



Investigation on CO₂ bio-mitigation using *Halomonas stevensii* in laboratory scale bioreactor: Design of downstream process and its economic feasibility analysis

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ARTICLE INFO

Keywords:

CO₂ bio-mitigation
Halomonas stevensii
 Downstream bio-processing
 Process economics
 Thermodynamic analysis

ABSTRACT

In the present study, *H. stevensii* was cultivated on a semi-continuous mode in a laboratory scale bio-reactor using CO₂(g) [15% (v/v)] as carbon source and thiosulfate (S₂O₃²⁻) as an energy source for the total duration of three days. Approximately, 100% CO₂(g) removal from gaseous phase was achieved. Leachate obtained was subjected to different downstream bio-processing strategies. Biomass harvesting using filtration and recovery of metabolites without cell disruption using solvent extraction from wet biomass was observed as the best downstream processing strategy. Qualitative analysis of products was carried out using gas chromatography and mass spectroscopy (GC–MS) and their results have indicated fatty alcohols (C₈–C₂₇) as primary metabolites. Fourier transform infrared spectroscopy (FTIR) analysis, approximate material balance and thermodynamic analysis have confirmed the intracellular assimilation of CO₂(g) as HCO₃⁻ and its metabolism into fatty alcohols. Economic feasibility of the process has suggested that the developed downstream process has the capability to replace coconut oil based process for the production of fatty alcohols (C₁₂–C₁₄) and it can be utilized for the production of dodecanol as compared to the tetradecanol.

1. Introduction

Carbon capture and utilization (CCU) involves the conversion of CO₂(g) into valuable chemicals and in a process contribute significantly towards CO₂ mitigation [1,2]. However, CO₂ is an inert molecule, due to which its chemical conversion into other compounds requires high input of energy and cost, which makes the mitigation benefits marginal. Thus, on the lines of CCU, biological route for CO₂ mitigation has gained much attention in the present decade. Biological route ensures the natural assimilation of CO₂(g) into biomass at the expense of very less energy penalty. Later, the assimilated CO₂(g) as cellular biomass has been metabolized to yield valuable chemicals such as bio-diesel, hydrocarbons, bio-surfactants, bio-hydrogen, glutamate, pyroglutamate, bio-methane, isopropanol etc. [3–7]. Thus, bio-mitigation of CO₂ has satisfactorily fulfilled the CCU requirements.

Fatty alcohols are one such chemicals which have commercial value. The market size of fatty alcohols was predicted to cross 3350 kt by 2023 with compounded annual growth rate of more than 4.2%. Thus, it has the total market value of more than 7.5 billion USD. Fatty alcohols have carbon chain length in the range of C₁₁–C₁₄ and are in

huge demand. They have approximately 55% share from the total market of fatty alcohols [8]. The utilization of fatty alcohols (C₈–C₂₂) include: detergents, surfactants, lubricant additives, de-foamers, solubility retarders etc. and are currently synthesized from plant oil or petrochemical sources [9,10]. In order to reduce their dependency on petrochemicals and carbon footprint, industries are shifting their focus on the synthesis of fatty alcohols using biomass of various microorganisms such as yeast, microalgae, bacteria etc.

Microbial synthesis of fatty acids is already established in different studies [3]. Microbes convert the cellular fatty acids into fatty alcohols, when cultivated under changing environment of salt concentration (S), pH and temperature (T). Fatty alcohols may act as an osmotic regulator and an insulator for the protection of microbial cell and hence, makes microorganisms to survive under extreme conditions [9]. Bacterial conversion of cellular fatty acids to fatty alcohols involves two step reaction mechanisms: 1) reduction of fatty acyl-CoA to the corresponding fatty aldehyde catalyzed by acyl-CoA reductase and, 2) the reduction of fatty aldehyde to the corresponding fatty alcohol catalyzed by fatty aldehyde reductase [11]. A study reported the possibility of single step fatty alcohol production by an enzyme analogous to fatty

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Nomenclature

ΔG°	Standard Gibbs's free energy (kJ mol ⁻¹)
ΔG_R	Overall Gibb's free energy change of the reaction (kJ mol ⁻¹)
ΔG_R°	Standard Gibb's free energy of the reaction (kJ mol ⁻¹)
ΔG_i°	Standard Gibb's free energy of formation of ith species (kJ mol ⁻¹)
a_i	Activity of ith species in solution
C	Cost of biomass (Rs kg ⁻¹)
C _C	Fraction of carbon content in the biomass
C _i	Molarity of ith species
C _M	Maximum acceptable biomass cost (Rs kg ⁻¹)
E _P	Economic potential (Rs)
F	Fraction of cost which represents the downstream processing costs
I	Ionic strength
M	Molality
M _A	Amount of biomass (g)
M _C	Atomic weight of carbon (g mol)
M _{C, go}	Carbon left in gaseous phase as CO ₂ at the end of batch study (g)
M _{C, in}	Mass of carbon supplied as CO ₂ (g)
M _{C,bo}	Carbon assimilated as biomass (g)
M _{C,CO2(l)}	Dissolved CO ₂ in aqueous phase (g)
M _{CO2}	Molecular weight of CO ₂ (g mol)
P	Total cost (Rs)
P _M	Total cost for the process using biomass as raw material (Rs kg ⁻¹)
P _{Max}	Biomass productivity (g L ⁻¹ d ⁻¹)
P _R	Cost of raw material as coconut oil (Rs)
Q _r	Product of the activities
R	Experimentally obtained actual product recovery (%)
R.R _{CO2}	Actual CO ₂ (g) utilization efficiency (%)
R _{CO2}	CO ₂ fixation rate (g L ⁻¹ d ⁻¹)

$v_{i,r}$	Stoichiometric reaction coefficient of the ith species
W _F	Weight of fatty alcohol obtained (g)
X _{Max}	Maximum biomass concentration (g L ⁻¹)
X _o	Biomass concentration at the initial (t _o) (g L ⁻¹)
X _t	Biomass concentration at the end of the cultivation period (t ₁) (g L ⁻¹)
Y	Factor correcting for the differences in the solvent recovery costs
y _{CO2, final}	Final (t = tx) CO ₂ (g) concentration (% v/v)
y _{CO2, in}	Initial (t = 0) CO ₂ (g) concentration (% v/v)
Z	Mass fraction of fatty alcohol in the biomass
Z _i	Charge present on the ionic species "i" in the solution

Greek Letters

α	Hydrated ion radius (pm)
Γ	Activity coefficient
η_{CO2}	CO ₂ mitigation efficiency
μ	Specific growth rate (d ⁻¹)

Abbreviations

ANOVA	One-way analysis of variance
ATR	Attenuated total reflectance
CCU	Carbon capture and utilization
FT-IR	Fourier transform infrared spectroscopy
GC-MS	Gas chromatography and mass spectroscopy
MSM	Minimal salt medium
OD	Optical density
SD	Standard deviation
SSL	Sambhar Salt Lake
CMP	Current market price
MAP	Maximum acceptable price
PLC	Programmable logic controller

acyl reductase (in eukaryotes) and is isolated from bacterium *Marinobacter aquaeolei* VT8 [12]. These studies have suggested that bacteria are capable of synthesizing long chain fatty alcohols.

At present, production of fatty alcohols using microorganisms gaining importance due to its economic competitiveness with the existing processes. Few studies have reported the production of fatty alcohols from genetically engineered bacterium utilizing different organic compounds as carbon substrate [10,13–15]. However, the employment of these bacterial strains for the production of fatty alcohols at industrial level is still under investigation.

In recent years, on the lines of CCU world community has focused towards the development of integrated bio-based green technology known as bio-refinery [16]. It involves the integration of CO₂ bio-mitigation system with suitable downstream processing strategy for the recovery of valuable chemicals [2]. However, most of the work related to conceptualize the bio-refinery using CO₂(g) as substrate is based on microalgae [16–18]. But, the commercial exploitation of micro-algae within bio-refinery concept still requires a dedicated research as the major challenges faced include: 1) low photosynthetic efficiency 2) hindrance of growth in presence of flue gas, 3) associated high energy penalty (light utilization), 4) slow growth rate and 5) high cost associated with harvesting and dewatering [19].

Few recent studies have investigated the prokaryotes for their ability to capture CO₂ in the absence of light and its simultaneous conversion into valuable products [20–22]. Therefore, the capability of bacteria utilizing CO₂ in dark and the production of valuable chemicals within the framework of bio-refinery is an exciting area to think upon and requires serious attention. Presently, very few or almost no studies

have been reported for the development of laboratory scale bacterial CO₂ bio-mitigation system focusing on the commercial production of targeted compounds. Hence, if the strategy for the fatty alcohol production using bacterium capable of utilizing CO₂(g) in absence of light is developed then it has potential to solve the purpose of CCU and will open door for making the concept of cost-effective bio-refinery into reality. This requires the development of cost effective downstream bio-processing methodology. Therefore, the present work deals with the analysis and development of an economic downstream bio-processing strategy for the production of fatty alcohols (C₁₂–C₁₄) using a CO₂ fixing strain isolated from extreme environment of Soda Salt lake. The economics feasibility analysis of developed downstream bio-process methodology is compared with existing bio-based commercial methods for fatty alcohol production.

2. Materials and methods**2.1. Bacterium strain and media**

The present study is carried out using *Halomonas stevensii* KP 163920 isolated from Sambhar Salt Lake (SSL), Rajasthan, India. The isolation, identification and CO₂ fixation ability of *H. stevensii* was already studied and reported [23]. In the present study, the minimal salt medium (MSM) utilized for the growth of *H. stevensii* was taken from the literature and composition (g L⁻¹) was given as: KNO₃ - 1, K₂HPO₄ - 1, NH₄Cl - 3 m M, Na₂S₂O₃ - 100 mM, and NaCl - 35 in distilled water. pH of the MSM was adjusted to 10 using 0.1 M NaOH stock solution. MSM stock solution was prepared, autoclaved and preserved for the

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