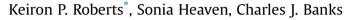
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# Comparative testing of energy yields from micro-algal biomass cultures processed via anaerobic digestion



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### A R T I C L E I N F O

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## ABSTRACT

Although digestion of micro-algal biomass was first suggested in the 1950s, there is still only limited information available for assessment of its potential. The research examined six laboratory-grown marine and freshwater micro-algae and two samples from large-scale cultivation systems. Biomass composition was characterised to allow prediction of potentially available energy using the Buswell equation, with calorific values as a benchmark for energy recovery. Biochemical methane potential tests were analysed using a pseudo-parallel first order model to estimate kinetic coefficients and proportions of readily-biodegradable carbon. Chemical composition was used to assess potential interferences from nitrogen and sulphur components. Volatile solids (VS) conversion to methane showed a broad range, from 0.161 to 0.435 L CH<sub>4</sub> g<sup>-1</sup> VS; while conversion of calorific value ranged from 26.4 to 79.2%. Methane productivity of laboratory-grown species was estimated from growth rate, measured by changes in optical density in batch culture, and biomass yield based on an assumed harvested solids content. Volumetric productivity was 0.04–0.08 L CH<sub>4</sub> L<sup>-1</sup> culture day<sup>-1</sup>, the highest from the marine species *Thalassiosira pseudonana*. Estimated methane productivity of the large-scale raceway was lower at 0.01 L CH<sub>4</sub> L<sup>-1</sup> day<sup>-1</sup>. The approach used offers a means of screening for methane productivity per unit of cultivation under standard conditions.

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# 1. Introduction

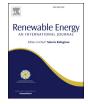
Much of the recent focus on micro-algae as a potential source of biofuel has been through the extraction of bio-oil for subsequent trans-esterification [1]. Other research approaches have considered the production of hydrogen through biophotolysis [2,3] and, more recently, by indirect photolysis and dark fermentation [4]. The potential for direct ethanol production appears limited, although the fermentation of starch storage products may be more favourable [5]. Research on anaerobic digestion of micro-algal biomass goes back more than 50 years [6] and the subject has been revisited a number of times since then [7-13]. More recently digestion has been considered as a means of improving the overall energy balance of biodiesel production [14-16]; as a substrate for codigestion to improve volumetric biogas yields in digestion of less favourable substrates [17,18], or with other carbon-rich wastes [19]; or as an adjunct to wastewater treatment [20,21].

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There are at least 30,000 known species of micro-algae, and one of the key research tasks for commercialisation for energy production purposes has been to screen for favourable composition and for ease of cultivation and processing. The main focus of this screening has been on lipid productivity [22], with less attention given to potential as a fermentation substrate. Micro-algal biomass could be used as the sole substrate in an anaerobic digestion process, and techno-economic and life cycle assessments for this appear favourable [23,24]. In practice, however, serious issues may be encountered in long-term continuous digestion processes. Micro-algae from marine environments are likely to cause difficulties associated not only with high salinity but also with high sulphate concentrations [4,25]. To date most micro-algae that have been tested for digestion have low carbon/nitrogen ratios that may contribute to high digester ammonia concentrations and toxicity [26]. Many micro-algal species have also shown low biodegradability, possibly due to the nature of the cell walls [27], although pre-treatments can improve methane yield [28]. When considering the potential for micro-algal digestion, it is therefore important that the origin and type of the micro-algal material is taken into consideration.







For micro-algae as a substrate for energy production it is also necessary to consider the overall energy balance associated with the production process. Although many factors contribute towards this, the overall biomass yield is a prime consideration as this determines the culture requirements in terms of reactor volume, mixing and harvesting energy per unit of production [29]. The current paper reports the specific methane vield of some common freshwater and marine micro-algal species in biochemical methane potential tests. These values are compared to the theoretical methane potential based on chemical composition and to the higher heat value measured by bomb calorimetry. The energy potential is then calculated based on the specific methane yield coupled with the growth rate and biomass productivity of each of the species considered. The approach thus provides a methodology for the initial screening of micro-algae before full kinetic evaluation of methane production and determination of any secondary limiting factors in semi-continuous digestion.

#### 2. Materials and methods

### 2.1. Micro-algal biomass

The marine species Isochrysis galbana, Thalassiosira pseudonana, Nannochloropsis occulata, and Dunaliella sp. were obtained from the culture collection of the National Oceanographic Centre (Southampton, UK) and the freshwater Chlorella vulgaris and Scenedesmus spp. from the Culture Collection of Algae and Protozoa (CCAP, www. ccap.ac.uk). These micro-algae were cultured on Jaworski's Medium (IM) with the following components (mg  $L^{-1}$ ): macro-nutrients and buffers Ca(NO<sub>3</sub>)<sub>2</sub>·4H<sub>2</sub>O 20.00, KH<sub>2</sub>PO<sub>4</sub> 12.40, MgSO<sub>4</sub>·7H<sub>2</sub> 50.00, NaHCO3 15.90, H3BO3 2.48, MnCl2·4H2 1.39, (NH4)6M07O24·4H2 1.00, NaNO<sub>3</sub> 80.00, Na<sub>2</sub>HPO<sub>4</sub>·12H<sub>2</sub>O 36.00; chelating agents EDTAFeNa 2.25, EDTANa<sub>2</sub> 2.25; vitamin supplements Cyanocobalamin 0.04, Thiamine HCl 0.04, Biotin 0.04. The JM was made up with deionized water when used with freshwater species, and with artificial seawater (Ultramarine Synthetica sea salts, Bristol UK) for marine species. For biomass production each species was grown over a period of 10 days in a 20-L glass culture vessel supplied with 10 L min<sup>-1</sup> of air filtered through a 2  $\mu$ m glass fibre filter (Whatman GF/F). The cultures were maintained under constant illumination of 120  $\mu$ mol m<sup>-2</sup> s<sup>-1</sup>. Cultures were harvested using a continuous centrifuge (Powerfuge Pilot CARR Centritech) operating at 17,000 g and at a flow rate of 1 L min<sup>-1</sup>. The harvested micro-algal centrifugate was then frozen at -17 °C until used.

Two samples of micro-algal material from large-scale bioreactors were also used. One was a culture of *Scenedesmus* grown in a 3000-L tubular photobioreactor (PBR), and the other a mixed culture primarily consisting of *Scenedesmus* and *Chlorella* spp. taken from a 100 m<sup>2</sup> raceway operating at a depth of 0.1 m: both reactors were located in Almeria, Spain [30]. Both cultures were grown using commercial fertiliser products and were harvested using a disc stack centrifuge (Alfa Laval Clara 15, LAPX 404 SGP-31G/TGP-61G). The *Scenedesmus* culture from the PBR was freeze-dried before shipping and use, whereas the raceway culture was frozen after harvesting and defrosted before use.

#### 2.2. Determination of micro-algal growth rate and yield

The growth rate for each of the laboratory-grown micro-algal species was determined in 100 ml working volume Erlenmeyer flasks. Each flask had an optical glass side-arm tube of 10 mm path length, allowing direct readings of the culture optical density (OD) at  $\lambda = 678$  nm to be taken using a spectrophotometer (Cecil 3000 series, Cecil Instruments, UK), without opening the flasks. The OD had been previously correlated with total suspended solids (TSS)

for each species (results not reported here). The growth rate  $\mu$  (day<sup>-1</sup>) on a volatile solids (VS) basis was calculated from Equation (1).

$$\mu = \frac{Ln((TSS_t \times VS\%)/(TSS_0 \times VS\%))}{t - t_0}$$
(1)

where  $TSS_0$  and  $TSS_t$  are the TSS concentrations based on OD at the start and end of the exponential growth phase, VS% is the percentage VS content of the total solids (TS), and *t* and *t*<sub>0</sub> are the start and end times in days.

Potential biomass yield in g VS  $L^{-1}$  day<sup>-1</sup> was calculated from the growth rate assuming a harvested solids concentration of 0.5 g VS  $L^{-1}$  and 12 h of daylight-driven growth per day.

## 2.3. Characterisation of micro-algal biomass

Total suspended solids (TSS) and total and volatile solids (TS and VS) were measured using Standard Methods 2540 D and G, respectively [31]. Total Kjeldahl N was determined using a Kjeltech block digestion and steam distillation unit according to the manufacturer's instructions (Foss Ltd, Warrington, UK). Elemental composition was determined using a FlashEA 1112 Elemental Analyser (Thermo Finnigan, Italy) following the manufacturer's standard procedures, with L-Aspartic Acid, Atropine and Nicotinamide as standards for C, H and N. Birch and Pasta were used as standards for sulphur determination with the addition of vanadium pentoxide catalyst and a desiccating column to remove the H peak. For marine samples the elemental composition of the ashed sample was also analysed. Higher heat value (calorific value, CV) was determined using a bomb calorimeter (Cal2K Eco, South Africa) standardised with 0.5 g benzonic acid.

#### 2.4. Biochemical methane potential (BMP)

This assay was carried out in 0.5-L digesters which were mixed manually once per day. Inoculum was taken from a mesophilic digester treating municipal wastewater biosolids (Millbrook wastewater treatment plant, Southampton, UK). The inoculum-tosubstrate ratios used were around 4:1 based on the VS content of the materials [33]. Initial tests were carried out in triplicate for a period of 28 days against blanks with no substrate added and against a positive cellulose control (C6288, Sigma-Aldrich Ltd, UK). Some of the samples were then tested a second time for a period of 90 days to confirm the ultimate BMP values. All tests were carried out at  $37 \pm 1$  °C. Biogas was collected in 1-L collection cylinders using a 75% sodium chloride barrier solution adjusted to pH 2 with sulphuric acid to minimise losses of CH<sub>4</sub> through dissolution. Biogas composition was analysed each time the collection cylinder was emptied. The methane content of the sample was measured by gas chromatography, using a Varian star 3400 CX Chromatograph with a mixed gas standard of 65%  $CH_4$  and 35%  $CO_2$  (v/v) for calibration (BOC, UK). The volume of methane was calculated by multiplying the dry biogas volume (i.e. after deduction of the calculated volume of water vapour) by the percentage of methane, corrected so that %CH<sub>4</sub> plus %CO<sub>2</sub> = 100%. All gas volumes reported are corrected to standard temperature and pressure (STP) of 0 °C, 101.325 kPa as described by Walker et al. (2009) [33].

### 2.5. Calculation of theoretical methane yield, calorific value and biodegradability

Measured BMP values were compared to the theoretical methane potential (TMP) calculated from the Buswell equation [34] shown in Equation (2), with elemental composition data taken

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