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Impact of heavy metals on hydrogen production from organic fraction of municipal solid waste using co-culture of *Enterobacter aerogenes* and *E. Coli*

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ABSTRACT

In the present study, the effect of heavy metals (lead, mercury, copper, and chromium) on the hydrogen production from the organic fraction of municipal solid waste (OFMSW) was investigated using co-culture of facultative anaerobes *Enterobacter aerogenes* and *E. coli*. Heavy metals were applied at concentration range of 0.5, 1, 2, 5, 10, 20, 50 and 100 mg/L. The results revealed that lead, mercury, and chromium negatively affected hydrogen production for the range of concentrations applied. Application of copper slightly enhanced hydrogen production at low concentration and resulted in the hydrogen yield of 36.0 $mLH_2/gCarbo_{initial}$ with 10 mg/L copper supplementation as compared to 24.2 $mLH_2/gCarbo_{initial}$ in control. However, the higher concentration of copper (>10 mg/L) declined hydrogen production. Hydrogen production inhibition potential of heavy metals can be arranged in the following increasing order: $Cu^{2+} < Cr^{6+} < Pb^{2+} < Hg^{2+}$. COD removal rate and volatile fatty acid generation efficiencies were also significantly affected by heavy metal addition. Thus, the present study reveals that the presence of heavy metals in the feedstock is detrimental for the hydrogen production. Therefore, it is essential to remove the toxic heavy metals prior to anaerobic digestion.

1. Introduction

The rapid consumption of non-renewable fossil fuels has resulted in environmental pollution and severe energy crisis, which emphasize the importance of renewable biofuel production. Biological hydrogen is a carbon-free renewable energy carrier, with high energy density (Marcoberardino et al., 2017). Biological hydrogen production via anaerobic digestion is a less energy-intensive, environmental-friendly and sustainable method compared to the current energy production methods (Gomez-Flores et al., 2015). Several types of substrate has been utilized for biological hydrogen production through anaerobic digestion such as sugarbeet (Urbaniec and Grabarczyk, 2014; Panagiotopoulos et al., 2015), kitchen waste (Gao et al., 2015), fruits and vegetables waste (Scano et al., 2014), brown algae (Fasahati et al., 2017), molasses (Han et al., 2016), organic waste (Han et al., 2017) and organic fraction of municipal solid waste (Barati et al. 2017), etc. Anaerobic digestion for hydrogen production offers numerous significant advantages such as low sludge production and low energy requirement (Chen et al., 2008). At the same time, waste disposal is a problem of the modern times. India generates municipal solid waste at an average rate of 0.11 kg/capita/day according to CPCB (GIZ, 2015). Most of this waste is dumped in open areas creating severe environmental problems. The organic fraction of municipal solid waste (OFMSW) is highly degradable (Rao et al., 2000), thus anaerobic fermentation of OFMSW can be one of the promising methods to generate hydrogen as it is abundant and free of cost (Kafle and Kim, 2013). It provides an eco-friendly solution to organic waste by converting waste to biofuel (Ionescu et al., 2013).

Co-culture of facultative anaerobes *E. coli* and *Enterobacter aerogenes* was used in the present study based on the results of our previous investigations (Sharma and Melkania, 2017). Previously *E. coli* and *Enterobacter aerogenes* have been isolated from sewage sludge and used for hydrogen production (Jame et al., 2011; Rachman et al., 1997). Both *E. coli* and *Enterobacter aerogenes* are very important hydrogen producers with high hydrogen production rate. Both are facultative anaerobes and can grow under aerobic as well as strict anaerobic conditions. Both the bacterial species has fast growth rate and ability to utilize a wide range of substrate and high tolerance to dissolved oxygen and hydrogen partial pressure (Zhang et al., 2011). Co-culture of *E. coli* and *Enterobacter* species has been used to produce hydrogen from biodiesel waste and a 3.1-fold higher hydrogen productivity was achieved as compared

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to hydrogen productivity with *Enterobacter* species alone (Maru et al., 2016). Similarly, Sivagurunathan et al. (2014) used facultative anaerobic bacteria *E. coli*, for hydrogen production using fructose as a carbon source and found the peak hydrogen yield of 1.17 mol-hydrogen /mol-fructose. Kumar et al. (2015) investigated hydrogen production from industrial wastewater using cultures of *Escherichia coli* XL1-Blue/*Enterobacter cloacae* DSM 16657 and found a remarkable increase in hydrogen production by using bacterial co-culture. Application of co-culture system offers advantage of improved hydrogen production and yield as compared to mono-culture systems (Pachapur et al., 2015). In co-culture system, different microbial strains are mixed which improves the individual characteristic that other strain lacks. Thus, co-culture system eliminates the need of pretreatment steps and use of expensive reducing agents. Thus, co-culture system is cost-effective as compared to mono-cultures. It offers various advantageous such as resistance to environmental fluctuation, reduction in lag phase and provides eight times more stability in hydrogen production rate as compared to mono-culture systems (Pachapur et al., 2015).

Heavy metals naturally exist in the environment and are widely used in the industrial and manufacturing processes. Consequently, they are transferred to organic residuals (Lineres, 1992). There are various sources of heavy metals in municipal solid waste, for example, batteries, household dust, disposable materials (eg. bottle caps), paints and inks, medicines, household pesticides, body care products, etc. (Bardos, 2004). Trchounian et al. (2017) observed that Mn^{2+} was stimulatory to the FOF1- ATPase activity at the concentration range of 10–50 μm, but it was inhibitory for the growth rate of *Escherichia coli*. Chromium is a heavy metal which is carcinogenic and imparts detrimental effects on the metabolism of almost all the microbial strains (autotrophic and heterotrophic), metazoans and protozoans (Luo et al., 2010; Kim et al., 2012). Wang et al. (2014) found that production of extracellular polymeric substances was increased when Cr^{6+} was added in the granular sludge from an aerobic granular sequencing batch reactor, which is supposed to be helpful for the strains to cope with Cr^{6+} induced stress. Heavy metals are of potential toxicity for anaerobic digestion. They can accumulate in the digesters to a potentially toxic level due to their non-biodegradable nature (Nayono, 2009). The toxicity of the heavy metals, to a large extent, is controlled by the physical and chemical nature of the medium in which they are present. This is correlated with the ion-specific physicochemical parameters such as electronegativity, reduction-oxidation potential, electron density, solubility product of the metal-sulfide complex, etc. (Workentine et al., 2008). Heavy metals are inhibitory to anaerobic microorganisms including acidogens (Zayed and Winter, 2000), acetogens (Li and Fang, 2007) and methanogens (Karri et al., 2006). Several studies have been done to evaluate the effect of heavy metals on a variety of microorganisms in anaerobic digestion. Tsai et al. (2005) applied 5mgCd/L in an anaerobic system involved in nutrient removal using betaproteobacteria and found a decrease of 30.7% to 2.1% in the activity of microorganisms. In an anaerobic medium, heavy metals may be precipitated as carbonates, sulfides or hydroxides (Gonçalves et al., 2007), can be chelated by compounds such as soluble microbial products produced during digestion (Walker et al., 2003) and might be adsorbed to sludge ligands (Alibhai et al., 1985). The failure of the anaerobic digestion occurs only when the concentration of metal ions exceeds a threshold level (Leighton and Forster, 1997). Heavy metals disrupt the enzyme structure and function by binding with thiol and other such groups on the protein molecules. Heavy metals may also replace natural metals present in enzyme prosthetic groups (Soldatkin et al., 2012). Oleszkiewicz and Sharma (1990) has suggested various mechanisms of metal toxicity as:(1) combining with sulfhydryl group (-SH) such as in cysteine (2) replacing metallic enzyme cofactors (3) mercapto group inactivation (4) binding with acid groups in the polypeptide chain. Oleszkiewicz and Sharma (1990) studied the toxicity of lead, copper, cadmium, zinc, and mercury on anaerobic digestion and reported that aluminum silicate enhanced the toxicity of other heavy metals and thus showed an antagonistic property. Several mechanisms have been proposed to remove heavy metal ions such as sorption, precipitation, and chelation by inorganic and organic ligands (Agrawal et al., 2011). In addition, heavy metal sorption onto activated carbon, bentonite, koline, diatomite and waste material such as cellulose pulp waste and compost can also mitigate toxicity (Ulmanu et al., 2003).

Anaerobic digestion of the OFMSW to produce hydrogen can reduce the organic pollution; while at the same time potentially offset the use of fossil fuels. However, the higher sensitivity of the anaerobic digestion to toxicants such as heavy metals negatively affects the hydrogen production efficiency. Therefore, it is necessary to evaluate the effect of the heavy metal concentration on the anaerobic fermentation the hydrogen in order to prevent hydrogen production efficiency reduction in presence of the heavy metals. The objective of the present study was to assess the impact of four heavy metal ions viz. Pb^{2+}, Hg^{2+}, Cu^{2+}, and Cr^{6+} on hydrogen production from organic fraction of municipal solid waste using co-culture of facultative anaerobes *Enterobacter aerogenes* and *E. coli*. These metals were selected for the present study as they have been reported to cause toxicity to the anaerobic digestion in the previous studies (Yang et al., 2016; Hussein and Mansour, 2014). Various concentrations of these heavy metals were taken ranging from 0.5 mg/L to 100 mg/L. The heavy metals are present in the municipal solid waste at various concentrations depending on the origin of the municipal solid waste (Zhao et al., 2012). Rawat et al. (2008) performed heavy metal quantification in the selected landfill areas in India and found that Cu, Cr, Pb and Hg were in the concentrations ranges of 34.4–45.4 mg/kg, 6.7–40.4 mg/kg, 5.6–100 mg/kg and 0.6–2.1 mg/kg respectively. Moreno et al. (2013) reported that concentration range for Cu, Cr, Hg and Pb in the municipal solid waste was from 15.64 to 422 mg/kg, from 12.66 to 144.87 mg/kg, from 0.84 to 2.41 mg/kg and from 26.69 to 171.48 mg/kg. Hence, a wide range of the concentrations of the heavy metals was applied in the present study. The present study is important for understanding the biological effects of various heavy metals on fermentation and hydrogen production in order to prevent loss of hydrogen yield due to the presence of the heavy metals in the feedstock.

2. Methods and material

2.1. Microorganisms and media

Facultative anaerobes *Enterobacter aerogenes* and *E. coli* were isolated from sewage sludge using selective and differential media Eosin Methylene Blue (EMB) Agar. The isolated strains were further grown in LB (Luria-Bertani) medium (10 g peptone, 5 g yeast extract and 10 g sodium chloride per liter) separately in an incubator at 37 °C. To ensure balanced growth the bacteria were cultured for more than five generations before they were used for the experiment (Stoke et al., 2012).

2.2. Feedstock preparation

The organic fraction of municipal solid waste (OFMSW) was collected from a local municipal landfill site located in Rudrapur, Uttarakhand, India. OFMSW consisted of household waste such as vegetables, fruits, rice, beans, bread and some meat and paper. OFMSW was ground using an electrical grinder without dilution which resulted in the particle size of <2 mm. The feedstock was