Indian J Microbiol (Oct–Dec 2018) 58(4):529–530
https://doi.org/10.1007/s12088-018-0759-4

SCIENTIFIC CORRESPONDENCE

Beyond the Theoretical Yields of Dark-Fermentative Biohydrogen

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Received: 6 August 2018 / Accepted: 9 August 2018 / Published online: 13 August 2018

Abstract Theoretical hydrogen (H_2) yield by dark fermentative route is 12 mol/mol of glucose. Biological H_2 production yields of 3.8 mol/mol of glucose by microbes have been reported. Transient gene inactivation in combination with adaptive laboratory evolution strategy has enabled the H_2 yield to exceed the stoichiometric production values.

Keywords Hydrogen · Dark fermentation · Sugar · Microbes · Transient gene inactivation · Adaptive laboratory evolution

Fossil fuel reservoirs are depleting faster than they can be replenished. An associated limitation on the usage of these non-renewable fuels is their high pollution causing capacity. The need is to search for novel fuels, which can be based on renewable resources [1]. In addition, it provides an opportunity to identify an ideal fuel. Here, bioalcohol, biodiesel, biohydrogen and methane are the most competitive and ecofriendly fuels [2–11]. Each of them has its own merits and demerits. Biohydrogen has been assessed to be most energy efficient and non-polluting fuels, easy to transport, and can be converted into other forms of energy [1]. Different H_2 production methods include: (i) Thermochemical, (ii) Electrolytic, (iii) Photolytic and (iv) Biological. Biologically, H_2 can be produced by dark- and photo-synthetic microbes from pure sugars and biowastes [2]. Theoretically, 12 mol of H_2 can be generated via the complete oxidation of 1 mol of hexose sugar [12]. However, stoichiometrically, a maximum to 4 mol H_2 per mole of hexose sugar can be achieved. Under dark-fermentative conditions, maximum of 4 and 2 mol of H_2 can be produced depending upon the acetate and butyrate as fermentative byproducts, respectively (Eqs. 1 and 2).

$$\text{Hexose} + 2H_2O \rightarrow 2\text{Acetate} + 4H_2 + 2CO_2 \tag{1}$$

$$\text{Hexose} \rightarrow \text{Butyrate} + 2H_2 + 2CO_2 \tag{2}$$

Overall, dark-fermentative H_2 production rates are significantly higher than those achieved through photo-fermentative process. In the past three decades, limited number of H_2 producers with little significant improvement in the biological H_2 yields have been reported. The dark-fermentative H_2 production by pure cultures such as: *Bacillus*, *Caldicellulosiruptor*, *Clostridium*, *Thermotoga*, and *Enterobacter* has been reported to be up to 3.80 mol/mol of hexose [12–14]. To achieve higher H_2 yields, various strategies have been adopted, including: (i) identification of novel producers, (ii) optimization of process parameters, and (iii) genetic engineering of native producers. In addition, different approaches have been suggested to improve the overall efficiency of the process by integrating various processes such as: dark- and photo-fermentations, polyhydroxyalkanoate production and biomethanation [15–19].

After a few decades of dedicated efforts, it has now been shown that we can go beyond the physiologic yields of dark-fermentative biohydrogen. It is remarkable that genetically modified extremophile *Thermotoga maritima* under dark-fermentation process showed a 1.9-fold higher H_2 yield of 11.54 mol/mol of maltose compared to the wild

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type [20]. Here, the strategy of transient gene inactivation to disrupt lactate dehydrogenase (*ldh*) to block lactate production was combined with adaptive laboratory evolution. After a few passage, strain Tma200 was evolved, which was slow growing and consumed maltose at a lower rate but could oxidize sugar more efficiency. It was found to be very effective in improving the H_2 yield to 5.77 mol/mol of hexose by competing the needs for cell biomass synthesis with metabolite (H_2) formation using maltose as feed. This novel strategy can be extended to other H_2 producers to improve the overall efficiency of the process using biowaste as an economical feed for the sustainable development.

Acknowledgements This work was supported by Brain Pool Grant (NRF-2018H1D3A2001746) by National Research Foundation of Korea (NRF) to work at Konkuk University. This research was also supported by KU Research Professor Program of Konkuk University. This work was supported by the Energy Efficiency & Resources Core Technology Program of the KETEP, granted financial resource from the Ministry of Trade, Industry, & Energy, Republic of Korea (20153010092130).

References

1. Das D, Veziroğlu TN (2001) Hydrogen production by biological processes: a survey of literature. Int J Hydrogen Energy 26:13–28. https://doi.org/10.1016/S0360-3199(00)00058-6
2. Kalia VC, Purohit HJ (2008) Microbial diversity and genomics in aid of bioenergy. J Ind Microbiol Biotechnol 35:403–419. https://doi.org/10.1007/s10295-007-0300-y
3. Patel SKS, Mardina P, Kim D, Kim S-Y, Kalia VC, Kim I-W, Lee J-K (2016) Improvement in methanol production by regulating the composition of synthetic gas mixture and raw biogas. Bioresour Technol 218:202–208. https://doi.org/10.1016/j.biortech.2016.06.065
4. Patel SKS, Lee JK, Kalia VC (2016) Integrative approach for producing hydrogen and polyhydroxyalkanoate from mixed wastes of biological origin. Indian J Microbiol 56:293–300. https://doi.org/10.1007/s12088-016-0595-3
5. Patel SKS, Selvaraj C, Mardina P, Jeong J-H, Kalia VC, Kang Y-C, Lee J-K (2016) Enhancement of methanol production from synthetic gas mixture by *Methylosinus sporium* through covalent immobilization. Appl Energy 171:383–391. https://doi.org/10.1016/j.apenergy.2016.03.022
6. Patel SKS, Lee JK, Kalia VC (2017) Dark-fermentative biological hydrogen production from mixed biowastes using defined mixed cultures. Indian J Microbiol 57:171–176. https://doi.org/10.1007/s12088-017-0643-7
7. Patel SKS, Singh R, Kumar A, Jeong J-H, Jeong S-H, Kalia VC, Kim I-W, Lee J-K (2017) Biological methanol production by immobilized *Methylocella tundrae* using simulated biohythane as a feed. Bioresour Technol 241:922–927. https://doi.org/10.1016/j.biortech.2017.05.160
8. Patel SKS, Kondaveeti S, Otari SV, Pagolu RT, Jeong SH, Kim SC, Cho BK, Kang YC, Lee JK (2018) Repeated batch methanol production from a simulated biogas mixture using immobilized *Methylocystis bryophila*. Energy 145:477–485. https://doi.org/10.1016/j.energy.2017.12.142
9. Patel SKS, Kumar V, Mardina P, Li J, Lestari R, Kalia VC, Lee J-K (2018) Methanol peoduction from simulated biogas mixtures by co-immobilized *Methylomonas methanica* and *Methylocella tundrae*. Bioresour Technol 263:25–32. https://doi.org/10.1016/j.biortech.2018.04.096
10. Patel SKS, Lee JK, Kalia VC (2018) Nanoparticles in biological hydrogen production: an overview. Indian J Microbiol 58:8–18. https://doi.org/10.1007/s12088-017-0678-9
11. Prakash J, Sharma R, Patel SKS, Kim IW, Kalia VC (2018) Biohydrogen production by co-digestion of domestic wastewater and biodiesel industry effluent. PLoS ONE 13:e0199059. https://doi.org/10.1371/journal.pone.0199059
12. Patel SKS, Kumar P, Kalia VC (2012) Enhancing biological hydrogen production through complementary microbial metabolisms. Int J Hydrogen Energy 37:10590–10603. https://doi.org/10.1016/j.ijhydene.2012.04.045
13. Kumar P, Patel SKS, Lee JK, Kalia VC (2013) Extending the limits of *Bacillus* for novel biotechnological applications. Biotechnol Adv 31:1543–1561. https://doi.org/10.1016/j.biotechadv.2013.08.007
14. Kalia VC, Chauhan A, Bhattacharyya G, Rashmi (2003) Genomic databases yield novel bioplastic producers. Nat Biotechnol 21:845–846. https://doi.org/10.1038/nbt0803-845
15. Kalia VC, Lal S, Ghai R, Mandal M, Chauhan A (2003) Mining genomic databases to identify novel hydrogen producers. Trends Biotechnol 21:152–156. https://doi.org/10.1016/S0167-7799(03)00028-3
16. Reddy CSK, Ghai R, Rashmi KVC (2003) Polyhydroxyalkanoates: an overview. Bioresour Technol 87:137–146. https://doi.org/10.1016/S09608524(02)002122
17. Porwal S, Kumar T, Lal S, Rani A, Kumar S, Cheema S, Purohit HJ, Sharma R, Patel SKS, Kalia VC (2008) Hydrogen and polyhydroxybutyrate producing abilities of microbes from diverse habitats by dark fermentative process. Bioresour Technol 99:5444–5451. https://doi.org/10.1016/j.biortech.2007.11.011
18. Patel SKS, Singh M, Kumar P, Purohit HJ, Kalia VC (2012) Exploitation of defined bacterial cultures for production of hydrogen and polyhydroxybutyrate from pea-shells. Biomass Bioenergy 36:218–225. https://doi.org/10.1016/j.biombioe.2011.10.027
19. Patel SKS, Kumar P, Singh S, Lee JK, Kalia VC (2015) Integrative approach to produce hydrogen and polyhydroxybutyrate from biowaste using defined bacterial cultures. Bioresour Technol 176:136–141. https://doi.org/10.1016/j.biortech.2014.11.029
20. Singh R, White D, Demirel Y, Kelly R, Noll K, Blum P (2018) Uncoupling fermentative synthesis of molecular hydrogen from biomass formation in *Thermotoga maritima*. Appl Environ Microbiol. https://doi.org/10.1128/AEM.00998-18