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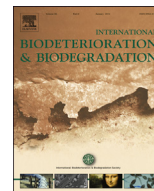

Improvement of biological sulfate reduction by supplementation of nitrogen rich extract prepared from organic marine wastes

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ABSTRACT

Organic marine waste was treated by both chemical and biological method to supplement it as nitrogen source in the growth media of sulfate reducing bacteria (SRB), and the resulting extracts were termed as marine waste extract (MWE) and *Lactobacillus* fermented MWE, respectively. Because of higher nitrogen content and other micro-nutrients, MWE could support higher sulfate reduction (83%) as compared to *Lactobacillus* fermented MWE (71%). The study further demonstrated that when compared to each optimized single process parameters (pH, MWE and sulfate concentration), optimization of multiple parameters based on Box-Behnken design and response surface methodology improved SRB growth and sulfate reduction by 5–6% and 10–11%, respectively. The experimentally optimized process parameters (1500 mg/L sulfate, pH 7.5 and 20% v/v MWE) resulted in 98% sulfate reduction which was close to the values derived from the theoretical model. This study highlights the use of MWE to improve the treatment of sulfate and metal rich wastewater.

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1. Introduction

Mining and extraction industries generate wastewaters which are often acidic in nature and rich in sulfate and metals (Lens et al., 1998; Kaksonen et al., 2004). Such wastewaters cause severe environmental threats such as ecological instability, contamination of water bodies and loss of biodiversity (Byrne et al., 2012). To reduce the detrimental effects, the wastewaters should be treated to remove acidity, metal contents, and sulfate before discharging into water bodies.

Sulfate reducing bacteria (SRB) mediated passive remediation is one of the potential alternative and widely practiced technologies used for the treatment of metal and sulfate rich wastewater (Kalin et al., 1991; Christensen et al., 1996; Chang et al., 2000; Johnson and Hallberg, 2005; Kaksonen and Puhakka, 2007; Cheong et al., 2010). SRB are anaerobic bacteria which utilize sulfate as terminal electron acceptor and reduce it to sulfide as a part of its energy metabolism. The resulting sulfide either evaporates as H_2S or precipitates the dissolved metals as metal sulfide (Johnson and Hallberg, 2005; Muyzer and Stams, 2008). The precipitated metal sulfide could be further used for metal recovery (Foucher et al., 2001; Tabak et al., 2003).

The activity of SRB depends on the availability of nutrients, specially, carbon and nitrogen sources (El Bayoumy et al., 1999; Robinson-Lora and Brennan, 2009; Teclu et al., 2009; Das et al., 2013). There are several reports on different carbon rich sources for SRB cultivation, but little information is available about potential alternatives for supplying nitrogen to these organisms in commercial treatment settings.

Very few reports are available on use of marine waste extract (MWE) as potential nitrogen source for growth of SRB (Dev and Bhattacharya, 2014). The MWE supplemented growth medium was able to better support SRB growth and sulfate reduction, compared to standard commercially available SRB growth media. The marine wastes are generated due to regular fishing activities and mainly composed of dead crabs, shrimps, mollusks, jelly fish and fish scraps. In the fishery industries, these wastes are disposed untreated into the sea. These wastes contain high COD (chemical oxygen demand) and their deposition on the seashore results in chocking of water channel and water pollution. Use of these wastes

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as nutritional supplement could not only help in waste management but also help in growth of SRB. The MWE was found cost effective compared to commercial nitrogen sources like NH_4Cl, tryptone and yeast extract.

Fermentation of shrimp and crab wastes with lactic acid bacteria results in deproteinization (Shirai et al., 2001; Bueno-Solano et al., 2009). In the present study the nitrogen rich extract was prepared by fermenting the organic marine waste with lactic acid bacteria. The aim of lactic acid fermentation was to generate protein rich extract containing high nitrogen and lactic acid. Lactic acid serves as good electron donor for SRB (Liamleam and Annachhatre, 2007). Therefore, the aim was to improve the SRB growth by supplementing nitrogen source along with lactic acid. The nitrogen rich extract prepared by this method was termed as *Lactobacillus* fermented MWE. Subsequently, MWE and *Lactobacillus* fermented MWE were compared for their ability to support SRB growth and sulfate reduction.

Different process parameters such as pH, sulfate and nitrogen concentration influence the treatment performance as they effect the growth of SRB (El Bayoumy et al., 1999; Oyekola et al., 2010; Sharma et al., 2014). In the present study, sulfate reduction was improved by optimizing the individual parameters like sulfate concentration, pH and MWE concentration. The mutual interactions of these three parameters on SRB growth and sulfate reduction have not been studied so far. Therefore, the present study was conducted to find out the optimized combination of these parameters that can support maximum sulfate reduction.

2. Materials and methods

2.1. Inoculum

2.1.1. Sulfate reducing bacteria

In the previous study, the bacterial mixed culture grown in SRB growth medium supplemented with MWE was found to contain 96% SRB population (Dev and Bhattacharya, 2014). The same bacterial culture was used as an SRB inoculum in the present study.

2.1.2. Lactic acid bacteria

Lactobacillus plantarum (MTCC 9495) was purchased from IMTECH, Chandigarh, India. The lyophilized culture was inoculated into MRS broth (De Man et al., 1960). The inoculum was developed after repeated subculture in MRS medium.

2.2. Preparation of marine waste extract by fermentation with lactic acid bacteria

The marine wastes were collected from different locations along the eastern seashore of Digha (West Bengal, India). The wastes were converted to 40 mesh size according to the procedure mentioned in the previous work (Dev and Bhattacharya, 2014). The ground marine waste was added as 10% (w/v) to 5% (v/v) glucose solution, mixed properly, and inoculated with *L. plantaram*. Fermentation was conducted at 37 °C for 5 days inside a shaking incubator. The fermented product was centrifuged (6440 g × 20 min) and supernatant was filtered (0.2 µm membrane filter, D121913, Whatman, GmbH, Germany) followed by storage in refrigerator.

2.3. Characterization of nutrients in Lactobacillus fermented MWE

To analyze the nutritional quality, *Lactobacillus* fermented MWE and its supplemented growth medium were characterized in terms of total kjeldahl nitrogen (TKN), total organic carbon (TOC), phosphorus (P), sulfur (S), potassium (K), calcium (Ca), sodium (Na), Manganese (Mn), Magnesium (Mn), iron (Fe), zinc (Zn), cobalt (Co) and copper (Cu). The lactic acid content of *Lactobacillus* fermented MWE was calculated because lactate serves as an efficient electron donor for SRB.

2.4. Comparison of MWE and Lactobacillus fermented MWE as nitrogen source

MWE and *Lactobacillus* fermented MWE were compared for their effectiveness to support SRB growth and sulfate reduction. The effectiveness of the two nitrogen sources was studied in terms of sulfate reduction (%), rate of sulfate reduction, sulfide generation, and total bacterial population. In the comparative analysis, the bacterial population is represented by ln (X/X_0). X_0 indicates the total bacterial number in cells/mL at time 0, where as X indicates total bacterial number at respective incubation time. The nitrogen sources (NH_4Cl and yeast extract) of Postgate B medium were substituted with MWE and *Lactobacillus* fermented MWE, respectively. The resulting growth media were termed as SRB growth medium supplemented with MWE and SRB growth medium supplemented with *Lactobacillus* fermented MWE, respectively. The composition of resulting growth media is presented in Table 1. The study was performed in batch mode. To correlate the effectiveness of both the extracts and their respective nitrogen contents, profiling of TKN throughout batch experiment was performed.

Both the media were prepared with deionized water, boiled and subsequently cooled under a continuous flow of nitrogen to remove the dissolved oxygen. The initial pH of the media was maintained at 7.2 by the addition of 0.1 N NaOH. Finally, the media were autoclaved at 103.4 KPa (15 lbs $inch^2$) pressure, cooled, and inoculated with SRB mixed culture. After the inoculation, the media was supplemented with 10% (v/v) reducing agent (0.7% sodium thioglycolate + 0.7% ascorbic acid).

2.5. Single parameter optimization

Improvement of biological sulfate reduction was observed by optimizing various process parameters such as sulfate concentration, pH and MWE concentration. The study was conducted in 300 mL serum bottle containing 250 mL media inside anaerobic chamber (Thermo scientific, model 1029) which was filled with 85% N_2, 10% H_2 and 5% CO_2. Three different sets of batches were employed each for sulfate concentrations, initial pH and MWE concentrations, respectively.

2.6. Multiple parameter optimizations

Multiple parameter optimization was performed to study the maximum sulfate reduction. This was achieved using Box-Behnken model and a response surface methodology (RSM) was generated using MINITAB15 software (Pan et al., 2008; Roy et al., 2012). Hsu et al. (2010) reported about the application of RSM for the optimized mutual interaction of Cu concentration and immobilized SRB biomass on sulfate reduction and Cu recovery. On the other hand, the optimized parameters obtained from this study were used to analyze sulfate reduction.

In the optimization process, the cumulative effect of three different parameters was studied. The variables were sulfate concentration (X_1), pH (X_2) and MWE concentration (X_3). The corresponding response variable was sulfate reduction (Y). A three factor Box-Behnken design with 12 unique sets and a triplicate on the center point was used and coded, and the actual levels of variable selected for the statistical design of the experiment are presented in Table 2. Sulfate concentration was varied from 500 mg/L to 2500 mg/L in steps of 500 mg/L, pH was varied from 6.5 to 8.5 in steps of 1 unit and MWE concentration was varied from 10% (v/v) to

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