Biochemical Engineering Journal 134 (2018) 129–135

Contents lists available at ScienceDirect

Biochemical Engineering Journal

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

Nutrient recovery from industrial wastewater as single cell protein by a co-culture of green microalgae and methanotrophs

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ARTICLE INFO

Article history:
Received 3 December 2017
Received in revised form 26 February 2018
Accepted 12 March 2018
Available online 13 March 2018

Keywords:
Algae-methanotroph interaction
Single cell protein
Methane oxidizing bacteria
Resource recovery
Wastewater treatment

ABSTRACT

Conventional water treatment technologies remove nutrients via resource intensive processes. However, new approaches for nutrient recycling are needed to provide food to the increasing population. This work explores the use of microbial biomass as a means to recover nutrients from industrial wastewater and upcycle them to feed grade single cell protein. Results demonstrated that both algae and bacteria could remove or assimilate most of the organic carbon present in the wastewater (~95% removal for monocultures and 91% for the algal-bacterial consortium). However, their growth stopped before nutrients and substrates in the gas phase (i.e., methane and oxygen for methanotrophs and carbon dioxide for algae) were depleted. Likely, algal growth was light limited and stopped after organic carbon was consumed. Methanotrophs growth could be limited by trace elements (e.g., copper). Nevertheless, for all cultures the protein content (45% of dry weight, DW, for methanotrophs; 52.5% of DW for algae; and 27.6% of DW for consortium) and amino acid profile was suitable for substitution of conventional protein sources. Further research should focus on increasing productivity of biomass grown on wastewater resources.

1. Introduction

Global population increase, climate change and industrialization are leading to alarming depletion rates of many resources worldwide. As an example, production of chemical fertilizers alone has increased by 500% over the last 50 years due to agricultural intensification to supply food for human consumption [1]. Consequently, there is an increasing interest on recovering resources from waste streams, which have been traditionally not utilized or negatively valued [2]. Common resource recovery strategies focus on the use of anaerobic digestion for carbon valorisation as biogas – energy recovery – combined with downstream nutrient recovery [3]. Most nutrient recovery technologies produce fertilizers, either mineral (e.g., struvite [4,5]) or organic (e.g., via microbe assimilation [6,7]), which are used for crop production. However, fertilizer application on land is not efficient because it leads large nutrient losses (about 50% of the total nitrogen input) as greenhouse gas emissions or due to run-off, thereby contaminating ground water [8].

The main protein sources for animal feeding are agricultural crops, principally soy meal protein. The increasing meat demand by growing population puts higher demands on protein supply [9]. However, vegetable protein production is not only inefficient, but generates large amounts of waste, has a high land and water footprint and is energy intensive [10]. Moreover, the agricultural soils are limited and if more protein should be produced to secure food supply new and lower-footprint modes to produce protein-rich feed or food ingredients are needed.

Single-cell protein (SCP) consisting of microbial biomass can generate nutritive proteins [11], with quality equal or better than conventional protein sources (e.g. soy or fishmeal [12]) at a lower cost. Some attempts have been made to produce SCP from synthetic or fossil fuel based resources (referred to as first generation processes). As example, methane oxidizing bacteria (MOB) are used at large scale for SCP production using natural gas and synthetic nitrogen sources [13]. Green microalgae have been also proposed as suitable protein source for animal feeds [14].

In this study we propose the use of a consortium of green microalgae and MOB for efficient nutrient recovery from wastewater. Whilst the concept has been previously proposed by Van der Ha et al. [15], their research focused on the production of lipids or polyhydroxibutyrate using synthetic media [16]. To the best of our knowledge, our study presents the first test of the concept using

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https://doi.org/10.1016/j.bej.2018.03.010
1369-703X/

industrial wastewater with the aim of protein production for use as feed ingredient, using synthetic biogas as methane source. First aim was to demonstrate the concept as a two stages system, whereby algae and MOB are cultivated separately in batch mode. The algae photobioreactor (PBR) was expected to consume carbon dioxide produced from anaerobic digestion and produce oxygen. The mixed methane and oxygen are then fed to the MOB batch cultivation. Second and main objective of the study was using a single stage system for nutrient upgrading using the algae-bacteria consortium, whereby algae produce oxygen for MOB, which at the same time provide the algae with carbon dioxide. The main advantage of the single stage process is the efficient and safe oxygen supply, which can minimize the risk of creating explosive atmospheres – main limitation of existing first generation processes [17].

2. Materials and method

2.1. Microorganisms and wastewater

Chlorella sorokiniana and *Methylococcus capsulatus* were purchased from the Culture Collection of Algae and Protozoa (CCAP, Scotland, UK) and NCIMB Culture Collection (GenBank accession number AE017282), respectively. Experiments were conducted using industrial wastewater from a potato processing plant. Wastewater was autoclaved and solids were removed via centrifugation at 3000 rpm for 10 min. We note this process is not suitable for full scale implementation. However, equivalent processes (e.g., ultrafiltration) have been successfully implemented in full scale as pre-treatment prior to algal cultivation [18]. Wastewater composition was 3000 mg L^{-1} of chemical oxygen demand (COD), 19 mg-N L^{-1} of ammonium, and 14 mg-P L^{-1} of total phosphorus. Prior to inoculation, 24 h batch experiments were ran with autoclaved wastewater to ensure non indigenous bacteria were active.

2.2. Reactor description

C. sorokiniana, *M. capsulatus* and a consortium of both of them were grown in triplicates in batch experiments carried out in 600 ml glass bottle at 37 °C, which was connected to an extra compartment of 1 L volume to provide extra gas phase (Fig.1). Light was supplied by 6 × 24 LEDs providing an intensity of 2700 μmol photons m^{-2} s^{-1} measured using a light meter (LI-COR LI-250 A). The gas was circulated using a peristaltic pump and injected into the wastewater using a ceramic diffusor. For *C. sorokiniana* and the bacterial-algal consortium cultivation, the gas composition was 60% CH_4 and 40% CO_2, mimicking biogas composition. For *M. capsulatus* the gas composition was 60% CH_4, 30% O_2 and 10% CO_2, which was equal to the gas composition at the end of *C. sorokiniana* batch cultivation.

Equipment was sterilized in an autoclave for 30 min at 121 °C. Previous to inoculation gas was recycled for one hour through the bioreactors to ensure that there were no gas leakages in the system. pH was adjusted at 7 using 99% H_2SO_4 or 50% NaOH.

2.3. Analytical methods

Biomass was tracked using optical density (OD) 440 nm for MOB and 680 nm for algae and the bacterial-algal consortium (Helios™ Epsilon visible spectrophotometer, Thermo Scientific, USA). Total and soluble COD, TP, and ammonium were monitored using commercial test kits (Hach Lange, Germany). Soluble compounds were measured after sample filtration (0.45 μm cellulose acetate filters, Sartorius, Germany). Methane, carbon dioxide and oxygen contents in the gas phase were monitored using gas chromatography (GC Trace 1310. Thermo Scientific), using two different columns for

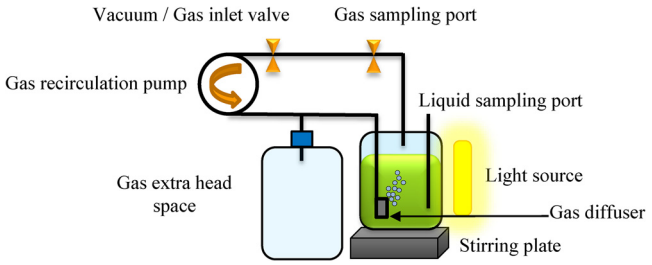

Fig. 1. Schematic representation of the photobioreactor used for all the experiments.

different gases. A HP-Plot/Q column (Agilent Technologies, USA, length 15 m, diameter 0.32 mm, film 20 μm) was used for methane and carbon dioxide, whilst HP-Molesieve column (Agilent Technologies, USA, length 30 m, diameter, 0.53, film 50 μm) was used for oxygen and methane analyses. Calibration was done by injecting gas mixtures of known concentrations (methane to carbon dioxide ratios (v v^{-1}) of 60/40, 40/30 and 5/5 for the first method; Air to methane ratios (v v^{-1}) of 100/0 and 50/50 for the second method).

Biomass macromolecular characterization (i.e., lipid, carbohydrate, protein and pigment content in biomass) determined and quantified as described by D'Este and colleagues [19]. Biomass at the end of each experiment was centrifuged at 6000 rpm for 10 min, supernatant was discharged and biomass was stored at −20 °C. Before analysis, biomass was freeze dried for 2 days (ScanVac CoolSafe freeze dryer, LaboGene ApS, Denmark). Freeze dried samples were ground to fine powder before further chemical analysis.

Total sugar determination was carried out on 25 mg of the freeze dried samples. Hydrolysis was performed by adding 0.25 mL of 99% H_2SO_4 for 30 min. Then, 2.25 mL of distilled water were added and the sample was autoclaved for 30 min at 120 °C. Supernatant was collected and filtered with a 0.2 μm pore size syringe filter. The resulting liquid was between 10–20 times diluted, depending on biomass source, using MilliQ water and transferred to 2 mL ion chromatograph (IC) vials. Vials were analyzed through IC (Thermo Scientific, Dionex ICS-5000, USA).

Determination of the amino acid composition was performed on 50 mg of the freeze dried biomass. This amount was placed into a microwave tube and 6 mL of 6N hydrochloric acid were added. Tubes were flushed with nitrogen to prevent oxidative degradation and closed with a cap afterwards. Hydrolysis of the sample took place in a microwave oven (Anton Paar Multiwave 3000, AT) at 500 W and 150 °C for 30 min. After that time, hydrochloric acid was removed from the tube by evaporation using the same microwave, at 600 W for 20 min. Then, 5 mL of distilled water were added to the pellet and mixed. Samples were filtered by using a 0.2 μm pore size syringe filter and then analyzed through HPLC. For amino acid quantification, a HPLC (Thermo Scientific Dionex UltiMate 3000 UHPLC, USA) equipped with a c18 reversed phase column (Agilent Technologies USA Eclipse Plus C18) and an in-line guard column (Macherey-Nagel Germany EC 4/2 Universal RP) was used. Calibration was done with a commercial amino acid mix (AAS18, Sigma-Aldrich) containing L-Alanine, L-Arginine, L-Aspartic acid, L-Cystine, L-Glutamic acid, Glycine, L-Histidine, L-Isoleucine, L-Leucine, L-Lysine, L-Methionine, L-Phenylalanine, L-Proline, L-Serine, L-Threonine, L-Tyrosine and L-Valine. Total protein concentration was determined by summing up all the amino acid masses retrieved after acid hydrolysis, minus the water mass (18 g H_2O mol^{-1} of amino acid) incorporated into each amino acid after the disruption of the peptide bonds.

Lipid content was determined as suggested by Bligh and Dyer [20]. Lipids were extracted in chloroform, methanol and water. GC-FID (Thermo Trace 1310 GC, USA) was used for detection. 100 μL