg/L to 800 mg/L) was used to generate electricity with the newly isolated electrogenic bacterium *Klebsiella* sp. C. The MFC produced the current density of 533 mA/m^2 and power density of 84 mW/m^2. The decolorisation efficiency by *Klebsiella* sp. C for RB19 was 90% within 24 h under optimised conditions i.e. pH 7, temperature of 37 °C, 6 g/L glucose concentration and glucose to microbe ratio of 60. The intermediates identified by GCMS analysis included oxalic acid, indene- 1, 3- dione and hydroquinone. These results indicated that *Klebsiella* sp. C could be considered as a new bacterium for electricity generation along with treatment of RB19 containing effluent.

1. Introduction

In developing countries, textile processing industries are well established sectors. In the textile industry, dyeing and printing processes use 100–350 m^3 of water for the production of a ton of textile product (Judd and Jefferson, 2003; Schoeberl et al., 2005). This water is mainly used for fixing dye and to remove unreacted dye from the fabric. Therefore, textile industries represent an important environmental problem due to such high water consumption and due to the presence of recalcitrant pollutants. According to the World Bank estimates, dyeing and finishing processes are responsible for generating around 17 to 20 percent of industrial waste water produced in a particular textile sector (Mahapatra, 2015).

Synthetic dyes are also used in other industrial processes like paper printing and colour photography. At present more than 100 thousand dyes are available and 7×10^5 tons of dyestuff are produced annually (Hung et al., 2012). Inefficiency of the printing and dyeing process results in 10–15% loss of all the dyestuff into the wastewater, which ultimately finds its way into the environment. There are several types of dye including reactive, acidic and basic, disperse and direct dyes. Reactive dyes based on azo and anthraquinone functional groups are the most commonly used dyes (Sudha et al., 2014). Reactive Blue-19 (RB19) is an anthraquinone based vinylsulphone dye, which is very difficult to degrade by chemical oxidation due to its anthraquinone structure being stabilized by resonance. It has 75–80% fixation efficiency on the cellulose (cotton textiles). This low dye absorption efficiency is due to the competition for cellulose in the dyeing solution between the native RB19 dye and the partially hydrolysed RB19 dye (RB19-OH), which has no affinity for cellulose. Hydrolysis of the vinyl sulfone moiety of RB19 dye (RB19-OH) before fixation (i.e. before formation of a covalent bond between dye and cellulose) is one of the fundamental problems associated with reactive dye technology. (Vidhyakalarani and Premaraj, 2013; Weber and Stickney, 1993). Textile industry effluent containing reactive blue (RB) 19 dye in a significant concentration (20–250 mg/L) has been successfully treated biologically (Holkar et al., 2014). Reactive blue 19 due its resistance to

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degradation persists in the environment for long periods. In aquatic environments, it reduces light penetration and increases oxygen consumption (Andrade et al., 2007).

Many treatment processes including Advanced Oxidation Processes (AOPs) such as ozonation (Fanchiang and Tseng, 2009), Ultra-Violet (UV) light/H_2O_2(Abbas Rezaee et al., 2008a) sonolysis, ozonation combined with sonolysis (He et al., 2008), electro-coagulation (Rajkumar et al., 2007), photo-oxidation (A. Rezaee et al., 2008b) and bacterial degradation by *Enterobacter species* (Holkar et al., 2014) have been developed to treat the textile wastewater containing RB19. These AOPs are highly effective for the treatment of RB19 but the energy required for the treatment is in the range of 500–760 kW/m^3 of treated water (Mahamuni and Adewuyi, 2010). Therefore, there is a need to find an alternative treatment process which requires less energy for operation. Microbial fuel cell is one such alternative treatment which generates the electricity from organic matter present in the wastewater while degrading and treating it simultaneously. The suitability of MFC for biodegradation of the most stable anthraquinone dye RB19 is not known. In the present study, an electricity generating strain belonging to the *Klebsiella* genus was isolated and identified by 16S rDNA sequencing. The influence of pH, temperature, glucose concentration and dye concentration on biodegradation and electricity generation has been studied.

2. Materials and methods

2.1. Reagents

Glucose, nutrient broth and agar were obtained from Sigma Aldrich, Mumbai (India). The textiles dye Reactive Blue 19 (RB19) having the purity of 95% was kindly donated by the Textile Department, Institute of Chemical Technology, Mumbai, India. Synthetic wastewater was prepared by adding RB19 at required concentration in M9 medium (Na_2HPO_4 31.7 g/L, KH_2PO_4 3 g/L, NH_4Cl 0.5 g/L, NaCl 0.5 g/L, $MgSO_4$ 0.12 g/L, $CaCl_2$ 4 mg/L, glucose 8 g/L, vitamin B 0.15 mg/L, pH 7.0) (Sathish et al., 2014).

2.2. Isolation and identification of RB19 dye degrading bacteria

A facultative anaerobic dye degrading strain was isolated from an activated sludge used in anaerobic digester to produce biogas from food waste at the Institute of Chemical Technology, Mumbai, India. Since, bacteria show good degradation of dye in textile effluent in the range of 50–400 mg/L, the M9 medium with 50 mg/L of RB19 dye was used in this study (Holkar et al., 2016). M9 agar plates were prepared by adding 25 g/L agar to M9 media. All media were autoclaved at 121 °C for 15 min prior to use. The 1 mL sludge collected from an anaerobic digester was inoculated into a 500 mL anaerobic bottle containing 200 mL of synthetic wastewater and 50 mg/L RB19 dye. Anaerobic conditions were induced by purging the headspace with nitrogen and sealing the bottle with rubber stoppers and aluminium cap. The anaerobic bottle was incubated at 37 °C under static condition until visual detection of the change in the color of the synthetic wastewater. Samples from well discoloured incubations were serially diluted and plated on M9 agar. After 24 h of incubation at 37 °C, different colonies having different decolorizing ability were inoculated into the synthetic wastewater and again streaked on M9 agar plate for purification. The bacterial strain with the strongest decolorizing ability labelled as C was preserved at - 20 °C in 25% glycerol solution. The 16S rDNA sequence of the isolate was determined at the National Collection of Industrial Microorganisms (NCIM) Resource Centre at National Chemical Laboratory (NCL), Pune. The sequence was deposited at National Centre for Biotechnology Information (NCBI) databank.

2.3. Bacterial degradation experiment

The isolated colony was inoculated in 250 mL Erlenmeyer flasks containing 100 mL nutrient broth cultivated at pH 7 and 30 °C for 12 h. An appropriate volume of this culture to achieve an optical density of 1.0 at 600 nm was transferred into an anaerobic bottle or an anaerobic flask containing 400 mL M9 medium. The required concentration of RB19 dye was added into the anaerobic bottle or flasks after incubating the subculture for 12 h under aerobic or anaerobic conditions. Effects of various parameters including aerobic, anaerobic condition, temperature (25, 30 and 37 °C), initial pH (5, 7 and 10), glucose concentration (2, 4, 6, 8 and 10 g/L), glucose to microbe weight ratio (20, 40, 60, 80 and 100) and RB19 dye concentration (50, 100, 200, 400, 600 and 800 mg/L) on the decolorisation were investigated. All these experiments were one factor a time experiments. Samples of 5 mL were collected every 4 h and centrifuged at 15000 × g for 20 min to remove biomass. Then, the absorbance of clear supernatant at 592 nm was measured using a UV-visible spectrophotometer. Decolorisation efficiency was calculated using the following equation (1)

$$Decolorisation\ \ efficiency(\%) = \frac{A_1 - A_t}{A_1} \times 100 \quad (1)$$

Where A_1 refers to the initial absorbance, A_t to the absorbance after incubation and t to the incubation time.

2.4. Microbial fuel cell experiment for electricity generation

A two-chamber Microbial Fuel Cell (2CMFC) having a total volume of 250 mL and a working volume of 200 mL in each section (anode or cathode) was used to check the electricity generating ability of isolated bacteria *Klebsiella* sp. C. A series of tests were performed in triplicate under the operational parameters giving conditions optimal for biodegradation of RB19. These operational parameters included aerobic and anaerobic conditions, temperature, initial pH, glucose concentration, glucose to microbe weight ratio and RB19 dye concentration. The 2CMFC as shown in Fig. 1 consisted of a graphite anode section and a graphite cathode section separated by a Nafion 212 Proton Exchange Membrane (PEM). The anode chamber of MFC was filled with synthetic wastewater. In the cathode chamber, air was bubbled through the water. The geometric area of each electrode was 3 cm^2. The area of PEM to transport protons from the anode to the cathode was 28 cm^2. The distance between the graphite anode and graphite cathode was 8 cm. The 2CMFC was operated in an incubator to maintain the specified temperature for thermostatization.

2.5. MFC characterization and electron transfer mechanism

The voltage was monitored with a digital multimeter at an interval of 24 h when the power generation reached the maximum and stabilized for several hours. The current and power was calculated according to equations (2) and (3):

$$\text{Current (A)} = \text{Voltage (V)/Resistance } (\Omega) \quad (2)$$

$$\text{Power (W)} = \text{Voltage (V)*Current (A)} \quad (3)$$

The current density and power density were obtained by dividing current and power, respectively, by the anodic surface area (m^2). Polarization and power density curves were used for the characterization of the MFC systems.

The electron transfer mechanism of the isolated strain was investigated by measuring the maximum currents of the same MFC system in the following three different conditions reported previously by Xia et al. (2010). In the first condition, the anode chamber was filled with the original anode medium. In the second condition, the original anode medium was centrifuged (10,000 × g for 15 min) and then the supernatant was refilled in the anode chamber. In the third condition,