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Metagenome changes in the mesophilic biogas-producing community during fermentation of the green alga *Scenedesmus obliquus*

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ABSTRACT

A microalgal biomass offers a potential alternative to the maize silage commonly used in biogas technology. In this study, photoautotrophically grown *Scenedesmus obliquus* was used as biogas substrate. This microalga has a low C/N ratio of 8.5 relative to the optimum 20–30. A significant increase in the ammonium ion content was not observed. The methane content of the biogas generated from *Sc. obliquus* proved to be higher than that from maize silage, but the specific biogas yield was lower. Semi-continuous steady biogas production lasted for 2 months. Because of the thick cell wall of *Sc. obliquus*, the biomass-degrading microorganisms require additional time to digest its biomass. The methane concentration in the biogas was also high, in co-digestion (i.e., 52–56%) as in alga-fed anaerobic digestion (i.e., 55–62%). These results may be related to the relative predominance of the order *Clostridiales* in co-digestion and to the more balanced C/N ratio of the mixed algal–maize biomass. Predominance of the order *Methanosarcinales* was observed in the domain Archaea, which supported the diversity of metabolic pathways in the process.

1. Introduction

The increasing global demand for energy heavily depends on fossil fuels such as oil, coal, and natural gas. With the anticipation of fossil fuels becoming exhausted in the foreseeable future, novel strategies need to be discovered for alternative energy generation. Photosynthetic biomass-based fuels are widely regarded as sustainable alternatives to fossil fuels. Biofuels and other forms of bioenergy are currently produced from terrestrial plants (Schenk et al., 2008). Microalgae may represent an alternative to terrestrial crops because they have higher photosynthetic efficiencies and higher growth rates, and can be grown in saline waters and marginal land areas (Posten and Schaub, 2009; Dębowski et al., 2013). Microalgae can be harvested practically all year round, which results in enhanced biomass-production efficacy. Cultivation can be carried out in closed photobioreactors or in open ponds. Open systems are usually considered economical, whereas closed systems are more efficient from the aspects of biomass production and control of the cultivation parameters (Edward, 2009; Edward, 2009), so that either concept may be competitive in the various applications (Guccione et al., 2014).

Microalgal biomass is of potential for anaerobic digestion (AD) as it can have high contents of lipids, carbohydrates, and proteins, and does not contain recalcitrant lignin (Chen et al., 2009; González-Delgado and Kafarov, 2011; Yen et al., 2013; Ward et al., 2014). With regard to the enormous biodiversity of microalgae and the recent developments in genetic engineering, this group of organisms is clearly one of the most promising sources for new-generation biofuels. Research on the AD of algal biomass goes back more than 50 years (Golueke et al., 1957). That early study made a comparison of sewage sludge and green algae (*Scenedesmus* sp. and *Chlorella* sp.). Following such pioneering experiments, relatively few investigations dealt with the anaerobic fermentation of microalgae (Uziel et al., 1974; Keenan, 1977; Binot et al., 1977; Samson and LeDuyt,

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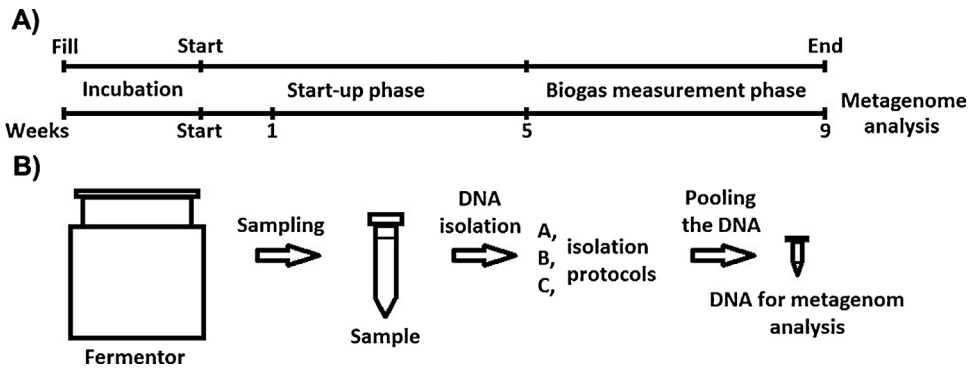

Fig. 1. The scheme and timeline of the experimental set-up. A. the time course of the various stages of the experiment. First a 2–3 weeks long "incubation" period indicates the set-up phase of the reactors. During the "start-up" phase the reactors, already producing biogas from a mixture of pig slurry and maize silage, were fed with the selected substrates, i.e., algal biomass, maize silage or a 1:1 mixture thereof. This lasted about 4 weeks. In the "biogas measurement" stage biogas yield from the selected substrates and metagenomic changes were monitored. B: The sample preparation steps for metagenomic studies. Note: the individual steps do not correspond to the time scale indicated in A.

1982; Becker, 1983; Hernández and Córdoba, 1993) until recently. Various freshwater and salt water algal strains were compared under mesophilic conditions (Mussgnug et al., 2010) and the biogas potential proved to depend strongly on the species and on the thickness of the cell wall. One noteworthy feature was that the CH_4 content of the biogas from the microalgae was 7–13% higher than that from maize silage (Mussgnug et al., 2010).

Intensive studies of the microbial communities of maize silage-fed anaerobic digesters (Schlüter et al., 2008; Krause et al., 2008; Kröber et al., 2009; Jaenicke et al., 2011; Wirth et al., 2012; Stantscheff et al., 2014; Ziganshina et al., 2014) have demonstrated that, although the anaerobic fermentation conditions (fermenter size, feedstock composition, and origin, mixing, inoculum composition, etc.) differed somewhat, but the substrates were essentially the same (maize silage and pig manure) and coherent data sets could be collected. Members of the phyla *Firmicutes* and *Bacteroidetes* played the most important roles in the hydrolysis of the plant biomass and in the secondary fermentation. In particular, many *Clostridium* species were identified which possess cellulolytic and H_2-producing activities, both properties probably being essential for the efficient degradation of the biomass. *Methanomicrobiales*, the most abundant order in the domain Archaea in large scale AD process, uses CO_2 as a carbon source and H_2 as an electron donor for methanogenesis. The general features of the community structure in the domain Bacteria appeared similar in the various studies, but alterations were noted in the domain Archaea. The most sensitive element in the microbiological food chain yielding biogas is the methanogenic group, changes in which may be associated with seasonal fluctuations or the variation of specific fermentation conditions (Rastogi et al., 2008; Lee et al., 2009). As an example, acetoclastic tend to predominate in biogas fermenters operated with wastewater sludge, while reactor communities fed with more diverse substrates prefer hydrogenotrophic methanogenesis (Sundberg et al., 2013).

Little is known about the microbial community of an anaerobic digester sustained with algal biomass. Ellis et al. (2012) employed 454 pyrosequencing to study the archaeal community during microalgal fermentation following the PCR amplification of *mcrA* gene regions. In alga-fed mesophilic AD inoculated with wastewater sludge, the majority of annotated *mcrA* sequences were assigned to the genus *Methanosaeta*. That investigation did not extend to the composition of the bacteria in the substrate algal biomass or within the anaerobic digester, although heavy bacterial representation could be expected in algal biomass cultivated in open ponds filled with wastewater. A more recent study (Wirth et al., 2014) analyzed the complete microbial community of a laboratory-scale AD fed with an algal–bacterial co-culture. A large proportion of bacteria belonging to the genera *Rhizobium* and *Burkholderia* lived in apparent syntrophic community together with the microalgal biomass, which changed the bacterial community composition significantly. This effect obscured the changes in the domain Bacteria as a result of the algal feedstock. The pronounced alterations observed in the domain Bacteria did not affect the microbial composition of the domain Archaea (Wirth et al., 2015).

Scenedesmus obliquus is a common freshwater microalga which can accumulate high amount of oil (Breuer et al., 2014; Mandal and Mallick, 2009) and starch (Batista et al., 2014). It can grow in various industrial wastewaters (Mata et al., 2013; Hodaifa et al., 2008) in a relatively wide temperature range (Xu et al., 2012). We report here an AD process involving the use of a photoautotrophically grown *Sc. obliquus* microalgal biomass with the aim of determining the response of the biogas producing microbial community to the novel substrate. The microbial community was monitored during the process by using high-throughput sequencing technology. The AD parameters and microbial community in an anaerobic reactor fed with *Sc. obliquus* and a co-digestion of maize silage and algal biomass were compared with the corresponding data on maize silage alone as control.

2. Materials and methods

2.1. Sc. obliquus *biomass production*

For biomass production, a culture of *Sc. obliquus* obtained from the culture collection of algae and protozoa (catalog no. CAAP276/72) was cultivated under natural light illumination at ambient temperature in a 4000 L tubular photobioreactor by first Hungarian Algatechnic Ltd. (ELMAT). BG11 medium was used (Stainer et al., 1971; Rippka et al., 1979). The biomass yield was approximately 2 g L^{-1}. The harvested biomass was stored at −20 °C until utilization.

2.2. *Anaerobic fermentation and biogas analysis*

Anaerobic fermentations were carried out in 5 L continuously-stirred tank reactors (CSTR) (Kovács et al., 2013a) in fed-batch operational mode. The experimental design and time course are summarized in Fig. 1. The reactors were operated with a pig manure + maize silage mixture (Wirth et al., 2012) until the biogas yield became stable prior to the commencement of feeding, i.e., start-up phase with the algal/maize silage substrates. The three reactors were fed with distinct substrates from the beginning of the start-up phase. One fermenter was fed with *Sc. obliquus* biomass at a loading rate of 1 g oDM L^{-1} day^{-1} (oDM = organic dry mat-

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