วารสารวิทยาศาสตร์บูรพา ปีที่ 25 (ฉบับที่ 2) พฤษภาคม – สิงหาคม พ.ศ. 2563
BURAPHA SCIENCE JOURNAL Volume 25 (No.2) May – August 2020
บทความวิจัย

การบำบัดน้ำทางชีวภาพในระบบน้ำหมุนเวียน โดยใช้สาหร่ายพวงองุ่น (*Caulerpa lentillifera* J Agardh)

Biological Water Treatment in Recirculating Aquaculture System by Using *Caulerpa lentillifera* J Agardh

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Received : 25 February 2019
Revised : 17 June 2019
Accepted : 12 November 2019

บทคัดย่อ

การบำบัดน้ำทางชีวภาพในระบบน้ำหมุนเวียนโดยใช้สาหร่ายพวงองุ่น (*Caulerpa lentilifera*) ประกอบด้วยถังเลี้ยงปลาซึ่งมีปลาการ์ตูน *Amphiprion clarkii* 12 ตัว/ถัง และถังบำบัดน้ำซึ่งมีสาหร่ายพวงองุ่นที่ความหนาแน่น 4 ระดับ (0, 2, 4 และ 6 กรัม/ลิตร/ระบบ) ในแต่ละระบบน้ำหมุนเวียนใช้น้ำทะเลความเค็ม 32 ส่วนในพันส่วน จำนวน 30 ลิตร มีอัตราการไหลเวียน 800 ลิตร/ชั่วโมง และให้แสงกับสาหร่าย 13,094.4±5,805.9 ลักซ์ 12 ชั่วโมง/วัน ส่วนชุดควบคุมถังบำบัดไม่ให้แสง ดำเนินการทดลองเป็นระยะเวลา 49 วัน เพื่อศึกษา 1) การเติบโตจำเพาะของสาหร่ายพวงองุ่น 2) การเติบโตจำเพาะ, อัตราการเปลี่ยนอาหารเป็นเนื้อ และอัตรารอดตายของปลาการ์ตูน และ 3) คุณภาพน้ำแต่ละระบบน้ำหมุนเวียน ผลการทดลองพบว่าชุดทดลองที่มีสาหร่ายบำบัด 2 กรัม/ลิตร สาหร่ายมีการเติบโตจำเพาะสูงสุด และทุกชุดทดลองที่สาหร่ายมีอายุมากกว่า 35 วัน มีการเติบโตจำเพาะลดลง ปลาที่เลี้ยงในระบบหมุนเวียนที่มีสาหร่ายบำบัดน้ำทุกชุดทดลองแสดงค่าอัตราการเปลี่ยนอาหารเป็นเนื้อลดลงและมีอัตรารอดสูงกว่าชุดควบคุมที่ไม่มีสาหร่ายบำบัด แต่การเติบโตจำเพาะของปลาทุกชุดทดลองและชุดควบคุมแสดงค่าไม่แตกต่างกัน คุณภาพน้ำในช่วงมีแสงของทุกชุดทดลองที่มีสาหร่ายบำบัดมีปริมาณออกซิเจนที่ละลายในน้ำ (DO), กรด-ด่าง (pH), ความเป็นด่าง (alkalinity) สูงกว่า และ คาร์บอนไดออกไซด์ที่ละลายในน้ำ ($CO_{2(aq)}$) ต่ำกว่าชุดควบคุมที่ไม่มีสาหร่ายบำบัด แต่ในช่วงไม่มีแสงคุณภาพน้ำของชุดทดลองที่มีสาหร่ายบำบัดมีปริมาณออกซิเจนที่ละลายในน้ำลดลงต่ำกว่า และ คาร์บอนไดออกไซด์ที่ละลายในน้ำเพิ่มสูงกว่าชุดควบคุมที่ไม่มีสาหร่ายบำบัด คุณภาพน้ำของชุดทดลองที่มีสาหร่ายบำบัดมีปริมาณสารประกอบไนโตรเจนต่ำกว่าชุดควบคุมที่ไม่มีสาหร่าย และชุดทดลองที่มีสาหร่ายบำบัดที่ความหนาแน่น 2 กรัม/ลิตร แสดงอัตราการลดลงของสารประกอบไนโตรเจนในน้ำได้สูงสุด แต่คุณภาพน้ำของทุกชุดทดลองที่มีสาหร่ายและชุดควบคุมไม่มีสาหร่ายบำบัดมีปริมาณฟอสเฟตที่ละลายน้ำไม่แตกต่างกัน

คำสำคัญ : สาหร่ายพวงองุ่น, บำบัดน้ำทางชีวภาพ, ระบบน้ำหมุนเวียน, ปลาการ์ตูนลายปล้อง

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Abstract

Biological water treatment using *Caulerpa lentillifera* was investigated in a Recirculating Aquaculture System (RAS) with 4 culture densities (0, 2, 4 and 6 g/L/system). Each RAS unit consisted of a fish tank stocked with 12 *Amphiprion clarkii* and a purification basin contained seaweed. Each RAS unit was filled with 30 L of seawater at 32 ppt, running at a circulation rate of 800 L/hr, and the seaweed culture was lighted at 13,094.4±5,805.9 Lux for 12 hr/day. The control without seaweed was not lit. The experiment ran for 49 days and the following parameters were recorded: (1) *C. lentillifera* specific growth rate (SGR), (2) *A. clarkii* SGR, food conversion ratio (FCR) and survival rate, and (3) water quality of each RAS unit. Results indicated that seaweed SGR at 2 g/L was significantly greater than those of others densities. Seaweed SGR for all treatments decreased after 35 days. Fishes reared in RAS units with seaweed resulted lower FCR and higher survival rate than those without seaweed. The fish SGR among RAS treatments were insignificantly different. Water qualities in all seaweed treatments during lighted periods including DO, pH and alkalinity were higher while $CO_{2\ (aq)}$ was lower than those without seaweed. However, during dark period DO of the former treatments were lower while $CO_{2\ (aq)}$ were higher than those of the latter treatments. The concentrations of N-compounds in water with seaweed treatments were lower than those without seaweed. The treatment with 2 g/L seaweed showed highest N-compounds reduction rate. The concentrations of soluble phosphate were insignificantly different among all treatments and control.

Keywords : *Caulerpa lentillifera,* biological water treatment, recirculating aquaculture system, *Amphiprion clarkii*

Introduction

Biological water treatment using seaweed for coastal aquaculture is commonly used. Seaweeds can be used as a primary component in the Recirculating Aquaculture System (RAS) due to its ability to convert waste products to useful biomass and dissolved oxygen (DO) (Tait, 1981; Clarke, 1967; Lalli and Parsons, 1997; Tchobanoglous and Stensel, 2003). Moreover using seaweed for water treatment is a low cost technology. There have been many research studies on the uses of *Caulerpa* sp. for food (Lewmanomont, 1978; Pugdeepun, 2001; Munlun, 2017; Sudtongkong, 2008), health (Dumay *et al*., 2002; Minh *et al*., 2019; Li *et al*., 2012; Sharma & Rhyu, 2014) and water treatment. (Pariyawathee, 2003; Mamat, 2004; Karthick *et al*., 2012; Perryman *et al*., 2017). Although using *Caulerpa* sp. for water treatment has been well studied, the potential of seaweed photosynthesis for supplying oxygen and decreasing carbon-dioxide in RAS has not been reported.

The main biotic factors of biological water treatment in a RAS are producers, consumers and decomposers. All of them need oxygen which is a limiting abiotic factor for their living activities and growth. Producers are the main supplying source of oxygen, so biomass and life-span of the producers are a significant carrying capacity

factor in the RAS (Khan Academy, 2018). In this experiment, *Caulerpa lentillifera* is used at different densities in order to study oxygen production in RAS. The levels of oxygen and carbon dioxide from photosynthesis and impact on fish health in the system during the culture period are monitored. The main objective is to determine suitable seaweed densities and appropriate rearing period for purifying water in the system effectively.

Methods

Experimental design

The experiment was designed with 4 treatments (3 replications) in a RAS. Each RAS unit consisted of a fish rearing tank with 12 *Amphiprion clarkii* in 30 L/unit and seaweed (*Caulerpa lentillifera*) purification basin in densities at 0, 2, 4 and 6 g/L/unit, respectively. No aeration was applied in all treatments except control (0 g/L/unit seaweed) (Table 1). A pretest was conducted and the results showed that in RAS units without fish the seaweed in all densities died within 3-5 days.

Table 1 Experimental design

treatment	Initial *A. clarkii* in rearing tank (fish)	Initial *C. lentillifera* in purification basin (g)	purification basin	seawater 32 ppt (L/system)	Lighted 13,094.4 Lux (hr/day)	Circulation rate (L/hr.)
1	12	0	Aeration	30	0	800
2	12	60	no aeration	30	12	800
3	12	120	no aeration	30	12	800
4	12	180	no aeration	30	12	800

Recirculating Aquaculture System (RAS) and experimental Setup

The RAS for each treatment, as shown in Figure 1, was filled with 30 L of filtered 32 ppt sea water added by 0.1 ml/L molasses. The system was run for 3 days in order to establish micro-organism and initial nutrients for the seaweed. At day 4, the acclimated *C. lentillifera* at different densities was introduced to the prepared purification units. The system was then run for 3 days to ensure the seaweed in all treatments were alive. Each treatment unit was stocked with 12 acclimated fishes (*A. clarkii*) with initial mean wet weight of 0.93±0.08 g/fish. The system was continually run for 2 days after that to ensure the fishes in all treatments were alive. The fishes in each treatment were fed four times a day (0.0559 g each time) with artificial feed - INVE 4/6 brand, with pellet size 0.4-0.6 mm, crude protein $\geq$ 55%, total fats $\geq$ 9% and moisture $\leq$ 8%.

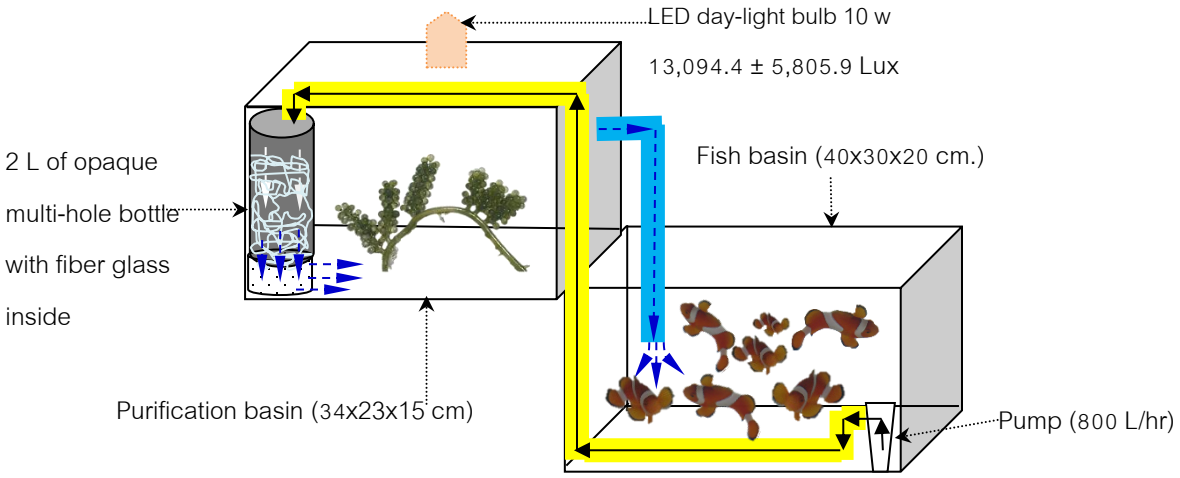

Figure 1 Model of Recirculating Aquaculture System

The seaweed and fishes from each treatment were weighed every week to calculate specific growth rate (SGR) using the method of Lobban *et al* (1985) as shown in Equation (1).

$$SGR\ (\%g/day) = [100Ln\ (final\ weight\ (g)/initial\ weight\ (g))]/rearing\ period\ (days) \quad (1)$$

Daily fish consumption and mortality were recorded. Water samples were collected at the outlet of purification basin 2 times a daily (at the beginning and the end of light period) for measuring pH, dissolved oxygen (DO), temperature, phenolphthalein alkalinity (Alk-P), total alkalinity (Alk-T), dissolved carbon dioxide ($CO_{2(aq)}$) and salinity. In addition water was sampled weekly for measuring N-compounds, soluble phosphate (PO_4^{-3}) and biological oxygen demand (BOD). The methods used for water quality analyses or measurements are summarized in Table 2.

Table 2 Water quality parameters and measure methods

Parameters	Measure Methods
pH	by electrometric pH meter model F-22 HORIBA
DO and temperature	by YSI DO meter model 5100
Alk-P and Alk-T	by titration method (APHA-AWWA-WPCF, 1980)
$CO_{2(aq)}$	by Moore nomographic method (Lind, 1974)
Salinity	by reflector salino-meter model S Mill-E ATAGO
N-compounds	NH_3-N+ NO_2^-+ NO_3^-
NH_3-N	by phenol-hypochlorite method (Strickland and Parsons, 1972)
NO_2^-	by diazotization method (Strickland and Parsons, 1972)
NO_3^-	by cadmium Reduction method (Strickland and Parsons, 1972)
PO_4^{-3}	by ascorbic acid method (Strickland and Parsons, 1972)
BOD	by 5 day BOD Test at 20 °C

Data analysis

The experimental results of *C. lentillifera* densities, rearing period, SGR and *A. clarkii* SGR, and water qualities were compared by using one way ANOVA and the difference of means were compared by using Duncan's New Multiple Range Test (Budsaba, 2010).

Results

C. lentillifera total weight gain and specific growth rate (SGR)

Total weight gain of *C. lentillifera* in each treatment was positive. The SGR of *C. lentillifera* at 2 g/L was significantly greater than those at 4 and 6 g/L (p<0.05). The seaweed reared less than 35 days were significantly higher than that reared more than 35 days (p<0.05) (Table 3).

Table 3 Biomass (wet weight g/basin) and specific growth rate (% g/day) of *C. lentillifera* in each treatment of RAS

Rearing periods (days)	Biomass (wet weight g/basin)			SGR (% g/day)		
	Treatment 2	Treatment 3	Treatment 4	Treatment 2	Treatment 3	Treatment 4
0	63±0.4 $^{a\ A}$	122±0.3 $^{a\ B}$	182±0.3 $^{a\ C}$	-	-	-
7	128±13.3 $^{ab\ A}$	211±10.2 $^{ab\ B}$	307±13.1 $^{ab\ C}$	10.09±1.494 $^{e\ B}$	7.81±0.675 $^{e\ A}$	7.45±0.575 $^{e\ A}$
14	200±24.0 $^{bc\ A}$	307±29.6 $^{b\ B}$	430±24.7 $^{b\ C}$	8.21±0.876 $^{d\ B}$	6.57±0.685 $^{d\ A}$	6.12±0.394 $^{d\ A}$
21	290±49.2 $^{cd\ A}$	415±61.9 $^{c\ B}$	582±24.9 $^{c\ C}$	7.22±0.844 $^{cd\ B}$	5.80±0.703 $^{cd\ A}$	5.52±0.197 $^{d\ A}$
28	376±69.3 $^{de\ A}$	513±61.8 $^{cd\ B}$	695±58.3 $^{cd\ C}$	6.33±0.693 $^{bc\ B}$	5.12±0.423 $^{bc\ A}$	4.77±0.294 $^{c\ A}$
35	477±92.5 $^{ef\ A}$	574±70.6 $^{d\ AB}$	764±110.1 $^{de\ C}$	5.74±0.567 $^{abc\ B}$	4.42±0.346 $^{ab\ A}$	4.07±0.410 $^{b\ A}$
42	554±109.1 $^{fg\ A}$	595±89.6 $^{de\ AB}$	814±134.3 $^{de\ C}$	5.14±0.475 $^{ab\ B}$	3.76±0.371 $^{a\ A}$	3.54±0.408 $^{ab\ A}$
49	654±93.4 $^{g\ A}$	684±88.0 $^{e\ AB}$	876±128.1 $^{e\ C}$	4.76±0.289 $^{a\ B}$	3.51±0.272 $^{a\ A}$	3.19±0.307 $^{a\ A}$

Remark : Values with different lowercase (a – b) superscripts in the same column were significantly different (p<0.05)
Values with different uppercase (A – B) superscripts in the same row were significantly different (p<0.05)

A. clarkii specific growth rate (SGR), food conversion ratio (FCR) and survival rate

The results of *A. clarkii* SGR showed that there were no significant differences among treatments (p>0.05). The FCR for fishes reared in seaweed at 6 g/L was significantly lower than the fishes reared in the units without seaweed (p<0.05), but not significantly different with fish reared in 2 and 4 g/L (p>0.05). The fish survival rates in all seaweed densities were significantly higher than those reared without seaweed (p<0.05) (Table 4).

Table 4 Specific growth rate (SGR), food conversion ratio (FCR) and survival rate of *A. clarkii* in each RAS

A. clarkii	*C. lentillifera* density (g/L/system)			
	Control	Treatment		
	0	2	4	6
SGR (% g/fish/day)	0.57±0.177	0.51±0.095	0.54±0.046	0.62±0.026
FCR	5.4±0.83 ^{B}	4.5±1.10 ^{AB}	4.2±0.25 ^{AB}	3.5±0.21 ^{A}
Survival rate (%)	78±4.6 ^{A}	95±4.6 ^{B}	100±0.0 ^{B}	95±4.6 ^{B}

Remark : Values with different uppercase (A – B) superscripts in the same row were significantly different (p<0.05)

Water qualities in each treatment of Recirculating Aquaculture System (RAS)

Water qualities in all treatments (RASs) for 49 days showed that temperature, salinity and BOD were not significantly different between control (no seaweed RAS) and treatments with seaweed at 2, 4 and 6 g/L (p>0.05). The average values of temperature, salinity and BOD were 27.53±0.644 ^{o}C, 33.11±0.575 ppt and 0.50±0.320 mgO_2/L, respectively, but the values of DO, $CO_{2(aq)}$, pH, Alk-P, Alk-T and nutrients (N-compounds and soluble phosphate; PO_4^{3-}) were significantly different between control and treatments with seaweed (p<0.05) (Table 5-7 and Figure 2-3). These results were explained in the following paragraph.

Water qualities including DO, pH, Alk-P and Alk-T in the seaweed treatments at 2-6 g/L during the light period increased but $CO_{2(aq)}$ decreased significantly compared with those without seaweed (p<0.05). However, during dark period DO of the former treatments were lower while $CO_{2\ (aq)}$ were higher than those of the latter treatments (Table 5 and Figure 2-3). The levels of N-compounds in all seaweed treatments were significantly lower than those in the control (p<0.05) (Table 6). The NH_3-N concentration decreased while the total concentration of N-compounds remained constant in the control. Among three seaweed densities, the amount of N-compounds in 6 g/L treatment was significantly lower than those in the 2 and 4 g/L treatments (p<0.05). The highest decreasing rate in N-compounds (0.08 mg N/g seaweed/L/system/day) was found in 2 g/L treatment while decreasing rate of 4 and 6 g/L treatments was 0.04 mg N/g seaweed/L/system/day (Table 6 and Figure 4). The soluble phosphate (PO_4^{3-}) concentrations between control and seaweed treatments were not significantly different (p>0.05) as shown in Table 7.

Table 5 Comparisons of DO, $CO_{2(aq)}$, pH, phenolphthalein alkalinity (Alk-P) and total alkalinity (Alk-T) in water during light and dark periods of each RAS treatment

Light period	Treatments (seaweed density; g/L)	DO (mg O_2/L)	$CO_{2(aq)}$ (mg CO_2/L)	pH	Alk-P (mg/L)	Alk-T (mg/L)
12 hr Light period	1 (0)	6.21±0.184^{c}	1.9±0.6^{c}	7.79±0.154^{b}	5.0±4.37^{a}	76.3±22.11^{a}
	2 (2)	6.34±0.226^{d}	1.1±0.4^{b}	8.02±0.104^{c}	10.2±3.38^{b}	88.8±17.51^{b}
	3 (4)	6.37±0.224^{d}	1.0±0.4^{ab}	8.05±0.088^{c}	10.3±2.81^{b}	92.7±13.43^{b}
	4 (6)	6.44±0.237^{e}	0.7±0.3^{a}	8.13±0.074^{d}	11.8±2.76^{b}	96.3±11.07^{b}
12 hr Dark period	1 (0)	6.13±0.156^{b}	1.9±0.4^{c}	7.80±0.132^{b}	5.6±4.07^{a}	75.8±20.21^{a}
	2 (2)	5.67±0.187^{a}	3.3±0.9^{d}	7.69±0.156^{a}	4.7±4.28^{a}	89.8±17.91^{b}
	3 (4)	5.72±0.151^{a}	3.3±0.9^{d}	7.70±0.120^{a}	5.0±4.53^{a}	91.4±10.22^{b}
	4 (6)	5.69±0.165^{a}	3.1±1.0^{d}	7.75±0.138^{ab}	5.8±4.95^{a}	95.8±9.36^{b}

Remark : Values with different lowercase (a – b) superscripts in the same row were significantly different (p<0.05)

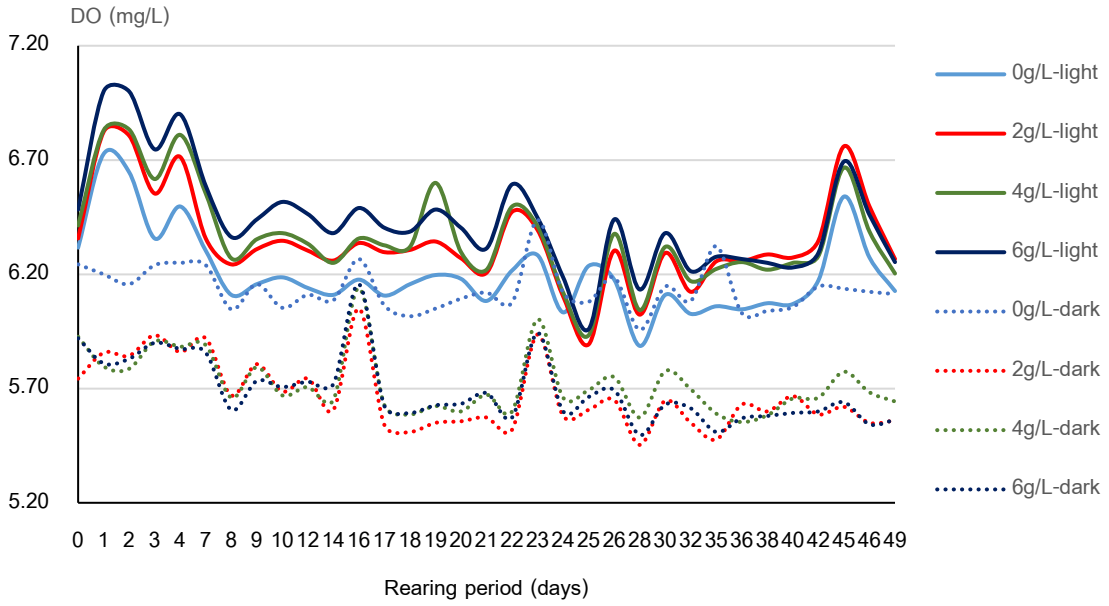

Figure 2 Dissolve oxygen (DO) of water during light and dark periods in each RAS treatment

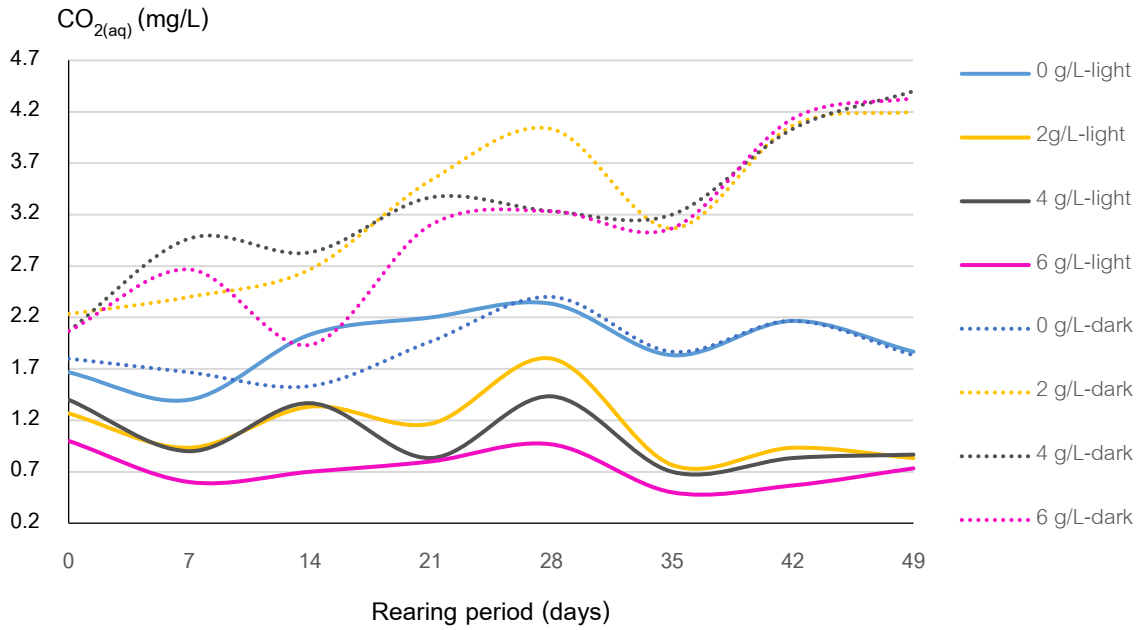

Figure 3 Dissolved carbon dioxide ($CO_{2(aq)}$) of water during light and dark periods in each RAS treatment

Table 6 Comparisons of N-compounds (mg N/L) of water in each RAS treatment.

Rearing periods (days)	*C. lentillifera* density (g/L of system)			
	Control	Treatment		
	0	2	4	6
0	0.342±0.0214 ^{a}	0.293±0.0539 ^{a}	0.340±0.0322 ^{a}	0.295±0.0394 ^{a}
7	1.990±0.6346 ^{b}	1.452±0.7585 ^{ab}	1.778±0.3850 ^{b}	1.385±0.0750 ^{b}
14	6.337±0.8079 $^{c\,C}$	3.382±1.6016 $^{bc\,AB}$	3.760±0.5423 $^{c\,B}$	2.358±0.2600 $^{c\,A}$
21	9.280±1.3091 $^{d\,C}$	5.115±2.1322 $^{cd\,B}$	5.258±1.0819 $^{d\,B}$	2.902±1.0155 $^{cd\,A}$
28	11.460±0.7896 $^{e\,C}$	6.317±1.9233 $^{de\,B}$	5.983±1.1665 $^{d\,B}$	3.857±0.7509 $^{d\,A}$
35	13.072±0.6393 $^{f\,C}$	6.735±2.1889 $^{de\,B}$	5.490±1.2817 $^{d\,B}$	3.782±0.9447 $^{d\,A}$
42	13.957±0.5954 $^{f\,D}$	7.202±2.2048 $^{de\,C}$	5.383±0.8730 $^{d\,B}$	3.510±1.0467 $^{d\,A}$
49	13.180±3.1939 $^{f\,C}$	8.117±2.9424 $^{e\,B}$	5.898±2.1003 $^{d\,AB}$	4.867±1.0978 $^{e\,A}$

Remark : Values with different lowercase (a – b) superscripts in the same column were significantly different (p<0.05)

Values with different uppercase (A – B) superscripts in the same row were significantly different (p<0.05)

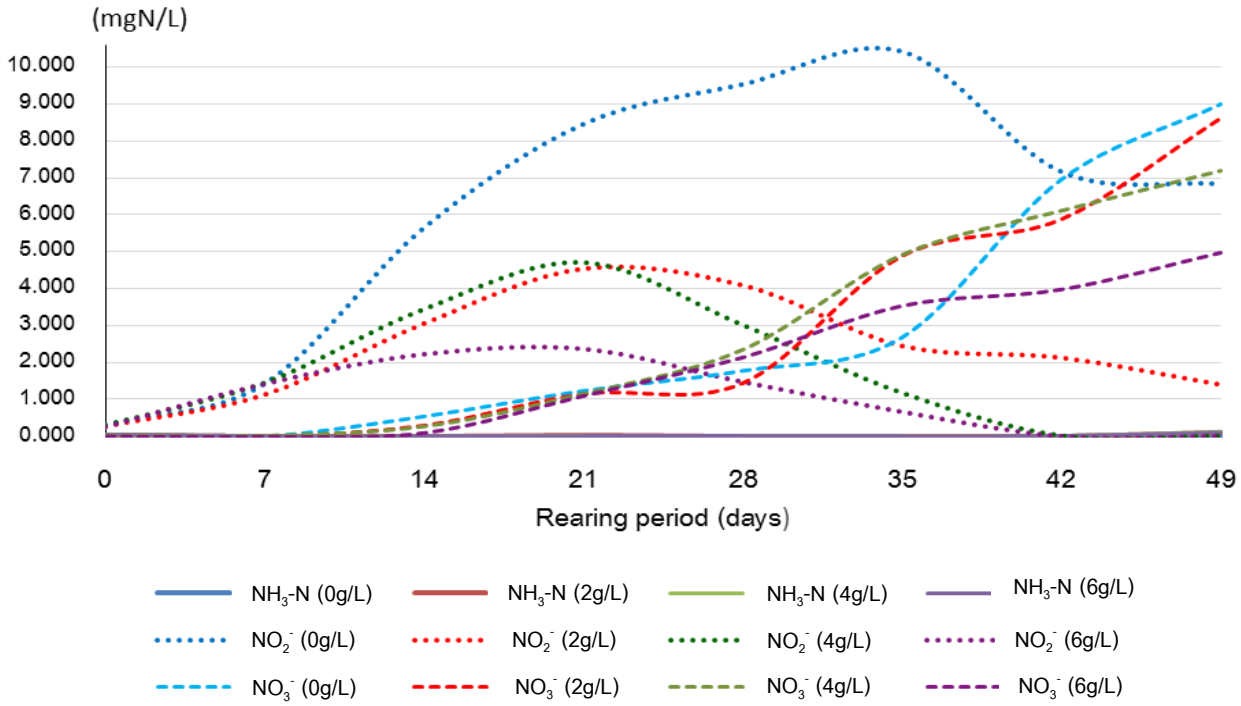

Figure 4 Nitrification processes of changing ammonia to nitrite and to nitrate in water of each RAS treatment.

Table 7 Comparisons of soluble phosphate (mg P/L) in water of each RAS treatment.

Rearing periods (days)	*C. lentillifera* density (g/L of system)			
	Control	Treatment		
	0	2	4	6
0	0.440±0.0390 ^{a}	0.440±0.0358 ^{a}	0.433±0.0258 ^{a}	0.467±0.0516 ^{a}
7	0.655±0.0295 ^{b}	0.627±0.0350 ^{ab}	0.638±0.0436 ^{b}	0.652±0.0674 ^{b}
14	0.860±0.0544 $^{c\,C}$	0.752±0.0436 $^{b\,A}$	0.790±0.0529 $^{c\,AB}$	0.828±0.0864 $^{b\,AB}$
21	1.508±0.0449 $^{d\,B}$	1.310±0.0732 $^{c\,A}$	1.400±0.0645 $^{d\,A}$	1.402±0.1165 $^{c\,A}$
28	1.693±0.0977 ^{e}	1.532±0.1930 ^{c}	1.693±0.1366 ^{e}	1.603±0.1732 ^{d}
35	1.895±0.0850 ^{f}	1.803±0.2581 ^{d}	1.947±0.1248 ^{f}	1.778±0.1113 ^{de}
42	2.077±0.0737 ^{fg}	2.020±0.3023 ^{d}	2.177±0.1637 ^{g}	1.972±0.1338 ^{f}
49	2.027±0.2955 ^{g}	1.955±0.3230 ^{d}	2.143±0.1815 ^{g}	1.862±0.3271 ^{ef}

Remark : Values with different lowercase (a – b) superscripts in the same column were significantly different (p<0.05)

Values with different uppercase (A – B) superscripts in the same row were significantly different (p<0.05)

Discussion

C. lentillifera specific growth rate (SGR)

The results of the SGR of *C. lentillifera* showed that the suitable initial rearing density of *C. lentillifera* for the RAS was not less than 2 g/L and harvesting period during 35-42 days. In practice the SGR of *C. lentillifera* could be higher or lower than that shown in this experiment depending on environmental conditions of the RAS, such as the area of seaweed expose to light or nutrients along with temperature, salinity, water movement. Those factors can determine seaweed growth (Mooney-McAuley *et al*., 2016; Nursidi *et al*., 2017; Harrison & Hurd, 2001). For water treatment, total weight gain was more important than SGR. In this experiment, the highest weight gain was 6 g/L at a rearing period of 42 days. Therefore, the starting seaweed density of 6 g/L, harvesting period during 35-42 days and replanting new seaweed before the old seaweed was harvested for a day or at the same time of harvesting are suggested for an efficient RAS.

A. clarkii specific growth rate (SGR), food conversion ratio (FCR) and survival rate

The results of *A. clarkii* SGR showed no difference among treatments. However, the FCR of the fishes reared in the seaweed treatment at 6 g/L was lower than that of the fishes reared in units without seaweed. There was no difference with the fishes reared in 2 and 4 g/L treatments. The fish survival rates in all seaweed densities were higher than those in the control. The fishes grew in all seaweed treatments were also more active in feeding than those in the control. This result can be explained by the fact that RAS with seaweed (*C. lentillifera* 2-6 g/L) purified water provides better environmental conditions for fish health and feeding efficiency (Roque d' Orbcastel *et al*., 2009; Zhang *et al*., 2011).

Water qualities in each treatment of Recirculating Aquaculture System (RAS)

Water qualities in seaweed treatments at 2-6 g/L during light periods, showed higher DO, pH, Alk-P, Alk-T and lower $CO_{2(aq)}$ when compared with those without seaweed. But water quality during the dark period showed increase in $CO_{2(aq)}$ to the level which did not reduce fish growth rate (Moran and Stottrup, 2011; Fivelstad, 2013; Ben-Asher et al., 2013) nor their health condition (Fivelstad et al., 1998). DO and pH (Figure 2 and 3) decreased during the dark period in the seaweed treatments to the level still acceptable for coastal aquaculture (Coastal Fisheries Research and Development Bureau, 2018). Most RASs exhibited problems of low DO, pH, Alk-T and high $CO_{2(aq)}$ (Zhang et al., 2011; Ben-Asher et al., 2013) but this experiment results show that using *C. lentillifera* 2-6 g/L to purify water in RAS gave increased DO, pH, Alk-T and decreased $CO_{2(aq)}$ in water during the light period, while water condition was not harmful to the fish in the dark period. This result indicated that the density of the seaweed at 2-6 g/L RASs can provide suitable oxygen and decrease $CO_{2(aq)}$ in the system water. DO aeration in the fish basin may solve the problems of low DO and high $CO_{2(aq)}$ during dark period. Using seaweed to supply oxygen and decrease $CO_{2(aq)}$ in a RAS during daytime (using sun light for the seaweed photosynthesis) would help to save

electricity cost. The densities of each seaweed treatment from the beginning to day 49 increased to 21±3.114 g/L, 22.81±2.932 g/L and 29.19±4.271 g/L, so there should be more DO during the light period and more $CO_{2(aq)}$ during the dark period. However, the results of water quality monitoring showed that in the seaweed treatment DO remained in the ranges of 6-7 mg/L and $CO_{2(aq)}$ was in the ranges of 3-4 mg/L (Table 4 and figure 2-3). This is because the saturated dissolved gas in water depends on temperature, salinity and pressure (Clot, 2012). Excess DO and $CO_{2(aq)}$ produced within the system then vaporize to the air.

The comparisons of the concentrations of N-compounds between the control without seaweed and seaweed treatments at 2, 4 and 6 g/L densities showed that the N-compounds in water of the seaweed treatments were lower than those in the control. Among the three seaweed densities, the amount of N-compounds in 6 g/L was lower than those in the 2 and 4 g/L treatments. The highest decreasing rate of N-compounds was 0.08 mg N/g seaweed/L/system/day in 2 g/L treatment comparing to 0.04 mg N/g seaweed/L/system/day in 4 and 6 g/L treatments. The nitrification process of changing ammonia to nitrite and to nitrate in the seaweed treatments were better than that in the control (Figure 4).

The soluble phosphate (PO_4^{3-}) concentrations between the control and seaweed treatments were not different. This means that *C. lentillifera* was unable to decrease PO_4^{3-} in RAS water (Table 7), corresponded with the results of Mamat (2004). In comparison, cultures of *Ulva intestinalis* was found able to decrease the PO_4^{3-} concentration in water (Kakhai *et al*., 2008; Kunawongdet *et al*., 2009).

Conclusions

When using *C. lentillifera* for biological water treatment in RAS, the density of the seaweed should be at least 2 g/L, harvesting period of the seaweed should be 35-42 days and replanting the new seaweed should take place a day before, or on the same day that the old seaweed is harvested. *A. clarkii* reared in 2-6 g/L of seaweed densities showed lower FCR and greater survival rate than the fishes reared in the control, but the fish SGR among treatments were not significantly different ($p>0.05$). Water qualities in the 2-6 g/L seaweed treatments during light periods resulted in higher DO, pH, ALk-P, Alk-T and significantly lower $CO_{2\ (aq)}$ than those without seaweed ($p<0.05$). During the dark period the DO was lower and $CO_{2\ (aq)}$ was significantly higher than the control ($p<0.05$). The concentrations of N-compounds in water of the 2-6 g/L seaweed densities were significantly lower than those in the control ($p<0.05$). The highest rate of decreasing N-compounds occurred at 2 g/L. The concentration of soluble phosphate was not significantly different ($p>0.05$) among treatments.

Acknowledgements

I would like to sincerely thank the following persons for their kindness to improve this research report to be more valuable. They are including my adviser Professor Dr. Kwei Lin from the Asian Institute of Technology for helping to improve English grammar, Dr. Thidarat Noiraksar, a senior scientist at the Institute of Marine Science Burapha University for helping to correct scientific words, Dr. Angus MacNiven, Managing Director Baan promong Co., Ltd., for editing the report draft, and Assistant Professor Dr. Anukul Buranapratheprat for editing and proofing the final report.

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