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Nutrients from anaerobic digestion effluents for cultivation of the microalga *Nannochloropsis* sp. — Impact on growth, biochemical composition and the potential for cost and environmental impact savings

Joshua J. Mayers[a,*], Anna Ekman Nilsson[b], Eva Albers[a], Kevin J. Flynn[c]

[a] Chalmers University of Technology, Department of Biology and Biological Engineering, Division of Industrial Biotechnology, SE-416 96. Göteborg, Sweden
[b] RISE Research Institutes of Sweden, Agrifood and Bioscience, Ideon, 223 70 Lund, Sweden
[c] Biosciences, Wallace Building, Swansea University, Singleton Park, Swansea SA2 0AP, UK

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ABSTRACT

Microalgal biotechnology has yielded a range of products for different consumer markets, but large scale production for bulk commodities is limited by the cost and environmental impact of production. Nutrient requirements for large-scale production contribute significantly to the cost and environmental impact of microalgal biomass production and should subsequently be addressed by more careful sourcing of nutrients. This study assessed the use of nitrogen and phosphorus contained in effluents from anaerobic digestion of food waste to cultivate the marine microalga *Nannochloropsis* sp. With suitable dilution, effluent could replace 100% of nitrogen demands and 16% of required phosphorus, without significant impacts on growth or biomass productivity. Additional phosphorus requirements could be decreased by increasing the N:P molar ratio of the media from 16:1 to 32:1. *Nannochloropsis* sp. accumulated lipid up to 50% of dry weight under N-stress, with significant increases in the content of saturated and mono-unsaturated fatty acids. Using empirical data generated in this study, the cost and environmental impact of nitrogen and phosphorus supply was assessed versus the use of fertilizers for biomass and biodiesel production. Nutrient requirements predicted by the Redfield Ratio overestimating impacts by as much as 140% compared to empirical data. By utilising residual nutrients and optimising nutrient supply, the cost and environmental impact of nitrogen and phosphorus were decreased by > 90% versus the use of artificial fertilizers. This study demonstrates the importance of using empirical data for process evaluation and how anaerobic digestate effluent derived nutrients can contribute to the sustainability of algal biomass production.

1. Introduction

Microalgae are considered an important feedstock for the production of a range of bulk and fine chemicals with applications in markets spanning food, feeds and cosmetics, as well as their much touted bioenergy potential [1,2]. However, expansion into production of bulk compounds with lower market values is limited, not least by profitability [3], but also the inherent energy requirements, greenhouse gas (GHG) emissions and other resource intensive aspects of production [4–7]. An approach to decrease the cost and environmental footprint of production is the integration of production with industrial infrastructure to make use of cheap inputs such as locally regenerated inorganic nutrients, CO_2, water and heat, possibly forming a more sustainable biorefinery [8].

Nutrient inputs for microalgal cultivation have a considerable impact on the economics and environmental sustainability of biomass production if high purity fertilizers are used [8,9] or if nutrients are not recycled effectively in these processes [6,10]. It has been estimated that fertilizer production can contribute between 10 and 60% of the cumulative energy required for biomass production [5,7,11–13]. The production of nitrogen (N) based fertilizers is heavily dependent on natural gas resources [14], subsequently the CO_2-equivalent (CO_2-eq) footprint of ammonia synthesis, the base of many N-fertilizers, is over 2 kg CO_2-eq produced per kg N [15]. If bioenergy production is the target of microalgal cultivation, then reliance on fossil reserves to produce fertilizers could result in significant problems in the future as demand increases and fossil reserves begin to wane [16], likely increasing the cost of N fertilizer production.

It is expected that due to the fertilizer requirements of large scale microalgal production, significant impacts will be felt by other markets,

* Corresponding author.
E-mail address: joshua.mayers@chalmers.se (J.J. Mayers).

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in particular, agricultural food production. This is especially critical in the case of P, which is derived from mineral extraction and is anticipated to become a limiting resource for agricultural and industrial sectors by the end of the century [17]. The U.S. Energy Independence and Security Act's (signed 2007) has set a target of producing 79 billion L of advanced biofuels per year by 2022 to aid in decreasing reliance on energy imports and establishing new sustainable energy targets. Canter et al. [6] calculated that to meet 24% of this target with microalgae derived fuel (19 billion L yr^{-1}), biomass production would consume 26–28% and 15–23% of current U.S. usage of N and P ($-P_2O_5$) fertilizer, respectively [6]. These requirements are on par with other large-scale agricultural requirements, such as for corn and soybean production [18], and could lead to increased fertilizer prices and decreased incentive for microalgal biofuel production. It is hence critical to develop strategies for efficient nutrient utilisation for large scale microalgal cultivation.

Research to decrease the use of costly fertilizer based nutrients take three main approaches: 1) optimisation of nutrient supply to avoid wastage [19,20], 2) establishing the use of more sustainable sources of nutrients, potentially from waste streams [21], and 3) the recycling of nutrients within the microalgal biorefineries themselves [6,22]. The use of nutrients from waste streams has received considerable attention for biofuel production from microalgae, but the cultivation of microalgal biomass for feed or food applications may be restricted by the source of nutrients that can be used. In particular, concerns may be raised over the presence of pathogens and high concentrations of metals or toxic compounds in the waste streams [23–25]. Some of these issues are discussed in the review of van der Spiegel et al. [24], but it is clear that there are still uncertainties in the regulation of waste nutrient usage in microalgal cultivation and these risks require further assessment and eventually suitable legislation.

A nutrient source that may avoid some of the issues associated with using municipal sewage or industrial wastes for biomass production are anaerobic digestion (AD) effluents. AD has been utilised for the stabilisation of municipal solid wastes, but has expanded to the agricultural and agri-food sectors for treatment of animal manures, food waste and horticultural wastes [26–28]. Anaerobic digestion as a technology is recognised as having the following benefits: GHG avoidance from organic waste matter going to landfill, renewable energy production from bio-gas, and recycling of residual organic materials as fertilizers – decreasing reliance on mineral fertilizers [29]. As such, the classification of digestate residuals as a by-product rather than a waste and finding new uses for this resource was seen as a key factor in aiding the development of the AD sector by the UK Department for Environment, Food & Rural Affairs [30]. Thus, the use of ADE as a source of nutrients contributes and exemplifies the principles of a circular economy, which is in line with a number of EU initiatives, including 'The Roadmap to a resource efficient Europe' (COM(2011) 574) and the Waste Framework Directive (Directive 2008/98/EC and 2011/753/EU) [31].

Digestates are a sludge that is typically separated into solid and liquid fractions via centrifugation. Solid fractions are enriched in organic nutrients and are composted or used as slow release fertilizer [26,32], whereas the liquid AD effluents (ADE) are a more concentrated source of inorganic N (mainly NH_4) and P-PO_4 [28,33]. As long as the inputs to AD processes are controlled, reactors are operated at high temperature (> 55 °C, thermophilic operation), and outputs are pasteurised (> 75 °C for 1 h) to decrease pathogen loads, these resources can be classified as high-quality fertilizers for use in agriculture for food and feed production [30,34]. Several studies have now demonstrated growth of microalgae on AD waste streams, with results comparable to that of cultures grown on synthetic media [33,35–37]. Coupling of microalgal biomass production to recovery of nutrient rich waste streams from AD is hence an attractive opportunity to mitigate the cost of nutrient inputs, decrease the energy and environmental impact of fertilizer production, while also avoiding competition for potentially limiting resources with other sectors [7,11].

In this study, the use of ADE from the digestion of food waste was assessed as a source of N and P for production of the widely exploited marine microalgae *Nannochloropsis* sp. and the potential effects on biochemical composition, specifically fatty acids. Using the generated empirical data, the cost and environmental savings of utilising ADE nutrients will be considered against a base case of fertilizer inputs.

2. Materials and methods

2.1. Anaerobic digestate effluent preparation and composition

ADE was collected from the commercial scale Biogen Gwyri AD plant in Gwynedd, North Wales (UK). The plant has an input capacity of 11,000 metric tonnes (t) yr^{-1} of municipal and commercial food waste. Reactors are mesophilic (35–40 °C) and generate 3500 MWh y^{-1} in electrical energy via combined heat and power generators, enough for ca. 700 homes yr^{-1}. The sludge removed from the reactor was pasteurised (70 °C for 1 h) by the operators upon removal to decrease pathogen numbers and then the solids and liquid effluent (ADE) were separated using a decanter centrifuge.

A 20 L batch of ADE that had been diluted 50% with deionised water was received in December 2012. The ADE was passed through mesh bag filters to remove solids (Nylon, 1 mm, 100 μm and 10 μm) and then stored at − 20 °C in 250 mL aliquots to prevent contamination and maintain a constant nutrient composition across all experiments. Aliquots of ADE were defrosted and decanted into 250 mL Erlenmeyer flasks before being sterilised via autoclavation (121 °C for 20 mins) for use as culture media. Autoclaved ADE was stored at 4 °C and used within 2 weeks.

The nutrient and metal composition of autoclaved ADE is shown in Table 1. Quantification of dissolved nutrients (NO_3, NH_4 and PO_4) was performed using the methods described in Section 2.5.2. The pH of the autoclaved ADE was 7.75 at room temperature. The total suspended solids content of the ADE after filtration through a 10 μm filter was measured by filtering 50 mL of the ADE onto pre-combusted (550 °C for 20 min), pre-weighed 0.7 μm GF/F filters (Whatman, GE Healthcare, Germany). Filters were rinsed with 100 mL of deionised H_2O and then dried at 70 °C for at least 18 h until a constant weight was recorded. This was performed in triplicate and was determined to be 2.14 ± 0.32 g L^{-1}. Quantification of ADE metal content was performed by inductively coupled plasma atomic emission spectroscopy

Table 1
Chemical composition of an anaerobic digestate effluent (ADE) derived from food waste.

Macro elements (mg L^{-1})	
Total nitrogen (TN)	5164
Ammonium	3192
Nitrate	0
Total phosphorus (TP)	136
Phosphate	71
Total Organic Carbon (TOC)	9800
Potassium	150
Calcium	82.2
Magnesium	11
Iron	6.9
Zinc	1.0
Trace elements (μg L^{-1})	
Arsenic	15.6
Boron	520
Cobalt	15.4
Copper	21
Lead	39
Manganese	73
Molybdenum	16
Sulphur	13
Dissolved sulphides (H_2S)	12.7
Tin	18
Titanium	1.8