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FERMENTATIVE BIOHYDROGEN PRODUCTION BY ANAEROBIC, THERMOPHILIC BACTERIUM *Thermoanaerobacterium aciditolerans* Trau DAt ISOALTED FROM VIETNAM

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ABSTRACT: *Thermoanaerobacterium aciditolerans* bacterium Trau DAt isolated in Vietnam had the hydrogen production capability under the anaerobic condition at 55^{o}C. Using glucose, the dark fermentation of strain Trau DAt for hydrogen production was performed in three different scales: the flask scales under the suitable (1) and optimal (2) conditions, and the automatic fermentor system Bio-Flo 110 (3). Under the suitable condition, the strain Trau DAt produced 330 ml volume total gas (L-1) and hydrogen occupied 42.95% of gas total. Under the optimal condition, the maximum volume total gas of 701 ml (L-1) was obtained and hydrogen occupied of 77.2% total volume gas. Based on results of RSM analysis and pH controlled examination, the dark fermentation of strain Trau DAt was performed under automatic fermentor system scale (Bio-Flo 110). 2.64 L total gas (L-1) was obtained by consuming 92.58% glucose and hydrogen volume occupied 94.85% of gas total. The maximum hydrogen yield of strain Trau DAt was 1.63 mol H2 (mol glucose)-1. Obtained results showed the remarkable potentiality of Trau DAt strain in application to the larger fermentation scale for biohydrogen production in Vietnam.

Keywords: *Thermoanaerobacterium aciditolerans*, anaerobic, biohydrogen, dark fermentation, thermophilic bacteria, Vietnam.

INTRODUCTION

Hydrogen is widely recognized as a clean and efficient energy resource for future. It is the only common fuel that is not chemically bound to carbon. When hydrogen burns in air, it gives off nothing worse than water vapor and heat energy. Therefore, burning hydrogen does not contribute to greenhouse effect, ozone depleting and acid rain. The capability for H_2 formation is widespread among microorganisms, but only a few of them have been investigated with a focus on biohydrogen production.

Both photosynthetic microorganism and fermentative bacteria can produce hydrogen. However, photobiological hydrogen production including photoautotropic and photoheterotrophic microorganism (purple-non-surfur-PNS) requires wide land to set up, need light along metabolism, and PNS lack the capacity for the efficient conversion of sugar to hydrogen [9]. On the other hand, fermentative bacteria represent a promising means not only to reclaim energy from wastes in the form of hydrogen but also to utilize the wastes as sources. In addition, the dark fermentation using fermentative bacteria has many advantages: (1) It can produce H_2 all day long without light; (2) A variety of carbon sources can be used as substrates even biomass; (3) It produces valuable metabolites such as butyric, lactic and acetic acids as by products; (4) It is anaerobic process, so there is no O_2. Thus, the dark fermentation for hydrogen production is the best choice for commercial biohydrogen production [1, 4, 5, 9, 12, 14, 20, 23].

Production of biohydrogen through microbial fermentation is well known processes in which thermophiles have many advantages compared to mesophilic microorganisms concerning fast growth rates and their ability to degrade a broad variety of substrates. Furthermore, many thermophiles produce fewer types of undesired end products compared to mesophiles [18, 20]. These advantages make the application of thermophiles for H2 production economical and technical feasible. By some above properties, hydrogen production of fermentative thermophilic bacteria has received

more and more attention [2, 8, 15, 16, 17, 19, 24].

Although a limited number of thermophilic bacteria can convert carbohydrate into H_2 with a satisfactory yield and productivity, an anaerobic fermentative hydrogen production process can be conducted by either pure cultures or mixed cultures. However, there are only few studies have been done by pure cultures of anaerobic, thermophilic bacteria to indicate the conversion of carbohydrates to hydrogen gas. High values of hydrogen produced per mol of utilized glucose have been reported by the hyperthermophiles *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii* [5, 20]. The hydrogen yields by thermophilic, anaerobic bacterium *Thermoanaerobacterrium aciditolerans* AK17 were up to 1.1 mol H_2 (mol glucose)-1 and 1.0 mol H_2 (mol xylose)-1. The maximum H_2 production yield was 2.53 mol H_2 (mol hexose)-1 by *Thermonanaerobacterium thermosaccharolyticum* PSU-2 [9, 12]. Results of earlier studies showed that anaerobic, thermophilic bacteria had a great potential to produce hydrogen in pure cultures. In this research, the hydrogen production capability of anaerobic, thermophilic bacterium *Thermoanaerobacterium aciditolerans* Trau DAt isolated in Vietnam was reported.

MATERIAL AND METHODS

Strain and Medium

The bacterium *Thermoanaerobacterium aciditolerans* Trau DAt belonging to culture collection of IBT (Institute of Biotechnology), VAST (Vietnam Academy of Science and Technology) was used in this study.

The basic medium used for enrichment and cultivation of H_2 producing bacterium *Thermoanaerobacterium aciditolerans* Trau DAt was NMV medium [7].

Cultivation and Analyses

Fermentation under the suitable condition: Experiments were performed in 600 ml serum bottles that contained 600 ml of suitable medium based on our previous result [22].

Fermentation under the optimal condition: Experiments were performed in 600 ml serum bottles that contained 600 ml of suitable medium based on our previous report [21].

Controlled pH experiments: T. aciditolerans Trau DAt was cultured in two 150 ml serum bottles which contained 150 ml medium under optimal condition with initial pH 6.5. pH media of fermentative processes were estimated 4 h per time. When the strain grew, pH would be decreased. When pH reduced at pH 6.0, one bottle was keeping at constant value pH 6.0, the other was not controlled pH during fermentative process.

Fermentation under the 7 L automatic fermentor system scale (Bio-Flo 110): Experiments for hydrogen fermentation was performed in automatic fermentor system with 7 L fermentation solution under optimal condition. pH was controlled during fermentative process (initial pH at 6.5 and then, controlled pH at 6.0) by using NaOH 3M.

For three above fermentative processes, 10% inoculums (v/v) that harvested after 16 h of pre-cultivation were added as inoculums. Fermentative processes were performed at 55^oC in anaerobic condition. The evolved gas mixture was collected in gas collector at normal temperature and atmospheric pressure. Bacteria growth and glucose consumption, gas volume were estimated during fermentative processes by OD measurement, DNS assay [10] and water displacement method, respectively. The gas products were analyzed by gas chromatography GC-TCD (Thermo Trace GC-Thermo Electro-USA) with a thermal conductivity detector. The batch experiments were continued until hydrogen production ceased.

RESULTS AND DISCUSSION

Biohydrogen production by dark fermentation under suitable condition

Based on results of suitable condition study [22], the dark fermentation by *Thermoanaerobacterium aciditolerans* Trau DAt was performed under the suitable condition at flask scale. Results showed that the strain Trau DAt entered stationary phase after 25h

cultivation and consumed about 7.3 g (L)-1 glucose (initial glucose concentration was 10 g (L)-1) (fig. 1). 330 ml volume total gas obtained and hydrogen volume was 141.7 ml (L)-1, occupying 42.95% total gas producer. Volume of 330 ml total gas was stabled until ending 28h fermentation (fig. 1). Hydrogen volume achievements in suitable condition were not as high as many earlier reports [2, 3, 13, 14, 16].

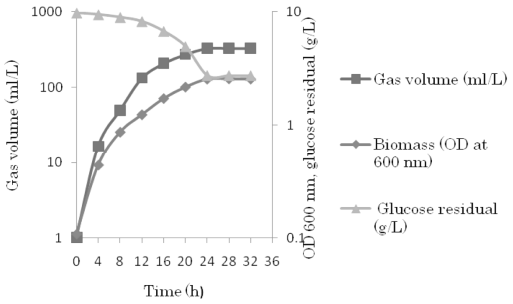

Figure 1. Capability of growth, hydrogen production, glucose degradation under suitable condition

Biohydrogen production by dark fermentation under optimal condition

Since the hydrogen yield of strain Trau DAt in suitable condition was not high in comparison to some other thermophilic strains, the response surface methodology (RSM) with central composite design was applied for three important factors including glucose, yeast extract, iron concentration to enhance hydrogen production yield. Experimental results showed that glucose, yeast extract and iron concentration all had significant influences and had a significant interactive effect on the hydrogen production potential. Based on RSM analysis, the optimal medium was NMV medium with optimal glucose, yeast extract, iron concentration [21]. Then, the dark fermentation was done in optimal condition based on the combination between the suitable condition and RSM results [21, 22]. Results in figure 2 showed that H_2 production was accompanied with growth and glucose degradation. H_2 production began when cell growth entered the early exponential phase (4 h) and rate of H_2 production reached a maximum in the late exponential phase. The volume of produced H_2 was high in the late exponential phase and early stationary phase. Strain Trau DAt consumed about 11 g $(L)^{-1}$ glucose (initial glucose concentration was 12 g $(L)^{-1}$) and produced 701 ml volume total gas per volume of 1 L media, volume of H_2 was 541 ml, occupying 77.2% total volume gas (fig 2). Hydrogen production of strain Trau DAt is still lower than the maximal hydrogen value of *T. thermosaccharolyticum* PSU-2, but higher than volume of H_2 producing by *Clostridium saccharoperbutylacetonicum* ATCC at the same glucose concentration [6, 12]. Volume of H_2 was produced under optimal condition was higher 3.8 fold than one was produced under suitable condition. This confirmed again that the capability of hydrogen production by Trau DAt highly depended on cultivation condition. It also indicated that RSM is a useful method to enhance the hydrogen production yield by bacterium *T. aciditolerans* Trau DAt. Furthermore, it was clear that the more improvable fermentative condition, the higher H_2 yield of *T. aciditolerans* Trau DAt was obtained.

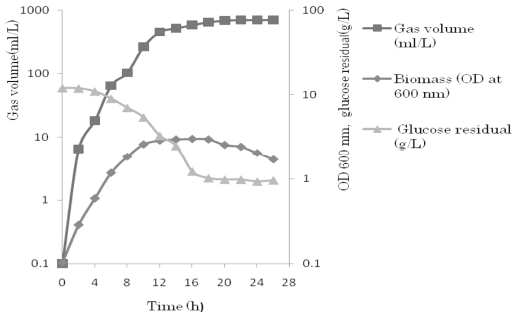

Figure 2. Capability of growth, hydrogen producer, glucose degradation under optimal condition at flask scale

Hydrogen production under controlled pH condition

Early research showed that capability of hydrogen producing bacteria depends on not only nutritional factors but also pH. pH is one of the most important factors in hydrogen production due to its effects on FeFe-hydrogenase activity, metabolic pathways, and

the duration of lag phase. Hydrogen fermentation processes produce by- product, which reduce pH of culture media [1, 7]. Khanal et al. (2004) reported that low pH values of 4.0-4.5 cause longer lag period. On other hand, high initial pH values such as 9.0 decrease lag time but have a lower yield of hydrogen production [7, 24].

Bacterium Trau DAt was cultured under optimal condition with maintainable pH and non-maintainable pH during fermentative processes. Results in figure 3 showed that pH of culture media started to reduce at pH 6.0 after 12 h. At this time, this train entered mid exponential phase, it also was time to produce H_2. Therefore, maintainable pH 6.0 was performed in controlled pH case. In non-controlled pH case, hydrogen fermentative process of *T. aciditolerans* Trau DAt was dropped when pH medium was reached to pH 4.0. In opposite case, H_2 yield obtained higher. 700 ml volume gas (L)-1 was produced in controlled pH case whereas 500 ml volume gas (L)-1 was released in non-controlled pH case. It implied that hydrogen fermentation condition was favorably maintained by pH control in the cultures. Stable pH 6.0 was optimal for *T. aciditolerans* Trau DAt to grow and produce H_2. Alalayah et al (2009) also reported that the maximum rate of hydrogen production of *Clostridium saccharoperbutylacotonicum* N1-4 was measured at pH 6.0 while the min rate of hydrogen production was recorded at pH 4.0 [1].

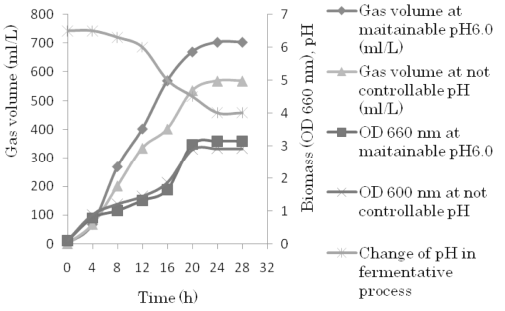

Figure 3. Capability of growth, hydrogen production with controlled pH and non-controlled pH under optimal condition

Dark fermentation for hydrogen production at fermentor scale

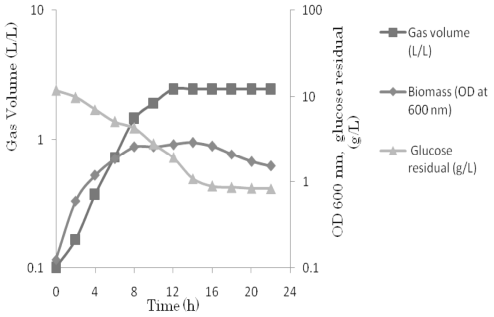

Figure 4. Capability of growth, hydrogen producer, glucose degradation under optimal condition in automatic fermentor system

Based on above result and our previous reports [21, 22], the dark fermentation of strain Trau DAt was carried out at automatic fermentor system scale (Bio-Flo 110) under condition: 12 g $(L)^{-1}$ glucose, 2.5 g $(L)^{-1}$ yeast extract, 400mg $(L)^{-1}$ $FeSO_4.7H_2O$, NaCl 0.5%, meat extract 0 g $(L)^{-1}$, with 10% inoculums (v/v), initial pH at 6.5 and then pH was automatically controlled at 6.0 during fermentative process. Result showed that lag time lasted about 2 hours (fig. 4). It meant that initial pH at 6.5 was suitable for starting hydrogen fermentation. Exponential phase lasted about 12 hours, H_2 was highly produced during this phase. This strain consumed 11.05 g $(L)^{-1}$ glucose (92.58%) to produce 2.64 L total gas $(L)^{-1}$. The gas chromatography GC-TCD analysis showed that hydrogen volume was 2.50 L $(L)^{-1}$, occupied 94.85% of gas total. It showed that H_2 gas component obtained at automatic fermentor system was much higher than those at flask scale. It meant that the automatic controlled system was better than non-automatic controlled one for hydrogen production of the strain Trau DAt. These results also indicated that the maximum hydrogen yield of strain Trau DAt was 1.63 mol H_2 (mol glucose)$^{-1}$. Maximum H_2 production yield from different reported strains were compared with that of strain Trau DAt. *Thermotoga elffi* [5], *Calidicellulosiruptor saccharolyticus* [20], *C. thermocellum* [10], *C. thermolacticum* [3],

T. thermosaccharolyticum [12] and *T. aciditolerans* AK17 [8] were known of process H_2 producing abilities under thermophilic and hyperthermophilic conditions corresponding to 2.7, 3.3, 1.95, 1.5, 2.53 and 1.1 mol $(hexose)^{-1}$, respectively. Strain Trau DAt had higher H_2 yield than those of *C. thermolacticum, T. aciditolerans* AK17.

CONCLUSION

Obtained results of the present study showed that the hydrogen production capacity of *Thermoanaerobacterium aciditolerans* bacterium Trau DAt highly depended on fermentation condition. At flask scale, strain Trau DAt produced 330 ml volume total gas, approximately 141.7 ml H_2 $(L)^{-1}$ media and 701 ml volume total gas, in proportion 541 ml H_2 $(L)^{-1}$ media under suitable and optimal condition, respectively. Hydrogen fermentation condition was favorably maintained by controlled pH at pH 6.0. The maximum volume of total gas produced by the strain Trau DAt was 2.64 L corresponding to 2.50 L H_2 $(L)^{-1}$, equivalent to 1.63 mol H_2 $(mol\ glucose)^{-1}$ under the optimal condition and maintainable pH 6.0 in automatic fermentor system.

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REFERENCES

1. Alalavah M. W., Kalil S. M., Kadhum H. A., Jahim M .J., Alauj M. N., 2009. Effect of Environmental Parameters on hydrogen Production using *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564). American J. Environ. Sci., 5 (1): 80-86.
2. Amorim C. L. E., Sader T. L., Silva, L. E., 2011. Effect of Substrate Concentration on Dark Fermentation Hydrogen Production Using an Anaerobic Fluidized Bed Reactor. Appl. Biochem. Biotechnol DOI, 10.1007: 9511-9519.
3. Collet C., Adler N., Schwitzguebel J. P., Peringer P., 2004. Hydrogen production by *Clostridium thermolacticum* during continuous fermentation of lactose. Int. J. Hydrogen Energy, 29: 1479-1485.
4. Defeng X., Nanqi R., Manli G., Jianzheng L., Qiubo L., 2005. Monitoring of microbial community structure and succession in the biohydrogen production reactor by denaturing gradient gel electrophoresis (DGGE). Sci. China Ser. C. Life Sci., 48 (2): 155-162.
5. De Vrije T., de Haas G. G., Tan G. B., Keijsers E. R. P., 2002. Classen PAM. Pretreatment of Miscanthus for hydrogen production by *Thermotoga elfii*. Int. J. Hydrogen Energy, 27: 1381-1390.
6. Ferchichi M., Crabbe E., Hintz W., Gil G., Almadidy A., 2005. Influence of culture parameters on biological hydrogen production by *Clostridium saccharoperbutylacetonicum* ATCC 27021. World J. Microbiol. Biotechnol., 21: 855-862.
7. Nguyen Thi Thu Huyen, Dang Thi Yen, Nguyen Thi Yen, Vuong Thi Nga, Lai Thuy Hien, 2012. Using of response surface methodology for optimization of biohydrogen production by Clostridium sp. Tr2 isolated in Vietnam. Tap chi Sinh hoc, 34(4): 497-484.
8. Khanal S. K., Chen W. H., Li L., Sung S., 2004. Biological hydrogen production: effects of pH and intermediate products. Int. J. Hydrogen Energy, 29: 1123-1131.
9. Koskinen P. E. P., Beck S. R., Orlygsson J., Puhakka J. A., 2008. Ethanol and hydrogen production by two thermophilic, ananerobic bacteria isolation from Icelandic geothermal areas. Biotechnol. Bioeng., 101: 679-690.
10. Levin D. B., Islam R., Cicek N., Sparling R., 2006. Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates. Int. J. Hydrogen Energy, 31: 1496-1503.

11. Miller G. L., 1959. Use of dinitrosalicylic acid reagent for determination of reducing sugar. Anal. Chem, 31(3): 426-428.
12. O-Thong S., Prasertsan P., Karakasshev D., Angelidaki I., Thermophilic fermentative hydrogen production by newly isolated *Thermonanaerobacterium thermosaccharo-lyticum* PSU-2. Int. J. Hydrogen Energy, 33: 1204-1214.
13. Puhakka J. A., Karadag D., Nissila, E. M., 2012. Comparison of mesophilic and thermophilic anaerobic hydrogen production by hot spring enrichment culture. Int. J. Hydrogen Energy, 37(21): 16453-16459.
14. Ren Q. N., Wang Y. D., Yang P. C., Wang L., Li. F. Y., 2010. Selection and isolation of hydrogen-producing fermentative bacteria with high yield and rate and its bioaugmentation process. Int. J. Hydrogen Energy, 35(7): 2877-2882.
15. Romano I., Dipasquale L., Orlando P., Lama L., Ippolito G., Pascual J., Gambaccorta A., 2010. *Thermoanaerobacterium thermostercus* sp. nov., a new anaerobic thermophilic hydrogen-producing bacterium from buffalo-dung. Extremophiles 14(2): 233-240.
16. Saratale D. G., Chen D. S., Lo C. Y., Saratale G. R., Chang S. J., 2008. Outlook of biohydrogen production from lignocellulosic feedstock using dark fermentation-a review. J. Sci. Indust. Res., 67: 962-979.
17. Sigurbjornsdottir A. M., Orlygsson J., 2012. Combined hydrogen and ethanol production from sugars and lignocellulosic biomass by *Thermoanaerobacterium* AK54, isolated from hot spring, Appl. Energy, 97: 785-791.
18. Jame R., Vilimova V., Lakatos B., Verecka L., 2011. The hydrogen production by anaerobic bacteria grown on glucose and glycerol. Acta. Chimica. Slovaca, 4(2): 145-157.
19. Ueno Y., Sasaki D., Fukui H., Haruta S., Ishii M., Igarashi Y., 2006. Changes in bacterial community during fermentative hydrogen and acid production from organic waste by thermophilic anaerobic microflora. J. Appl. Microbio., 101(2): 331-343.
20. van Niel E. W., Budde M. A., de Haas G. G., van der Wal F. J., Claassen P. A., Stams A. J., 2002. Distinctive properties of high hydrogen producing extreme thermophiles, *Caldicellulosiruptor saccharolyticus* and *Thermotoga elfii*. Int. J. Hydrogen Energy, 27: 1391-1398.
21. Nguyen Thi Yen, Lai Thuy Hien, Nguyen Thi Thu Huyen, 2013. Optimization of medium components for hyrogen production of anaerobic bacterial strain Thermoanaerobacterium aciditolerans Trau DAT isolated from Vietnam by using response surface methodology. Tap chi Sinh hoc, 35(4): 469-476.
22. Nguyễn Thị Yên, Lại Thúy Hiền, Nguyễn Thị Thu Huyền, 2014. Nghiên cứu khả năng tạo khí hydro sinh học trong điều kiện kị khí của vi khuẩn ưa nhiệt *Thermoanaerobacterium aciditolerans* Trau DAt phân lập ở Việt Nam. Tạp chí Khoa học và Công nghệ, 52(1): 73-82.
23. Yokoyama H., Moriya N., Ohmori H., Waki M., Ogino A., Tanaka Y., 2007. Community analysis of hydrogen-producing extreme thermophilic anaerobic microflora enriched from cow manure with five substrates. Appl. Microbiol. Biotechnol., 77: 213-222.
24. Zhang T., Liu H., Fang H. H. P., 2013. Biohydrogen production from starch in wastewater under thermophilic condition. J. Environ. Manage., 69:149-156.

QUÁ TRÌNH LÊN MEN SINH HYDRO SINH HỌC CỦA VI KHUẨN LÊN MEN KỴ KHÍ, ƯA NHIỆT *Thermoanaerobacterium aciditolerans* Trau DAt, PHÂN LẬP TẠI VIỆT NAM

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TÓM TẮT

Vi khuẩn, *Thermoanaerobacterium aciditolerans* Trau DAt, phân lập tại Việt Nam có khả năng sinh hydro trong điều kiện kỵ khí ở 55^{o}C. Trong nghiên cứu này, quá trình lên men tối sinh hydro của chủng Trau DAt lên men tối sinh hydro được thực hiện ở ba cấp độ khác nhau: (1) lên men bình thí nghiệm trong điều kiện phù hợp, (2) trong điệu kiện tối ưu và (3) lên men trong thiết bị lên men tự động Bio-Flo 110 (5 L). Trong điều kiện phù hợp, chủng Trau DAt tạo được 330 ml (L^{-1}) khí và khí hydro chiếm 42,95% tổng lượng khí thu được. Trong điều kiện tối ưu, lượng khí tối đa thu được là 701 ml (L^{-1}) và khí hydrogen chiếm 77,2%. Sau cùng, quá trình lên men tối sinh hydro của chủng Trau DAt được thực hiện trong bình lên men tự động Bio-Flo 110 (5 L) trong điều kiện lên men tối ưu và pH được kiểm soát ở pH 6,0. Chủng Trau DAt đã tiêu thụ 92,58% lượng glucose ban đầu để sản xuất 2,64 L (L^{-1}) khí và lượng khí hydrogen volume chiếm 94,85% tổng thể tích khí thu được. Sản lượng hydro cao nhất của chủng Trau DAt đạt 1,63 mol H2 (mol glucose)-1. Các kết quả thu được đã chỉ ra tiềm năng đáng kể của chủng Trau DAt trong việc ứng dụng để lên men sản xuất hydro sinh học ở qui mô lớn hơn tại Việt Nam.

Từ khóa: *Thermoanaerobacterium aciditolerans*, hydro sinh học, lên men tối, vi khuẩn kỵ khí, ưa nhiệt, Việt Nam.

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