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Anaerobic digestion of macrophytes algae for eutrophication mitigation and biogas production

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Abstract

An economy based on biofuels requires production methods which are economically and technically competitive. The production of biogas from biomass by microorganisms is an attractive and ambitious possibility. Marine and lake algae represents a biomass source that could be used for biogas production and their extraction is of benefit for the aquatic environment reducing eutrophication. The goal of the experimental work is to evaluate the algae biomass naturally present in lagoon environment and analyze the biogas resulting from the anaerobic digestion. The Biomethane Potential (BMP) of macrophytes with inoculums from a sewage sludge treatment plant was measured at the University of Perugia. The CH₄ content of biogas was approximately 52%, cumulative CH₄ yield of 217 Nm³ / t SV was observed after 41 days of digestion.

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Keywords: BMP tests; macrophytes; eutrophication; biogas production.

1. Introduction

Among different biofuels, algae, can provide raw material for different types of renewable fuels such as biodiesel, methane, hydrogen and ethanol [1]. As photosynthetic organism, algae require sunlight, carbon dioxide and water. CO₂ sequestration by algae mass cultures represent a significantly positive environmental impact, 1,8 kg of CO₂ are required to produce 1 kg of algae biomass. This stage can enable the utilization of CO₂ streams produced by concentrated sources. A significant reduction in Greenhouse Gases (GHG) emissions would result from the utilization of algae biomass for renewable biofuels production [2]. Aquatic macrophytes, however, many excessively propagate causing stress freshwater environments around the world due to eutrophication [3, 4], especially in areas facing the placing of

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rivers. Environmental stress caused by eutrophication also has effects on public health [5] being often a consequence of human activities such as industrialization, agriculture, and, over the last 20 years, aquaculture [6].

This paper focuses on a test case in southern Italy: the Lesina Lagoon, where a large quantity of biomass is causing water stagnation, ecosystem change, foul odor, fishing interference. Every year a large amount of biomass is removed from the lagoon with significant costs for harvesting and disposal. Biomass and wastes are energy resources that could be used locally as a supply for small scale CHP application, hence increasing sustainability. Available technologies are used to transform both biomass and waste into usable fuels (liquid or gaseous) through several processes ranging from thermochemical to biochemical [7-11]. Gaseous products (syngas, biogas, landfill gas, etc.) can be used in gas turbines and engines requiring costly systems to clean the gas and to modify combustion chambers, which further burdens small scale business plan scenarios [12-17]. Therefore, residual biomasses and wastes, with next-to-zero economic value, or else showing a disposal cost, would be interesting for small to micro scale conversion. Anaerobic digestion can be applied on harvested macrophyte wastes with high moisture content to produce biogas. This process shows different yields depending on algae strain used, reaching values from 0,2 m³/kg to 0,5 m³/kg [18, 19]. Similar results were obtained by digestion of *Laminaria* sp. and *Ulva* sp. seaweeds mixed with other organic waste (milk) [20]. Other works investigated pre-treatment techniques (mechanical, ultrasound, micro-wave, thermal, chemical and biological) to disrupt the structure of cellulose, to increase its surface, to improve biodegradability through cell wall disruption, so that micro-organisms can more easily access the algae and to improve biogas production [21, 22]. S.M. Lee et al., evaluated the use of *Laminaria japonica* for hydrogen and methane production in a mixed culture with microorganisms. The hydrogen and methane gas production levels under these conditions were 356.03 mL/L and 2243.59 mL/L, respectively [23].

Operating conditions for algae biogas production using anaerobic digestion must be optimized, taking into account reaction conditions (mesophilic or thermophilic), temperature, time, organic loading, solid retention time and pH. Main challenge of this technology is also related to the reduction of production costs. Given the algae blooming and disposal costs at the lagoon, anaerobic digestion was proposed for energy production from biogas and to evaluate the methanation potential of the macrophytes a BMP test was carried out.

Nomenclature

BMP	Biochemical Methane Potential
C	Carbon (%)
H	Hydrogen (%)
M	Moisture (%)
N	Nitrogen (%)
VS	Volatile Solids (%)

2. Materials and methods

The Lesina lagoon (41.88°N and 15.45°E), is situated on the southern Adriatic coast of Italy (Fig. 1) and is about 22 km long, with a total area of about 51 km² due to their exposure to rapid nutrient enrichment, coastal lagoons are vulnerable to eutrophication. The concentrations of nutrient salts are influenced by weather conditions, and especially during periods of high rainfall, the waters of the lagoon show large amounts of nitrates, from waste water runoff from surrounding land cultivated with fertilizers

[24]. The trophism of the lagoon depends also on the reduced depth that allows the penetration of light to the bottom making photosynthetic processes possible along the whole water column.



Fig. 1. Lesina Lagoon

In 2009, the Natural Park of Gargano where the Lagoon is situated led a campaign of survey and inventory of species present in the Lagoon [25]; results showed that the ecosystem of the lagoon is characterized by a production of macroalgae covering the bottom and sides resulting in a majority of species *Cladophora* sp., *Enteromorpha* sp., *Gracilaria confervoides*, *Valonia* sp. and *Zostera noltii* (Table 1).

The experimental campaign was carried out at the University of Perugia; several tests were carried out on the considered substrates: chemical and physical analysis, in order to characterize the substrate; BMP tests, in order to evaluate the biogas production and the methane yield. The inoculum and pH effects were also investigated and results were compared to data from the Literature.

Table 1. Macrophytes community in Lesina lagoon

Angiospermae	Rhodophyta	Chlorophyta
Nanozostera noltii	Acrochaetium virgatulum	Blidingia ramifera
Ruppia cirrhosa	Ceramium virgatum	Blidingia minima
	Chrodactylon ornatum	Cladophora fracta
	Lithophyllum pustulatum	Cladofora laetevirens
	Polysiphonia denudate	Cladophora rupestris
	Colaonema daviesii	Cladophora vadorum
	Gracilaria gracilis	Derbesia tenuissima
	Dasya baillouviana	Entocladia viridis
	Hydrolithon boreale	Rhizoclonium tortuosum
	Hydrolithon farinosus	Rhizoclonium lubricum
	Enteromorpha sp	Ulva intestinalis
	Valonia sp	Ulva lens

Sample of biomass were gathered by personnel from the National Research Council, Institute of Marine Sciences of the lagoon of Lesina (Foggia); in different areas of the Lagoon. The samples were then chopped to particle size of 0.5-1.5 cm, and a statistically significant sample was extracted and preserved at -20°C for the tests. Eventually the algae sample was defrosted at room temperature and were prepared by inoculating 30 g of biomass with 90 g of inoculum and diluting, the mixture with 45 g of water.

The preparation of the substrate and the ratio between inoculum and biomass was determined based on tests of digestion previously carried out; water was added to obtain a solid content lower than 15% [26-28].

2.1 Characterization of the substrate and inoculums

The samples of algae were analyzed and loaded into the batch digesters with digestate from a sewage sludge treatment plant operating under mesophilic conditions. Chemical-physical properties were measured by means of TGA 701 LECO for Proximate Analysis and Truspec CHN LECO for Ultimate Analysis. Moisture, Ash and Total and Volatile Solids Content, according to CEN/TS 14774 Methods for determination of moisture content - Oven dry method – Part 3: Moisture in the analysis sample; CEN/TS 14775 Method for the determination of ash content and CEN/TS 15148 Solid Biofuels – Method for the determination of the content of volatile matter were determinate. The percentage of Total Solid was calculated as the difference between 100 and the moisture value.

The chemical and physical characteristics of algae and digestate are shown in table 2.

Table 2. Chemical and physical characteristics of algae and the digestate

	Algae	Digestate
Moisture(%)	84.34 SD±0.06	97.47 SD±0.12
Ash (%)	2.00 SD±0.08	0.91 SD±0.04
VS(%)	13.66 SD±0.02	1.62 SD±0.01
C (%)	5.33 SD±0.01	6.86 SD±0.03
H (%)	9.80 SD±0.05	6.78 SD±0.05
N (%)	0.53 SD±0.02	0.44 SD±0.02
C/N	10.06	15.59

3. BMP tests

Biochemical methane potential test (BMP) were carried out on specifically designed 1 l capacity vessels (Fig.2b) in boron-silicate glass, in specific conditions [29]. They are equipped with probes for pressure, temperature and pH measurement (Hanna Instruments HI9124, double junction electrode, resolution 0.01) and biogas sampling, in order to analyze its composition. Screw plugs guarantee the anaerobic conditions during the test. The vessels are maintained at constant temperature in a thermostatic bath covered with a PVC lid to avoid water evaporation (Figure 2a).

Biogas production is measured with pressure sensors (UNIK 5000, accuracy 0.04% and stability 0.05%), connected to a system (NANODAC) for data acquisition and record. Excess biogas is vented and sampled with an air tight syringe HAMILTON 1025SL; gas composition were analyzed chromatographically using a gas chromatograph (Varian Inc. CP-4900) equipped with 2 columns: a Molsieve 5A Backflush heated column (20 m x 0.53 mm), and a Pora PLOT U heated column (10 m x 0.53 mm). Argon and Helium were used as carrier gases. Injection temperature, column temperature and column pressure were set to 110°C, 120°C and 3.5 atm respectively for column 1, and 110°C, 150°C and 1.54 atm for column 2, respectively. The test was conducted in triplicate and one bottle was filled with only inoculum as blank (control assay). After filling the bottles, they were flushed with nitrogen gas, tightly sealed and incubated at 35°C. The bottles were shaken daily in order to create a homogeneous substrate preventing stratification of organic material and to improve biogas production.

The methane yield of inoculum was also measured as control and subtracted from that of each reactor.

It was not necessary to correct the initial pH of the mixture with acidic or with basic substances, since the measured values were included in the optimum range (Table 3). Batch reactors were operated with a residence average time of 41 days.

During the experimental tests both the quantity and the quality of the biogas produced was monitored and methane content of the biogas was periodically analyzed.



Fig. 2. (a) Thermostatic bath; (b) Bottle for BMP tests

Table 3 Substrate/Inoculums ratios

BOTTLE	COMPOSITION	M (%)	SV(Kg)	pH initial
1, 2, 3	Algae 30 g	94.19 SD±0.09	0.0046 SD±0.00	7.45 SD±0.01
	Dig. 90 g			
	water 45 g			
4 Blank	Dig. 200 g	97.47 SD±0.12	0.0016 SD±0.00	7.50 SD±0.01

4. Results and discussion

The biogas production curve is reported in Fig. 3, which shows an immediate start up and a continuous production. A correct balance between bacteria and organic matter allowed a regular and continuous degradation of the substrate. The peak of production was on the 9th day, after the biogas production rate decreased and the generation of methane mostly completed in 41 days. The rapid biogasification was probably due to rapid biodegradation of intracellular soluble organic matter. In general, hydrolysis of celluloses is a limiting step during anaerobic digestion of plant materials, since recalcitrant lignin protects cellulose against enzymatic attack by coating them [30].

However the body rigidity and structure of macrophytes is different from other plants, macrophytes have more flexible and softer body structure in order to adapt to the water flow [31].

The organic solid and lignocelluloses content of macrophytes significantly varies with species, lignin is a polyphenol that implies lower delignification rate [32].

Biogas composition during the test was measured: maximum methane percentage (51.4%) coincided with the maximum biogas production. The biogas and methane yields resulted 0.423 Nm³/kgSV and 0.217 Nm³/kgSV respectively in agreement with data from the Literature [33, 21] showing yields slightly lower for production of methane and slightly higher for biogas, probably due to absence of continuous mixing and to substantial lignin content, others studies have reported that lignin content influences the methane recovery [34, 35] in the range 156.4 - 444.3 mL biogas/g TS and from 0.13 to 0.17 L CH₄/g VS methane yield. The total CH₄ yield of submerged macrophytes greatly varied from 161.2 to 360.8 mL g-VS⁻¹ depending on species. [18]. However the presence of inoculum and the reduction of organic load, has ensured a complete performance of the process with an important methanogenic phase.

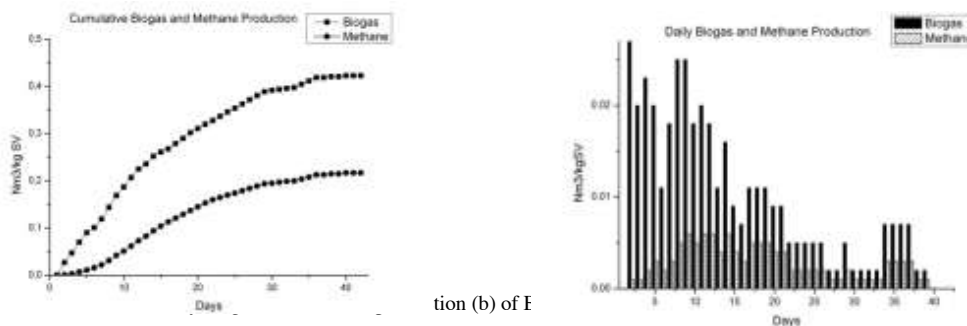


Fig. 3. (

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5. Conclusions

Eutrophic water areas generate large amounts of algae that spread across the surface of the water, and are washed on shore on the coast. The algae problem is evident for a large part of the year, to keep the coastal clean, large quantities of algae need to be removed, and macrophytes may be used for the production of biogas. This study measured the productivity of biogas and methane from macrophytes obtained from Lesina Lagoon, through the biogasification potential of macrophytes inoculated with digestate from anaerobic sewage sludge treatment plant. The cumulative methane yield obtained was $0.217 \text{ Nm}^3/\text{kgSV}$ which is comparable to most of other aquatic weeds conducted in previous studies.

Overall biomass analyzed presents itself as a substrate suitable for anaerobic fermentation and characterized by good yields in terms of biogas and methane. The result obtained suggest that the biogas produced from macrophytes can be used for energy purposes due to a high methane content equal to 52%. This process could be applied to eutrophic ecosystems affected by algal blooms as an alternative to biomass transportation and landfilling.

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Biography

Annarita Pugliese graduated in Biological Sciences at the University of Perugia, specializing in Chemistry at the University of Pisa and a PhD in Energy Engineering. She is involved in biomass and waste to energy technologies. She has participated in several national and regional projects on bioenergy.