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Anaerobic Codigestion of Algal Material with Two Different Co-Substrates, Biowaste and Sewage Sludge: Process Yields and Behaviour Comparison

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The problem of algae disposing, especially in coastal and lagoon areas where eutrophication occurs in all its gravity, makes necessary to seek appropriate methods for the treatment of this organic material. The anaerobic digestion for the treatment of this biomass is currently of considerable interest, due the low environmental impact and the energy recovery at the same time. After a pre-screening analysis, it is possible to say that algal biomass completely meets the requirements needed for anaerobic digestion process. In this paper are reported the feasibility results of the anaerobic codigestion treatment of algal biomass. Pilot-scale continuous stirred tank reactors with working volumes of 3 and 1 m^3 were used, and full-scale digestion operational conditions were applied. Anaerobic digestion process with algae and organic fraction of municipal solid waste was inhibited by high concentration of hydrogen sulfide. This process was feasible if the algae content in the feeding mixture was equal to 10 % (in terms of solids) and with addition of iron powder; on the other hand the co-treatment of sewage sludge appeared very interesting, in fact the biogas production rate increased from 0.4 to 0.8 $m^3/m^3_{reactor}d$ and methane content reached 71 %.

1. Introduction

Nowadays algae disposing is a real problem, especially in the Venice lagoon (North-East Italy), where eutrophication is presented in all its severity. Several studies reported the feasibility of anaerobic digestion of algal biomass already in the 50s (Golueke et al., 1956). On the other hand the availability of this kind of substrates is seasonal and continuous feeding of a digester with only algae is very difficult. Considering the feasibility problems to sustain anaerobic digestion of sole algae and the integrated approach proposed on waste and wastewater treatment (Bolzonella et al., 2006), existing facilities can be used such as wastewater treatment plants with sewage sludge (SS) and organic fraction of municipal solid waste (OFMSW) anaerobic digestion. In 2010 almost 200 plants were running in Europe with these kinds of wastes (Gottardo et al. 2013). Algae can be mixed with main substrate and fed to reactors with consequently several benefits. In fact anaerobic codigestion, simultaneous digestion of two or more organic substrates, is more attractive than single-substrate process due to several advantages such as the improvement of the nutrients balance, dilution of toxic compounds and inhibitors (Mata-Alvarez et al., 2011). The aim of this study was to test different anaerobic codigestions conditions in terms of type and ratio of mixed substrates, organic loading rate (OLR) and hydraulic retention time (HRT) applied, and to determine biogas yields and solid removal efficiencies. In order to compare experimental results with those obtained by pilot-scale studies conducted using OFMSW (Pavan et al., 2000) and SS (Bolzonella et al., 2005), it seemed appropriate to test the process on a pilot plant already functioning with these substrates and located inside the Treviso wastewater treatment plant (WWTP,

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north of Italy). The experimental test with OFMSW was carried out at thermophilic temperature (55°C); this temperature, as suggested by Cecchi et al. (1991), optimizes the anaerobic digestion OFMSW and its biogas yields.

2. Materials and methods

2.1 Substrates characteristics

A mixture of two types of algae (total volatile solids were 72 % of total solids) was collected in Venice lagoon: green algae with very large leaf (*Ulva rigida*) and, in lesser extent, filamentous red algae (*Gracilaria confervoides*). The biomass collected reflected the real availability of these algae in the environment and moreover the biomass was affected by presence of sediment and salts, because they were not washed and drained. The algae were reduced to fine particles by a cutting mill before mixing with other substrate.

The OFMSW derived from street collection in Treviso city and was pretreated in a dedicated mechanical selection for metals and plastics removal and shredding of the organic material, as reported by Bolzonella et al. (2006).

Sewage sludge (SS) was collected directly in the full scale WWTP of Treviso Municipality. It was originated from a 70,000 people equivalent WWTP adopting a biological nutrients removal process (Johannesburg scheme). The daily dry flow is some 19,000 m^3 /d of municipal wastewater while the solid retention time and food to microorganism ratio applied in the activated sludge process were 15 d and 0.15 kg COD/kg MLVSS d respectively, where MLVSS was mixed liquor volatile suspended solids.

Substrates were characterized in triplicate and their average chemical characteristics are reported in Table 1. Comparing the sulphur concentration in the different substrates, the algae had significant content of sulphur probably due to presence of sediment and salt impurities, collected along with the algae. Instead the sludge had interesting concentration of nitrogen and phosphorus because of nutrients uptake during wastewater treatment.

*Table 1: Characteristics of the substrates (*Mix about 9:1 of Ulva rigida and Gracilaria confervoides): total solids (TS), total volatile solids (TVS), total Kjeldhal nitrogen (TKN), total phosphorus and sulphur.*

Substrates	TS (%)	TVS (%TS)	TKN (%TS)	P (%TS)	S (%TS)
Algae*	25.4	32.0	1.3	0.4	3.5
Biowaste	29.1	52.4	0.7	0.3	0.5
Sewage Sludge	7.4	49.0	2.4	1.3	1.2

2.2 Pilot plants and operational conditions

The anaerobic codigestion trials, with OFMSW and SS, were carried out in parallel. The operational conditions tested were chosen considering those applied in the full scale reactors treating these substrates.

The experiment regarding the anaerobic codigestion of the organic fraction of municipal solid waste and algae ground was conducted in a continuous stirred reactor (CSTR) with working-volume of 3 m^3. Pressure inside the digester was controlled at about 100-300 mm water column (w.c). The feeding was semi-continuous (twice per day) and the hydraulic retention time (HRT) adopted was 8 d. The organic loading rate (OLR) was maintained at 14 kgVS/m^3 d and various algae/OFMSW ratios were evaluated (50 %, 40 %, 10% and 0 % of total solids due to algae). The algal biomass and the biowaste were mixed with liquid recirculation and tap-water to reach correct volumetric flow. Thermophilic temperature (55 ± 1 °C) was chosen for experimentation with OFMSW.

For the codigestion study of SS and algae a conventional CSTR, with a working volume of 1 m^3 was used. The reactor was inoculated with approximately 0.5 m^3 of digestate from WWTP of Treviso. The digester was filled up using tap-water, the temperature was adjusted to 37 °C. The pressure inside the reactor was maintained around 300 mm w.c. The OLR increased from 1.7 kg VS/m^3 d (with sole sewage sludge) to 2.8 kgVS/m^3 d by addition of algae. Tested OLRs were lower than those used treating OFMSW because of low solid content of SS and of longer HRT (14 d). The feeding mixture tested was composed by 20 % (on TS basis) of algae and 80 % of SS.

2.3 Analytical methods

The process stability parameters called pH, volatile fatty acids (VFAs) content and speciation, as well as total and partial alkalinity and ammonium concentration, were checked daily in both reactors. All the analyses, except for volatile fatty acids (VFAs), were carried out in accordance with the Standard Methods (APHA–

AWWA–WEF, 2011). Volatile fatty acids content was determined using a gas chromatograph (Carlo Erba instruments) equipped with a Fused Silica Capillary Column (Supelco NUKOLTM, 15 x 0.53 x 0.5 μm film thickness) and with a flame ionization detector (200 °C), using hydrogen as gas carrier. The temperature during the analysis started from 80 °C and reaches 200 °C trough two other steps at 140 and 160 °C, with a rate of 10 °C/min. The analysed samples were centrifuged and filtrated on a 0.45 μm membrane before injection. Gas productions were monitored continuously by a gas flow meter (Ritter Company, drum-type wet-test volumetric gas meters), while the biogas compositions, in terms of CH_4, CO_2 and H_2, were determined by a gas-chromatograph (GC Agilent Technology 6890 N) equipped with HP-PLOT MOLESIEVE, 30 x 0.53 mm ID x 25 um column, using a thermal conductivity detector and argon as gas carrier. The reactors effluents were monitored 2/3 times per week in terms of total and volatile solids content (TS and TVS), chemical oxygen demand (COD), total Kjeldhal nitrogen (TKN) and total phosphorus.
Monitored parameters allowed to evaluate processes stability, verify mass balances and determined processes efficiencies.

3. Results and discussion

3.1 Codigestion with OFMSW and Algae

Initially the reactor was fed with sole OFMSW for 65 d in order to reach stationary conditions, with performances similar to literature values (Cecchi et al., 1991). The feed had a solid content of 20%. Although the high organic loading rate (14.1 kg VS/m^3 d), the process was steady and was characterized by biogas production of 0.25 m^3/kg TVS with 70 % of methane, corresponding with 29% of removed volatile solids.
After the 65th day, a part of OFMSW in the feeding was substituted by algae. The mixture consisted of algae and OFMSW in proportion of 50 % (on TS basis) and the final solid concentration of the mixture was about 200 g TS/ kg. The choice of this high percentage of algae was adopted in order to accentuate changes in yield and in digester stability parameters, related to the change of the substrate. In this way it was not only possible to check the presence of any inhibitions for microorganisms, but also to study the possibility of a quick recovery of the digester in case of process inactivation. This condition was carried out until day 90 with many critical issues. In fact after a promising start (days 65-66) the digester began to show signs of instability in the control parameters. The pH dropped down to acid value (5.2) and the percentage of CH_4 decreased under value of 35 % in the biogas. The remained part of biogas was composed mainly by CO_2 while hydrogen was not detected. These values have dropped much lower than those in optimal ranges of normal operation with sole bio-waste (Table 2). In the same periods, the alkalinity of the digestate measured at pH 6 (partial alkalinity) reduced, while at pH 4 (total alkalinity) increased. This indicated an increase of the VFAs concentration in the digester, linked to methanogenic activity reduction (Graef et al., 1974). These parameters showed a likely effect of inhibition of methanogenic archaea (Chen et al., 2008). However, overloading could be excluded because, during the previous experiments, the digester has been working at same organic load, without recording similar problems. The sudden stoppage of methanogenic activity seemed to be attributed to a large amount of sulphuric impurities in the algae substrate. In fact, a large amount of hydrogen sulphide was monitored in the gas (1.8 - 2 % H_2S) corresponding to concentration of 200 ppm in the digestate. Both the values were much higher than that normally observed in the gas produced through sole OFMSW anaerobic digestion and normally present in the digestate (about 12 ppm) (Dewil et al., 2008),
In order to verify a possible recovery of the digester and acclimation of microorganisms, the feed supply conditions described above were maintained for a complete hydraulic retention time. Determined the irreversibility of the process, which would lead in these operating conditions to acidogenesis state, some steps were taken to process recovery. For 5 d the digester was fed with sole OFMSW, with a concentration of about 10 % in TS. OFMSW was diluted exclusively with tap water, in order to speed up the lowering of the percentage of sulphur in the digestate. Solid concentration of the feed was then increased to about 20 % (on TS basis). The digester responded properly to this treatment, production yields increased step by step and stability parameters were recovered. In few days the production of biogas returned almost equal to previous trial yields (average values about 64 %-66 % CH_4 and 36 %-34 % CO_2).
In order to dilute the pollutant in the algae substrate, on day 96 a new start-up of the digester with a mixture algae/OFMSW was done using a concentration of algae equal to 10 % of the total solids present in the mixture. At the time of these new conditions applied, the digester lowered its yields both in terms of biogas production and removal percentages, reaching values comparable with those of the previous failure.
For verify if the failure of the process was due to the sulphur compounds present in the algae, by day 109, fine iron powder was mixed in the feed, in an amount equal to about 3 % of the total solids introduced with the algae. The iron reacted with H_2S, as reported in Eq(1), and consequently reduced the concentration of H_2S in the reactor.

$Fe + H_2S \rightarrow FeS + H_2$ (1)

The response of the digester to this treatment was immediate: the gas production rose up to 0.31 m^3/kg TVS_{fed} (Table 2), value comparable with that obtained with the digestion of sole OFMSW (De Beare, 2000). No problems were reported in these working conditions with regard to the stability parameters of the digester, whose values tended rather to increase (Table 2). Also the percentages of solid removal increased until normal values (near 50 % of solids).

On the basis of these observations, it was investigated if the addition of iron powder in such quantity allowed to apply higher organic loads of algal biomass in the feeding (Xiang et al., 2000). From day 126, the percentage of algae increased to approximately 40 % of the total solids fed. Looking at the graph of gas production (Figure 1) it is clear that digester yields dropped again, although a slower rate than the first failure (the latter on days 66-76). Finally on days 135 - 140 biogas production stabilized at a value of about 0.15 m^3/kg TVS_{fed}. Therefore the process in these conditions seemed feasible, pH was around 7.5 and average ammonium concentration was 1244 mgN-NH_4^+/l, but biogas productions were lower than those obtainable with sole OFMSW.

Some interesting observations can be obtained from analysis on the specific gas production compared to ammonia nitrogen concentration in the digestate. During the periods of failure of the digester such parameter increases almost three times, rising from an average value in normal conditions of about 600 mg N-NH_4^+/l to over 1,600 mg N-NH_4^+/l. The greater content of nitrogen in the algae probably increased the concentration of ammonium that could have contributed, with hydrogen sulphide, to inhibit the bacteria in the digester.

Considering that removal rate was proportional to biogas production, an improvement of performance of the digester can be seen in the last days of the trial. Even with caution, it can be said that this effect is probably in relation to an acclimatization of the bacteria to the substrate rather than the increased percentage of iron in the mixture. The determination of the precise causes of this increase and the optimal operating conditions for the digestion of mixtures of algal biomass/OFMSW can however only be achieved by programming the microbiological analysis of the substrates, or a long term project where to apply different operational conditions for sufficiently long periods so as to ensure a complete acclimatization of the bacterial flora to feeding substrate.

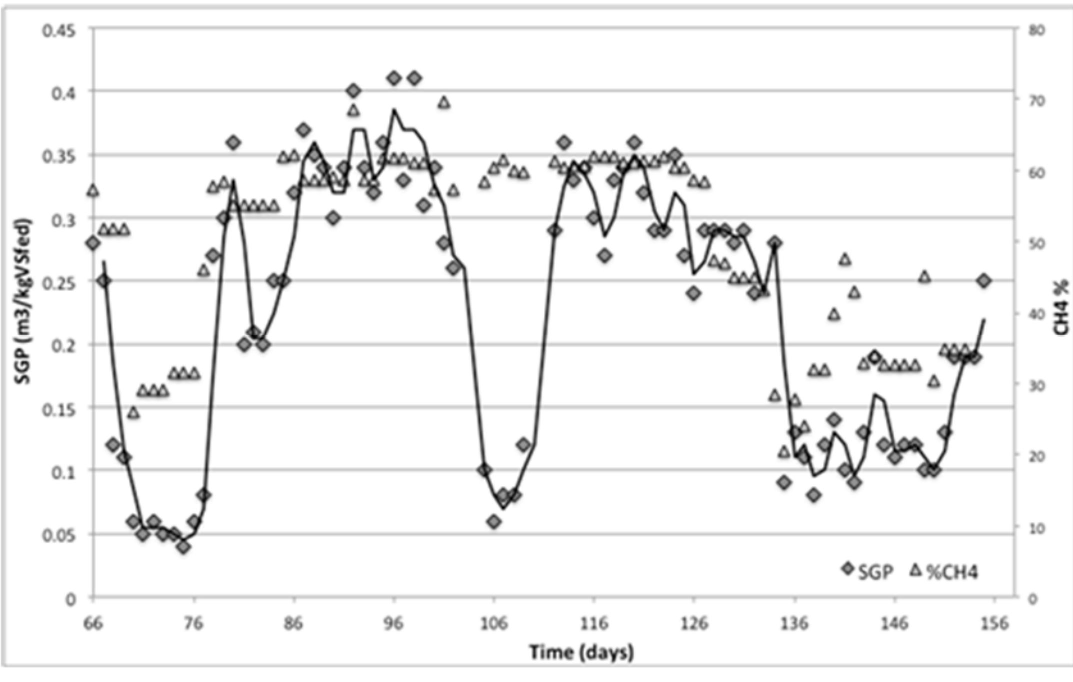

Figure 1: Specific gas production and methane percentage during the AD biowaste/algae trial

*Table 2: Yields and Parameters of biowaste and algae AD studies in stable process periods (*10 % of total solid due to algae and 3% to iron powder)*

Yields & Parameters	Biowaste (AD)	Biowaste – Algae (Co-AD)*
HRT, d	7.9 ± 1.0	7.5 ± 0.4
OLR, kgTVS/m^3 d	14.1 ±1.8	14.7 ± 1.9
GPR, $m^3/m^3_{reactor}$ d	3.5 ±0.2	4.6 ± 0.3
SGP, m^3/kg TVS $_{fed}$	0.25 ± 0.04	0.31 ±0.04
CH_4, %	69.1 ± 3.7	61.0 ± 0.9
TS digestate, g/kg	167.7 ±15.6	137.6 ± 13.6
pH digestate	6.8 ±0.2	7.6 ± 0.1
Part.Alkalinity, $gCaCO_3$/L	1.3 ± 0.5	5.0 ± 0.5
Tot.Alkalinity, $gCaCO_3$/L	9.5 ± 2.1	9.0 ± 0.7
N-NH_4^+ digestate, mg/L	323 ± 211	670 ± 70.3

3.2 Codigestion with SS and Algae

The second part of the study concerns the possibility of codigestion of mixtures of sewage sludge and algae determining the yields in different working conditions of the anaerobic digester.
Sole sewage sludge was fed to the reactor for the first 37 days. The stability parameters were in the typical range (Table 3). The examination of the stability parameters of the process showed no sign of imbalance within the microbiology of the digester. Considering the process stability and low solid content of feed (35.7 gTS/kg), the organic load could increase by co-substrate addition (algae to sewage sludge ratio of 20/80 on TS basis). Hence the algae were fed to the reactor increasing the solids content in the feed to 61.4 gTS/kg, and consequently the OLR ranged from 1.7 to 2.8 kg TVS/m^3 d. Transient period to reach fixed conditions lasted 20 d, when the OLR increased step by step and biomass acclimated to new type of feed. Stability parameters (pH, partial and total alkalinity, ammonium concentration) generally increased and didn't underline process problems. The steady state condition was reached around the 57th day and maintained for more than 2 HRTs. In this period the changes of parameters were not remarkable except for ammonium concentration (840 mgN- NH_4^+/l) due to greater nitrogen input and higher hydrolysis of algae compared with sludge one. Although the solid increased from 36 to 47 gTS/kg, the organic matter removal (in terms of volatile solids) ranged from 27 to 29 %.
The yields obtainable by the digestion of sole excess sludge and the mixture of substrates were comparable (Table 3) but the methane content ranged from 61 % to 71 % improving energetic process balance of the plant. Operational conditions change didn't created problems and biomass easily adapted to higher ammonium concentration, that didn't reach worrying level. Also hydrogen sulphide didn't accumulate in the digester. Probably because of metals presence in the sludge that created insoluble sulphide salt, thus species less available to microorganisms and consequently not toxic for methanogens.
Considering implementation in full scale plants, the algal biomass use in WWTP digester seems easier and more economical advantageous. The process should improve biogas production without increase management cost. In fact the HRT applied didn't change and existing reactors can be used.

Table 3: Yields and Parameters of algae and sewage sludge AD study

Yields & Parameters (Average)	Sewage Sludge (AD)	Sewage Sludge – Algae (Co-AD)
HRT, d	14.5 ± 1.4	14.7 ± 1.1
OLR, kgTVS/m^3 d	1.7 ± 0.4	2.8 ± 0.4
GPR, $m^3/m^3_{reactor}$ d	0.4 ± 0.1	0.8 ± 0.1
SGP, m^3/kg TVS $_{fed}$	0.25 ± 0.07	0.28 ± 0.09
CH4, %	61.5 ± 1.6	71.1 ± 3.1
TS digestate, g/kg	35.9 ± 3.3	46.7 ± 4.9
pH digestate	7.2 ± 0.1	7.5 ± 0.1
Part.Alkalinity (6), $gCaCO_3$/L	2.2 ± 0.5	3.7 ± 0.2
Tot.Alkalinity (4), $gCaCO_3$/L	3.5 ± 0.2	5.7 ± 1.3
N-NH_4^+ digestate, mg/L	418 ± 75	840 ± 79

4. Conclusions

This study investigated anaerobic codigestion of algal biomass with OFMSW and SS. Sulphides and ammonium concentration affected process with OFMSW. In order to avoid inhibition problems, algal biomass fraction should be maintained around to 10 % (in terms of solids) of feed mixture. Addition of iron powder (3 % of fed solids) reduced hydrogen sulphide toxicity by precipitation as FeS. In these operational conditions average specific biogas production was 0.28 m^3/kg TVS_{fed} with about 61% of methane.
Anaerobic process of SS and algae appeared more interesting, in fact the addition of algal biomass allowed to increase organic load from 1.7 to 2.8 kgTVS/ m^3 d without change HRT. The biogas production ranged from 0.4 to 0.8 $m^3/m^3_{reactor}$ d and sulphides did not accumulate in the reactors: sulphide reacted with sludge metals and precipitated, while ammonium concentration was below 900 mg $N\text{-}NH_4^+$/l.
Both proposed processes are feasible approaches to algal mass recovery and to renewable energy production. Application in WWTP appears more economical advantageous maintaining the same reactor volume and avoiding metals addition, as well as biogas production should increase significantly.

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