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High Biomass Production and Starch Accumulation in Native Green Algal Strains and Cyanobacterial Strains of Thailand

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ABSTRACT

Microalgae (including blue-green algae or cyanobacteria) have been actively studied regarding their potential to use as a future alternative renewable energy source due to the accumulation of starch in their biomass. The starch accumulated in microalgae can be hydrolyzed to organic acids by lactic acid bacteria prior to converting to hydrogen by photosynthetic bacteria through various pathways. The aims of the current study are to investigate the biomass production and starch accumulation in native strains of microalgal strains in Thailand. A total of 25 strains of microalgae comprising 14 strains of green algae and 11 strains of cyanobacteria were determined. Among these, a cyanobacteria strain, *Nostoc* sp. TISTR 8872 showed the best results in respect to biomass production of 0.26 g (DW)/l, while the highest starch accumulation was obtained from a cyanobacteria strain, *N. muscurum* TISTR 8871 of up to 32.97% starch. It is recommended that the time course of starch, protein and fatty acid accumulation in *Nostoc* sp. TISTR 8872 and *N. muscurum* TISTR 8871 cultivated in various kinds of media and wastewater should be further investigated.

Key words: biomass production, starch accumulation and microalgae

INTRODUCTION

Biomass has been a traditional energy source in the rural world across the globe for decades. Modernization, instead of reducing biomass energy consumption, has continuously increased its utilization for both households and the production of modern energy (Prasertsan and Sajjakulnukit, 2006). A variety of biomass resources can be used to convert to energy such as microbes, plants, animals and their organic waste products (Levin *et al*., 2007). Several alternatives of algal biomass are potentially a reliable energy source for hydrogen production (Kim *et al*., 2005). It is renewable, abundant and easy to use.

The previous research on biomass production and starch accumulation in microalgae, *Chlamydomonas reinhardtii* UTEX 90 accumulated 1.45 g (DW)/l and 0.77 g starch/l during photosynthetic growth using TAP media at 25 °C in presence of 2% CO_2 for 3 days (Kim *et al*., 2006). Ike *et al*. (1997) reported in the green algae, *C. reinhardtii* and *Dunaliella tertiolecta* cultured in a modified Bristol medium and f/2

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medium the dry weight of algal biomass were 7.62 and 7.27 g/l and starch content were 41.80 and 17.20 g/l, respectively. Starch in microalgae can be found in the form of carbohydrate, glucose, sugars and other polysaccharides. The starch content in green algae, *C. reinhardtii, Chlorella vulgaris*, *Dunaliella salina*, *Scenedesmus obliquus* and *Synechococcus* sp. were 17, 12-17, 32, 10-17 and 15% of dry weight, respectively, while in cyanobacteria, *Anabaena cylindrical* and *Spirulina maxima* were 25-30 and 13-16% of dry weight, respectively (Spolaore *et al*., 2006).

The development of energy sources and devices will emerge more aggressively to address the world's energy and environmental situation. Molecular hydrogen is an optimal fuel for a future climate-constrained world (Wunschiers and Lindlad, 2002), since it can be generated from water by biophotolysis of algae and cyanobacteria, photobiological (Laurinavichene *et al*., 2002) or fermented by anaerobic bacteria (Kapdan and Kargi, 2006). Algal biomass has been focused on as a future renewable energy source and alternative energy. Algae photobiological produced starch which can be used as a substrate for hydrogen production by various systems. At present an alternative method for hydrogen production by a three-step microbial hydrogen-producing system has been proposed. The first step is to use microalgae for starch production; the second is to convert the starch by hydrolyzing lactic bacterium in fermentation process to lactic acid, which is a suitable substrate used for hydrogen production by photosynthetic bacteria in the third step (Ike *et al*., 1997; Kawaguchi *et al*., 2001 and Kim *et al*., 2006). It seems that this system is one of the most suitable systems for hydrogen production. Hence, this experiment was initiated to investigate the biomass production and the starch accumulation in microalgal strains deposited at MIRCEN, TISTR.

MATERIALS AND METHODS

Preserved microalgae at Microbiological Resources Centre (MIRCEN), Thailand Institute of Scientific and Technological Research (TISTR), comprising 14 green algal strains and 11 cyanobacterial strains, were investigated for their biomass production and starch accumulation. Each green algal and cyanobacterial strains at initial concentration of 0.20 at OD_{1000} was grown in 300 ml BG-11 medium under illumination at 60 μmole photon $m^{-2}s^{-1}$ by cool white fluorescence lamps with 12 h dark and 12 h light cycle and shaken at 150 rpm on an orbital shaker at 28±1°C. The strains were sampled every 2 days for determination of cell growth by spectrophotometer at OD_{1000}. After 20 days of cultivation, algal biomass was determined as total dry weight. Starch accumulation was detected as glucose based on the method of Kochart (1978) after hydrolysis in 2M $HClO_4$ for 2 h at 100°C, using glucose (VWR International Ltd., England) as a standard. The mean of dry biomass and starch accumulation from triplicate tests of each strain were compared by a one-way ANOVA, separating the significantly different mean values with by means of Tukey's multiple comparison tests (Zar, 1996).

RESULTS AND DISCUSSIONS

After 20 days of cultivation the highest mean cells concentrations of green algal and cyanobacterial strains were obtained from *Scenedesmus* sp. TISTR 8597 and *O. okeni* TISTR 8549, respectively (Figure 1 and 2).

The mean of final dry biomass ranged between 0.05-0.25 and 0.13-0.26 g (DW)/l in green algae and cyanobacteria, respectively. The green algae, *S. obliquus* TISTR 8522 showed the best biomass production of 0.25 g (DW)/l with significant difference from other strains (Table 1). In the case of cyanobacteria, *Nostoc* sp. TISTR 8872 revealed the highest biomass production of

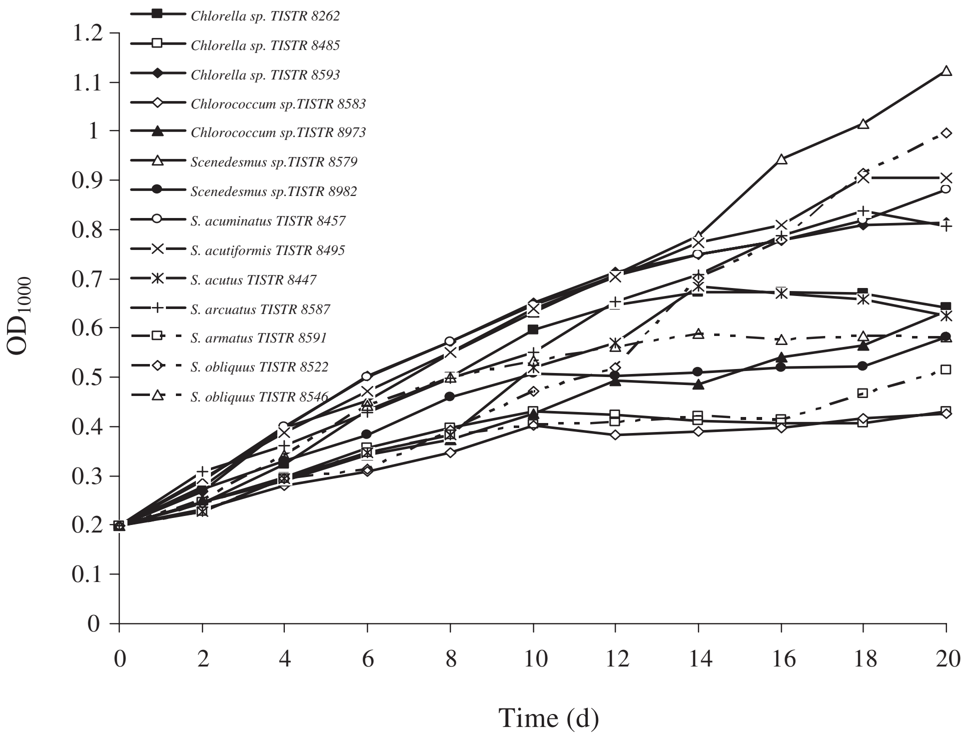

Figure 1 Time courses of growth of green algal strains cultivated in BG-11 medium.

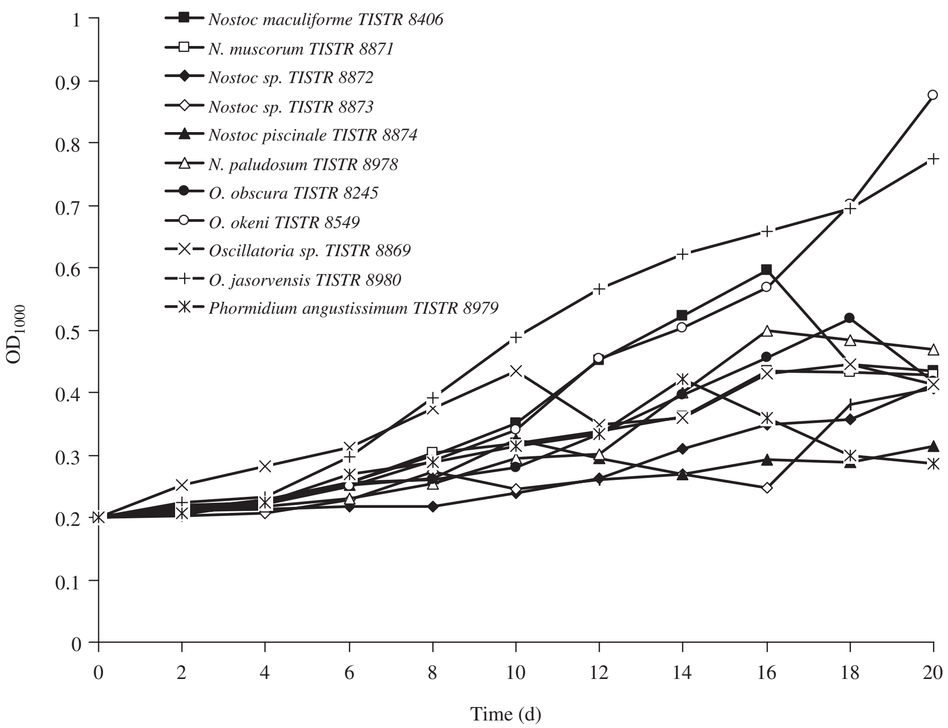

Figure 2 Time courses of growth of cyanobacterial strains cultivated in BG-11 medium.

0.26 g (DW)/l with no significant difference from other strains except for *Nostoc piscinale* TISTR 8872 (Table 2). However, the biomass production between *S. obliquus* TISTR 8522 and *Nostoc* sp. TISTR 8872 were not significantly different. Kim *et al*. (2006) reported biomass in the green algae, *C. reinhardtii* UTEX 90 accumulated 1.45 g (DW)/l during photosynthetic growth using TAP media at 25°C in presence of 2% CO_2 for 3 days and 7.62 and 7.27 g (DW)/l in *C. reinhardtii* and *Dunaliella tertiolecta* cultured in modified Bristol medium and f/2 medium, respectively (Ike *et al*., 1997).

Table 1 Biomass production and starch accumulation of green algal strains cultivated in BG-11 medium for 20 days.

Strains of microalgae	Biomass (Mean±SE; g dry weight l^{-1})	% Strach (Mean±SE)
Chlorella sp.TISTR 8262	0.10±0.00^{abc}	21.47±0.72^{defg}
Chlorella sp.TISTR 8485	0.09±0.00^{abc}	27.00±1.10^{g}
Chlorella sp. TISTR 8593	0.08±0.01^{ab}	21.97±1.28^{defg}
Chlorococcum sp.TISTR 8583	0.11±0.01^{bcd}	25.98±1.12^{fg}
Chlorococcum sp.TISTR 8973	0.20±0.03^{ef}	16.80±1.71^{bcd}
Scenedesmus sp.TISTR 8579	0.13±0.00^{cd}	20.35±0.36^{cdef}
Scenedesmus sp.TISTR 8982	0.08±0.01^{abc}	13.34±2.29^{b}
S. acuminatus TISTR 8457	0.12±0.02^{bcd}	7.32±0.92^{a}
S. acutiformis TISTR 8495	0.17±0.00^{de}	16.38±1.08^{bcd}
S. acutus TISTR 8447	0.11±0.01^{bcd}	18.57±1.11^{bcde}
S. arcuatus TISTR 8587	0.10±0.00^{abc}	12.88±0.99^{ab}
S. armatus TISTR 8591	0.05±0.00^{a}	15.43±0.74^{bc}
S. obliquus TISTR 8522	0.25±0.01^{f}	23.66±0.56^{efg}
S. obliquus TISTR 8546	0.12±0.06^{cd}	23.37±0.49^{efg}

Means in the column followed by the same letters are not significantly different as determined by means of Tukey's multiple comparison tests (α=0.05)

Table 2 Biomass production and starch accumulation of blue-green algal strains cultivated in BG-11 medium for 20 days.

Strains of microalgae	Biomass (Mean±SE; g dry weight l^{-1})	% Strach (Mean±SE)
Nostoc sp. TISTR 8872	0.30±0.00^{b}	30.66±0.58^{d}
Nostoc sp. TISTR 8873	0.20±0.04^{ab}	32.85±1.52^{d}
N. maculiforme TISTR 8406	0.17±0.03^{ab}	30.07±1.50^{d}
N. muscorum TISTR 8871	0.38±0.00^{c}	33.49±0.89^{d}
N. paludosum TISTR 8978	0.20±0.03^{ab}	32.14±0.15^{d}
N. piscinale TISTR 8874	0.13±0.02^{a}	17.37±1.15^{bc}
Oscillatoria sp. TISTR 8869	0.26±0.01^{b}	19.32±0.81^{c}
O. jasorvensis TISTR 8980	0.21±0.02^{ab}	9.74±0.40^{a}
O. obscura TISTR 8245	0.21±0.03^{ab}	12.61±1.59^{b}
O. okeni TISTR 8549	0.20±0.01^{ab}	8.08±0.27^{a}
Phormidium angustissimum TISTR 8979	0.15±0.02^{ab}	28.48±0.35^{d}

Means in the column followed by the same letters are not significantly different as determined by means of Tukey's multiple comparison tests (α=0.05)

The starch accumulation was analyzed to determine the content of carbohydrate. Table 1 shows that starch accumulation of *Chlorella* sp. TISTR 8485 was 27.00%±1.10, which significantly higher than any other strains of green algae, but not significantly different with *Chlorella* sp. TISTR 8262, *Chlorella* sp. TISTR 8593, *Chlorococcum* sp. TISTR 8583, *S. obliquus* TISTR 8522 and *S. obliquus* TISTR 8546. However, Ike *et al*. (1997) reported on green algae, *C. reinhardtii* cultured in modified Bristol medium, that the starch content was 4.18%. Other macroalgae stains, *Enteromorpha* sp., *Ulva rigida*, *Caulerpa geminate*, *Gigartina livida* contained 10.61%±0.19, 3.52%±0.42, 2.88%±0.03 and 2.40%±0.20 of starch content, respectively (Zemke-White and Clements, 1999). This difference may come from strains and the culture medium. In the cyanobacteria, the starch content in *N. muscurum* TISTR 8871 was the highest (32.97%±1.07), but not significantly different from *N. maculiform* TISTR 8406 (30.07%±1.50), *Nostoc* sp. TISTR 8872 (28.97%±0.24), *Nostoc* sp. TISTR 8873 (32.85%±1.52), *N. paludosum* TISTR 8978 (32.14%±0.15) and *P. angustissimum* TISTR 8979 (28.48%±0.35). Other investigators observed that with a cyanobacteria, *Spirulina fusiformis* growing on Zarouk medium with the salinity ranging from 0.00 to 16.00 ppt., the starch content varied from 37.30 to 56.10% (Rafiqul *et al*., 2003), 44.00% in *Spirulina maximas* and 16.00% in *Phormidium* sp. grown on pretreated and diluted swine wastewater (Canizares-Villanueva *et al* ., 1995).

CONCLUSION

In this study, the cyanobacterial strain, *Nostoc* sp. TISTR 8872 showed the best result for biomass production, while the highest starch accumulation was obtained from *N. muscurum* TISTR 8871. To obtain the highest level of starch, both biomass production and percentage of starch content should be considered. Therefore, *Nostoc* sp. TISTR 8872 and *N. muscurum* TISTR 8871 were recommended for future study on optimization of biomass production and starch accumulation using defined medium, fertilization medium and wastewater.

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