Current Topics in Biotechnology
Vol. 7, 2012

Original Communication

Production of methane using microalgae biomass from a wastewater treatment plant

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ABSTRACT

Two recurrent topics among the scientific community are the use of microalgae for biofuel production, and as a biological agent for nutrient removal in wastewater treatment plants. We have analysed the possibility of coupling these two processes, using microalgae that naturally form biofilms on wastewater treatment tanks to produce methane. A methane assay using microalgae biofilms from the primary (T1) and secondary (T2) treatment tanks from a wastewater treatment plant (WWTP) on the Canary Islands showed that, when this substrate is added to suitable methanogenic bacteria, in this case the microbial community in marine sludge from a fish farm, it gives a methane yield of 0.104 Nm^3 kg^{-1}VS (biofilm from T1), and 0.076 Nm^3 kg^{-1}VS (T2). We also checked the microalgal biomass production of each tank (40.13 g AFDW d^{-1} and 106.41 g AFDW d^{-1}, for the T1 and T2 respectively), and the growth of this biofilms in photobioreactors (PBRs). When grown in PBRs, the algae community from the primary tank was dominated by a unicellular Chlorophyta (specific growth rate: 0.42 d^{-1}), while biofilm from the secondary tank was dominated by filamentous Chlorophyta (specific growth rate: 0.25 d^{-1}). The biofilms growing in the WWTP in Gran Canaria are a free, naturally available source of biomass, and we have shown that this biofilm, besides being a natural agent for nutrient removal in a WWTP, has also the potential of being used as a low cost, green source of biomass for methane production when used in combination with other substrates.

KEYWORDS: biogas, methane production, microalgae, wastewater treatment plant, bioenergy

INTRODUCTION

The rapid increase in human population in the last century, and the continuous increase of the global demand for energy since the industrial revolution combined with the use of fossil fuels have caused a series of environmental problems (eg. green house effect and climate changes, pollution and depletion of water resources), which the human race has now to face and find appropriate solutions. Finding solutions that could tackle more than one environmental problem at a time would be highly attractive, both from an environmental and an economical point of view.

Eventually, fossil fuels will have to be substituted by renewable, carbon-neutral sources of energy. However, the first generation of biofuels produced from plant sugars (ethanol) and lipids (biodiesel) have comparatively bad energy balance, besides

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displacing agricultural land designated to food crops [1, 2]. Second generation energy crops solved part of these problems, such as avoiding competition for arable agricultural lands [3]. The use of microalgae, which has the potential of fast growth and can be cultivated on non-arable areas or directly in water bodies, for biofuel production (by the conversion of the whole plant, as done for the second generation biofuels) is far more promising. For this reason, microalgae, together with other high energy crops, forms a third generation of substrates [1, 2, 3, 4, 5].

Integrated approaches, i.e., the production of algal biomass with more than one purpose, increase the economic value of this commodity. Two of the most prominent niches are its use for wastewater treatment and biofuel production [6]. Microalgae enhance the removal of nutrients, organic contaminants, heavy metals, and pathogens from wastewater, while providing an interesting raw material for the production of high-value chemicals or biofuel [7]. Thus, microalgae is actively introduced and cultivated in wastewater treatment plants, but it may also grow naturally in some treatment tanks, providing a cost-free source of biomass.

Therefore, the objectives of this study were to analyse the possibility of using microalgae biofilms that naturally grow on wastewater treatment tanks to produce methane. Besides studying the possibility of directly using the biofilm from the treatment tanks, we also studied the possibility of growing these microalgae in photobioreactors (PBRs), estimated their growth rates, and determined what species would dominate the community under the tested conditions. The aim was to test a low cost, environmental friendly alternative for the use of microalgae in wastewater treatment plants, with the economical and environmental advantages of enhancing the removal of nutrients from wastewater, and producing sustainable biofuel.

MATERIALS AND METHODS

Sampling of the algal material

The algal materials used in the methane production tests were biofilms that naturally grow on the walls of the tanks of a wastewater treatment plant (WWTP). The WWTP sampled in this study was the EDAR (Estación Depuradora de Aguas Residuales) del Sureste, Mancomunidad Intermunicipal del Sureste de Gran Canaria, managed by ACCIONA Agua. The algal biofilms were collected from the primary and secondary treatment tanks (from now onwards referred to as T1 and T2, respectively). These tanks have an inside wall (71 and 78 cm inwards from the external wall, respectively), with an upper edge, where wastewater is always running over, which are 33 and 29 cm wide, respectively. The biofilm was collected from the upper edge of the inside wall from both T1 and T2. Biofilm was collected with a shovel, sieved with a nylon net (50 µm mesh-size) to concentrate the biomass, and put on sampling bottles.

Immediately after sampling, the algal material was brought to the laboratory at the Marine Biotechnology Centre (ULPGC), where subsamples were taken for taxonomic identification of the microalgae present, inoculation in photobioreactors (PBR), and to estimate its dry weight (ash-free dry weight - AFDW). A fraction of the biomass was frozen to be used in methane production assays. Taxonomic identification was made by microscopic observation using an inverted microscope. Identification was made up to genus level, whenever possible.

Growth of the biofilms in photobioreactors

An inoculum of the samples from T1 and T2 was taken to grow the algae in 1.5 L photobioreactors (PBR) under direct sunlight and outdoor temperature. Besides the possibility of directly using the algal biofilm that naturally grows on the treatments tanks, we wanted to study the possibility of growing these algae in PBR, to estimate their growth rate, and to determine what species from the biofilm community would dominate under these conditions. Five different treatments were applied:

1. Biofilm from T1 grown with culture medium: the algae were inoculated in four 1.5 L PBRs, containing 1.3 L of fresh-water MBA medium (Table 1). The algae were grown under direct sunlight and natural conditions in a green house with no roof at the Marine Biotechnology Centre, ULPGC, in the Canary Islands.

Table 1. Composition of the MBA culture medium.

Nutrients	mM	g l^{-1}
KNO_3	30	3
KH_2PO_4	3	0.4
$MgSO_4$ *$7H_2O$	1	0.25
Na_2EDTA	0.3	0.13
$FeSO_4$ *$7H_2O$	0.3	0.08
Trace metals		
$MnCl_2.4H_2O$	$9.1\ 10^{-4}$	$1.8\ 10^{-4}$
$ZnCl_2$	$3.8\ 10^{-4}$	$5.2\ 10^{-5}$
$Na_2MoO_4.\ 2H_2O$	$2.6\ 10^{-4}$	$6.3\ 10^{-5}$
$CoCl_2.6H_2O$	$7.7\ 10^{-5}$	$1.8\ 10^{-5}$

2. Biofilm from T2 grown with culture medium: algae from T2 grown at the same conditions as above.

3. Biofilm from T1 grown with water collected from the primary treatment tank: we inoculated the algae from the biofilm grown in T1 with water collected directly from T1.

4. Biofilm from T1 grown with water collected from the secondary treatment tank: we inoculated the algae from the biofilm grown in T1 with the water from T2.

5. Biofilm from T2 grown with water collected from the secondary treatment tank: we inoculated the algae from the biofilm grown in T2 with water collected directly from T2.

Microalgal growth was determined in these PBR by monitoring fresh weight (FW) or optical density (OD), depending on the algae present. Fresh weight was measured by harvesting total culture biomass with a 20 µm mesh-size nylon net, weighting the biomass, and returning it to the culture medium. Optical density was measured in a spectrophotometer at 680 nm, diluting it when necessary so the values would range between 0.2 and 0.8. The values of FW and OD were converted to dry weight (ash-free dry weight, AFDW). Conversion factors FW to AFDW and OD to AFDW were obtained by measuring both parameters (FW and AFDW or OD and AFDW) from 3 samples harvested from the cultures during exponential phase. AFDW was determined by filtering a known volume of culture through triplicate pre-combusted (450ºC for 4 h), pre-weighed glass fiber GF/C filters. Filters containing the algal material were then dried at 60ºC for 24 h, weighed, and further combusted at 450ºC for 4 h to remove volatile solids. AFDW was calculated as the difference between the weight of the filter after combustion at 450ºC. Specific growth rate (r) of the cultures was calculated as $r = (\ln Nt_1 - \ln Nt_0)/t_1 - t_0)$, where Nt_0 and Nt_1 are the biomass at times t_1 and t_0, respectively.

Methane potential bioassays

Methane potential bioassays were done using the Automated Methane Potential Test System (AMPTS), developed by Bioprocess Control AB (BPC), Sweden. The AMPTS makes on-line measurements of biomethane flows of any biological degradable substrate (both solid and liquid forms) at laboratory scale, that is, the methane production is directly measured by the machine and the data automatically stored in a computer. The AMPTS consists of: 1) a water-bath with controlled temperature and space for 15 bottles were the biodigestion occurs; 2) 15 CO_2-fixing bottles, each one connected to one of the 15 digestion bottles, these are then connected to; 3) a volume measuring device, which makes on-line measurements. The AMPTS follows the same measuring principles as conventional methane potential tests, making the results comparable

with standard methods. Methane released from the digestion bottles is analyzed using a wet gas-flow measuring device with a multi-flow cell arrangement (15 cells, one for each bottle). This measuring device works according to the principle of liquid displacement and can monitor an ultra low gas flow, where a digital pulse is generated when a defined volume of gas flows through the device. It only registers methane flow, since several gas fractions, such as CO_2 and H_2S, are removed by the alkali solution in the CO_2-fixing bottles. A data acquisition system is used together with the flow cells in order to record, display and calculate data [8].

Inoculum and substrate used in the methane potential bioassays: two kinds of bacterial inoculum were used: cow manure and sludge from a marine fish farm. Both inocula received the following previous treatment: they were pasteurized (70ºC for 2 hours), then left for 20 days at room temperature and vented from time to time to release the gases that these bacterial community were still producing (from the original nutrients that it contained). After this conditioning, this substrate has been used ever since in our methane potential bioassays.

As stated above, the substrate used in the tests was the algal biofilm collected directly from the primary and secondary treatment tanks.

Assays: the assays were conducted by mixing, separately, the algal biofilm from T1 and T2 with cow manure or marine sludge, in duplicate bottles, making therefore, four treatments: a) Biofilm T1 + cow manure; b) Biofilm T2 + cow manure; c) Biofilm T1 + marine sludge; d) Biofilm T2 + marine sludge. Controls were made by adding only bacterial inoculum, with no substrate, to the digestion bottles.

The results of the MP bioassay are expressed in Nm^3 (volume normalized by standard temperature and pressure) of methane per kg of volatile solids (VS) (Nm^3 $kg^{-1}VS$). VS is the same as ash-free dry weight (AFDW), i.e., the dry weight minus the inorganic part. All our biomass values (of fresh weight or optical density) where transformed to AFDW, i.e., VS. We chose to use the VS nomenclature to normalized methane production because this (Nm^3 $kg^{-1}VS$) is the unit most commonly found in the literature to express methane potential.

pH was measured at the beginning and at the end of the assays, which lasted for 30 days. Salinity was also measured in the bottles, since we added marine sludge bacteria. This was made to check for possible inhibiting factors.

Biomass production and methane potential of the biofilms from the treatment tanks

After the initial biomass sampling at the WWTP, two more samplings were conducted to measure and calculate the biomass production and methane potential of the tanks. To measure the biomass productivity of the tanks, an area of 957 cm^2 (33 x 29 cm), and 928 cm^2 (32 x 29 cm) were collected from T1 and T2, respectively, on two occasions. The same area was collected in each occasion, with an interval of 7 days between each sampling, and thus, the increase in the biofilm biomass could be measured, giving the biomass productivity of the tank (g AFDW m^{-2} d^{-1}).

The area of the internal wall of the tank (the upper edge, where we collected the biofilm) was used to estimate the daily and monthly biomass production of each tank. With these data and with the data from the methane production tests, we estimated the methane potential of the tanks.

RESULTS AND DISCUSSION

Microalgal community composition of biofilms

Primary treatment tank (T1):

The biofilm growing on the primary treatment tank was dominated by filamentous cyanobacteria. Unicellular cyanobacteria and chlorophyta were also present, but were less abundant. The algae present were as follows:

- Cyanobacteria:
  - Oscillatoriales (filamentous): *Oscillatoria* sp., *Leptolyngbya* sp., *Phormydium* sp., *Limnothrix* sp.
  - Chroococcales (unicellular): *Synechococcus* sp., *Chroococcus* sp.
- Chlorophyta:
  - Chlamydomonadales: *Chlamydomonas* sp.
  - Chlorellales: *Chlorella* sp., *Oocystis* sp.
  - Sphaeropleales: *Scenedesmus* sp.

Secondary treatment tank (T2)

The filamentous Chlorophyta *Stigeoclonium* sp. dominated the biofilm on the secondary tank. Filamentous and unicellular cyanobacteria, unicellular chlorophyta, and diatoms (Bacillariophyta) were also present, but in lesser quantity. The microalgae present were as follows:

- Cyanobacteria:
  - Oscillatoriales (filamentous): *Oscillatoria* sp.
  - Chroococcales (unicellular): non-identified to genus
- Chlorophyta:
  - Chaetophorales (filamentous): *Stigeoclonium* sp.
  - Chlamydomonadales: *Chlamydomonas* sp.
  - Chlorellales: *Oocystis* sp.
- Bacillariophyta:
  - Fragilariales: *Synedra* sp.

Growth of the algal biofilms in photobioreactors (PBR)

From the five treatments used (see Material and Methods section), algal growth was detected only in the treatments were culture medium was added (i.e., Biofilm T1 + medium and Biofilm T2 + medium) (Figure 1). The growth of the biofilm in the PBR for these two treatments was as follows:

- Biofilm T1 + medium

The growth of the biofilm from T1 in culture medium showed two distinct phases: first, the culture was dominated by filamentous cyanobacteria, as it occurred *in situ* in the primary wastewater treatment tank, and had a growth rate of 0.28 d^{-1}; afterwards, in a second phase, unicellular Chlorophyta (*Scenedesmus* sp. and *Chlorella* sp.) dominated the culture, with a growth rate of 0.42 d^{-1} (Figure 1).

- Biofilm T2 + medium

Filamentous Chlorophyta (*Stigeoclonium* sp.) dominated the algal community in the PBR inoculated with the biofilm from T2, as it did in the treatment tank, having a growth rate of 0.25 d^{-1} (Figure 1).

- Treatments grown with wastewater

In the treatments where water from the treatments tanks was added to the algae (Biofilm T1 + water

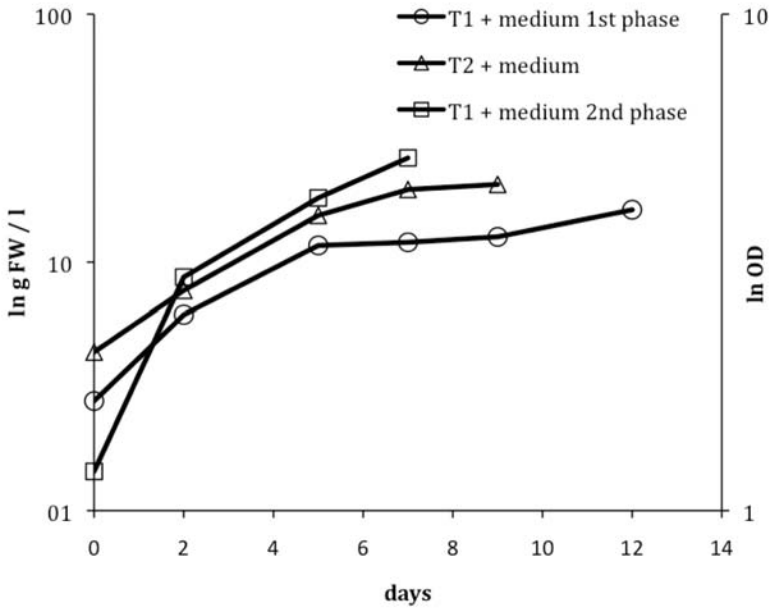

Figure 1. Growth curve for the treatments Biofilm T1 + medium (phase 1 (⊖) and phase 2 (⊟)), and for Biofilm T2 + medium (⧋). The curve of Biofilm T1 + medium phase 1 and Biofilm T2 + medium is shown in log scale for the ln g of fresh weight per litre; and for Biofilm T1 + medium phase 2 the curve is shown in log scale for the ln of the optical density.

T1, Biofilm T1 + water T2, Biofilm T2 + water T2), the culture maintained itself for about 7 days in stationary phase, and start to decrease in biomass (Figure 2). Even during these 7 days, microscopic observations showed that most of the microalgal cells had no or little chlorophyll-*a*, an indication of cell death.

The algae could not grow in these waters probably due to the lack of available nutrients. The growth of the algae in the treatments where MBA culture medium (with nutrients) was added, support the hypothesis that the lack of nutrient limited the growth of the algae grown directly with water from the treatments tanks. The fact that the algal biofilm naturally grows in the WWTP can be explained because, in that case, there is a constant flow of wastewater in the tanks, continually bringing new loads of nutrients, which can, in this case, support algal growth.

Since both resources were available for free use (both the algal biofilm and the water from the tanks) we wanted to test if we could use both to produce algal biomass, with the minimum resource input, and which could be used for biogas production. However, as shown, the growth of the algae with wastewater in PBR was not viable. Nevertheless, we still could grow the algae with artificial medium (MBA), under direct sunlight. MBA is a very cheap medium, made with commercially available fertilizer as sources of N and P, and which requires no vitamin addition, thus making the growth of these algae still economically viable.

To make the use of microalgae for biofuel production to be economically viable, it is necessary to use a low cost method of cultivation and harvesting. In this study, we were using techniques that would not increase the production cost of algal cultivation and methane production too much, aiming at a viable bioenergy alternative. For these reasons this study may be more significant than if we had used more expensive culture media, applied pre-treatments to increase the methane production, and algae that require expensive harvesting techniques, which may produce high methane yields [1], but may not be economically viable.

Methane potential of biofilm from Tank 1 and 2

Methane potential assays: the potential of the algal biofilm from the WWTP for methane production was observed when this substrate was added to marine sludge. When added to this inoculum, biofilm from T1 showed a methane production of 0.104 Nm^3 $kg^{-1}VS$, while biofilm

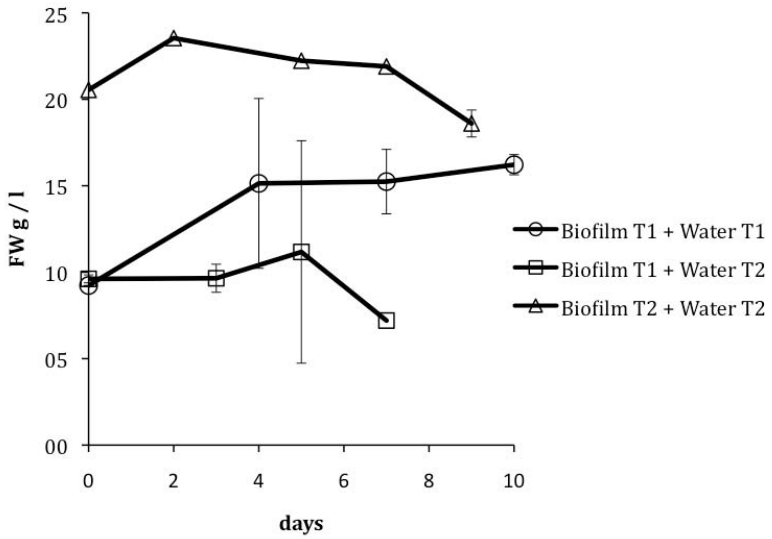

Figure 2. Growth curve (in g of fresh weight per litre) of the algae for the treatments where water from the treatment tanks were added (i.e., Biofilm T1 + water T1, Biofilm T1 + water T2, Biofilm T2 + water T2).

from T2 produced 0.076 Nm^3 kg^{-1}VS (Table 2). Although we observed production of methane when the algal biofilm was added to cow manure (Table 2, see Volume of methane produced), the production in the cow manure control was higher than the production observed when algal substrate was added to this inoculum. Thus, when correcting the value for the methane production in the control, the methane potential of the treatments with cow manure was negative. One of the bottles of the *Biofilm T2 + cow manure* treatment had a production a little higher than in the control, but in average, the methane production was negative. The fact that the treatments with biofilm + cow manure produced less methane than in the control, indicates that there should be something inhibiting the process in these treatments, since, even if no methane would have been produced by the digestion of the algal material, it should have, at least, produced the same amount of methane as in the control (by the digestion of remaining substrate present in the cow manure). We measured the pH in the digestion bottles at the beginning and at the end of the test (Table 3), since low pH may inhibit the methanogenic process. Initial pH in the cow manure treatments were around 6.1, which is close to the zone of pH inhibition to methane production. However, the fact that the initial pH in the cow manure control was also at this level, and we observed methane production in the control, added to the higher pH observed in these treatments at the end of the experiment (out of the zone of inhibition), points to an inhibiting factor other than pH. Also, there was no difference in salinity between the treatment with cow manure and its control. Probable causes of inhibition include the algal material itself. The fact that biofilm from T2 had positive methane production compared to control may indicate that the inhibition in T1 treatments was caused by the dominance of cyanobacteria in this biofilm. Cyanobacteria are known producers of potent toxins and bioactive compounds [13], which may have affected the methanogenic bacteria in cow manure, whereas marine sludge bacteria community could have been more resistant.

Nevertheless, if we observe that the anaerobic digestion of the algal biofilm both from T1 and T2 produced methane when added to the marine sludge bacteria, we can see that this material has potential to be used for methane production, as

Table 2. Volume of methane (l) produce, and methane potential (MP) for each replicate of each treatment. Column 4 shows the average MP for each treatment with their respective standard deviation (SD). The volume of methane produced is transformed in MP by subtracting the volume produced by the control and normalized by the grams of ash-free dry weight (i.e., VS) added to each bottle.

Treatment	Vol. Methane produced (Nl)	MP (Nm^3 kg^{-1} VS)	Average ± SD MP (Nm^3 kg^{-1} VS)
Biofilm T1 + cow manure	0.201	-0.071	-0.071 ± 0.00
Biofilm T1 + cow manure	0.204	-0.071	
Biofilm T2 + cow manure	0.847	0.013	-0.009 ± 0.03
Biofilm T2 + cow manure	0.665	-0.030	
Biofilm T1 + marine sludge	1.438	0.117	0.104 ± 0.02
Biofilm T1 + marine sludge	1.119	0.091	
Biofilm T2 + marine sludge	0.470	0.059	0.076 ± 0.02
Biofilm T2 + marine sludge	0.689	0.092	
Cow manure control	0.769		
Marine sludge control	0.031		
Marine sludge control	0.034		

Table 3. Initial and final pH in every test bottle and salinity.

Treatment	Initial pH	Final pH	Salinity (ppt)
Biofilm T1 + cow manure	6.03	7.18	5.86
Biofilm T1 + cow manure	6.08	7.08	5.19
Biofilm T2 + cow manure	6.21	7.06	5.59
Biofilm T2 + cow manure	6.14	7.06	5.72
Biofilm T1 + marine sludge	6.74	7.35	24.4
Biofilm T1 + marine sludge	6.93	7.26	28.1
Biofilm T2 + marine sludge	6.44	7.46	25.0
Biofilm T2 + marine sludge	6.79	7.41	26.2
Cow manure control	6.19	7.03	5.69
Marine sludge control	7.22	7.19	37.5
Marine sludge control	7.21	7.12	39.8

long as it is added to a suitable inoculum. We did not have access to inoculum directly from a biogas plant, which can be considered a strong and more stable inoculum, and which could produce a higher methane yield.

Anaerobic digestion in the treatments with marine sludge was more stable, with low methane production in the control. The methane production in these treatments was comparable to values of methane production of macroalgae, especially comparing with similar batch experiments (Table 4). Batch digestion of macroalgae produced methane in the range of 0.1 to 0.22 m^3kg^{-1} VS. Biofilm from T1 added to marine sludge produced 0.104 m^3kg^{-1}VS.

Biomass production and methane potential of algal biofilm from tank 1 and 2

The wall surface of the wastewater treatment tanks that the algal biofilm has to grow is not very large: 12.7 m^2 for T1, and 13.8 m^2 for T2. Because of the small area, the total daily and monthly production of each tank is also not very high (Table 5) (we can compare it with the average annual productivity of microalgae biomass in a tropical region which is around 1.5 kg m^{-3} d^{-1} of dry weight [14]), and, consequently, the amount of methane that could be produced using only algal biomass from the tanks (Table 5). Nevertheless we should consider that obtaining algal biomass was not the objective when the wastewater treatment tanks were constructed.

Biogas plants are often constructed coupled to wastewater treatment plants, because this is very cost effective, since wastes are used for biogas production. Coupled to these wastes, the algal biofilm that grow on the wastewater treatment plant is a possible substrate that can be exploited. If this is considered, there are several techniques that could be applied to increase the area for biofilm growth (from increase the internal walls of the tanks, to implement curtains specific for algal growth). By this way, besides increasing the algal biomass yield of the tanks (which could be used for methane production), it would also increase the removal of nutrients by the algae. In fact, this alternative is already being used in some places. In Stockholm, a consortium made by Clear Water Energy AB, Stockholms University, and the wastewater treatment plant, is testing the use of microalgae that grows in the treatment tank, and increases the nutrient removal of the wastewater, to produce biogas [15].

The biofilms growing in the WWTP of the EDAR del Sureste, in Gran Canaria, are a free naturally available source of substrate. Nowadays, this biomass is being periodically removed from the tanks and discarded. This available material could be used, if not as a sole source of substrate for biogas production, at least in combination with

Table 4. Methane yield for some macroalgae in continuous and/or batch systems.

Species	Temp. ^{o}C	System	Methane yield	Reference
Laminaria saccharina	35^{o}C	continuous	0.230 $m^3kg^{-1}VS$	[9]
Macrocystis pyrifera	35^{o}C	batch continuous	0.103 $m^3kg^{-1}VS$ 0.277 $m^3kg^{-1}VS$	[10]
Macrocystis pyrifera	n.p.	continuous	0.39-0.41 $Nm^3kg^{-1}VS$	[11]
Gracilaria tikvahiae	29-35^{o}C	batch	0.220 $m^3kg^{-1}VS$	[12]
Ulva sp.	29-35^{o}C	batch	0.220 $m^3kg^{-1}VS$	[12]

n.p. = data not provided

Table 5. Daily and monthly algal biomass production of the primary (Tank 1) and secondary (Tank 2) treatment tank (considering the area where the biofilm grows); and the amount of methane produced using the algal biomass produced during one month for each tank.

Treatment	Daily tank biomass production (g AFDW d^{-1})	Monthly tank production (g AFDW $month^{-1}$)	MP of tank (Nm^3)
Tank 1	40.13	1200	0.13
Tank 2	106.41	3180	0.25

other material. Since it implicates no production cost, a better use of this material would only be beneficial.

Microalgae have been used in wastewater treatment plants to enhance the removal of nutrients, organic contaminants, heavy metals, and pathogens from the wastewater [7, 16]. Therefore, the use of this biomass means a low cost, environmental friendly process, with the economical and environmental advantages of enhancing the removal of nutrients from wastewater, and producing sustainable biofuel.

As a next step we are attempting to isolate the algae present in both biofilms. Samples for isolation were taken at the same time the material was collected for the assays. When we have enough biomass of isolated cultures, we intent to use this biomass to estimate the percentage with which each of the algae present in the biofilm were contributing for the production of methane.

CONCLUSIONS

The biofilms that naturally grow on the walls of the wastewater treatment tanks of the EDAR del Sureste, in Gran Canaria contain a consortium of microalgae dominated by filamentous cyanobacteria (T1) and chlorophyta (T2). The methane potential of these biofilms is equivalent to those of macroalgae biomass. This material is freely available, and currently being discarded when cleaning the tank walls. Based on our results, we suggest that the use of this material as substrate for biogas plants should be considered, especially in combination with substrates of marine origins (e.g. residues from fish industry or aquaculture farms) that could be added to marine sludge. These biofilms, besides being used in WWTP as a natural agent for nutrient removal, have the potential of being used as a low cost, green source of biomass for biogas production. Furthermore, we have demonstrated that it is possible to grow the algae present in the biofilms from T1 and T2 in PBR, with low cost, and producing high biomass yields. Thus, growing the algal biofilms in PBR can be used as a complement or alternative method to produce biomass, in addition to harvesting from the WWTP tanks, for methane production.

ACKNOWLEDGEMENTS

The authors would like to thank Eva Maria Garcia Romero and the personnel from the WWTP EDAR del Sureste, Mancomunidad Intermunicipal del Sureste de Gran Canaria; Agueda Angulo Montesdeoca, for helping with the algae cultivation in PBR; Moneiba Suarez Lozano, for helping with biofilm sampling; Barbara Surek and Michael Melkonian for helping identifying the microalgae. Financial support was provided by the project "Microalgas-Bioprospección" from the Special State Funds for the Revitalization of the Economy and Employment (MINECO).

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