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Potential of *Ulva* sp. in biofiltration and bioenergy production

Tiềm năng rong Ulva sp. *trong lọc sinh học và sản xuất năng lượng sinh học*

Research article

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In order to evaluate the effect of seaweeds in bio-filtration for removing nitrogen from marine aquaculture and in bioenergy production, *Ulva* sp. was used in this study. Experiments were triplicated and run in 3-day incubation at salinities with 30 psu, 10 psu and 5 psu in different initial ammonium nitrogen concentrations from 100 µM to 10,000 µM, equivalently to marine aquaculture conditions. The highest concentrations of ammonium removed were about 690 µmol (12.42 mg) NH_4^+ at 30 psu, 410 µmol (7.38 mg) NH_4^+ at 10 psu and 350 µmol NH_4^+(6.3 mg NH_4^+) at 5 psu in three days of incubation, while highest growth rates of *Ulva* sp. were 49% and 150% per day at 500 µM of initial ammonium concentration, similarly to the growth rate reported in microalgae. Moreover, after these experiments, biomass of *Ulva* sp. has been tested for bioenergy producing goals, because the carbohydrate concentration of this alga was very high, reaching 60-70% of DW. Thus, *Ulva* sp. can be cultured to remove nitrogen concentration in eutrophication conditions at aquaculture systems in combination with the purpose of bioenergy production after harvesting.

Để đánh giá hiệu quả của tảo biển trong việc lọc sinh học loại bỏ hợp chất ni tơ từ việc nuôi trồng thủy sản và trong việc sản xuất năng lượng sinh học, Ulva sp. đã được sử dụng trong nghiên cứu này. Các thí nghiệm được lặp lại 3 lần và chạy trong 3 ngày trong tủ ổn nhiệt tại các điều kiện độ mặn 30psu, 10psu, 5psu ở các nồng độ NH_4^+*-N từ 100µM đến 10.000µM, tương đương với điều kiện nuôi trồng thủy sản nước mặn. Nồng độ cao nhất của* NH_4^+*-N được loại bỏ khoảng 690 µmol* NH_4^+*(12,42mg* NH_4^+*) tại 30psu, 410µmol* NH_4^+*(7,38mg* NH_4^+*) tại 10psu và 350 µmol* NH_4^+*(6.3mg* NH_4^+*) tại 5psu, trong đó tỉ lệ sinh trưởng của Ulva sp. là rất cao, sinh trưởng từ 49 đến 150% mỗi ngày tại nồng độ ammonium ban đầu 500 µM tương đương với sinh trưởng của vi tảo. Hơn nữa, sau các thí nghiệm trên, sinh khối của Ulva sp. được thử nghiệm sản xuất năng lượng sinh học vì hàm lượng carbohydrate trong tảo rất cao, chiếm khoảng 60-70% trọng lượng khô của tảo. Như vậy, Ulva sp. có thể được nuôi trồng để loại bỏ hợp chất ni tơ trong điều kiện phú dưỡng của các hệ thống nuôi trồng thủy sản, kết hợp với mục tiêu sản xuất năng lượng sinh học sau thu hoạch.*

Keywords: *Ulva* sp., biofiltration, aquaculture, bioenergy production

1. Introduction

There are many worldwide interests in the potential of microalgae and macroalgae in bioenergy production, particularly for oil and biodiesel production. The potential of bioenergy resources from macroalgae relates to that of alga feedstock. This was evaluated by growth rate of alga biomass in optimal conditions. In the Southeast Asia area, macroalgae which are the available resources in the brackish or marine medium need to be studied deeply as a strategy of sustainable environmental protection. Many species of macroalgae such as Ulva sp., Gracilaria sp., Porphyra sp. have been studied as object for biofiltration in eutrophicated water of aquaculture (Pedersen, 2004; Zhou et al. 2006; Kang et al. 2011; Dong Xu et al. 2011).

Macroalgae are considered as potential materials for biofuel production, because they have high carbohydrate content suitable for transformation processes into bioethanol and other products (Reith et al., 2005). Macroalga utilization offers many benefits by enhancing economic production through mitigation of eutrophication in aquaculture ponds and using harvested biomass for bioenergy. In the present study, we estimated the growth rate of macroalga *Ulva* sp. under laboratory conditions for re-

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moving nitrogen from eutrophic water equivalent to polluted aquaculture conditions and we evaluated the potential of bioenergy production from this algal biomass.

2. Materials and Methods

Ulva sp., classified as *Enteromorpha* sp. before, was collected from the Ishigaki-Jima Island, Okinawa Prefecture, Japan and cultured in IMK medium for microalgae (Nihon-Seiyaku Co., Ltd). The seawater taken from Hiroshima Bay, Seto Inland Sea, Japan and filtered by 0.45µm membrane filter was used for preparing the culture medium. The filtered seawater of Hiroshima Bay was diluted by distilled water at 30, 10, 5 psu, and the modified IMK medium and K_2HPO_4 solution were added, so the final concentration of DIP was 30 µM. The initial ammonium nitrogen was added like 100, 500, 1000, 2000, 5000 and 10000 µM concentrations for 30 psu experiments; 200, 500, 1000, 2000 and 5000 µM of concentrations for 10 psu experiments; 200, 500, 1000, 2000 and 5000 µM for 5 psu experiments, respectively.

About 0.11-0.35 g of wet weight alga was taken, depending on each batch of experiments. All batches of experiments were inoculated in 2 L plastic bottles with 1.8 L of the culture medium. All tanks were incubated at 25°C, with light intensity of 800 µmol/m^2/s where the L: D is 14 h: 10 h, and getting bubbled by the air.

The water samples for ammonium nitrogen analysis were collected every 24 hours, and filtered by glass fiber filter (Whatman, GF/F). After a 3-day incubation, the algal biomass was collected and weighted in order to calculate growth rate and to evaluate chemical composition.

Ammonium nitrogen and phosphorus concentrations in the medium were measured by HACH test kit (Hach Co., Ltd). The collected algal biomass was weighted, and the water content of the algae was measured by weight before and after dry-freezing process, following determination of ash content, total nitrogen and total phosphorus concentrations.

The chemical compositions of the algal biomass and monosugar were measured by HPLC method to evaluate the potential for bioenergy production.

3. Results and Discussion

3.1 Growth rate of *Ulva* sp.

The growth rates of *Ulva* sp. in the various salinities, initial NH_4-N concentrations were different (Figure 1). Growth curves at 30 psu differed from 10 psu and 5 psu and values of growth rates were decreasing gradually whereas initial ammonium concentrations were increased. Initial ammonium of 500 µM was the best condition for growth of *Ulva* sp. at 30 psu and 10 psu experiments, in which the growth rate of the algae was extremely high, reaching about 149% and 49%/day, respertively.

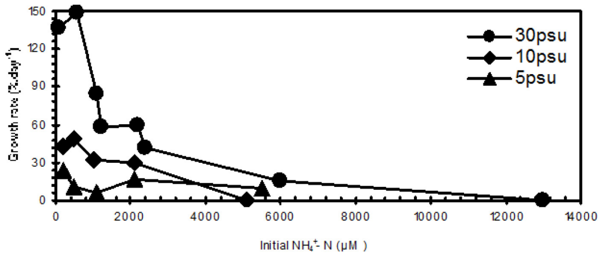

Figure 1. The growth rate of *Ulva* sp.

The growth rate of *Ulva* sp. tended to decrease when initial ammonium concentration was increased, and salinity was decreased. The *Ulva* sp. can grow even in conditions of 2,000 to 5,000 µM of initial ammonium concentrations, which is an extremely high ammonium concentration. They can not grow at 10,000 µM of initial high ammonium concentration at 30 psu. With 2,000 and 5,000 µM of initial ammonium concentrations in 30 psu experiments, the growth rates of *Ulva* sp. were 42% and 16% per day, respectively.

In low salinity concentrations, at 10 and 5 psu experiments, the growth rates were lower than that in higher salinity. However, *Ulva* sp. can still grow in 5,000 µM of initial ammonium concentrations from 5 to 30 psu experiments. It should be noted that growth rates of some other macroalga species (E.Msuya, Amir Neori, 2000) were lower than that of *Ulva* sp. cultured in this experiments. Growth rates of *Ulva reticulata, Gracilaria crassa, Chaetomopha crassa* in E.Msuya's research were from 0,5 to 3% per day. Growth rate of *Ulva lactuca* (Pedersen and Borum, 1996) reached up to 30%/day in northern temperate regions. It was proved that growth rates of *Ulva* sp. in various initial NH_4^+-N concentrations at 30 psu, 10 psu and 5 psu were extremely high, and the highest growth rate in 500 µM of initial ammonium at 30 psu reached 149% per day. Therefore, *Ulva* sp. could be suitable for water treatment by ammonium uptake.

3.2 Ammonium nitrogen uptake rate of *Ulva* sp.

Table 1 shows that the capacity of ammonium nitrogen uptake by the algae in a 3-day incubation was extremely high. At the 30 psu experiments with 100 µM of initial ammonium nitrogen concentration, ammonium was rapidly removed by the algae. The ammonium nitrogen was uptaken highly in all experiments. Ammonium nitrogen uptake of this alga were 160 µmol to 690 µmol in 3-day incubation depending on initial ammonium concentrations.

Table 1. Ammonium nitrogen uptake rate of *Ulva* sp.

Salinity (psu)	Initial ammonium nitrogen (µM)	Uptake rate of ammonium nitrogen (µmol/ 3 days)
30	100	310
	500	690
	1,000	600
	2,000	600
	5,000	450
	10,000	nd
10	200	360
	500	410
	1,000	250
	2,000	360
	5,000	nd
5	200	350
	500	310
	1,000	160
	2,000	nd
	5,000	nd

* nd: not definited

Figure 2 demonstrates the ammonium nitrogen uptake rates of *Ulva* sp. At 30 psu experiment, capacity of ammonium nitrogen uptake of this alga was highest, about 4-5 mmol/g initial wet weight in 3-day incubation. This was equivalent to about 64-70 mg ammonium removed by one gram of algal wet weight in 3-day incubation. However, at 10 psu and 5 psu experiments, ammonium uptake of *Ulva* sp. was smaller, about 1 mmol/g initial wet weight in 3-day incubation. In this condition, value of ammonium uptake of *Ulva* sp. was also high.

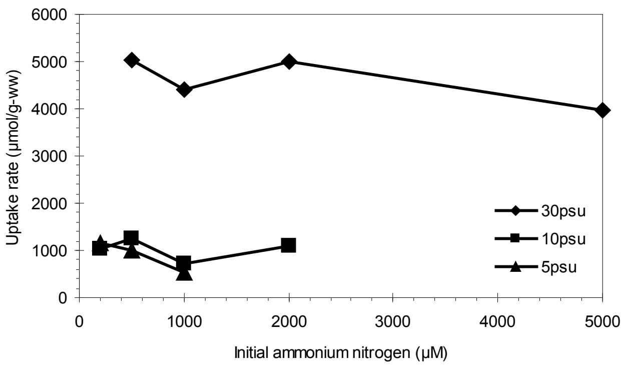

Figure 2. Uptake rate of ammonium nitrogen (µmol/3days).

There are many factors influencing the growth rate of green algae and their removing ability for nutrients like physical condition (pH, salinity, temperature, etc.) and chemical condition (nutrients, metals, etc.) in cultivation media. However, nitrogen and phosphorus were two main nutrients that limit algal growth and yields in most natural environments (Harrison and Hurd, 2001). Nitrate and ammonia were important available nitrogen sources for growth of seaweeds. After these ions are absorbed from the environment, they are generally incorporated into amino acid and proteins, or used in the synthesis of pigments such as phycoerythrin, which is considered to be a store of available nitrogen (Lapoine, 1985; Ryther et al., 1981). However, in many types of seaweeds, nitrogen in the form of ammonium was frequently absorbed and assimilated more rapidly than nitrate and urea (De Boer, 1981). In this report, the carried out experiments showed that capacity of NH_4^+ uptake was very good by *Ulva* sp. in different salinities.

In general, ammonium uptake rates of *Ulva* sp. were different depending on initial NH_4-N concentrations and sanilities. In media with initial high concentrations of ammonium (5,000 µM and 10,000 µM), NH_4^+ uptake by *Ulva* sp. was limited. In initial lower concentrations of ammonium, (500 µM to 2000 µM), nutrient uptake by the alga was very good at different salinities.

3.3 Chemical composition of *Ulva* sp.

After experiments, water content of the alga was determined. Water contents in almost of the algal samples were very high, about from 80% to 92%, depending on initial conditions of culture. Phosphorus content of the algal biomass was about from 0.1% to 1% depending also on initial experimental conditions. The obtained data showed that phosphorus contents were higher in *Ulva* sp. cultured in conditions of lower salinity (These values were from 0.1% to 0.3% at 30 psu, from 0.59% to 0.78% at 10 psu and about 1% at 5 psu).

The data about ash, protein and carbohydrate (C-H) contents of *Ulva* sp. biomass presented in Table 2 showed that they changed depending on the initial ammonium concentrations and salinities.

Table 2. Chemical composition of *Ulva* sp.

Sanility (psu)	**Initial NH_4^+-N** (µM)	**Ash** (% dw)	**Protein** (% dw)	**C-H** (%dw)
30psu	100	28.88	11.44	59.68
	500	24.87	14.48	60.65
	1,000	16.19	18.69	65.12
	2,000	15.06	19.63	65.31
	5,000	8.55	20.33	71.12
	10,000	6	23.42	70.58
10psu	200	17.72	23.93	58.35
	500	16.03	27.35	56.62
	1,000	11.54	27.98	60.48
	2,000	11.31	27.96	60.73
	5,000	10.68	25.23	64.09
5psu	200	7.65	32.33	60.02
	500	5.19	31.23	63.58
	1,000	2.44	29.92	67.64
	2,000	2.34	30.62	67.04
	5,000	2.24	30.98	66.77

In general, carbohydrate content containing 60-70% of DW increased a little, while the ash content decreased indicating advantages of this alga species serving as a source for producing biofuel.

4. Conclusions

The green alga *Ulva* sp. can be cultured, and survived in medium having high ammonium concentration and showed quickly growth rate from 6% to 149% per day.

The obtained results indicated that *Ulva* sp. could be useful for nitrogen removing as biofiltration with 690 µmol (12.42 mg) NH_4^+ at 30 psu, 410 µmol (7.38 mg) NH_4^+ at 10 psu and 350 µmol (6.3 mg) NH_4^+ at 5 psu in 3-day incubation. Moreover, it's biomass could be a suitable source for bioenergy production because of the very high content of carbohydrates (60-70% of DW).

So, the nutrient uptake ability of the alga combined with its biomass after harvesting for potential energy production may offer an economically viable solution for sustainable development.

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