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Biovalorization of Red Radicchio (Treviso Variety) By-products by a Two-stage Anaerobic Digestion

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Italian horticulture is characterized by a large number of vegetables. Such products comprise red or variegated chicories, called “radicchio” which are cultivated mainly in northeastern Italy. The increased consumers’ demand for these products has led to an increased production which reaches approximately 250000 t/y. A major problem though is the by-products produced from their elaboration in the farm, reaching approximately 10 t/ha. Therefore the bio-valorization of these by-products may be a useful tool to give an additional value to a product otherwise deemed to be wasted.
In this work the bio-valorization of red radicchio (Treviso variety) waste was evaluated in a two-stage anaerobic digestion (AD) laboratory-scale apparatus. The tests were made also by mixing this waste with maize to check the possible bio-valorization of the mixture.
Two coupled 5 L batch-fed completely stirred reactors, one for the hydrolytic-acidogenic step and one for the acetogenic-methanogenic step, kept at mesophilic temperature, were adopted. All runs were performed without the addition of chemicals.
After the evaluation of the retention time of each stage, samples of hydrolized substrates from the first hydrolytic bioreactor were sent to the second methanogenic bioreactor to assess the specific gas production, the methane yield in the biogas produced and the specific methanogenic activity.

1. Introduction

The Italian food industry is notorious for its diversity and quality. According to the Italian National statistics institute (ISTAT), at the end of 2012 Italy had the highest number of quality certifications in Europe, such as the Protected Designation of Origin (PDO) or the Protected Geographical Status (IGP). (ISTAT, 2013) Therefore, the presence of agricultural by-products and processing wastes is statistically relevant enough to present an environmental and management challenge.
Food waste is a worldwide problem: a research carried out by the Swedish institute for Food and Biotechnology for the Food and Agriculture Organization of the United Nations, has highlighted that at least one-third of the food produced is wasted globally, which amounts to $1.3 \cdot 10^9$ t/y. (FAO, 2011)
The reduction of this waste is becoming one of the top priorities in modern food industry, while the implementation of alternative treatments of the food waste instead of landfill disposal is heavily promoted.
In Italian horticulture the production of Radicchio (a type of chicory) is one of the leading agricultural products. Among the different types of chicories, the production the Red Radicchio (Treviso variety) involves many steps, during which several amounts of by-products are discarded and left to waste.
Radicchio plants (as most vegetables) have a high content of water (over 94 %) with a high cellulosic content, and presence of minerals and vitamins. The high biodegradability and the poor organic content wouldn’t make red radicchio by-products a feasible substrate for single substrate digestion. But the mineral content and vitamins may make this substrate an ideal co-digestion target.
In this study the anaerobic digestion of Red Radicchio by-products was carried out in single stage and two stage processes, with the by-products used as a single substrate and in co-digestion.

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2. Materials and methods

2.1 Experimental set up

The bench scale plant used for the assays was the same described previously by Colussi et al. (2013) and used for the two stage anaerobic digestion of maize silage (see Figure.1).

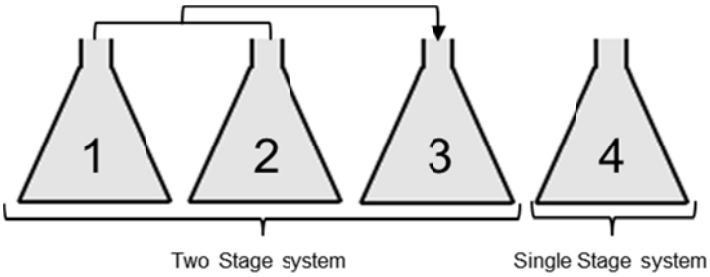

Figure 1: Experimental concept used in the present study, each first stage reactor (1 and 2) contributed to 50 % of the total organic load added to the second stage reactor (3)

Of the four available reactors three were used for the two stage digestion process (reactors 1 and 2 for the first stage and reactor 3 for the methanogenesis) and one (reactor 4) was used for the single stage digestion process. Samples taken from the two first stage reactors would be used to provide the organic load for the methanogenic reactor. Temperature, pH and pressure data were sampled by a data logger with a temporal scan of 2 min and sent to a PC.
The assays were divided into 4 stages: initially, the first stage reactors were fed with maize silage in order to increase the content of precursors needed for the second stage, then assays using maize silage were carried out to confirm the stability of the process. Next the Red Radicchio waste (RRW) was used as the sole digestion substrate, and in the last part a co-digestion with both substrates was established.

2.2 Analytical methods

Total solids (TS), total volatile solids (TVS), total suspended solids (TSS), total volatile suspended solids (TVSS) and total chemical oxygen demand (CODtot) analyses were performed as described in standard methods. (APHA, 2005)
A PerkinElmer Autosystem XL gas chromatograph, equipped with a FID detector and a HP-PLOT/U column with 30 m of length and 0.52 mm diameter, was used for the analysis of the volatile fatty acids content of the first stage reactor samples that would be fed into the second stage reactor.
The produced gas was analysed with a landfill gas analyser (GA2000 plus by Geotechnical Instruments).

2.3 Substrate and anaerobic sludge

The Maize silage was obtained from a silo after approximately 6 months of ensiling. Samples of the silage were collected for characterization, while the rest was stored in airproof bags at 4 °C. The RRW was provided by producers of the Treviso area.
The results of the characterization of the substrates are presented in Table 1.

Table 1: Substrate characterization

Sample	TS [%]	TVS [%]	COD_{tot} [g/g TS]
Maize silage	33.35	32.53	1.48
RRW	5.43	4.81	1.74

The seeding cultures were obtained from a full scale two stage industrial reactor run by Conserve Italia (Codigoro, Italy) and preserved at room temperature prior to use. The sludge used for the first stage reactors was passed through a sieve with lumen Ø of 2 mm, in order to remove all gross solids from the sludge.
After characterization (see Table 2), 2.5 L of the first stage sludge were used to seed the first stage reactors, and 2 L of methanogenic sludge to seed the second stage reactors; tap water was added to reach the final reactor volume of 4.5 L.

Start-up of the reactors was achieved with a sludge stabilization process, to ensure, in the first stage reactors, a sufficiently grade of digestion of the substrate: three consecutive loadings were performed in the course of three weeks. During this period, the methanogenic reactors were fed with the residual sieved first stage sludge, in order to maintain the activity and specialization of the microorganisms.

Table 2: Sludge characterization

Sample	TS [g/L]	TVS [g/L]	COD_{tot} [g/L]
First stage sludge	47.63	42.35	68.00
Methanogenic sludge	34.02	25.30	22.83

3. Results and Discussion

Through the assays the first stage reactors did not produce any gas. Initially this was believed to be a sign of reactor failure. Gas production was null even after increasing the organic loading rate and reducing the hydraulic retention time of the reactors, which is considered a standard procedure in achieving ideal first stage digestion conditions, as introduced by Pohland et al. (1971) and Ghosh et al. (1977).
In spite of the lack of gas production, an increase in soluble COD was observed (indicating a conversion of the solid substrate into soluble matter), which was confirmed by the gas chromatographic analysis that revealed a high content of acetic acid and traces of other volatile fatty acids.
The pH within the first stage reactors was lower than 5.0 through the assays: with a minimum of 3.3 within reactor 1 at the beginning of the stabilization stage, after which the pH maintained values around 3.9 ± 0.1, while reactor 2 went from a minimum pH of 3.9 at start-up to a stable value of 4.3 ± 0.1.
After confirmation of the acidogenic activity of the first stage reactors, a volume of effluent of these reactors was fed to the methanogenic reactor of the two stage system, which was replaced with tap water and fresh substrate. The single stage reactor was loaded with fresh substrate and tap water.
Both systems would be loaded with comparable amounts of organic matter, in order to present a reliable comparison between the two processes. The hydraulic retention time of the two stage system was 7 days for each reactor, while the hydraulic retention time of the single stage process would vary between 7 and 20 days depending on the substrate fed. A run would be considered over, when the biogas production rate would be less than 15 mL/h. At this point the reactors would be fed with more substrate.
The results obtained from the two stage digestion of maize silage were comparable with the results reported by Colussi et al. (2013) with a specific methane yield (SMY) of 0.27 m^3 CH_4/kg COD_{loaded}.
The results obtained from the single stage digestion of maize silage were in the average range of those available in literature with a SMY of 0.25 m^3 CH_4/kg COD_{loaded}. (Hermann et al. 2012)
The methane content in the two systems was very different, going from a 59.6 % of methane in the biogas produced by the two stage process and 41.4 % methane in the biogas obtained from the single stage process.
After verifying the reliability of the system the assays of Red Radicchio waste digestion were started. The first stage reactors were fed with fresh RRW and tap water: samples used to load the second stage reactor were taken after 7 days, while the reactors were being fed with fresh substrate.
Figure.2 presents the methane production curves of the two systems in the assays with RRW as substrate. The methane production of the first reactor loadings is not shown, as it was much lower than in following assays indicating that it was the transitional acclimation phase that the microorganisms need in case of a substrate change.
While the production curves of the two stage system nearly overlap each other proving a stable steady state digestion, the methane production curves of the single stage process are very distant from each other, particularly after the first day mark. This indicates the lack of achieving a stable steady state in the single stage digestion process in the time available for the assays performed in this research. A prolonged assay time with the same substrate would probably stabilize the methane production process. Nevertheless these results can be considered positive in the frame of this research, showing that the two stage digestion system reaches a stable steady state in a much shorter time, when compared to a single stage process operated under the same conditions.

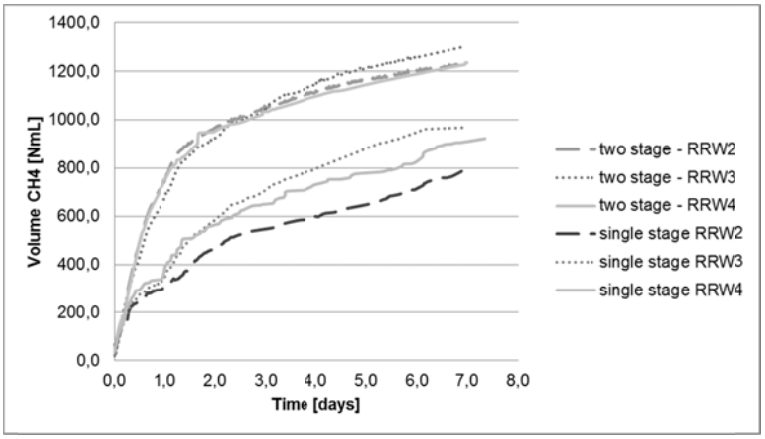

Figure 2: Methane production curves for the methanogenic reactor of the two stage digestion process and the single stage digestion process, in the assays carried out with Red Radicchio waste as substrate

Gas chromatographic analysis of the first stage reactors revealed a high content of ethanol with traces of acetic acid, a completely different result from maize silage, that had a very high content of acetic acid with almost no ethanol, thus indicating a possible specialization of the first stage reactors to the type of substrate added. The chart on the left of figure 3 is the one obtained during the assays carried out with maize silage, with a high content of acetic acid (recorded at 7.5 min) and only traces of ethanol. The chart on the right of figure 3 was obtained during the assays with RRW, note the high content of ethanol (recorded at 3.5 min), and traces of acetic acid.

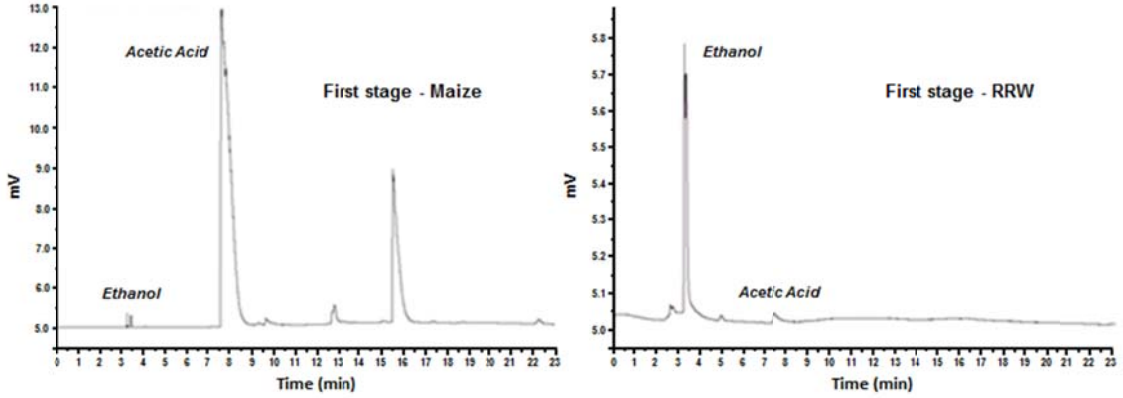

Figure 3: Gas chromatographic charts obtained from the content analysis of the first stage reactors

The SMY of the two stage system was 0.25 m^3 CH_4/kg COD_{loaded} with a CH_4 content in biogas of 56.5 %. The SMY of the single stage digestion was 0.20 m^3 CH_4/kg COD_{loaded} with a CH_4 content of 45.7 %.
After using each substrate separately in the anaerobic digestion assays, the two base substrates were combined for the co-digestion assays, with each substrate contributing to 50.0 % of the total organic load added to the reactors. The chart in Figure.4 compares the last runs obtained from the three loading stages of the assays. As can be observed, there is a marked decrease in methane production in the co-digestion assays. This effect was also observed in the single stage system, although not as noticeable as in the two stage system. These results seem to suggest a possible inhibitory effect of the two substrates on the methanogenic process.
The SMY is slightly worse than that obtained from each substrate separately, in both the single stage and the two stage digestion systems. With a value of 0.24 m^3 CH_4/kg COD_{loaded} and a methane content in the biogas of 55.7 %, the two stage system had still better performance results than the single stage digestion process whose SMY was 0.19 m^3 CH_4/kg COD_{loaded}, with 47.9 % of the biogas produced being methane.
The gas chromatographic analysis of the first stage reactors revealed the presence of both ethanol and acetic acid in significant amounts with a slightly higher content of acetic acid.

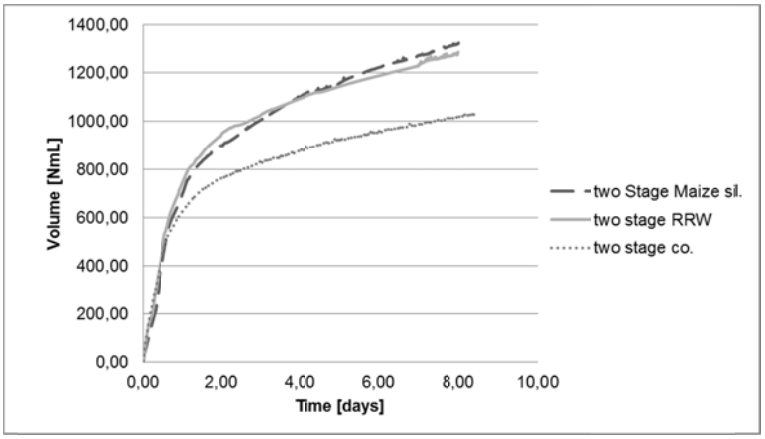

Figure 4: Methane production curves for the methanogenic reactor of the two stage digestion process when loaded with Maize silage, Red Radicchio waste and a mixture of the two substrates (co-digestion)

This detrimental effect to the methane production rate with the co-digestion of Maize silage and Red Radicchio by-products could be due to various factors: firstly there is the chance of sludge exhaustion. The co-digestion assays were carried out seven months after reactor start-up, although there were no problems with sludge activity in the assays with maize silage and RRW separately, the reactor life time could've been close to the final part of sludge activity. This seems to be confirmed by the volatile solids content of the reactors, that went from an initial content of 80 % of the total solids, to a final content of about 66 % (notably this variation happened abruptly and as soon as the co-digestion assays started).
Another possible reason could be of biochemical nature. Studies carried out by Hovey et al. in 2005 and then by Li et al. in 2007 have reported that the methanogenic Archea adapted their biochemical pathways depending on the substrate they were exposed to. These studies were carried out with acetic acid and methanol, while the assays presented in this article contained ethanol and acetic acid as precursors. In the current consensus on the biochemistry of anaerobic digestion, ethanol is a precursor to acetic acid and is not a direct precursor for methane. There is a possibility that the lack of transformation of ethanol into acetic acid in the first stage, could trigger the adaptation of populations that can carry out this metabolic step in the second stage reactor. While the adaptation was total in the digestion assays carried out solely with RRW (thus not hindering the methane production rate), it may have been only partial in the co-digestion assays, thus possibly inefficient for the achievement of a good methane production rate.

4. Conclusions

In this article, the results of the anaerobic digestion of Red Radicchio by-products as single substrate and in co-digestion with maize silage are reported. The results have highlighted a possibility of a negative effect of a co-digestion of the RRW with Maize silage, with a significant loss in methane production rate and content. There could be many reasons for this effect, sludge exhaustion being seemingly the most probable. Further studies are needed to pin point the actual cause of the reduction in methane production rates.
Running the single stage digestion assays parallel to the two stage digestion assays, allowed to verify the actual improvement of the methane production in the two stage digestion, along with experimental data about the stability and reliability of the process. The two stage digestion assays have a higher yield than the single stage system. The two stage process responded better than the single stage to substrate change thorough the assays. This confirms the buffer effect provided by the first stage reactor where adaptation to new substrate can occur quickly without hindering methanogenic activity.

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