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Aquacultural characteristics of *Rhizoclonium riparium* and an evaluation of its biomass growth potential

Kuo-Ping Chao[1], Chung-Sing Chen[2], Eugene I-Chen Wang[3] & Yu-Chang Su[3,*]
[1]*Department of Biological Science and Technology, Chung Hwa College of Medical Technology, Tainan, Taiwan;* [2]*Department of Aquaculture, National Taiwan Ocean University, Keelung, Taiwan;* [3]*Division of Wood Cellulose, Taiwan Forestry Research Institute, Taipei, Taiwan*

*Author for correspondence: e-mail: ycsu@serv.tfri.gov.tw phone: +886-2-2301-8366

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Abstract

A study was made of environmental factors affecting the growth of *Rhizoclonium riparium* in order to evaluate its suitability for large-scale culturing. The results indicate that under the natural conditions prevailing at Taishi, Taiwan, this species can grow year-round, with a monthly biomass production (oven-dried) of 945–1540 kg ha^{-1} pond surface (assuming a pond depth of 1 m). The specific growth rate ranged from –2.1 to 10.4% per day. Salinity and temperature, both influenced the rate significantly, with optimal values at 20‰ and 25 °C, respectively. Short (2-mm) lengths of filaments had a higher specific growth rate than longer (20 mm) filaments. Under rotational culturing conditions, the specific growth rate was reduced when flow was increased.

Introduction

Rhizoclonium riparium is a cosmopolitan filamentous alga, which occurs in a variety of habitats in the subtropical environment of Taiwan, including semi-enclosed intertidal zones, marshy areas of estuaries and abandoned aquaculture ponds. It prefers brackish water, such as the intertidal zone, to full marine conditions and is especially abundant in standing water. It poses a big threat for aquaculturists, as it removes dissolved oxygen at times, reduces space for cultured animals and hinders their growth (Caffrey, 1992). Aquaculturists continue to make various attempts to suppress growth of this alga (Lin & Lin, 1981; Caffrey & Monahan, 1999; Ridge et al., 1999), but this requires large investments in manpower, time and chemicals. Several studies have also investigated how filamentous algae might be used for the treatment of wastewater (Mulbry & Wilkie, 2001; Erler et al., 2004).

Rhizoclonium can be substituted for a portion of the wood fiber currently obtained from forests usually used to make paper (Chao et al., 1999, 2000); however, a steady supply of sufficient quantities is needed if *R. riparium* is to serve as a substitute on an industrial scale. The aim of the study reported here was to investigate some of the practical aspects of culturing *R. riparium* as a raw material for use in paper-making. This involved a series of small-scale growth experiments to estimate its potential for producing biomass in such an environment.

Materials and methods

Measurement of growth under natural conditions in outdoor experiments

The outdoor experiments took place at the Taishi Branch Station of the Taiwan Fishery Research Institute from July 1997 to August 1998. (One of the reasons for the delay in the presentation of these results is that Taishi Station was at the time a military-restricted area.)

Taishi is in south-central Taiwan, adjacent to the Taiwan Strait (ca. 1.5 km). Apart from typhoons, it has little rain but strong sunshine. Because of dilution by

effluent from a nearby clam culturing farm, the salinity of seawater drawn at Taishi can often be less than 25‰. Seawater for these experiments was therefore taken only at high tide, when salinity is back to normal levels.

First, 1-m^3 culturing tanks (made from fiberglass reinforced plastics and 120 cm diameter, 95 cm high, $n = 5$) were filled with clean seawater adjusted to a salinity of 20‰ with clean groundwater and exposed to the atmosphere. Samples of *R. riparium* (100 g wet weight; equivalent to an oven-dried weight of about 20 ± 5 g) were placed in these tanks located outdoors at the Research Institute. Every 2 weeks, salinity was recorded and all the *R. riparium* was removed from the tank using a 300 mesh plankton net. The alga was first rinsed with 20‰ seawater to remove small entrained algae, and then centrifuged for 2 min to remove surface moisture. Samples (2–3 g wet weight) were taken from random locations in the mass, placed in weighing bottles ($n \geq 3$) and heated in a 105 °C oven for 8 h. The oven-dry weight of these samples were measured and used to estimate the oven-dried weight of the entire mass. The tanks were then emptied, cleaned and refilled with 20‰ seawater and were re-seeded with 100 g (wet weight) of *R. riparium.* This 2-week experiment cycle continued from July 1997 to August (summer) 1998 for 28 cycles. Climatic data were collected for this period.

During July and August 1998, a parallel experiment was run using five additional 1-m^3 tanks in which the seawater was completely replaced weekly.

Also from July 1997 to August of 1998, to determine maximum growth with no alga replacement, 100 g (wet weight) alga was put in 2.5-m^3 culture tanks (200 cm diameter 85 cm high, $n = 3$) and cultured with 2 t of 20‰ seawater in an outdoor environment. Salinity was measured and adjusted every 2 weeks. Growth was measured every 2 weeks as described earlier, but all *R. riparium* was returned to the tanks, and culturing continued until the biomass did not increase further. To determine maximum growth (as oven-dried weight) in all of these experiments, specific growth rate was calculated from the raw data as follows:

$$\text{specific growth rate} = 100 \times [(\ln W_f - \ln W_i)/\ln W_i]\, t^{-1} \qquad (1)$$

where W_i is the initial and W_f the final weight after t days of incubation.

Effects of salinity and temperature on specific growth rate

After selectively culturing *R. riparium* for over 1 year to arrive at a purified strain, seven cleaned snippets of the alga 2 ± 0.2 mm in length, were put into each of the wells (diameters 3.4 cm; height 1.72 cm) of covered six-well petri culturing dishes (one dish per salinity). Ten milliliter of filtered (0.45 μm filtration membrane), sterilized seawater diluted with sterilized distilled water to a salinity of 10, 15, 20, 25, 30 or 33‰ were added to each well and covered. No nutrient salts were added. The petri dishes were then placed in walk-in culturing rooms with the temperature adjusted to a constant 15, 20, 25 or 30 °C and a 12-h light–12-h dark regime (light phase = 100 μmol photon m^{-2} s^{-1}). The seawater culture medium was drawn off and replaced every 2 days. Each culturing condition had at least three replications. Every week for the 4-week experimental period, growth of the alga was measured under a dissecting microscope. For this experiment, length was used instead of weight to calculate the specific growth rate:

$$\text{specific growth rate} = 100 \times [(\ln L_f - \ln L_i)/\ln L_i]\, t^{-1} \qquad (2)$$

where L_i is the initial and L_f the final length after t days of incubation.

Effects of rotational culturing and algal snippet length on the growth of the alga

For the rotational culturing experiment, conditions were the same as above (2 ± 0.2 mm in alga length) except that the temperature was maintained at 25 °C and the covered six-well petri culturing dishes were put on rotating tables with the speed set to 0, 50 or 100 rpm. The rotational culturing experiment was continued for 2 weeks.

The effect of original length on the growth of the algal snippet at 25 °C was evaluated by placing seven snippets of cleaned alga (20 ± 2 mm) and 100 mL filtered, sterilized diluted seawater (conditions and salinities as described earlier, but did not use rotational culturing) into petri dishes (diameter 14.12 cm; height 1.18 cm). The length effect experiment was continued for 4 weeks. For both experiments, there were three replicates for each salinity condition. Growth

measurements were made weekly with a dissecting microscope. The specific growth rate was determined using Equation (2).

Statistical analysis

Using PC-SAS version 6.12 (SAS Institute), multivariate analysis was used to compare the various environmental factors and the corresponding specific growth rates. Significance for salinity and temperature was set at $p < 0.01$, and Tukey's test was performed to compare the differences across treatments ($\alpha = 0.05$).

Results

Growth rate in outdoor culture

At the Taishi outdoor culturing site, the average sunlight ranged from 4.07 to 26.1 MJ m^{-2}, and the mean daily air temperature ranged from 10 to 32 °C (Figures 1A and B). After 2 weeks of exposure to dilution by rainwater and evaporation by sunlight, the salinity of the water in the tanks varied from 10 to 22‰ (Figure 1C). Under these conditions, as shown in Figure 1D, mean specific growth rates reached a maximum of 8% per day in early April, 1998 (week 35), and a minimum of approximately -1.5% during mid-December 1997 (week 20). The specific growth rates for the weekly water replacement experiment were between 5.2 and 1.5% per day, which are represented by the heavy black lines in Figure 1D.

Maximum biomass and growth rate

Figure 2 shows the biomass data for the *R. riparium* in the 2.5-m^3 outdoor tanks. Growth increments were highest early in each experiment, and tended to decrease with time. From an initial *R. riparium* algal mass of 100 g (wet weight), the maximum biomass attained was 458 g (oven-dried). The maximum specific growth rate was 16% per day, and the minimum was –0.4% per day. As shown in Figure 3, an analysis of the specific growth rate versus the ratio of liquid (i.e., the number of liters of seawater for each gram oven-dried alga) revealed a linear correlation ($R^2 = 0.804$). Growth rates were high when the algal density was low and low when the algal density was high.

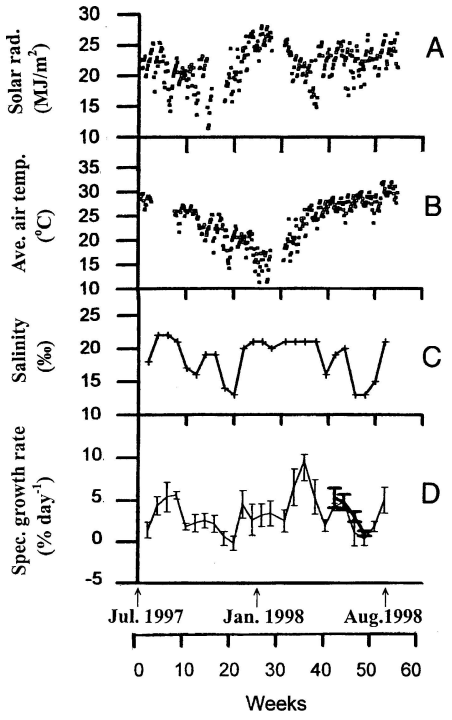

Figure 1. Mean specific growth rates of *R. riparium* in outdoor culture. (A) Sunlight. (B) Mean daily air temperature. (C) Salinity before water replacement. (D) Specific growth rate (the heavy line in (D) shows the specific growth rates for the weekly water replacement experiment, five replicates).

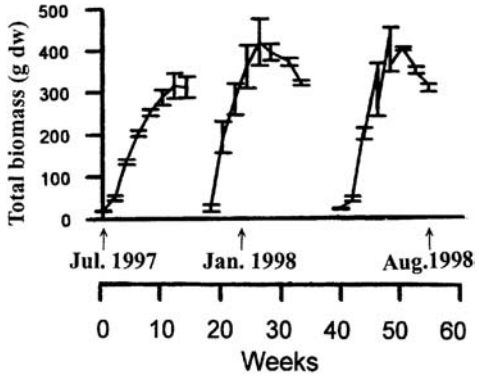

Figure 2. Maximum growth of *R. riparium*. in 2.5-m^3 tanks at Taishi. Dry weight of algal mass in grams; three replicates.

Effects of salinity, temperature and snippet length on growth

Figure 4 shows the average specific growth rates of algal snippets under continuous batch culture for 4 weeks at different salinities (10–33‰) and temperatures (15–30 °C). Both salinity and temperature significantly

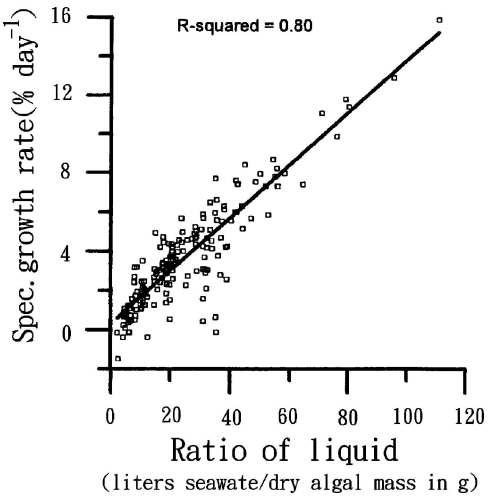

Figure 3. Specific growth rate vs. ratio of liquid (liters seawater/dry algal mass in grams) (based on results in Figures 1 and 2).

Table 1. Summary of statistical analysis of specific growth rates showing the significance of both temperature (*T*; 15–30 °C) and salinity (*S*; 10–33‰) for a culture period of 4 weeks and an initial algal length of 2 mm.

Source	d.f.	ANOVA.SS	Mean square	*F* value	Pr > *F*
T	3	314.8	104.9	710.3	0.0001
S	5	33.3	6.66	45.0	0.0001
T ∗ *S*	15	11.3	0.76	5.11	0.0001

Table 2. Statistical significance of both algal length (*L*; 2 mm vs. 20 mm) and salinity (*S*; 10–33‰) on specific growth rate over a 4-week culture period at 25 °C.

Source	d.f.	ANOVA.SS	Mean square	*F* value	Pr > *F*
L	1	122.67	122.67	1121.72	0.0001
S	5	23.67	4.73	43.28	0.0001
L ∗ *S*	5	3.15	0.63	5.76	0.0012

($p < 0.0001$) affected specific growth rate (Table 1). Tukey's tests showed that the best specific growth rate (9.47 ± 5.06) occurred at 25 °C, followed by 20, 30 and 15 °C, respectively. The optimal salinity for algal growth was 20‰, followed by 30, 25, 15, 10 and 33‰. After optimal temperature of 25 °C, the original length of the snippet (2 mm vs. 20 mm) was also significant ($p < 0.0001$), with shorter snippets having a higher specific growth rate than longer ones (Figure 4; Table 2).

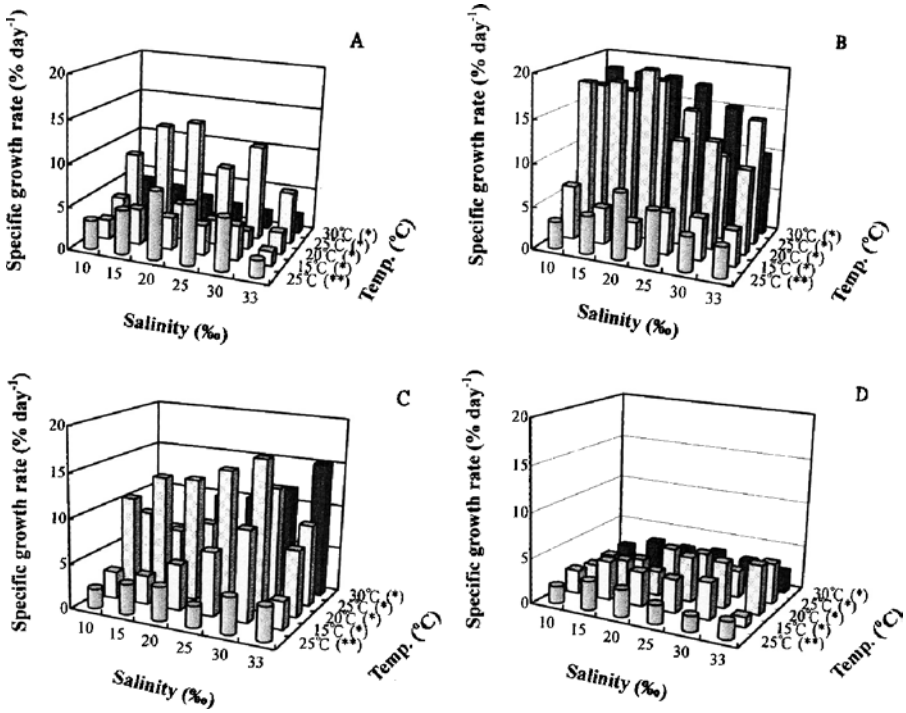

Figure 4. Average specific growth rate (measured by length) of *R. riparium* during 4 weeks of continuous culture at different salinity and temperature. Initial algal snippets were either 2 mm (*) or 20 mm (**) in length. Error bars omitted. (A) Week 1; (B) Week 2; (C) Week 3; (D) Week 4.

Table 3. Statistical significance of rotational culturing speed (R; 0–100 rpm) and salinity (S; 10–33‰) on the specific growth rate of the *R. riparium* over a 2-week period at 25 °C.

Source	d.f.	ANOVA.SS	Mean square	F value	Pr > F
R	3	293.59	97.86	236.81	0.0001
S	5	234.50	46.90	113.49	0.0001
$R * S$	15	26.20	1.75	4.23	0.0001

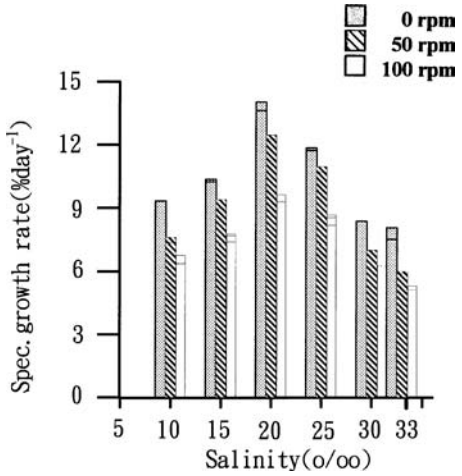

Figure 5. Effect of rotational culturing on the mean specific growth rate of *R. riparium* (2-mm snippets) at 25 °C and various salinities (10–33‰).

Effect of rotational culturing

Figure 5 shows the mean specific growth rates of 2-mm snippets at the optimal temperature (25 °C) and various salinities (10–33‰) during 2 weeks of culture at different rotational speeds. Rotational culturing had a significant influence on the specific growth rate of the alga ($p < 0.0001$; Table 3). The higher the rotational speed, the slower the alga grew.

Discussion

Results from the outdoor experiments (Figures 1 and 2) showed that the *R. riparium* could grow year-round and that salinity strongly influenced its growth. When dilution by rainwater reduced salinity to 13‰, growth deteriorated, and the specific growth rate became negative. Conversely, when salinity remained between 15 and 23‰ (increased by evaporation), *R. riparium* grew well, with biomass gains of 5.0–14% per day.

Statistically significant results were also obtained under the more controlled conditions of the indoor studies (Figure 4; Tables 1–3), which, unlike the outdoor cultures, eliminated competition from other algae, such as diatoms, blue-green algae and other plankton-like algae. High specific growth rates under all six salinity conditions in the laboratory experiments demonstrated the wide salinity tolerance of *Rhizoclonium*, which in the case of *R. riparium* ranges from 0.1 to 34‰ (Imai et al., 1997). Here, the optimum 20‰ salinity for growth of the *R. riparium* suggests that this species is well suited to the conditions in aquacultural ponds near the Taishi estuary. This result is also consistent with other reports that salinity is an important factor for the growth of *R. riparium* (Imai et al., 1997) and *R. implexum* (Phillips et al., 1994, 1996).

The effects of solar radiation and air temperature are not apparent from Figure 1, but the indoor study showed that temperature was significant (Table 1), with 25 °C being optimum for the growth of this species. Again, this is recognized in earlier reports (e.g., den Hartog & Polderman, 1975; Hall & Walmsley, 1991, Bischoff & Wiencke, 1993, McIntire et al., 1994; Mealta et al., 2002). On the other hand, although von Wachenfeldt (1981) has shown that frequent hourly water replacement can enhance the growth of *Chaetomorpha*, Figure 1D suggests that doubling the frequency of water replacement did not have any significant effect under the present conditions.

The maximum-growth experiments (Figure 2), given mean water depth of 70 cm and surface area of 3.14 m^2 then converting to a standard depth of 1 m, give an estimated maximum biomass of between 199 and 229 oven-dried g m^{-2}. This mass was reached on average in about 2.5 months, and equates to between 796 and 916 oven-dried kilogram biomass per hectare of water per month. Since biomass increased fastest when the total biomass was low (Figures 2 and 3), shortening the culture period should further increase the monthly yield. Thus, for a culture period of 1.5 months, between 863 and 1320 oven-dried kg ha^{-1} per month could be harvested. For a 1-month culture period, the yield would be 945–1540 oven-dried kg ha^{-1} per month, and harvesting every 2 weeks would yield between 1200 and 2180 oven-dried kg ha^{-1} per month. These results are comparable to those of Eiseltova and Pokorny (1994), who found that *Cladophora fracta* produced 4900 oven-dried kg ha^{-1} after an average of 2 months' growth, but are rather less than the field report for *Enteromorpha intestinalis*, which

was 26,000–30,000 wet weight kg ha^{-1} (or, assuming a dry mass of 15%, 3900–4500 dry weight kg ha^{-1} per month; Parchevskii & Rabinovich, 1992).

The biomass yield of *R. riparium*, however, far exceeds the 2 m^3 ha^{-1} per year (83 kg ha^{-1} per month, assuming that 2 m^3 is approximately 1000 kg), which is the average growth rate of Canadian forests, and the 4 and 6 m^3 ha^{-1} (based on averages for 1980–1985) annual growths of the US and German forests, respectively (Su and Wang 1998), some current sources of fiber for paper production.

However, both increased snippet length (2 mm vs. 20 mm; Figure 4) and rotational culturing (Figure 5) adversely affected specific growth rates. Other things being equal, longer snippets will have a lower specific surface area compared to shorter snippets, and will thus be less effective in absorbing nutrient salts and gases and ejecting wastes.

As Hein et al. (1995) have pointed out a lower surface area to volume ratio will tend to result in reduced growth rate and a lower production. This effect can be offset by increasing the flow rate of the medium. In general, faster-flowing water increases both the dissolution of oxygen and carbon dioxide and the diffusion and mass transfer of nutrient salts (Ghosh & Gaur, 1994; Saravia et al., 1998). This is because increasing the flow rate causes eddy currents around the alga and thins the viscous boundary layers around the alga (Raven, 1970; Wheeler 1980; Jones et al., 2000), but, at high flow rates above 4 cm s^{-1}, the carbon-fixing capability of *Macrocystis pyrifera* algae is impaired (Wheeler, 1980), and physical damage may be sustained. However, even before these limits are reached, water flow will necessarily increase drag and skin friction coefficients and this can cause reduction of algal masses for some species (Peterson & Stevenson, 1990; Biggs et al., 1998; Finlay et al., 1999; Schultz, 2000).

In the present case, in which rotational speed of the culture vessel lowered the specific growth rate (Figure 5), it is clear that *R. riparium* is better adapted to bodies of water with low current velocity, which is consistent with the observation that this alga is often the dominant alga in stagnant ponds.

In conclusion, it has been shown that *R. riparium* can grow year-round, that it has great biomass production capacity, and that its growth rate is superior to that of terrestrial plants traditionally used in paper-making. If this *R. riparium* is used as a fiber supplement, it could help to ease competing land-use demands and reduce the impetus to harvest forests. Additionally, as an absorber of free carbon dioxide, and through its ability to take up carbon dioxide *via* bicarbonate cultivation (data not shown), *R. riparium* should also help to lower atmospheric carbon dioxide levels. Our future work will focus on the practical details of culturing and harvesting this potentially environmentally friendly alga.

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