https://doi.org/10.30799/jespr.162.19050203 J. Environ. Sci. Pollut. Res. - Volume 5 Issue 2 (2019) 342–344

Share Your Innovations through JACS Directory

Journal of Environmental Science and Pollution Research

Visit Journal at http://www.jacsdirectory.com/jespr

ISSN: 2455-0272

CO_2 Sequestration by Thermophiles with Ethanol Production

Bhatia Ruchi[1], Kataria Rosy[2], Bhatnagar Tripti[2,*]

[1]*Noida International University, Gautam Budh Nagar – 203 201, Uttar Pradesh, India.*
[2]*Codon Biotech Pvt. Ltd., Noida – 201 301, Uttar Pradesh, India.*

ARTICLEDETAILS

Article history:
Received 05 March 2019
Accepted 18 May 2019
Available online 08 June 2019

Keywords:
Thermophiles
Ethanol
CO_2 Sequestration
Flue Gas

ABSTRACT

The present work involved screening of two thermophilic bacterial cultures N2 - *Bacillus vietnamensis* and N3 - *Bacillus stearothermophilus* for production of ethanol as a result of CO_2 sequestration. The selected bacterial strains were initially screened by providing CO_2 gas and later by providing flue gases which are given out due to combustion of coal in industries. Confirmatory analysis of ethanol production done by GC revealed the presence of higher levels of ethanol production when flue gases were directly bubbled in the thermophile containing media. The thermophiles were initially tested for ethanol production at higher temperatures since the industrial flue gases are released at a very high temperature. The study thus establishes the production of ethanol by both the thermophiles bacterial cultures: *Bacillus vietnamensis* and *Bacillus stearothermophilus*.

1. Introduction

Global warming has reached an alarming level due to the changes in global environment [1]. Unprecedented anthropogenic emission of greenhouse gases (GHGs) are probably the cause of the current alteration in climatic pattern across the world [2]. Emission of CO_2 in particular have grown exponentially and the prevailing CO_2 level has surpassed the safety limit of 350 ppm which is said to be dangerous to sustain life on earth [3,4]. Due to industrialization, atmospheric CO_2 is rapidly increasing and the excess contributes significantly to global warming [5].

The climate change that is taking place due to increase in CO_2 concentration is largely irreversible for 1000 years even if the emission stops. Industries related to electricity generation, natural gas processing, cement iron and steel manufacturing are the major contributors to atmospheric CO_2. Sequestration of CO_2 from the industries is today's demand in order to reduce the impact of CO_2 on global warming.

Sequestration strategies adapted so far are broadly divided into Physical or abiotic & Biological or Biotic means. Physical means include injecting the CO_2 into subsurface geological reservoirs [6,7], chemical absorption method [8], membrane technology [9], molecular sieve [10] etc. Physical means have high cost associated with it and so the best alternative are the biological methods.

The biological sequestration can be grouped into two categories, terrestrial and aquatic ecosystem. The word terrestrial ecosystem includes natural and plantation forest crops and grassland food and biomass crops. While algae comprise the dominant photosynthetic organism in aquatic ecosystem and are responsible for major CO_2 sequestration [11,12]. Using algae for CO_2 sequestration has certain disadvantages like; high cost of infrastructure, the limited availability of land space near power plants for algal growth, high CO_2 concentrations results in acidic algae suspensions, thereby stunting algae growth [13,14].

This has thus, led to isolation and studies on chemoautotrophic bacteria which have the ability to fix CO_2. These organism in addition to deriving energy from CO_2, produces other carbon-based product of interest (for e.g. biofuel) from inorganic carbon that is the greenhouse gases or flue gases [15].

The present study aims to optimize ethanol production by thermophilic bacterial cultures which would utilize flue gases and would sequester CO_2 as their carbon and energy source and in turn produce ethanol.

*Corresponding Author:tripti.codonbt@ gmail.com(Bhatnagar Tripti)

https://doi.org/10.30799/jespr.162.19050203

2. Experimental Methods

Two thermophilic bacterial cultures N2 - *Bacillus vietnamensis* and N3 - *Bacillus stearothermophilus* [16] were used in the study to optimize production of ethanol as a result of CO_2 sequestration.

2.1 Fermentation Media

To study the utilization of CO_2 and production of ethanol, different fermentation medias were prepared From the different medias four medias resulted in ethanol production and were named as -FM-I,II,III, IV.

FM I - NaCl - 8 g/L, NH_4Cl - 2 g/L, $MgSO_4$ - 2 g/L, $Cacl_2$ - 0.1 g/L, yeast extract - 10 g/L, glucose - 20 g/L, Na_2HPO_4 – 4.2 g/L, NaH_2PO_4 – 4.7 g/L
FM II - K_2HPO_4 - 1.5 g/L, KH_2PO_4 - 3 g/L, $MgSO_4$- 0.3 g/L, $CaCO_3$ - 0.05 g/L, NH_4Cl - 1.0 g/L, $NiCl_2.6H_2O$ – 2 mg/L, $FeSO_4.7H_2O$ – 1 mg/L, glucose - 20 g/L, yeast - 10 g)
FM III - Tryptone - 20 g/L, yeast ext - 10 g/L, K_2SO_4 -1.3 g/L, $MgSO_4$ - 0.27 g/L, $MnCl_2$ - 0.015 g/L, $FeCl_3$ - 0.007 g/L, citric acid - 0.32 g/L, glucose - 20 g/L
FM IV - NH_4Cl- 0.3 g/L, NaCl - 0.3 g/L, $CaCl_2$ - 0.11 g/L, $MgCl_2$ - 0.1 g/L, yeast- 10 g/L, glucose - 20 g/L, $NaHCO_3$- 0.8 g/L, Na_2HPO_4 - 4.2 g/L, NaH_2PO_4 - 4.7 g/L

2.2 Inoculation and Fermentation for Utilization of CO_2 and Production of Bioethanol

The two Thermophilic bacterial strains namely: N2 - *Bacillus vietnamensis* and N3 - *Bacillus Stearothermophilus* present in slants were subcultured in nutrient broth containing sodium thioglycolate and were inoculated in the different fermentation media and placed in CO_2 chamber at 70 °C and the estimation of bioethanol was done by Nucon gas chromatograph using FID and Porapack columns. To maximise the utilization of CO_2 and production of ethanol by the thermophiles different changes were done in fermentation media FM-IV and accordingly seven sets were prepared.

A1 - FM- IV + 70 °C + aerobic condition.
A2 - FM- IV + 70 °C + aerobic condition for first 24 h & then anaerobic condition in a CO_2 chamber.
A3 - FM- IV + 70 °C + 5 g/1000 mL glucose + aerobic condition for first 24 h & then anaerobic condition in a CO_2 chamber.
A4 - FM- IV + 70 °C + 10 g /1000 mL glucose + aerobic condition for first 24 h & then anaerobic condition in a CO_2 chamber.

A5 - FM- IV + 70 °C + 10 g/1000 mL glucose + anaerobic condition
A6 - FM-IV + 70 °C + 5 g/1000 mL glucose + anaerobic condition
A7 - FM-IV + 70 °C + 5 g/1000 mL glucose + aerobic condition

To maximise the utilization of CO_2 the amount of carbon source glucose was reduced and incubation condition were altered in different sets of fermentation media IV. All the seven sets were inoculated with the strain N2 & N3- *Bacillus vietnamensis* and *Bacillus Stearothermophilus* and kept it in a CO_2 chamber at 70 °C and daily estimation of ethanol was done by gas chromatography. The main aim of the study was Sequestration of CO_2 emitted from the industries related to electricity generation, natural gas processing, cement iron and steel manufacturing so that the impact of CO_2 on global warming could be reduced and some commercially important product can be produced (ethanol). Fermentation media IV was added with 5 g/L of glucose so as to just enhance the growth of the bacterial cultures. To study the effect of flue gases and CO_2 gases in nature, a prototype of flue gas emitter or chimney of an industry was designed. The flue gas was directly bubbled into the fermentation media inoculated with both the thermophiles. Intermittently the media was exposed to H_2 gas also and every day ethanol produced was estimated.

3. Results and Discussion

Initially the two thermophiles when inoculated in fermentation media (FM – I, II, III, IV) showed ethanol production at varying concentrations. *Bacillus vietnamensis* produced lesser amounts of ethanol as compared to *Bacillus Stearothermophilus*. Highest amount of ethanol was produced by *Bacillus Stearothermophilus* (5.38 g/L) in fermentation media IV. This media was further used to standardize and increase the production of ethanol while sequestering CO_2 produced by combustion of coal. The ethanol amounts are shown in Table 1.

Table 1 Ethanol production by thermophiles under different medias

S.No.	Fermentation media	Day of maximum ethanol production	Ethanol production (g/L)	
			N2	N3
1	FM I	3rd	1.17±0.2	1.82 ±0.03
2	FM II	3rd	1.6 ± 0.4	3.06 ±0.12
3	FM III	3rd	1.08 ±0.1	2.89 ±0.11
4	FM IV	3rd	2.04 ±0.45	5.38 ±0.18

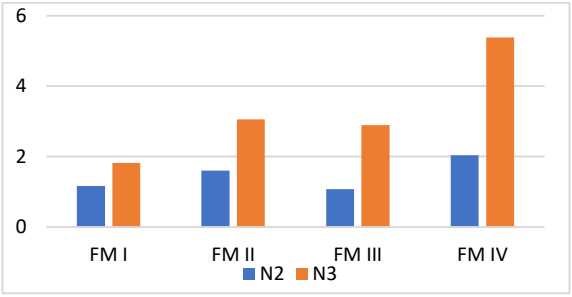

Fig. 1 Comparative ethanol production by thermophiles under different medias

Table 2 Ethanol production by thermophiles under different conditions

S.No.	Fermentation media	Day of maximum ethanol production	Ethanol production (g/L)	
			N2	N3
1	A1	3rd	0.98±0.21	1.63 ±0.07
2	A2	3rd	1.18 ± 0.08	1.13 ±0.02
3	A3	3rd	1.45 ±0.16	2.47 ±0.12
4	A4	4th	0.88 ± 0.14	0.92 ±0.10
5	A5	4th	0.32 ±0.0	0.44 ±0.22
6	A6	4th	0.33 ±0.03	0.26 ±0.1
7	A7	4th	0.56 ±0.10	0.35 ±0.08

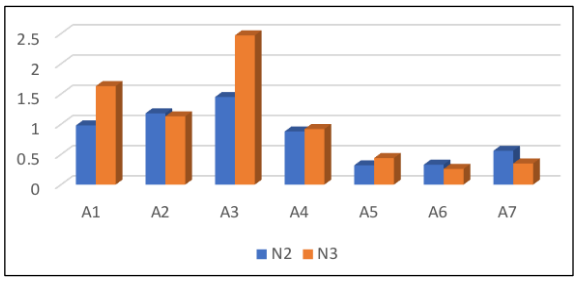

Fig. 2 Ethanol production by thermophiles at different conditions

Fermentation media IV gave the maximum amount of bioethanol production so further optimization of this media was done with lowered glucose level so as to increase the utilization of CO_2. Few alterations in the incubation conditions were also done. Seven sets were prepared and inoculated with the thermophiles.

3.1 Production of Bioethanol using Flue Gases (Atmospheric Pollutant) as Source of CO_2 in Fermentation Media IV

Fermentation media IV with glucose concentration of 5 g/L gave the best result so we proceeded with the same media but the source of CO_2 was flue gases (atmospheric pollutant) produced by combustion of Coal. Strain N2 - *Bacillus vietnamensis* and N3 - *Bacillus stearothermophilus* when inoculated in fermentation media IV with 5 g/L of glucose and kept in a chamber where CO_2 and other flue gases were pumped into the media on the 2nd day of inoculation. Both strain N2 - *Bacillus vietnamensis* as well as strain N3 – *Bacillus stearothermophilus* gave the maximum production of 8.4 g/L and 8.34 g/L ethanol respectively on the 5th day.

Table 3 Ethanol production by thermophiles when flue gases as carbon source

S.No.	Samples	Retention time	Area	Amount of ethanol g/L
1	N2	2.95	2020890	8.40±0.08
2	N3	3.05	1303849	8.34±0.14

The result of the study showed that when the strain N2 - *Bacillus vietnamensis* and N3 – *Bacillus stearothermophilus* were inoculated in fermentation media I and kept in a CO_2 chamber at 70 °C the strain N3 - *Bacillus stearothermophilus* showed the maximum production of 1.82 g/L. For our further studies, in order to enhance the utilization of CO_2 and maximize the production of bioethanol further different fermentation media namely FM- I, FM-II, FM-III, FM-IV were taken. After inoculation and incubation in a CO_2 chamber at 70 °C it was seen that the strain N3 - *Bacillus stearothermophilus* gave a maximum production of 5.38 g/L.

Fermentation media FM-IV was further standardized using lower level of carbon source so that utilization of CO_2 could be maximized. For this seven variants were prepared, the one with lower glucose concentration of 5 g/L and change in incubation condition showed enhanced utilization of CO_2 and N3 - *Bacillus stearothermophilus* produced 2.47 g/L of bioethanol. It was also seen that when the strain N2 - *Bacillus vietnamensis* and N3 - *Bacillus stearothermophilus* were inoculated in fermentation media FM-IV with 5 g/L of glucose and bubbled with flue gas directly from a coal burning unit, the bacterial cultures could sequester the CO_2 into higher concentration of ethanol which could serve as a dual role of reducing the atmospheric pollutant CO_2 and also producing commercially important ethanol. Another study [17], compared the effect of temperature, solvents, and cultural conditions on the fermentative physiology of an ethanol – tolerant (mutant) (56 g/liter at 60 °C) and parent strain of *Clostridium thermohydrosulfuricum*. The mutant produced ethanol at high concentrations and displayed an ethanol/glucose ratio (mole/mole) of 1.0 in media where initial ethanol concentrations were C 4.0% (wt./vol), whereas when ethanol concentration was changed from 0.1% to1.6% (wt./vol), the ethanol/glucose ratio for the parent strain changed from 1.6 to 0.6.

Thermophilic bacteria have many properties making them suitable for second generation ethanol production. Thermophiles degrade a much wider range of carbohydrates as compared with both *S. cerevisiae* and *Z. mobilis* and their cultivation does not require extensive mixing, cooling, or heating of the fermentation vessel. Additionally, direct ethanol recovery from the fermentation broth is possible by in situ vacuum distillation. Apart for the broad operating temperature ranges, thermophiles often tolerate extremes of pH and salt concentrations during fermentation while having low nutritional requirements. Also, thermophiles are generally recognized as safe (GRAS) with all known thermophiles being classified in the lowest microbial risk class [18].

4. Conclusion

From this study it can be concluded that the screened anaerobic thermophilic chemoautotrophs can sequester CO_2 and convert it into useful product: ethanol. Thus, the cultures screened have great capacity of utilizing the CO_2 and other flue gases and producing ethanol. CO_2 produced from power plants could thus be converted into economically important ethanol for industrial uses and thus resulting in reducing the impact of CO_2 on global warming.

References

[1] D.J. Shakun, P.U. Clark, H. Feng, S.A. Marcott, A.C. Mix, Global warming preceded

https://doi.org/10.30799/jespr.162.19050203

by increasing carbon dioxide concentrations during the last deglaciation, Nature 484 (2012) 49-54.
[2] M.E. Huntley, D.G. Redalje, CO_2 mitigation and renewable oil from photosynthetic microbes - A new appraisal, Mitigat. Adapt. Strategies Global Change 12 (2007) 573-608.
[3] L.H. Treut, R. Somerville, V. Cubasch, Y. Ding, C. Mauritzen, et al., Historical overview of climate change, Climate Change 12 (2012) 129-127.
[4] J. Hansen, M. Sato, P. Kharecha, D. Beerling, R. Berner, et al., Target atmospheric CO_2: Where should humanity aim?, Open Atmos. Sci. J. 2 (2010) 217-231.
[5] N.S. Lewis, D.G. Nocera, Powering the planet: Chemical challenges in solar energy utilization, Proc. Nat. Acad. Sci. 103 (2006)15729–15735.
[6] J. Reardon, T. Bucholz, M. Hulvey, J. Tuttle, A. Shaffer, et al., Low energy CO_2 capture enabled by biocatalyst delivery system, Energy Procedia. 63 (2014) 301-321.
[7] H. Herzog, What future for carbon capture and sequestration?, Environ. Sci. Tech. 35(7) (2001) 148A–153A.
[8] H. Herzog, D. Golomb, Carbon capture and storage from fossil fuel use, Encycl. Energy 1 (2004) 1–11.
[9] H. Herzog, The economics of CO_2 separation and capture, Technology 7 (2000) 1-19.
[10] T.D. Burchell, R.R. Judkins, M.R. Rogers, A.M. Williams, A novel process and material for the separation of carbon dioxide and hydrogen sulfide gas mixtures, Carbon 35 (1997) 1279-1294.
[11] M. Giordano, J. Beardall, J.A. Raven, CO_2 concentrating mechanisms in algae: Mechanisms, environmental modulation, and evolution, Annual Rev. Plant Biol. 56 (2005) 99-131.
[12] J.R. Beneman, Mitigation with microalgal systems, Energ. Covers Manage. 8 (1997) 475-479.
[13] K.L. Kadam, Environmental implications of power generation via coal-microalgae cofiring, Energy 27 (2002) 905-922.
[14] I.M. Machado, S. Atsumi, Cynobacterial biofuel production, J. Biotechnol. 162(1) (2012) 50-56.
[15] M.L. Gray, T.Y. Soong, K.J. Champagne, H. Pennline, J.P. Baltrus, et al., Improved immobilized carbon dioxide capture sorbents, Fuel Proces. Tech. 86 (2005) 1449-1455.
[16] R. Bhatia, Deeksha, T. Bhatnagar, Isolation and characterization of thermophiles producing bioethanol, Int. J. Adv. Multidiscip. Res. 3-4 (2016) 32-37.
[17] R.W. Lovitt, R. Longin, J.G. Zeikus, Ethanol production by thermophilic bacteria: Physiological comparison of solvent effects on parent and alcohol-tolerant strains of *Clostridium thermohydrosulfuricum*, Appl. Environ. Microbiol. 48 (1984) 171-177.
[18] M.P. Taylor, K.L. Eley, S. Martin, M.I. Tiffin, S.G. Burton, D.A. Cowan, Thermophilic ethanologenesis: Future prospects for second-generation bioethanol production, Trends Biotechnol. 27 (2009) 398–405.

https://doi.org/10.30799/jespr.162.19050203

Cite this Article as: Bhatia Ruchi, Kataria Rosy, Bhatnagar Tripti, CO_2 sequestration by thermophiles with ethanol production, J. Env. Sci. Pollut. Res. 5(2) (2019) 342–344.