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Rumen anaerobic fungi create new opportunities for enhanced methane production from microalgae biomass

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ABSTRACT

The aim of this study was to investigate the effects of bioaugmentation with anaerobic rumen fungi at varied ratios of inoculums on the performance of anaerobic digesters of microalgae biomass for increasing methane production. Anaerobic rumen fungi, *Anaeromyces*, *Neocallimastix*, *Orpinomyces* and *Piromyces*, were used in this study and have groups of genes that originate from bacteria by the way of horizontal gene transfer. The results imply that rumen fungi improved the fermentation and degradation of microalgae biomass because they fostered cell wall degradation while methane production increased of 41% because of bioaugmentation with rumen fungi during anaerobic processes. Overall, the findings here indicate that bioaugmentation with a combination of rumen fungi in anaerobic process can represent an appropriate alternative to the use of chemical pretreatments of microalgae biomass. Thus, anaerobic rumen fungi have promise for enhancing biogas production from different microalgae and macroalgae species and also various lignocellulosic substrates.

1. Introduction

As microalgae have high photosynthetic performance, advanced growth rates and the characteristic of not requiring an external organic carbon source, it is thought that microalgae are potential sources for bioenergy and biofuel production [1]. Production of biogas comprising hydrogen or methane from anaerobic digestion of algae is currently rather conspicuous technology because they have the capacity for energy conservation and environmentally friendly features. In addition to environmental conditions promoting microbial activity, degradation of substrates is quite an important parameter through anaerobic process [2]. Anaerobic digestion can take place directly on algae following new collection or microalgal wastes after lipid extraction. With regard to the former, the resistance of the microalgal cell wall may be a significant restrictive factor for cell digestibility [3] Certain microalgal species, like *Haematococcus* sp. and *Chlorella sp.*, feature recalcitrant cellulose in the cell wall, and it protects microalgae against invasion of enzymes and also limits algal biodegradability [4].

It was discovered that roughly 50% of biomass of *Chlorella vulgaris* was removed through methanogenic fermentation [5]. Certain pretreatment methods, such as physical, chemical, mechanical and thermal, have been studied in order to enhance digestion efficiency [6]. Despite the fact that these pre-treatment methods could improve biogas, especially methane, yield from algae with tough cell walls, the energy cost of these technologies was quite high [7]. Moreover, if thermochemical pre-treatment methods are employed, they can cause probable configuration of inhibitory substances. Enzymatic hydrolysis, a well-recognized biological pre-treatment method, induces high methane production in comparison to mechanical methods. It has also been observed that cellulose enzymes lead to maximum methane efficiency versus other enzymes [8]. Nevertheless, enzymes are generally efficient only during the first step of the process after their addition. They become completely ineffective a while later. On the other hand, materials can be consistently hydrolyzed during growth and proliferation by fungi and living bacteria [9]. However, the most convenient species of bacteria and fungi should be carefully determined in order to support maximal microalgal hydrolysis and be complementary to sequential or simultaneous anaerobic digestion [10].

Microalgae biomass can be biodegraded at about 50% of maximum level in anaerobic digester systems - the limiting stage is the hydrolysis. Ruminant's digestion may be more effective because of faster degradation and shorter retention time. While several months are required for anaerobic digestion retention time, several days are sufficient for rumen fermentation [11]. Therefore, the hydrolysis yield of rumen fermentation is considerably superior. Ruminal fermentation is an improved system where cellulose is degraded [12]. It was shown that anaerobic systems of ruminants can enhance transforming cellulosic biomass into biofuels, especially ethanol. The inspiration for biogas

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production performance in rumen is a considerably evolved cellulose-degrading system in nature. Complex microbial populations involving protozoa, bacteria and fungi hydrolyze plant materials in the rumen under anaerobic conditions. Although widespread natural relationships between protozoa and bacteria are symbiotic, advantageous cohabiting with filamentous fungi is not frequent [13]. Symbiotic relationships with anaerobic fungi (AF) is one characteristic of ruminants and certain non-ruminant herbivores. Active plant biomass hydrolyzing enzymes, consisting of cellulases, hemicelluloses, pectinases, and phenolic acid esterase, are produced by rumen AF and can be located in cellulosomes [14]. Rumen fungi can influence algae cell walls, reach fermentable substrates not appropriate for surface-acting bacteria, colonize further recalcitrant plant materials, degrade plants and decrease the size of recalcitrance particles owing to effective hydrolysis [15,16].

Assuming that cellulose is conducive to cell wall resistance in microalgae, enhancement of digestibility of microalgae biomass in order to improve archael microbial variety and the effectiveness of methane production were analyzed by bioaugmentation with rumen anaerobic fungi. This research examined the effects of bioaugmentation of anaerobic rumen fungi at varied ratios of inoculums on microalgae *Haematococcus pluvialis* for biomethane production, which was determined by standardized digestion testing. In addition, high-throughput sequencing technology with Illumina Miseq and qPCR were applied to evaluate community dynamics under fungal bioaugmentation for identifying hydrolytic bacteria and methanogen community structures. To the best of our knowledge, this study is the first report on the improvement of degradation of algal biomass by bioaugmentation using rumen anaerobic fungi. Moreover, not only degradation of microalgae *H. pluvialis* is focused on, but also different types of algae and various substrates featuring high-lignin content can be provided by anaerobic rumen fungi, and so biogas production can be improved by anaerobic rumen fungi holds much promise.

2. Materials and methods

2.1. The experimental approach

Several serious studies were conducted in order to understand the effect of rumen fungi for improvement of biogas production from microalgae, *H. pluvialis*, on anaerobic digesters. Initially, rumen fluid was taken via rumen fistulae from a cow. The fluid was analyzed through metagenomic analysis in order to determine the specific anaerobic rumen fungi within it. Isolated and cultivated rumen fungi were evaluated with strain identification and phylogenetic analysis techniques so as to characterize the species of anaerobic rumen fungi. Four species were isolated that had high lignocellulose-degrading enzyme expression and were selected and mixed. Afterwards, this mixture was added in the anaerobic digesters fed with microalgae *H. pluvialis* at different inoculums ratios: %1 (F_1), 5% (F_2), 10% (F_3), 15% (F_4) and 20% (F_5) (v/v). In order to understand the effect of anaerobic rumen fungi on biogas production, one digester was not biougmentated with anaerobic rumen fungi as a control digester: 0% (F_0). Anaerobic digesters fed with microalgae were set up semi-continuously with 2000 mL volumes over 40 days at 41 °C. Reactors were operated in duplicate under the same conditions. Performance of anaerobic digesters was evaluated via biogas and biomethane production. The inhibitory effect of the digesters was controlled with measurement of volatile fatty acids (VFAs). Finally, microbial community dynamics during the anaerobic digestion process were distinguished according to Illumina Miseq and qPCR analyses.

2.2. Rumen

2.2.1. Rumen sampling

The use of ruminant animals, which features husbandry and particular experimental procedures, and the collection of the rumen samples for this research was approbated by the Animal Ethics Committee of Veterinary Faculty of Istanbul University. Rumen samples that contained anaerobic rumen fungi were collected from the Veterinary Faculty of Istanbul University. Samples for all rumen content comprising fluid and solids were taken via rumen fistulae from a cow (live weight: 400–450 kg) with confidential techniques by veterinarians. Cows were older than two years and fed with alfalfa hay, barely grass, legumes, silage and soybean meal during the summer and winter periods. All samples of ruminal fluid were flushed with nitrogen gas (N_2) so as to provide anaerobic conditions after loading and sealing. A number of the samples of rumen fluid were stored at −20 °C in order to extract DNA for subsequent metagenomic survey of rumen fluid.

2.2.2. Metagenomic survey

Metagenomics, also known as environmental genomics, was employed for determination of the abundance and identity of rumen fungi in a sample. The metagenomic survey, based on total purified DNA, was comprehensively investigated in species of rumen fungi classified by gene function and pathway analysis at the DNA level [17]. The experimental workflow for it was conducted as outlined in Fig. 1. Firstly,

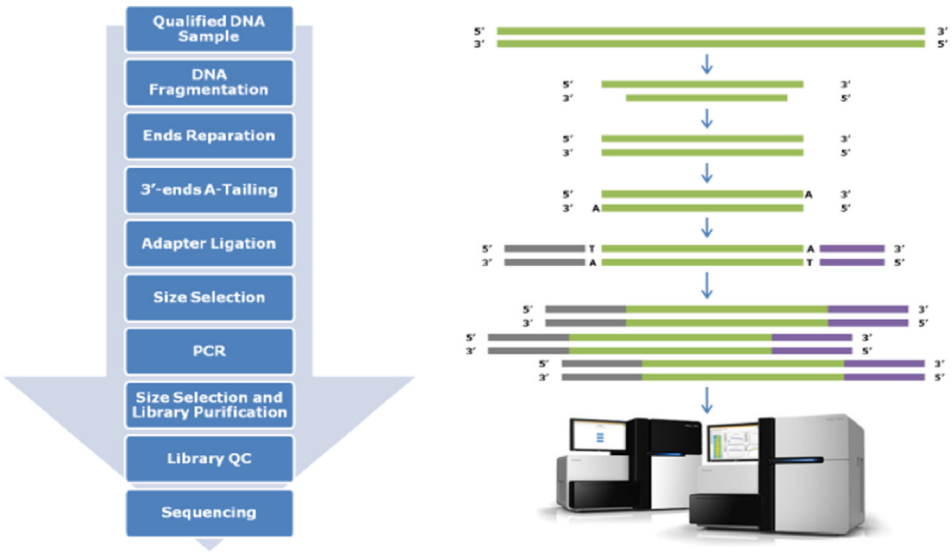

Fig. 1. Experimental Workflow of Meta genomic survey.