Algal Research 30 (2018) 73–78

Contents lists available at ScienceDirect

Algal Research

journal homepage: www.elsevier.com/locate/algal

Improvement of the seeding of filamentous *Ulva tepida* on free-floating surfaces

Christina Praeger*, Rocky de Nys

MACRO — The Centre for Macroalgal Resources and Biotechnology, College of Marine and Environmental Sciences, James Cook University, Townsville, Queensland, Australia

ARTICLE INFO

Keywords:
Macroalga
Aquaculture
Aonori
Land-based cultivation
Bioballs
Areal biomass yield

ABSTRACT

A key to the diversification of seaweeds produced in land-based systems is to enable the cultivation of high-value species which require attachment to surfaces. Approaches to enable this have been investigated with the seeding of small floating surfaces, or 'bioballs', being successful in an initial proof-of-concept study. The present study improves this method by directly seeding bioballs with *U. tepida* under aerated outdoor cultivation, thereby eliminating the more complex laboratory-based step, where bioballs are seeded and maintained. This study quantified the effect of density of bioballs in combination with seeding density of zoids on the settlement onto bioballs when seeded under aerated outdoor cultivation. Subsequently, the seeded bioballs were cultivated for 31 days to determine growth and productivity, thereby identifying the optimal time point of harvest for maximum productivities. Settlement was significantly affected by the density of zoids and bioballs used during the seeding process, with generally higher numbers of settled zoids on bioballs seeded at high zoid densities in combination with low bioball densities. However, productivity at harvest after 11 days onwards was not significantly different across treatments with no carry-over effect from settlement. The highest productivity (12.3 ± 1.8 g dw m^{-2} d^{-1}) was achieved if the bioballs were harvested within 19 days of outdoor cultivation and decreased with extended cultivation periods with lower growth rates, and reproductive events on day 27 of outdoor cultivation. Overall, the method significantly improved the baseline method of seeding bioballs by minimising the steps in the seeding and maintenance of bioballs. The requirement of controlled laboratory conditions can be eliminated facilitating scalable on-land production of biomass for bioremediation and bioproducts of *U. tepida* and likely a wider range of seaweed species.

1. Introduction

Land-based algal cultivation systems allow for control over abiotic and biotic factors which directly affect the quantity and quality of biomass. The free-floating cultivation of seaweeds improves the efficacy of land-based production of biomass [1–4] and enabling this method for species that normally depend on attachment to surfaces is important for the diversification of species in land-based systems [5,6]. The seeding of small floating surfaces is a successful method which allows for broader options for land-based cultivation including tanks, raceways and high rate algal ponds [6].

The green filamentous macroalga *Ulva tepida* has been a focus for this approach as it is effective in bioremediation systems due to its broad temperature and salinity tolerances [7–9]. Importantly, it is a high-value food product (aonori) and contains ulvans [10] which have nutritional, health, and biomedical applications [11]. In addition, the life cycle has been closed enabling a reliable source of seedlings for the artificial seeding of surfaces [12,13] including floating surfaces [6].

There are currently three major steps involved in the seeding and cultivation of *U. tepida* on floating surfaces: 1) obtaining a zoid suspension for seeding, 2) seeding and maintenance of surfaces for three days under static laboratory conditions, and 3) transfer to an outdoor system for cultivation [6]. Notably, the established baseline method of the initial proof-of-concept study requires improvement to achieve industrial application. This can be realised by minimising the number of steps involved, while maintaining high productivities. The elimination of the seeding and maintenance step under laboratory conditions (step 2) resulting in the direct seeding of surfaces in culturing tanks under aeration would simplify the process significantly and thereby improve the resource efficiency of the biomass production.

Therefore, the aim of this study was to improve the previously developed baseline method by seeding surfaces in outdoor culturing buckets under aeration and thereby eliminating maintenance under laboratory conditions. A range of densities of surfaces and zoids were tested to quantify their effect on settlement and productivity.

* Corresponding author.
E-mail address: tine.praeger@jcu.edu.au (C. Praeger).

https://doi.org/10.1016/j.algal.2017.12.008
Received 31 March 2017; Received in revised form 21 December 2017; Accepted 21 December 2017
Available online 06 January 2018

Subsequently, growth and productivity were determined over 31 days to identify the optimal time point of harvest for maximum productivities.

2. Materials and methods

2.1. Collection of algal biomass and preparation of reproductive material

To produce reproductive material (zoids), biomass of the green filamentous species *U. tepida* [14] (syn. *U. sapora* [15,16]) was collected by hand from an aquaculture facility near Ayr (19°29′S, 147°29′E), Queensland, Australia. Samples were transported within 2 h to a laboratory at James Cook University in Townsville, Australia, where they were gently washed with filtered seawater (0.2 μm and UV sterilised) to remove debris, epiphytes and invertebrates. To induce the release of zoids, *U. tepida* was temperature shocked [12] and then chopped using a blender in the early afternoon on the day of collection [8]. Cut filaments were maintained in a culture cabinet (Sanyo MLR-351) in a 12 h light:12 h dark photoperiod at 25 °C. The peak release of zoids occurred after three days around 11 am and the density of zoids was calculated using a haemocytometer.

2.2. Experimental set-up

To determine the effect of the density of surfaces, in combination with seeding density of zoids, on the settlement and productivity of *U. tepida* both factors were manipulated in a fully factorial experiment. Bio-media (Kaldnes Type C1 Media; Aquasonic Pty Ltd., Australia) was used as the settlement surface, referred to hereafter as 'bioball' (see [6] for more details).

To test the effect of the interaction between the density of bioballs and zoids on the settlement a range of bioball densities was tested. Bioballs were added to 15 L buckets (AP15LWHT/E; People in Plastic) and each bucket filled with 8 L filtered seawater (FSW; 0.1 μm; Mini water Filtration System, Sawyer) and gently aerated through multiple inlets around the base. The lowest density was 90 bioballs per bucket (hereafter referred to as bioball treatment 'B 1×'). Bioballs were individually counted and subsequently weighed to quantify the average weight of the B 1× treatment (14.0 ± 0.1 (S.D.) g). The number of bioballs for higher bioball densities was based on weight and was increased two- (hereafter 'B 2×'), four- (hereafter 'B 4×'), eight- (hereafter 'B 8×') and 16-fold (hereafter 'B 16×') resulting in approximately 180, 360, 720, and 1440 bioballs, respectively (n = 15 replicate buckets for each bioball density).

To test the effect of seeding density of zoids for each bioball density, the bioballs were seeded with a range of densities of zoids using an initial zoid suspension of 450 × 10^3 zoids mL^{-1}. Three volumes of zoid suspension were used to provide a low, medium and high seeding density by adding 100 mL, 300 mL and 600 mL of the zoid suspension, respectively, to the buckets holding the bioballs. The water level was immediately topped up to 9 L using FSW for each bucket. Consequently, the low, medium and high seeding density of zoids per cultivation bucket (9 L) corresponded to 5, 15, and 30 × 10^3 zoids mL^{-1}. The buckets were placed in a recirculating water bath to minimise temperature fluctuations and maintained on batch culture conditions with no water exchange for three days to ensure sufficient time for zoids to settle and germinate. To compensate for water lost by evaporation dechlorinated water was added daily to maintain a volume of 9 L. There were five replicates for each combination of bioball density and seeding density of zoids. After three days, three bioballs were randomly sampled from each bucket and preserved in 10% Lugol's solution to determine the number of settled zoids according to [6]. In brief, settled zoids which had germinated were counted at a surface area of 1.755 mm^2 at three random spots of each bioball.

Subsequently, the remaining bioballs were cultivated at the same stocking density among treatments to minimise any interactions with growth and consequently productivity [6]. Therefore, the number of bioballs was reduced to 87 in each bucket which was equal to the lowest bioball density used for seeding (B 1×) minus the three bioballs sampled for the quantification of settlement. Buckets were then placed on flow-through (5 μm filtered seawater) with an exchange rate of approximately 4 volumes per hour and maintained for 31 days in outdoor culture under ambient light. All culturing buckets were placed in a circulating water bath to minimise temperature fluctuations and cleaned weekly.

After 7 days of outdoor cultivation, the seeded bioballs were repeatedly sampled every fourth day to measure the fresh weight (fw) of *U. tepida* following [6] and then returned to each bucket. Algal productivity per surface area (P; g dw m^{-2} d^{-1}) and specific growth rate (SGR; % day^{-1}) were calculated for each replicate. Productivity was calculated using the equation $P = \{[(fw / (fw:dw))_2 - (fw / (fw:dw))_1] / A\} / (t_2 - t_1)$, where *fw* and *dw* are, respectively, the fresh and dry weights at time t_1 and t_2 of outdoor cultivation (days) and A is the area of buckets used for outdoor cultivation. Specific growth rates were calculated as follow: $SGR = \ln(B_2 / B_1) / (t_2 - t_1) \cdot 100$, where B_1 and B_2 are the algal biomasses (g fw) at time t_1 and t_2 of outdoor cultivation (days).

To determine the fw:dw ratios at each sampling point, an additional 21 buckets were maintained under the same conditions with each containing 87 bioballs seeded with a medium density of zoids from the bioball densities B 2×, B 4× and B 8× (n = 7 for each bioball density). At each harvest above (every four days) all seeded bioballs were removed from three of the additional buckets, one from each of the bioball densities, and spun to constant weight using a spin dryer (Koh-I-Noor), weighed (fw) and subsequently dried in a dehydrator at 65 °C for 2 days to then quantify the dry weight (dw). Growth rates were also determined to ensure they were similar to those of the major experiment and therefore provide a valid proxy for the experimental fw:dw ratio [8].

Experiments were conducted from April to June 2016. The average water temperature was 26.7 °C (± 1.2 S.D.) with an average photosynthetically active radiation of 22.0 (± 6.1 S.D.) mol photons m^{-2} day^{-1}. The concentration of nutrients was measured three times a week and adjusted when necessary with MAF growth medium (Manutec Pty Ltd., 13.4% N, 1.4% P) to maintain a concentration of nitrogen as nitrate of 1.5 mg L^{-1} and phosphorous of 0.2 mg L^{-1}.

2.3. Statistical analysis

To formally test the effect of bioball density and zoid density on the settlement of *U. tepida*, data were analysed by two-factor PERMANOVA using PRIMER 6 (v. 6.1.13) and PERMANOVA + (v. 1.0.3) [17] with both as fixed factors. The effects of zoid density on the SGR over time was analysed as fixed factors using a three-factor PERMANOVA. The effect of bioball density and zoid density as fixed factors on the productivity was assessed for each harvest period using a two-factor PERMANOVA. All PERMANOVA tests presented here used the Euclidean distance measure on normalised data and p-values were calculated using permutation of residuals under a reduced model with 9999 random permutations. If there was a significant difference, pairwise a posteriori comparisons were made among the significant groups (α = 0.05) [18]. PERMANOVA is the equivalent of an ANOVA performed on similarity values and p-values are obtained by permutation methods. The method is non-parametric and distance-based pseudo-F statistics are calculated for each term. All data are reported as mean ± 1 standard error (S.E.) unless stated otherwise.

3. Results

3.1. Settlement

The average number of settled zoids varied between treatments and there were significant differences in the number of settled zoids