Algal Research 24 (2017) 81–88

Contents lists available at ScienceDirect

Algal Research

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

Seeding filamentous *Ulva tepida* on free-floating surfaces: A novel cultivation method

Christina Praeger *, Rocky de Nys

MACRO – the Centre for Macroalgal Resources and Biotechnology, College of Science and Engineering, James Cook University, Townsville, Queensland, Australia

ARTICLE INFO

Article history:
Received 24 November 2016
Received in revised form 3 February 2017
Accepted 16 March 2017
Available online xxxx

Keywords:
Macroalgae
Aquaculture
Aonori
Seaweed
Cultivation
Bioballs

ABSTRACT

The selection of seaweed species for the integrated cultivation in land-based aquaculture systems is primarily based on the ability to grow in free-floating cultures thereby increasing stocking densities and areal biomass productivity. We assessed a novel approach to enable the free-floating cultivation of seaweed species that are dependent on the attachment to surface structures by seeding zoids of *Ulva tepida* onto small surfaces which float in the water column. In this study we firstly assessed how the density of settlement surfaces (hereafter referred to as 'bioballs') and density of zoids influenced the settlement onto bioballs, and secondly how the stocking density of these seeded bioballs affected the biomass yield in outdoor cultivation over a 35 day period. Settlement was not affected by the density of bioballs with zoids settling evenly across bioball treatments. The number of zoids that settled successfully increased with increasing density. However, both seeding factors (density of bioballs and zoids) had a minimal effect on growth rates, yield of biomass per bioball and total biomass harvested, which were primarily affected by stocking density. Low stocking density generally resulted in higher growth rates and yield of biomass per bioball, although the total yield was lower compared to higher stocking densities. Overall, growth rates decreased over time for all stocking densities with a sharp decrease from 27 days of outdoor cultivation and onwards due to reproductive events. Our study demonstrates that the rate of biomass production of *U. tepida* is primarily driven by the stocking density of seeded bioballs, and underlines the importance of short cultivation cycles with harvest prior to reproduction. This novel cultivation method enables the free-floating cultivation of species that normally depend on attachment to fixed surfaces and thereby expands the range of seaweed species for land-based cultivation.

1. Introduction

The concept of algal bioremediation is widely accepted as an efficient remediation strategy for nutrient-rich waste water streams from land-based aquaculture [1,2]. The effective removal of excess dissolved nutrients requires robust species of algae with high growth rates which translate to high bioremediation capabilities [3]. Ideally, the biomass produced should also add economic value through high-value applications [4] in particular food and functional food products [5]. However, the initial focus for the selection of algal species for bioremediation has been based on nutrient uptake and ease of cultivation at a large-scale, while the value of the biomass produced has generally been subordinate [3]. Consequently, seaweeds which have been successfully integrated into existing land-based aquaculture to sequester nutrients are easily cultivated unattached in ponds and tanks using vegetative fragmentation [6–9]. In contrast, many high-value seaweed species require cultivation after attachment to a surface [10] and consequently have not been integrated with land-based aquaculture.

The green filamentous macroalga *Ulva tepida* has recently become a target for scalable on-land production of biomass for bioremediation and bioproducts due to its high growth rates and exceptional environmental tolerance [3,11,12]. This robust species is an ideal candidate for year-round cultivation in tropical environments which are typically characterised by strongly seasonal rainfall associated with challenges for the consistent mass-cultivation of less robust species of algae [13]. In addition to its high bioremediation capability [11], *U. tepida* is differentiated by the potential to add value as the food product aonori [14]. It also holds potential for the production of natural mineral salts [15] and functional biopolymers as ulvans with food, pharmaceutical, biomedical and agricultural applications [14]. This species therefore incorporates value into the production chain in the form of both bioremediation and value-added bioproducts.

U. tepida, and filamentous species of *Ulva* more generally, are cultivated by seeding surface structures such as nets [10,16,17] and ropes for subsequent grow-out [11,14]. In a process to enable the free-floating

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

http://dx.doi.org/10.1016/j.algal.2017.03.003
2211-9264/

cultivation of filamentous species of *Ulva*, the 'germling cluster' method has been proposed where propagules attach to one another and form aggregates [18]. However, in this method the propagules not only attach to each other but also to the bottom of the culture container, and consequently require manual detachment for subsequent free-floating cultivation [18]. Therefore, in a novel approach to diversify methodologies to enable the free-floating cultivation of species that are dependent on attachment to surfaces we propose a cultivation technique based on the conventional method of seeding surfaces. However, rather than seeding conventional structures such as nets and ropes which have a fixed position for cultivation, small surfaces which float in the water column are seeded thereby enabling broader options for cultivation including land-based tanks, raceways and high rate algal ponds.

Therefore, the overall objective of this study was to achieve the free-floating cultivation of *U. tepida*, as a model species, by seeding small floating surfaces to deliver high biomass yields through high stocking densities and growth rates. A range of densities of settlement surfaces and zoids, and subsequently stocking densities, were tested to optimise this method. The number of settled and germinated zoids was quantified on settlement surfaces three days post seeding and then transferred into flow-through outdoor cultivation where the growth rate and biomass yield per unit surface was determined over a cultivation period of 35 days to identify treatments with maximal biomass yield and efficiencies.

2. Materials and methods

2.1. Collection of algal biomass and preparation of reproductive material

The species used in this study was *U. tepida* Masakiyo & S. Shimada [19] (syn. *Ulva sapora* [20,21]) and was collected in the morning 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 [22] and then chopped using a blender in the early afternoon on the day of collection [11]. The release of zoids occurred after two days at 11 am and the density of zoids was calculated using a haemocytometer.

2.2. Experimental set-up

2.2.1. Seeding of bioballs (bioball density and seeding density)

Bio-media (Kaldnes Type C1 Media; Aquasonic Pty Ltd., Australia), hereafter referred to as 'bioball', was used as the settlement surface for the experiment and made of polyethylene. Each bioball was slightly positively buoyant and wheel-shaped (mean diameter of 10.3 mm) with a protected surface within the wheel (Fig. 1). The total surface area of each bioball was 673.4 (±26.7 S.D.) mm^2 of which the external surface area was 223.8 (±22.2 S.D.) mm^2 and the protected internal surface area 449.6 (±11.3 S.D.) mm^2.

Firstly, the effect of the interaction between the density of bioballs and zoids on the settlement of *U. tepida* was tested. The density of bioballs was tested in 1 L containers (VP1LWH; People in Plastic, Australia) filled with 90 (hereafter referred to as bioball treatment 'B 1×'), 180 (hereafter 'B 2×') and 360 bioballs (hereafter 'B 4×') ($n = 30$ for each bioball treatment). The bioballs of treatment B 1× covered the bottom of a container in a single layer and higher densities of bioballs resulted in two and four layers, respectively (Fig. 2). Bioballs were maintained at the bottom of each container using a mesh (Gutter guard; Saxon, Australia) and weighted down with a PVC ring.

The effect of the seeding density of zoids was then tested in a factorial design against each bioball density. A total of 300 mL of a zoid suspension with a density of 5, 10 or 20 × 10^3 zoids mL^{-1} in nutrient enriched (f/2; AlgaBoost™, Australia) autoclaved filtered seawater was added to each container filled with bioballs, corresponding to a total number of 1.5, 3 and 6 × 10^6 zoids for each container. There were 10 replicates for each seeding density and bioball density, resulting in a total of 90 containers for the nine treatments. Containers were then randomly placed in culture cabinets (Sanyo MLR-351; VWR, Australia) in a 12 h L: 12 h D photoperiod (irradiance of 80 μmol photons m^{-2} s^{-1}) at 25 °C. After three days, three random bioballs were sampled from the top layer of each treatment and preserved in 10% Lugol's solution to determine the number of settled and germinated zoids. In addition, three bioballs were collected from the bottom layer of the bioball densities B 2× and B 4× to determine any differences in the settlement of zoids with depth of the layer of bioballs. To quantify the number of settled zoids, each bioball was carefully cut using a scalpel. Settled zoids which had germinated were counted at a surface area of 1.755 mm^2 at three random spots of the protected surface of each bioball using a stereo microscope (Olympus SZ61), camera (Olympus DP26) and labSens software (V1.4; Olympus). Pilot studies showed that zoids settle evenly on the bioball, but detach from the external unprotected surface during the early stages of cultivation.

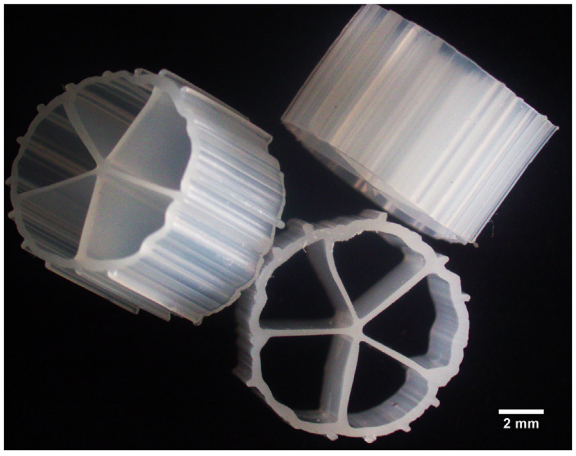

Fig. 1. Light micrograph of wheel-shaped bioballs used as settlement surface.

2.2.2. Cultivation of seeded bioballs (stocking density)

Subsequently, the effect of stocking density in combination with seeding factors (density of bioballs and zoids as above) on growth rate

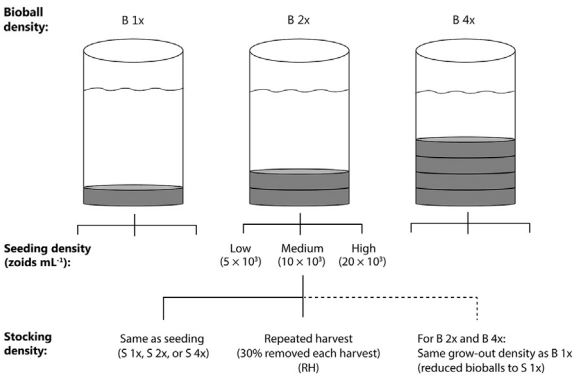

Fig. 2. Experimental design. Bioball density represents different numbers of bioballs in containers (B 1× = 90 bioballs, B 2× = 180 bioballs, and B 4× = 360 bioballs) resulting in single and multiple layers of bioballs. The effect of seeding density of zoids was tested for each bioball density by adding 300 mL of zoid suspension with a low (5,000 zoids mL^{-1}), medium (10,000 zoids mL^{-1}) and high density (20,000 zoids mL^{-1}) to each container. Subsequently, seeded bioballs were cultivated at a range of stocking densities: (1) the same number of bioballs used for the seeding (S 1× for B 1×, S 2× for B 2×, and S 4× for B 4×), (2) a repeated harvest treatment where the number of bioballs was reduced by 30% at each harvest, and (3) the bioball treatments B 2× and B 4× were cultivated the same density as B 1× (S 1×).