High methane yields in anaerobic digestion of the cyanobacterium *Pseudanabaena* sp.

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Abstract

Microalgae are a novel biomass feedstock for the production of biogas through anaerobic digestion. However, anaerobic digestion of microalgal biomass often suffers from low methane yields due to their rigid cell walls. In contrast to eukaryotic microalgae, prokaryotic cyanobacteria have a weaker cell wall and might be more attractive as feedstock for anaerobic digestion. Nevertheless, feedstock biomass composition influences the anaerobic digestion process. In cyanobacteria, nitrogen is a key nutrient for which availability induces changes in the biomass composition. In this study, the cyanobacterium *Pseudanabaena* sp. CY14-1 was cultivated at different nitrogen concentrations (14, 42 and 56 mg N L⁻¹) to achieve biomass with a variable carbohydrate content (61, 38 and 23 % dry weight, respectively). Anaerobic digestion of these three types of *Pseudanabaena* sp. CY14-1 biomass was performed in batch experiments to evaluate the effect of biomass composition on methane yields. During the first days of the experiment, the methane yield of the biomass was comparable irrespective of the carbohydrate content. The final methane yield, however, was higher for the biomass with 23 % carbohydrate

content ($251 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}_{\text{in}}$) than for the biomass with 38 and 61 % carbohydrates (226 and $215 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}_{\text{in}}$, respectively). In contrast, 61 % carbohydrate-rich biomass showed the highest hydrolysis constant (0.21 day^{-1}) among the three types of *Pseudanabaena* sp. CY14-1 biomass. Methane yields achieved with these three biomasses are higher than those reported for some eukaryotic microalgae species, highlighting the potential of cyanobacteria as biomass feedstock for anaerobic digestion.

Keywords: *Cyanobacteria, nitrogen concentration, carbohydrates, biogas.*

1. Introduction

Biogas production through anaerobic digestion (AD) is a simple and low-cost method to convert biomass feedstocks into a renewable source of energy [1]. Substrates commonly used for AD include animal manure, lignocellulosic materials from agricultural waste, food waste, or sludge generated during wastewater treatment [2,3]. Eukaryotic microalgae and prokaryotic cyanobacteria represent an alternative renewable biomass feedstock for biogas generation through AD [4,5]. Microalgae and cyanobacteria are highly productive photosynthetic microorganisms that can be cultivated on non-arable lands [5]. Moreover, production of microalgae or cyanobacterial biomass can be coupled to CO_2 removal from flue gasses and recovery of N and P from wastewater [5].

Most studies on AD of photosynthetic microorganisms have focused on eukaryotic microalgae species belonging to the genera *Chlamydomonas*, *Chlorella*, *Scenedesmus*, and *Nannochloropsis*

[6–10]. Their methane yields ranged from 81 to 185 mL CH₄ g⁻¹ COD_{in} [7,9]. Relatively few reports have been published on AD of prokaryotic cyanobacteria species, namely *Anabaena*, *Aphanizomenon*, *Arthrospira*, *Borzia*, and *Synechocystis* [8,9,11]. Their methane yields range from 160 to 261 mL CH₄ g⁻¹ COD_{in}, which are higher than those of eukaryotic microalgae [8].

The first stage of the AD process, the hydrolysis stage, is often described as the limiting step of the AD process [12]. Therefore, efficient hydrolysis of the organic matter is key to improve methane yields [12]. Prokaryotic cyanobacteria have a relatively simple cell wall composition when compared to eukaryotic microalgae or lignocellulosic materials, which facilitates the hydrolytic stage of AD [4]. The use of lignocellulosic materials and eukaryotic microalgae requires biomass pre-treatments to enhance hydrolytic rates, resulting in an increase of the biogas production costs [8,13–15].

In addition to cell wall complexity, the methane yield of AD is influenced by the biomass macromolecular composition, more specifically, the relative content of proteins, carbohydrates, and lipids in the biomass. For instance, the use of biomass with high protein content releases ammonia during AD, leading to inhibition of methanogens. The use of carbohydrate-rich biomass can result in the accumulation of volatile fatty acids, decreasing the pH in the digester and, thus, inhibiting methanogens. Both situations would destabilize the AD microbiome resulting in low methane yields [16]. While lipids are easily degradable, at high concentrations they can cause microbial inhibition resulting in mass transfer problems in the digesters [17]. Therefore, biomass macromolecular composition is a key parameter to ensure high methane yields. The macromolecular composition of cyanobacterial biomass is highly variable and is influenced by the

nutrient supply. For instance, the cyanobacterium *Arthrospira* accumulates up to 68 % dry weight protein when nitrogen concentration in the cultivation medium is sufficient or 73 % dry weight carbohydrate when its concentration is markedly reduced [18,19].

In this study, we investigated AD of biomass of the cyanobacterium *Pseudanabaena* sp. CY14-1. We selected this strain because this species occurs naturally in microalgae-based wastewater treatment systems and is recognized for its high nutrient uptake capacity [20–23]. Interestingly, this species has a weak cell wall [24], which should facilitate the AD process. Therefore, *Pseudanabaena* sp. CY14-1 biomass cultivated in wastewater is a promising feedstock for biogas production using AD. Like many other prokaryotic cyanobacteria, *Pseudanabaena* species have been reported to accumulate carbohydrates or proteins depending on the available nitrogen concentration in the medium [25]. In this study, *Pseudanabaena* sp. CY14-1 was cultivated under variable nitrogen concentrations to modify its macromolecular biomass composition. We subsequently evaluated the methane yield from the types of biomass and compared it to published data on prokaryotic cyanobacteria and eukaryotic microalgae.

2. Methods

2.1. *Pseudanabaena* cultivation and biomass production

The cyanobacterium *Pseudanabaena* sp. CY14-1 (from now *Pseudanabaena*) was isolated from a freshwater surface sediment of a reservoir at KU Leuven campus Kortrijk as described by [26].

For identification, genomic DNA was extracted, and sequencing of the 16S rRNA gene was performed (GenBank accession number MF029647). The cyanobacteria primers CYA106F and CYA781R were used to amplify a partial sequence of the 16S rRNA gene [26,27]; sequencing was performed by GATC, and the obtained sequence was compared to gene sequences available in GenBank using NCBI Blast [26].

Pseudanabaena was cultivated in 1 L glass bottles stirred and mixed by aeration with 0.2 μm filtered air (5 L min^{-1}). Cultures were placed in a temperature-controlled room (22 °C) and irradiated with daylight fluorescent tubes, providing a light intensity of 100 $\mu\text{E m}^{-2} \text{s}^{-1}$ at the surface of the bottle. Three nitrogen concentrations (56, 42 and 14 $\text{mg L}^{-1} \text{N}$), were supplied into the cultures, run in duplicate, in the form of NaNO_3 (treatment A, B, and C, respectively). These different nitrogen concentrations were selected based on exploratory experiments to achieve biomass with different carbohydrate content. The concentration of the rest of the nutrients as in the standard WC medium was: 36.8 $\text{mg L}^{-1} \text{CaCl}_2 \cdot 2\text{H}_2\text{O}$, 36.9 $\text{mg L}^{-1} \text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 12.6 $\text{mg L}^{-1} \text{NaHCO}_3$, 28.4 $\text{mg L}^{-1} \text{Na}_2\text{SiO}_3 \cdot 9\text{H}_2\text{O}$, 8.7 $\text{mg L}^{-1} \text{K}_2\text{HPO}_4$, trace metals and vitamins [28]. Medium's pH was set to 8.0 but not further controlled. Biomass was harvested at day 6 of cultivation. Aeration in the glass bottles was stopped allowing cells settling. Settled biomass was collected and centrifuged at 2 000 g for 5 min prior to AD tests.

Pseudanabaena's specific growth rate μ (day^{-1}) for each treatment was calculated using the following equation (**Eq. 1**):

$$\mu = \frac{\ln(X_t/X_0)}{(t - t_0)} \quad \text{Eq. 1}$$

Where X_0 (mg L^{-1}) is the initial biomass concentration at day zero (t_0) and, X_t (mg L^{-1}) is the accumulated biomass concentration at a specific time (t) of six days.

2.2. Anaerobic digestion

Anaerobic sludge was collected at the wastewater treatment plant of Valladolid, Spain. The sludge had a total solids (TS) concentration of 36 g L^{-1} and volatile solids (VS) with respect to TS of 60 %. AD experiments were conducted in batch mode during 29 days in 120 mL glass digesters containing 70 mL working volume. Mesophilic temperature (35°C) and 150 rpm were set to promote the growth of anaerobic microorganisms. To ensure anaerobic conditions, oxygen was removed from digesters by purging helium. Freshly harvested *Pseudanabaena* biomass was mixed with anaerobic sludge in order to obtain a chemical oxygen demand to volatile solids (COD/VS) ratio of $0.5 (\text{g g}^{-1})$. Digesters were run in triplicate. The initial pH was set to 7.5 but was not controlled during the experiment. However, a final concentration of $1.3 \text{ g L}^{-1} \text{ CaCO}_3$ was added as buffer per digester. The volume of biogas produced by the substrates was calculated under standard temperature and pressure (0°C and 1 atm) by measuring the pressure of the headspace in the bottles. Bottles containing only anaerobic sludge were run as blanks for quantification of endogenous methane production, and control tests using ethanol as substrate were run to check the correct performance of the anaerobic microorganisms. In order to calculate methane yields, biogas composition was analyzed by gas chromatography (Clarus 580 GC, PerkinElmer, USA) coupled to a thermal conductivity detector equipped with HSN6-60/80 Sulfinert P ($7' \times 1/8''$) and MS13X4-09SF2 40/60 P ($9' \times 1/8''$) packed columns (PerkinElmer, USA). Helium was used as carrier gas at

a flow rate of 30 mL min⁻¹. The injector, oven and detector temperatures were 80, 62, and 200 °C, respectively. The injected sample volume was 100 µL.

The hydrolysis constant for AD of *Pseudanabaena* biomass was calculated based on the first-order equation (**Eq. 2**), as reported by [29]:

$$B(t) = B_{\infty} [1 - \exp(-k_H (t - L_P))] \quad \text{Eq. 2}$$

Where $B(t)$ is the accumulated methane yield (mL CH₄ g⁻¹ COD_{in}), B_{∞} is the theoretical potential methane yield (mL CH₄ g⁻¹ COD_{in}), k_H the hydrolysis constant (days⁻¹), L_P the lag phase (days) and t the elapsed time (days).

2.3. Analytical methods

To quantify the biomass produced, *Pseudanabaena* growth was monitored by measuring absorbance at 750 nm corresponding to optical density. Dry weight (DW) was gravimetrically determined by filtration of a culture volume with a known optical density over a pre-weighed Whatman filter (GF/C) followed by overnight drying at 105 °C. Optical density (OD) and biomass DW were linearly correlated following **Eq. 3**.

$$\text{Biomass concentration (mg L}^{-1}\text{)} = 584(OD_{750 \text{ nm}}) - 17 \quad \text{Eq. 3}$$

Carbohydrate content in the biomass was determined by the phenol-sulphuric acid method using glucose as standard [30]. Protein content was determined by the Lowry assay using bovine serum albumin as standard [31]. Lipid content in biomass was determined as reported by Mishra et al.,

[32] using canola oil as standard. Nitrogen concentration in the cultivation medium was determined by the salicylic-sulphuric acid method using NaNO_3 as standard [33]. Chemical oxygen demand (COD) was determined by colorimetric analysis using commercial test kits (Merck, ISO 15705).

2.4. Statistics

Statistical differences among treatments were evaluated by one-way analysis of variance (ANOVA) and the post hoc Tukey test (IBM SPSS Statistics, USA). A p -value lower than the significance level (α) 0.05 was considered to be significant.

3. Results and discussion

3.1. *Pseudanabaena* cultivation and biomass production

Biomass production, as estimated from optical density at 750 nm, in the three nitrogen treatments was not affected by the nitrogen concentration supplied in the medium, and the stationary phase of each treatment started on day 6 (**Figure 1.A**). The growth rate as estimated from the increase in optical density was also not statistically different among the treatments ($p = 0.392$). However, the nitrogen concentration in the culture medium had a strong effect on the macromolecular biomass composition in terms of proteins and carbohydrates (**Table 1**).

Treatment A had sufficient nitrogen during cultivation (**Figure 1.B**) and was mainly composed of protein (61 ± 10.1 % DW) with only 23 ± 6.8 % DW carbohydrate. By day 5, treatment B consumed the 42 mg L^{-1} nitrogen supplied (**Figure 1.B**), accumulating 38 ± 3.2 % DW carbohydrate. Finally, treatment C with the lowest initial nitrogen concentration (14 mg N L^{-1}), accumulated 61 ± 2.1 % DW carbohydrate and was nitrogen-limited from day 2 of cultivation (**Figure 1.B**). Similar to our results, up to $10 \text{ mg L}^{-1} \text{ day}^{-1}$ consumption of nitrogen has been previously reported for *Pseudanabaena* [22]. In addition, carbohydrate accumulation as a response to nitrogen limitation has been reported in different cyanobacteria species, including *Pseudanabaena* [18,25]. Comparable to our results, biomass accumulation during nitrogen starvation was not affected in the cyanobacteria *Arthrospira* sp., accumulating up to 73 % DW carbohydrate after 10 days of cultivation despite the nitrogen concentration tested [18]. Lipid content in *Pseudanabaena* biomass ranged from 8 to 10 % DW and was not statistically different among the treatments ($p = 0.190$). Thus, nitrogen limitation did not promote lipid accumulation in *Pseudanabaena*, as similarly reported for other cyanobacteria species [11,18,34].

Figure 1: (A) *Pseudanabaena* growth measured by absorbance (optical density) at 750 nm (n=2). (B) Nitrogen concentration in *Pseudanabaena* treatments during cultivation (n=2). Data represent averages and standard deviations.

Table 1: *Pseudanabaena* growth parameters and biomass composition determined at day 6 of cultivation. Data represent averages and standard deviations (n=2). Superscripts represent a significant difference in the results ($\alpha = 0.05$).

	Treatment A	Treatment B	Treatment C
Initial nitrogen concentration (mg L ⁻¹)	56 ± 0.1	42 ± 0.4	14 ± 0.2
Biomass concentration (mg L ⁻¹)	440 ± 33 ^a	461 ± 36 ^a	409 ± 42 ^a
Growth rate (day ⁻¹)	0.34 ± 0.01 ^a	0.36 ± 0.001 ^a	0.34 ± 0.02 ^a
% Carbohydrates (DW)	23 ± 6.8 ^b	38 ± 3.2 ^b	61 ± 2.1 ^a
% Proteins (DW)	61 ± 10.1 ^b	46 ± 5.6 ^{ab}	18 ± 1.1 ^a
% Lipids (DW)	11 ± 0.1 ^a	10 ± 1.6 ^a	8 ± 0.2 ^a

DW refers to dry weight

3.2. Anaerobic digestion

Methane yield was evaluated in batch AD assays for 29 days. In all treatments, a rapid increase in methane yield from 60 to 140 mL CH₄ g⁻¹ COD_{in} was observed between days 2 to 5 (**Figure 2**). This rapid increase in methane yields indicated that *Pseudanabaena* biomass was highly digestible, regardless of the carbohydrate and protein content. However, from day 5 onwards, methane yields differed statistically ($p = 0.01$) among the three different *Pseudanabaena* biomass (**Table 2**). Biomass from treatment A with 23 % DW carbohydrate produced the highest methane yield of 251 ± 7.2 mL CH₄ g⁻¹ COD_{in}. In contrast, methane yield reached only 226 ± 5.3 mL CH₄ g⁻¹ COD_{in} and 215 ± 1.4 mL CH₄ g⁻¹ COD_{in} for biomass from treatments B and C with 38 % and 61 % DW

carbohydrates, respectively. Methane concentration in biogas is commonly reported around 50-70 % v v⁻¹ [35]. Despite the differences in methane yields among *Pseudanabaena* biomass, methane concentration in biogas was not statistically different among treatments ($p = 0.158$) and contained around 61-70 % v v⁻¹ methane for all substrates (**Table 2**). Ash content for *Pseudanabaena* was below 5 % DW for the three types of biomass (data not shown), similar to that of other cyanobacteria species, including *Arthrospira* and *Synechocystis* [36,37]. AD experiments were conducted based on the COD/VS ratio (0.5 g g⁻¹). Given the low ash content, the cyanobacterial biomass used was suitable for AD since only the organic matter (represented by VS or COD) is converted to methane.

Figure 2: Methane yields achieved when digesting *Pseudanabaena* biomass with different carbohydrate content. DW refers to dry weight. Data represent averages and standard deviations:

Treatment A and C n=3, Treatment B n=2.

Table 2: Parameters obtained from AD using *Pseudanabaena* biomass. Data represent averages and standard deviations. Treatment A and C n=3, Treatment B n=2. Superscripts represent a significant difference in the results ($\alpha = 0.05$).

Treatment	Carbohydrate in biomass (% DW)	COD/VS ratio* (g g ⁻¹)	Methane yield (mL CH ₄ g ⁻¹ COD _{in})	Methane concentration in biogas (% v v ⁻¹)	k _H (day ⁻¹)**	B _∞ (mL CH ₄ g ⁻¹ COD _{in})***	R ² model
A	23 % ± 6.8 ^a	1.8	251 ± 7.2 ^a	70 ± 6.3 ^a	0.18 ± 0.003 ^a	241 ± 6.7 ^a	0.99
B	38 % ± 3.2 ^b	1.7	226 ± 5.3 ^b	65 ± 3.5 ^a	0.20 ± 0.01 ^b	215 ± 5.5 ^b	0.99
C	61 % ± 2.1 ^b	1.5	215 ± 1.4 ^b	61 ± 7.0 ^a	0.21 ± 0.006 ^b	203 ± 1.0 ^b	0.99

* Chemical oxygen demand to volatile solids ratio.

** Hydrolysis constant.

*** Theoretical potential methane yield.

Methane yields of lignocellulosic materials, evaluated in batch mode, range from 50 to 500 mL CH₄ g⁻¹ VS_{in}, as reviewed by [38]. This high variability can be explained by the biomass composition diversity of the organic substrates, AD conditions (digestion time and temperature, and substrate to inoculum ratio) evaluated or the use of biomass pre-treatments to improve methane yields. A COD/VS ratio of 1.8 g g⁻¹ was obtained for *Pseudanabaena* with 23 % DW carbohydrate (biomass from treatment A), equivalent to 452 mL CH₄ g⁻¹ VS_{in}. Consequently, *Pseudanabaena*'s digestibility is as high as that reported for the former organic substrates; however, additional biomass pre-treatments in *Pseudanabaena* are not necessary to improve methane yields.

When compared to eukaryotic microalgae species, *Pseudanabaena*'s methane yields are higher than the reported values of 81-185 mL CH₄ g⁻¹ COD_{in} for the species *Chlorella* and *Scenedesmus*, and their consortium including *Chlamydomonas* (**Table 3**). Methane yields around 200 mL CH₄ g⁻¹ VS are reported for *Nannochloropsis salina* [6], and higher methane yields around 500 mL CH₄ g⁻¹ VS have been reported for the species *Dunaliella salina* and *Chlamydomonas reinhardtii* [4]. Compared to cyanobacteria species, *Pseudanabaena*'s methane yields are similar to that of *Aphanizomenon ovalisporum*, *Ananabaena plactonica*, and *Synechocystis* sp. with reported methane yields between 218-261 mL CH₄ g⁻¹ COD_{in} (**Table 3**). Methane yield of *Arthrospira platensis* digestion is reported between 318-481 mL CH₄ g⁻¹ VS [4,39], similar to that of *Pseudanabaena*'s with 452 mL CH₄ g⁻¹ VS. The high methane yield of prokaryotic cyanobacteria species, including *Pseudanabaena*, and few eukaryotic microalgae species, including *Chlamydomonas* and *Dunaliella*, may be explained by the presence of a weaker cell wall poor in complex carbohydrates such as hemicellulose, cellulose, or algaenan typically found in most of eukaryotic microalgae species and lignocellulosic materials [4,40]. Although, increases in methane yields (10 - 60 %) are attained when biomass is pre-treated [41], additional procedures prior to AD decrease process energy output and increase production costs [14]. Thus, the high digestibility and fast nutrient uptake of *Pseudanabaena* would represent an advantage for wastewater treatment combined with bioenergy production.

As initially discussed, the highest methane yield of 251 mL CH₄ g⁻¹ COD_{in} was reached for *Pseudanabaena* biomass with low carbohydrate content (treatment A). In contrast, methane yields decreased while carbohydrate content in biomass increased. Therefore, biomass composition does affect the methane yield. Similarly, as listed **Table 3**, the methane yield of *Anabaena plactonica*

with 63 % DW content of carbohydrate was lower ($187 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}_{\text{in}}$) than that of *Anabaena plactonica* with 42 % DW carbohydrate ($261 \text{ mL CH}_4 \text{ g}^{-1} \text{ COD}_{\text{in}}$). Theoretical methane yields of proteins are higher than for carbohydrates: 496 and 415 $\text{mL CH}_4 \text{ g}^{-1} \text{ VS}$, respectively [42]. Thus, the higher concentration of proteins in *Pseudanabaena* biomass (treatment A) is related to the higher methane yield observed in these batches. Even though protein-rich substrates are claimed to be detrimental for methanogens' activity due to ammonia toxicity, the low substrate to inoculum ratio used in batch systems reduces this negative effect [43,44]. Thus, the combination of these factors explained the higher methane yield obtained from *Pseudanabaena* with high protein content (treatment A).

In contrast, semi-continuous and continuous AD of carbohydrate-rich substrates are reported to improve methane yield. *Arthrospira platensis* co-digestion with beet silage almost doubled its methane yield in a continuous system compared to *A. platensis* mono-digestion [44]. In another study, when operating a semi-continuous reactor at a hydraulic retention time of 15 days, *A. platensis* carbohydrate-enriched biomass increased methane yield by 35 % for biomass with 60 % DW carbohydrate [11]. It is well known that the hydrolysis stage is the rate-limiting step of the AD process for a given substrate [12]. Similar to a higher methane yield of carbohydrate-rich biomass in continuous AD, the hydrolysis constant calculated for *Pseudanabaena* digested in batch mode was higher for biomass with higher carbohydrate content (**Table 2**). Biomass from treatment A, with the lowest carbohydrate content (23 % DW), had the lowest hydrolysis constant (k_H) of 0.18 day^{-1} . This value was statistically different ($p = 0.008$) from the k_H of the biomass with higher carbohydrate content: 0.20 and 0.21 day^{-1} for 38 % (treatment B) and 61 % DW (treatment C) carbohydrates, respectively. Thus, carbohydrate-rich biomass was hydrolysed faster than the

protein-rich biomass. This behaviour is explained by the fact that a higher C/N ratio enhances substrate hydrolysis during AD [41], as observed for *Pseudanabaena* and other cyanobacteria species when carbohydrate-rich substrates are digested [44,45].

Hydrolysis constants of lignocellulosic material are highly variable due to their diverse biomass composition, values from 0.06 to 0.24 day⁻¹ are reported [46]. In case of eukaryotic microalgae and prokaryotic cyanobacteria, very few studies report their hydrolysis constants: González-Fernández et al., (2016) reported hydrolysis constants of 0.04-0.20 day⁻¹ for the digestion of *Chlorella*, *Scenedesmus* and *Chlamydomonas* consortium cultivated under different conditions, while for the cyanobacterium *Chroococcus* sp. and *Arthrospira platensis*, hydrolysis constants of 0.03 and 0.04 day⁻¹, respectively, were reported [44,45]. Thus, hydrolysis constants of 0.18-0.21 day⁻¹ found in this study confirm *Pseudanabaena* as a high hydrolysable substrate suitable for AD. Nonetheless, further studies evaluating AD of microalgae and cyanobacteria biomass must be implemented to evaluate the influence of biomass macromolecular composition and digestion operational conditions in methane yields.

Table 3. Literature comparison among cyanobacteria and microalgae species' performance in anaerobic digestion.

Microorganism	Major biomass component (% DW)	Methane yield (mL CH ₄ g ⁻¹ COD _{in})	Anaerobic digestion time (days)	Methane concentration in biogas (% v v ⁻¹)	Reference
<i>Anabaena planctonica</i>	63 % carbohydrate	187	22	74	[8]
<i>Anabaena planctonica</i>	42 % carbohydrate	261	35	NA	[9]
<i>Aphanizomenon ovalisporum</i>	42 % carbohydrate	223	22	74	[8]
<i>Aphanizomenon ovalisporum</i>	34 % carbohydrate	218	35	NA	[9]
<i>Arthrospira platensis</i>	60 % carbohydrate	160	15	NA	[11]
<i>Borzia trilocularis</i>	63 % protein	168	22	76	[8]
<i>Chlorella vulgaris</i>	65 % protein	125	22	76	[8]
<i>Chlorella vulgaris</i>	34 % carbohydrate	185	35	NA	[9]
<i>Chlorella vulgaris</i> , <i>Scenedesmus obliquus</i> and <i>Chlamydomonas reinhardtii</i>	57 % protein	171	35	NA	[10]
<i>Chlorella vulgaris</i> , <i>Scenedesmus</i>	50 % carbohydrate	146	35	67	[47]

obliquus and

Chlamydomonas

reindhardtii

<i>Pseudanabaena</i> sp.	23 % carbohydrate	251	29	70	This study
<i>Synechocystis</i> sp.	65 % protein	220	22	75	[8]
<i>Scenedesmus</i> sp.	NA	81	34	62	[7]

NA refers to not available information.

Conclusions

Nutrients availability in the cultivation medium affects the macromolecular biomass composition of cyanobacteria. In this study, the cyanobacterium *Pseudanabaena* was cultivated under limiting nitrogen concentrations to promote the accumulation of carbohydrates. When only 14 mg N L⁻¹ were supplied in the cultivation medium, up to 61 % DW carbohydrate were accumulated in *Pseudanabaena* biomass. In contrast, nitrogen availability of 42 and 56 mg N L⁻¹ decreased carbohydrate content in biomass to 38 and 23 % DW, respectively. Nevertheless, biomass with the lowest carbohydrate content (23 % DW) resulted in the highest methane yield of 251 mL CH₄ g⁻¹ COD_{in}, followed by 226 and 215 mL CH₄ g⁻¹ COD_{in} for the biomass with 38 and 61 % DW carbohydrates, respectively. Despite biomass composition, high hydrolysis constants of 0.18-0.21 day⁻¹ were determined indicating that *Pseudanabaena* biomass does not hamper the hydrolytic stage of AD. Thus, contrary to eukaryotic microalgae or lignocellulosic materials, cyanobacteria exhibit a high methane yield and require no pre-treatments prior AD.

Author's contributions:

Study's conception and design: CGF, KM, SPCB, JAM. Collection and assembly of data: SPCB, JAM. Data interpretation, manuscript drafting, and final approval of the article: SPCB, JAM, CGF, KM. Administrative and technical support, and obtaining funding: GCF, KM.

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