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CO_2 sequestration potential of halo-tolerant bacterium *Pseudomonas aeruginosa* SSL-4 and its application for recovery of fatty alcohols

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ABSTRACT

Bio-mitigation of CO_2 utilizing prokaryotes and simultaneous extraction of valuable bio-molecule is fast gaining interest now-a-days. Present work discusses the thermodynamic assessment of CO_2 bio-mitigation capability of *Pseudomonas aeruginosa* SSL-4 isolated from halo alkalophilic habitat in the absence of light. The maximum specific growth (μ_{Max}, h^{-1}) of isolate was found to be 0.425 (±0.0025) and 0.34 (±0.0063) at 3% (w/v) salt concentration and 35 °C, respectively. The isolate was cultivated in the environment having initial CO_2 (g) concentration of 17(±0.8) % (v/v) using Fe[II] as an energy source (0, 50 and 100 ppm) for evaluating CO_2 fixing ability of microorganisms. The maximum CO_2 removal efficiency of 92.37 (±2.46) % (v/v) was obtained at 100 ppm of Fe[II] concentration. The isolate has shown the maximum CO_2 fixation rate (R_{CO_2}) of 0.04 (±0.003) and 0.06 (±0.001) g/L/d at 50 and 100 ppm of Fe[II] concentration, respectively. FT-IR and GC–MS analysis of obtained leachate revealed the presence of fatty alcohols (C_{12}–C_{28}) and total product recovery (C_{12}–C_{18}) of 0.371 g per g of biomass. The thermodynamic assessment revealed the actual CO_2 utilization efficiency of 41.16%. Thus, the isolated strain from extreme hyper saline environment has shown the potential for research dedicated to carbon capture and utilization.

1. Introduction

The escalating level of carbon dioxide (CO_2) globally due to anthropogenic activities is a cause of major concern (Edenhofer et al., 2014). The biological route is one of the most viable solutions to mitigate CO_2 and is mainly focused on the use of photo-autotrophic microorganisms (Ho et al., 2011). Cultivation of microalgae offers a dual advantage with its ability to utilize CO_2 and convert the same in various industrially or environmentally useful products such as bio-fuel, ingredients for pharmaceutical industries, cosmetics, etc. Therefore, in the past few decades, the paradigm has shifted towards carbon capture and utilization (CCU) through mass scale cultivation of algae and other phototrophic microorganisms (Jajesniak et al., 2014). However, carbon capture via microalgae process is still expensive and has limitations which restrict its application in real-time situations (Leite et al., 2013). The application of phototrophic, chemolithotrophic and some of the mixotrophic bacteria for efficient utilization of CO_2 may prove to be a better alternative for CCU and production of useful by-products at large scale.

Few recent studies have investigated the prokaryotes for their ability to capture CO_2 in the absence of light and its simultaneous conversion into valuable products (Alonso-Sáez et al., 2010; Saini et al. 2011; García et al., 2016). One of the study have shown the fixation of CO_2 by *Sulfurovum lithotrophicum* 42BKT^T under high pressure of $CO_2 + N_2$ [$p^o_{CO_2} : p^o_{N_2} = 2:8$ (atm)] at 29 °C. The study confirmed the utilization of 37% of total CO_2 and its fixation into metabolites such as glutamate and

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pyroglutamate (Kwon et al., 2015). The other study in the field of CCU reported the CO_2 fixing ability of chemolithotrophic bacterium *Serratia* sp. ISTD04 and 94% conversion of obtained lipids to fatty acid methyl esters (Bharti et al., 2014). However, the above reported literatures do not take into account the actual CO_2 fixation efficiency from gaseous phase to biomass. Also, thermodynamic assessment of CO_2 utilization by bacterial species may provide the better understanding and useful information in terms of product formation. The earlier studies have not given much focus on the thermodynamic assessment of microbial reactions involving CO_2 fixation and conversion into value added products.

Energy required for the conversion of CO_2 into the cellular organic carbon can be obtained either from light or inorganic compounds depending on the type of organisms (Berg, 2011). In the absence of light source, the microbial fixation of CO_2 is represented by oxidation-reduction or electron donor–acceptor scheme of dissolved inorganic compounds (Amend and Shock, 2001). Considering constant temperature and pressure for a biological system, it is possible to predict the fate of chemical reaction by using a measure of potential energy called the free energy (ΔG). If the chemical reaction is thermodynamically unfavorable ($\Delta G > 0$) then cells are able to carry out a reaction by coupling it to a reaction that has a negative ΔG of larger magnitude, so that the sum of the two reactions has a negative ΔG. This way thermodynamic assessment of the microbial system w.r.t electron acceptor/donor component helps in the development of an appropriate mitigation strategy (Inskeep et al., 2005). The prior information w.r.t electron donor/electron acceptor may also aid in estimating the products being formed (McCollom and Amend, 2005). These calculations if carried out well, would lead in the implementation of the biological system in the real industrial scenario. Hence, the present work was focused on the thermodynamic assessment of the biological systems by varying the concentration of an electron donor which is Fe[II] in the present study. The emphasis is placed on the overall reaction rather than the stepwise reactions which constitute CO_2 metabolic process.

Iron (Fe) is one of the most abundant metal on Earth's crust which has a potential to participate in the oxidation-reduction reaction and is used as an energy source by microorganisms for cellular CO_2 assimilation (Mignone and Donati, 2004). Naturally iron exists in two readily inter-convertible redox states: ferrous ion (Fe[II]) and ferric ion (Fe[III]). The bio-usable state of iron in any environment depends on the pH and the redox potential of that environment. As the pH of the environment increases from 7 to 14, the dissolved iron (Fe[II] or Fe[III]) hydrolyzes to form precipitates in the oxic environment (Tekerlekopoulou et al., 2006). Therefore, there is a limited availability of bio-usable form of iron due to the low solubility of the Fe[III] in oxic environment. Prokaryotes have developed a specific strategy to meet the cellular iron demand which include (1) secretion of high-affinity extracellular ferric chelators, called siderophores (iron carriers) to solubilise iron prior to transport, (2) reduction of naturally occurring Fe[III] complexes by reductases located on the cell surface followed by uptake of the reduced iron (Andrews et al., 2003). Thus, prokaryotes have developed different approaches to adapt in conditions of limited bio-usable form of iron. However, very few studies have reported the Fe-metabolizing bacterial isolates from hypersaline environments (Switzer et al., 1998; McBeth et al., 2011; Emmerich et al., 2012).

The another advantage of CCU by bacterial species is a possibility of obtaining the useful products. Several studies have reported the use of halophiles for the recovery of products and its potential for the industrial application (Horikoshi, 1999; Oren, 2010). One such product is fatty alcohol which is one of the most widely used chemicals in industries. It is currently derived from plant oils and petrochemical sources. The microbial production of fatty alcohols involves two steps mechanism: (1) reduction of fatty acyl-CoA to the corresponding fatty aldehyde catalyzed by acyl-CoA reductase then, (2) the reduction of fatty aldehyde to the corresponding fatty alcohol catalyzed by a fatty aldehyde reductase. However, an enzyme (Fatty acid reductase) analogous to fatty acyl reductases (in eukaryotes) isolated from bacterium *Marinobacter aquaeolei* VT8 revealed possibility of the single step fatty alcohol production (Hofvander et al., 2011; Zheng et al., 2012). Thus, bacteria are capable of synthesizing fatty alcohols which have chain length ranging from C_9 to C_{20}. The production of fatty alcohols via synthetic biological route has gained interest in recent years as it can also be used as fuel additives due to its excellent fuel properties (Yao et al., 2014). The natural route for production of fatty alcohols seems advantageous. This may be due to the fact that millions of years of evolution have engineered these pathways for fast kinetics and thermodynamic efficiency. Therefore, isolates which are isolated from the extreme environments and having CO_2 fixation capability along with fatty alcohols production, can provide an economic and efficient solution. Thus, the present work is focused on the isolation and characterization of a bacterium obtained from extreme hypersaline environment and its utilization for the CCU.

The aim of the present study was (i) to isolate chemolithotropic Fe[II] oxidizing bacterium from a halophilic environment, (ii) to test the CO_2 fixation capability of the isolate by varying Fe[II] concentration and (iii) extraction and quantification of value added product. In the present work, a bacterial strain was isolated from Sambhar Salt Lake which is considered as a hypersaline environment. The isolated strain was acclimatized and enriched under laboratory conditions. The batch studies were carried out using the enriched culture to check its ability for CO_2 fixation from the gaseous phase. Fourier Transfer Infrared Spectroscopy (FT-IR) and Gas Chromatography–Mass Spectroscopy (GC–MS) studies of liquid phase were carried out to explore the possibility of products formation. All the studies were carried out in the dark to restrict the presence of photoautotrophic microorganisms.

2. Materials and methods

2.1. Sample collection site

The sludge and lake water sample from Sambhar salt lake in Rajasthan, India, were collected separately in sterilized sample vials. The samples were stored under dark and cold (4 °C) conditions until used. The geographical coordinates of the lake are 26° 58′ N, and 75° 5′ E, in the middle of a closed depression in the Aravalli schist, approximately 65 km northwest of Jaipur, with its axis northwest to southeast. The total salinity of the lake is 7% (w/v) with salt concentration in the range of 12–30% (w/v). The major contributors to Sambhar lakes's salinity are sulfates, carbonates, bicarbonates, chlorides, sodium and smaller amounts of potassium salts. The pH and total suspended solids of the water sample obtained from the lake are in the range of 9.5–10 and 0.7–2.8% (w/v), respectively (Joshi and Seth, 2011). Thus, the Sambhar salt lake is a natural habitat for the halophilic microorganisms.

2.2. Chemicals and culture media composition

The minimal salt medium (MSM) was prepared for the enrichment and isolation of iron oxidizing bacteria as well as for carrying out the batch studies. Composition of MSM used in this study is as follows (in g/L): NaCl-29.22, KCl-0.2, KNO_3-1, NH_4Cl-0.2, $MgCl_2{\cdot}6H_2O$-0.2, K_2HPO_4-0.5, KH_2PO_4-0.2. 1000 ppm stock solution of Fe[II] was prepared by dissolving 4.96 g of $FeSO_4{\cdot}7H_2O$ in distilled water and the volume of solution was made to one liter. The solution was filter sterilized. The pH of stock Fe[II] solution was adjusted to 3.5 using 0.1 M HCl acid solution. All the chemicals used in the present study were of biological and analytical grade. These chemicals were obtained from different commercial sources (Himedia Mumbai, India, Alfa Aesar Hyderabad, India). The CO_2 and He gas cylinders (purity 99.99%) were purchased from Sigma Aldrich Pvt. Ltd., India.