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Biodegradation and bioelectricity generation by Microbial Desalination Cell

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ABSTRACT

This study highlights the application potential of Microbial Desalination Cell in effective bioremediation of dye house effluent by biodecolourisation of industrial dyes. The study showed, for the first time, that the dye house effluent could be used as an organic substrate in MDC, achieving biodecolourisation of effluent along with considerable desalination and power production. Utilization of these wastes in MDC can protect the environment from dye-containing wastewater contamination and the treated water can be reused for other purposes. Two bacterial strains, a novel isolate *Bacillus subtilis moh3* and a MTCC strain *Aeromonas hydrophila* subsp. *hydrophila* 8049 were used for decolourisation of two model dyes-Malachite Green (C.I.42,000) and Sunset Yellow (C.I.15,985). Decolourisation of dyes by these cultures was studied in batch cultures and optimized the growth conditions. The same cultures when used in Microbial Desalination Cell, achieved complete biodecolourisation along with considerable desalination (62.2 ± 0.4% and 57.6 ± 0.2%) and power production.

1. Introduction

Dyes are widely used in textile, leather and paper industries. Up to 50% of the dye applied during industrial processing may be lost depending on dye type and may lead to the release of intensely coloured effluents (Fernando et al., 2012). Currently, the discharge of wastewaters containing dye is an important environmental hazard. These dyes are highly stable in light and are also resistant to microbial degradation. Major classes of synthetic dyes include azo, triphenyl methane, and anthraquinone dyes, some of them are known to be very toxic and mutagenic to living organisms (Ren et al., 2006). Azo dyes constitute the largest class of synthetic dyes used in commercial applications (Pandey et al., 2007). The discharge of dyes into environment is undesirable, not only because of their colour but also because many dyes and their breakdown products are toxic and/or mutagenic. Discharge of these coloured effluents into rivers and lakes results in a reduced dissolved oxygen concentration, thus creating anoxic conditions that are lethal to resident organisms (Saratale et al., 2011).

A range of physicochemical methods exists to remove colour from dye containing effluents. The most extensively used are coagulation and flocculation processes. They require significant quantities of chemicals and produce notable amounts of sludge, requiring further handling and disposal. Adsorption and membrane filtration techniques lead to secondary waste streams which need further treatment and/or disposal. Both advanced oxidation processes (AOPs, such as ozonation, UV/H, Fenton, etc.) and membrane filtration methods are quite effective for colour removal but are energy and cost intensive. Enzymatic decolourisation is now widely used for the decolourisation of dye wastewater. However, this method is also facing several problems such as cost of enzymes, enzyme stability and product inhibition (Husain, 2010). On the other hand, biological processes provide a low-cost and efficient alternative for simultaneous colour and organic matter removal. However, it is difficult to achieve complete degradation only using aerobic biological processes (Méndez-Paz et al., 2005). Anaerobic treatment may be a feasible alternative for treatment of wastewater containing dye (Solanki et al., 2013).

Shortage of pure drinking water and energy crisis are the two equally pressing issues the world is facing today. One of the solutions to increase potable water availability is desalination of brackish water and seawater but the current desalination

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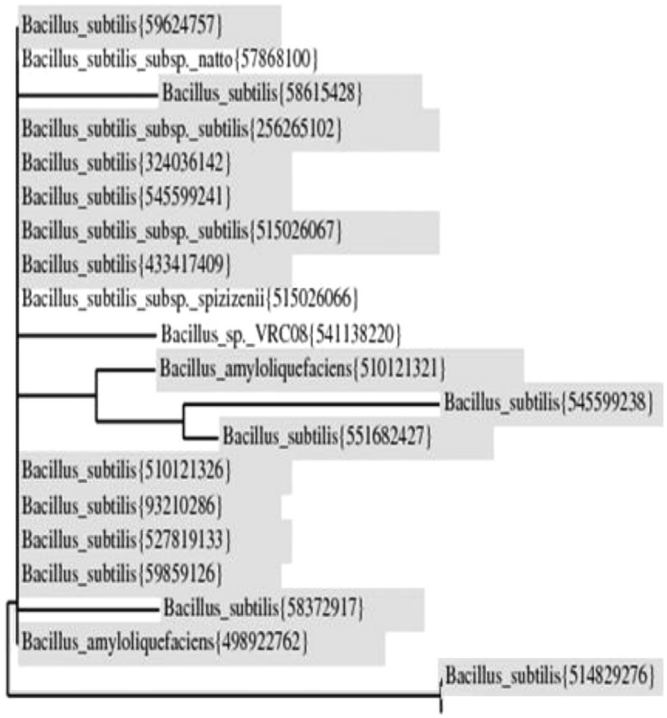

Fig. 1. Phylogenetic tree of *Bacillus subtilis moh3.*

technologies are energy intensive. Hence the authors here suggest a method for solving all these problems in a single step by using a Microbial desalination cell (MDC). MDC is a recently discovered new type of bioelectrochemical system developed from Microbial fuel cell (MFC), which could be used to partially or completely desalinate water while at the same time generating electrical power and biodegrading wastewater (Cao et al., 2009). The bioelectricity produced from degradation of organic compounds in wastewater (here dyes) acts as a driving force for desalination and incorporates salt removal as a part of the energy producing process. The complementary functions of energy production and desalination during wastewater MDC operation can improve the overall system performance and make the system more applicable for full scale applications (Luo et al., 2012).

Most of the substrates used in MFC and MDC remain in the form of glucose, oxalate, butyrate, and other easily degradable substrates. Some toxic and complex organics such as p-nitrophenol, quinoline, acidogenic food waste leachate and hydrocarbon-contaminated sediments have also been employed as organic substrates in the MFCs (Catal et al., 2009; Zhang et al., 2010; Rikame et al., 2012; Morris and Jin, 2012). More recently, MFC has shown the ability to decolourise certain azo dyes, while simultaneously generating bioelectricity (Fernando et al., 2012; Sun et al., 2013). To our knowledge, MDC has not yet been employed for such toxicant biodegradation studies. The authors here suggest that, the use of MDC instead of MFC for decolourization purpose increases the efficiency of biodecolourization and power production along with an added advantage of saltwater desalination. A number of electrochemically active bacteria such as *Shewanella putrefaciens*, *Geobacter sulfurreducens*, *Bacillus subtilis, Aeromonas hydrophila* etc. have been successfully employed in MFC studies (Kim et al., 2002; Bond and Lovely, 2003; Pham et al., 2003; Nimje et al., 2009). In this study, we have used MDC to investigate the biodecolourisation as well as biodegradation of model dyes Malachite Green and Sunset Yellow using two microbial strains, *B. subtilis moh3* and *A. hydrophila* along with desalination and power generation.

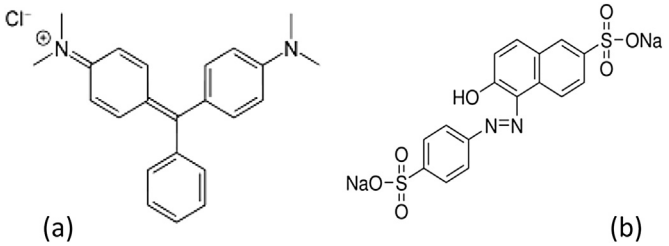

Fig. 2. Chemical structures of (a) Malachite Green and (b) Sunset Yellow.

2. Material and methods

2.1. Bacterial cultures and dyes

Two bacterial cultures were used in the decolourisation studies. One is a novel strain *B. subtilis moh3*, which was already isolated by the authors, from oil-contaminated sludge and deposited in Genbank (Accession No. KF021537) (Fig. 1). The other one is a standard strain *Aeromonas hydrohila* subsp. *hydrophila* 8049 obtained from MTCC (Microbial Type Culture Collection Centre, Chandigarh, India). The dyes used in the study were Malachite Green (C.I.42000), a triphenyl methane dye and Sunset Yellow (C.I.15985), an azo dye, which were kindly donated by CLRI, Chennai, India (Fig. 2). A simulated dye house effluent was prepared using M9 synthetic medium containing 0.1% yeast extract (Ren et al., 2006) with 50 ppm of each dye.

2.2. Decolourisation study

The stock solution of both the dyes, 1000 mg/l was prepared and from that required concentrations of dye solutions were made. The bacterial inoculums (2% v/v) from overnight cultures of each bacterium at the log phase of growth were transferred to 250 ml conical flasks, each containing 100 ml of sterile M9 synthetic media containing 0.1% yeast extract and 50 ppm of each dye. The flasks were incubated at 30 °C under static conditions. The uninoculated M9 medium containing the same amount of dye was kept as control under the same experimental conditions. Small aliquots (3 ml) of the solution were taken at regular intervals (3 h intervals) and analysed using UV–Visible spectrophotometer for Optical Density at 600 nm ($O.D_{600}$), centrifuged at 10,000 rpm for 10 min, and the supernatant was analysed for OD_{600} and for the maximum absorbance at 620 nm for Malachite Green and 485 nm for Sunset Yellow. The experiments were conducted in triplicate. The decolourisation percentage was calculated as follows:

$$\%\ \text{decolourisation} = \frac{(\text{Initial absorbance} - \text{Final absorbance}) \times 100}{\text{Initial absorbance}} \quad (1)$$