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Original Article

Culture system for *Wolffia globosa* L. (Lemnaceae) for hygiene human food

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Abstract

This study aimed to develop a suitable culture system for the mass production of *Wolffia globosa* for human consumption. *W. globosa* was grown in five different culture systems (static, vertical aeration, horizontal surface agitation, system with top water spraying and layer culturing system with top water spraying). Dry weight of *W. globosa* determined every 7 days indicated that a horizontal surface agitation provided the highest mass of 42.94±2.17 g/m^2 and significantly difference with others in 28 days ($p<0.05$). Twenty one days-culture of *W. globosa* in the horizontal circulation produced the highest yield of 1.52±0.04 g dry weight/m^2/d and was significantly higher than yields in other systems. Frond size of *W. globosa* in 7 days culture was the biggest of all the culture systems; however, no significant difference was found among the culture systems. The biomass had 48.2% protein with complete essential amino acids, 9.6% fat and 14.5% crude fiber with low bacterial contamination.

Keywords: culture system, *Wolffia globosa,* water meal, duckweed, mass culture

1. Introduction

Development of new foods is vital to the needs of rapid expanding in Asia because of rapid human population growth. *Wolffia* spp., water meal or duckweed, is an aquatic plant generally found throughout Thailand and the neighbor countries. *Wolffia arrhiza* is used as food ingredient by Burmese, Laotian and Thai especially in the northeast and north of Thailand. *Wolffia* or Khai-nam in Thai is an oval shape plant floating on pond water surface. Khai-nam is generally regarded as poor people's food and has attracted little attention as a potentially significant source of human food (Bhanthumnavin and McGary, 1971).

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Furthermore, *W. arrhiza, W. columbiana* and *W. globosa* (Figure 1) exhibit high growth rates and consequently absorb large amounts of nitrogen and phosphorus and the vegetative frond on dry weight contains 34-45% protein with

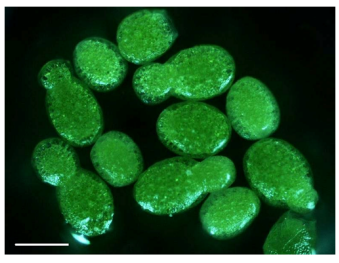

Figure 1. *W. globosa* collected from a natural pond in Mueang district, Sakon Nakhon province, Bar = 0.5 mm

complete essential amino acids, 5-7% fat, 10-11% crude fiber (Rusoff *et al.*, 1980; Fujita *et al.*, 1999; Ruekaewma, 2011). Moreover, it may be feasible to use *W. arrhiza* and *W. globosa* to produce high protein animal feed (Naskar *et al.*, 1986; Chantiratikul *et al.*, 2010; Chantiratikul and Chumpawadee, 2011). *Wolffia* spp. also has a potential for utilization in the treatment of wastewater (Hillman and Culley, 1978; Edward *et al.*, 1991).

In addition, researchers are using these plants to study basic plant development, plant biochemistry, photosynthesis, toxicity of hazardous substances, and much more. Environmental scientists are using *Wolffia* spp. to remove unwanted substances from water (Cross, 2006: online). Although *Wolffia* spp. has been widely studied, the hygienic mass production of *Wolffia* spp. has received only little attention. In this research, we elucidate a suitable culture system for hygienic mass production of *W. globosa* for human consumption.

2. Materials and Methods

Outdoor mass culture of *W. globosa* for growth rate and quality determination was designed in 2 experiments. Firstly, five different culture systems were designed to determine production rate of *W. globosa.* Secondly, the culture system yielding the higher production was selected for *W. globosa* quality study. The experiments were conducted at Mueang District, Sakon Nakhon Province, Thailand, using a selected stain of local *W. globosa*.

Five different culture systems; 1) a static culture, 2) a vertical aeration culture, 3) a horizontal surface agitation culture, 4) a system with top water spraying, and 5) a layer culturing system with water spraying on the top (see Figure 2 for details) were used for mass culture of *W. globosa*.

The static culture had no circulation during culture period. In the vertical aeration culture system, an air stone (400 l/hr flow) was used to circulate the water vertically. In the horizontal surface agitation culture, a blade paddle wheel driven by a mini-motor (3,500 rpm) was used to circulate the water horizontally. For the system with top water spraying and the layer culturing system with top water spraying, water was moved from the bottom of the culture to the top and sprayed (900 l/hr) over the surface area. For the layer culture system, a sheet of plankton net was placed few centimeters over water surface and water spraying above the net provided. All culture systems were run in black cylindrical plastic tanks with an area of 0.152 m^2 and 40 cm high and were setup in a warehouse covered with transparent plastic sheet to prevent rain water.

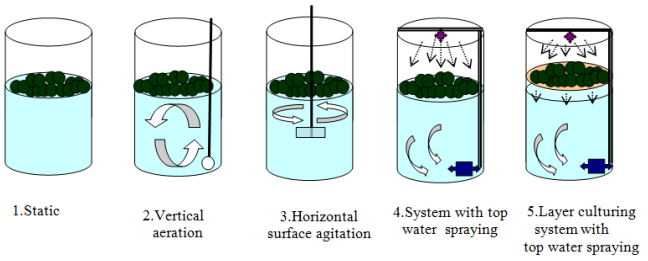

Figure 2. Diagrams of five different culture systems for mass production of *W. globosa*

At culture, 50 l of Modified Hutner's medium (Hutner, 1953) with pH 6 was prepared. Depth of the culture was maintained at 30 cm by adding freshwater to recover from evaporation. The plastic tank was inoculated with *W. globosa* frond at a density of 15% surface area (24 g/tank). The experiments were run in October 2010 to February 2011 under ambient temperature and light conditions.

Environmental parameters such as light intensity, temperature, pH, dissolved oxygen, NO^-_3-N and PO_4-P (APHA, 1995) of all the outdoor culture systems were monitored every 7 days. Wet weight and frond size of *W. globosa* were investigated after a culture of 28 days. They were measured every week and then were returned to the systems. The dry weight was determined after oven drying at 70ºC for 24 hrs (Driever *et al.*, 2005).

The five culture systems were run in triplicates. Data were analyzed using descriptive statistics and one way analysis of variance (ANOVA) followed by Duncan's multiple comparison tests. F-values with a probability level < 0.05 were considered to be statistical significant.

The samples of *W. globosa* in the horizontal surface agitation were analyzed for proximate analysis (AOAC, 2005), amino acid profile (Petritis *et al.*, 2002) and microbial determination (USFDA/CFSAN/BAM, 2008: online, Chapter 3, 4, 12).

3. Results

Mass culture of *W. globosa* was evaluated in five different culture systems, a static, a vertical aeration, a horizontal surface agitation, a system with top water spraying and a layer culturing system with top water spraying (Figure 3), throughout the entire period of outdoor cultivation (28 days). *W. globosa* was cultivated under the laboratory conditions and then transferred to the outdoors in five different culture systems. Growth of *W. globosa* was evaluated by measurement in dry weight and the result showed that the horizontal surface agitation culture system provided the highest mass production of 42.94±2.17 g/m^2, which was significantly different from the others ($p<0.05$). The dry weight in other systems was 35.12±3.47, 31.32±4.93, 29.06±7.61 and 25.65±1.63 g/m^2 in system with top water spraying, static, vertical aeration and layer culturing with top water spraying, respectively (Table 1).

Production rate, at 21 days--culture of *W. globosa* in the horizontal surface gave the highest yield of 1.52±0.04 g dry weight/m^2/d which was significantly higher than that in other systems (1.18±0.17, 0.96±0.27, 0.94±0.04 and 0.86±0.09 g dry weight/m^2/ d for system with top water spraying, vertical aeration, static and layer culturing with top water spraying, respectively) (Table 2).

Frond size of *W. globosa* in 7 days was 0.59±0.09, 0.53±0.03, 0.49±0.03, 0.48±0.08 and 0.47±0.06 mm^2 in vertical aeration, system with horizontal flow, system with top spraying, layer culturing system with top spraying and static,

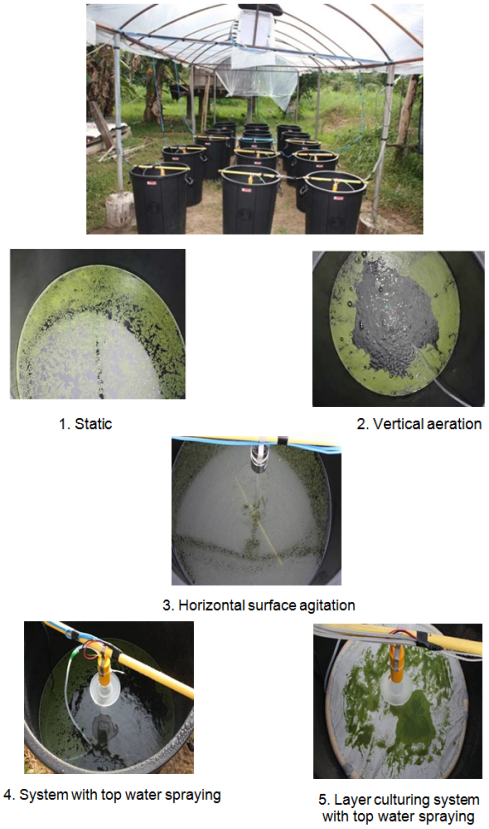

Figure 3. *W. globosa* cultured outdoor in five different culturing systems and setup all culture systems in a warehouse

respectively, with no significant difference (Table 3).

The environmental parameters during outdoor cultivation are shown in Table 4. There were temperature variation due to seasoning effect, pH and dissolved oxygen variations due to light effect.

A proximate analysis and microbial determination for the system with horizontal flow is shown in Table 5. It indicated that *W. globosa* contained 48.2% protein, 9.6% fat, and 14.5% crude fiber. The amino acids indicated a complete essential profile for human need. In microbial determination, only a limited number of contaminated microbial and no pathogen was found in *W. globosa* samples.

4. Discussion and Conclusions

In the present study, the horizontal surface agitation produced higher dry weight than other systems in 28 days (42.94 g/m^2). Moreover, the horizontal surface agitation provided higher production rate than other systems in 21 days-culture (1.52 g dry weight/m^2/d). After day 21, production rate in all treatments declined due to these being over dense. A similar trend was reported by Suppadit *et al.* (2008), Suppadit (2011) and Cheng and Stomp (2009).

Production rate in the vertical aeration system was not significantly different throughout 28 days of culture. This might be due to vertical movement in water column interfering normal living of *W. globosa*, since this plant always floats only on the water surface in calm areas with less vertical movement. The system provided low yield. Vertical aeration

Table 1. Dry weight (g/m^2) of *W. globosa* cultivated in five different culture systems

Culture systems	Days-culture				
	0	7	14	21	28
Static	6.32	10.38±0.31^{c}	15.14±0.24^{cd}	26.14±0.87^{bc}	31.32±4.93^{bc}
Vertical aeration	6.32	10.69±0.85^{c}	16.77±3.22^{bc}	26.52±5.71^{bc}	29.06±7.61^{bc}
Horizontal surface agitation	6.32	12.97±0.22^{a}	20.97±0.01^{a}	38.17±0.89^{a}	42.94±2.17^{a}
System with top water spraying	6.32	11.63±0.51^{b}	18.74±1.49^{ab}	31.11±3.63^{b}	35.12±3.47^{ab}
Layer culturing system with top water spraying	6.32	9.17±0.20^{e}	13.16±0.76^{d}	24.48±1.86^{c}	25.65±1.63^{c}

$^{a, b, c, d}$ significant difference in mean of column at $p < 0.05$

Table 2. Production rate (g dry weight/m^2/d) of *W. globosa* cultivated in five different culture systems

Culture systems	Days-culture			
	7	14	21	28
Static	0.58±0.04^{c}	0.63±0.02^{cd}	0.94±0.04^{bc}	0.89±0.18^{bc}
Vertical aeration	0.62±0.12^{c}	0.75±0.23^{bc}	0.96±0.27^{bc}	0.81±0.27^{bc}
Horizontal surface agitation	0.95±0.03^{a}	1.05±0.00^{a}	1.52±0.04^{a}	1.31±0.08^{a}
System with top water spraying	0.76±0.07^{b}	0.89±0.11^{ab}	1.18±0.17^{b}	1.03±0.12^{ab}
Layer culturing system with top water spraying	0.41±0.03^{d}	0.49±0.05^{d}	0.86±0.09^{c}	0.69±0.06^{c}

$^{a, b, c, d}$ significant difference in mean of column at $p < 0.05$

Table 3. Frond size (mm^2) of *W. globosa* cultivated in five different culture systems

Culture systems	Days-culture				
	0	7	14	21	28
Static	0.42	0.47±0.06	0.36±0.05^{b}	0.34±0.01^{c}	0.31±0.06
Vertical aeration	0.42	0.59±0.09	0.39±0.22^{ab}	0.36±0.03^{bc}	0.35±0.02
Horizontal surface agitation	0.42	0.53±0.03	0.41±0.02^{ab}	0.38±0.03^{ab}	0.35±0.09
System with top water spraying	0.42	0.49±0.03	0.44±0.04^{a}	0.33±0.01^{c}	0.41±0.07
Layer culturing system with top water spraying	0.42	0.48±0.08	0.43±0.02^{a}	0.41±0.02^{a}	0.43±0.06

$^{a, b, c}$ significant difference in mean of column at $p<0.05$

Table 4. Environment condition during the cultivation (October 2010 - February 2011) in five culture systems

Parameters	value
Light intensity	Max 98,000 lux
Light : Dark period	About 12:12 hrs
Temperature (air)	20-36.5°C
Temperature (medium)	17- 31°C
pH	5.8-7.4
Dissolved Oxygen	5.5-15.5 mg/l
NO_3 - N	40-50 mg/l
PO_4 - P	30-40 mg/l

Table 5. Proximate analysis and microbial determination of *W. globosa* (dry matter) in the horizontal surface agitation culture system

Component	value
Protein (%)	48.2
Fat (%)	9.6
Crude fiber (%)	14.5
Amino acid (mg/100g of protein)	
Aspatic acid	4137
Threonine *	1124
Serine	2048
Glutamic acid	4378
Proline	2450
Glycine	2530
Alanine	3213
Cystine	1928
Valine *	2410
Methionine *	843
Isoleucine *	1205
Leucine *	3896
Tyrosine	1365
Phenylalanine *	924
Histidine *	402
Lysine *	3333
Arginine *	2369
Tryptophan *	120
Microbial analysis	
Total plate count, cfu/g	1.7×10^6
MPN *E.coli /g*	< 3
Staphylococcus aureus, cfu/g	< 10 (ND)
Salmonella spp. / 25 g	ND

*the essential amino acid for human

system gave a lower production rate of *W. globosa* than the static water culture.

The system of layer culturing with top water spraying provided the lowest yield (both dry weight and production rate) throughout the cultivation period. It indicated that a layer culturing system with top water spraying is unsuitable for mass culture of *W. globosa.* This might be because the fronds of *W. globosa* piled up very much on the layer (Figure 3) and was not spread by the water. Therefore, frond surface of *W. globosa* has low photosynthesis effecting yield and production rate.

Fedler and Duan (2011) studied biomass production for bioenergy using recycled wastewater in a natural waste treatment system and reported the biomass production of duckweed (containing both *Lemna* and *Wolffia*) in the tank with a TN concentration of about 2 mg/l, with water surface area of 2.54 m^2 and height of 0.9 m. The average daily growth rate of duckweed was 99-127 g wet weight/m^2/d. The mean long–term extrapolated yield of *Lemna* and mixed *Lemna–Wolffia* was 0.003 g dry weight/m^2/d (Edwards *et al.*, 1991). Maximum yield of duckweed was 15 g dry weight/m^2/d using domestic sewage (Oron *et al.*, 1984, 1988; Gaigher and Short, 1986). Naskar *et al.* (1986) reported a dry weight yield of *W. arrhiza* grown in different concentrations of sewage, ranging from 0.002 to 0.003 g/m^2/d. For yield of *Wolffia* in this experiment, the different culture systems provide 0.51 to 1.90 g dry weight/m^2/d.

Protein, fat, crude fiber content and essential amino acid profile of *W. globosa* were compared to *W. arrhiza* (Jairakphan, 1999), *W. columbiana* (Rusoff *et al.*, 1980) and *W. globosa* (Ruekaewma, 2011) as shown in Table 6. The result revealed that *W. globosa* grown in a system with

Table 6. Comparison of a proximate analysis of *Wolffia* spp. (dry matter)

Components	*Wolffia columbiana* (Rusoff *et al.*, 1980)	*Wolffia arrhiza* (Jairakphan, 1999)	*Wolffia globosa*	
			Culture system (the present study)	Natural pond (Ruekaewma, 2011)
Protein (%)	44.7	20.15	48.2	33.3
Fat (%)	6.6	2.43	9.6	5.0
Crude fiber (%)	11.0	14.72	14.5	10.7
Amino acid (mg/100g of Protein)				
Aspatic acid	5630	1209	4137	3539
Threonine	2550	641	1124	662
Serine	2280	565	2048	982
Glutamic acid	5760	1669	4378	2557
Proline	2410	674	2450	1279
Glycine	3040	831	2530	1507
Alanine	3750	1595	3213	3128
Cystine	-	104	1928	5457
Valine	3490	944	2410	1849
Methionine	870	201	843	571
Isoleucine	3060	685	1205	685
Leucine	5830	1300	3896	2032
Tyrosine	2170	374	1365	890
Phenylalanine	3600	758	924	502
Histidine	1180	309	402	2281
Lysine	3370	751	3333	530
Arginine	3780	804	2369	139
Tryptophan	-	201	120	346

Hutner's medium provided higher protein and fat content of 48.2% and 9.6%, respectively, and higher than those of *W. columbiana* collected from anaerobic dairy waste lagoons in the Louisiana State University (LSU) campus, the lagoons contained from 20 to 40 mg/l of TKN during the collection period. *W. arrhiza* and *W. globosa* which were collected from natural pond, however, crude fiber content in *W. arrhiza* was higher than others.

The essential amino acid concentration in *W. columbiana* was higher than that found in *W. arrhiza* and *W. globosa*. Cystine and tryptophan were found only in *W. arrhiza* and *W. globosa*.

It is clear that *W. globosa* grown in enriched culture system produced higher protein, fat, crude fiber and essential amino acid profile than *W. globosa* in natural pond (except Cystine). The crude protein content of *Wolffia* obtained from natural water (ponds, lakes, ditches, streams and paddy fields) has been reported in the range 7 to 20% (Tan, 1970; Bhanthumnavin and McGarry, 1971; Jairakphan, 1999). *Wolffia* spp. grown in enriched nutrients media or effluents from agricultural and municipal waste lagoons, can provide protein content up to 30-45% much greater than those grown in natural waters with low nutrients (Rusoff *et al.*, 1980; Fujita *et al.*, 1999; Chantiratikul *et al.*, 2010).

In the horizontal surface agitation culture outdoors, *W. globosa* showed high yield at 48.2% protein with complete essential amino acids, 9.6% fat and 14.5% crude fiber. Low bacterial contamination was found in this culture system. This system then may be a recommended technique for *W. globosa* culture for hygienic human consumption product.

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