Biological sulphate reduction using watermelon rind as a carbon source

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ABSTRACT

Biological treatment of sulphate rich waste waters with dissimilatory sulphate reducing bacteria (DSRB) has gained importance in the last few years. Usually, sulphate rich effluents are deficient in electron donors / carbon sources and thus require the addition of these through an external source. In this study, some pure cultures of SRB were employed in the decontamination / reduction of sulphates using watermelon rind as a carbon source. About 69% sulphate reduction was achieved in a 50 days trial of anaerobic incubation. The findings of this study will be very helpful in developing economical and environmental friendly bioremediation process(es).

Key words: Carbon source, DSRB, Economical bioremediation, Electron donor, Sulphate reduction, Watermelon rind.

INTRODUCTION

Sulphate reducing bacteria (SRB) make a group of obligatory anaerobes showing diversity in morphological and physiological characteristics and have the potential to dissimilate sulfate to sulfide while utilizing various types of growth substrates (Willis, et al., 1997). These are widely distributed among terrestrial, sub-terrestrial and marine ecosystems and may have an autotrophic, litho-autotrophic or heterotrophic respiration type of life under anaerobiosis. Possible microaerophilic natures of these bacteria have also been reported (Fauque & Ollivier, 2004). This group of bacteria exhibit autotrophic as well as heterotrophic modes of nutrition. Autotrophic SRB metabolize CO₂ and H₂ in order to fulfill their metabolic needs of carbon and electrons, respectively, while heterotrophic ones utilize organic compounds as substrates (Lens & Kuennen, 2001). Recent advances in biochemical as well as microbiological studies suggest the utilization of a wide variety of substrates by SRB as electron acceptors and donors (Rabus, et al., 2006).

In the last few years, SRB have been extensively employed in the decontamination of acid mine drainage and other sulphate rich effluents (Steed, et al., 2000; Lima, et al., 2001; Burgess & Stuetz, 2002; Johnson & Hallberg, 2005; Neculita, et al., 2007). According to Barnes (1998) sulphate reduction requires energy, so, an efficient and economical application of these bacteria in bioremediation process(es) is dependent on the selection of an energy source. In general, sulphate reducing bacteria prefer low molecular weight organic compounds, however, some can utilize different contaminants in the environment including halogenated compounds and constituents of petroleum hydrocarbons as a source of energy (Fauque, et al., 1991; Hao, et al., 1996; Harms, et al., 1999; Morasch, et al., 2004). Carbon source of most common use in culturing of
SRB at laboratory scale is the lactate (Postgate, 1984; Barnes, 1998; El Bayoumy, et al., 1999) and cannot be used at large scale processes being too much expensive. Ethanol is considered to be another cost effective substrate (Tsukamoto, et al., 2004; Huisman, et al., 2006). Several types of natural organic materials serving as carbon sources have been investigated already and include animal manure, bagasse, leaf mulch, molasses, mushroom compost, sawdust, sewage sludge, vegetal compost, whey, wood chips and other agricultural wastes (Dvorak, et al., 1992; Hammack, et al., 1994; Christensen, et al., 1996; Waybrant, et al., 1998; Annachhatre & Suktrakoolvait, 2001; Costa & Duarte, 2005; Coetsier, et al., 2006).

Selection of a cheaper carbon source is of great significance in economizing bioremediation process(es) especially for developing countries that cannot afford much cost in the protection of their local environments. The purpose of the present work was to investigate the effectiveness of watermelon rind as a carbon source in the biological treatment of sulphate rich waste waters. Watermelon rind is also one of the remarkable wastes of agricultural lands. So, concomitant use of both above mentioned wastes in bioremediation processes would achieve double benefits.

MATERIALS AND METHODS

Sample collection
Anaerobic sludge samples were collected from the bed of a sewage channel in Lahore, Pakistan. The collected samples were stored in sterile, screw capped containers and were transported to the laboratory as soon as possible.

Enrichment culture development
Collected samples were enriched in medium B (Postgate, 1984). All enrichments were made by seeding 10% sludge in sterilized serum bottles of 20ml capacity. These bottles were filled up to the brim with fresh medium B and sealed with rubber stoppers and aluminium crimp seals so that no air was trapped in the bottles and incubated at 30°C till blackening of the medium. Sulphate reducing bacterial growth was confirmed by the formation of black precipitates in addition to production of rotten egg smell of H₂S.

Isolation of pure cultures of SRB
Pure cultures of SRB were isolated following the method of Postgate (1984). Accordingly about 4ml lots of medium E (Postgate, 1984) were distributed into six long test tubes (15×1cm) and kept them at 40°C. Inoculum from already enriched cultures was taken and diluted by dipping a sterile closed Pasteur pipette into the enrichment and then successively into tubes 1 to 6. Agar was allowed to set and then the tubes were incubated at 30°C for 48 hours. After successful incubation, a suitable tube was broken at a convenient point and 2 or 3 colonies were withdrawn. Each colony was broken in sterile saline and inspected the cell suspension under the microscope. Pure cell suspensions were transferred to medium B again and growth was obtained for using this as inoculum in batch experiments.
**Batch experiments**

Batch experiments were carried out for each isolated strain in triplicate in serum bottles of 120ml capacity. The growth medium used was the modified Postgate B medium containing sulphate (3.5g/litre) and dried watermelon rind powder (2%) as a carbon source instead of lactate while in control experiments lactic acid (sodium salt) was used as a carbon source. The inoculum size used was 5% (v/v) having almost $4.1 \times 10^6$ CFU/ml. pH of the medium was maintained at 6.0 for each experiment. Diffusion of oxygen in inoculated media was prevented by adding a layer of autoclaved liquid paraffin. The inoculated bottles were sealed with fine rubber stoppers and aluminium crimp seals and incubated at 30°C for 50 days.

**Analytical procedures**

Periodically, 5ml samples were taken with the help of a syringe for measuring pH, sulphate and colony forming units (CFU/ml). pH was measured with the help of a digital pH meter while sulphate was estimated following the method as described by Cha, *et al.* (1999).

**RESULTS AND DISCUSSION**

This study reports for the very first time the utilization of watermelon rind as a carbon source by four sulfidogenic bacterial strains isolated from the local environment of Pakistan. Sulphate reducing pattern appeared quite different when watermelon rind was used instead of sodium lactate. In the initial stages of incubation, sulphate was reduced drastically. About half of the total added sulphate was consumed/reduced in the first 10 days of incubation (Fig 1). But in the latter 40 days of incubation, only 29% sulphate was reduced as a whole. Such types of results were consistent for all isolated strains. Basically, watermelon rind is a mixture of many simple and complex molecules (Kumar, *et al.*, 2012). In the early stages of incubation, low molecular weight molecules of the mixture were in abundance with the complex molecules and frequently available for sulphate reducing bacterial growth but with the passage of time simpler molecules became exhausted from the medium due to consumption of these in the growth of SRB and thus became unavailable or very less available to be utilized by SRB in the coming period. That’s why the sulphate reducing rate became very slow in the latter days of incubation. The preference of SRB for simple organic molecules has already been investigated (Gibert, *et al.*, 2004; Tsukamoto, *et al.*, 2004; Zaguary, *et al.*, 2006).
Fig. 1: Sulfidogenic bacterial isolates HDW-1, HDW-2, HDW-3 and HDW-4 showing sulphate reducing efficiencies in accordance with pH and CFU/ml of the media in figures A, B, C and D, respectively, while using watermelon rind as a carbon source.
Fig. 2: Sulfidogenic bacterial isolates HDW-1, HDW-2, HDW-3 and HDW-4 showing sulphate reducing efficiencies in accordance with pH and CFU/ml of the media in figures A, B, C and D, respectively, while using sodium lactate as a carbon source.
The relation of sulphate reduction with CFU seemed ambiguous and unpredictable when the periodically calculated values of CFU/ml appeared almost the same throughout this study in accordance with the values of sulphate reduction of latter incubation periods. This might be due to the predominant viability of the bacterial cultures showing no / or lesser growth in the absence of simpler organic molecules and thus resulting in negligible sulphate reduction.

In control experiments, maximum sulphate reduction (98%) was observed when lactic acid (sodium salt) was used as a carbon source. In almost all cases, about 68% and 89% of the total added sulphate was reduced within 10 and 20 days, respectively (Fig 2). Maximum sulphate reduction was probably due to ideal pH of the medium exhibiting neutral to basic range and simplicity of lactate molecules. Similar findings regarding efficient sulphate reduction in alkaline pH have been reported already by various researchers (Martins, et al., 2009; Singh, et al., 2011).

In this study, maximum 69% sulphate was reduced when watermelon rind was used as a carbon source. This whole study for assessing watermelon rind as a carbon source was carried out with pure cultures of sulfidogenic bacteria. However, the use of mixed cultures is advantageous over the use of pure cultures in having bacterial consortia that facilitate the development of reducing conditions and these are also more easily available (Gibert, et al., 2004). So, in case of mixed cultures, the efficiency of sulphate reduction must be increased using the same carbon source and thus demanding the need of further studies in future for the evaluation of watermelon rind as a carbon source while using mixed cultures of SRB.

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REFERENCES


