Bioresource Technology 192 (2015) 97–104

Contents lists available at ScienceDirect

Bioresource Technology

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

Sustainable production of toxin free marine microalgae biomass as fish feed in large scale open system in the Qatari desert

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HIGHLIGHTS

- Areal productivity of 20.37 g/m^2/d achieved for an incremental salinity culture.
- During extreme evaporation water loss, batch cultivation time should be minimum.
- Absence of vitamins can prevent contamination of toxic microalgae.

ARTICLE INFO

Article history:
Received 18 March 2015
Received in revised form 7 May 2015
Accepted 8 May 2015
Available online 21 May 2015

Keywords:
Microalgae biomass
Feed
Evaporation loss
Toxin

ABSTRACT

Mass cultivation of microalgae biomass for feed should be cost effective and toxin free. Evaporation loss in Qatar can be as high as 2 cm/d. Hence, production of marine microalgae biomass in Qatar would also require mitigating water loss as there was only very limited groundwater reserve. To address these issues, a combination of four growth conditions were applied to a 25,000 L raceway pond: locally isolated microalgae strain was selected which could grow in elevated salinity; strain that did not require silica and vitamins; volume of the culture would increase over time keeping denser inoculum in the beginning, and evaporation water loss would be balanced by adding seawater only. A local saline tolerant *Nannochloropsis* sp. was selected which did not require silica and vitamins. When the above conditions were combined in the pond, average areal biomass productivities reached 20.37 g/m^2/d, and the culture was not contaminated by any toxic microalgae.

1. Introduction

Anticipating the insufficiency of future protein supply, almost six decades ago, scientists explored the potential of microalgae as an alternative protein source (Anupama and Ravindra, 2000; Becker, 2007). Nutritional value of some the studied microalgae have shown that the intrinsic proteins are of high quality and are equal, sometimes superior in quality compared to common vegetable proteins (Austic et al., 2013; Becker, 2007; Hemaiswarya et al., 2011). In addition to protein, microalgae have other cell compounds like carbohydrate (mostly simple sugars), lipids, peptides, pigments, vitamins, minerals and trace elements (Brown et al., 1997; González López et al., 2010). Despite all these advantages, the cost of producing the biomass and the technical difficulties to incorporate algae into palatable food, use of microalgae biomass as protein source was not employed extensively (Becker, 2007). In the year 2000, worldwide commercial production of algae biomass was around 5000 metric ton/yr at an estimated average price of 300 US$/kg (Muller-Feuga, 2000). However, a recent hike of crude oil price and pressure from sustainable feed and food production, intensify the research on mass scale microalgae production (Brennan and Owende, 2010; Pittman et al., 2011; Taelman et al., 2013). Over the last decade significant progress was made and more researches are ongoing to bring down the production cost and make the overall process sustainable (Delrue et al., 2013; Kiron et al., 2012; Leite et al., 2013; Weschler et al., 2014). Very recently, it was estimated that the cost of producing 1 kg of biomass in the open raceway pond could be 2.71 $, and there was provision to reduce the cost further. (Kang et al., 2015)

On an average, fish contributes 17% of worldwide consumption of animal proteins (Muller-Feuga, 2000). There are numerous scientific evidences that global catches of wild fishes are declining at an alarming rate (Pontecorvo and Schrank, 2012). On the other hand, fish demand will increase over time. Aquaculture, mainly the inland fish farming can play a significant role to meet the

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http://dx.doi.org/10.1016/j.biortech.2015.05.019
0960-8524/

future demand. It was estimated that in the year 2000, aquaculture contribute 40% of the wild fisheries and by the year 2030 the yield of aquaculture will surpass the yield from the wild (Brander, 2007). Hence to increase the capacity of the aquaculture industry, fish feed production should increase accordingly. Fish meal and fish oil are two of the important ingredients of fish feed. Unfortunately, these two products are processed from the wild catch. Hence to achieve future sustainability alternative options are needed. On an area basis, microalgae can produce more protein than any other terrestrial plant. Additionally microalgae can be grown anywhere using non-potable water (e.g. seawater).

Qatar has neither any river nor any water body above the ground. According to Environmental Statistics Annual Report 2012 (or, ESAR 2012) by the State of Qatar Statistics Authority, annual average rainfall from 2009 to 2011 was around 52.2 mm. Underground water is the main fresh-water source and currently being used in few farms within the country; however, because of declining water level, it might be difficult to get any freshwater form here. Almost 99% of the municipal potable water is supplied by desalination of seawater (Darwish and Mohtar, 2012). Several wastewater treatment facilities produce almost 11.88 million m^3 of treated wastewater or TWW (ESAR 2012). Microalgae biomass grown in TWW may have trace amount of toxic and heavy metals or even pathogens both as cell components and also adsorbed onto to the biomass (Wilde and Benemann, 1993); hence such biomass quality may vary and also may not be suitable for use as feed. The state of Qatar has a length of 160 km and width of 90 km; the length of its coastal line is about 1000 km (EASR 2012). Hence, a collection of required seawater would be easier.

Almost 60% of the algal biomass is carbon which is mainly derived from dissolved carbon dioxide (CO_2). After CO_2, the second most important element that needs to be supplied is nitrogen. Nitrogen requirement, both the source, and concentration, for microalgae is very strain-specific. In the literature, a wide range of nitrogen requirement (2–10% of the biomass weight) for the microalgae was reported. Although $NaNO_3$ is commonly studied as the nitrogen source for many microalgae species, an ideal strain should be able to utilize a wide range of nitrogen sources specially the ones which are cheaper and also provide higher nitrogen utilization.

Open pond cultivation of microalgae biomass is often susceptible of contaminations from both competing microalgae and or predators like ameba and zooplankton (Bartley et al., 2013; Parmar et al., 2011). Although monoculture of the selected strain is desirable, for feed purpose the culture should be at least free from toxin-producing organisms. Tang et al. (2010) reported that all the known toxic microalgae would not be able to grow in the absence of the three vitamins (e.g., B3, B7 and B12). In addition to these toxic algae group, many other microalgae require these vitamins (Croft et al., 2006). Thus, growing any microalgae strain that doesn't require any vitamin will eliminate the possibility of contamination by toxic microalgae and also minimize the risk of competition from many other microalgae. Additionally, if any strain doesn't require silica, it would also prevent the growth of a large group of microalgae (i.e., most of the diatoms).

From the open pond, there will be loss of water through evaporation. The extent of evaporation in Qatar can vary from 0.2 cm in winter to even 2.0 cm in the summer time (our own observation). Maintaining a fixed salinity and volume in the culture it is customary to add freshwater to the culture (Murphy and Allen, 2011; Yang et al., 2011). It was estimated that such freshwater requirement in the seawater culture could be 370 kg/kg biodiesel (Yang et al., 2011); hence to produce 1 kg of biomass almost 111 kg of freshwater would be required, considering 30% of biomass was lipid and converted to biodiesel. There are also some strains that can grow at elevated salinity. Hence, if the daily evaporation loss is balanced by adding seawater, it might still be possible to grow these microalgae. In addition, for the elevated salinity, many other competing microalgae, and the predators might not grow or, at least, minimize their growth. In such cases, microalgal biomass production should run in batch mode and for minimum number of days.

Qatar falls in low elevation coastal zone (0–10 m above sea level); if seawater needs to be pumped from the sea it would require less energy than pumping groundwater for cases where the groundwater level is much deep. For instance, the average groundwater level in US is around 183 m (Murphy and Allen, 2011). Therefore, the energy requirement to pump this groundwater is significant; when such groundwater is extracted for use in the open microalgae pond it becomes very important to recycle the culture water. Since there is a shortage of freshwater in Qatar and no provision of extracting the underground water in future, production of microalgae biomass must rely on 100% seawater. Additionally, the extent of evaporation in Qatar is also much higher than many other places. Hence, at the end of a batch cultivation the salinity of the water will be higher in the absence of any freshwater addition, and any subsequent culturing in this elevated saline water may affect the biomass productivity significantly. Thus, large-scale microalgae cultivation in Qatar should be in batch mode.

Previously no attempts were made to produce mass scale microalgae biomass for fish feed in Qatari desert. The objectives of this research were to (1) study the possibility of producing toxin-free microalgae biomass in large scale open raceway pond, and (2) study the feasibility of minimizing or even avoiding use of fresh water for biomass production.

2. Methods

2.1. Microalgae strain and media

For this study a locally isolated *Nannochloropsis* sp. was used. The strain was isolated from a local fish pond which was using brackish groundwater (salinity 17). The growth of this strain was monitored by optical density (OD) measurement by a Jenway 6800 spectrophotometer at 750 nm wavelength. A calibration curve was made between OD @ 750 nm and biomass density ($y = 0.629x - 0.0305$; y was measured as g/L and x was OD @ 750 nm and $0 \leqslant x \leqslant 1$, when x value for any culture was greater than 1 it was diluted with seawater such that final OD became lesser than 1). For small scale indoor study artificial sea-salt was mixed with DI water to prepare the desired salinity; whereas filtered seawater (salinity 40) was used for all outdoor experiment. All the indoor experiments were conducted in a temperature controlled room which was maintained at 25 °C. All the small scale experiments were conducted in replicates.

2.2. Measurement of light intensity

All the indoor experiments were carried out with blue fluorescent lights. 6 tubes were provided from each side of bottles, and average optical path was around 4 cm for all the cultures. Indoor light intensity was measured by a handheld LICOR LI-1400 Light meter whereas outdoor sunlight was measured on the surface of the pond by a PAR quantum sensor connected to a computer for continuous data logging.

2.3. Measurement of nitrogen and phosphorus

Total Nitrogen concentration in the discharge water was measured using HACH method 10242. Phosphorus content in the discharge water was measured by molybdenum blue method.