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Integrating photobiological hydrogen production with dye–metal bioremoval from simulated textile wastewater

Anubha Kaushik, Sharma Mona *, C.P. Kaushik

Department of Environmental Science and Engineering, Guru Jambheshwar University of Science & Technology, Hisar 125001, India

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ABSTRACT

The study reports production of hydrogen in photobioreactors with free (PBR_{Fr}) and immobilized (PBR_{Imm}) *Nostoc* biomass at enhanced and sustained rates. Before running the photobioreactors, effects of different immobilization matrices and cyanobacterial dose on hydrogen production were studied in batch mode. As hydrogen production in the PBRs declined spent biomass from the photobioreactors were collected and utilized further for column biosorption of highly toxic dyes (Reactive Red 198 + Crystal Violet) and metals (hexavalent chromium and bivalent cobalt) from simulated textile wastewater. Breakthrough time, adsorption capacity and exhaustion time of the biosorption column were studied. The photobioreactors with free and immobilized cyanobacterium produced hydrogen at average rates of 101 and 151 μmol/h/mg Chl a, respectively over 15 days, while the adsorption capacity of the spent biomass was up to 1.4 and 0.23 mg/g for metals and 15 and 1.75 mg/g for the dyes, respectively in continuous column mode.

1. Introduction

Hydrogen (H_2), a clean and high energy carrying resource is considered to be an efficient energy alternative for the future, which can be fed into the fuel cell systems for efficient conversion of chemical energy to electricity (Rosenbaum et al., 2005). Microalgal systems are emerging as promising H_2 producers of the future (Benemann, 2000), which show direct involvement of nitrogenase and hydrogenase enzymes. To deal with the oxygen sensitivity of these enzymes, indirect biophotolysis involving separation of H_2 and O_2 evolving reactions into separate stages, coupled through CO_2 fixation has been explored (Song et al., 2011). Efforts are underway to increase H_2 production by manipulating various physico-chemical conditions including composition of buffer or nutrient medium and entrapment of the cells in different matrices (Burgess et al., 2004; Davila-Vazuez et al., 2011; Kaushik and Anjana, 2011). Besides aiming to increase hydrogen production, it was proposed by Benemann (2000) that efforts should be made to integrate H_2 production by the microalgae with wastewater treatment to reduce overall cost. Very little work has been done on this aspect and we have come across one preliminary batch study by Shah et al. (2001) using a marine species for H_2 production and dye removal. A recent study reports H_2 production by *Clostridium* in toxic wastewater (Ho and Lee, 2011). This is the first lab-scale study reporting possible integration of continuous hydrogen production by a fresh water cyanobacterium in photobioreactors and biosorption of some toxic and carcinogenic dyes and metals from simulated textile wastewater in continuous system.

Keeping into consideration the need of the small scale textile units to have economically and environmentally sustainable treatment techniques, the aim was to explore a system integrating energy production and upgradation of the wastewater. This industry contributing largely to the national economy, is however, decentralized as small scale cottage industry in most of the developing countries. Several synthetic dyes, heavy metals and other chemicals are used by textile and dyeing industries, many of which reach the waste stream (Jacob and Azariah, 2000). Reactive dyes with azo-based chromophores combined with different reactive groups are much in use in textile industry (Aksu and Tezer, 2005) and azo dyes are known to release toxic amines due to reactive cleavage of azo groups (Joshi et al., 2004). However, one of the recent findings indicates that aromatic amines could also slightly stimulate the biodecolorizer *Aeromonas hydrophila* in the presence of azo dyes (Chen et al., 2009). Many triphenyl methane dyes, also used extensively in textile dyeing, are also hazardous due to their carcinogenic nature. Reactive red 198 (RR 198) dye and Crystal violet (CV) dye, a triphenyl methane dye widely used by textile mills were selected for bioremoval in this study. Besides dyes, textile wastewaters also contain several heavy metals that are constituents of metallized dyes or are used as binding agents. Cr(VI) and Co(II) selected in the present study, exist in the textile wastewaters causing several adverse impacts on aquatic systems and human health. While use of low cost adsorbents for biosorption of dyes

* Corresponding author. Tel.: +91 1662 263153; fax: +91 1662 277942.
E-mail address: monasharma140@gmail.com (S. Mona).

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and heavy metals has been widely studied and reviewed (Crini and Badot, 2008), there has been increasing interest on exploring various microbial systems for wastewater treatment, considering the environmentally safe, toxic sludge-less process in contrast to the conventional methods (Forgacs et al., 2004).

We have used, an indigenous strain of cyanobacterium isolated from contaminated textile mills sites, suited to the sub-tropical climatic conditions, for the dual purpose of hydrogen production and biosorption of some toxic dyes and heavy metals present in textile wastewaters. Since metals and dyes coexist in the textile wastewaters, and their co-existence change the biosorption pattern (Sadettin and Donmez, 2007), we have used simulated textile wastewater in the present study. After exploring the hydrogen production and biosorption potential of several native cyanobacteria and green algae (Anjana et al., 2010; Mona et al., 2011a), we selected *Nostoc linckia* HA-46, a promising strain for the study. H_2 photo production by the cyanobacterium was investigated in photobioreactor (PBRs) both in free (PBR_{Fr}) and immobilized (PBR_{Imm}) forms. Immobilization matrices being natural, non-toxic and cheap are not only suitable for industrial use (Karube et al., 1986), but also the enzyme system and pH inside the matrix are more stable (Kosourov and Seibert, 2009). Prevention of clump formation and filament breakage add to its merits. Spent biomass from PBR_{Fr} was dried and immobilized prior to its use as a biosorbent, while that from PBR_{Imm} was used as such. Since biosorption could be metabolism dependent or independent based on the fact whether the cells are live or dead/dormant, hence it was hypothesized that overall functioning of the two types of integrated systems using different forms of biomass would vary.

2. Methods

2.1. Cyanobacterial strain and growth conditions

Nostoc linckia HA 46 strain isolated from a textile mill oxidation pond was selected as a model system. Identification and culturing details of the organism are described in our earlier report (Mona et al., 2011a). The cells were harvested in late logarithmic phase (7 d) by centrifugation at 1200 g for 15 min and used for hydrogen production studies in free or immobilized forms.

2.2. Immobilization of Nostoc for hydrogen production

Nostoc linckia (7 d stage) were immobilized by entrapment method using different matrices. A dense 7-mL cell suspension (containing 0.1 g dry wt. of the cyanobacterium) was re-suspended in double distilled water and mixed thoroughly followed by immobilization. Entrapment of *Nostoc* in alginate gel was done using sodium alginate as described elsewhere (Kiran and Kaushik, 2008). Immobilization in carrageenan gel was done following the procedure described by Karube et al. (1986) and in agarose gel following Aksu (1998). Entrapment of *Nostoc* biomass in Poly vinyl alcohol alginate (PVA–A) gel and Polyvinyl alcohol alginate cross-linked with glutaraldehyde (PVA–A–G) gel was done following the methods described by Bagai and Madamwar (1998). The cyanobacterium immobilized in various matrices was in the form of small beads (3 ± 0.1 mm) and the rates of hydrogen production were expressed in nmol/h/mg dry wt. for batch studies.

2.3. Hydrogen assay and hydrogenase activity

H_2 production experiments in batch mode for the immobilized *N. linckia* beads were performed in triplicates in sealed vials (15-mL) under conditions of continuous stirring (100 rpm). Immobilized cyanobacterial beads entrapped in different matrices were incubated for hydrogen production and hydrogenase activity with BG-11 medium (2 mL cyanobacterial beads + 1 mL culture medium). All the air in the vial was replaced with argon to provide anaerobic conditions. Hydrogen assay was carried out at 18 h light (3000 l×)/6 h dark, 31 °C temperature and pH 8.0, based on our earlier study (Mona et al., 2011a). Hydrogen production by the immobilized *Nostoc* cells was estimated after 24, 48, and 72 h of incubation. *In vitro* hydrogenase activity of the immobilized cyanobacterium was estimated following the method of Kosourov et al. (2002) using methyl viologen that was reduced by sodium dithionite in dark anaerobic conditions and the rates of H_2 production were estimated on a calibrated gas chromatograph (Agilent 6890N) equipped with a 13× molecular sieve column, thermal conductivity detector (TCD) using nitrogen as the carrier gas as described in our earlier paper (Mona et al., 2011a). Hydrogen production rates were expressed in nmol/h/mg dry wt.

2.4. Optimization of cell dose in alginate immobilized beads

After obtaining results on hydrogenase and hydrogen production in different immobilization matrices, alginate was found to be most efficient and hence used for further studies. Effect of cyanobacterial dose (0.1–2.5 g) in the immobilized beads on H_2 production was studied during 24, 48, and 72 h of incubation.

2.5. Photobioreactor (PBR) studies for hydrogen production

Photobioreactors (1-L) made up of borosilicate glass with a working volume of 0.9 L were used for continuous hydrogen production at 31 °C. The reactors contained either free or immobilized biomass of *N. linckia*, referred to as PBR_{Fr} and PBR_{Imm}, respectively. Alginate immobilized *Nostoc* (1.1 g), in the form of beads, filled 0.6 L of the reactor (PBR_{Imm}) while the rest was occupied by BG-11 medium. In PBR_{Fr} 0.6 L of concentrated 7 d culture of *Nostoc* (containing 8.5 g dry wt.; 5.1 μg Chl a/mL) was suspended in 0.3 L of BG-11 medium. The reactors were stirred intermittently. Argon was sparged in the beginning (for 10 min) to replace all the air in the reactors. Illumination (3000 l×) was done from three sides with white fluorescent light and a light/dark cycle of 18/6 h was maintained. Each bioreactor was connected to a vessel containing 50% KOH solution for absorbing CO_2 produced by the cyanobacterium in the gas phase. The CO_2 free hydrogen was further passed through a vessel (fitted with septum) containing NaCl and a few drops of HCl to absorb any moisture or alkaline vapors (Pandey and Pandey, 2008). After every 24 h gas sample was withdrawn through a gas tight syringe from the head space of the vessel to analyze the concentration of H_2 produced. Additional glucose (20 mM) was added after 5 d and a mixture of CO_2 + Ar (02:10 v/v) were sparged for making the environment anaerobic at 10 d when the rates of hydrogen production started declining. Different concentrations of exogenous glucose added to the culture were earlier tried in batch mode and 20 mM concentration gave the best results for hydrogen production (detailed results not included here). Previously, we studied the effect of different combination of inert gases on rate of hydrogen production by *N. linckia* and CO_2 + Ar (02:10 v/v) was found to be most suitable (Mona et al., 2011a) and was therefore used in the present photobioreactor studies.

Changes in chlorophyll a of the cyanobacterial beads for immobilized system and that of the cyanobacterial culture for free system were studied every 24 h by estimating the pigment concentration spectrophotometrically by hot extraction method using methanol at 60 °C. Hydrogen production rates were expressed in μmol/h/mg Chl a for the PBR studies. In case of immobilized cells it was not possible to determine dry wt. of the cyanobacterium at each sampling day, hence hydrogen production