Biorefinery potential of flue gas wastewater by thermophilic cyanobacteria isolated from a Malaysian hot springs

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Biorefinery potential of flue gas wastewater by thermophilic cyanobacteria isolated from a Malaysian hot springs

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Abstract: *Thermophilic cyanobacteria are known for their ability to conduct carbon fixation and carbon mitigation. This study investigates the potential of Synechococcus sp. and Thermosynechoccos elongatus isolated from Malaysia hot springs for biorefinery of flue gas wastewater. Notably, Synechococcus sp. and Thermosynechoccos elongatus grew better in 1% and 2.5% flue gas wastewater, respectively. Biomass production, growth rate, carbon fixation rate and CO_2 removal rate of both Synechococcus sp. and Thermosynechoccos elongatus were optimal in the presence of light/dark cycles. Thermosynechococcus elongatus displayed better biorefinery potential for flue gas wastewater than Synechococcus sp., with a CO_2 removal rate of 38.77%, compared to 12.37% and 23.81% by Synechococcus sp. These thermophilic cyanobacteria could be incorporated in the flue gas treatment system in the industry.*

Keywords: Flue gas; Thermophilic cyanobacteria; Carbon dioxide reduction.

1. INTRODUCTION

Flue gas, a byproduct of combustion in industries and power plants, is a significant contributor to environmental pollution. While primarily composed of carbon dioxide (CO_2), flue gas also contains harmful pollutants like nitrogen oxides (NO_x) and sulphur dioxides (SO_2) [1]. According to statistics by the International Energy Agency (IEA) in 2021, power plant flue gases contribute more than 28% of global CO_2 emissions from energy use. These emissions pose a serious threat to both our environment and human health.

FGD systems on the market are highly effective at removing SO_2 and NO_x from flue gas [2], the water used in these processes can still contain the harmful SO_2 and NO_x residuals. This water is typically stored in a holding pond before being released into the environment. Bioremediation, the use of biological systems to remove pollutants, offers a promising approach for mitigating flue gas emissions [3]. Reportedly, one solution to this problem is the use of sulphur-bacteria biofilters, which, can only enhance SO_2 removal from flue gas and produce elemental sulphur [4].

It has been suggested that cyanobacteria can be an excellent microbial host for bioremediation of flue gas due to their capabilities in CO_2 and NO_2 fixation [5]. Moreover, previous studies have demonstrated the ability of certain mesophilic cyanobacterial strains to utilize flue gas components such as NOx and SO_2 [6], [7] to produce organic chemicals, including fatty acids and lipids. To date, only one study has reported the ability of *Thermosynechococcus elongatus* PKUAC-SCTE54, a thermophilic cyanobacterium, to grow in 15% CO_2 and 200 ppm of NO and SO_2 at temperatures above 50°C (as reviewed by Dalvi et al. [5]).

On the other hand, previous studies have used other thermophilic bacterial species to treat hazardous compounds in different sources, for example, a laboratory-scale biofilter can effectively treated 1000 ppm v/v SO_2 [8], a two-stage consists of a biotrickling filter and a biological post-treatment unit can remove SO_2 from flue gases and produce elemental sulphur [4], and a biofilter for ethylacetate removal has shown a higher removal efficiency (>100 $g/m^3/h$) than a mesophilic biofilter at 45–50 °C [9]. Besides, volatile compounds, including methanol, α-pinene [10], BTEX [11], and other organics, have been successfully treated using thermophilic biofiltration.

Besides, previous studies have reported on the development of microbial systems for wastewater treatment [12,13]. The microbial fuel cells had a removal efficiency of chemical oxygen demand (COD) between 38.88% and 52.40%, which is a promising technology for wastewater treatment [12]. Moreover, *Galdieria sulphuraria*, a red microalgae, is also another promising microbial host for wastewater treatment due to its ability for the efficient biological oxygen demand (BOD) and heavy metals (HMs) removal from wastewater [13].

This study investigates the biorefinery potential of thermophilic *Synechococcus* sp. and *Thermosynechococcus elongatus* in treating flue gas wastewater using optimized BG-11 media with varying flue gas wastewater concentrations, as well as the effects of photoperiods on the process. Findings on biomass production and CO_2 bioremediation by the species provide insights into their biorefinery capacity of flue gas wastewater for applications of the strains tolerant to high temperatures in industrial flue gas wastewater treatment.

2. MATERIALS

2.1 Cyanobacteria strains

Thermosynechococcus elongatus and *Synechococcus* sp. were isolated from Kg. Air Panas, Hulu Slim hot spring, and Kg. Lada hot spring, Malaysia, respectively.

2.2 Chemicals

Cyanobacteria were maintained in 1xBG11 medium according to Rippka et al. [14] with modification (from 10xBG11 consists of 176 mM $NaNO_3$, 2.3 mM K_2HPO_4, 3.0 mM $MgSO_4.7H_2O$, 2.4 mM $CaCl_2.2H_20$, 0.3 mM Citric acid, 0.4 mM $(NH_4)_5[Fe(C_6H_4O_7)_2]$, 0.03 mM Na_2EDTA, 1.9 mM Na_2CO_3, 0.01 mM H_3BO_3, 2.3 µM $MnCl_2.4H_2O$, 0.19 µM $ZnSO_4.7H_2O$, 0.4 µM

$CuSO_4.5H_2O$, 0.08 µM $Na_2MoO_4.2H_2O$, and 0.06 µM $Co(NO_3)_2.6H_2O$).

3. METHODS

3.1 Growth condition

For optimisation of growth in flue gas wastewater, cyanobacteria strains were grown in 1xBG-11 at 40± 2°C under continuous light (white light 120 µmol m^{-2} s^{-1}) under continuous light (24L:0D). For analysis of biomass production and CO_2 bioremediation, cyanobacterial cultures were grown under continuous light (24L:0D) and a photoperiod of 12-hour light: 12-hour dark (12L:12D) as well as 8L:16D. The L:D represents the ratio of the light and dark periods from this point onwards. Cultures were grown for 21 days prior to analysis.

3.2 Analysis of Biomass

After cultivation of 21 days, cells from 150ml culture were harvested by centrifugation at 3,500 rpm for 20 min at room temperature. The cells pellet was dried in an oven at 70°C overnight. The dried biomass was weighed several times until there was no fluctuation in the weight. The biomass production was measured based on the cell dry weight (CDW) using the formula:

$$\frac{([\text{DCW after dehydration (g)} - \text{DCW before dehydration (g)}] \times 1000\ \text{mg})}{150\ \text{ml} \times 10^{-3}\ \text{L}}$$

All data were recorded and used to plot a graph of dry cell mass (mg/L) versus flue gas concentration (% v/v) to identify the optimum flue gas concentration for cultivation of cyanobacteria.

3.3 Growth rate and CO_2 biofixation rate analyses

Optical density (OD) of cultures was used to determine growth rate, biomass productivity and CO_2 fixation rate. A 200 µL sample from the growing culture was taken every three days and measured at OD 730nm. The OD readings were used to plot the growth curve.

The growth rate was determined using the equation:

$$\mu = \frac{\ln(OD_2) - \ln(OD_1)}{(t_2 - t_1)}$$

Where,
OD_1 = Initial OD
OD_2 = Final OD
t_1 = Initial time (h)
t_2 = Final time (h)

To calculate volumetric biomass productivity (P_{max}), the following equation was used:

$$P_{max} = \mu \times X$$

Where,
µ: Specific growth rate (h^{-1})
X: Biomass concentration (g/L)

CO_2 fixation rate was measured using CDW (g/L) of culture according to [15]. The cells were harvested using centrifugation at 6,000 rpm and dried in an oven at 70°C overnight. The CDW was used to determine the CO_2 biofixation rate using the equation:

$$RCO_2 = M_{cbm} \times P_{max} \times (m_{CO_2}/m_c)$$

Where,
M_{cbm} = the fraction of carbon in biomass
m_{CO_2} = the molecular weight CO_2
P_{max} = the maximum volumetric biomass productivity
m_c = molar mass of carbon
According to [16], cyanobacteria have relatively high carbon content, approximately 50% of their dry weight. Thus, the fraction of carbon in biomass (M_{cbm}) is equal to half of the sample dry cell mass. The molecular weight of CO_2 (m_{CO_2}) is equal to 44.009 g/mol, while the molar mass of carbon (m_c) is equal to 12 g/mol. Maximum volumetric biomass productivity refers to the highest rate at which biomass can be produced per unit volume of culture per unit time.

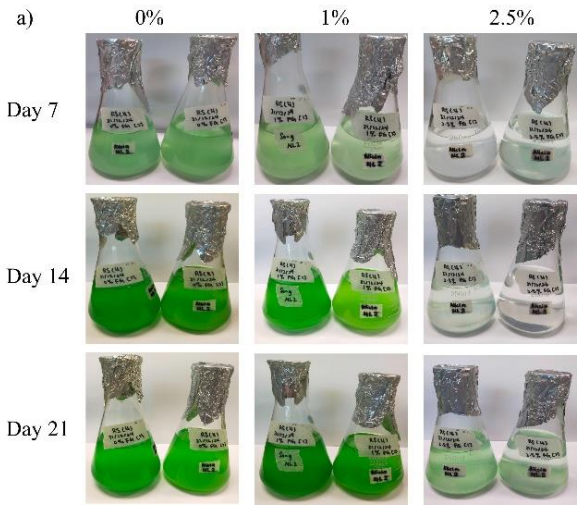
4. RESULTS AND DISCUSSION

4.1 optimization of cyanobacteria growth in flue gas wastewater

Synechococcus sp. and *Themosynechococcus elongatus* were cultivated in BG-11 medium without flue gas wastewater (known as control onwards) and with either 1% or 2.5% flue gas wastewater to determine the optimal concentration at which the species can survive.

After 21 days of cultivation, *Synechococcus* sp. showed better growth in BG-11 media with 1% flue gas wastewater compared to BG-11 media with 2.5% flue gas wastewater (Figure 1a). Moreover, the biomass production of *Synechococcus* sp. was the highest in BG-11 with 1% flue gas wastewater (0.23 g/L), although there was no significant difference between control (0.21 g/L) (P= 0.84) and culture in 2.5% flue gas wastewater (0.03 g/L) (P= 0.10) (Figure 2a).

Whereas *Themosynechococcus elongatus* grew better in both BG-11 with 1% and 2.5% flue gas wastewater compared to the control (Figure 1b). The biomass production of this species was also high in both media, 0.1 g/L 0.11 g/L in BG-11 media with 1% and 2.5% flue gas wastewater, respectively (Figure 2b). A previous study reported that *Thermosynechococcus elongatus* PKUAC-SCTE54 can grow in 15% CO_2 [3]. Whereas *Thermosynechococcus elongatus* TA-1 showed better growth in 10% and 20% CO_2 [17]. Thus, the studied *Themosynechococcus elongatus* could potentially grow in a higher percentage of CO_2, which is worth investigating in the future.



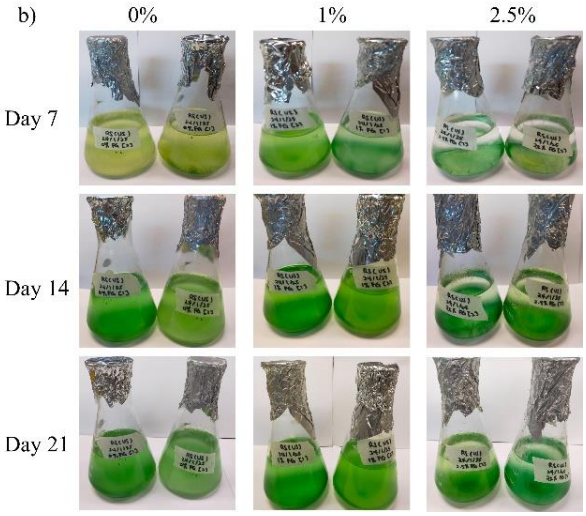


Fig. 1. Optimizations of cyanobacteria species growth in BG-11 media supplied with 1% and 2.5% flue gas wastewater. a) S*ynechococcus* sp. b) *Thermosynechococcus elongatus*. 0%: BG-11 without flue gas wastewater. Experiments were done in duplicate.

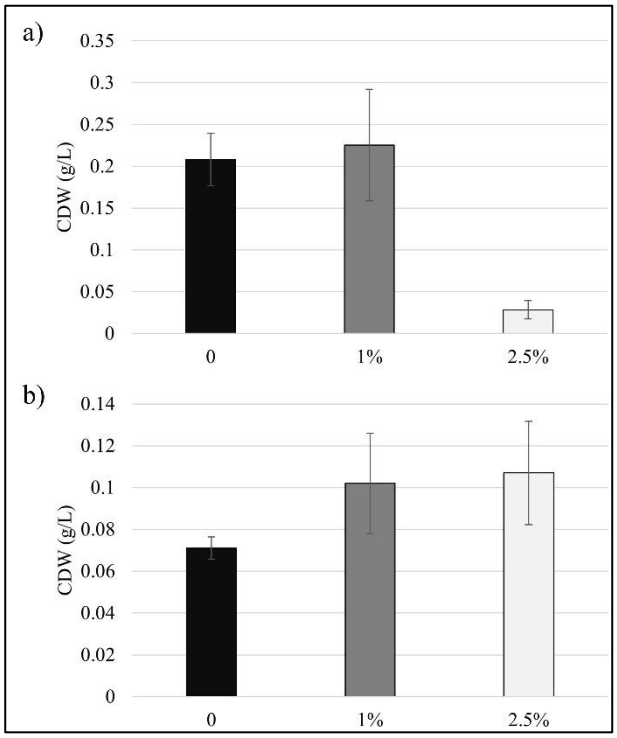


Fig. 2. Biomass production of a) *Synechococcus* sp. and b) *Thermosynechococcus elongatus* in BG-11 media supplied with 1% and 2.5% flue gas wastewater. Culture in BG-11 without flue gas wastewater labelled as 0.

4.2 Effects of photoperiod to biomass production and CO_2 bioremediation of *Synechococcus* sp.

Synechococcus can perform carbon fixation, but not all *Synechococcus* strains can fix nitrogen. Most marine *Synechococcus* strains cannot fix nitrogen [18] while some freshwater *Synechococcus* strains possess nitrogen fixation capabilities [19].

This study found that light/dark cycles play an important role in the growth of *Synechococcus* sp., both in the control and in cultures with flue gas wastewater (Fig. 3). *Synechococcus* sp. grown in both the control media and BG-11 media with 1% flue gas wastewater had the fastest growth rate of 0.0888 μ and 0.0907 μ, respectively, under the 12L:12D photoperiod (Fig. 4). Besides, the culture's growth rate was also significantly higher ($p < 0.05$) under the 8L:16D photoperiod compared to constant light (24L:0D) (Fig. 4). The growth rate results corroborate the biomass production of Synechococcus sp., which was highest under the 12L:12D photoperiod in both the control and BG-11 media with 1% flue gas wastewater, although no significant differences were observed across all photoperiods (Fig. 5). Previous studies showed that light/dark cycles regulate the circadian clock in *Synechococcus elongatus*, with the clock gating its cell division, which occurs during the day [20, 21]. Similar to the previous studies [20, 22], here the biomass production of *Synechococcus* sp. was higher in the presence of light/dark cycles compared to constant light conditions. Remarkably, biomass production of *Synechococcus* sp. was higher in the BG-11 with 1% flue gas wastewater than in the control media under both 8L:16D and 12L:12D photoperiods.

Moreover, CO_2 removal rate by *Synechococcus* sp. cultured in the BG-11 with 1% flue gas wastewater was the highest under the 12L:12D photoperiod compared to the 8L:16D photoperiod and constant light condition (Table 1). The fact that cyanobacteria perform oxygenic photosynthesis during the day to harvest light energy for carbon fixation, whereas nitrogen fixation occurs in the evening when there is little photosynthesis activity and low or absence of O_2 [19], supports the roles of the light/dark cycle for CO_2 fixation by the studied *Synechococcus* sp.

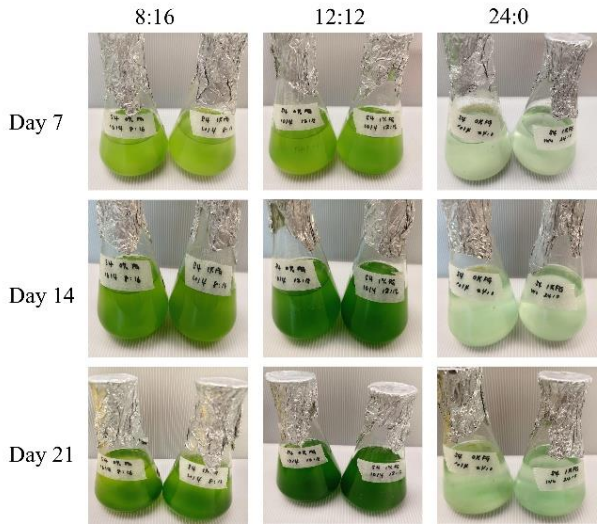


Fig. 3. Effects of photoperiod to growth of a) *Synechococcus* sp. in BG-11 media supplied with 1% flue gas wastewater. Left: BG-11 without flue gas wastewater (Control). Right: BG-11 with 1% flue gas wastewater.

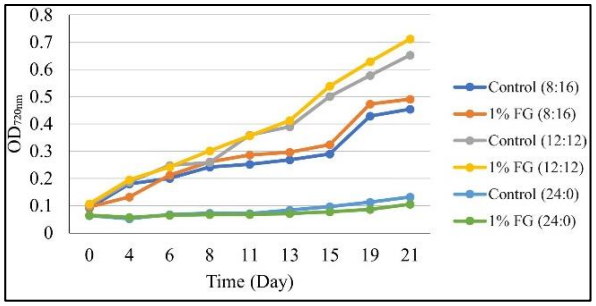


Fig. 4. The growth of *Synechococcus* sp. in BG-11 media supplied with 1% flue gas wastewater under different photoperiods (8L:16D, 12L:12D and 24L:0D). Culture in BG-11 without flue gas wastewater (control) and with 1% flue gas wastewater (1% FG).

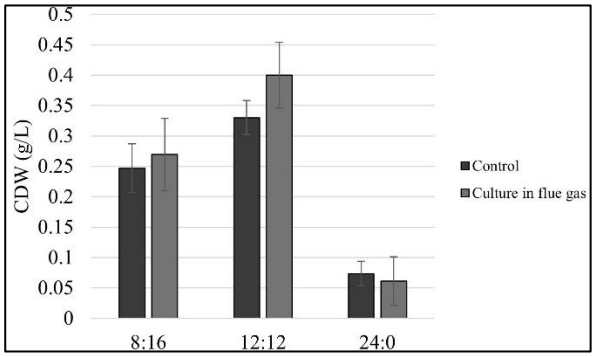


Fig. 5. Biomass production of *Synechococcus* sp. growth in BG-11 media supplied with 1% flue gas wastewater under different photoperiod.

Table 1. CO_2 bioremediation by *Synechococcus* sp.

Photoperiod	Culture	Carbon fixation rate (g/L/d)	CO_2 removal rate (%)
8:16	Control	0.03089	12.37
	Synechococcus sp.	0.03471	
12:12	Control	0.04835	23.81
	Synechococcus sp.	0.05986	
24:0	Control	0.00424	Ineffective
	Synechococcus sp.	0.00238	

4.3 Effects of photoperiod on biomass production and CO_2 bioremediation of *Thermosynechococcus elongatus*

Growth of *Thermosynechococcus elongatus* in both control and BG-11 media with 2.5% flue gas wastewater was monitored under 8L:16D, 12L:12D and 24L:0D photoperiods (Fig. 6). Interestingly, the growth of *Thermosynechococcus elongatus* in both media was optimal under an 8L:16D photoperiod. Although the growth rate of *Thermosynechococcus elongatus* was highest under the 12L:12D photoperiod (0.1169 μ) when grown in the control media, its growth rate in the BG-11 media with 2.5% flue gas wastewater showed the opposite trend (-0.0428 μ) (Fig. 7).

Similar to the results recorded for *Synechococcus* sp., the biomass production of *Thermosynechococcus elongatus* was higher in the BG-11 with 1% flue gas wastewater than in the control media, except when grown under a 12L:12D photoperiod (Fig. 7). The slight increase in biomass production under the 8L:16D photoperiod compared to continuous light highlights the role of light/dark cycles, specifically a longer dark period, in promoting a higher rate of cell division in the studied *Thermosynechococcus elongatus*; the difference was not statistically significant.

Thermosynechococcus elongatus showed a 38.77% of CO_2 removal rate in the BG-11 media with 2.5% flue gas wastewater under an 8L:16D photoperiod, which was the highest compared to *Synechococcus* sp. across all photoperiods (Table 1 and Table 2). On the other hand, CO_2 removal was ineffective under both 12L:12D photoperiod and constant light. An opposite result was reported in a previous study using *Thermosynechococcus* CL-1, which had a carbon fixation rate of 3.9 g/L/d under continuous light [23]. However, continuous cultivation was used to investigate the fixation rate by *Thermosynechococcus* CL-1 which may result in that opposite trend with a higher carbon fixation rate.

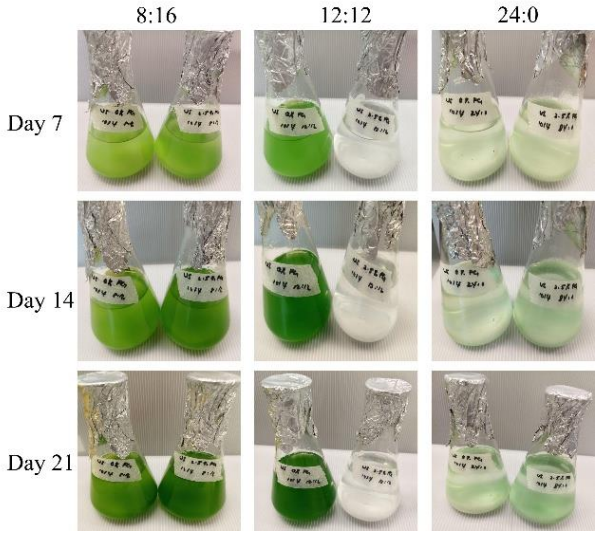


Fig. 6. Effects of photoperiod to growth of *Thermosynechococcus elongatus* in BG-11 media supplied with 2.5% flue gas wastewater. Left: BG-11 without flue gas wastewater (Control). Right: BG-11 with 2.5% flue gas wastewater.

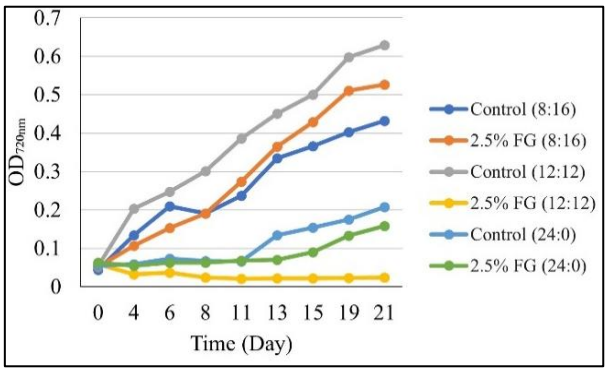


Fig. 7. The growth of *Thermosynechococcus elongatus* in BG-11 media supplied with 2.5% flue gas wastewater

under different photoperiods (8L:16D, 12L:12D and 24L:0D). Culture in BG-11 without flue gas wastewater (control) and with 2.5% flue gas wastewater (1% FG).

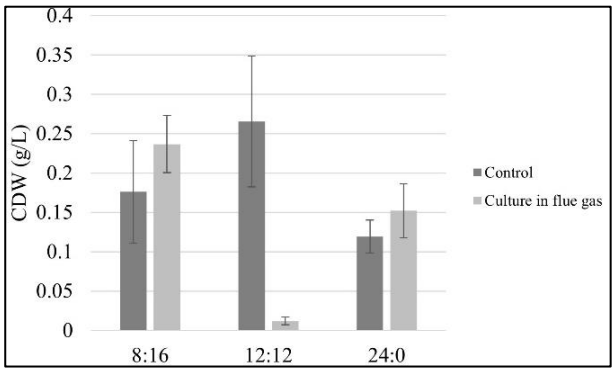


Fig. 8. Biomass production of *Thermosynechococcus elongatus* growth in BG-11 media supplied with 2.5% flue gas wastewater under different photoperiod.

Table 2. CO_2 bioremediation by *Thermosynechococcus elongatus*.

Photoperiod	Culture	Carbon fixation rate (g/L/d)	CO_2 removal rate (%)
8:16	Control	0.03131	38.77
	T. elongatus	0.04345	
12:12	Control	0.05111	Ineffective
	T. elongatus	-0.00007	
24:0	Control	0.01261	Ineffective
	T. elongatus	0.01124	

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