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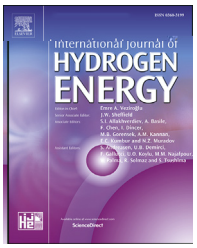

Biohydrogen production with lipid-extracted *Dunaliella* biomass and a new strain of hyper-thermophilic archaeon *Thermococcus eurythermalis* A501

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HIGHLIGHTS

- *T. eurythermalis* A501 *Dunaliella* 2 .
- - *Dunaliella* 2 .
- A 2.5 / - 2:1 2 .
- 192.35 / 2 *T. eurythermalis* A501 .

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ABSTRACT

D *k* *k* - *Dunaliella* - *Thermococcus eurythermalis* A501, 2.5 / 2:1 , 192.35 183.02 / ( ) *Dunaliella primolecta* *D. tertiolecta* , , 19 *k* .

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Introduction

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k 7 .

A ,

8 .

C

Dunaliella

9,10 . *Dunaliella*

k 11,12 .

k 13 ,

Dunaliella ,

15 , - (30), ( ) 600 °C 4 .

Microorganism fermentation

T. eurythermalis A501 - ( ) 22 , ( ) , 1 ; , 4 ; C , 23 ; C ·6 2 , 5 ; (4)2 4, 0.5 ; C , 0.7 ; , 3.3 ; B , 0.05 ; C 2, 0.01 ; C C 2·2 2 (2%), 1 ; 4 (10), 1 ; C 3·6 2 (25), 1 ; 2 4 (6%), 1 ; 2 4 (6%), 1 .

Dark fermentation

B 150 , A . A 85 °C 18 - *T. eurythermalis* A501 (10^6 /). ( ) *Dunaliella* , - . 1. (/) C . 23 .

, - 0.625 10 / , κ 1:5, 1:2, 1:1, 2:1 5:1. A , ± D.

Analytical procedure for hydrogen

A 50 - κ 24 . (C-14B, AD ,) (CD) 1 (D -02, AD ,). A 120 °C, 100 °C. (; /) ( ) ( ). , (. (1)) 25 :

$$= H_m exp\left\{-exp\left[\frac{R_m e}{H_m}(\lambda - t) + 1\right]\right\} \quad (1)$$

, H (/), λ ( ), H_m (/), R_m κ - (/ /), t ( ), (1) = 2.718.

Results and discussion

Microalgal biomass characterization

C κ .

Table 1 – Different added concentrations of dry and wet and lipid-extracted microalgal biomass under the conditions of same initial biomass concentration, same initial carbohydrate concentration and same initial protein concentration.

A	(, /)		
Dunaliella primolecta D	5	5	5
	5	5	5
-	5	3.96	4.2
Dunaliella tertiolecta D	5	5	5
	5	5	5
-	5	2.75	4.85

D *D. primolecta* : D (/) = 0.4909 × D_{680}+0.0955 (^{2} = 0.9903).
D *D. tertiolecta* : D (/) = 0.3573 × D_{680}+0.1340 (^{2} = 0.9954).
D. primolecta *D. tertiolecta* 1.05 / 1.02 / ,
D. primolecta *D. tertiolecta* 1.67 / 1.53 / , .

11,12 . , 12 . k D (2). *D. primolecta* *D. tertiolecta* (20%). (30%), (2). . D. *primolecta* *D. tertiolecta* 26.47% 37.10%, (2), . A - - - 26 . A 2, *Dunaliella* (26.39% 32.85% *D. primolecta* *D. tertiolecta*,). , , k -

Hydrogen production from different algal biomass

- *Dunaliella* *T. eurythermalis* A501,

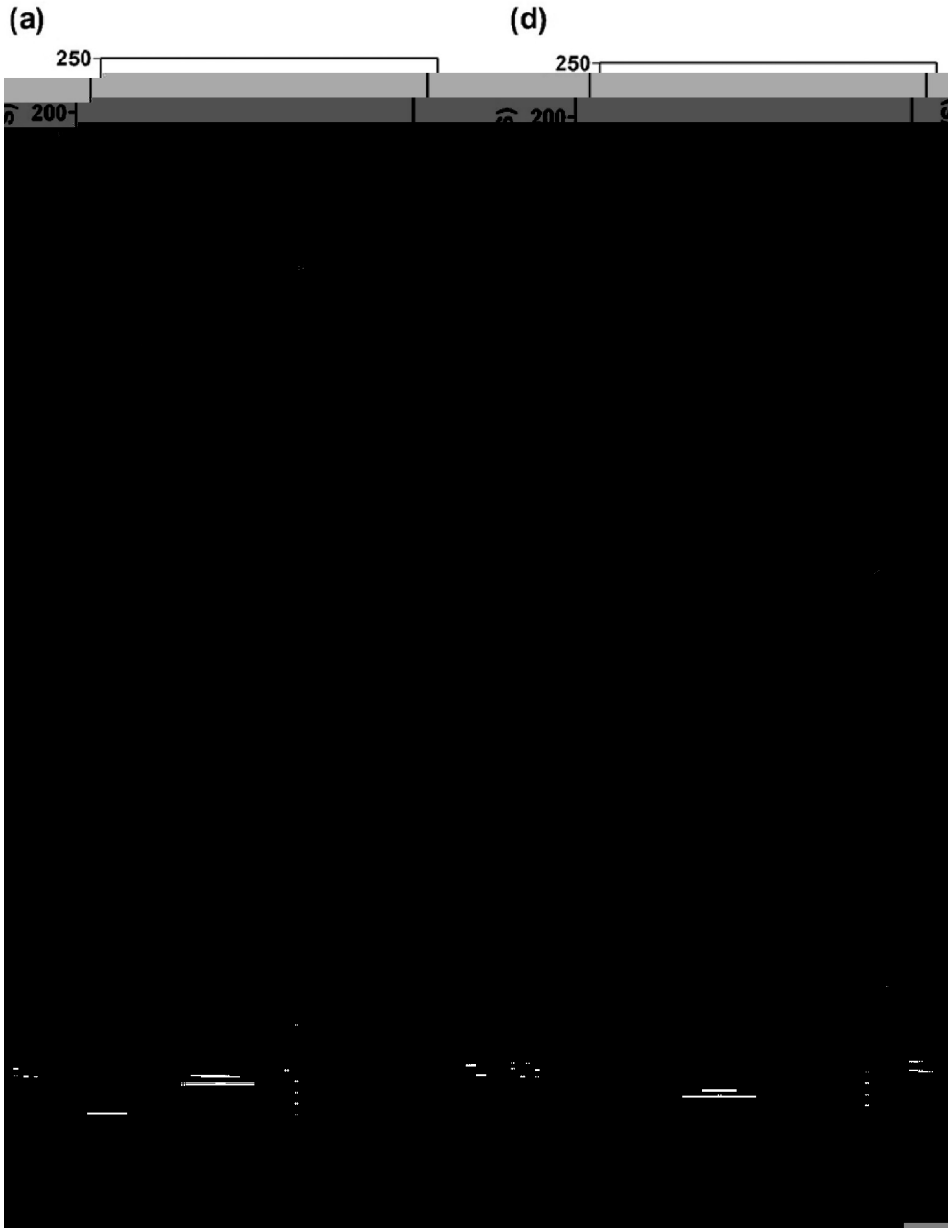

Fig. 1 – SHYs from different algal biomass of *Dunaliella primolecta* (a, b, c) and *D. tertiolecta* (d, e, f) by *Thermococcus eurythermalis* A501. a, d: Different algal biomass under the same initial biomass concentration; b, e: different algal biomass under the same initial carbohydrate concentration; c, f: different algal biomass under the same initial protein concentration.

C

35 ,

( )

36 . ,

Optimization of initial algal concentration for hydrogen production

k 37,38 . , . 2. *D. primolecta* *D. tertiolecta* 0.625 / 2.5 / , (. 2). A 2.5 / , *D. primolecta* *D. tertiolecta* 209.04 / 178.65 / , . 10 / , *D. primolecta* *D. tertiolecta* 97.47 / 94.18 / , . B . 39 *S. obliquus* 2.5 50 / , 56.5 10.8 / . , k . A , 40 , (A) k 41 . A , , k 42 19 , , 43 . 44 *Pyrococcus furiosus*, . A , *P. furiosus* 45 .

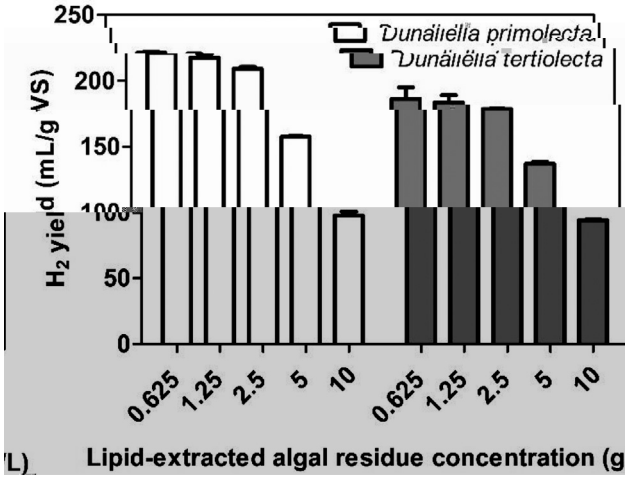

Fig. 2 – Effects of algal residue concentrations on SHY of *Thermococcus eurythermalis* A501.

, k 31 . 0.625 / 2.5 / (. 2). , 2.5 / .

Optimization of initial gas-liquid volume ratio for hydrogen production

46 . , (. 3). 1:5 2:1, (. 3). A 2:1, *D. primolecta* *D. tertiolecta* 1.7- , 1:5. , 47,48 . B . 43 AD , , , 49 .

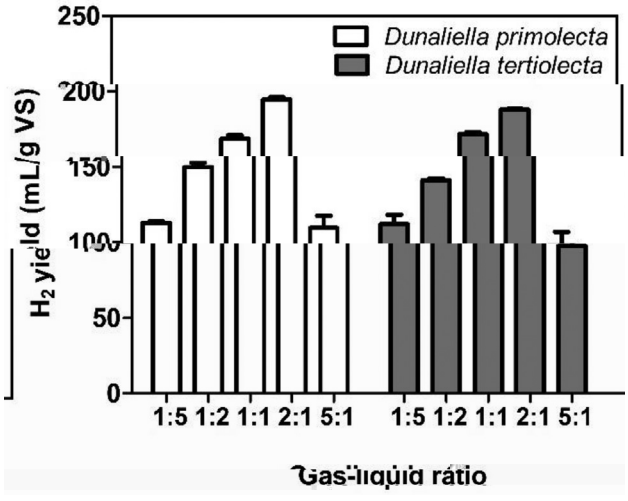

Fig. 3 – SHYs of *Thermococcus eurythermalis* A501 under different initial volume ratios of gas to liquid.

However, as the initial ratio of gas to liquid further increased to 5:1, the SHYs of D. primolecta and D. tertiolecta algal residues signiÞcantly decreased to 109.74 mL/g VS and 97.41 mL/g VS, respectively (Fig. 3). When the reactor volume is Þxed, the further increase of gas-liquid ratio will lead to the decrease of the volume occupied by the fermentation broth, which is not conducive to the growth of hydrogen-producing bacteria.

Overall, the highest SHYs were achieved when the initial volume ratio of gas to liquid was 2:1 (Fig. 3). Therefore, it was decided to maintain the gas-liquid ratio of 2:1 in subsequent experiments.

Biohydrogen production kinetics

In order to further analyze the parameters in the process of dark fermentation and verify the hydrogen production potential of T. eurythermalis A501, kinetic analysis was carried out using a modiÞed Gompertz equation (Eq. (1)). The conditions set in the simulation were the optimal conditions, that is, the algal residue concentration was 2.5 g/L and the initial volume ratio of gas to liquid was 2:1. The hydrogen generation kinetic Þtting results of D. primolecta and D. tertiolecta are shown in Fig. 4.

With the extension of fermentation time, the dynamic curve Þrst went through a lag phase, and then hydrogen production increased rapidly until the hydrogen accumulation Þnally reached a stable phase (Fig. 4). The equation Þts the experimental data well, since the R^2 is higher than 0.99 (Table 3). The estimated kinetic parameters H_m, R_m and l are in Table 3. The maximum SHYs with D. primolecta and D. tertiolecta as substrates for T. eurythermalis A501 were 201.423 mL/g VS and 184.038 mL/g VS, respectively. These values are quite high when compared with previous studies. Yang et al. [50] obtained a maximum SHY of 45.5 mL/g VS using lipid-extracted S. obliquus microalgal residues as a substrate for fermentation by an anaerobic digested sludge. Nobre et al. [30] also reported a SHY of 97.7 mL/g VS using oils and pigments extracted Nannochloropsis sp. microalgal biomass as feedstock through dark fermentation by Enterobacter aerogenes

Short lag periods were also observed during fermentation (6.569 h and 5.652 h when using D. primolecta and D. tertiolecta as substrates, respectively), indicating that T. eurythermalis A501 has strong adaptability to the operating fermentation conditions. To further estimate the time required to complete the fermentation process, the kinetic parameter t_{95}, which represents the time required to complete 95% of the hydrogen production reaction [25], was also estimated by equation (2):

$$t_{95} = l + \frac{H_m}{R_m e}(1 - \ln(0.95)) \quad (2)$$

The values of t_{95} of D. primolecta and D. tertiolecta were 18.807 h and 16.045 h, respectively (Table 3). This is relatively short when compared with previous studies, including one where more than 70 h fermentation time was needed by Clostridium acetobutylicum using A. platensis as feedstock [51]. This is a beneÞcial result considering that short lag period and fermentation time can reduce energy expenditure and increase hydrogen production efÞciency.

Fig. 4 e Biohydrogen production kinetics of lipid-extracted algal residues of Dunaliella primolecta (a) and D. tertiolecta (b) by Thermococcus eurythermalis A501. Kinetic analysis conditions were as follows: microalgal residue concentration 2.5 g/L, initial volume ratio of gas to liquid 2:1.

High SHY and short lag phase obtained by T. eurythermalis A501 using Dunaliella algal residue as a substrate can be related to the advantages of thermophiles. This is consistent with a previous study by Kumar et al. [52], who showed that the lag phase is greatly shortened under thermophilic

Table 3 e Fitting parameters of dark fermentation from Thermococcus eurythermalis A501 combined with Dunaliella algal residue substrates. Kinetic analysis conditions were as follows: microalgal residue concentration 2.5 g/L, initial volume ratio of gas to liquid 2:1.

Parameter	Dunaliella primolecta	Dunaliella tertiolecta
H_m (mL/g VS)	201.423 ± 4.720	184.038 ± 1.887
R_m (mL/g VS/h)	24.040 ± 2.071	25.864 ± 1.216
l (h)	6.569 ± 0.362	5.652 ± 0.175
t_{95} (h)	18.807	16.045
R^2	0.996	0.999

conditions, and therefore more suitable for hydrogen production than under mesophilic conditions. In addition, Dunaliella algal biomass, which lacks a cell wall, may also promote substrate utilization by T. eurythermalis A501, thus increasing the SHY.

Effects of autoclave pretreatment on hydrogen production

It is necessary to sterilize the culture medium before a fermentation reaction when the hydrogen-producing microorganism is in pure culture. In this study, Dunaliella biomass did not undergo any pretreatment, except the autoclaved process necessary for fermentation medium (with addition of microalgal biomass). This study used a new hyperthermophilic archaeon, T. eurythermalis A501 as hydrogen-producing catalyst, which may have the advantage of preventing contamination due to the high temperature (85 C) maintained by its fermentation process. In this case, the effect of autoclave pretreatment was investigated (Fig. 5).

The SHYs of D. primolecta and D. tertiolecta without autoclave sterilization were 192.35 mL/g VS and 183.02 mL/g VS, respectively (Fig. 5). The SHYs were only slightly lower than that with sterilization, indicating that the high SHY was almost unaffected by autoclave treatment. This can be explained by the high temperature maintained during fermentation. In addition to preventing contamination, the purpose of autoclaving Dunaliella biomass is to destroy cell structure and hydrolyze the released polysaccharides into monosaccharides [53,54]. Dunaliella have no cell walls, meaning they do not require drastic pretreatment. Moreover, the oil extraction process also destroyed the structure of Dunaliella cells, leading to easier release of intracellular compounds. During dark fermentation, maintaining a high temperature can also promote the utilization of algal residue by T. eurythermalis A501. The results suggested that the temperature of the fermentation process may be high enough that the algal residue is fully utilized by T. eurythermalis A501, and thus high SHYs were obtained.

Dark fermentation can be inoculated with either single or mixed culture. Compared with single culture, mixed culture such as anaerobic sludge is easier to use because of its simple operation and because sterilization is not required [55]. Sterilization on an industrial scale is economically inefficient. However, special treatments are still needed for the initial inoculum of mixed culture, so as to select hydrogen-producing microorganisms and inhibit the activity of other competitors or hydrogen consumers. This was confirmed by the work of Cai and Wang, who observed that the hydrogen production effect of pre-treated intertidal sludge was significantly better than that of untreated sludge [56].

At present, the most common treatment method is heat shock [57], but during the treatment process, the diversity of hydrogen-producing bacteria will decline (lack of mesophilic hydrogen-producing bacteria that do not form spores), and the activity of hydrogen consumers cannot be completely inhibited, which will eventually decrease hydrogen production [58,59]. In this study, high SHY was still obtained without sterilization when T. eurythermalis A501 used Dunaliella algal residues as a substrate. This is favorable, considering that the hydrogen production mode of T. eurythermalis A501 combined with lipid-extracted Dunaliella algal residues can achieve high SHY while reducing sterilization costs. Moreover, the SHY obtained with this method lacking any pretreatment was quite high, considering that the range of hydrogen production by fermentation of microalgal biomass without any pretreatment in previous studies is 0.37 e 97.7 mL/g VS [60].

It should be noted that the high temperature required by T. eurythermalis A501 in the fermentation process still requires a high energy input, which may also increase the cost of dark fermentation. For possible future industrial use, additional heat demand required in thermophilic fermentation may be achieved at a large-scale via the use of waste heat with efficient heat exchange and recovery units. Ljunggren and Zacchi [61] reported that the use of heat recovery devices can reduce heat demand by 88%, thus greatly reducing the cost of thermophilic fermentation. On the other hand, they also found that the addition of yeast extract as a nutrient to the fermentation medium significantly increased the total cost. In our study, T. eurythermalis A501 produced hydrogen in the basal TRM medium (without yeast extract or tryptone) with Dunaliella algal residue as the sole substrate. Moreover, high value chemicals such as VFAs were also produced when hydrogen is produced by dark fermentation. The fermentation effluent can be used as a feedstock for further biogas production (combined with anaerobic digestion or photo fermentation) or biomass production (microalgal culture) in a biorefinery way, thus further enhancing comprehensive utilization and feasibility of this model [62e 64].

Conclusions

In this work, lipid-extracted Dunaliella residue was found to be a potential fermentation substrate utilized by T. eurythermalis A501 that can promote comprehensive utilization of microalgal resources in an integrated biorefinery approach. Substrate concentration and initial volume ratio of gas to liquid were both key factors in the fermentation process. The highest hydrogen yield of 192.35 mL/g VS with D. primolecta algal residues was obtained under optimal conditions without any pretreatment. The results indicated the superiority of T.

Fig. 5 e Effect of autoclave sterilization on SHY of Thermococcus eurythermalis A501.

eurythermalis A501 -
Dunaliella ,
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k C 41476122 ; D (863) C 2013AA065805 .

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