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Short Communication

The influences of the recycle process on the bacterial community in a pilot scale microalgae raceway pond

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HIGHLIGHTS

- Bacteria were present over the whole harvest/recycle process at large scale.
- Electroflocculation had little effect over the bacterial community.
- Bacteria found within this study are a symbiotic partner to *Tetraselmis* sp.

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ABSTRACT

The use of recycled media has been shown to be a necessary step within the lifecycle of microalgal biofuels for economic sustainability and reducing the water footprint. However the impact of the harvesting of microalgae on the bacterial load of the recycled water has yet to be investigated. Within this study PCR–DGGE and real-time PCR was used to evaluate the bacterial community dynamics within the recycled water following harvest and concentration steps for a pilot scale open pond system (120,000 L), which was developed for the production of green crude oil from *Tetraselmis* sp. in hyper saline water. Two stages were used in the harvesting; Stage 1 electroflocculation, and Stage 2 centrifugation. Electroflocculation was shown to have little effect on the bacterial cell concentration. In contrast bacterial diversity and cell concentration within the centrifugation step was greatly reduced.

1. Introduction

Improvements in the flocculation methods to increase the efficiencies of harvesting microalgae have been well researched and has been demonstrated with dissolved air flocculation (DAF)(Chu et al., 2011; Haarhoff and Edzwald, 2013), electroflocculation (Lee et al., 2013) and centrifugal force (Pahl et al., 2013). Combinations of these technologies has been shown to shorten the time required for recovering microalgae; for example by combining electroflocculation with dissolved air flotation, the flocculation time was reduced from 30 min to 14 min (Xu et al., 2010). Flocculation methods have been shown to be both effective for low concentrations of microalgae and inexpensive, this makes it suitable for the initial concentration of the biomass (Molina Grima et al., 2003). The wastewater industry has long made use of centrifugation for the dewatering of solids. Similarly, the recovery of microalgal cells via centrifugation results in the rapid harvesting of up to 94% of the algal biomass (Molina Grima et al., 2003).

Reduction and reuse of waste is a key part of the environmental and economic sustainability for the production of microalgal biofuels (Cho et al., 2011). One aspect of this is the use of recycled water from the harvest process. The large volumes of water which are found in open systems together with the low density of microalgae provide challenges in efficiencies and cost effectiveness; therefore it is essential to reclaim the water from the harvest process. An example of why recycling water is essential was shown by Yang et al. (2011); their life cycle analysis of biodiesel production from microalgae showed that the recycling of water from harvest reduces the water and nutrient usage by 84% and 55% respectively. These authors also showed that by reclaiming the water following microalgal harvest no further additions of potassium, magnesium and sulphur to the open pond system were required (Yang et al., 2011). Furthermore the use of recycled water collected from harvesting stages prevents new input of water from external sources (which may also contain undesired organisms), significantly reducing the costs associated with the acquisition of water.

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Previous research has focused on the ability to use recycled water, with a main focus on nutrient recycling. To date minimal research has been conducted on the bacterial community dynamics within the recycled water (Cho et al., 2011). When developing a harvesting system it is important to influence the growth towards the desired organism; previous studies have shown that recycled water can enhance the growth of unwanted microorganisms during the flocculation process, which is not desired when growing organisms of interest (Guo et al., 2011). Bacteria have shown to have a varying effect on microalgal growth: the symbiosis between microalgae and bacteria has shown to be beneficial due to bacterial ability to produce B12, an essential vitamin for microalgae (Goecke et al., 2013; Kazamia et al., 2012); particular negative aspects of enhanced bacterial growth are the introduction of competition for nutrients and loss of nitrogen through denitrification processes (Christenson and Sims, 2011). To maintain a large scale open pond in a sterile condition is impractical as it is exposed to the environment; however ensuring that the conditions are selective towards desired microorganisms is achievable. Monitoring the bacterial population in terms of biomass and diversity within the recycled water from a harvesting system is essential to prevent an increase in the bacterial load with each harvest cycle.

The aim of this investigation was to determine the effects on the bacterial community dynamics during the harvest of *Tetraselmis* sp. This study also assessed the effectiveness of using molecular biological methods such as polymerase chain reaction (PCR) combined with denaturing gradient gel electrophoresis (DGGE) and real time PCR (RT-PCR) as tools to effectively determine the overall efficiency of a pilot scale microalgal biofuels harvest system. These tools have been commonly used to evaluate microbial communities and effects of treatment in other systems (Erkelens et al., 2012).

2. Methods

2.1. Process description and plant design

Harvesting of *Tetraselmis* sp. in hyper saline water was conducted on a daily basis from an open pond system (120,000 L) (Muradel, Australia). The harvest of the microalgae was conducted over a two stage system involving firstly electroflocculation and secondly continuous centrifugation. The electroflocculation unit consisted of aluminium sheets with a separation of 0.15 m between each electrode and a DC power supply. The electroflocculator unit was 2.4 m × 1.2 m × 0.15 m with a linear flow velocity 0.075–0.08 m s^{-1} with an electroflocculation duration of 30 s. A voltage between 10 and 20 V was used. This electroflocculation unit has been previously described (Lee et al., 2013). Water recycled from this process was returned to the open pond system. The harvested *Tetraselmis* sp. would then undergo stage 2 of the harvesting process. The centrifuge system which was used in this harvesting process was an Evodos type 10 (Evodos, Netherlands). Harvested microalgae from stage 2 would then undergo downstream processes for bioconversion to green crude oil, while the supernatant from the centrifuge was discharged.

2.2. Experimental design and sampling plan

Samples were collected from the 120,000 L open pond system developed for the continuous production of microalgae biomass. An initial water sample of 5 L was taken from the ponds and a final water sample was taken from the open pond system after five days as a comparison over time to observe bacterial community changes. Throughout the five days the harvesting process was continuous with recycled water reintroduced into the open ponds. In addition, samples from the electroflocculation (100 mL) and centrifugation (100 mL) stages were taken.

2.3. Molecular analysis

The extraction of DNA was conducted using a Mo Bio Ultra Clean DNA extraction kit (Mo bio USA) with 1.2 mL of sample used. Extracted DNA was stored at −20 °C for future use if not used immediately. The presence of DNA was confirmed on a 0.8% agarose gel with SybrSAFE. The eubacterial PCR primers set 341FGC and 518R were used to determine changes in community dynamics (Erkelens et al., 2012). DNA template (2 μL) was used together with a KAPA master mix. PCR–DGGE was performed using a D-Code system (BioRad) with a 6% polyacrylamide gel (35.5:1 acrylamide/bisacrylamide) with a denaturing gradient of 40–60%. The DGGE run conditions were 60 V at 60 °C for 20 h. Following electrophoresis the gel was stained with silver (Girvan et al., 2003).

2.4. Identification of bands of interest

Bands of interest were excised from the PCR–DGGE gel; excised bands were left overnight at 55°Cc in 50 μL molecular grade water. The bands where then reamplified using 2 μL of template using primers 341F 518R (Rölleke et al., 1996). Reamplified PCR amplicons underwent the MoBio PCR clean-up kit (MoBio, USA). Clean PCR amplicons were quantified using a Nano-drop lite (Thermofisher Scientific Australia) and then sent to the Australian Genomics Research Facility (AGRF, Australia) according to submission requirements. Sequences were analysed using National Centre for Biotechnology Information using a BLAST algorithm (<http://www.ncbi.nlm.gov.library.vu.edu.au/BLAST/>), and a comparison was formed against a nucleotide sequence database for identification.

2.5. Quantification of bacteria with Real-Time PCR

For real-time PCR analysis for bacterial quantification, DNA template (1 μL) was used together with a 20 μL reaction of KAPA Sybrgreen Master Mix on a Qiagen Rotor Gene 6000. Bacterial selective primers 314F and 518R were used to quantify the number of gene copies (Rölleke et al., 1996). After the run was completed a melt curve was conducted to ensure the product of interest was produced, the melt curve conditions were: 50–95 °C with a 0.5 °C increase every 5 s. Two negative controls were added within the samples where added within the run to screen for contamination.

2.6. Statistical analysis

PCR–DGGE gels were digitalised and quantified to determine the changes within the bacterial community. Digitalised gels were analysed using Total Lab Quant to determine the bacterial community dynamics occurring at each stage (Total Lab, UK). A Shannon Weaver index was applied to the PCR–DGGE gels to determine the diversity within each bacterial community sample (Marzorati et al., 2008). Equitability index was also assessed to determine the evenness of the bacterial community (Marzorati et al., 2008). The results from the real time PCR analysis of samples were statistically analysed using a *T*-TEST to determine significant difference between bacterial cell counts.

3. Results and discussion

3.1. Bacterial cell counts with real time PCR

The results of the QPCR analyses showed that the microalgal harvesting stage had a significant impact on the number of bacteria