Algal Research 17 (2016) 217–226

Contents lists available at ScienceDirect

Algal Research

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

In-situ disinfection and a new downstream processing scheme from algal harvesting to lipid extraction using ozone-rich microbubbles for biofuel production

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ARTICLE INFO

Article history:
Received 24 November 2015
Received in revised form 21 April 2016
Accepted 7 May 2016
Available online xxxx

Keywords:
Algal biomass
Algal biofuel downstream process
Microflotation
Microbubble
Ozonation
Algal lipid extraction

ABSTRACT

The scaling up and downstream processing costs of biofuels from microalgae are major concerns. This study focuses on reducing the cost by using energy efficient methods in the production of microalgae biomass and the downstream processes (biomass harvesting and lipid extraction). Ozonation of *Dunaliella salina* (green alga) and *Halomonas* (Gram-negative bacterium) mixed cultures for 10 min at 8 mg/L resulted in a reduction in the bacterial contaminant without harming the microalga. Harvesting of *D. salina* cells through microflotation resulted in a 93.4% recovery efficiency. Ozonation of the harvested microalgal cells for 60 min produced three main saturated hydrocarbon compounds (2-pentadecanone, 6, 10, 14-trimethyl; hexadecanoic acid; octadecanoic acid) consisting of 16 to 18 carbons. By systematically switching the carrier gas from CO_2 to O_3, the microbubble-driven airlift loop bioreactor (ALB) delivers nutrient to the culture and in-situ disinfection respectively. Further, modulating the bubble size to match particle size ensures recovery of the cells after culture. All three key operations (disinfection, harvesting and lipid extraction) are assembled in a scalable, relatively energy efficient process.

1. Introduction

Over the past decade, the majority of the research on sustainable, environmentally friendly energy sources has focused on biofuels. However, pharmaceuticals and nutraceuticals are other crucial co-products in addition to biofuels that are obtainable from microbial biomass [1]. The production of biofuels and their associated co-products from microalgae basically consists of three main unit operations: culturing (including sterilisation), harvesting (including dewatering) and lipid extraction. All of these operations are largely uneconomical due to the high energy cost of processing [2]. Harvesting and extracting lipids from the microalgal biomass are the most expensive processes. The cost of harvesting itself contributes up to 30% of the cost of the entire process [3]. Brentner et al. [4] has reported that the process of microalgal biomass harvesting through centrifugation and press filtration, requires 90% and 79%, while lipids extraction through supercritical CO_2 and ultrasonication requires 66% and 110% of the total energy gained from the biofuel production.

For biofuels to be sustainable, current practices must seek to increase the production efficiency of all key unit processes and increase the profitability of integrated processing plants with co-products. First, algae are known to thrive within a given level of dissolved carbon dioxide and generally grow faster at higher dissolved CO_2 levels [5]. Conversely, the presence of oxygen (a metabolic by-product) can adversely limit growth at high concentrations. Conventional systems typically achieve mixing using motorized impellers or sparge CO_2 into the bioreactors using perforated membranes or pipes; in contrast, little attention has been paid to the bubble size and the resulting hydrodynamic effects on the microbial consortia, which can be deleterious [6].

The successful production of the microalgal biomass is hugely dependent on an axenic (bacteria-free) culture. However, ensuring contaminant-free cultivation can prove challenging because conventional methods can be ineffective. Ozone is one of the most widely used disinfectants employed to effectively regulate smell, taste and biological growth and eradicate pigments [7]. Khadre et al. [8] also demonstrated the application of ozone as a powerful antimicrobial agent for food processing and to decontaminate food contact surfaces, equipment and environments. Prior to conversion to useful end-products, the biomass is harvested from cultures and dewatered. Several methods to achieve this goal exist, including filtration, centrifugation and flotation. Recovery by flotation is the most effective industrial technique for colloidal particle recovery. The process entails generating bubbles that attach to the cells and results in the rise of the consortium to the surface of the column, where skimming is performed [9,10].

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http://dx.doi.org/10.1016/j.algal.2016.05.006
2211-9264/

Gas bubbling in liquid media has been widely applied in many fields, including the above-mentioned operations. Due to their high surface area to volume ratio, microbubbles can be effectively applied in an algal culture to substantially enhance the CO_2 dissolution rates. Introducing microbubbles enriched in CO_2 with negligible oxygen content at the bottom of an algal production tank will alleviate both of these limiting transfer rates [11]. The microbubbles will create a rapid influx of CO_2 and simultaneously extract dissolved oxygen due to the high mass transfer coefficient and oxygen gradient between the phases, so that the bubbles bursting at the top surface completely bypass the boundary layer limitations [11]. This unique property of microbubbles can equally be exploited for culture sterilisation and disinfection using ozone. These effects are strongly influenced by the size of the bubbles. Conventional means are relatively inefficient in making small bubbles and often settle for millimeter-sized bubbles. In rare instances when microbubble production is successful, it is not cheap [12]. The typical mechanisms all add external fields with high energy density. In dissolved air flotation for example, pressure levels of 6–8 bars are employed, including the use of saturators to make microbubbles. These combined with the vacuum pumps required to push water into saturators, further increases both the capital and operating costs associated with microbubble production [13]. With the fluidic oscillation approach by contrast, only air, approximately 1000 times less dense than water, is pushed at less friction loss than steady flow through the same piping [11]. So the energy efficiency is a crucial benefit. But capital efficiency is nearly as important as only a low pressure blower is required rather than a compressor. These capital and electricity savings are replicated on just about any scale.

This paper reports the development of a novel airlift loop bioreactor where the microbubble dispersal can be switched from a nutrient gaseous input (i.e., CO_2-rich stack gas) to air blown through the ozone generator to disperse ozone. Furthermore, by only tuning the bubble size, the rig is readily adaptable to harvesting the algae. The expectation is that the algae will grow to a greater density and exhibit higher growth rates with intermittent disinfection but will also be sufficiently axenic to address the high demand for secondary metabolites and lipids for the pharmaceutical and biofuel industries. We believe that our novel process can contribute to in-situ disinfection and the development of a cost-efficient disruption method that can be applied on an industrial scale. Therefore, the aims of this paper are to report: (1) the development of axenic conditions at the beginning of the process and intermittent disinfection during the growth phase to eliminate or reduce contamination and (2) the development of an easy and cheap disruption and lipid extraction method using ozonation that is applicable to a large scale.

2. Materials and methods

2.1. Microalgae culture and bioreactor set-up

The *Dunaliella salina* strain 19/30 used in the study was obtained from the Culture Centre of Algae and Protozoa, Oban, UK. The culture was grown for 14 days in 250 mL shake flask with 100 mL of working volume during preparation of inoculum. While for the mass production, the strain was grown in 2 L photobioreactor with 1.5 L working volume and both cultivation methods used artificial seawater as the culture medium [11]. A 10% (*v*/v) inoculum size (14 days old) was used in all *D. salina* culturing processes. A mixture of 5% CO_2 and 95% N_2 was directed into the photobioreactor for 30 min every day to serve as a carbon source and agitation. Continuous illumination of the shake flasks and photobioreactors culture was accomplished using a fluorescent lamp at 90 μmol quanta m^{-2} s^{-1}; this measurement was obtained using a quantum sensor (Hansatech Instrument Ltd., UK). The experimental set up was based on previous studies [14]. The *D. salina* cultures were maintained at room temperature (23–25 °C).

Generally, there were 4 different bioreactors employed in this study (Fig. S1 in Supplementary material). Firstly, in the study of contaminant effects on algal growth performance, 0.1 L small bioreactor was used to perform the 10 min ozonation. Secondly, 2 L airlift loop bioreactor (ALB) was used to study the different gas flowrate effects towards *D. salina*

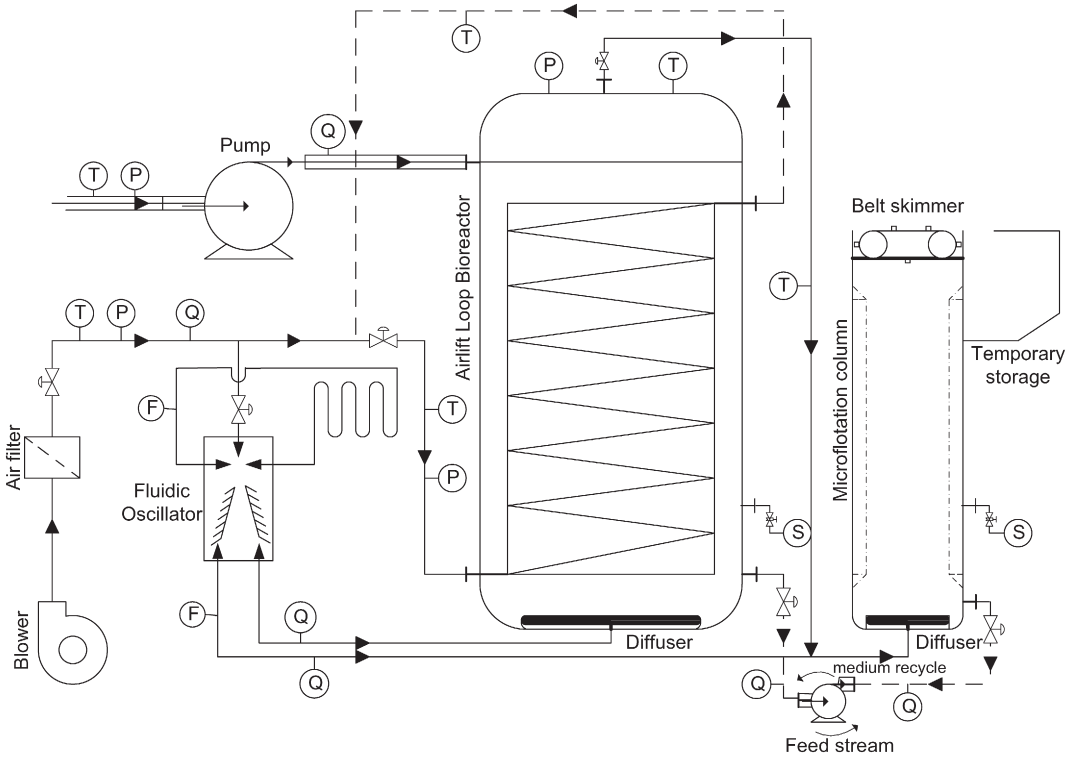

Fig. 1. Piping and instrumentation schematic for the novel bioreactor rig for biomass production. The main units in the production system are the Airlift Loop Bioreactor (ALB) and the Microflotation (MF) units. Where Q, T P and S represent Air Flowrate, Temperature, Pressure and Sampling port respectively. The zig zag line is a heat exchanger pipe around the draft tube.