A BIOREFINERY PLATFORM FOR BIOFUELS AND BIOPOLYMERS FROM ORGANIC WASTE

Hydrogen, Methane and PolyHydroxyAlkanoates

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1. ABSTRACT

This thesis aims a bio-refinery platform that derives from the union of different projects that have been concatenated together in order to develop an integrated approach for the treatment of wastes of organic origin.

The novelty of this thesis is the proposal of a waste treatment plant where multi-feedstock will be managed and multi-bio-products will be produced. A further developing interpretation of the anaerobic digestion processes is proposed in order to consider the waste management as a real production process. Therefore, the production should be maximized and its quality standardised. Starting from organic waste of different origin (food waste and sewage sludge), a selection of different bio-based compounds and bio-fuels will be produced.

First aim of the bio-refinery focused on the municipal organic waste pre-treatment; the application of a press systems for the separation of segregated biowaste into liquid and solid fraction. This pre-treatment implements initiatives to support and improve the quality of the biowaste treated, to enhance energy production by anaerobic digestion and reduce energy costs to manage this increasing urban waste stream. It's a new paradigm for biogas plant pre-treatment configuration.

Moreover, there is a vast interest for orienting anaerobic digestion towards biohythane (10% H_2, 60% CH_4 and 30% CO_2) or biomethane (>90% CH_4) for their potential use both for automotive sector and grid injection; also following several governments and EU directives, a number of EU funded projects are now focusing on these themes, such as GasHighWay (IEE), Valorgas (FP7-Energy), and Alt-Hy-tude and MHyBUS (Life+).

The second aim of the thesis is the production of hydrogen and methane by double-phase anaerobic digestion (fermentation coupled with methanogenesis) and the development of an automatic pilot scale process control.

By controlled fermentation volatile fatty acids can be produced and other products with a larger added-value can be also obtained using VFAs as building blocks. Bio-products with added-value are liquid biofuels, platform chemicals and biopolymers. Polyhydroxyalkanoates (PHAs) have a good potential for the market, provided that a) their present cost decreases; b) their environmental impact is further reduced. Both objectives could be achieved by using the organic fraction of municipal solid waste (post controlled fermentation process) as the PHA feedstock, since it has no cost and no competition against the food-chain. Therefore, the third aim of the thesis is the production scenario of PHAs. Controlled fermentation will be developed in order to produce organic acids. Volatile fatty

acids are of particular interest as they constitute a key group among the building-block chemicals that can be produced via fermentative pathways by mixed microbial cultures (MMC).

This research project will develop a range of new industrial bio-based processes for processing and managing the food waste and sewage sludge from wastewater treatment plants. The ambition is to obtain valuable and sustainable products, along with reducing the volume of MSW requiring ultimate disposal by landfill.

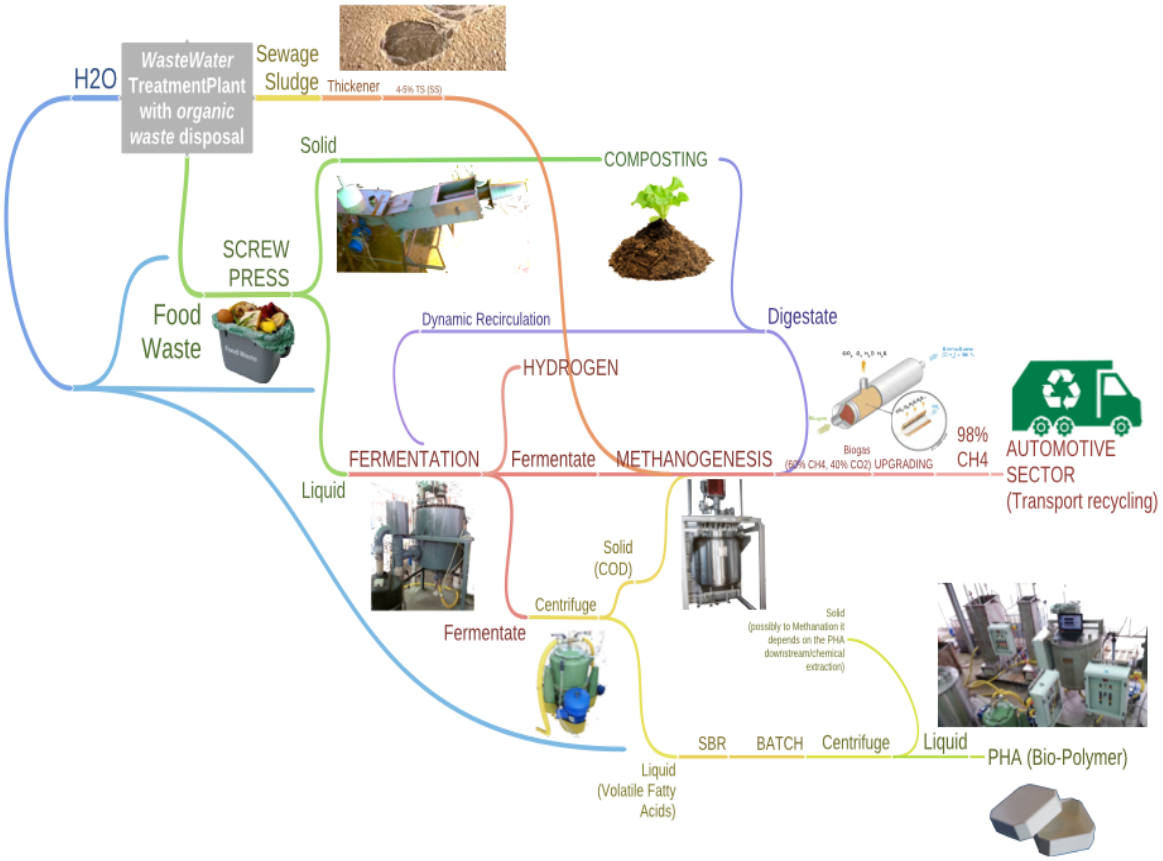
Some techniques are innovative applications to the waste sector (increased conversion into bio-fuels), as well a novelty approach is proposed (pilot-scale production of biopolymers from organic waste) and a further innovation is in the way they are combined together.

2. GENERAL INTRODUCTION

Waste production is strongly linked to concepts such as demography, urbanization and prosperity. The intense demographic and urban growth, resulting in the high increase of welfare from the second half of the twentieth century, has highlighted the importance of proper waste management, especially those of an organic nature. The production of energy and bio-products from mediums without an economic value, which is waste, is therefore a challenge we have to face.

In recent decades the international and scientific community have shown great interest in a promising renewable branch of energy sources: the biomasses. The term biomass includes organic waste and sewage sludge as points of interest of this thesis but also agricultural and industrial waste or energy crops that will not be discussed. The organic fraction of municipal solid waste in particular and the activated sludge from the treatment of civilians waters, their use and their treatment, their energy yield and their potential reuse for the production of biofuels and bio-based products will be the subject of this thesis that opens the scenario to a proposition of cycles treatment integration; a Biorefinery platform for the production of biofuels and biopolymers from organic waste.

Biorefinery process diagram:



The rapid global population growth associated to the intensive urbanization is rising every year. An inevitable expansion in waste production arises as a consequence as well as increases in the quantity of sludge from wastewater treatments and energy demand.

In wastewater treatment plants, anaerobic digesters (approximately 36,000 operate nowadays in Europe [1]) are generally oversized due to low organic matter content in the civil sewage sludge and due to the by-pass of the primary sedimentation tanks. The result is a higher energy expenditure for the slowly biodegradable organic load oxidation.

Organic waste from municipalities and sewage sludge from waste water treatment plants (WWTP) are usually separately handled or sent to anaerobic digestion process (AD), which provides

stabilization and produces biogas. This separation creates an interesting opportunity to identify processes and strategies that allow for the effective conversion of organic carbon contained in urban wastes into energy carriers and useful bio-based products, while also reducing the global impacts on water and climate caused by their treatment and disposal. For this reason, a proper and "smart" management of the waste streams must be accomplished.

The key issue is the co-treatment with other organic wastes along with stabilization and waste reduction in order to obtain benefits; energy and furthermore bio-products.

In order to integrate water and waste cycles, the organic fraction of municipal solid waste separately collected may be treated in wastewater treatment plants (WWTP) in association with the sludge produced in the water treatment mud-line. The integrated system approach in a novel “bio-waste bio-refinery” presented in this study is the key point to implement synergic treatment of bio-waste streams of urban origin.

3. SCOPE OF THE THESIS

The overall objective of the thesis is to integrate different treatments into a single facility and to use one main technology chain for the conversion of several types of urban bio-wastes into valuable bio-based products, while also optimizing energy yields and minimizing any residual or consequent waste to be disposed of. The process control automation of each stage of the productive pattern will be developed. Considering the novelty of this approach, specific objectives have been evaluated:

1) Physical, chemical and biological substrates characterisation. Biowaste is coming from a source sorted collection approach, and subsequently a pre-treatment “squeezing” phase will be evaluated.
2) The entire study has been evaluated at pilot scale, in which each step of dark fermentation, anaerobic digestion and polyhydroxyalkanoates production was tested in a scale where fluid dynamics properties and their effect can be considered and be directly used for a bright upscale.
3) Economic and statistical analysis, based on mass and energy balances has been performed by the data acquired during the long term management of the different platforms considered in this thesis.

4) Eco-biotechnology concept was regularly pondered, which aims to produce bio-products and energy by exploiting mixed culture and ecological selection principles (avoiding the use of pure cultures and sterile reactors), in this way the thesis assessed a methodology of environmental biotechnology, bringing down costs, with the goal of industrial biotechnology.

This integrated and flexible Biorefinery concept presented has several advantages.
It allows to achieve the critical operating capacity of the bio-waste Biorefinery even in small "waste basins". In order to define appropriate strategies, it is necessary to take into account that driving forces and constrains depend on the territorial conditions.

4. STATE OF THE ART

4.1 Organic Waste management in Europe

The demographic growth results in greater organic waste production which assigns a crucial importance to improve waste management in a more appropriate way.
Before addressing the specific waste management topic presented in this thesis, it is advisable to focus on the substrate: the municipal solid waste (MSW).
It represents one of the most significant solid streams to be disposal, from both quantitative and qualitative standpoints.
In the European context the definition of MSW is not univocal among all countries. In fact, it differs according to the management strategies adopted in each Member State. Eurostat [2] defines Municipal Waste, the waste produced from domestic and commercial activities, offices and public places. Waste collection is the primary responsibility and duty of municipal authorities, who should furthermore ensure its appropriate disposal in accordance with their own *waste management* program.
Total Municipal solid waste production in EU- State Members earned 252 million tons in 2011 [3] with an average per capita generation of 541 kg per year, almost 1.5 kg of MSW per day.
Almost 40% of 252-million-ton waste/year produced in EU arises as high-quality organic material (high biodegradability and low inert material content) as a consequence of an outstanding separate collection program successfully set up in the municipalities [4], and in a global perspective, the amount of wasted food is definitely expected to increase (about 44% between 2005 and 2025); MSW

Landfilling can enlarge world methane emissions from 31 million to 43 million tons. Figure 1 shows the per capita production of waste in EU.

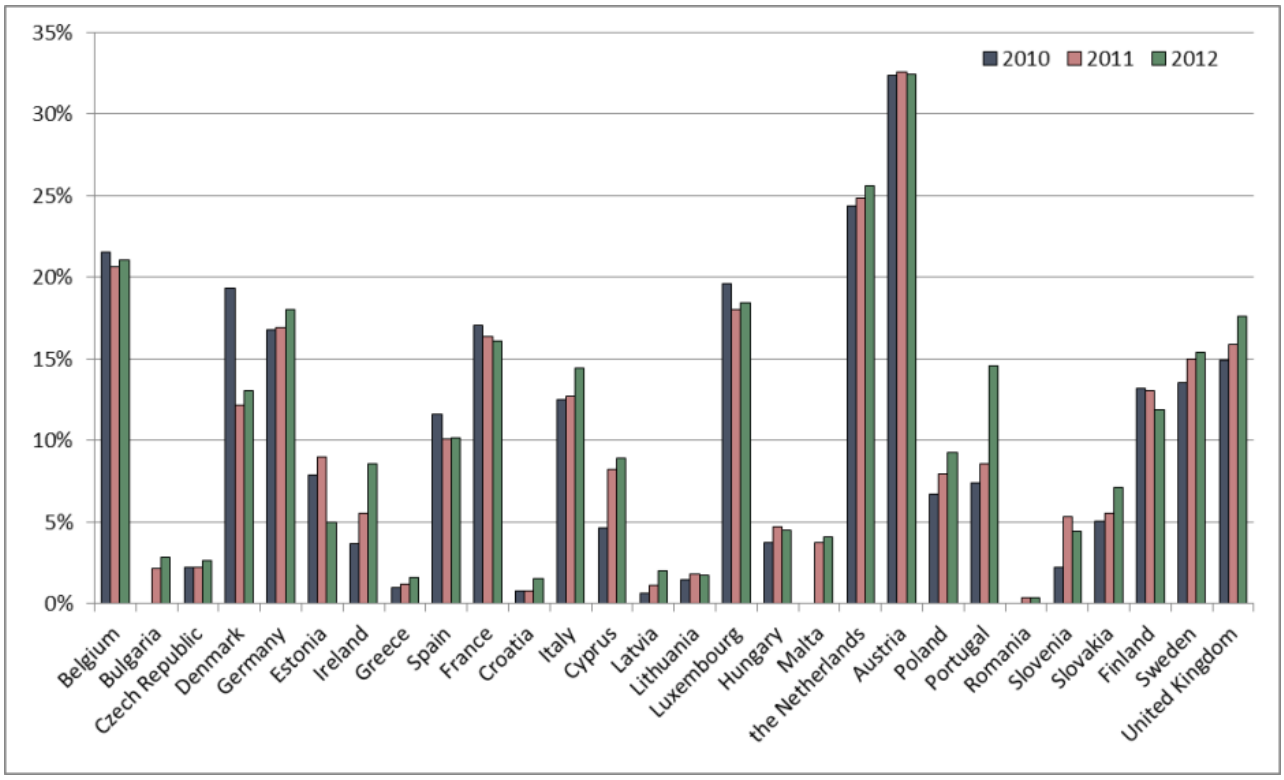


Figure 1. Per capita EU waste production

Interesting is to determine whether that reduction is related either to international economic crisis that inevitably affects consumption or to prevention and regulation of urban waste in line with EU policies.

In this concern, the yearly edition of Urban Wastes Report (ISPRA, 2014) revealed in 2014 a remarkable analysis carried out between 2007 – 2012. The relation among data from waste production and spending households' consumption was examined. It was noticed a dissociation tendency of both parameters; reduction of 0.7% on household consumption, evidently generated by the international economic crisis, in contrast to reductions over 6.5% of waste production. Concerning municipal waste management in the European context ([5], 2012), about 33% of all MSW has been landfilled, while 24% has been incinerated. In addition, 28% of this residue has been recycled whereas over 15% has been treated through biological processes, composting and anaerobic digestion.

It is essential underline such a great variability between different approaches to achieve MSW management among the different countries in EU.

Waste collection can be contemplated as the primarily pre-treatment step for a proper municipal solid waste management: different collection strategies may bring about quite different organic streams which might require different pre-treatment technologies consequently [6]. In the past, the unsorted MSW has been mechanically treated while the sorted OFMSW has been sent to biological treatments. Recently, yields obtained from both separately and source sorted OFMSW collection have proven these approaches arise the most promising way to obtain valuable substrates [7].
The organic waste, before being sent to biological treatment, either aerobic or anaerobic ones, must be pre-treated in order to ensure them adequate for these bio-processes. Several authors in scientific literature reported the collection as the first and foremost pre-treatment for the organic fraction derived from municipal solid waste. Separate collection efficiency determines the complexity of the subsequent waste processing and the main substrate characteristics influence the choice of waste treatment. The organic waste removed from the door-to-door waste collection brings more quality for biological treatments than that one derived from unsorted material. This is assigned by the existence of high organic fraction and few inert aggregates.

Separate collection of food waste from households is an efficient instrument to avoid organic material to landfill and direct it to biological treatment. Source-segregation concept is adopted by more and more municipalities in European Countries and elsewhere.
Along with physic-chemical analyses, this present "state of the art" chapter is also aimed to provide a European overview of differences and similarities in food wastes entering the respective source segregation system. Factors related to suitability of materials for AD is also discussed.
Aside process technology, stability AD problems can be linked to the composition of food waste, which might vary in different regions and with different collection schemes. Characterisation of food waste in household composition studies is not an easy task, and the data is often hardly comparable due to different classification systems [8].
Food waste entering the source segregation stream in selected regions in the UK, Finland, Portugal and Italy was analysed for its major components in the Valorgas project (2010). We compared these results with other obtained during this Ph.D. project, in Italy and Greece. Compositional analysis of different organic wastes was evaluated. Beside the fact of gaining knowledge of the nature and properties of food waste, and in particular of any major regional differences in composition that could

impact the anaerobic digestion and fermentation, it was one aim to provide information on properties and quality to complement assessment of collection schemes.

The data comparison is based on the results compiled in the report of the Deliverable "compositional analysis of food waste from study sites in geographically distinct regions of Europe" of the FP7 EU project "Valorisation of food waste to biogas" [9], Treviso (Italy) [10] and Kifissia, Athens and Tinos (Greece) data [11].

A variety of categorisation systems exists for the main components of waste streams, including organic fraction of municipal solid waste, source segregated organic waste or food waste from households.

Table 1. Comparison chart of the characterization of organic waste in five European countries.

(Fractions in % wet wight)	UK (5 cities)	Finland (1)	Italy (2)	Portugal (3)	Greece (3)
Fruit and Vegetables	60.9	44.5	69.0	59.2	61.86
Bread and Bakery	9.0	3.8	4.8	3.1	6.26
Meals	12.3	6.3	1.4	29.0	2.73
Pasta/Rice/Flour/Cereals	1.5	0.4	12.4	0.2	0.70
Meat and Fish	6.1	4.3	7.3	6.2	3.32
Dairy and Eggs	1.7	2.0	1.4	0.7	1.13
Cake/Dessert/Confectionery/Snacks	0.7	3.2	0.2	0.3	1.34
Drinks (Coffee and Tea bags)	7.1	27.5	0.3	0.2	0.52
Other Biowaste	0.5	7.8	3.0	1.0	21.80
SUM (%)	99.8	99.8	99.8	99.9	99.7

The comparison of the 5 Countries showed that "Fruit and vegetables wastes" fraction is the largest proportion, with an average 45% - 69% of the total wet weight in each case. The fraction of "Meat and fish" was similar in all countries. It is an important aspect due to the fact that this category is likely to make a major contribution to the high protein and nitrogen content of food waste, which might lead to stability problems in anaerobic digestion. The percentage of "Bread and bakery" products was similar in Finland, Portugal, Italy and Greece and only higher in the UK; differences in this category will tend to be enhanced on a wet weight basis as these products have a high capacity to absorb any liquid present or generated as the waste begins to degrade during transport and storage steps. Only the waste from Italy showed a high proportion of the category Pasta/rice/flour/cereals. "Mixed meals" and "Drinks" showed a particularly wide range, probably reflecting both national differences and aspects (e.g. tea bags in the UK, coffee in Finland) of the waste collection system.

One conclusion of the five studied European countries is the variations in the fractions “Fruit and vegetable waste”, “Drinks” and “Mixed meals” is most influential for changes in the composition of source-segregated food waste.

Table 2. Physical-chemical chart of the characterization of organic waste in five European countries.

	UK (5 cities)	Finland (1)	Italy (2)	Portugal (3)	Greece (3)	St. Dev.
pH	5.15	5.34	6.04	5.93	5.27	0.3
Total Solid (% w.w.)	25.54	27.02	25.95	31.00	22.54	0.2
Volatile Solid (% w.w.)	23.57	24.91	21.88	27.60	21.31	-
VS/TS (%)	92.29	92.19	84.32	89.03	94.54	-
TKN (gN/kgTS)	30.82	23.90	27.65	33.90	28.91	0.4
TP (gP/kgTS)	4.49	2.73	3.47	4.88	2.98	0.1
Lipids (g/kgVS)	151.40	156.00	202.00	225.00	189	-
Crude Protein (g/kgVS)	208	162	186	-	-	-

Results of the physic-chemical analyses showed a strong tendency to similarity in the samples, especially from the standpoint of key parameters in anaerobic digestion. Total and volatile solids contents were generally similar (225 – 310 g TS / kg w.w.). Total Kjeldahl nitrogen (TKN) values were similar as well (24 – 34 g N / kg TS), with a concentration indicating this substrate as a potential inhibitor of anaerobic digestion (ammonia toxicity). Concentrations of plant nutrients (Nitrogen N, Phosphorus P, Potassium K) suggested that the digestate from this feedstock has significant potential for fertiliser replacement. The elemental analysis and the measured calorific value confirmed this is an energy-rich substrate.

Despite some variation in the waste compositions, the values for key analytical parameters showed a high degree of similarity. While food preferences and cuisine may vary from region to region, the fundamental requirements of human diet and therefore of domestic food waste are likely to remain similar. This is essential if we want to asses a Biorefinery concept adaptable in every European Country.

4.2 Food Waste valorisation

In order to achieve a sustainable food waste system, the task is to establish the valorisation of not avoided food waste, along with diminution of organic waste production.

The term food waste that belongs to the broader set of the organic waste, but we could compare it with the organic fraction of household waste. It may refer to lost or wastage material which was initially destined for human consumption, or might incorporate comestible material purposely fed to animals or by-products of food processing deflect from human consumption, or might even refer to over-nutrition as the gap between consumed and needed per capita food energy [12]. Food waste represents the all losses and inedible by-products in the food supply chain (FSC) and is generated at all stages of the food supply chain (e.g. pre-consumer waste, earlier stages of the FSC and post-consumer waste).

At all stages, not all food losses would potentially be avoidable. Food is a biological material susceptible to degradation and which requires processing before it can be consumed, therefore part of the food that can not be considered as such is necessarily unusable and therefore inevitable [12].

In industrialized countries, substantial food losses occur as post-consumer waste, mainly in households after purchase, but retail, distribution and processing remain accountable for substantial quantities of wastage [13]. There are losses along the whole FSC although post-consumer waste is the most visible part of all waste. Through literature data indicated wastage in the range of a quarter to a third of all food grown [12] [14].

Food wastage represents a loss of embedded energy and other resources such as water and fertilizer. It is therefore evident, that avoidance is the most important step when looking at food waste. It has been assessed that by more efficient utilization of already available systems and technologies around half of the worldwide food losses could be avoided [14]. Valorisation of not avoided food waste contributes to improved overall energy balance of food systems and holds potential to be highly beneficial towards protection of productivity of agricultural soils. Valorisation of not avoided food waste should be given high priority [15] [16]. Efficient waste management among others is an element that can unlock food-bioenergy synergies [16]. Transition toward sustainability will require changing to systemic perspectives, the means to reduce food waste would be the most efficient food waste valorisation strategy. But not all food waste is potentially avoidable and it is not realistic that all potentially avoidable waste will actually be prevented. It is therefore essential to establish reliable and efficient valorisation pathways for generated wastes.

Despite the variations or non essential variations of the material, food waste is typically to be classified as highly biodegradable biomass with generally high water content. It is hence appropriate for biogas production through anaerobic digestion (AD), provided that favourable process conditions are enabled. The biogas is a resourceful energy carrier, while digestate (effluent of the reactor

containing liquefied or solid material) is a valuable fertilizer and soil amendment when spread to agricultural land [17].

Food waste digestion is a well known process, and experiences are available both from laboratory research and from full-scale practical implementation. However, successful operation of food waste AD involves specific knowledge and key factors controlling to be considered. Alternative food waste valorisation strategies (e.g. bio-hydrogen production, bio-polymers production) are subject to on-going research and development, but at present AD represents the only established state-of-the art technology well beyond pilot stage.

Digestion of food waste is vulnerable to incidence of both high concentrations of volatile fatty acids (VFAs) and of ammonia [18] [19]. Thermophilic process increases the risk of digester failure due to VFA and / or ammonia inhibition [18]. Ammonia remains a critical issue in AD [20] [21]. Knowing these potential issues the present thesis aimed to develop pilot-scale processes mainly following the thermophilic anaerobic regime (and moreover mesophilic comparison) with the purpose of maintaining stability and furthermore automating the process so as to increase and optimize the production of energy and bio-products.

Avoidance of food wastage needs to be put on top of the agendas in order to advance progress towards more sustainable food systems.

There is full consensus that not avoided food waste is a potential source for bioenergy generation. Anaerobic digestion with biogas production is an already well-known and in practice adopted technology for valorisation of food waste. This thesis will develop the concept that domestic organic waste of MSW is the substrate for a new production chain not only linked to energy production.

5. A NEW BIOREFINERY APPROACH

It is necessary to extend and to improve available options for resource recovery from organic fraction of MSW, especially towards higher value products than energy and compost. Another AD technique is considered; the use of a fermenter reactor. The two-phase anaerobic digestion approach physically divides the biochemical step namely hydrolysis-acidogenesis in the first phase (dark fermentation) and the second step acetogenesis-methanogenesis process in the second phase. The phases separation gives the optimal growth rates and pH requirements for acidogenic (between 5.5 and 6.5) and methanogenic microorganisms (around pH 7), and thus different requirements regarding reactor process conditions are needed [22].

The first fermentation phase in fact constitutes a valuable external carbon source (soluble COD, volatile fatty acids) that can be used as basic units to further PHA synthesis. Moreover, through fermentation hydrogen can be produced. The application of anaerobic co-digestion allows to obtain two energy carriers for the automotive sector, biomethane and biohythane.
Hydrogen mixed with biogas, both produced via renewable energy resources, can be considered as an alternative fuel to traditional fossil fuels. At present, a feasible scenario is the Hydrogen Fuel Injection (HFI). With HFI is meant to mix a gaseous fuel with hydrogen to obtain a mixture with improved combustion characteristics.
Full-scale biohythane process implementation is currently under evaluation.
In sum, the co-digestion SS-OFMSW once considered an integrated approach between the water treatment and waste cycles, supports harness the benefits from a well known anaerobic digestion process.
Proceeding the separated phase anaerobic digestion process can make feasible the possibility to produce bio-hydrogen, bio-methane and volatile fatty acids.
The volatile fatty acids are the building blocks for the construction of additional products, the bio-polymers.

Plastics have many societal benefits, but it also gives rise to certain environmental problems. Durability and a massive use connected to inappropriate waste management is a high potential risk that leads accumulation of this material in Nature and landfills [23].
Poly-hydroxy-alkanoates (PHA) is a biodegradable renewable biopolymer, which is produced naturally in several different groups of bacteria. During natural biosynthesis monomeric units of PHA are produced and polymerized by ester linkage. Then the polymers aggregate by accumulation into cytoplasmic inclusions bounded by monolayer envelopes.
These inclusions are often referred to as granules [24] and function as intracellular energy and carbon reserves in stages of starvation and can account for 80% of the total dry weight of microbial biomass [25]. Because the PHA is naturally polymerized during biosynthesis it can be extracted directly in its polymerized form.

PHA is already known as a fully biodegradable and commercially available bio-plastic [26]. It has similar properties to the synthetic polymers produced in the petrol chemical industry such as polypropylene (PP). Although biological production of PHA can be used to produce substitute

polymer similar to those produced in a petrochemical industry. There is still a higher production cost accompanying the production of PHA. This economical bottleneck is an obstacle that has to be taken into consideration before investigating commercial production of PHAs as a feasible substitution of petrochemical production [27]. It is commonly recognized that the high production cost associated with PHA production is due to around 50% of the production cost is directly related to the expensive carbon source [28]. However several other aspects such as material (chemicals, production strain etc.) and also the culture conditions and fermentation types (batch, fed batch etc.) can add to the high production cost. One obvious way to decrease the PHA production cost could be to find und utilize a cheap renewable and readily available carbon source in the production of PHA polymers instead of using refined organic substrates.

One carbon source that is considered a viable substitute in PHA production is bio-waste (food waste – OFMSW). The attractive solution could be convert the bio waste by microbial fermentation to value added products in the form of organic acids, solvents or biopolymers.

Presently several biotechnological processes are utilizing single strain cultures, which require well defined substrate and sterile process conditions [29], this attributes to a higher production cost. These factors impose a financial burden on the industrial production and makes single strain cultures unfavourable for large scale production of PHA [30] [31].

A more financially attractive method for the PHA production is to implement eco-biotechnology. Eco-biotechnology aims to produce products (PHA) by exploiting mixed culture and ecological selection principles, in this way it links the methodology of environmental biotechnology with the goal of industrial biotechnology. The principle of eco-biotechnology is based on the biological selection and competition instead of genetic or metabolic engineering [32].

Due to the diversity of microorganism in mixed cultures they can deal with a range of substrates and of variable compositions (e.g. the heterogeneity of bio-waste). The conditions in these systems are designed so the metabolic conversion of interest insures an ecological advantage for the microorganisms and determine which catabolic product allows the most efficient growth and thereby dominate the community of the mixed culture [30] [33].

Biowaste is first converted into organic acids (mainly butyric acid, acetic acid, propionic acid and valeric acid), hydrogen gas, carbon dioxide and cells.

Secondly the organic acids are used as substrate and consumed, during nitrogen restriction leading to accumulation of poly-hydroxy-alkanoates (PHA) inside the cells. Production of the PHA usually takes place in a batch or fed batch reactor [34].

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6. CHAPTER 1

Pre-Treatment

Research Paper

Mesophilic and thermophilic anaerobic digestion of the liquid fraction of pressed biowaste for high energy yields recovery

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Abstract

Deep separate collection of the organic fraction of municipal solid waste generates streams with relatively low content of inert material and high biodegradability. This material can be conveniently treated to recovery both energy and material by means of simplified technologies like screw-press and extruder: in this study, the liquid fraction generated from pressed biowaste from kerbside and door-to-door collection was anaerobically digested in both mesophilic and thermophilic conditions while for the solid fraction composting is suggested. Continuous operation results obtained both in mesophilic and thermophilic conditions indicated that the anaerobic digestion of pressed biowaste was viable at all operating conditions tested, with the greatest specific gas production of 0.92 $m^3/kgVS_{fed}$ at an organic loading rate of 4.7 $kgVS/m^3d$ in thermophilic conditions. Based on calculations the authors found that the expected energy recovery is highly positive.

The contents of heavy metals and pathogens of fed substrate and effluent digestates were analyzed, and results showed low levels (below End-of-Waste 2014 criteria limits) for both the parameters thus indicating the good quality of digestate and its possible use for agronomic purposes. Therefore, both energy and material were effectively recovered.

Key words

Anaerobic digestion, mesophilic, thermophilic, biogas, pressed biowaste, transient conditions

Abbreviations

AD: Anaerobic Digestion, ALK tot: Total Alkalinity, COD: Chemical Oxygen Demand, CSTR: Continuous Stirred Tank Reactor, DM: Dry Matter, DW: Dry Weight, GP: Gas Production, GPR: Gas Production Rate, HRT: Hydraulic Retention Time, MSW: Municipal Solid Waste, OFMSW: Organic Fraction of Municipal Solid Waste, OLR: Organic Loading Rate, Ptot: Total Phosphorus, SGP: Specific Gas Production, SMP: Specific Methane Production, SSC: Steady State Condition, TKN: Total Kjeldahl Nitrogen, TS: Total Solids, TVS: Total Volatile Solids, CFU: Colony Forming Unit, VFAs: Volatile Fatty Acids, VS: Volatile Solids, WW: Wet Weight

1. Introduction

Anaerobic digestion is a proven and widespread technology for the management of organic waste of different origin (Mattheeuws and De Baere, 2014). There are currently more than 14,000 plants running in Europe, 28% of which are dedicated to the treatment of wastewater sludge, municipal and industrial organic waste, while the remaining 72% use agro-waste as feedstock (EBA, 2014).

With specific reference to municipal waste management, the success of this technology in recent years has been determined by the implementation of deep separate collection schemes: this determined the possibility of handling streams with a reduced amount of inert material and high moister content and biodegradability like segregated food waste (Valorgas 2010; Bernstad et al., 2013).

Beside anaerobic digestion, the other biological process widely diffused within EU for the management of organic wastes is the aerobic stabilization, or composting: at present a compost production of around 10.5 million tonnes of organic waste is reported (COM(2008), 811 http://eur-lex.europa.eu).

Noticeably, the two processes, i.e., anaerobic digestion and composting, can be integrated together since the solid fraction of digestate can be treated aerobically (Nayono et al., 2009) so to recovery both renewable energy and nutrients from organic waste. At present some 8 million tons of biowaste are anaerobically digested within EU Countries and normally the biowaste is pre-treated and prepared for the AD process by means of several mechanical steps. A large number of plants treating organic waste started their operations in the 1980s, when both the amount and the composition of biowaste were quite different from the present situation. This has resulted in the need for some modifications both in plant management and operating conditions (Di Maria et al., 2012). In fact literature showed

that during conventional pre-treatment methods around 30% of the initial wet material could be rejected without any treatment (Pognani et al., 2012). The pre-treatment of the organic fraction of municipal solid waste is in fact one of the main challenges in mechanical–biological treatment plants equipped with anaerobic digesters (Romero-Güiza et al., 2014).

Recent literature highlights the observations related to the loss of biodegradable organic matter during the pre-treatment steps (Müller et al., 1998; Bolzonella et al., 2006; Ponsá et al., 2010b). Moreover, these steps are time and energy consuming (Tonini et al., 2014) and generally are not able to achieve high removal yields for inert materials like small pieces of plastics and fine heavy materials like crashed glass, sea shells and sand. These materials could then accumulate inside the reactor determining a reduction of the reaction volume and a possible risk of process failure (Angelidaki and Boe, 2010).

Another important aspect to be considered is then the reduction of the energy demand for pre-treatment processes and if possible enhance the biogas production of the anaerobic digestion plants that treat the municipal biowaste (Morais et al., 2007).

In order to address all these issues an interesting option is the use of very simple pre-treatment steps like presses and extruders: in these machines the size of the organic material is reduced while inert material (mainly plastic) is eliminated.

Biowaste pressing produces two streams: one semi-liquid to be digested and a second one solid to be composted (Hansen et al., 2007). Nowadays another advanced energy saving pre-treatment approach has been developed: biowaste squeezing. This is a mechanical pre-treatment process. The advantages of mechanical pretreatment include an easy implementation, better dewaterability of the final anaerobic residue and a moderate energy consumption (Ariunbaatar et al., 2014). Pretreatment and digester design are the key techniques for enhanced biogas optimization (Shah et al., 2014).

Only few examples of such approach can be found in literature at the best of our knowledge.

Satoto Nayono et al. (2009) studied AD of pressed off leachate from OFMSW and the co-digestion of press water and food waste (Satoto Nayono et al. 2010) for improvement of biogas production. The co-digestion of press water and food residues with defibred kitchen wastes (food waste), operated at an OLR in the range 14-21 kgCOD/m^3d, reported greater biogas production rates then sole biowaste. An increment of the OLR of biowaste by 10.6% with press liquid fraction increased the biogas production as much as 18%, with a biogas production rate of 4.2 m^3/m^3d at an OLR of 13.6 kgCOD/m^3d. These experimentations were conducted through laboratory scale reactors, from 1 to 8 liters working volume.

According to the scenario reported above and the evidences of recent studies, this study was dedicated to the anaerobic digestion of the liquid fraction of pressed biowaste at pilot scale so to identify bottlenecks, consumes and yields of interest for a possible process scale-up. The use of a screw press allows for the production of two streams, one liquid, clean and very biodegradable, easy to convert into biogas (thus energy), and another one semi-solid with a level of biodegradability and water content and C/N ratio suitable for composting.
Clearly, the liquid stream, because of its characteristics, is particularly suitable also for co-digestion with sludge in wastewater treatment plants.

In this study particular attention was paid to the definition of the optimal operating conditions and yields for the anaerobic reactor.
Beside this the digestate characteristics were considered in detail also in order to respond to the requirements defined in the "End of Waste Criteria" technical proposal by the Joint Research Center of Sevilla (2014). Based on suggested criteria, pathogens and metals in the digestates were analyzed in order to evaluate the necessity of further anaerobic digestate treatment, for example in a co-composting process.

2. Materials and Methods

2.1 Pretreatment strategy and experimental set up description

A pilot-scale press, specifically designed for this experimentation, was used in order to pre-treat separately collected biowaste and split it into two streams, one liquid to be anaerobically digested and a second one solid to be composted.
Door-to-door collected biowaste from Treviso area (Italy) was first shredded into a knife mill and treated in a press for solid-liquid separation. Only the liquid fraction was then sent to the anaerobic process while the semi-solid part, characterized by a higher content of dry matter, was suitable for aerobic stabilization process.
The semi-liquid stream was then sent to two pilot scale CSTR anaerobic digesters, one mesophilic (37°C ± 0.1) and the other thermophilic (55°C ± 0.1), working with an organic loading rate in the range 3 - 6 kgVS/m^3 per day and a hydraulic retention time of 20 days to simulate the best operating conditions expected for a full-scale treatment plant. Organic matter degradation at increasing OLR (and decreasing HRT) was investigated. The research was carried out using two pilot scale reactors

completely equal in terms of electro-mechanics, working volume (0.23 m^3) and heating system. The reactors were made of stainless steel AISI-304 and the mixing was ensured by mechanical anchor-bars agitators in order to maximize the mixing degree inside the reactor, thus avoiding the typical stratification of floating materials on the top and of sinking heaviest materials on the bottom of the reactor. The temperature of 37 °C (mesophilic thermal range) and 55 °C (thermophilic thermal range) of the reactors was maintained constant by an external jacket; in which heated water was recirculated. The biogas produced was sent to a hydraulic guard with the purpose of maintaining an operating pressure of 0.1 m water column inside the reactor. Reactors were fed once a day.

2.2 Analytical methods

Biowaste commodity class was analyzed in accordance with the procedure reported by MODECOM™ (1998). The reactor effluents were monitored 3 times per week in terms of TS, TVS, COD, TKN and P total. For TS determination, 105 °C drying temperature was adopted and no losses were caused (Peces et al., 2014). The process stability parameters, namely pH, volatile fatty acids (VFAs) content and distribution, conductivity, total and partial alkalinity and ammonium (NH_4^+–N), were checked daily. All the analyses performed according to the Standard Methods for Water and Wastewater Analysis (1998). The analysis of the volatile fatty acids was carried out with a Carlo Erba™ gas chromatograph equipped with a flame ionization detector (T = 200 °C), a fused silica capillary column Supelco NUKOL™ (15 m x 0.53 mm x 0.5 μm thickness of the film), while hydrogen was used as carrier gas. The analysis was conducted using a temperature ramp from 80 °C to 200 °C (10 °C / min). The samples were analyzed before being centrifuged and filtered with a 0.45 μm filter. Biogas production was monitored by a flow meter (Ritter Company™), while methane, carbon dioxide and oxygen in biogas were determined through a portable infrared gas analyzer GA2000™ (Geotechnical Instruments™) continuously and a Gas Cromatograph 6890N, Agilent Technology™, once a day.

The content of heavy metals and pathogens of fed substrate and digestates, in the two experimentations (mesophilic and thermophilic), were analyzed (EPA 3051A 2007 + EPA 6020A 2007).

3. Results and discussion

3.1 Biowaste pretreatment and composition

The biowaste collected in Treviso area and used in this experimentation showed the composition reported in Table 1: fruit and vegetable waste were typically half of the waste material, a result in line with previous studies on this topic (see Valorgas D2.1 http://www.valorgas.soton.ac.uk/deliverables.htm) while pasta/bread and meat/seafood represented another 25% of the wasted food. Some 10% of the material was un-classified (melt material).

Table 1. Components of biowaste

Composition	WW, %	DW, %
Fruits & vegetables	46-58	38-42
Other kitchen waste *	16-25	15-22
Paper & cardboard	9-14	7-12
Not classifiable	8-14	6-12

* Putrescible material non-vegetable (eg pasta, cakes, meat, etc..). WW = wet weight. DW = dry weight

Biowaste compositional analysis (of five samples) showed that food waste was more than 82% of the total (on wet weight) while the remaining parts were paper (11%) and inert materials (7%) like glass and metals or textiles. The different fractions for each type of material in terms of total and volatile solids are presented in Table 2.

Table 2. Characteristics of the different parts of biowaste

Fractions	TS	VS	VS/TS
	g/kg ww	g/kg ww	%
Kitchen waste	371	346	93
Fruits & vegetables	236	215	91
Paper	391	365	93
Plastic	431	399	92
Inert	640	229	35
Not classifiable	371	288	77

Table 3. Characteristics of biowaste, and pressed liquid and solid fractions

Substrate	TS g/kg biowaste	VS g/kg biowaste	TVS/TS %	COD g/kg TS	TKN g/kg TS	P g/kg TS
Biowaste	298±44.2	267±32.5	89.8±3	1,090±449	27±4	4.0±0.2
Liquid $_{bw}$	186±49.3	169±24.0	91.0±2	1,189±357	24±4	4.2±0.3
Solid $_{bw}$	378±34.3	343±18.9	89.6±1	764±498	23±6	3.9±0.4

As for the general chemico-physical characteristics, biowaste showed an average dry matter content of 298 gTS/kg, 90% volatile solids. The COD values were typically greater than 1,090 gCOD/kgTS with a low nitrogen content.

The liquid phase obtained was particularly suitable for AD because of its total and volatile solids (91% of dry matter on average) with a very high COD content, most of it being soluble, and COD/N ratio of 49.

3.2 Performance of the pilot scale reactors

The start-up phase of both mesophilic and thermophilic reactors was characterized by a gradual increase of the organic loading rate (OLR) starting from 1 kgVS/m^{3}d onwards with a fixed hydraulic retention time of 20 days; when a steady state condition was achieved the OLR applied was ranging between 3.0 and 3.5 kgVS/m^{3}d.

In order to verify the process resilience also transient conditions were tested: transient conditions were obtained testing the system at different organic loading rates. In particular, the OLR was tripled (from 2 to 6 kgVS/m^{3}d) on alternate days and stopping the feed once a week. Every day pH, ammonium, alkalinity (partial and total) and VFAs in the effluent as well as biogas production and its composition were analyzed before the addition of fresh substrate.

Table 4. Experimental runs and organic loads applied

	RUN I – Start up	**RUN II – SSC**	**RUN III – Transient**
OLR (kgVS/m^3_rd) Average Value (M)	1	3.5	3 – 6
RUNs time (d) MESOPHILIC	40	40	60 started from (day 110)
OLR (kgVS/m^3_rd) Average Value (T)	1	3.5	3 – 6
RUNs time (d)	80	40	60

THERMOPHILIC			
HRTs (d)	20	20	18-20
Meso and Thermo			

Reported values show how the two systems needed different time in order to conclude the start up phase and reach a steady state condition. The duration and the OLR fluctuations applied during the transient period was the same in both reactors, in order to have the best comparison of the results of the stressing tests.

3.2.1 Performances of the mesophilic anaerobic digestion process

The inoculum used for the mesophilic trials was anaerobically digested sludge originated from a full-scale anaerobic digestion process (Treviso Municipal Wastewater Treatment Plant). The pilot scale reactor was maintained at the operating temperature of 37 °C with low loading rate (1 kgVS/m^3d) for a week, in order to acclimatize the biomass to the liquid pressed organic stream. Initially the reactor was fed with an OLR of 4.19 kgCOD/m^3d.

Once the methanogenic biomass was active and responding appropriately in terms of biogas quality, the reactor was fed daily, and the OLR was increased stepwise from 1 kgVS/m^3d to 3.5 kgVS/m^3d in 2 HRTs (RUN I, start up, from day 1 to day 40).

In steady state conditions the pH of the mesophilic AD was in the range 7.1 - 7.7 favoring the metabolic activities of fermentative bacteria and the growth of methanogens. The pH drop observed during the start up period (days 1-7) was related to the high VFA concentration due to the sharp increase of acetate and propionate acids. However, the overall anaerobic process and methane production is not inhibited presumably because of the balanced presence of both acids (Zhang et al., 2014). After day 7 concentrations of volatile fatty acids decreased indicating the adaptation of the anaerobic biomass to the new environmental conditions which signals the initiation of steady state phase (Gallert and Winter, 2005) during which VFA concentration remained at an average value of 912 mgVFA/L (predominantly acetic acid). The system didn't show any upset to its stability, thus indicating good robustness of the process also in transient conditions, a relevant aspect for the full-scale implementation of the process. As for pH, this remained constant, particularly in steady state conditions, with an average value around 7.7 because of the high buffer capacity of the system: this is highlighted by an average total alkalinity value of 5,177 mg$CaCO_3$/L (Figure 1). The ratio between VFA concentration and alkalinity was also evaluated. Soluble COD (sCOD) showed an average value

of 2,294 mgCOD/L. In steady state conditions the concentrations of the partial alkalinity ranged between 2000 to 4500 mg$CaCO_3$/L (determined at pH 5.75), while the total alkalinity was greater and in the range 4500 to 6000 mg$CaCO_3$/L (determined at pH 4). These figures ensured sufficient buffer capacity: the ratio of VFA to total alkalinity (i.e. the difference between total and partial alkalinity compared to total alkalinity) was constantly below 0.3 indicating the stability of the AD processes in terms of volatile fatty acid accumulation (Ripley et al., 1986). The concentration of volatile fatty acids and alkalinity are the two parameters that show a more rapid variation when the system tends to be upset (Ahring et al., 1995; Bolzonella et al., 2003) since in case of organic overload, the concentration of fatty acids increases while the alkalinity tends to decrease. The relationship between these two parameters (Cecchi et al., 2005; Bolzonella et al., 2003) is a useful stability indicator to be considered: ratio values around 0.3 indicate a stable operation of the AD process, while greater values may indicate the inception of instability. During organic loading increase the ratio was around 0.22, thus the system achieved a perfect steady state even with the transient increasing of the organic loading rate.

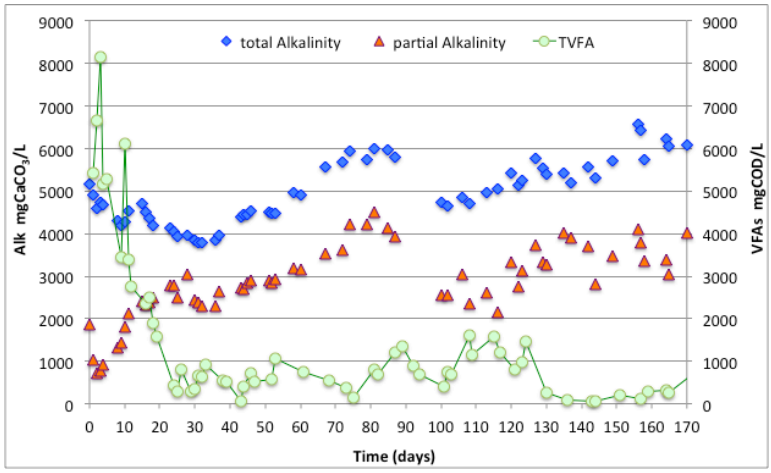

Figure 1. Alkalinity and VFAs trend during the mesophilic trial.

The use of a real substrate and the heterogeneity of biowaste, determined inevitable variations in the solid content in the reactor: the observed standard deviation for this parameter was 186 ± 49.3 gTS/kg). Alkalinity and ammonium concentrations increased slightly but constantly along the experimentation. This behavior is related to the nature of substrate (Cecchi et al., 1993): in fact, the high degradation of pressed biowaste leads to a quick release of ammonium in liquid phase due to proteins deterioration, as reported also in other studies (Converti et al., 1999; Cavinato et al., 2012).

The monitoring of the total ammonium showed an average value of 878 mgN-NH_4^+/L (St.Dev. ± 79), 64 mgN-NH_3/L free ammonia, well below the typical critical level for inhibition (Chen et al., 2008). The average content of total solids in the reactor remained almost constant with an average value of 22.5 gTS/kg (St.Dev. ± 1.9) and an average volatile solids content of 16.1 gVS/kg (St.Dev. ± 0.7). The ratio between total and volatile solids shows an average value of 71.5% (TVS/TS), it is thus highlighted the capability of the system of converting the organic matter into biogas, leaving a residual dry matter content lower than 3% in digestate.

The biogas composition in terms of average percentage of methane detected in steady state condition (SSC) was 66% CH_4 and the remaining part CO_2. The average specific gas production (SGP) was found equal to 0.79 m^3biogas/kgVS and the average specific methane production (SMP) was 0.47 m^3CH_4/kgVS, while the average gas production rate (GPR) was 2.3 m^3biogas/m^3_rd. Profiles of specific methane production (SMP) during the experimental trials are shown in Figure 2.

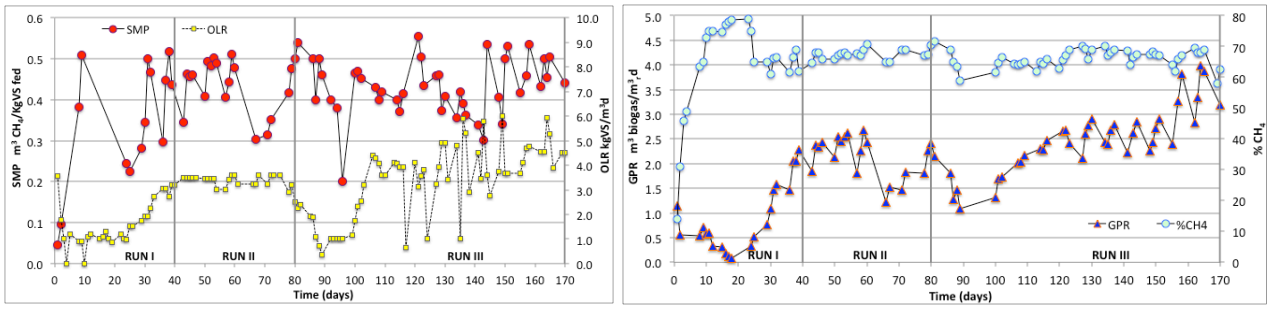

Figure 2. Specific methane production and OLR variations in mesophilic anaerobic digestion and methane percentage compared to the Gas Production Rate

Overall the mesophilic digestion process of the semi-liquid fraction of biowaste in steady state conditions showed great strength and resilience with reference to the process parameters (pH, alkalinity and VFA concentration, and biogas composition). Based on the aforementioned figures for each ton of biowaste semi liquid fraction, the biogas and methane production in AD mesophilic conditions equals to 148 and 98 m^3 respectively, corresponding to an expected power generation of around 300 kWh electrical energy (assuming biogas LHV=6.6 kWh/m^3biogas and 30% energy conversion efficiency). A maximum value of 0.82 m^3biogas/kgVS in terms of SGP was observed at an OLR of 4.5 kgVS/m^3d. Interesting values in the velocity of biogas production were achieved in the latter period, with an average value of 3.1 m^3biogas/m^3_rd.

With specific reference to the mass balance of the AD system the average VS content of the influent was 790 gVS(in)/d, and the VS content of the output materials, namely biogas and digestate accounted for 512 and 259 gTS/d respectively. According to the above mentioned figures the mass balance accounts to 93%, with a 11.8% error, while the VS reduction was approximately 65%. Also the resulting digestate contains less than 3% residue expressed as dry matter.

Similar results were found for dry matter and COD thus confirming the quality of the calculation. The COD balance showed a deficit of 9.8%.

Also the mass balances for nutrients (nitrogen and phosphorus) were good:

nitrogen balance reached 89.5% with an error (deficit) of 11.5% while the ammonification degree of the mesophilic system was 56.2%.

Phosphorus was slightly higher in the digester output with a balance of 105% and an excess of 5%.

The removal efficiency of organic compounds was measured daily by determining the elimination of COD and Volatile Solids (VS) (Nayono et al., 2009). In the first 22 days of operation the system reached a COD elimination greater than 78% and then remained around 74% during SSC. In Figure 3 the relationship between solids elimination, total solids and volatile solids compared to OLR trend is presented.

Compared to previous results of Nayono et al., (2009) we can emphasize that during SSC with an applied OLR of 3.5 kgVS/m^{3}d the mesophilic reactor showed a high efficiency on VS removal while during transient conditions, raising the OLR from 4.5 to 6 kgVS/m^{3}d, the anaerobic process appeared to be less efficient. This is due to the stress condition the reactor had to endure, even if a VS elimination of 50% is considered close to the optimum for anaerobic degradation of press water (Nayono et al., 2009).

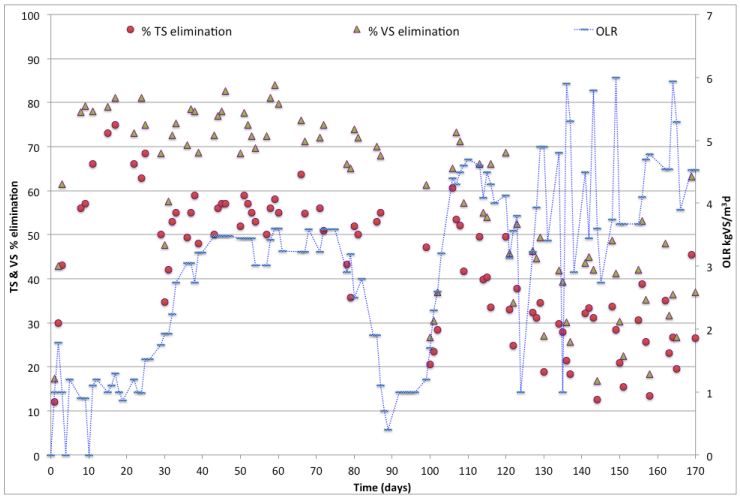

Figure 3. Total solids and volatile solids elimination during all the trials in mesophilic conditions.

3.2.2 Performances of the thermophilic anaerobic digestion process

The inoculum used for the thermophilic reactor was the same of used for the mesophilic tests. After the first days of operation in mesophilic conditions the working temperature was increased from 37 °C to 55 °C using the single-step strategy (Cecchi et al., 1993) while feeding was stopped. The thermophilic conditions were reached in a couple of days and maintained for about ten days without feeding. In order to acclimate the biomass to the organic material, the reactor was started up with a low organic loading rate (1 kgVS/m^3d) for one week, then the OLR was increased to 3.5 kgVS/m^3d and maintained for about 4 HRTs (RUN I from day 0 to day 80).

The short chain volatile fatty acids concentration remained constantly below 1000 mg/L, with an average value of 489 mgVFA/L; acetate was the main compound found. Maintaining VFA at this level prevents potential process inhibition due to VFA accumulation, which in turn leads to a decrease in pH. Average pH was around 8.1. The low pH fluctuations indicated the good buffer capacity of the system, which maintained pH at compatible levels with the methanogenic thermophilic levels.

Average total alkalinity (determined at pH 4) in steady-state conditions was 5,380 mg $CaCO_3$/L. Partial alkalinity (determined at pH 5.75) showed a profile in line with the trend of the volatile fatty acids, and consequently the difference between partial and total alkalinity, which is directly proportional to the concentration of VFA, remained constant. The values of total and partial alkalinity were 5,200 and 3,900 $CaCO_3$/L respectively, corresponding to a ratio value of 0.23 (VFA/alkalinity). Even in the thermophilic reactor the value of this ratio was stable, thus it justifies the possibility to increase the system to OLR of 4 - 4.5 kgVS/m^3d. During the transient period (RUN III from day 120 to day 170) the best specific biogas productions were obtained at OLR in the range 4 - 4.5 kgVS/m^3d, with a SGP average value of 0.9 m^3biogas/kgVS. Transient OLR conditions from 2 to 6 kgVS/m^3d were evaluated.

The VFA/alkalinity ratio of the transient OLR period had an average value of 0.27 as shown in Figure 4.

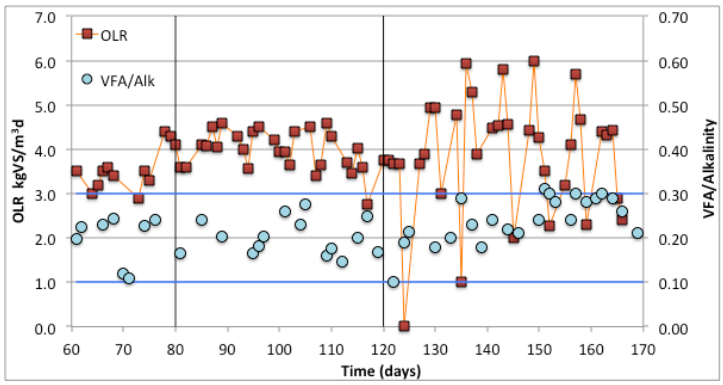

Figure 4. Trend of the thermophilic ratio volatile fatty acids and alkalinity with OLR variations

The average total ammonium concentration (as mgN-NH_4^+/L) was 1,004 mgN-NH_4^+/L, with maximum values of 1,086 mgN-NH_4^+/L. When OLR was increased to 4-4.5 kgVS/ m^3_rd, the corresponding value of free ammonia was 374 mgN - NH_3/L, a value below the inhibition limit, normally reported in literature (about 700 mgN/L, Angelidaki et al., 1994).

The content of total solids in the reactor remained almost constant with an average value of 16.3 gTS/kg and a volatile solids content of 12.3 gVS/kg. The ratio between total and volatile solids shows an average value of 76.5% (TVS/TS), it's thus highlighted the large capacity of the system to convert the organic matter into biogas, leaving a residue of dry matter lower than 2% in digestate.

The average composition of the biogas in terms of methane percentage was high, equal to 68.8%, and the specific methane production (SMP) was 0.55 m^3CH_4/kgVS (Figure 5). With regard to biogas and energy yield, the average specific gas and methane production equals to 0.90 m^3biogas/kgVS and 0.55 m^3CH_4/kgVS respectively, while the average gas production rate was 3.0 m^3biogas/m^3_rd. Based on the above figures for each ton of biowaste semi liquid fraction, the biogas and methane production in AD thermophilic conditions equals approximately 166 and 113 m^3 respectively, corresponding to around 350 kWh electrical energy (assuming biogas LHV=6.6 kWh/m^3 biogas and 30% energy conversion efficiency). Overall the thermophilic digestion process of biowaste semi-liquid fraction in steady state condition showed increased buffer capacity and higher biogas production potential compared to mesophilic digestion.

SGP reached a value as high as 0.94 m^3biogas/kgVS at an OLR 4.5 kg VS/m^3d transient period (RUN III, duration 4 HRTs), reporting an average increased value in biogas production of 0.9 m^3biogas/$kgVS_{fed}$.

These values indicated the capability of the system of converting most of the organic material into biogas and are of particular significance.

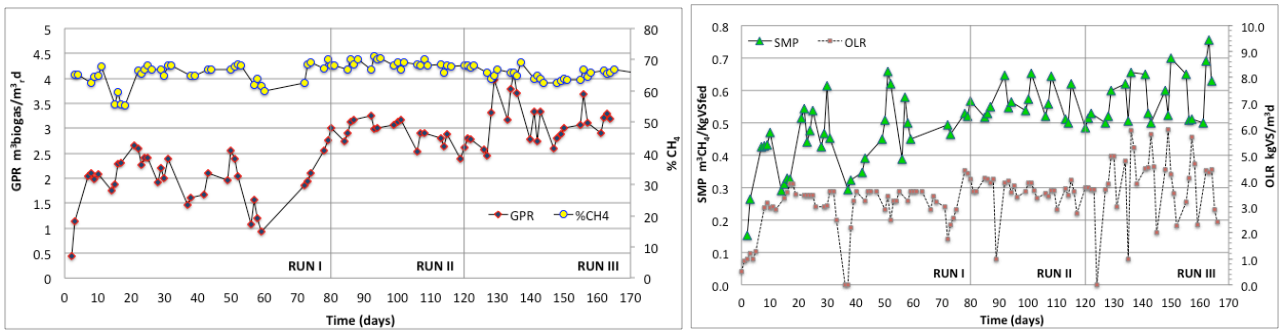

Figure 5. Specific methane production and OLR variations in thermophilic anaerobic digestion and methane percentage compared to the Gas Production Rate

During the steady state condition of the thermophilic reactor (RUN II from day 80 to day 120), with OLR between 3 and 3.5 kgVS/m^3d, the mass balance around the system was calculated: the influent and effluent (as both digestate and biogas) quantity of volatile solids accounted for 790, 141 and 578 g/d, respectively. The balance was therefore 91%, with a 9% error. Similar results were found for dry matter and COD (errors of 11% and 11.4%, respectively) thus confirming the accuracy of the monitoring analysis data.

Also the mass balances for nutrients (nitrogen and phosphorus) closed properly.

Nitrogen balance was 89.5% with a deficit of 10.5%. It is interesting to note here that ammonification degree, that is the conversion of organic nitrogen (proteins) into ammonium nitrogen, was 60.2% in thermophilic conditions.

Phosphorus balance was 104% with a surplus of 4%.

In conclusion we can highlight that the mass balances of the thermophilic anaerobic system showed deficits or surpluses in matter transformation all within the margin of error in order to consider the system trial to be admissible from scientific opinion (Banks et al., 2011).

3.3 Energetic considerations

Due to the high moisture content and biodegradability, the treatment method that allows for an effective stabilisation of this organic material with a positive energy balance is anaerobic digestion followed by or combined with composting. The anaerobic digestion of the liquid fraction of pressed waste (roughly equivalent to two thirds of the biowaste) can produce 200-220 kWh with a slight superiority for the thermophilic process (Pavan et al., 2000; Van Lier et al., 2000).

With reference to energy consumption, an advanced industrial press machine for the treatment of biowaste is characterized by an installed power in the range 375-400 kW for a treatment capacity of some 12 ton/h. The typical energy consumption is therefore some 33 $kWh_{consumed}$ per ton of treated biowaste.

Considering the energy requirements for composting (moving, air pumping, curing) of the solid residual material originated from the screw press system it was calculated an energy consumption of some 25 kWh considering an electricity power request of 50 kWh/ton (Kubler & Rumphorst, 1999) while other 25 kWh should be accounted for all the other plant facilities (blowers, digester mixing, pumping …) (Correia et al., 2010).

The global energy balance is therefore highly positive: the energy production is nearly double compared to energy consumption.

3.4 Digestate characteristics

Heavy metals concentrations of biowaste and mesophilic and thermophilic digestate were checked five times during steady state conditions. The average concentrations of heavy metals in both the input material (biowaste) and in mesophilic/thermophilic digestates, were much lower than the limits of the technical proposals End-of-Waste criteria (EoW-2014) elaborated by the Joint Research Center of Sevilla, as showed in Table 5. Concentrations referred to dry matter were typically greater in digestate because of the high conversion capability of organic matter into biogas in the anaerobic process. Determined concentration for the two digestate samples (e.g. thermophilic and mesophilic) are of the same level of magnitude and should be considered equivalent also considering the reported standard deviations.

If digestate undergoes composting a further reduction of organic matter will be observed with consequent increase of metal concentrations. However, digestate is an already stabilized organic material therefore the reduction of dry matter is limited. Also the addition of bulking agent can not change the concentrations because of its low metals content (Eftoda et al., 2004; Cavinato et al., 2013).

Table 5. Heavy metals concentrations (mesophilic and thermophilic conditions)

	EoW 2014	BIOWASTE	MESOPHILIC DIGESTATE	THERMOPHILIC DIGESTATE

Cu d.m.	mg/kg	200	47±5	68.1±3.2	52.5±7.8
Zn d.m.	mg/kg	600	112±28	155±13	129±32
Pb	mg/kg d.m.	120	1.54±0.8	17.3±2.4	12.9±4.3
Ni	mg/kg d.m.	50	43.7±3	42.1±1.6	27±0.5
Cr tot d.m.	mg/kg	100	61.5±9	85.9±4.1	74.6±6.4
Cd d.m.	mg/kg	1.5	0.4±0.2	0.23±0.14	0.26±0.08
Hg d.m.	mg/kg	1	0.055±0.005	0.24±0.09	0.08±0.02
As d.m.	mg/kg	10	0.24±0.10	0.25±0.09	0.19±0.03

Biowaste is known to contain pathogenic bacteria such as Salmonella and other microorganisms that may be a health risk for both people and animals (Sahlström, 2003). The content of pathogens of fed substrate and both effluents digestates, in the two experimentations, was analyzed through five replicates. While Salmonella spp was never found, the limit of 1000 CFU/g for E.coli proposed in the End of Waste Criteria technical report (2014) was reached only occasionally (Table 6). This suggests the opportunity to treat digestate in a post-composting process in order to reduce the presence of enteric bacteria (Cekmecelioglu et al., 2005). Several experimental investigations demonstrated that rapid inactivation of Escherichia coli and Salmonella spp. occurs by thermophilic digestion (Smith et al., 2005; Wagner et al., 2008).

As for fertilizing properties, AD allowed getting a final product (digestate) with very good fertilizing properties because of the high nutrient content (C, N, P, K) in available forms (Tambone et al., 2010).

Table 6. Pathogens analysis

SAMPLE	TBC 37°C ISS 004A	TBC 22°C ISS 004A	*E.coli*	total Coliform	*Salmonella spp* ISS 011A
Biowaste	$4 \cdot 10^8$ CFU/g	$8 \cdot 10^8$ CFU/g	$7 \cdot 10^5$ CFU/g	$6 \cdot 10^5$ CFU/g	absent
Thermophilic digestate	$1 \cdot 10^7$ CFU/g	$1 \cdot 10^7$ CFU/g	$4 \cdot 10^3$ CFU/g	$1 \cdot 10^3$ CFU/g	absent
Mesophilic digestate	$3 \cdot 10^6$ CFU/g	$4 \cdot 10^6$ CFU/g	$3 \cdot 10^3$ CFU/g	$2 \cdot 10^4$ CFU/g	absent

4. Conclusions

Biowaste from door-to-door separate collection was pressed and the liquid fraction underwent to mesophilic and thermophilic anaerobic digestion. Mesophilic digestion gave an average biogas production of 0.79 m^3biogas/kgVS with 66.0% methane content while in the case of thermophilic conditions the average biogas production was 0.90 m^3biogas/kgVS with 68.8% methane.

The application of press systems for the separation of segregated biowaste into semi-liquid and semi-solid fractions can be beneficial for further optimizing biowaste treatment in integrated anaerobic-aerobic systems.

The energy balance of the system, considering the energy revenue form biogas and the energy input for composting, is clearly positive.

Heavy metals concentrations and pathogens were below the limits reported by "End-of-Waste" criteria (2014) for future legislative developments, thus indicating the good digestate quality and its possible use for agronomic purposes.

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7. CHAPTER 2

Double phase and mono phase anaerobic digestion comparison

Research Paper

Comparison of single and double-phase thermophilic anaerobic digestion of food waste: process performance, statistical analysis and energy evaluation

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ABSTRACT

This study compared the performances of single- and two-stage anaerobic digestion processes of food waste. The processes were monitored by taking into account both the start-up and steady state process performances and considered the transient conditions. In addition to a conventional univariate analysis, we also performed a multivariate analysis to increase the validity of the results of the comparison study. The transient states caused peaks due to a high organic loading rate, simulating possible overloading events and the recovery capacity of both processes (resilience). The specific gas production of the methanogenic reactor of the two-stage process was higher (0.89 m^3_{biogas}/kg_{VS}) than for the single-stage process (0.76 m^3_{biogas}/kg_{VS}). This finding was related to the increase in the removal efficiency (of 17%). We performed mass balances to evaluate which system was more resilient and energetically more sustainable. Considering our pilot-scale results, a final overall assessment of a 100.000 PE basin and a comparison of the energy yields and biogas upgrade are also discussed.

Keywords

anaerobic digestion, food waste, energy comparison, biogas upgrading, pilot scale, statistical analysis

1. Introduction

The municipal solid waste produced within the EU was 252 million tons in 2011 [1] with an average

per capita production of 541 kg/inhabitant per year. Up to 40% of this waste was organic material of good quality (high biodegradability and low content of inert material), which is possible due to the successful implementation of separate collection systems in recent years [2] [3]. Noticeably, the amount of food wasted is expected to increase by 44% globally between 2005 and 2025 [4]. Landfilling of this type of material can cause an increase in methane emissions from 31 million to 43 million tonnes on a global scale.

Organic waste, such as segregated food waste, can be conveniently treated via anaerobic digestion (AD). Currently, more than 16,000 AD plants are running within the EU, 20% of which treat organic waste [5].

Anaerobic digestion performed at the single stage in which four microbiological reactions, hydrolysis, acidogenesis, acetogenesis and methanogenesis, occur concurrently in the same reactor has been extensively studied [6] [7]. Historically, the single-stage reactor has been the most used process for organic waste treatment using the different anaerobic processes [8] [9] [10] [11].

However, it has been demonstrated that the two-stage process is a valuable option [12]: in these hydrolysis processes, the limiting step of the entire process and acidogenesis are performed in the same (first) reactor, whereas methanogenesis is performed in a second, specifically dedicated reactor. This allows for an increase in the organic loading rate and the simultaneous reduction of the hydraulic retention time; thus, globally, an improvement in the performances of these reactors is expected [13]. Although the use of two-stage processes for substrates with a low biodegradability, such as waste-activated sludge, has been largely demonstrated as beneficial [14] [15] [16], the use of one- or two-stage processes for high biodegradable substrates, such as food waste, organic fraction municipal solid waste or similar waste, is controversial [17] [18].

The use of one- versus two-stage anaerobic digestion processes for food waste has been tested on a laboratory scale by different researchers. Ghanesh et al. [19] performed an AD process in a single-stage reactor and reported a methane yield of 0.45 m^3 CH_4/kg_{VS} and a volatile solids (VS) removal rate of 83%. AD was also performed in a two-stage system and showed significant reduction in VS; however, the energy and mass balance showed that the single-stage process was 33% superior in terms of biogas production and energy yield compared with the two-phase process. The lower energy yield of the two-phase system was due to the loss of energy during hydrolysis in the first-phase reactor, and the deficit in methane production in the second-phase reactor attributed to the COD loss due to biomass synthesis and adsorption of slow biodegradable COD onto the flocks. Schievano et al. [20] compared the one- and two-stage AD process by applying the same organic loading rate in

the two systems and focusing on both chemical and microbiological aspects. The results showed an average methane concentration of 68% and 55% in the two-stage and single-stage systems, respectively. The specific methane production was 351 LCH_4/kg_{VS} for the two-stage system and 404 LCH_4/kg_{VS} for the one-stage system.

Later, however, the same authors [21] emphasized that two-stage AD can increase energy recovery from biomass compared with one-stage AD.

Additionally, this result was evaluated using laboratory-scale reactors with a 300 mL operating volume. Nowadays with regard to our knowledge there are no comparative studies at pilot scale. The aim and the novelty of this study was to verify the process performances of single- and two-phase anaerobic digestion of food on a pilot scale to obtain robust data for comparison.

In particular, our research considered the mass balances and yields of the two systems. Moreover, insights on the start-up phase, transient conditions and steady state conditions were part of the study. In addition, a multivariate analysis was performed to support the comparison study.

2. Materials and Methods

The research was performed in the experimental hall in the wastewater treatment plant (WWTP) at Treviso (North Italy). Reactors were operated both under steady state conditions (SSC) and at transient conditions, as determined by the hydraulic retention time (HRT) and the fast organic loading rate (OLR) to verify the resilience of the systems.

2.1. Substrate and inoculum

The anaerobic digested sludge that was used as inoculum for the methanogenic reactors (single-stage and second-phase reactors) was collected in the WWTP where a 2000 m^3 anaerobic digester treats the collected biowaste at 35 °C. The sludge was acclimatized for two weeks to the thermophilic temperature [10].

The substrate used in these experimental tests was food waste, which originated from door-to-door collection within Treviso Municipality.

The fermentative reactor (first phase) was inoculated with food waste and water and then regularly fed into the reactor to reach the OLR required.

2.2. Reactor set-up

Stainless steel AISI-304 reactors were used with a working volume of 230 L for the one-stage digester, whereas the two-stage system included two reactors of a volume of 200 and 380 L. Mechanical anchor agitators ensured mixing occurred to maximize the degree of homogenization inside the reactor [22]. The working temperature was set at 55 °C ± 0.1 and maintained by hot water running through an external jacket.

2.3. Experimental set-up

After the initial adaptation step, the two different AD systems were operated by applying an organic loading rate of approximately 3.5 $kg_{TVS}/m^3_r d$ (single-stage and two-stage) and a hydraulic retention time of 20 days. They were maintained under stable condition for 2 HRTs (40 days - RUN I)
After RUN I (steady state conditions), a high organic loading rate (stress tests) was applied to both systems according to the following pattern:
- RUN II: Doubling the OLR for one day;
- RUN III: Doubling the OLR for two consecutive days;
- RUN IV: Doubling the OLR for three consecutive days.
The influent and effluent streams of the processes were monitored during the entire experimentation. Analyses of the parameters and biogas yields were conducted in parallel for the two systems. A comparison was performed that considered the biogas productions in terms of yield and composition and total solid removal (TSr), and we analysed the system instability via measurements of pH, ammonia, alkalinity and volatile fatty acids (VFAs).

2.4. Sampling and analysis

The reactor effluents were monitored 3 times per week for total solids (TS), total volatile solids (TVS), chemical oxygen demand (COD), total Kjeldahl nitrogen (TKN) and total phosphorous (P). For the TS determination, a drying temperature of 105 °C was adopted, and no losses were caused [23]. The process stability parameters, i.e., the pH, volatile fatty acid content and distribution, conductivity, total and partial alkalinity and ammonia nitrogen (NH_4^+–N), were measured daily. All the analyses were performed according to the Standard Methods for Water and Wastewater Analysis

[24]. The analysis of the volatile fatty acids was conducted using a Carlo Erba™ (Milano, Italy) gas chromatograph equipped with a flame ionization detector (T = 200 °C), a fused silica capillary column, Supelco NUKOL™ (15 m x 0.53 mm x 0.5 μm thickness of the film), and hydrogen was the gas carrier. The analysis was conducted by increasing the temperature from 80 °C to 200 °C (10 °C/min). The samples were filtered using a 0.45 μm filter. The biogas production was monitored using a flow metre (Ritter Company™), and methane, carbon dioxide and oxygen in the biogas were determined continuously using a portable infrared gas analyser GA2000™ (Geotechnical Instruments™) and once a day using a Gas Chromatograph 6890N, from Agilent Technology™. It was equipped with an HP-PLOT MOLESIEVE column with a 30 x 0.53 mm ID x 25 μm film using a thermal conductivity detector and argon as the gas carrier (79 ml/min). The H_2, CH_4, O_2 and N_2 were analysed using a thermal conductivity detector (TCD) at a temperature of 250 °C. The injector temperature was 120 °C. There was a constant pressure in the injection port (70 kPa). Samples were taken using a gas-type syringe in 200-μL biogas amounts. Once the entire sample was vaporized, separation of the peaks occurred within the column at a constant temperature of 40 °C (8 min).

2.5. Multivariate data analysis

Descriptive statistics and exploratory data analysis were performed using the open-source program, R (The R Foundation for Statistical Computing, version 3.1.3). Datasets for both experiments, including the results of the analytical procedures, were obtained three times per week (Monday, Wednesday and Friday) for 8-week pseudo-stable periods, which corresponded to more than 2 HRT of monitoring.

Clustering analysis was performed using the Principal Component Analysis (PCA) approach. The statistical process control chart is defined as a group of methods that evaluate whether a singular process remains efficient and not susceptible to specific problems, which can change and jeopardize the entire course of the process [25] [26] [27]. For an acceptable region that is limited by an upper (UCL) and a low (LCL) control limit, a control statistic should be calculated and tested to accept or reject the null hypothesis (H0: process control) with a certain probability of obtaining a Type I error.

3. Results and discussion

3.1. Composition and characterization of the food waste used in this study

An analysis of the composition of the food waste is showed in Table 1.

Table 1. Composition of the food waste collected in Treviso (Italy)

Fractions	Wet weight
	%
Fruit and Vegetable	56.3 – 66.9
Fish and Meat	17.8 – 24.3
Pasta, Bread and Rice	9.3 – 14.8
Paper and Cardboard	4.9 – 6.6
Inert and Unclassified Materials	1.8 – 3.8

The table shows a food fraction (fruit, vegetable and other organic waste) greater than 85% for the wet weight of the food waste, whereas the remaining percentage is mainly composed of paper (approximately 6%), which is still anaerobically biodegradable, and inert material (approximately 3%). The fraction of fruit and vegetable was approximately 60% of the overall organic waste. Comparing these values with values from reference [28], in this study, the fraction of fruit and vegetable was lower, whereas the fraction due to other organic waste (e.g., bread, pasta, dairy, etc.) was higher. Because of these differences in the composition, the total solid content of the organic waste fraction used in this study (table 2) was higher than that used in reference [28].

The observed results are, however, typical of the Mediterranean Region, as confirmed by data reported in a Greek study [29] in which the organic fraction (fruits, vegetable and other organic waste) was 86.2%, with vegetable and fruits at approximately 60%. These values are in agreement with our study. However, the amount of inert material for the Greek municipalities used in that study was 4% less than in our study.

Table 2. Food waste characterization

Parameters	Units	Average ± S.D.	Min	Max
TS	$g/kg_{w.w.}$	298.2 ± 44.2	244.4	332.3
TVS	$g/kg_{w.w.}$	267.6 ± 32.5	239.8	299.5
TVS/TS	%	89.8 ± 3	87.2	92.8
COD	$g\ O_2\ /kg_{TS}$	1,110 ± 254	966	1,380
TKN	$g\ N\ /kg_{TS}$	27 ± 4	31	25
P	$g\ P\ /kg_{TS}$	4.0 ± 0.2	3.7	4.2

With specific reference to data reported in Table 2, it is clear that this material was particularly suitable for the AD process. The VS/TS ratio was 90%, and the COD:N ratio was an average value of 40.

3.2. Comparison of the single- vs. two-stage system for steady state conditions

The comparison of the processes was conducted by considering the data obtained during approximately 50 days of stable operating conditions (2 HRTs).
In Table 3, the single-stage (SS) and first- (F1) and second-phase (F2) effluents and respective gas yields are reported.

Table 3. Process parameter data

Parameter	Unit	SS	F1	F2
TS	$g/kg_{w.w.}$	27 ± 1	56 ± 29	15 ± 5
TVS	$g/kg_{w.w.}$	17 ± 1	52 ± 27	10 ± 4
COD	$gO2/kg_{TS}$	806 ± 74	829 ± 55	643 ± 42
TKN	g/kg_{TS}	33 ± 1	30 ± 5	44 ± 7
P	g/kg_{TS}	12 ± 2	11 ± 0.8	12 ± 2
pH		8.0 ± 0.1	4.6 ± 0.3	8.0 ± 0.1
Partial Alkalinity	mg CaO_3/L	3414 ± 91	-	2715 ± 113
Total Alkalinity	mg CaO_3/L	5311 ± 117	1685 ± 229	4943 ± 241
NH_3	mg NH_3-N/L	443 ± 35	28 ± 9	501 ± 28
VFAs	g COD/L	892 ± 68	9997 ± 3962	548 ± 96
Specific Gas Production (SGP)	m^3_{biogas}/kg_{TVS}	0.75 ± 0.1	0.12 ± 0.02	0.88 ± 0.01
Specific Methane Production (SMP)	m^3_{CH4}/kg_{TVS}	0.45 ± 0.02	0.020 ± 0.008	0.55 ± 0.01

Gas Production Rate (GPR)	m^3_{biogas}/m^3d	2.2 ± 0.4	-	2.8 ± 0.5

Regarding the TVS removal efficiency and biogas production, Table 3 shows that the SGP for the second phase was higher (t-test, $p < 0.01$) than for the single-stage. Furthermore, the mass balance exhibited an 11% increase for TVS removal efficiency for the second phase and single phase. Considering all results from the two-phase system, we determined an efficiency removal increase of 16% compared to the single-stage system. Thus, the fermentation played the role in "pretreatment" of the food waste, which was designed to increase the conversion efficiency of the volatile fraction to biogas.

In accordance with the higher TVS removal efficiency, a higher ammonification rate was revealed. In fact, the ammonification rate in the second phase was 4.4% higher than for the single-stage system (62.0% for the single stage, 66.4% for the double phase). This led to a higher ammonia release in the second phase (table 3) than in single stage; however, in both experiments, the free ammonia concentration never exceeded 600 mg $N\text{-}NH_3/L$, and inhibition of the methanogenic activity was not detected during this period. Absence of inhibition was also demonstrated by the biogas composition in both experiments. In fact, the single-stage reactor and the second phase exhibited an average percentage of methane that was detected in the SSC of 56 ± 2 $\%CH_4$ and 61 ± 2 $\%CH_4$, respectively, and they were almost constant during the 50-day trial. The decrease in the overall production of biogas and the increase in the percentage of CO_2 could have been caused by the presence of inhibition phenomena that was detrimental to the methanogenic component, for example, due to the excessive presence of volatile fatty acids [30].

The average values for the specific gas production (SGP) and gas production rate (GPR) during the period of one-stage stability (RUN I) were 0.76 m^3_{biogas}/kg_{VS} and 2.2 m^3_{biogas}/m^3d, respectively, with maximum values achieved in terms of SGP of up to 0.92 m^3_{biogas}/kg_{VS}.

The gas production (GP) during the stable period remained between 476 L/d and 526 L/d.

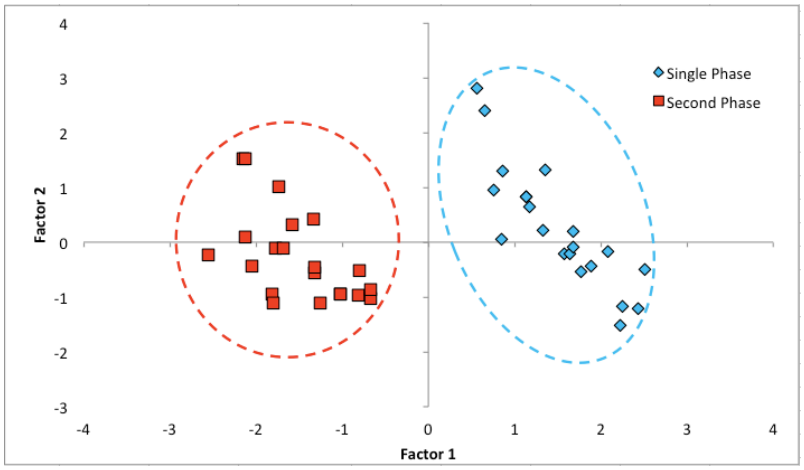
In the two-stage process, the average percentage of methane detected was 61 ± 2% and was almost constant, suggesting the stability that the methanogen reactor reached under these operating conditions.

The two-phase process exhibited a remarkable resilience; the average values of the SGP and GPR during the stable period (RUN I) were 0.89 m^3_{biogas}/kg_{VS} and 2.83 m^3_{biogas}/m^3d, respectively.

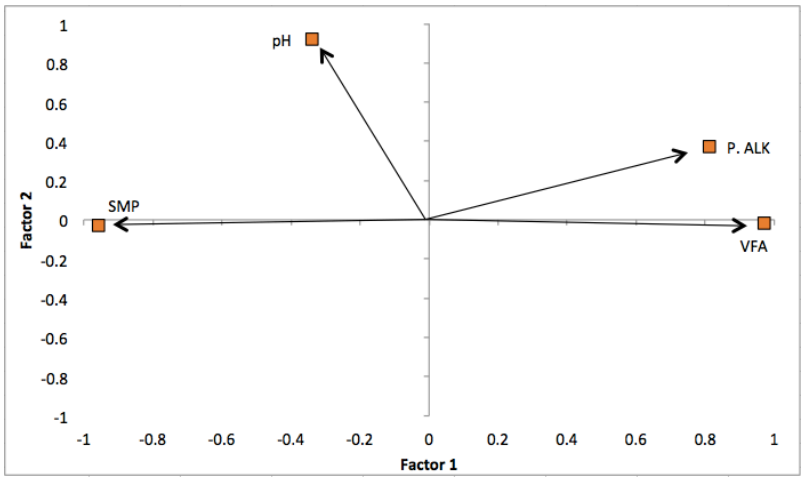
For the other parameters used to characterize the methanogenic process (pH, partial alkalinity and volatile fatty acid concentration), Table 3 shows the buffering capacity for the second phase on average was lower than for the single stage (t-test, $p<0.01$). Regardless, the VFA concentration for the second phase was on average higher than for the single stage (t-test, $p<0.01$), and the average pH values were similar for both experiments (t-test, $p = 0.35$).

Considering these latter parameters and the specific methane production, cluster analysis was performed to evaluate, from a multivariate point-of-view, the differences in the steady state conditions for the two systems (single stage and second phase). The cluster analysis showed that the two experimental performances were divided in two different clusters (Figure 1a of the score plot). The variables used to identify the two clusters followed what is observable in Table 3. As shown in Figure 1b (loading plot), pH contributes less significantly than the other variables to the division of the two classes, whereas the VFA and SMP exhibited the inverse correlation, suggesting that the second phase had a lower VFA and a higher SMP than the single stage. In contrast, the VFA concentration and partial alkalinity showed a direct correlation; therefore, the second phase exhibited a lower buffer capacity than the single stage.

Figure 1. a) Score plot and b) loading plot



a



b

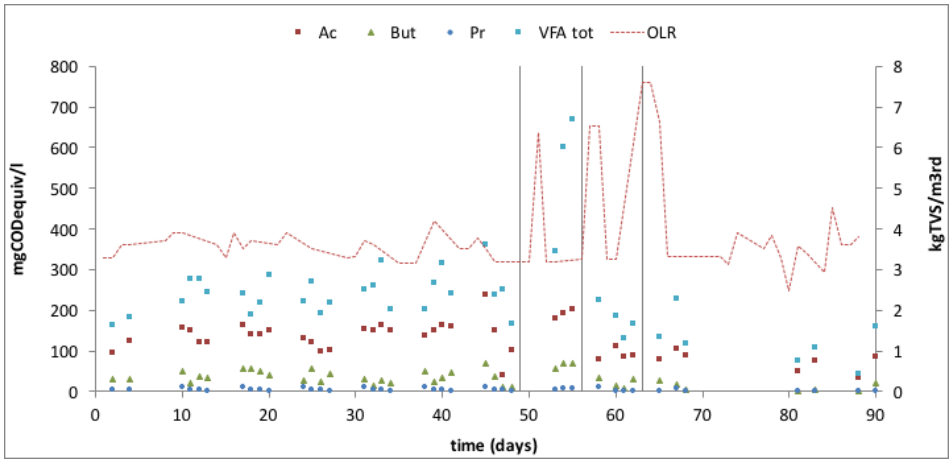
The score plot described 91% of the information via the first factor (67%) and the second factor (24%). Ultimately, the two experiments showed that employing a two-phase rather than a single-stage configuration significantly affects the methanogenic process. This is mainly due to the different chemical-physical characteristics of the food waste fed into the one-stage digester and the fermented waste fed into the two-stage digester. In the fermentation reactor, the pH was approximately 4.6 or below; hence, no partial alkalinity was present, but the acid concentration was high (at approximately 10 g COD/L of VFAs). Feeding this effluent into the methanogen stage reduced the partial alkalinity inside the reactor; however, the high efficiency of VFA conversion to biogas allowed the two-stage process to maintain its stability.

3.2. Stress tests: process-monitoring results

Upon observing the variation in the VFAs concentration in the single-stage system after the first OLR increased (day 51th, RUN II), the system reported an initial accumulation of VFAs, and in particular, propionic acid, possibly indicating a potential change in the metabolic pathways [31]. This was followed by a fast biodegradation of the rapidly hydrolysable material; the volatile fatty acid concentration decreased from 666 mg COD/L to less than 200 mg COD/L. Conditions far from stability were also subsequently monitored during the following increases of OLR during RUN III. During this period and after two consecutive days of overloading (56th day, RUN III), the soluble chemical oxygen demand (SCOD) content rose to 2,289 mg COD/L, although during the following days the system showed clear signs of rapid degradation of the organic fraction with a decreased concentration of approximately 1,000 mg COD/L.

The system, therefore, did not report critically unstable conditions, and maintained average values for VFA and SCOD of 232 mg COD/L and 1,030 mg COD/L, respectively.

Figure 2. VFA single stage



While observing the variations in the SCOD concentration in the single-stage system (Figure 3) during the first OLR increase (day 50th, RUN II), the system did not report a significant accumulation of SCOD. Instead, during this period an increase in biogas production was detected (Figure 4), and the SGP was near the average value that was determined during the stable period. During the two and three consecutive days of overloading (57th and 58th day, RUN III, 64th, 65th and 66th day, RUN IV) the SCOD trend showed a small increase than previous trend. As in the latter overloading, biogas production increased but the SGP was slightly lower than average value determined in the stable period. The average percentage of methane was 59% ± 4, and there were no substantial fluctuations in the CH_4 percentage.

Figure 3. SCOD single stage

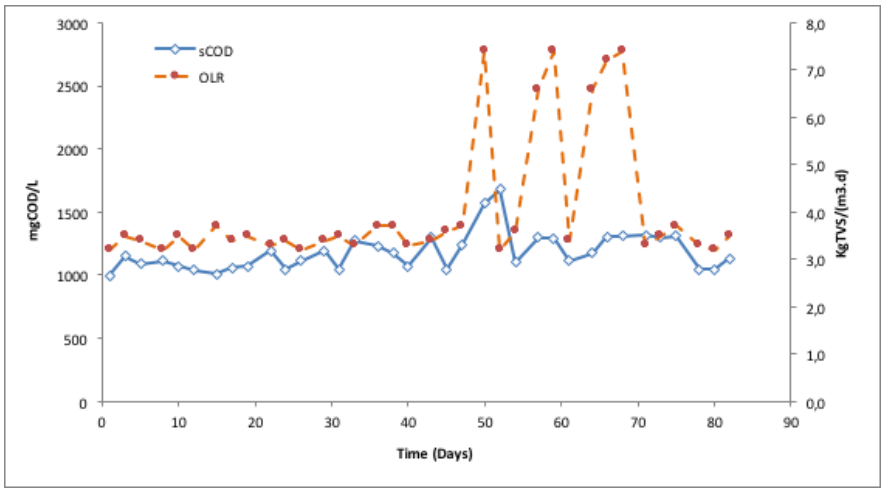


Figure 4. Gas production during the single-stage trial.

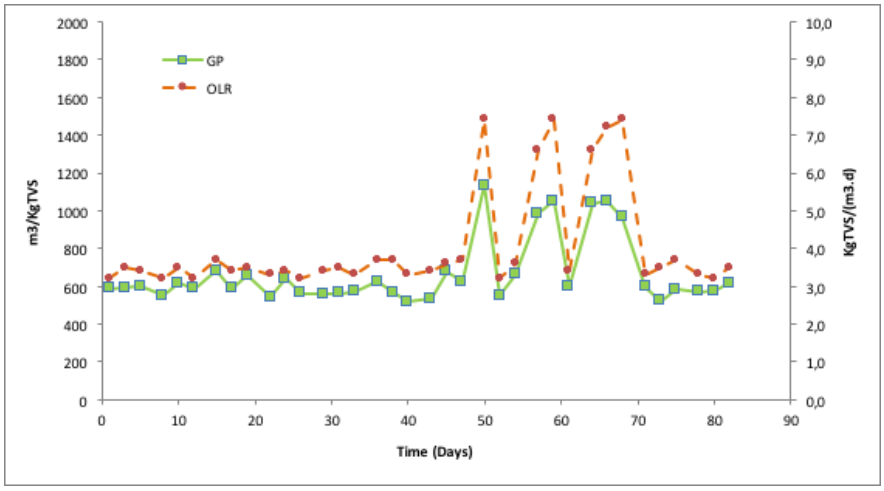


Figure 4 shows that the system increased its GP consistently with every increase in organic load, thus exhibiting variations in the percentage of methane. Although not entirely critical in the long term, this outlines how the reactor was resistant to perturbations.

The total ammonia in the methanogenic reactor also was below the potential inhibition value at an average of 403 mg N-NH_3/L.

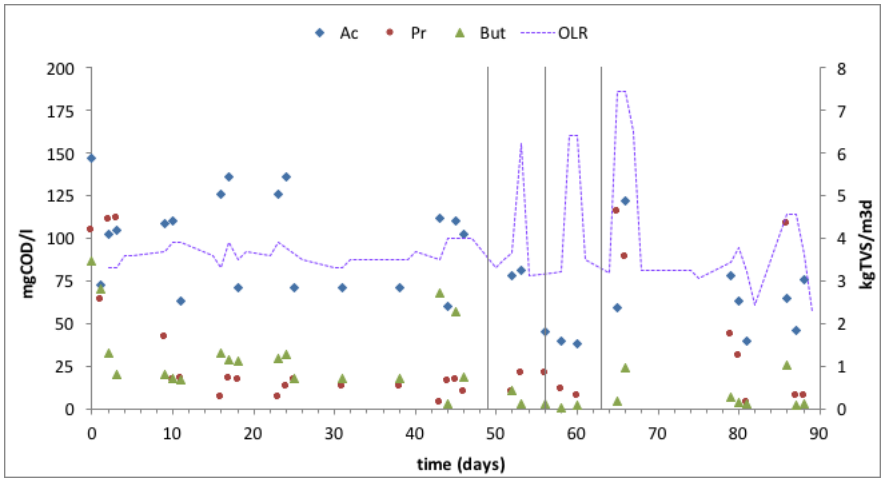
In the two-stage system, the effect of the fermentation step on the solubilisation of the organic matter was evaluated. The VFA concentrations increased up to 14 g COD/L (during RUN I) with a dominance of acetic and butyric acid. The first phase exhibited significant changes in the VFA and SCOD concentrations due to the waste variability, but this fermentation step withstands a high OLR and acts as a real "buffer system" for the methanogenesis process. Of note, the maximum VFA

concentration was detected after two consecutive days (RUN III) at approximately 15,890 mg COD/L.

For the methanogenic step, during transient conditions, the system reported no critical stability issues, and it maintained average SCOD and SGP values close to the averages determined during the stable period.

By analysing these RUNs in particular, we found that during the days when the organic load was doubled up to 6-7 kgTVS/m^3d, the methanogenic reactor found no substantial increases of the SCOD concentration. The organic fraction that was fed was well degraded and converted to biogas without accumulation in the reactor, maintaining an average VFA and SCOD of 219 mg VFA/L and 844 mg COD/L, respectively.

Figure 5. VFA in the second stage (transient conditions)



Low VFA concentration detection brings an important implication. The system reported no disturbances to its internal stability, and it exhibited a good tolerance to transient conditions and a high removal efficiency. The average biogas percentage did not substantially change during the period of increased OLR.

Figure 6. SCOD in the second-phase reactor

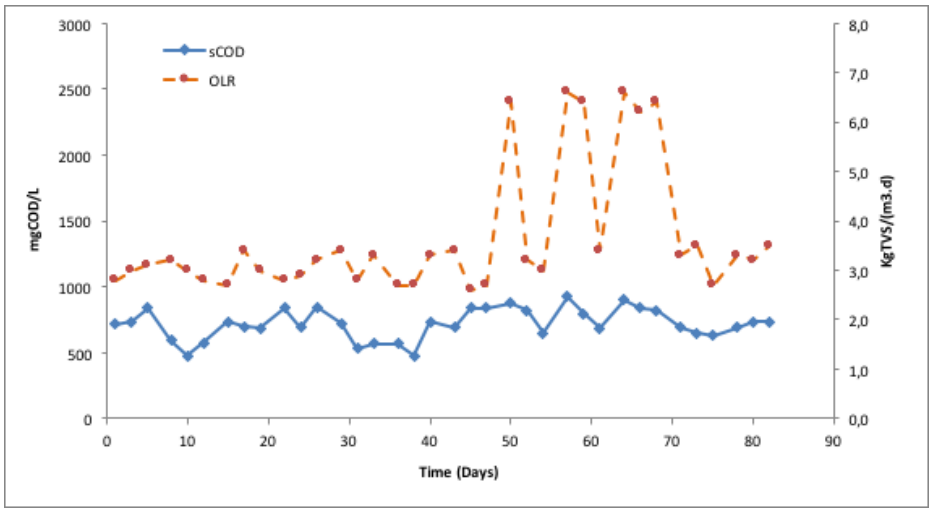
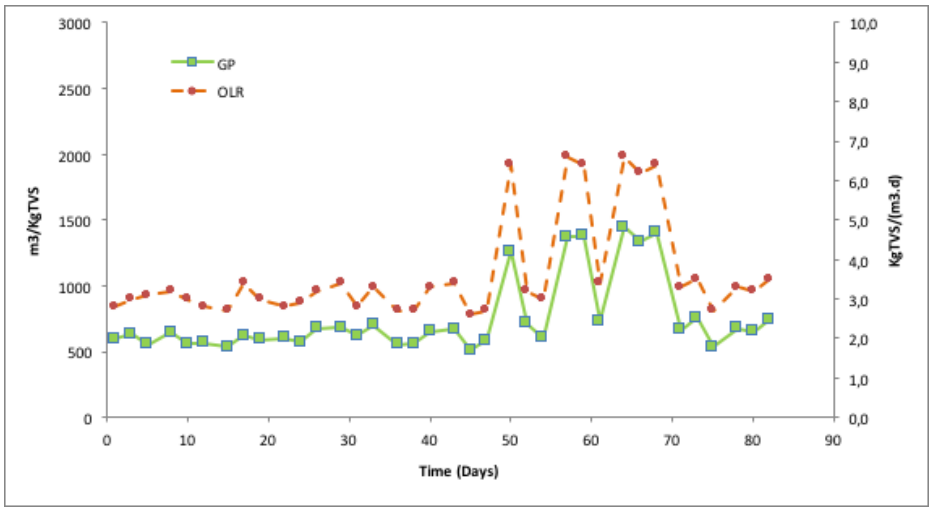


Figure 7. Gas production compared with OLR in the second-phase reactor



During the transient days (RUN II–III–IV), the average percentage of methane was 61% ± 2. The biogas production confirmed that the system was able to recover for a hypothetical organic overload, which is relevant from the point-of-view of the upgrading process to a full-scale process. During the long activity of a full-scale reactor, there may be periods of alteration.

The total ammonia in the fermentation reactor has typically been reported below the inhibition limit with an average value of 505 mg N-NH_3/L during transient conditions. The value of free ammonia depends on the temperature and pH of the system, and thermophilic conditions could cause problems, but this concentration is well below the level of inhibition [32]. The total ammonia in the methanogenic reactor has typically been reported below the potential inhibition value at an average value of 522 mg N-NH_3/L.

Generally, no evidence of instability was observed during the transient days (RUN II–III–IV) for both experiments, as demonstrated by the VFA and partial alkalinity ratio (Figures 8 and 9). The VFAs and alkalinity are two parameters that show a rapid variation when the AD system gradually moves away from stable conditions. The volatile fatty acids concentration tends to increase and the alkalinity tends to decrease, thus, a useful parameter to consider is the ratio of these two amounts [33]. In general, a ratio of approximately 0.3 – 0.4 indicates stable operation of the digester, whereas higher values may indicate the onset of instability issues. During the transient days of our experiments (RUNS II, III, IV), the ratio mentioned above never went above the threshold value; therefore, the systems showed no signs of instability (Figure 8 and 9).

Figure 8. Single-stage VFA/alkalinity ratio

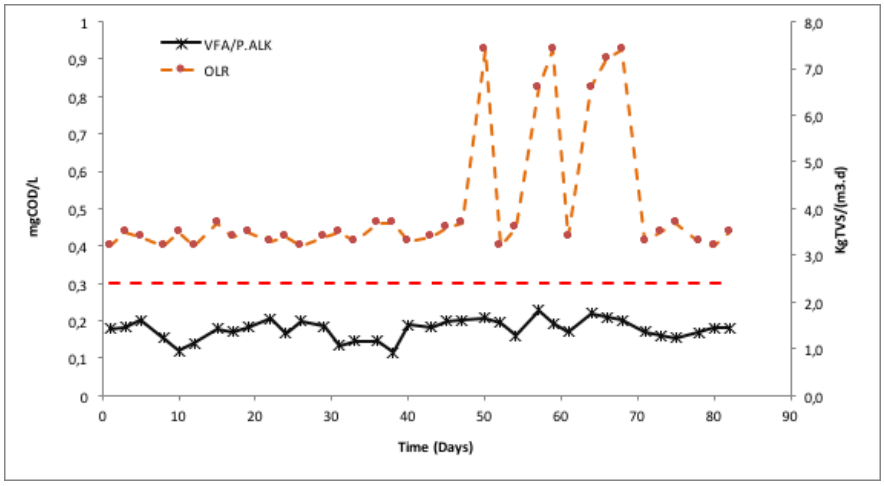
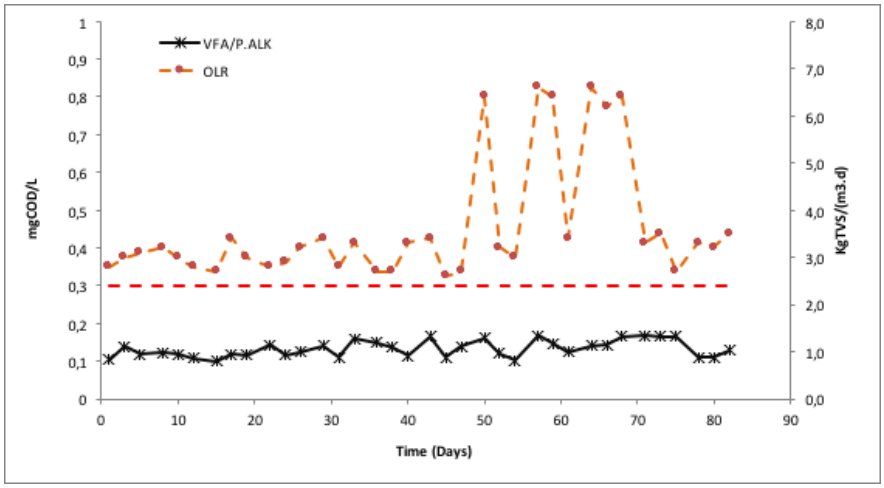


Figure 9. Double-stage VFA/alkalinity ratio



To understand if the increase in OLR was due to the natural variability of the processes, a control chart (Shewhart control chart coupled with principal component analysis) was used. The principal components included the four variables used for the cluster analysis described previously (pH, VFA, SMP, partial alkalinity). Using several Rank analysis approaches (scree plot, Kaiser Guttan criterion, corrected average eigenvalue criterion), the first principal component (factor) was determined to be significant. Moreover, the model formed using the first factor was evaluated to be correct via residual analysis [34].

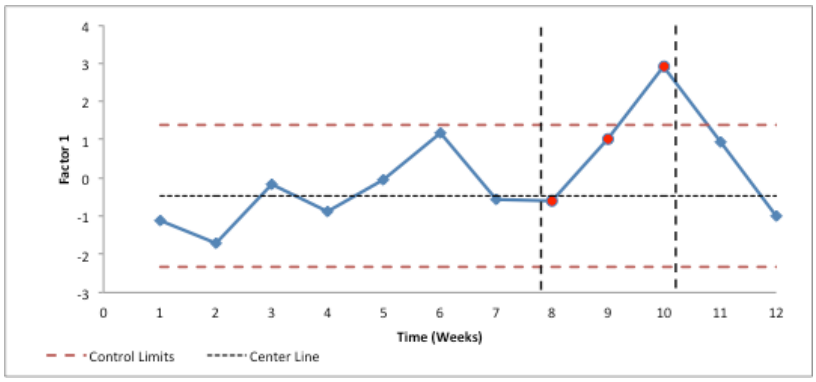
The control limits were calculated using the steady state period data from RUN I (49 days equal to 2.3 HRTs).

By analysing the control chart for the single-stage system (Figure 10a), we observed extraneous data during RUN IV. The extraneous point indicates the anomalous variability of the process; this anomalous variability is due to external causes, specifically, the increase in OLR. In fact, by observing the loading variables for the first factor (pH –0.74, P.Alk –0.83, VFA +0.94, SMP –0.89), we clearly observed an increase in the VFAs concentration and decrease in the remaining variables during the transient period, which may be due to a possible imbalance at the start of the system in favour of an acidogenic process.

Instead, concerning the second phase system, no extraneous data were observed (Figure 10b); therefore, the increases in OLR produced no change in the natural variability of the process.

Figure 10. Control charts; a) single stage, b) double stage

a



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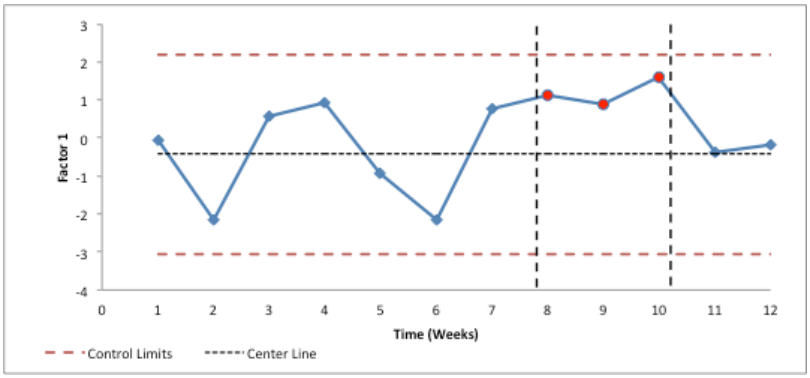

3.3. Energy yield comparison

To compare the energy yields of the two systems, a scaled-up version of these processes was evaluated using the analytical data of the pilot-scale experiments. We assumed a typical specific food waste production of 300 g/PEd for the wet weight of food waste [35] [36] and a potential basin of 100,000 PE/d.

The efficiency of typical mechanical waste pretreatment was considered to be approximately 90%; thus, the mass flow of food waste in the AD system was approximately 27,000 $kg_{foodwaste}$/d. The total volatile solids in the food waste, assuming the concentration obtained from these experiments (0.267 $kg_{VS}/kg_{foodwaste}$) was 7.209 kg_{VS}/d. The OLR relative to the one-stage reactor was 3.5 kg_{VS}/m^3d, i.e., 13 $kg_{foodwaste}/m^3$d. By applying a hydraulic retention time of 20 d, the reactor volume was 2,060 m^3. The total flow rate into the one-stage reactor was 103 m^3/d of feed. Assuming that the specific gas production of the system is 0.76 m^3/kg_{VS}, the biogas production in a full scaled-up plant could be 5,479 m^3/d with 5,531 kg_{VS}/d removed (77% removal efficiency).

Therefore, the flow rate of the digestate coming from the one-stage was 1,750 kg_{VS}/d (2,779 kg_{TS}/d). Based on the same input data, the two-stage process had a biogas production of 865 m^3/d (SGP 0.12 m^3/kg_{VS}), which was obtained during the fermentation stage; this flow rate, on a VS basis, was calculated to be 1,235 kg_{VS}/d with a VS removal of 17%. Applying a hydraulic retention time of 3.3 d and 16.7 d for the first and second phase, respectively, the volumes of the reactors were 405 m^3 and 2,060 m^3.

The fermentation effluent flow rate was 6,381 kg_{VS}/d (7,170 kg_{TS}/d) and was feed for the second stage. The methanogenic reactor had an SGP of 0.88 m^3/kgVS, hence a biogas production of 5,551 m^3/d. This corresponds to 86% of the VS that were fed as fermentate; hence, the transformation of the biogas produced into VS removed was 5,495 kg_{VS}/d. The two reactors, therefore, converted into

biogas 6,730 kg_{VS}/d of the total input amount of 7,209 kg_{VS}/d fed with a substrate removal efficiency of 93%. The output digestate flow rate from the second phase was 1,233 kg_{VS}/d (1,868 kg_{TS}/d). Comparing the two AD systems, we observed: 1) the two-stage system had a removal efficiency that was 17% higher than the one-stage system, and 2) taking into account a digestate dewatering post-treatment, the two-stage system had 33% less sludge for disposal.

Primarily, we compared the energetic and economic analyses of the two processes. We used a specific heat request of 1 kcal/kg °C, a temperature of the feed flow rate of 10 °C, and an average lower heating value (LHV) for the biogas of 5,500 Kcal/m^3. The thermal and electrical yields of the combined heat and power unit (CHP) had an overall efficiency of 0.9 (0.5 Heat efficiency and 0.4 Electrical efficiency), and the rest was lost.

Specific yields were determined from the experimental data. The thermal energy that could be produced from the single-stage system through the CPH was approximately 63,039 MJ/d, and an electric energy of 50,432 MJ/d could have been possible, which corresponds to 14 MWh/d. The energy request for the one-stage reactor corresponds to 23,463 MJ/d. Approximately 83% of the total thermal energy request came from the heating power for the organic waste by pre-heating from 10 °C to 55 °C for the thermophilic process. The remaining 17% was due to the 9.5 °C added to the 55 °C to support the heat dissipation phenomena.

Therefore, the thermal balance had a net production of 39,577 MJ/d.

With waste treatment facilities, such as AD facilities, a gate fee offsets the operation, maintenance, labour costs, capital costs of the facility and any profits and the final disposal costs of any unusable residues. The gate fee (or tipping fee) is the charge charged upon a given quantity of waste received at a waste processing facility. Currently, the gate fee is 85 €/ton waste. However, digestate has a cost of 60 €/ton when sent for composting.

Assuming only 130 €/MWh (no incentives) [37], the annual increased revenues from electricity (IRE) could be 656,050 €/year. The digestate (after dewatering treatment, approximately 25% TS) disposal costs, assuming100 €/ton [37], are estimated at 400,173 €/year. Hence, the one-stage system can produce a net profit of 255,877 €/year.

Total heat losses were estimated considering the dimension of the two reactors and the typical construction specifications. Specific yields were determined from experimental data. The thermal energy produced from the system through the CPH was approximately 73,822 MJ/d, and electric energy of 59,058 MJ/d was produced, which corresponds to 16,4 MWh/d. The thermal energy request for the two-stage reactors corresponds to 28,557 MJ/d. From the total thermal energy request, 81%

comes from the heating power for the food waste by pre-heating from 10 °C to 55 °C for the thermophilic process. The remaining 9% is due to the 10.6 °C added to the 55 °C to support the heat dissipation phenomena.

Therefore, the thermal balance has a net production of 45,265 MJ/d. Assuming 130 €/MWh (no incentives), the annual increased revenues from electricity (IRE) could be 768,269 €/year. The digestate (after dewatering treatment, approximately 25% TS) disposal costs for 100 €/ton for the two-stage system are 272,835 €/year. Hence, the two-stage system can produce a net profit of 495,434 €/year.

Capex costs are also required. The cost to actuate a first fermentation reactor with a necessary volume of 405 m^3 is 347,325 € [37] (750 €/m^3 reactor plus heat exchanger and pumps/piping costs). Thus, the payback time should be 1.45 years.

The final consideration for the one- and two-stage comparison is that the two-stage reactor has a net 55% higher energy production compared with the one-stage reactor. This amount is due to the higher removal efficiency of the two-stage system that leads to a higher biogas production and some reduced total solid concentration of the resulting digestate.

The upgrading process has been evaluated to achieve bio-methane production at a concentration above 98% [38] for the automotive sector.

Single-stage:

The energy required for AD is 5,607,764 Kcal/d for the single stage (substrate heating), and as specified above, the boiler efficiency is approximately 0.9. This leads to a net biogas production of 4,346 m^3/d, i.e., 181 m^3_{biogas}/h. Assuming a cost to upgrade of 0.25 €/m^3 biomethane produced (98% CH_4) using the pressure swing adsorption (PSA) technique [38], the expenses needed per day are 437 €/d with a biomethane production of 102 m^3/h (1,750 kg CH_4/d). Assuming a price of 0.99 €/kg, the annual net income (for 360 d/year) would be 466,182 €/year.

Two-stage:

The energy required for anaerobic digestion would be 6,825,284 Kcal/d for the double stage (substrate heating), and as specified above, the boiler efficiency is approximately 0.9. This leads to a net biogas production of 5,037 Nm^3/d, i.e., 181 Nm^3_{biogas}/h. Assuming the cost to upgrade is 0.25 €/Nm^3 biomethane produced (98% CH_4) using the pressure swing adsorption (PSA) technique, the expenses

needed per day would be 508 €/d with a biomethane production of 118 Nm^3/h (2,030 $kgCH_4/d$). Assuming a price of 0,99 €/kg, the annual net income (for 360 d/year) could be 540,874 €/year.

4. Conclusions

We determined how the anaerobic digestion of an organic substrate with a COD content of approximately 1 g/L of food waste or organic fraction of municipal solid waste could be utilized in both single-stage and double-stage approaches. The systems showed resilience and high biogas yields, although interesting issues for possible instability prevention have emerged via our multivariate analysis.

- By comparing the two AD systems, we observed that the two-stage has a removal efficiency that is 17% higher than the one-stage, and using a digestate dewatering post-treatment, the two-stage system has 33% less sludge for disposal.
- The SGP in the second phase was higher (0.89 m^3_{biogas}/kg_{VS}) than in the single-stage reactor (0.76 m^3_{biogas}/kg_{VS}).
- The overall double-phase system efficiency for removal augmentation was 16% more than for the single-stage system.
- Employing a two-phase rather than single-stage configuration process significantly affects the methanogenic process. Fermentation played the role of "pretreatment" for the food waste; it promotes the conversion efficiency of the volatile fraction to biogas. The payback time for introducing a fermenter is less than 1.5 year.
- Generally, no evidence of instability was observed during the transient conditions for both experiments, even though upon analysing the control vs. the single-stage system, we observed extraneous data during RUN IV. This extraneous data indicates the anomalous variability of the process.

In Italy, the profit from the production of electrical energy is 130 €/MWh (no incentives); hence, approximately 656,050 €/year and 768,269 €/year can be achieved for single- and double-stage systems, respectively, compared to a profit from bio-methane production of 466,182 €/year and 540,874 €/year for single- and double-stage systems, respectively. For biomethane to become

competitive with electric energy production and to drive biomethane production, in Italy, it is necessary to provides incentives for biomethane production.

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8. CHAPTER 3

Pilot scale fermentation coupled with anaerobic digestion

Research Paper

Pilot scale fermentation coupled with anaerobic digestion of food waste - effect of dynamic digestate recirculation

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Abstract

The anaerobic digestion in double stage is a known and adopted system, but the process productivity and optimization still remain an aspect to investigate. The accumulation of organic acids (produced during fermentative metabolism) in the first stage generally decrease the pH below the optimal values (5.5). A pre-evaluation strategy by control charts for further pH control is proposed. The process combines in series the 1st Fermentation process and the 2nd Anaerobic Digestion process, using the recirculation of the anaerobic digestion effluent, rich in buffer agents, to control the pH in the 1st stage. The recycle ratio becomes a further operating parameter that should be properly managed. A proper management as dynamic recirculation flow allows to maintain the pH of the first phase to values higher than 5. Specific hydrogen production, specific methane production and volatile fatty acid production; 170 LH_2/kgVS at 40% H_2, 710 LCH_4 at 67% CH_4, 14 gCOD/L VFA were obtained respectively.

Keywords

Hydrogen, methane, volatile fatty acids, process control, control chart, multivariate analysis

1. INTRODUCTION

Anaerobic digestion (AD) is a widespread and well known technology to treat organic waste of diverse stocks [1]. In the past it was considered as a system to manage the municipal waste. Nowadays the development of door-to-door separated waste collection makes the food waste an interesting source for energy and material production, and the AD becomes the main bio-refinery process able to answer to increasing energy demand. A further developing view of the AD process is to consider and manage it as a real production process [2]; therefore, the production should be maximized and its quality standardised.

AD involves different microorganism that through synergic way allow not only the production of methane but also other valuable products, hydrogen and volatile fatty acids [3]. In order to extract these different products, AD has to be split into two main phases [4][5][6].

The first phase of fermentation includes the step of hydrolysis, acidogenesis and part of the acetogenesis, instead the second phase substantially optimizes the last step, the methanogenesis. Therefore, through the optimization of the fermentation, hydrogen (gas), volatile fatty acid (liquid) and other low weight organic compounds such as alcohols and lactic acid [7] can be obtained. The VFAs can be used as external carbon source for biopolymers production, such as poly-hydroxyl-alkanoates [8][9].

Double stage AD is a known and adopted system, but the process productivity depends on HRT distribution between the two phases and pH control in the fermentation (1^{st} stage). In fact, HRT and pH control can affect (inhibit or promote) several metabolic pathways and consequently the production of volatile fatty acids and hydrogen.

At the first two stages AD process have been suggested to adjust the physiological conditions requirements by the respective microbes involved in the different process stages. The optimal pH values for the 1^{st} and 2^{nd} stage have, for example, been identified as pH 5.0-6.5 (for VFAs production), pH 5.5 (for Hydrogen production) and pH 7-8, respectively [10][11].

The accumulation of organic acids (produced during fermentative metabolism) in the first stage generally decrease the pH [12] below the optimal values. Recently some authors [13][14] proposed a strategy for pH control, coupling in series the 1^{st} fermentation process with a 2^{nd} anaerobic digestion

process and using the recirculation of the anaerobic digestion effluent, rich in buffer agents, to control the pH in the 1st stage.

The recycle ratio becomes a further operating parameter that should be properly managed. The literature points out how to work with an excessive recirculation may result in a gathering of ammonia in the system and consequently into an inhibition of methanogenic [15] and hydrogenogenic processes [12]. Conversely recirculation ratios too low may be insufficient to control the pH of the reaction medium where the hydrogenogenic process occurs. Many process variables to control the process are involved, hence the process monitoring and fault detection are very important tasks in this biological engineering systems since they aim to ensure the success of the planned operations and to improve the productivity [2]. Since the complexity of AD process, many highly correlated variables are measured and should be subject to considerable misleading in a non-statistical data mining. Further, [16] stated that important information lies not only in any individual variable but also in how the variables change with respect to one another. On basis of these observations AD requires the application of analytical multivariate statistical methods. Multivariate analysis is a method to detect patterns and correlations in large datasets [17] such as the several parameters monitored in anaerobic process. This approach has been used for a long time in the chemical processing, but was only introduced into the industrial wastewater treatment plants in the late 1990s. However, our understanding of the multivariate statistical methods as evaluation to further control the AD processes is lacking in literature.

The aim of this work was the study of recirculation ratio effect by multivariate methods in order to further develop an optimized automatic control able to optimize the hydrogen and/or VFAs production in the first phase, and methane generation in the second methanogenic one. Multivariate analysis, focus on pH role, allowed to better understand the behaviour on recirculation ratio variance.

2. MATERIAL AND METHODS

Initially, it has to be focused which region within the domain of possible values of the recirculation ratio to consider. This way can eliminate a variable from the system. In the case to operate in the region marked by high recirculation ratios, close to 1, the process control attention will be paid exclusively to the content of ammonia in the system, that accumulates persistently. Conversely, in the case to operate in the region characterized by low circulation ratio, next to 0.3, the goal will be to verify if this ratio is largely sufficient to ensure an effective and lasting control of the pH in the reaction medium of the fermentation process.

For this purpose, the experimental test was divided in three periods (RUNs): in RUN1 the recirculation ratio was kept on 0.4 during overall period while in RUN2 and RUN3 it was kept variable between 0.4 - 0.6 and 0.5 – 0.7 respectively, with a frequency of three weeks. In each trial of this study we wanted to understand the influence of each recirculation ratio choice has exercised alongside the fermentation process and the methanogenic process.

Table 1: Operational conditions applied during the experimental test

Parameters	units	RUN1	RUN2	RUN3
HRT 1 phase	d	3.3	3.3	3.3
HRT 2 phase	d	12.6	12.6	12.6
OLR 1 phase	KgTVS/(m^3.d)	17	17	17
OLR 2 phase	KgTVS/(m^3.d)	3.5	3.5	3.5
Recirculation Ratio	-	0.4	0.4 - 0.6	0.5 - 0.7

2.1.Analytical methods

Substrates and digestates of both reactors were monitored three times a week in terms of total and volatile solids (VS), soluble (sCOD) and total chemical oxygen demand (COD), total nitrogen (TN) and total phosphorus (TP). Process stability parameters, namely pH, VFAs, free ammonia (NH_3), total (T.ALK) and partial alkalinity (P.ALK) were checked daily. All the analyses, except for VFA and NH_3, were carried out in accordance with the Standard Methods [18].

NH_3 was determined from the equilibrium relationship with N-NH_4^+ (AMM) in soluble in the aqueous fraction (Anthonisen et al. 1976). VFAs content was monitored using a gas chromatograph (GC) (Carlo Erba instruments) with hydrogen as gas carrier, equipped with a Fused Silica Capillary Column (Supelco Nukol TM, 15 m x 0.53 mm x 0.5μm film thickness) and with a flame ionization detector (200 ^oC). The temperature during the analysis started from 80 ^oC and reaches 200 ^oC through two other steps at 140 ^oC and 160 ^oC, with a rate of 10 ^oC/min. Samples were centrifuged and filtrated on a 0.45 μm membrane prior analysis. Biogas production was measured with two flowmeters (Ritter Company, drum-type wet-test volumetric gas meters), fitted on the reactors. The specific methane production (SMP) was determined using the methane concentration in biogas which was measured by a GC equipped with a HP-Molesieve column (30 m x 0.3 mm x 0.25 μm film thickness) employing thermal conductivity detection (TCD).

2.2.Data analysis

According to [19] PCA is intended as a worthwhile chemometric technique when an effective reduction of the multidimensional space into few components is achieved, maintaining data variability. PCA provides an approximation of a dataset bringing back two matrices in reply: the matrix of scores and the matrix of loadings. In summary, these matrices capture the essential data patterns of the original dataset. Plotting the columns of the scores matrix gives a graph named score plot, where the relationship between observations is displayed and so clusters can be identified. Plotting the columns of the loading matrix returns another graph named loading plot, where the relationship between variables is showed. In this way, the power importance analysis of variables to identify clusters is accomplished.

2.3.Substrate and inoculum

The anaerobic digested sludge used as inoculum for the methanogenic reactors (single stage and second phase) was collected in the WWTP located in Treviso (northern Italy) where a 2000 m^3 anaerobic digester treats the source collected biowaste at 35 ºC. The sludge was acclimatized for one week to thermophilic temperature [20].

The substrate used in these experimental tests was the food waste from door-to-door collection of Treviso Municipality. The amount of total solids of biowaste used was 28% with a total volatile solids (TVS) on TS content of 92%. Regarding the content of nutrients, table 2 shows how food waste used in this study was characterized by an adequate nutrients ratio, particularly COD:N ratio with an average value of 41.

The fermentative reactor (first phase) was inoculated with food waste and water and then regularly fed with separately collected food waste and water in order to reach the volume required.

2.4.Reactor set-up

The reactors used were made of stainless steel AISI-304 with a working volume of 230 L for one-stage digester, and with reference to the two-stage process of 200 L for the fermentation unit and 380 L for the digester unit. Mechanical anchor agitators ensured the mixing in order to maximize the degree of homogenization inside the reactor. The working temperature was set at 55°C ± 0.1 (thermophilic temperature range) and maintained by an external jacket. The reactors were slightly pressurised at 0.01 atm.

2.5.First stage (Hydrolysis) batch tests

Batch tests were carried out to determine the hydrolysis step of food waste fermentation.

This part of the study was performed in order to investigate the hydrolysis in batch tests and the effective amount of volatile acids (VA) produced in relation to the pH. Hydrolysis potential batch tests (HPB) were carried out to determine the amount of VFAs and Lactic Acid (LA) production of the food waste with tap water in thermophilic condition.

First batch test was set up in triplicate mimicking the fermenter using different food waste to water ratio in order to determine the amount of VFAs and LA produced and observe the change in pH while the hydrolysis proceeds. Afterwards all the vials were flushed with a mixture of N_2 and CO_2 (80% and 20% respectively). These batch tests were run for one week. Everyday, samples were taken for pH, VFAs and LA analysis and hydrogen production. The pH was measured using pH meter and VFAs analysis performed. As suspect of lactic acid production, some representative samples were analysed with the HPLC. The procedure for lactic analysis, 2M H_2SO_4 was used during sample preparation and the analysis was conducted using a HPLC (Ultimate 3000 Dionex^{TM}); HPLC on a Dionex Ultimate 3000-LC system (Dionex Corporation, Sunnyvale, CA, USA) with an Aminex® HPX-87H column coupled to a refractive index detector. As mobile phase H_2SO_4 (4 mM) was used, with a flow rate of 0.6 ml/min at 60°C. All chromatograms were integrated using the Chromeleon software (Dionex Corporation).

The bio-hydrogen produced was also measured with the GC abovementioned. Total alkalinity was measured during the trial. Methane (CH_4) production in the different vials was analysed by injecting gas samples from the headspace of each vial into the abovementioned GC for methane analysis and the batch vials were degasified whenever over-pressure of more than 1 bar was detected. Methane was analysed in order to understand when the hydrolysis in batch switched to a methanogenic activity.

3. RESULT AND DISCUSSION

3.1.First phase

The scope to find a suitable management of the process led to an accurate analysis the proper recirculation ratio to adopt. To control the pH in the first stage by means of the digestate recirculation is advantageous economically, however it must be operated appropriately, otherwise the process itself leads to instability. [14] have shown how working with a high recirculation ratio would lead to an accumulation of ammonia in the system, able to inhibit both the methanogen consortium that the hydrogenogenic process. For this aim, three runs were tested; in each run a different strategy for the controlling of the pH were applied. In RUN1 the recirculation ratio was maintained to 0.4 for overall

period, instead the RUN2 and RUN3 were operated with a variable recirculation ratio between 0.4 – 0.6 and 0.5 – 0.7 respectively, by varying this parameter alternately with a three-week frequency.
In figure 1, the trend of pH for three RUNs is presented.

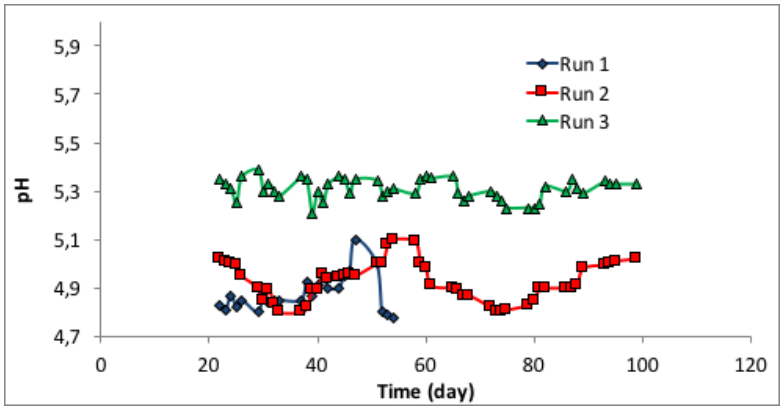
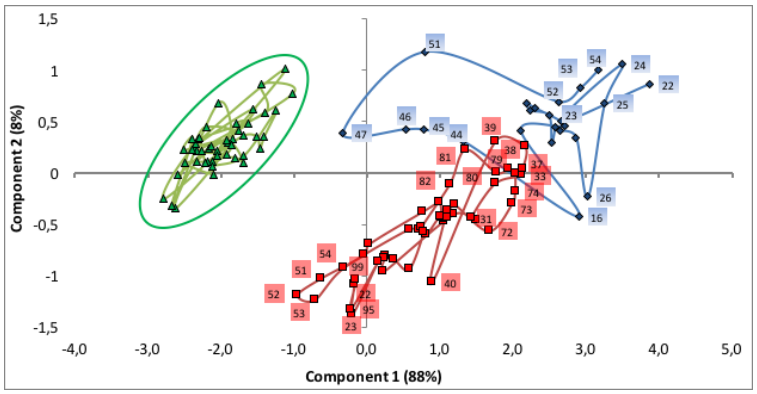
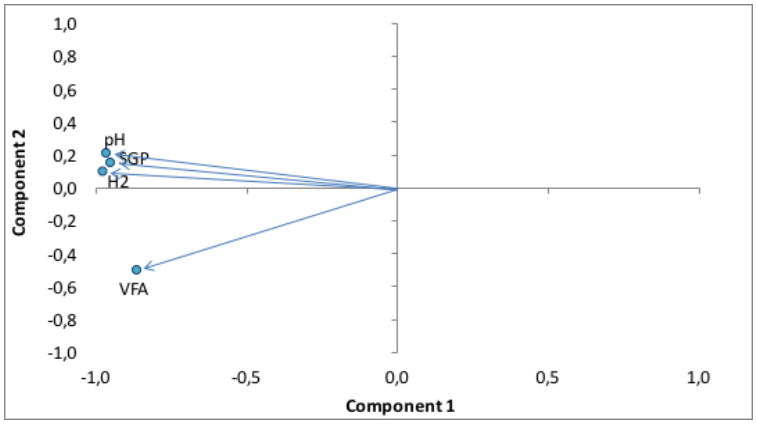


Figure 1: first phase pH trend during the three RUNs.

The figure 1 shows how the RUN3 was the sole run where the pH was kept above 5 for the overall experimental trial. During the RUN1 the pH of the reaction medium has exceeded the value 5 only towards the end of the trial and moreover it was able to remain in this condition for a very short time. The low pH value of the reaction medium has adversely affected the hydrogenogenic activity reporting a low production of hydrogen (27 LH_2/kgVS) and VFAs (7241 mg/L).
Observing the pH trend of RUN2 (figure 1) it is possible to note the average pH was lower than 5, values above 5 were detected only for a few days at the end of the 0.6 recycle period ratio. In other words, during the RUN2 and RUN3 the alkalinity contribution provided by the digestate was not able to buffer acids produced during the fermentation phase. Also in this case the performance of the VFA and the hydrogen production were affected by the variability of the pH, 37 LH_2/kgVS and 9185 mg/L respectively.
To understand the different strategies effects of recirculation ratio applied on the production variables, principal component analysis (PCA) was used. PCA allows to reduce the multidimensional space into few components and therefore to study the relationship among variables and objects in the modelled space formed by principal components (PCs), saving data variability. Figure 2 shows the score and loading plots formed by the first and second PC (explained variances were designated in parenthesis).



(a)



(b)

Figure 2. Score (a) and loading (b) plot

Observing the loading plot (figure 2b) we can note how the pH was directly correlated with volatile fatty acids and hydrogen production. These evidences are in according with [13] and [21]. The latter authors showed how the acetic acid can inhibit the metabolic activity of *Clostridium thermoaceticum* when the pH of reaction medium was lower than 5. Thus, the lower production of VFAs could been related to a detoxification mechanism of the cell to avoid the inhibitory effects.

From the Score plot (figure 2a) we can just identify the cluster associated to the RUN3 (green ellipse) while a portion of the cases associated to the RUN1 and RUN2 showed themselves not distinguishable. Higher pH, VFAs yields, SGP and $\%H_2$ characterize RUN3 than the other RUNs. Moreover, in RUN1 and RUN2 we note a higher and non-random variability than RUN3.

For the study of the variability of the RUN1 and RUN2, the multivariate control chart approach was adopted [22].

RUN1 the control chart shows that in the period in which the reaction medium has exceeded value 5, which is returned to the desired range, the process has highlighted very different characteristics

compared to the previous condition. In particular, in the production of volatile fatty acids and the specific biogas production.

To understand the direction these variables have been taking in order to determine the shift of the process, a reduction of the dimensionality of the problem was performed, through the use of the principal components and the application of the Shewhart control chart.

The first principal component extracted 77% of the total information and it was sufficient to describe the problem based on Rank Analysis criteria.

The x-bar chart RUN1 (x bar chart figure 3) confirmed an outside control signal which is much above the Control Limit (3σ). Whereas the loadings of the first component we can underline as the out of control signal was due to high values of all the variables considered: pH 0.90 (first PC), VFA 0.88, 0.83 SGP, H_2 0.85.

On reaching the pH value of the first phase to values greater than 5, the fermentation process has highlighted an important change of condition. The system switched from a purely solvatogenic condition, characterized by a low production of VFA and hydrogen, to an acidogenic one, vice versa characterized by an increased production of volatile fatty acids and hydrogen. It is finally noted that the increase of the hydrogen production is mostly due to the increase of the SGP, instead of the hydrogen percentage in the biogas produced. In general, there was a positive correlation between the pH, the specific hydrogen production and VFAs production, which confirmed what we wanted to demonstrate.

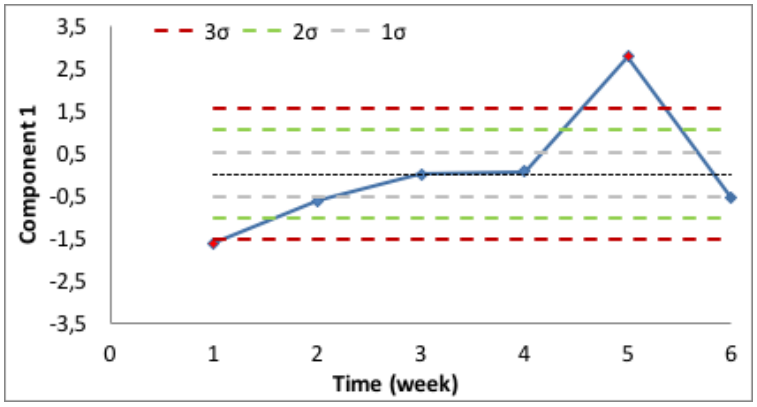


Figure 3. X bar chart of the RUN1

As a result of the accumulation of volatile fatty acids in the reaction medium, the alkalinity fed with the recirculated digestate was not able to maintain a condition of pH to a value greater than 5. A

consequence of this the process switched back to the previous condition. In conclusion of this first RUN1 it was not possible to maintain the pH of the fermentation process above 5 through the use of a constant recirculation ratio equal to 0.4.

Through the RUN2 the first principal component describes the data to 90% and is therefore also in this case sufficient to describe the process.

The x bar chart (figure 4) confirmed the hypothesis expressed in the previous RUN1. The oscillatory trend of the principal component in the x-bar shows how the process does not respond to a single distribution but two partially overlapping. On the basis of the considerations in the RUN1, also in the RUN2 is possible to consider that the recirculation ratio strategy adopted in RUN2 swung the process in two different conditions, one acidogenic and one solvatogenic. Also in this trial is decisive the pH contribution to promote the two processes.

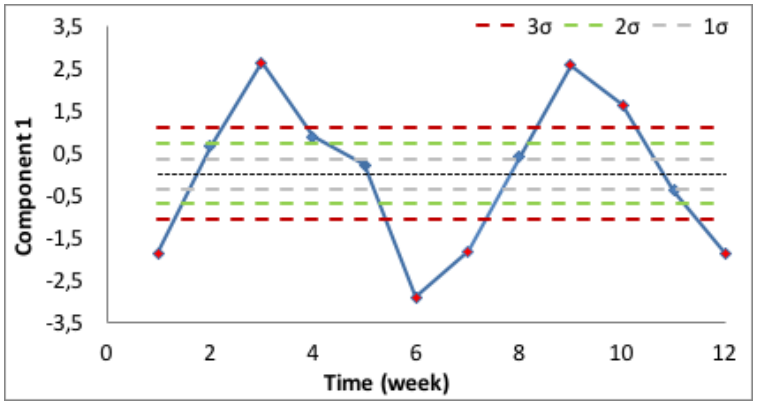


Figure 4. x bar chart of the RUN2

The fermentation process in RUN3 is most suitable for the production of VFA and hydrogen. The choice to operate with a variable recirculation ratio of 0.5 and 0.7 has allowed the accumulation of HCO_3^- in the reaction medium. It has favoured the establishment of a buffer capacity which ensured the process stability even in the period of 0.5 recirculation ratio.

The trial RUN3 never left its optimum fermentation environment (cluster analysis figure 3), one that is within the pH above 5. Moreover, in this case a control chart does not show points out of control due to metabolic switch toward solvatogenic neither methanogenic conditions. Unlike other approaches, the pH of the first stage is maintained for the entire experiment above 5 and it was not affected by the fluctuation of the recirculation ratio. Better performance on VFAs production (table 3 shows the main chemical - physical characteristics of the reaction medium, the

stability parameters and production yields related to the fermentation process hydrogenogenic during RUN3), biogas composition and yields on the first stage.
The fermentation of RUN3 process is more suitable for the production of hydrogen and VFAs.

Table 3: stability parameters, chemical-physical characteristics and process yields for the Ist phase RUN3

	Parameter	M.U.	Average ± St.Dev.	Min	Max
I° PHASE	TS	gTS/Kg	53 ± 5	46	61
	TVS	gTVS/Kg	44 ± 4	39	46
	COD	gO_2/Kg	52 ± 9	41	63
	TKN	gN/Kg	1.6 ± 0.7	0.9	2.6
	P tot	gP/Kg	0.48 ± 0.10	0.45	0.50
	pH	-	5.3 ± 0.1	5.21	5.39
	VFA	mgO_2/L	13,920 ± 488	11,616	14,957
	Total Ammonia	$mgN\text{-}NH_4^+$/L	687 ± 5	678	696
	SGP	Nm^3/KgTVS	0.170 ± 0.010	0.165	0.172
	GPR	$Nm^3/(m^3.d)$	2.88 ± 0.04	2.72	2.95
	H_2	%	40 ± 2	36	44
	CO_2		52 ± 2	47	58
	CH_4		7 ± 1	5	10

3.2.Hydrolysis batch tests

Different amounts of water were added to mimic a higher water saturation of the waste. The results in table 4 reveal that the VFA concentration increased with adding less water, but the total amount of VFA released from the waste decreased with lower water saturation. The highest VFA release (1.52 g) corresponded to a conversion efficiency of the total organic matter (with a VS content of 27%) into VFA of 24.8%.

Table 4. VFA release in batch hydrolysis setup with different amounts of water added

Set-up	OFMSW (g)	H_2O (mL)	Waste/percolate ratio	VFAs (g/L)	VFA (g)
A	23.4	300.0	7.8%	5.05	1.52
B	23.4	221.0	10.6%	6.17	1.36
C	23.4	158.0	14.8%	7.55	1.19
D	23.4	78.8	29.7%	11.30	0.89

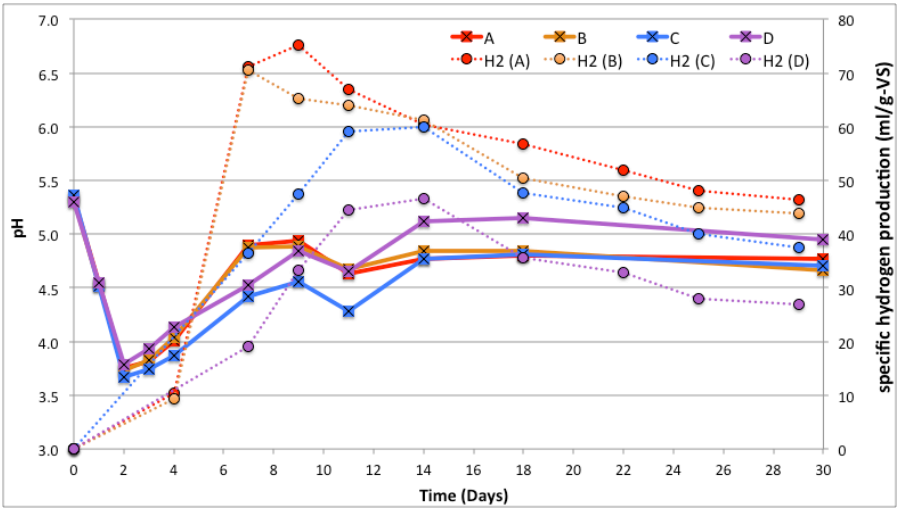


Figure 5. Hydrogen production during the second HBT trials.

The production of hydrogen was detected and the data showed great variability amongst the batch set-up. All samples showed an increase in hydrogen production over the first 10 days and eventually a decrease. No hydrogen production was detected when pH was below 4.5.

For all the samples, the pH fell rapidly in the first 2-3 days to around 3.70 before it rose again and reached a plateau (figure 5). The anomalous pH drops for all samples around day 10 could be due to pH calibration error. The overall pH of each setting, after 30 days, has no significant differences; data after 30 days showed a standard deviation of 0.13. As expected, there is evidence of high production of lactic acid in all samples of the first 4 days that is responsible for the rapid drop in pH of all batch setup (figure 5). The maximum concentration of lactic acid ranges from 4.5 to 14 g/L. The more concentrated the food waste biomass is, the higher the concentration of lactic acid. There is a high possibility that there would be lactic acid production taking place in the reactor setup. It is therefore important to not have too high the organic loading in the reactor setup as this would result in unwanted lactic acid production or to control the pH of the fermentation phase above pH 5. There is a high possibility that lactic acid is produced in the reactor setup below pH 5 [21]. However, it can be seen that the high lactic acid concentration for every batch setup correlate to a pH below 4.5 [23]. Therefore, lactic acid production could have already been avoided in the reactor as the pH will be keep strictly in the range of 5-5.5, by a dynamic digestate recirculation.

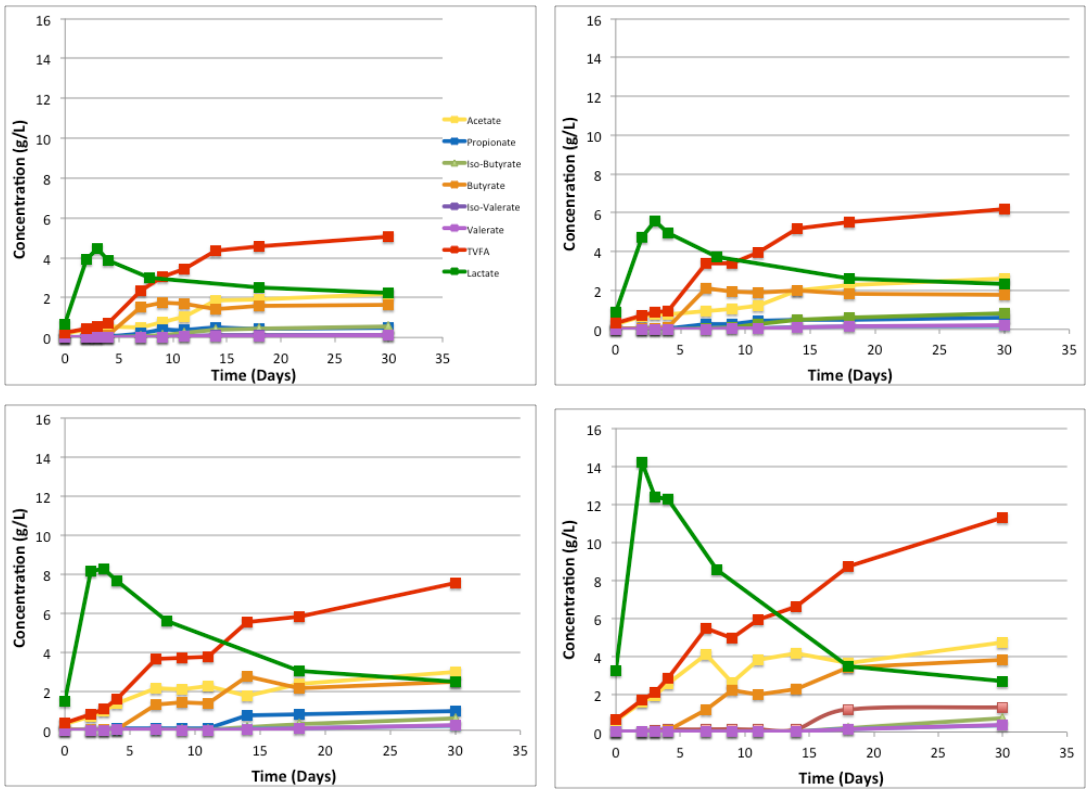


Figure 6. Lactic acid and VFAs production of the four hydrolysis batch tests.

Likewise, the VFA concentration increased as the water concentration decreased. The concentration of VFA for A, B, C, D after 30 days were 5.05 g/L, 6.17 g/L, 7.55 g/L and 11.30 g/L respectively. However, the VFAs in grams for A, B, C and D were 1.51 g/L, 1.36 g/L, 1.19 g/L and 0.89 g/L.

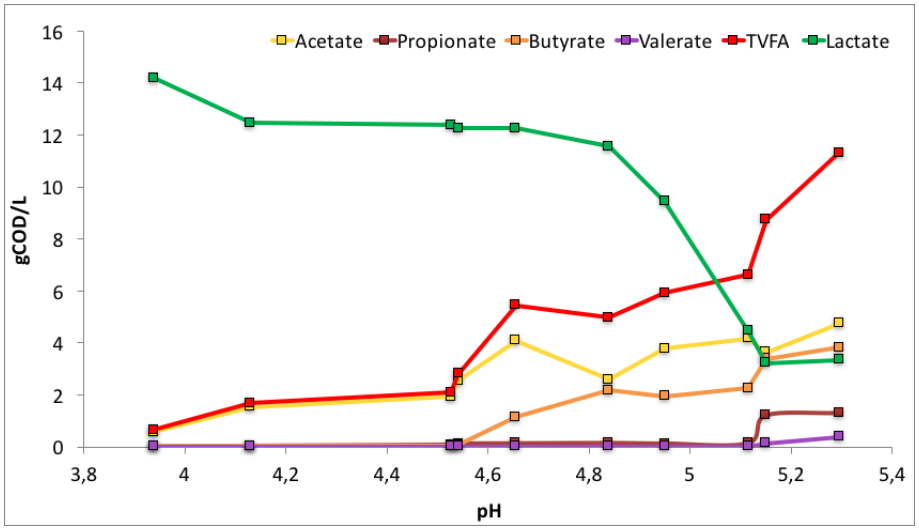


Figure 7. Lactic Acid and Volatile Fatty Acid production related to pH tendency on HBT.

In figure 7, the volatile acids on pH function are reported. It is possible to underline in what way below pH 5 the lactic acid is predominant, on the contrary the VFAs production, particularly acetic acid and butyric acid is noticeably incremented at pH values higher than 5.
It is demonstrated how above pH 5 volatile fatty acid production is enhanced, this is well correlated with literature data of [24].

3.3.Second phase

Figure 8 shows the results of analysing agglomerative hierarchical cluster.

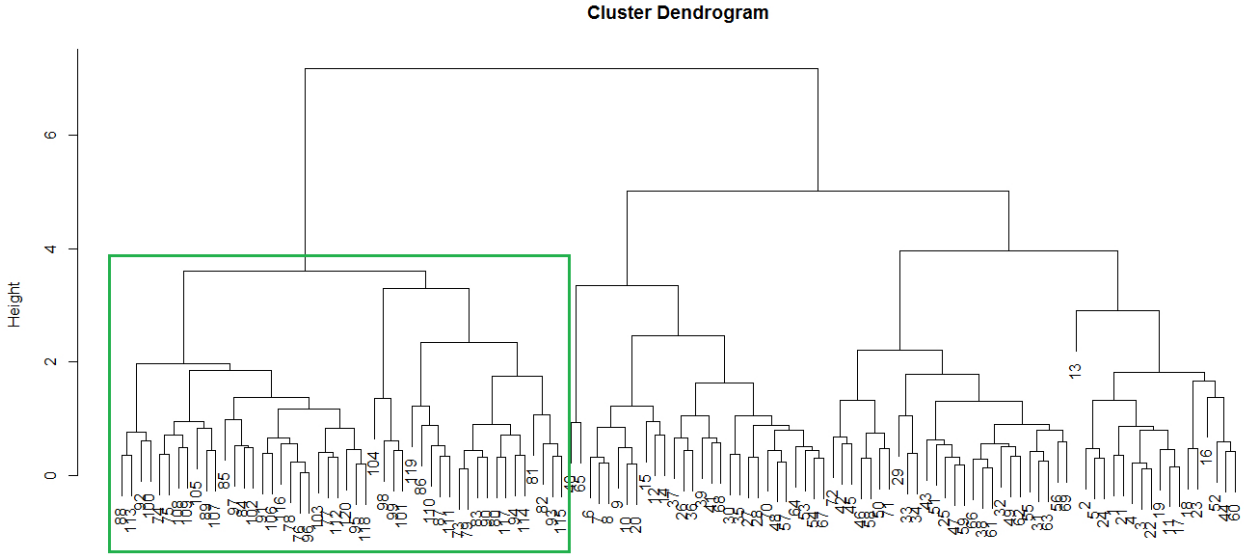
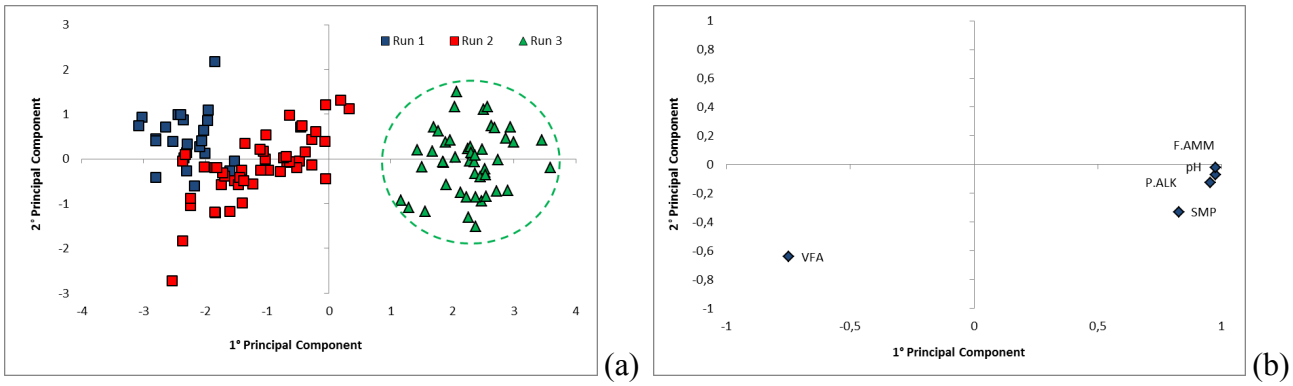


Figure 8. Cluster Dendrogram 1-24 match RUN 1, 25-73 RUN 2, 74-120 RUN 3

The objects of the dendrogram from 1-24 match RUN 1, 25-73 RUN 2, 74-120 RUN 3.
The algorithm performed the fusion of objects considered by increasingly larger cluster size as the distance among the objects (decreasing similarity).
One grouping on the RUN 3 is detectable. The other two groups (RUN 1, 2) are indistinguishable. This analytical methodology highlights how recirculation ratio 0.4 and ratio 0.4 – 0.6 (variable) did not produced a visible change in the characterization of the methanogenic process. On the other hand, the recirculation ratio of RUN 3 allowed to obtain a distinguishable process among the previous RUNs, based on the 5 variables considered (pH, NH_3, alkalinity, SMP, VFA). The obtained result underlined that it was necessary to analyse the role of these variables that helped to distinguish the methanogenic process RUN 3. This agglomerative hierarchical analysis does not allow to obtain this information, which is possible to obtain through the principal component analysis instead. By

principal components analysis it is in fact possible to comprehend the relevance of the original variables have had in the clusters analysis by Loading Plot graph.



(a) (b)

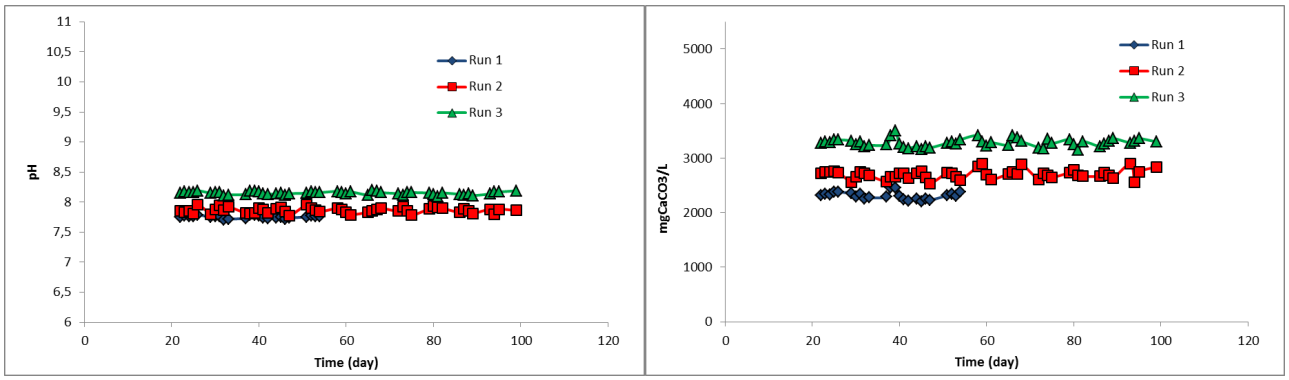
Figure 9. Score plot (a) and Loading plot (b) of the second methanogenic phase RUNs.

The Score Plot allows the first principal component, which extracts 78% of the information overall, to show a clear separation among the observations on the RUN3 among the other RUNs.

The analysis of the Score plot jointly the Loading Plot highlights the role of the five variables.

As previously, also by means of the use of the main components it was able to isolate only the cluster relative to the RUN3. Interpreting jointly the Score plot with the plot Loading is possible to notice how the second stage of RUN3 is distinct from the remaining tests; it is characterized by higher pH, partial alkalinity and higher SMP and minor VFAs content, which indicates a better efficiency of the process.

As regards the second phase, in the next figure the stability parameters trends are reported.



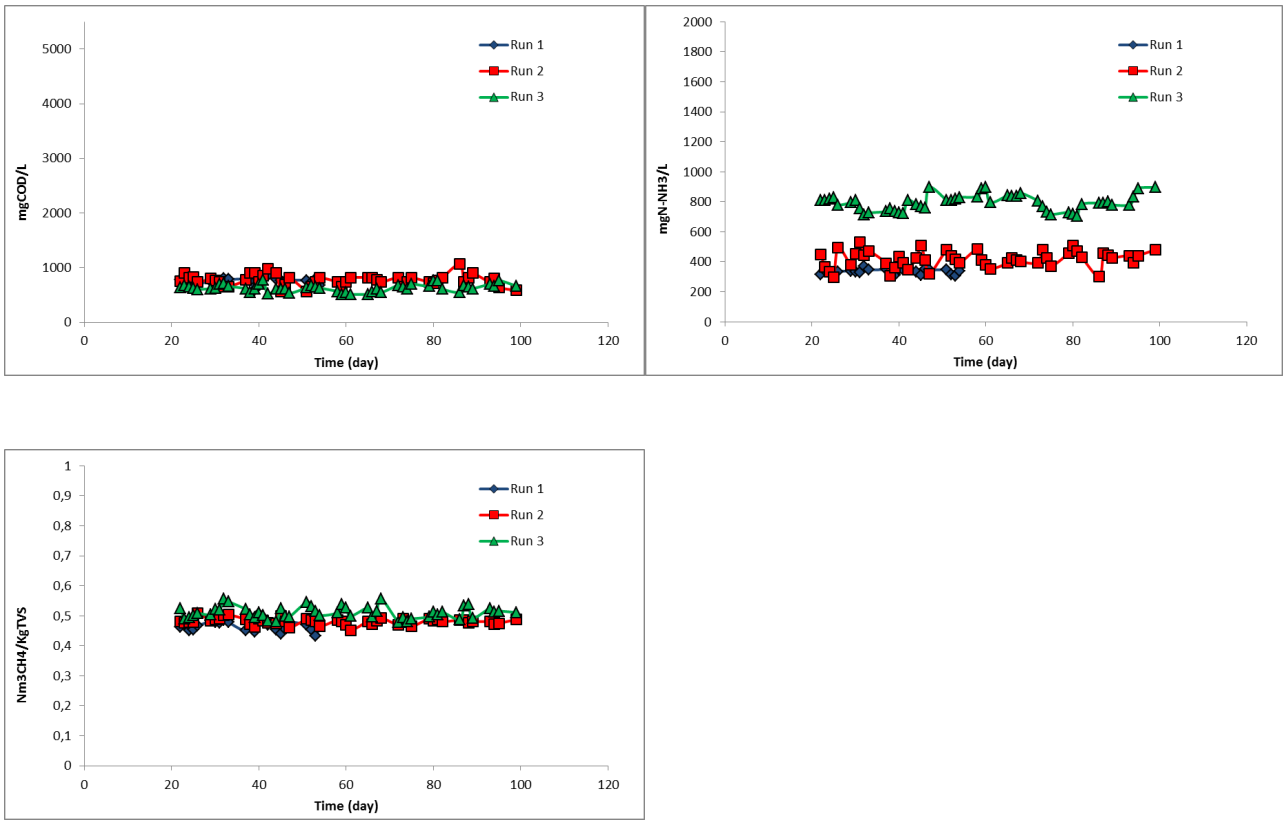


Figure 8. Stability parameters during the three RUNs in the second methanogen phase.

Table 5 shows the main chemical - physical characteristics of the reaction medium, the stability parameters and the production yields related to the methanogenic process during RUN3.

Table 5. Stability parameters, chemical-physical characteristics and process yields for the IInd phase RUN3

	Parameter	M.U.	Average ± St.Dev.	Min	Max
II° PHASE	TS	gTS/Kg	23.2 ± 4	26	30
	TVS	gTVS/Kg	16 ± 3	10	21
	COD	gO_2/Kg	20 ± 2	19	23
	TKN	gN/Kg	1.5 ± 0.2	1.0	1.8
	P tot	gP/Kg	0.21 ± 0.01	0.10	0.25
	pH	-	8.15 ± 0.10	8.10	8.20
	P. Alkalinity	$mgCaCO_3$/L	3,283 ± 73	3,145	3,498
	T. Alkalinity		5,256 ± 50	5,157	5,376
	VFA	mgO_2/L	631 ± 72	449	781
	Total Ammonia	$mgN\text{-}NH_4^+$/L	1,539 ± 148	1,290	1,885
	Free Ammonia	$mgN\text{-}NH_3$/L	794 ± 52	706	898

SGP	$Nm^3/KgTVS$	0.75 ± 0.02	0.71	0.79
GPR	$Nm^3/(m^3.d)$	2.50 ± 0.10	2.37	2.77
CH_4	%	67 ± 2	64	70
CO_2		32 ± 2	29	35

4. CONCLUSIONS

In conclusion, Cluster analysis allowed to understand how among the three processes studied only the RUN3 has shown a different condition, in the direction of a better efficiency of the process, both from yields point of view and through stability process parameters, in particular the higher alkalinity amount in the reaction medium.

A proper management of the recirculation allows to maintain the pH of the first phase to values higher than 5. It allows to foster metabolic hydrogenogenic processes and it seems also to improve the environmental conditions occurring the methanogenic processes, in particular by increasing the alkalinity of the reaction medium.

Acknowledgments

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Research paper

Automatic process control for stable bio-hythane production in two-phase thermophilic anaerobic digestion of food waste

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Abstract

The paper reports the results of a long term (310 days) pilot-scale trial where food waste as sole substrate was treated in a two-phase thermophilic anaerobic digestion process. This was optimized for concurrent hydrogen and methane production. First phase's optimization for hydrogen production was obtained recirculating the effluent coming from the methanogenic phase and without the addition of external chemicals. A drawback of such approach is the recirculation of ammonia into the first phase reactor for hydrogen production with possibility of consequent inhibition.
Therefore this study was focused on the development of a control protocol based on ammonia concentration. The first part of this paper illustrates how the use of a variable recirculation flow makes possible to control the whole process, preventing the ammonia inhibition in the system. In order to lay down the groundwork for an automatic control of the process, in the second part of the study a preliminary statistical study is presented. In the latter are developed models to predict ammonia levels in system using the measure of Electrical Conductivity, Volatile Fatty Acids and Alkalinity.
During steady state conditions, managed by a variable recirculation flow, the system produced a mixture of gas that met the standards for the biohythane mix with an average composition range of

7% H_2, 58% CH_4 and 35% CO_2. The overall average specific gas production (SGP) reached 0.69 m^3_{Biogas}/kgTVS and gas production rate (GPR) of 2.78 $m^3/m^3_r d$.

Keywords: bio-hythane, hydrogen, process control, ammonia, food waste, anaerobic digestion.

Abbreviations

AD: Anaerobic Digestion, ALK tot: Total Alkalinity, BHy: Biohythane, COD: Chemical Oxygen Demand, COND: Conductivity, CSTR: Continuous Stirred Tank Reactor, DF: Dark Fermentation, DW: Dry Weight, GP: Gas Production, GPR: Gas Production Rate, HPR: Hydrogen Production Rate, HRT: Hydraulic Retention Time, MPR: Methane Production Rate, OLR: Organic Loading Rate, Ptot: Total Phosphorus, SDEC: Standard Deviation Error in Calculation, SDEP: Standard Deviation of Prediction Errors, SGP: Specific Gas Production, SHP: Specific Hydrogen Production, SMP: Specific Methane Production, SSC: Steady State Conditions, TKN: Total Kjiendhal Nitrogen, TS: Total Solids, TVS: Total Volatile Solids, VFAs: Volatile Fatty Acids, WW: Wet Weight, WWTP: Waste Water Treatment Plant

1. Introduction

The use of anaerobic digestion (AD) for treatment of biowaste and other organic waste/residues has been growing consistently for the last 30 years in Europe. A step forward for the common anaerobic digestion process of biowaste, which has gained interest among the researchers, is the two-stage approach finalized to the production of hydrogen in the first phase reactor and methane in the second one [1].

Today the hydrogen production by fermentative processes of carbohydrate-rich substrates (like biowaste, food waste and similar), named Dark Fermentation (DF), is one of the most promising technologies for high yield hydrogen production. Several studies showed that DF could be coupled with AD in order to obtain a mixture of gases to be used separately or mixed together: the typical average composition for commercial porpoises is 10% H_2, 30% CO_2 and 60% of CH_4, to achieve a second generation biofuel that can be of great interest for combined heat and power (CHP), cogenerator motors or the automotive industry [2], as a result of the upgrade for the elimination of CO_2.

In DF processes the activity of enzyme hydrogenase is strongly influenced by environmental factors,

such as pH and temperature, whose optimal values for maximum activity were identified to be 5.5 and 55 °C, respectively [3, 4]. The pH range affects hydrogen production greatly. Maintaining pH in a given range of values for a prolonged exercise is often a hitch without an external chemical control, because the high loads applied to this type of processes involve an accumulation of VFA, resulting in an increase of acidity. Moving to values below 5, the process is controlled by fermentative metabolism, giving typical products of solventogenesis (alcohols and lactic acid) [5].

If we take a look at the literature on this topic, there are several cases in which an external control of pH is provided. Talking about this, in recent years a perceptive practice has been developing as a less expensive alternative to the use of chemicals for external control of the pH in the phase of Dark Fermentation to maintain the pH within the optimal range (5 – 6) for the hydrogenase catalysed reactions [5]. It consists in applying a recirculation to the head to the process from the stage of methanogenesis in order to exploit the residual buffer capacity (ammonia and bicarbonate) of this substrate [6]. Moreover, the application of a recirculation flow allows to balance the nutrients intake and helps dilute the feedstock [7, 8].

Therefore, also from an economical point of view, it is convenient to develop a pH control system which allows to manage and optimize the process in a sustainable approach, because neither chemical addition nor high costs devices would have to be used to reach the target. Therefore, this research deals with the optimization of a two-phase anaerobic digestion process that treats food waste for bio-hythane production without additional external chemicals.

Considering a long term management of the process, the main problem that can occur in a two-phase system with recirculation flow relates to the accumulation of ammonia: in thermophilic condition, free ammonia leads to inhibition of methane production in concentration exceeding 700 mg/l [9]. It is also important to point out that the rate of hydrogen production can be inhibited by the presence of ammonia at high concentrations [10, 11].

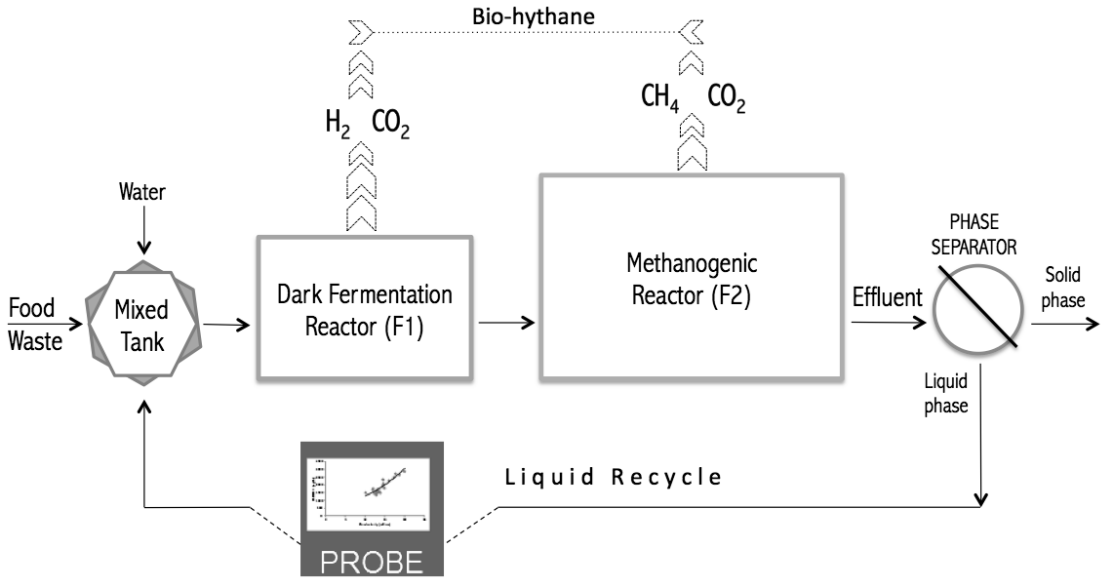
According to this strategy the decisive step was to affirm the possibility to set the stability parameters to maintain the process strong and durable, performing a control system (possibly an automatic device) which allows to maintain the right amount of recycle according to the change of the stability parameters of the reactors in real time.

The main problem linked to this approach regards the choice of control parameters to be measured on line. In fact, ammonia concentration probes in such heterogeneous media could be difficult to use and, on the long-run, may prove to be not reliable. Thus, the approach used here considers to use an indirect measure of the main control parameter, using simpler ‘predictors’. These indirect predictors

can be also measured in easier way using on line probes.

Therefore, this paper is dedicated to the definition of the best control parameters for process control.

Figure 1. Two-phase process



2. Material and methods

2.1 Experimental set-up

Two stainless steel CSTR reactors (AISI 304) were used for biohythane production. The first reactor (F1), dedicated to the fermentative step, had a 200 l working volume, while the second reactor (F2), dedicated to the methanogenic step, had a 380 l working volume. Both reactors were heated by a hot water recirculation system and maintained at 55°C using electrical heater controlled by a PT100-based thermostatic probe. The feeding system was semi-continuous, arranged once per day. The organic waste was reduced in size using a grinder, mixed with tap water and liquid fraction of sludge recirculation from the methanogenic reactor and then fed to the first reactor.

The process was maintained in operation for 310 days. The operational conditions we applied were for the DF phase a HRT of 3.3 days and a OLR of 18.4 kgVS/m^{3}d; for the Methanogenic phase a HRT of 12.6 days and a OLR of 4.8 kgVS/m^{3}d. Regarding the recirculation ratio, the entire experimental test has been divided in three periods: during the first and second working periods the recirculation ratio was maintained steady (0.5 and 0.25 respectively), during the third working period

the recirculation ratio was variable according to the ammonia concentration in the system.

2.2 Substrate and inoculum

The first reactor, devoted to hydrogen production, was not inoculated with an active biomass but it was filled up with a mixture of organic waste coming from the municipality of Treviso and tap water, in order to obtain a total solids content of about 8% [10]. Biowaste has a high carbohydrate content that can be converted into hydrogen and organic acids through the action of fermentative bacteria [6]. The use of biowaste generates an inoculum capable of producing hydrogen in short times, for the presence of indigenous bacterial communities. Afterwards, the first reactor was daily fed with a liquid mixture of organic waste, sludge recycled from second phase and water in order to reach the required volume. The typical composition of the collected waste and main chemical – physical characteristics of the biowaste used as fed are shown in Table 1.

Table 1. Food waste composition [10]

Commodity class	Treviso Plant	
	% WW	% DW
Fruits & Vegetables	38-46	30-38
Kitchen waste *	13-16	12-19
Paper & paperboard **	13-18	15-19
Plastic **	5-10	7-14
Aggregates/Inerts **	6-12	14-19
Not classifiable	10-20	13-25

Table 2. Chemical-physical characteristics of treated food waste

Parameter	Unit	Average Value	min	Max
Total Solid	gTS/kg waste	260 ± 44	151	331
Volatile Solid	gVS/kg waste	216 ± 36	133	283
VS/TS	%	83 ± 3	80	88
P-PO_4	gP/kgTS	3.2 ± 0.5	2.0	3.7
TKN	gN/kgTS	29 ± 8	13	44
COD	gO_2/kgTS	954 ± 83	880	1103

* Putrescible material non-vegetable (eg pasta, cakes, meat, etc..). ** Fraction gray (paper and board + plastic + aggregates) WW = wet weight. DW = dry weight

The methanogenic reactor was inoculated with the anaerobic digested sludge coming from the full-

scale digester of Treviso WWTP [17] and maintained at 55°C for one week and daily fed with the effluent from the first phase.

2.3 Analytical methods

The effluents of the reactor were monitored 2 to 3 times per week in terms of total and volatile solids content, chemical oxygen demand, TKN and total phosphorus. The remaining parameters, namely pH, conductivity, volatile fatty acids content and speciation, total and partial alkalinity and ammonia, were checked daily. All the analyses, except for VFAs, were carried out in accordance with the Standard Methods [12]. The analysis of the volatile fatty acids was carried out with a Carlo Erba™ gas chromatograph equipped with a flame ionization detector (T = 200 °C), a fused silica capillary column Supelco NUKOL™ (15 m x 0.53 mm x 0.5 μm thickness of the film), while hydrogen was used as carrier gas. The analysis was conducted using a temperature ramp from 80 °C to 200 °C (10 °C / min). The samples were analyzed before being centrifuged and filtered with a 0.45 μm filter.
The production of gas for both reactors was monitored by two flow meters (Ritter Company™, drum-type wet-test volumetric gas meters). The percentages of methane, carbon dioxide and oxygen were determined by an infrared gas analyzer portable GA2000™ (Geotechnical Instruments™). The percentage of hydrogen and methane was determined by a gas chromatograph GC Agilent Technology 6890N™ equipped with a column HP-PLOT MOLESIEVE™ (30 m x 0.53 m ID x 25um thickness of the film), using a thermal conductivity detector (TCD) and Argon as gas carrier.

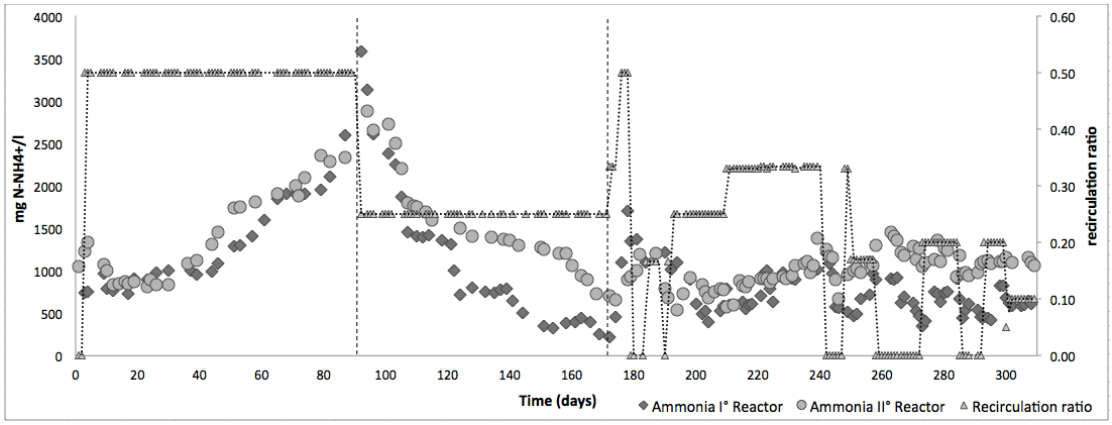
2.4 Chemometric approach (statistical analysis)

The study has evolved to find which relationship may connect the total ammonia to conductivity. Throughout all the data-base of the experimental long term process of bio-hythane production was used, 180 data-set of the anaerobic digestion process.
The statistical analysis was outwardly validated in order to compare and determine the best possible prediction of the parameter ammonia nitrogen. These operations of calculation and statistical development were carried out with the aid of the software "STATISTICA", "XLSTAT" and "R".

3. Results and discussion

The average amount of total solids of biowaste used as feedstock in this experimentation was 26%, 83% of whom volatile, with a COD/TS ratio of 0.9. The COD:N:P ratio was about 298:9:1 (table 2). The process has lasted 310 days with the aim to determinate the best recirculation ratio that allows for the control of the system and, at the same time, the best yield in terms of biohythane. For this purpose three conditions were tested: in the first period the recirculation ratio used was 0.5 v/v, in the second period the recirculation ratio used was 0.25 and, finally, in the third period the recirculation ratio was maintained variable in according of the ammonia concentration in the system. Below, in Figure 2, the trend of recirculation ratio versus ammonia concentration in the first and second reactor is shown.

Figure 2. Total ammonium trend and recirculation ratio during experimentation trial

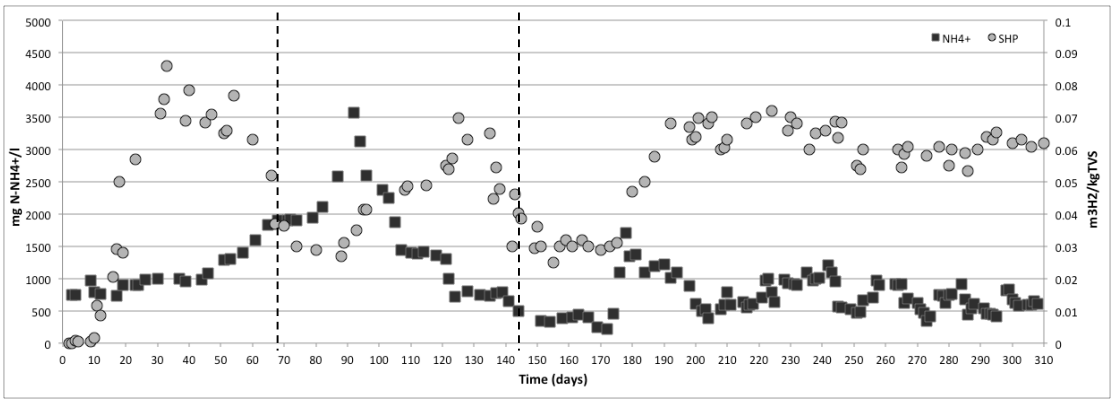


As shown in figure 3 (a) (b), in the first period (1 – 90 working days), the maximum yields of gas production, in terms of specific hydrogen and methane production, were achieved: 0.085 $m^3H_2/kgTVS_{fed}$ and 0.52 $m^3CH_4/kgTVS_{fed}$, respectively. However, with this recirculation ratio, as shown in Figure 2, the ammonia concentration in the system continued to increase to the point it exceeded the threshold values of inhibition of the whole biological process, as demonstrated by the decrease of specific hydrogen and methane production (Figure 3) and by the accumulation of volatile fatty acids [18] in the second reactor (up to 9.5 g/l). A clear evidence of the process upset can be easily observed considering the final part of the period, around days 80-90 in which the trends of ammonia concentration and hydrogen specific productions (SHP) are clearly in a opposite way. When ammonia passed a threshold of 2 gN/l SHP decreased from 0.075 $m^3H_2/kgVS$ down to 0.03 $m^3H_2/kgVS$. Also methane specific production (SMP) showed a similar trend.

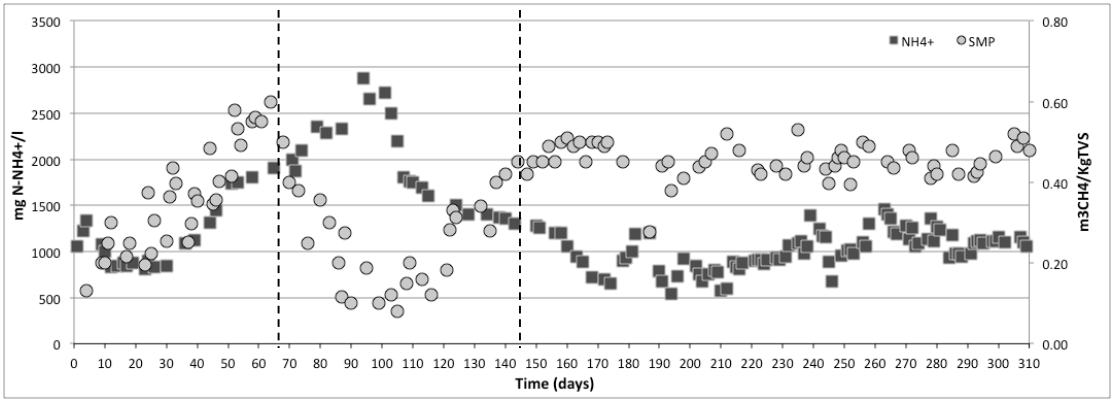
Figure 3. (a) Total ammonium and SHP trend in reactor F1 and (b) Total ammonium reactor and SMP

trend in reactor F2

a



b

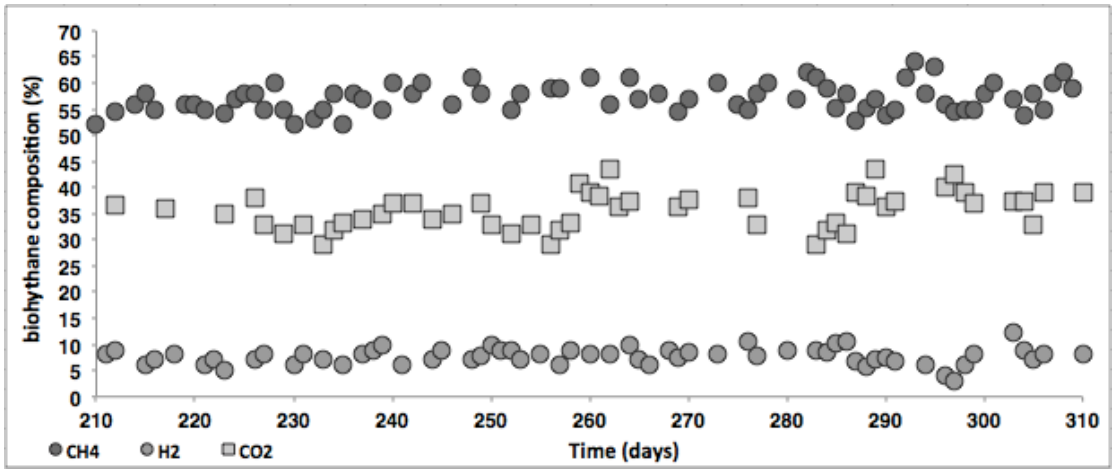


After day 90 the reduction of the recirculation ratio together with the increase of water in the feeding mix determined a clear reduction of ammonia as shown in Figure 2. Therefore, at the same time, the whole process come back to a good balanced condition, as demonstrated by the increase of specific hydrogen and methane production (Figure 3). Carrying on the system this way, the specific hydrogen production decreased again to some 0.025 m^3H_2/kgTVS fed. This fact could be associated with an inhibition of the biological hydrogen production, which could be due by the low pH values reached, reported in the same working period (about 4.5). These values are lower than the range of functionality of the hydrogenase enzyme [5, 13]. The low ammonia concentration in the first reactor (below 500 mg $N\text{-}NH_4^+$/l) could explain these pH values: in fact, such values of ammonia concentration in the system are not able to buffer the acids produced from fermentative process (like VFAs, that reached a value of about 14 gCOD/l).

In the third period (172 – 310 working days), after a period of adjustment, the whole system reached a stable biogas production that met the characteristics of bio-hythane, as shown in Figure 4. In particular, methane, carbon dioxide and hydrogen shown a constant concentration around 55%, 35%

and 10%, respectively.

Figure 4. BioHythane composition during dynamic recirculation rate



In Table 3, the characteristics of the two reactors effluents, and the corresponding gas yields, during the third period, are shown.

Table 3. Dark Fermentation (F1) and Methanogenesis (F2) stability parameters, macronutrients and yields production in SSC

Parameter	m.u.	Average ± sd (F1)	Average ± sd (F2)
TS	g/kg waste	46 ± 11	35 ± 3
VS	g/kg waste	37 ± 9	21 ± 3
VS/TS	%	81 ± 3	62 ± 5
COD	gO_2/kgTS	39 ± 8	11 ± 2
TKN	g/kgTS	26 ± 7	27 ± 9
Ptot	g/kgTS	6.5 ± 0.6	7.4 ± 1.2
Alkalinity pH 4	mg$CaCO_3$/l	-	5184 ± 551
Alkalinity pH 6	mg$CaCO_3$/l	-	3527 ± 408
pH		5.2 ± 0.2	8.1 ± 0.1
NH_4^+-N	mg/l	705 ± 261	1190 ± 152
VFA	mgCOD/l	12241 ± 5643	640 ± 350

Yields Production	**m.u.**	**Average ± sd (F1)**	**Average ± sd (F2)**
GPR	m^3/m^3d	3.32±0.42	2.31±0.84
H_2	%	25±9	-
CH_4	%	16±7	67±3
CO_2	%	53±3	36±2
SHP	m^3/kgVS	0.06±0.02	-

SGP	m^3/kgVS	0.22±0.14	0.71±0.09

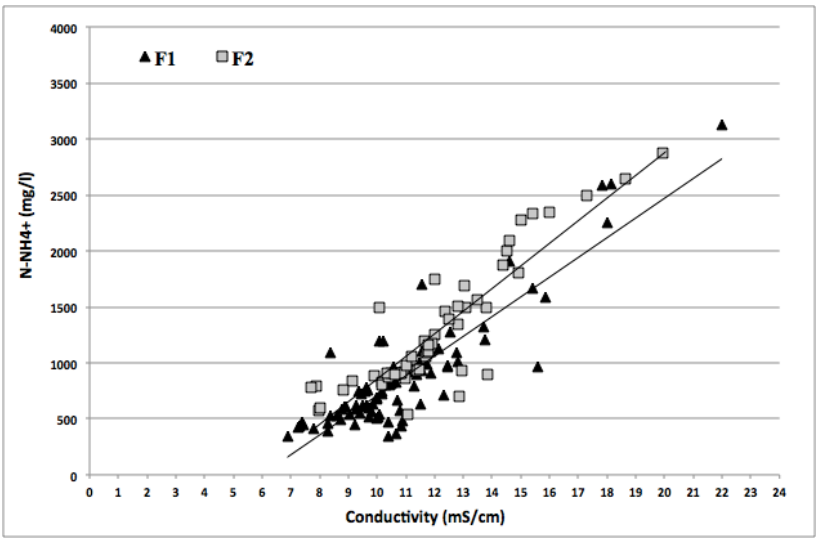
At steady state conditions (SSC) the highest specific production of hydrogen was 99 l/kgVS (SHP) with peaks percentages in the gas composition of 45.5% H_2. The highest production of biogas in methanogenic phase was 1200 l/d with a typical average composition of 63% of CH_4 and 36% CO_2. The average specific production of biogas was 0.71 m^3/kgTVS with a methane content of 67% (V/V). The second part of this study was devoted to investigate the possibility to "control" the concentration of ammonium in both reactors. This was obtained by reading other key parameters defined in order to predict the ammonia concentration. Subsequently, the possible reduction of the ammonia concentration within the volume of recirculation expected was lowered by dilution. The simulated system for automatic control of the process, with variable recirculation flow, led to an average production of the specific total gas produced of 0.69 m^3/kgTVS (SGP) with a production rate of approximately 2.78 $m^3/m^3{}_r$d (GPR). In this period, the average composition of the gas was re-aligned to the typical range of biohythane, recording average percentages of H_2, CH_4 and CO_2 respectively 7%, 58% and 35%.

Table 4. Mass Balance (SSC)

Parameter	IN	OUT gas	OUT liquid	OUT tot	% Removal	Closing Balance
TS g/d	4628	1997	2100	4097	43	-11
VS g/d	3845	1997	3289	3289	52	-14
N tot g/d	133	-	-	131	-	-2
P tot g/d	14.8	-	-	15.6	-	+5

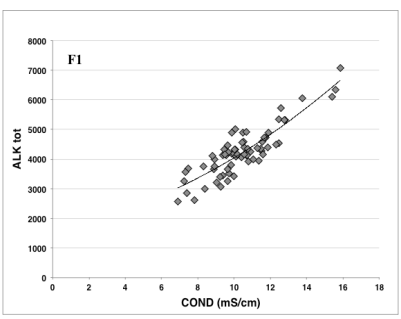
The heterogeneity of the substrates has led to a good mass balance, but not without losses: this is why solids have been lacking, during the feeding, from the first to the second phase. During the experiments, correlation between ammonia and conductivity was evaluated. The graph in Figure 5 is obtained during the experimental period of the whole study. The two beelines describe, for dark fermentation (F1) and methanogenesis (F2) processes, the correlation between two parameters, conductivity and ammonia.

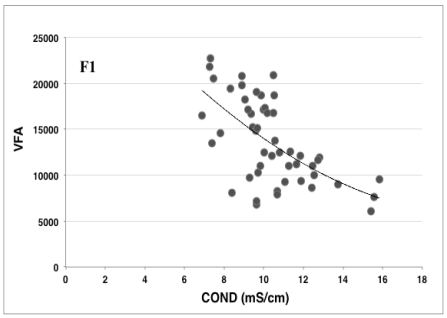
Figure 5. Correlation between total ammonium and conductivity in both reactors

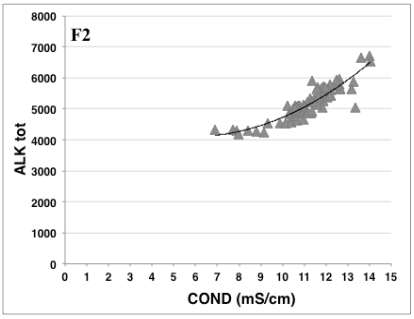


As it can be seen the two parameters, ammonia and electrical conductivity, are linearly correlated for both reactors. The measurement of the conductivity is therefore a good candidate in terms of control: in fact, it is a nonspecific measure, measurable with strong and reliable probes which demand for relatively low maintenance procedures. Also alkalinity showed a good correlation with conductivity while VFA showed an inverse correlation.

Figures 6 a-b-c. Show the correlation between total alkalinity, VFA and conductivity.







Regarding VFA behaviour, it was found that there was a decrease when the ammonia reaches a critical high level (as also shown in literature, [10, 11]) and high conductivity indicates an increase of ammonia (Figure 6), so it can be argued that all these aspects are linked together. Concerning ammonia inhibition, it has to be reminded that high levels of ammonia correspond to changes of intracellular pH, which require a higher energy expenditure [14]. This leads to a depletion of intracellular potassium and the inhibition of specific reaction enzymes [15, 16]. As final remarks, experiments done suggest how the control of inhibition can be based on ammonia content, and this parameter can be easily linked to the other, such as alkalinity, VFA, but especially conductivity, as

shown below by the chemometric analysis.

3.1 Chemometric results

The data-set of parameters for elaboration was taken on a daily basis; data were then used for the chemometric approach. Any single data-set, composed by 180 days each, was characterized by six variables that described precisely the sample.
Firstly the values were auto-scaled. This was done calculating the Pearson correlation matrix, which leads to the information described below.

Table 5. Pearson Correlation matrix (n)

Variables	pH	VFA	ALK Tot	COND	$N\text{-}NH_4^+$	NH_3
pH	1	0.16	0.26	0.43	0.39	0.63
VFA	0.16	1	0.70	0.71	0.73	0.67
ALK Tot	0.26	0.70	1	0.87	**0.95**	0.83
COND	0.43	0.71	0.87	1	**0.94**	0.87
$N\text{-}NH_4^+$	0.39	0.73	**0.95**	**0.94**	1	0.89
NH_3	0.63	0.67	0.83	0.87	0.89	1

Significance alfa level 0.05

Ammonia nitrogen and conductivity exhibit a strong correlation (0.94); the conductivity is strongly correlated with other parameters. Also the ammonia and alkalinity are strongly correlated (0.95). In fact, during titration for the calculation of total alkalinity, free ammonia is titrated. So it is correct that ammonia and total alkalinity are related. These are all data that reinforce the concepts expressed along this experimentation.
According to the study, the experimental data of these evaluations are chemometric modeled for the reactor of methanogenesis, F2, important from the point of view of the possible prediction of the ammonia nitrogen concentration in the recirculation flow.
The data set consists of n objects (daily substrate samples), each described by p variables (ammonia nitrogen, free ammonia, conductivity, alkalinity, pH and VFA). It is modeled using the ammonia content as dependent variable Y, and a number of relevant variables as predictors, including the conductivity. This in order to find the best equation to predict parameter Y, ammonia concentration. The criteria adopted are those of the linear regression, simple and multiple. Subsequently, the models were compared.
The data set was divided into training set and validation set, divided in 90/90 selected samples.

Simple linear regression

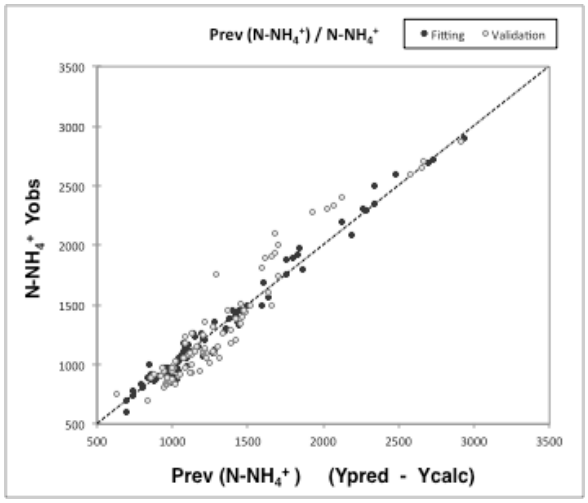
The first equation was calculated by a simple linear regression between the parameter Y ammonia through the parameter X conductivity, leading to:

$$\mathbf{N\text{-}NH_4^+ = -\ 1170 + 205\ x\ COND} \qquad \textbf{(eq. 1)}$$

the predictions of this equation were calculated through the training set and validation set, with external validation and then reversed the sets.

The results were the following: $R^2 = 0.84$ $Q^2 = 0.80$ $R^2 = 0.83$ $Q^2 = 0.78$

Figure 7. First linear equation. Fitting vs Validation



TRAINING SET	
SDEC	**R²**
120	0.83
VALIDATION SET	
SDEP	**Q²**
202	0.78

The model was stable to perturbation, after having reversed the training set and validation set: in fact, the parameter of predictability Q^2 are close to 0.8.

The Standard Deviation of Prediction Errors (SDEP) is a very important parameter since it estimates the error in prediction of the model to predict values of the response Y of the class objects whose response is not noted. The values Y_{pred} will therefore be accompanied by ± SDEP parameter to indicate the uncertainty (average) of the prediction. In this case, an uncertainty value of 202 (or 202 mg/l $N\text{-}NH_4^+$) shows how the predictive value needs to be increased.

The limit to use a system based only on conductivity for the determination of ammonia nitrogen consists in the fact that, since it provides information on the total amount of dissolved ions in the

medium, its performance depends on numerous factors. For this reason, in order to improve the effectiveness of the approach, the data were then subsequently analyzed from a chemometric multivariate analysis point of view.

Two different multiple linear regressions were evaluated, with the use or less of the parameter VFA as a predictor, jointly with alkalinity and conductivity.

First Multiple linear regression

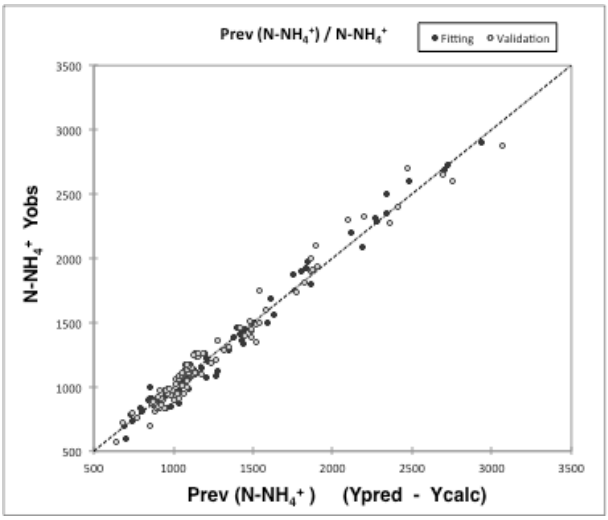
The equation of the multivariate model, using the predictor variables VFA, Alkalinity and conductivity, to predict the concentration of ammonia, is the following:

$$\mathbf{N\text{-}NH_4^+ = - 697 + 0.014 \times (VFA) + 0.135 \times (ALK\ tot) + 92.7 \times (COND)} \quad \mathbf{(eq.\ 2)}$$

The relative goodness of fit coefficients improves a lot: $R^2 = 0.94$ $Q^2 = 0.93$

as can be also seen by graphical representation of the model "externally validated", Standard Deviation Error in Calculation (SDEC) and SDEP, Figure 8:

Figure 8. Second linear equation. Fitting vs Validation



TRAINING SET	
SDEC	**R²**
114	0.94
VALIDATION SET	
SDEP	**Q²**
116	0.93

This multivariate model, set at 3 predictors, holds excellent ability to calculate itself, or the ability to describe complex data with which it was created (fitting), and its predictive ability (validation).

The relative fit goodness coefficients were

$R^2 = 0.94$ and $Q^2 = 0.93$

By reversing the training set of data with the validation set were obtained

$R^2 = 0.94$ and $Q^2 = 0.92$

It can be concluded that the multivariate model calculated using multiple linear regression, 3 chosen predictor variables, has a better ability to predict the data "out layer". It also has good stability, since crossing the training set with the validation set the coefficients R^2 and Q^2 remained virtually unchanged.

The values Y_{pred} will therefore be accompanied by ± SDEP parameter to indicate the uncertainty (average) of the prediction (Figure 8).

An uncertainty of 116 (or 116 mg/l $N\text{-}NH_4^+$) is considered a good result, although the uncertainty in the measurement between the calculated values (observed) in the laboratory was calculated to be about ± 86 mg/l $N\text{-}NH_4^+$.

At this point was evaluated another model without the VFA predictor parameter.

Second Multiple linear regression

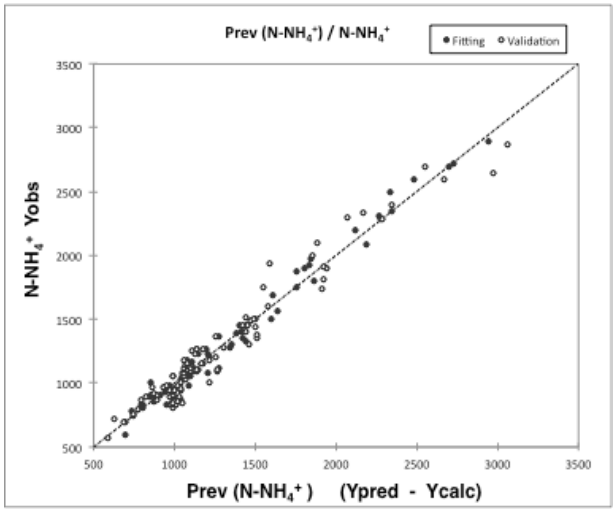
The equation of the multivariate model, using the predictor variables Alkalinity and conductivity, to predict the concentration of ammonia is the following:

$$\mathbf{N\text{-}NH_4^+ = -733 + 95.4 \times (COND) + 0.138 \times (ALK\ Tot)} \qquad \textbf{(eq. 3)}$$

The relative goodness of fit coefficients was: $R^2 = 0.93$ $Q^2 = 0.91$

A graphical representation of the model "externally validated" with SDEC and SDEP values on Figure 9.

Figure 9. Third linear equation. Fitting vs Validation



TRAINING SET	
SDEC	**R²**
116	0.93

VALIDATION SET	
SDEP	**Q²**
121	0.92

This multivariate model, chosen to 2 predictors, holds high ability to calculate itself and its predictive ability.

The relative fit goodness coefficients were

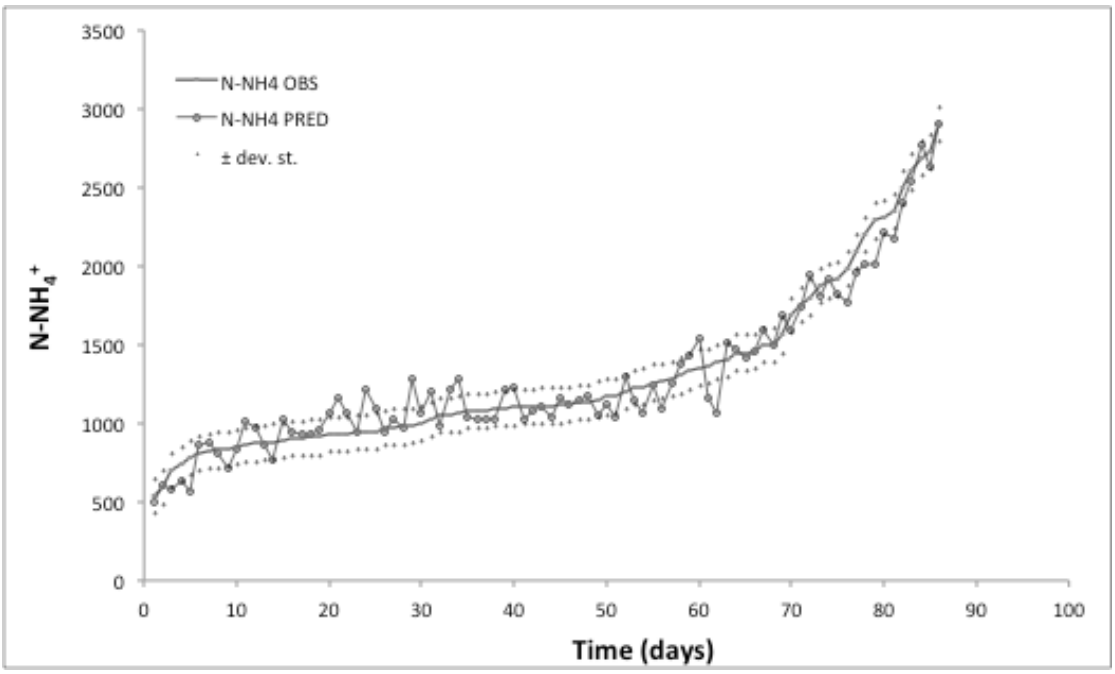
$R^2 = 0.93$ and $Q^2 = 0.91$

By reversing the training set of data with the validation set were obtained

$R^2 = 0.93$ and $Q^2 = 0.92$

Interestingly, the stability of the model is given by the values of R^2 and Q^2. In this regard a graph about the progression of ammonia observed (real) was developed, in relationship with the one predicted by the latter equation of the multivariate model.

Figure 10. Graphical analysis of predictive ability of the proposed model



In that case an uncertainty of 121 (or 121 mg/l N-NH_4^+) is still a good result, the uncertainty in the measurement between the observed values in the laboratory, as mentioned, is about ± 86 mg/l N-NH_4^+.

The stability of the model is given by the values of R^2 and Q^2 due to the external validation, since crossing the training set with the validation set the coefficients R^2 and Q^2 remained virtually unchanged. Good prediction is subsequently demonstrated in the graph above (Figure 10).

We have demonstrated the possibility of implementing an automatic control for the proper functioning of the process and this control can use as algorithm the multiple linear regression developed statistically.

4. Conclusions

Considering the experiments carried out and the whole set of data obtained, some considerations can be drawn:

- This pilot scale study shows that it is possible to obtain a stable hydrogen production by dark fermentation without physical or chemicals pretreatments when biowaste is used as sole substrate;
- The optimization was reached with only partial recycle of digested sludge from second reactor

(methanogenesis) after a mild solid separation, which allows to maintain the pH at an optimal level (5-6) for hydrogen evolution in the first reactor (dark fermentation);

- A stable Biohythane production was obtained with GPR 2.78 $m^3/m^3_r d$ and SGP 0.69 $m^3/KgTVS_{fed}$
- Comparing the predictive capabilities of the models (SDEP) and the economic feasibility, the best model seems to be the one based on two predictors, conductivity and alkalinity, with a SDEP of 121. Sometimes the prediction is outside measurement uncertainty, but its standard deviation doesn't exceed 121 mg/l, that represents a good result from an analytical error point of view. This demonstrates that the use of electrical conductivity and alkalinity measured on-line could be the best model option for on-line monitoring of this process.

As a final remark the development of a real semi-automatic control of the whole process, using basic models able to predict the concentration of ammonia, is developed and validated.
This can be done using the on-line measurement of easily quantified parameters, allowing the use of low cost probes, reaching high degree analytical strength at the same time.

Acknowledgements

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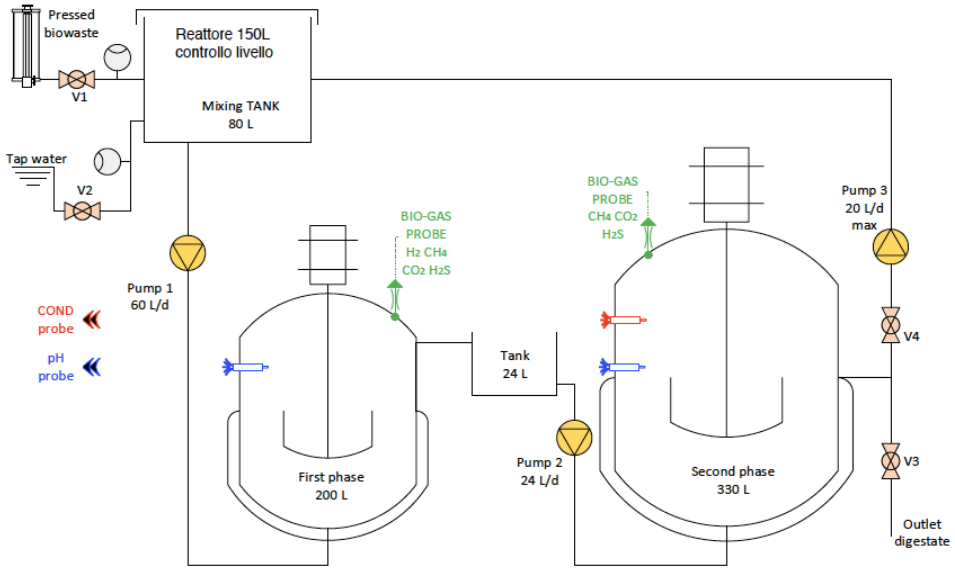
APPENDIX

Automatic double phase control in pilot scale

The novelty of this subsequent study was the experimentation applied in pilot scale in order to demonstrate how the ammonia prediction model can control a double phase anaerobic digestion. The new model is based on the use of two industrial pH probes and 1 industrial conductivity inductive loop probe (Metler Toledo^{TM}, value of significance 0.00). This new algorithm can be used to monitor and through a PLC (cRIO, National Instruments^{TM}) control the anaerobic digestion processes, adapted to produce hydrogen gas and an effluent rich in volatile fatty acids by fermentation. Volatile fatty acids will be necessary for the upcoming step chain of the bio-refinery from food waste (e.g. polyhydroxyalkanoates production).

Figure 1. The pilot scale process flow diagram.



Material and methods are reported in the previous article [Micolucci et al., 2014].

The process was dimensioned according to the following operations:

1) calculation of the ammonia content in the second phase
2) feeding of the second phase (methanogenic) from the first phase (fermentation)
3) filling of the mixing tank with water, digestate (if necessary) and pressed food waste
4) feeding of the first phase (fermentation)

The recycling of digestate was regulated through the 3 monitoring probes. The pH probe placed in the fermentation reactor allowed to adopt a range of pH to be maintained in the first phase; if the pH of the first phase was below the value 5.2, the recirculation was done accordingly applying the maximum recycle ratio, if the pH value was greater than 5.7 the recirculation was avoided. When the pH value of the first stage was placed within the range 5.2 – 5.7 then the system applied the calculation of the recirculation volume according to the prediction of the ammonia concentration in the second phase. The calculation was done through the use of the values (predictors) from the pH and conductivity probes placed in the second phase.

The experimentation trial was carried out in 3 stages (RUNs). Initially, the model was built through a statistical analysis of the data, leading a dedicated experimentation to obtain a wide range of ammonia values ($[NH_4^+$-N] in the second phase) in order to correlate it with pH and conductivity values.

After the creation of the model (RUN1), the trial lasted about 120 days. From day 1 to day 80 (RUN2) the organic waste from urban door-to-door selection with grinding pre-treatment (Wet-Refine) was used. The third part of the trial (RUN3, 40 days) is characterised by the change of the pre-treatment method of the substrate, using the pressed organic waste (Screw-Press).

Results and discussion

The different characteristics of the organic waste pre-treated are presented below.

The organic waste used came from door-to-door collection was conferred in the experimental area (Treviso WWTp) on a weekly basis. The incoming waste had an average dry matter content of 271 gTS/kg of which about 78% is volatile solids.

Table 1. Chemical - physical characteristics of the collected biowaste (wet weight).

Parameter	M.U.	Average ± St.Dev.	Min	Max
TS	gTS/Kg	271 ± 27	221	312
TVS	gTVS/Kg	213 ± 20	176	246
COD	gO_2/Kg	256 ± 26	206	296
TKN	gN/Kg	6.7 ± 1	4.6	7
P tot	gP/Kg	1.5 ± 0.7	0.8	2.8

The biowaste conferred was pre-treated by Wet Refine approach before being fed into the reactors; Following the table shows the chemical - physical characteristics of biowaste as a result of the above pre-treatment.

Table 2. Chemical - physical characteristics of the pre-treated biowaste (Wet Refine).

Parameter	M.U.	Average ± St.Dev.	Min	Max
TS	gTS/Kg	244 ± 32	202	300
TVS	gTVS/Kg	216 ± 27	175	270
COD	gO_2/Kg	230 ± 34	185	299
TKN	gN/Kg	6.7 ± 1	4.6	11
P tot	gP/Kg	1.1 ± 0.5	0.6	2.2

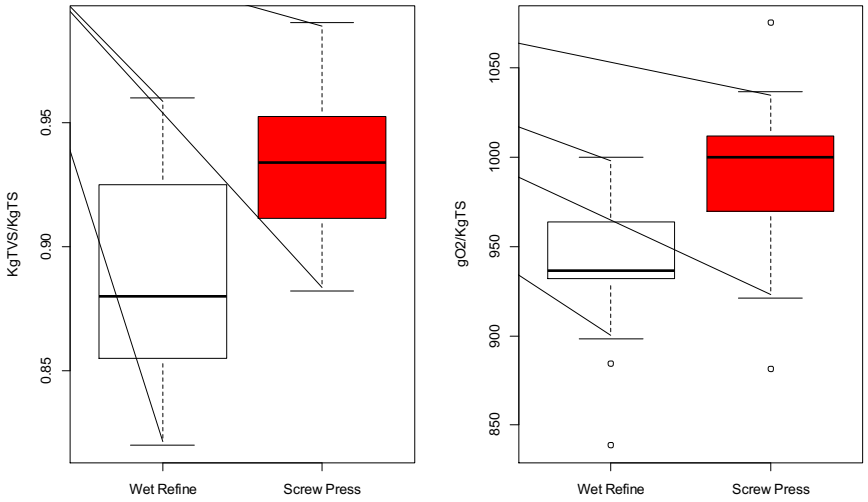
During the RUN3 the biowaste conferred, whose characteristics are described in the paragraph above, was pre-treated using Screw Press approach. The table below shows the chemical and physical characteristics of the pre-treated biowaste by Screw Press.

Table 3. Chemical - physical characteristics of the pre-treated biowaste (Screw Press).

Parameter	M.U.	Average ± St.Dev.	Min	Max
TS	gTS/Kg	213 ± 18	174	242
TVS	gTVS/Kg	199 ± 19	170	230
COD	gO_2/Kg	211 ± 17	174	245
TKN	gN/Kg	3.89 ± 1	3	4.6
P tot	gP/Kg	0.57 ± 0.3	0.35	0.98

Looking at the tables relating to the characteristics of biowaste pre-treated by Screw Press (Table 3) and by type Wet Refine approach (Table 2), can be seen easily that the dry content in biowaste juice is lower by almost 13% compared to the pre-treated biowaste with Wet Refine method. In spite of the major dry-volatile and COD concentrations on pre-treated biowaste by Wet Refine, the fraction of volatile and COD, based on TS, were significantly higher (t test, $p < 0.05$) in the biowaste juice.

Figure 2. Box - Plot TVS and COD on dry basis of the organic fraction pre-treated with Wet Refine and Screw Press.



It follows that, by means of the squeezing treatment, the biodegradable fraction is increased in solids present in the biowaste. In terms of the nutrients, the pressed biowaste showed a COD/N ratio of 53, higher than that on the biowaste treated with Wet Refine (COD/ N = 35), which makes it more suitable for biological treatment.

A new model has been developed to control a pilot scale double phase anaerobic digestion.

A first trial of about 300 days was dedicated to increase the ammonia concentration in the methanogenic reactor, from 500 mg/L to 3000 mg/L, in order to develop a model that can predict ammonia concentration in the second stage reactor through the use of two probes, pH and Conductivity.

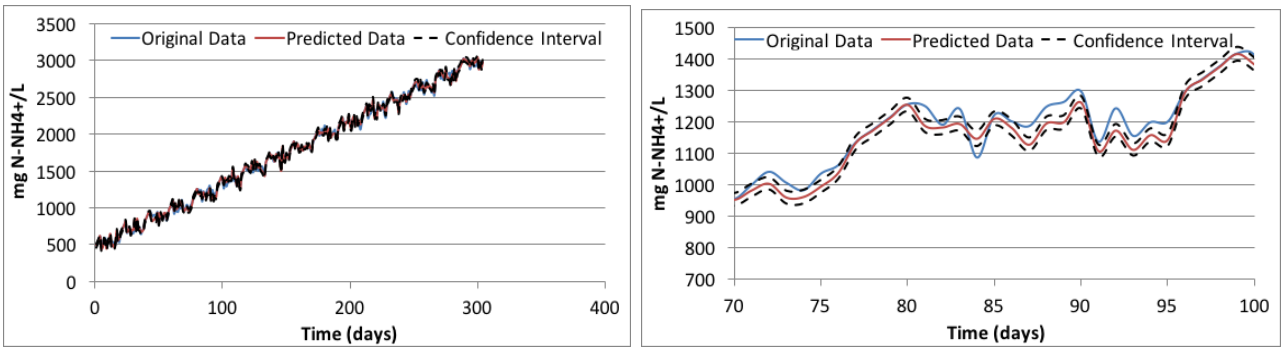
Using "R" software for the multiple linear regression calculation the original matrix of the data showed the following model (MOD_0): $AMM = f(pH, COND)$

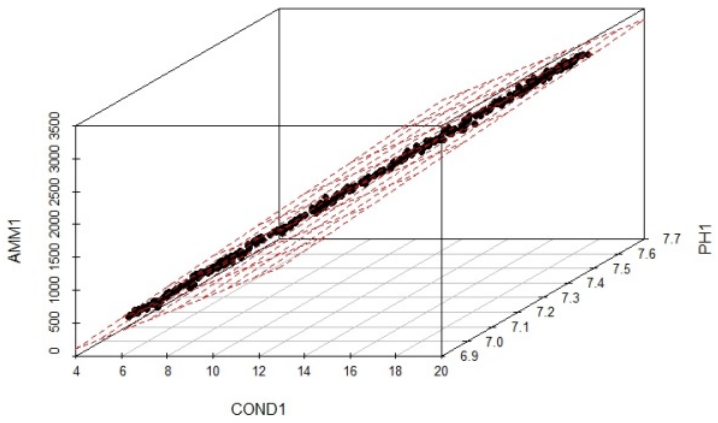
```
Coefficients:
             Estimate Std. Error t value Pr(>|t|)
(Intercept) 3874.920    645.809   6.000 8.37e-09 ***
COND1        235.543      5.059  46.557  < 2e-16 ***
PH1         -682.244     95.205  -7.166 1.24e-11 ***
---
Signif. codes:  0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1

Residual standard error: 65.99 on 213 degrees of freedom
Multiple R-squared:  0.9883,    Adjusted R-squared:  0.9882
F-statistic:  8965 on 2 and 213 DF,  p-value: < 2.2e-16
```

Both the Multiple R^2 and the adjusted R^2 (which takes into account the number of predictors used) is 0.988. In other words, the model is suitable to describe the 98.8% of the data used. Furthermore, the value of p in the statistical F Fisher (< 2.2e-16) informs that the regression model is identified as significative.

From the Anova test it is evident that for both independent variables, the regression coefficient is significantly different to 0 therefore the regression is significant for both variables ($p < 2e\text{-}16$ for the CONDuctivity and 8.37e-09 for the pH; both less than 0.001).

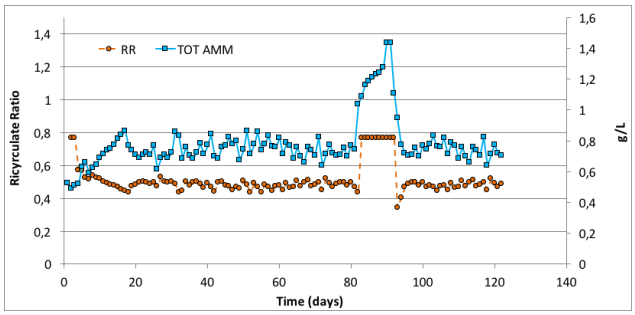
Figure 3. Predicted data compared to original data, fitting model representation.

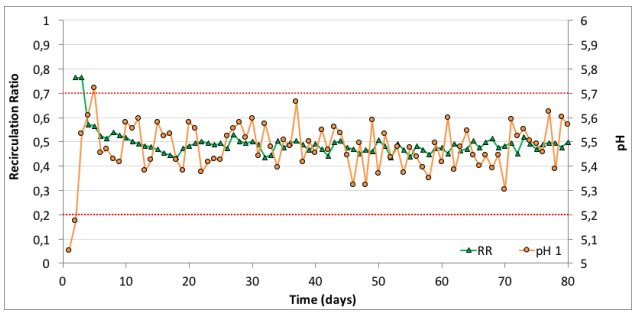


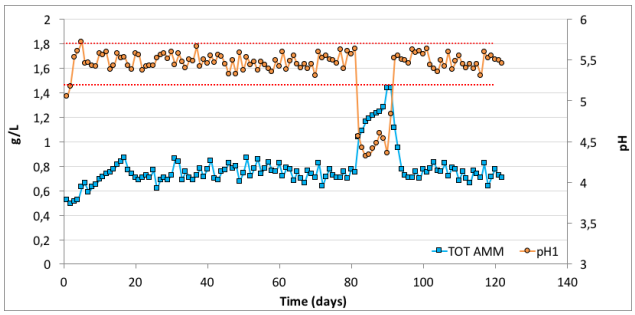


RUN2 and RUN3 are an experimental trial for about 120 days at pilot scale in order to obtain the evaluation of the software control and the model external validation.

Figure 4. Recirculation ratio, ammonia concentration (second phase), pH (first phase)

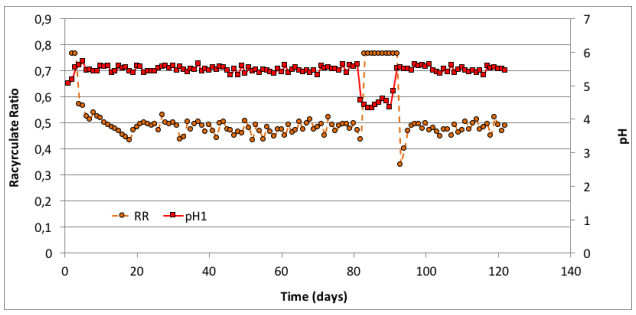


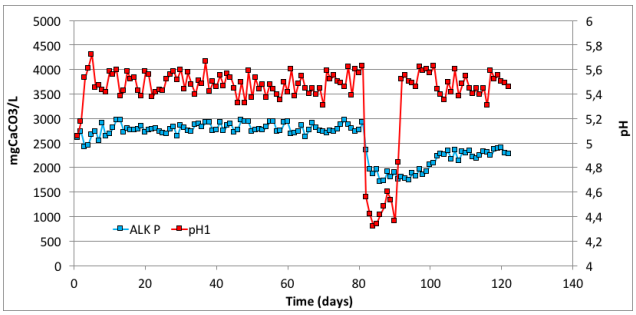




In the following charts is highlighted as the variable recirculation ratio is maintained within the range 0.4 – 0.6. During the beginning of the RUN2 the recirculation ratio was around 0.8 value so as to bring the pH of the first phase within the desired pH range (5.2 – 5.7). Moreover, the concentration of ammonia in the second phase has fluctuated remaining constant within a value of 600 – 900 mg/L during the trial of the RUN2.

The following charts (figure 5) show how the pH1 of the first phase has remained almost constant throughout the RUN2. When the pre-treatment of the substrate changed to Screw pressed biowaste (RUN3) the control algorithm has reacted so as to recirculate the maximum ratio as the pH1 was dropped below the value of 5. The increase in the recirculation brought pH1 to re-set within the desired range (figure 5) but this has increased the concentration of ammonia in the second step (figures 4). After day 90 the system has dynamic recirculation according to the calculation through the modelling algorithm and Ammonia values reported were around 800 mg/L for the entire duration of RUN3.





Conclusions

The pilot system's ability to regulate itself, furthermore within changes of substrate, highlights an important research step on automation and control of anaerobic digestion processes.

Through the use of chemometric tools and the statistic analysis it is possible to obtain efficient models for the prediction of some necessary parameters for the control and the optimization of the anaerobic digestion systems.

9. CHAPTER 4

Biofuels production and biogas upgrading evaluation

Research Paper

Anaerobic co-digestion of wastewater sludge and organic waste for the production of automotive sector biofuels

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Abstract

The following study deals with the application of anaerobic co-digestion process for biofuels production (biomethane and biohythane) in order to cope fossil fuel used for the automotive sector. The aim of the paper is to investigate and compare performances in single and two-phase thermophilic anaerobic co-digestion processes applied on waste activated sludge and organic fraction of municipal solid waste.

Hydrogen and methane production reached values of 24 LH_2/kgTVS and 272 LCH_4/kgTVS respectively when operating with a total hydraulic retention time of 20d (2.5d in the first phase and about 17d in the second phase) and organic loading rate 3.5 kg TVS/m^3d, respectively. Specific gas production in single and double-stage were 493 L/kgTVS and 572 L/kgTVS respectively. Biofuel yields were compared in order to consider an integrated approach on waste and wastewater cycles for the automotive waste collection transports. An overall assessment coming from the implementation of the approach on a 100,000 PE basin was also presented, based on pilot scale results obtained.

KEYWORDS: anaerobic co digestion, methane, hydrogen, fuel, wastewater, organic waste, automotive

1. INTRODUCTION

Co-digestion is the simultaneous anaerobic decomposition of two or more organic substrates mixture. Several studies showed benefits of the co-digestion, e.g. dilution of potential toxic compounds, nutrients balance improvement, synergistic effects of microorganisms, increased load of biodegradable organic matter and better biogas yield.

Currently sewage sludge and organic wastes co-digestion is a common practice in Europe and US. Among the different organic substrates studied, the anaerobic co-digestion of sewage sludge (SS) and organic fraction of municipal solid waste (OFMSW - biowaste) is the most popular co-digestion research subject [1] because of the possible exploitation of existing infrastructures; the anaerobic digesters of waste water treatment plants (WWTPs). In fact, because of over-sizing design or the treatment of very diluted streams, these reactors are very often operating at low organic loading rate (OLR); large spare volumes are therefore available for the co-treatment of sludge and other organic waste in WWTPs.

Moreover, the carried out studies showed the N content of sewage sludge can supplement a possible deficit of nutrients in the other co-substrate (e.g. OFMSW), whereas the higher biodegradability of the biowaste allows an increase in biogas production potential. Co-digestion is a process whereby energy-rich organic waste materials are added to dairy or wastewater digesters with excess capacity. The advantages that allowed OFMSW and sewage sludge co-digestion to establish this treatment in the European scenario are manifold. Anaerobic co-digestion can be considered as one of the most promising way to give a proper treatment of the organic fraction of municipal solid waste, considering both the economic and environmental aspects. This approach allows to recover renewable energy and also bio-products: each tonne of organic waste sent to the anaerobic treatment in fact, can produce up to 150-250 m^3 of biogas, depending from the quality of the treated substrate (mainly linked to collection approach), which can be conveniently converted into useful energy forms: heat, electricity and cogeneration (combined production of electricity and heat). The actual tendency, at European level, is to move towards an additional approach upgrading, considering the anaerobic digestion (AD) as the base to produce a real biofuel, to be used not only in situ (cogeneration), but also in the automotive sector.

The aim of the work is to demonstrate, through experiments at pilot scale, upgrading costs evaluations and an accurate calculation of the kerbside collection-transport-consumption, how produced bio-fuels can help to reduce costs and gaseous emissions, in order to achieve a possible smart cycles integration. In particular, the application of anaerobic co-digestion to obtain two energy carriers for the automotive sector, biomethane and biohythane, was investigated. Different scenarios related to single

stage (product: biomethane) or double stage (product: biohythane) anaerobic co-digestion showed how the biofuel produced can substitute fossil fuel generally used for the organic waste collection-transportation.
Comparative energy and mass balances have been performed.

2. MATERIAL AND METHODS

2.1 Experimental set-up

Three stirred reactors (CSTR) with 230L, 760L, 200L working volume respectively were exploited. The reactors were heated by hot water recirculation system and maintained at 55 °C using electrical heater controlled by a PT100-based thermostatic probe. The feeding system was semi – continuous, arranged once per day.
The biowaste was reduced in size using a grinder, mixed with waste activated sludge (WAS) and then fed to single stage reactor (230L) or to 1st stage reactor (200L – fermenter). During the whole experiment (365 days), the OLR and Hydraulic Retention Time (HRT) were maintained at about 17 KgTVS/m^3d and 2.5 d for 1st stage, and about 3.5 KgTVS/m^3d and 17 d for the 2nd stage. The single stage operated at 3.5 KgTVS/m^3d OLR and about 20 d HRT.
OFMSW / WAS ratio has been chosen considering the production of 200g_{WET}/d PE for biowaste and 60gTS/dPE for SS. OFMSW / WAS ratio adopted in this study was 50/50 on VS basis.

2.2 Substrate and inoculum

The anaerobic digested sludge used as inoculum for the methanogenic reactor was collected in the WWTP located in Treviso (northern Italy) where a 2000 m^3 anaerobic digester treats the source collected biowaste at 35 ºC. The sludge was acclimatized for two weeks to thermophilic temperature [2] [3].
The fermentative reactor was inoculated with separately collected biowaste, coming from the municipality of Treviso and waste activated sludge (WAS), coming from the WWTP above mentioned, then regularly fed once a day. Table 1 and 2 show the main characteristics of these substrates.

Table 1. WAS characterization.

Parameter	M.U.	Average	Std.Dev	Min	Max
TS	g/Kg	47.86	14.28	20.91	96.34
TVS	g/Kg	33.06	10.10	13.24	63.41
TVS/TS	%	69.06	4.10	46.67	80.95
COD	g/Kg	51.30	11.50	28.75	73.13
TKN	g/Kg	3.29	0.75	1.63	4.50
P_{TOT}	g/Kg	0.91	0.33	0.49	1.50

The thickened activated sludge was characterized by an average content of total solids to approximately 4.7% of the wet weight and volatile solids fraction 70% of TS.
The COD/TKN ratio was 17.

Table 2. Organic Waste characterization.

Parameter	M.U.	Average	Std.Dev	Min	Max
TS	g/Kg	259.9	38.8	198.97	334.10
TVS	g/Kg	226.1	41.3	153.45	282.15
TVS/TS	%	90.7	2.58	82.08	96.84
COD	g/Kg	241.3	48.9	165.43	306.41
TKN	g/Kg	6.7	1.3	4.87	10.98
P_{TOT}	g/Kg	1.5	0.7	0.84	2.85

The organic fraction of municipal solid waste used in the experimental trial was characterized by an average content of total solids approximately 25% of the wet weight and from a fraction of volatile solids 90% of TS. These data highlighted the high degree of biodegradability of OFMSW, which combined with a good ratio of macronutrients (COD : TKN : P) makes it a very suitable substrate for biological treatment processes.
In particular, the COD/TKN ratio turned out to be on average equal to 36, more than twice that of the WAS considered.

2.2.1 Punctual analysis of OFMSW collection and fuel consumption

The data collection work has taken into account the municipality of Vedelago (TV), a town of 17,000 inhabitants. The surface of the municipality is 61,66 km^2. This gives a rather low population density (270.5 PE/km^2), suitable for an estimate calculation to larger urban areas. The collection of wet waste within the City took place every two weeks and is divided into five rounds of collection through operator trucks. The data were collected during the months of May, June, July 2015, for a total of 17

pathways in the territory. The collection of organic waste within the City took place every two weeks and it was divided into five rounds of collection through the operators (trucks). The data collection was carried out three times for each highlighted kerbside path (5 routes on a total area of 62 km^2) (through a device called transponder). For each path carried out, were collected data such as: the total and actual kilometres collection, the total time of the collection and discharge service, the fuel consumption, the total weight of the wet waste and the number of bins collected.

2.2.2 T-Test and statistical data analysis

Comparison on biogas yields among the single stage option and the double phase was evaluated. To determinate how specific gas productions (SGP) are different from each option adopted, statistical data analysis and t-test were performed. This is the key on SGP comparison to determine the diverse resources obtainable among the single phase and the double phase anaerobic co-digestion systems.
Statistical data analysis was performed using the open-source program R (The R Foundation for Statistical Computing, version 3.1.3).
Normal distribution and variance homogeneity were checked by Shapiro–Wilk test [4] and F test [5] in order to ensure the applicability of t test.

2.2.3 Heat requirements and energy balances

In order to estimate heat balances for the AD system, operating in hypothetical WWTP integrated to OFMSW treatment with a size of 100,000 PE was considered. A specific heat request of 1 kcal/kg °C, a temperature of the sludge (WAS+OFMSW) of 10 °C, a combustion heat for biogas of 5,500 kcal/m^3, with an efficiency of 90% on combustion. To define the heating request for sludge and OFMSW flow the sludge production was set at 60g dry matter per person equivalent day [6]. Total heat losses were estimated considering the dimensions of the reactors and the typical construction specifications.

2.3 Analytical methods

The effluents of the reactors were monitored 2/3 times per week in terms of total and volatile solids content, chemical oxygen demand, Total Kjeldahl Nitrogen (TKN) and total phosphorus. The process stability parameters, namely pH, volatile fatty acid content and speciation, total and partial alkalinity and ammonia, were checked daily. All the analyses, except for VFAs, were carried out in accordance with the Standard Methods [7]. Volatile fatty acids content was monitored using a gas chromatograph

(Carlo Erba instruments) with hydrogen as gas carrier, 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). 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. Gas productions were monitored continuously by a gas flow meter (Ritter Company, drum-type wet-test volumetric gas meters), while the hydrogen content was measured by a gas-chromatograph (GC Agilent Technology 6890 N) equipped with the column HP-PLOT MOLESIEVE, 30 x 0.53 mm ID x 25 um film, using a thermal conductivity detector and argon as gas carrier.

3. RESULT AND DISCUSSION

3.1 Process performances and yields comparison

From the data collected we can first provide an assessment on different distances covered by the collecting tracks for each turn carried out. The following table shows the data collected regarding the distance, fuel consumption and weight of waste per track-path effected (table 3).

Table 3. Organic Waste collection-transportation.

Collection (triplicates) (km)	Fuel Consumption (L)	Organic Waste Collected (kg)
126 (path 1)	27.80	2,833
111 (path 2)	25.88	2,053
119 (path 3)	26.00	2,193
125 (path 4)	25.91	2,900
117 (path 5)	27.75	2,124
120 (average)	26.68 (average)	2,421 (average)

The average distance (km) per litres of fuel is about 4.48 km/L (22,3 L/100km, close to the European average 26,6 L/100km). The average litres of fuel used per ton organic waste is about 11,02 L/ton. This value is well interrelated with other European studies of door-to-door collection (figure 1; [8]).

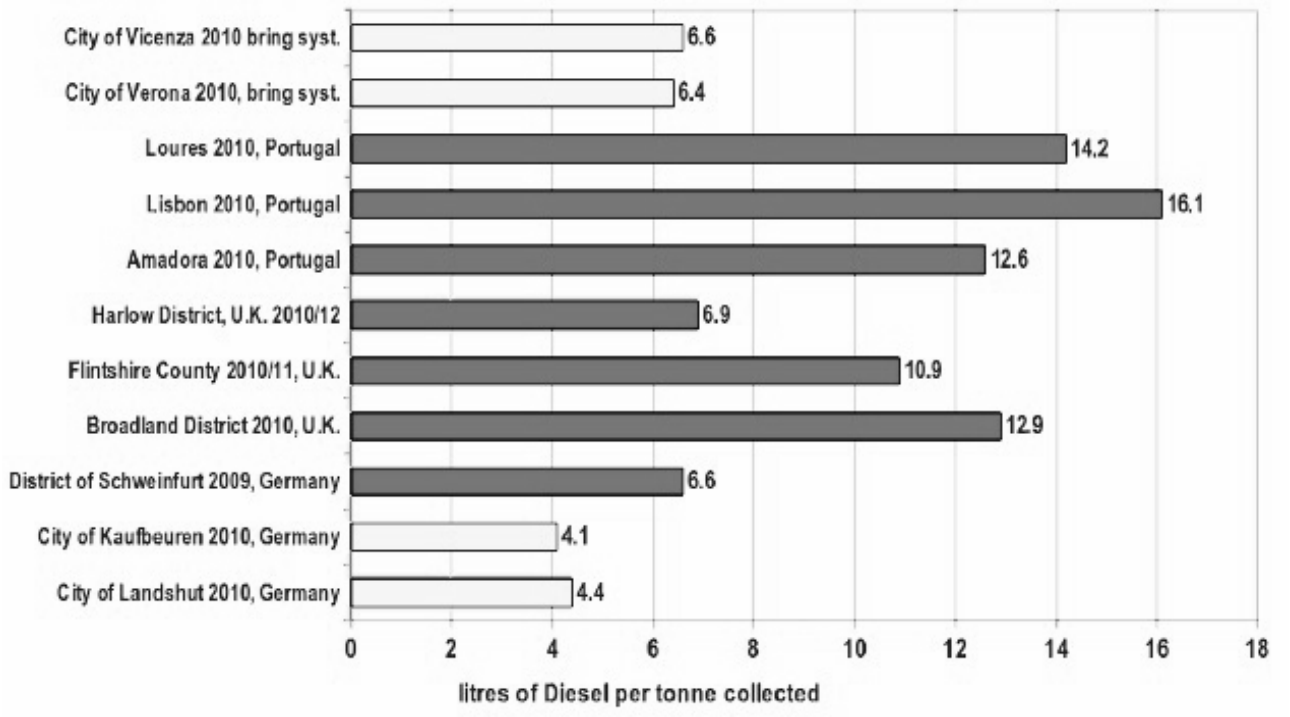

Figure 1. Fuel consumption per ton waste collected. Green bars for door-to-door system collection, yellow bars household street collection (Valorgas, 2012).

The pro-capite production was obtained considering only the household waste collection, about 90 kg/PE*y (average of years 2013-2015). This value corrisponds to 0,24 kg/PE*d, analogous with Veneto data of the cities of Padova (0,21 kg/PE*d), Rovigo (0,21 kg/PE*d), Vicenza (0,17 kg/PE*d), Verona (0,21 kg/PE*d), Treviso (0,19 kg/PE*d), Venezia (0,17 kg/PE*d) [9]. From literature this estimated value is about 0,30 kg/PE*d [10]. For the economic and environmental impacts evaluation we decided to use the value 0,20 kg/PE*d (average of the Veneto cities).

3.2 Process performances and yields comparison

The experimental period lasted a total of one year. The overall performances of the two-phase thermophilic anaerobic co-digestion process and the single stage process are summarized in table 4.

Table 4. Characterization of the effluents and process yields (thermophilic temperature 55±0.1 °C).

Parameters	Units	First phase	Second phase	Single stage
Total Solids	g/kg	48±5	25±4	26±2
Total Volatile Solids	g/kg	37±4	16±2	17±1
COD	g/kg TS	40±3	19±2	18±2
TKN	g/kg TS	34±1	35±1	34±1
P tot	g/kg TS	11±0	12±1	10±1

pH		5.3±0.01	8.2±0.5	7.97±0.26
Yields				
Hydrogen	%	36±8	-	-
Methane	%	-	64±2	60±9
SHP	Nm^3 H_2/kg TVS	0.024±0.005	-	-
SMP	Nm^3 CH_4/kg TVS	-	0.29±0.04	0.27±0.03
SGP WAS	Nm^3 CH_4/kg TVS	0.06±0.005	0.25±0.02	0.75±0.03
SGP OFMSW	Nm^3 CH_4/kg TVS	0.12±0.005	0.88±0.01	0.22±0.03
SGP CO-DIGESTION	Nm^3 CH_4/kg TVS	0.09±0.005	0.57±0.01	0.49±0.04

3.1.1 T-Test on SGP (I and II stage)

Shapiro test: single (p = 0.4476); double (p = 0.6525) accept H0: normal distribution

F test: p <0.01 refusal H0: heteroscedasticity

Welch's T.test: H0 rejected with p <0.01 significantly different averages.

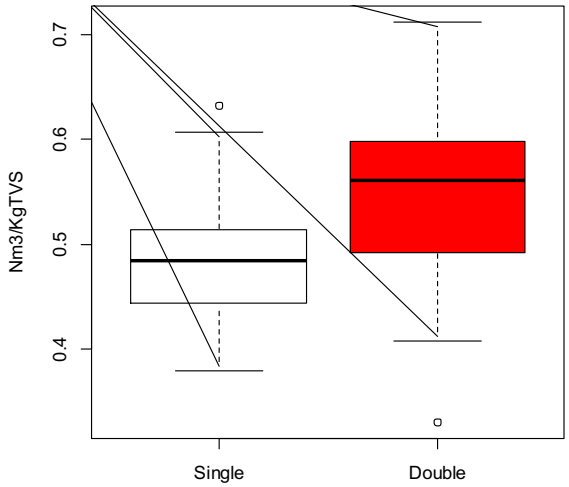

Figure 1. T-test on SGP comparison, single stage and double stage systems.

3.2 Cost-effective evaluation on biomethane produced for the automotive recycling sector

A final overall assessment on a 100,000 PE basin was evaluated, considering the pilot- and full-scale results obtained of different wastewater treatment plants combined with anaerobic co-digestion for

cycles integration strategy. To compare the substrate removal efficiency of the two systems, a scale-up of these processes was evaluated through the use of the analytical data of these pilot scale experiments, 200 g/PE*d organic waste was calculated as average with Veneto Region data, instead of considering the typical specific food waste production of 300 g/(PE*d) of wet weight food waste [10] and several potential basins to treat 100,000 PE sludge amount / day. In order to estimate the energy balances for the AD systems, a hypothetical equal distribution of the full-scale plants was considered. Upgrading process has been evaluated in order to achieve a bio-methane production with a concentration above 98% aimed to the automotive sector. The equitable distribution of treatment plants, the costs allocated to the maintenance of these facilities and the transport consumption for recycling have been carefully calculated.

Considering to revamp a basin of 100,000 PE, single stage and double stage co-digestion options are evaluated. Door-to-door waste collection still needs pre-treatment of OFMSW in order to remove any plastic, metals and inert materials. To this scope it was evaluated a treatment efficiency equal to 90% [11]. To treat 100,000 inhabitants waste flows, 2 CSTR 2000 m^3 each were considered. Specific gas production considered were 0.49 Nm^3 CH_4/kg TVS for single stage and 0.57 Nm^3 CH_4/kg TVS for double stage. The hydraulic residence time of the fermenter (first phase) have been evaluated as optimum at 2.5 days.

A heating value of. 5,500 kcal/m^3 for biogas was considered [12]. It was considered the use of a boiler (90% efficiency) to heat the substrate as feed for continuous digesters and maintain them at a temperature of 55° Celsius (thermophilic). Subsequently were calculated consumption for the digester heating, expenditure on upgrading biogas (through pressure swing adsorption process, PSA) amounted to € 0.24 / m^3 biomethane produced [13] and biomethane cost € 0.99 / kg.

3.2.1 Single stage evaluation

For the single stage system an OLR of 2.1 kgTVS/m^3d was considered, hence with a total volatile solid flow of 8,412 kgTVS/d (total wet flow rate 218 m^3/d). The TVS ratio was 50% OFMSW, 50% WAS. The digestate that must be conducted to disposal is 6,938 kgTS/d (25% TS), wet mass flow 28 ton/d. The cost for the digestate disposal is about 100€/ton [14], hence calculated for this system is about 2,775 €/d with TS concentration of the digestate as 25% TS following a filtration press treatment. The TVS removal efficiency of the single stage system is about 35% of the incoming TS flow. Because of the SGP there was a biogas production of about 4083 m^3/d, 170 m^3/h.

The biogas required to heat the sludge (OFMSW + WAS) and the digester is about 2004 m^3/d, 83 m^3/h.

The biogas that could be sent to upgrading is about 87 m^3/h with an output of biomethane at 98% (PSA 91% efficiency) of 49 m^3/h, 35 kg/h, 838 kg/d. The upgrading cost with PSA is 0.24 €/kg [15] [16] hence 282 €/d to treat the incoming biogas flow from the digester, that is still cheaper than the expenses for the transport using diesel fuel (around 1.2 €/L). Through the detailed study of the municipality of Vedelago (17,000 PE) it was seen as about 600 km/d is needed to cover the recycling requirements of the organic fraction of the waste. Via proportion it can be estimated that, with evenly distributed plants throughout the country, 3,518 km/d must be paths to a 100,000 PE territory. The kilometres passable through the production of biomethane are 3,150. This determines how it is still necessary a small integration of diesel to be integrated to complete the collection.

3.2.2 Double stage evaluation

Double stage system had an OLR in the second methanation phase same as the single stage (2.1 kgTVS/m^3d). Two methanation reactor with same volume of 2000 m^3 each were considered (HRT 18 d). The new integration is a fermentation reactor with a volume of 545 m^3 and HRT of 2,5 days.

The TVS ratio was 50% OFMSW, 50% WAS, same as the single stage. The digestate that must be conducted to disposal is 6,321 kgTS/d (25% TS), wet mass flow 25 ton/d. The cost for the digestate disposal is about 100€/ton [14], calculated for this system as about 2,528 €/d with TS concentration of the digestate as 25% TS following a filtration press treatment. The TVS removal efficiency of the single stage system is about 41% of the incoming TS flow (6% more efficient than the single stage). Because of the SGP there was a biogas production of about 4,757 m^3/d, 198 m^3/h (14% enhanced biogas yield compared to single stage system).

The biogas required to heat the sludge (OFMSW + WAS) and the digester was about 2,104 m^3/d, 88 m^3/h (5 m^3/h because of the new installed fermenter).

Even though the biogas required to heat the 3 reactors is higher than the single stage (2 digester) the biogas that could be sent to upgrading was still higher about 110 m^3/h with an output of biomethane at 98% (PSA 91% efficiency) of 62 m^3/h, 45 kg/h, 1,068 kg/d.

The upgrading cost with PSA is 0.24 €/kg, hence 359 €/d to treat the incoming biogas flow from the digester. Through the detailed study of the 17,000 PE municipality, it was seen as about 600 km/d is needed to cover the recycling requirements of the organic fraction of the waste. Via proportion it can be estimated that, with evenly distributed plants throughout the country, 3,518 km/d must be paths to a 100,000 PE territory. The kilometres drivable through the production of biomethane are 4,016. This determines how a smart integration of the first phase digester can overcome the fuel efficiency for the organic waste collection transportation, thus avoiding the use of fossil fuel.

3.3 Environmental impacts (CO_2 NO_x PM)

As environmental impacts in this study, pollutants such as CO_2, NO_X, PM into the atmosphere were evaluated; according to the use of diesel or bio-methane produced and used as a fuel substitute to the non-renewable fuel for transport on separate collection of organic waste.

According to the European Environmental Agency [17] about the contribution of transport to air quality diesel fuel impacts are the following: 139 g/kg CO_2, 0.433 g/kg NO_X, 0.018 PM. Methane air quality impacts: 108.68 g/kg CO_2, 0.045 g/kg NO_X, 0.017 PM. Hythane impacts: same as methane for CO_2 and particulate matter PM, lower for NO_X 0.036 g/kg.

Through these data it has developed a calculation to obtain a comparison of impacts on the air by the use of diesel, bio-methane and biohythane (table 5).

Table 5. Air quality impacts, CO_2, NO_X and PM calculated on 100,000 PE waste collection transports

Environmental Air Quality Impact		**Fossil Fuel**	**Single Stage co-AD**	**Double Phase co-AD**	
Pollutants	Unit measure	Diesel	Bio-Methane + Diesel	Bio-Methane	Bio-Hythane
CO_2	Kg/d	489	393	382	382 – 0.1%
NO_X	Kg/d	1.523	0.301	0.158	0.128
PM	Kg/d	0.063	0.060	0.060	0.060

In the first scenario single stage co-AD using bio-methane produced and diesel, CO_2 emissions can be reduced of about 20% instead of the use of sole diesel as fuel. NO_X can be reduced of about 80% instead of using sole diesel, PM reduced of about 5%.

In the second evaluated scenario, double phase co-AD can reduce pollutant emission of about 22% on CO_2 with biomethane or biohythane, 90% and 92% NO_X if using biomethane or biohythane respectively and 5% on PM with biomethane or biohythane.

4. CONCLUSIONS

Anaerobic double phase co-digestion of WAS + OFMSW may guarantee, beside the complete energetic sustainability of the process, to sustain the fuel consumption of the organic waste collection transportation. The experiment carried out and the linked simulations showed interesting results coming from the application of the integrated waste/wastewater treatment approach. Biogas produced

covers the expenses for the maintenance of the digesters and sludge heating. Fossil fuel consumption necessary for the separate collection can be produced and covered by the production of biomethane through the co-digestion of sludge and OFMSW. NO_X are reduced considerably (over 90%) because of the use of bio-methane instead of diesel.
The integrated approach considered gives considerable advantages, which lead this option in the field of the 'smart' opportunities for the urban services management.

AKNOWLEDGEMENTS

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10. CHAPTER 5

Polyhydroxyalkanoates production

Pilot scale mixed culture polyhydroxyalkanoates production from food waste

Plastic has many societal benefits, but it also gives rise to certain environmental problems. Durability and a massive use connected to inappropriate waste management is a high potential risk that leads accumulation of this material in Nature and landfills [1].

Poly-hydroxy-alkanoates (PHA) is a biodegradable renewable biopolymer, which is produced naturally in several different groups of bacteria. During natural biosynthesis, monomeric units of PHA are produced and polymerized by the ester linkage. Then the polymers aggregate by accumulation into cytoplasmic inclusions bounded by monolayer envelopes.

These inclusions are often referred to as granules [2] and function as intracellular energy and carbon reserves in stages of starvation and can account for 80% of the total dry weight of microbial biomass [3]. Because the PHA is naturally polymerized during biosynthesis it can be extracted directly in its polymerized form.

PHA is already known as a fully biodegradable and commercially available bio-plastic [4]. It has similar properties to the synthetic polymers produced in the petrol chemical industry such as polypropylene (PP). Although biological production of PHA can be used to produce substitute polymer similar to those produced in a petrochemical industry. There is still a higher production cost accompanying the production of PHA. This economical bottleneck is an obstacle that has to be taken into consideration before investigating the commercial production of PHAs as a feasible substitution of petrochemical production [5]. It is commonly recognized that the high production cost associated with PHA production is due to around 50% of the production cost is directly related to the expensive carbon source [6]. However several other aspects such as material (chemicals, production strain etc.) and also the culture conditions and fermentation types (batch, fed-batch etc.) can add to the high production cost. One obvious way to decrease the PHA production cost could be to find und utilize a cheap renewable and readily available carbon source in the production of PHA polymers instead of using refined organic substrates.

One carbon source that is considered a viable substitute in PHA production is food waste. The attractive solution could be to convert the bio-waste by microbial fermentation to value added products in the form of organic acids and moreover building blocks for biopolymer production.

Presently several biotechnological processes are utilizing single strain cultures, which require well-defined substrate and sterile process conditions [7], this attributes to a higher production cost. These factors impose a financial burden on the industrial production and make single strain cultures unfavourable for large scale production of PHA [8] [9].

A more financially attractive method for the PHA production is to implement eco-biotechnology. Eco-biotechnology aims to produce products (PHA) by exploiting mixed culture and ecological selection principles, in this way it links the methodology of environmental biotechnology with the goal of industrial biotechnology. The principle of eco-biotechnology is based on the biological selection and competition instead of genetic or metabolic engineering [10].

Due to the diversity of microorganism in mixed cultures they can deal with a range of substrates and of variable compositions (e.g. the heterogeneity of bio-waste). The conditions in these systems are designed so the metabolic conversion of interest ensures an ecological advantage for the microorganisms and determine which catabolic product allows the most efficient growth and thereby dominate the community of the mixed culture [8] [11].

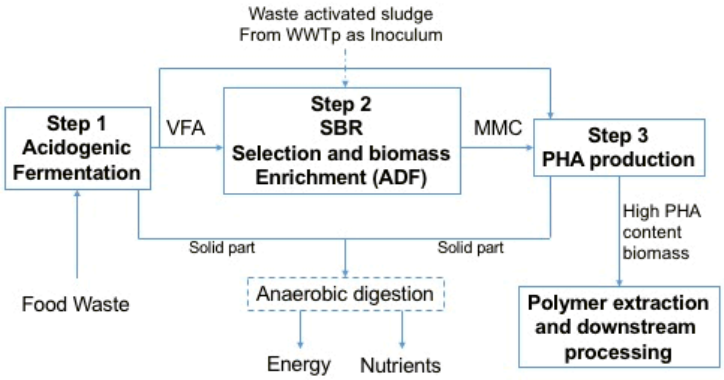
Food waste is first converted into organic acids (mainly butyric acid, acetic acid, propionic acid and valeric acid), hydrogen gas, carbon dioxide and cells.

Secondly, the organic acids are used as substrate and consumed, during nitrogen restriction leading to accumulation of poly-hydroxy-alkanoates (PHA) inside the cells. The process applied the alternation of aerobic feast and famine accomplished the selection of PHA storing biomass. Production of the PHA took place in a second batch reactor [12].

The novelty and the aim of the study were to produce biopolymers through food waste as substrate for VFA production through fermentation and the use of microbial mixed culture (MMC), avoiding the use of sterile reactors.

Organic fraction of municipal solid waste from household (food waste) mixed with sewage sludge from the Waste Water Treatment plant can be easily converted into bio-polymers (PHA) taking advantage of the waste activated sludge potentialities by a process specifically designed. In theory any fermentable organic waste can be converted into PHA by the multistage process showed in figure 1.

Figure 1. pilot scale PHA production process and flow chart diagram.



This innovative pilot scale process includes a first fermentative phase in which biowaste is converted into volatile fatty acids (VFA), utilized as building blocks substrate to feed the next process stages. VFA aimed at selection / enrichments of the microbial mixed culture (MMC) PHA-producers. This second MMC grow stage, the selection of PHA storing biomass [13], is developed under aerobic dynamic feeding (ADF) in a sequencing batch reactor (SBR) and waste activated sludge is used as inoculum. In the third stage, a batch process to maximise the PHA accumulation [13], the mixture of VFA and the selected biomass are used to maximize the PHA production and the intracellular content of the bio-polymer.

Material and methods

Process operation

The pilot scale process was built as composition of a 200L tank (S1) for pressed biowaste storage, a fermentation reactor (F) (HRT 3 - 4 d, fed with 16 Kg_{WET}/d corresponding to an average organic load OLR of 19 KgTVS/m^3d or 21.7 kgCOD/m^3d), one industrial centrifuge (C) (8,000 rpm) in order to divide the liquid fermented fraction from the solid part (200 μm ∅ pores), a 200L tank for the liquid fermented fraction storage, a SBR1 with a volume 576L utilised to culture the activated sludge under a dynamic feeding condition (*feast and famine*). A batch (B2) reactor volume 140L was used to exploit the storage performance of the biomass selected in each SBR1 cycle and to verify and control the biomass dynamic response to PHA accumulation. A storage biomass tank (S3) as last process step was used to receive the biomass from B2 and mix it with NaClO (7% Cl_2) used as extracting agent, in order to stop the biomass activity and break cells membrane. It was dosed at the end of accumulation step in a volumetric ratio equal to 0.2 NaClO / biomass.

SBR operation

The SBR1 was inoculated with 20L of activated sludge from the "Treviso Municipal Waste Water Treatment plant" (with an initial volatile suspended solids concentration in the range of 1000-3000 mg/L). The working SBR1 volume was 140L. Two RUNs were conducted under the same process condition, except for the substrate, in the first start-up phase (RUN I) a synthetic substrate as feed, acetic acid, was used. Acetic acid was diluted by water from the WWTp and anaerobic digestate for the contribution of micro- and macro-nutrients needed for cell growth. Given that the content of nitrogen and phosphorus in the anaerobic digestate was not enough to get a typical balanced growth ratio (COD:N:P = 100:5:1) [14], nitrogen and phosphorus were provided following the addition of a rate fixed (1.0L) of concentrated solution of NH_4Cl and KH_2PO_4. The feed solution was characterised by a soluble COD of 21.0 gCOD/L and a pH of 6.0 (with NaOH addition). The initial OLR applied was 2.5 gCOD/L in SBR1 and after a couple of weeks of operation enhanced to 3.5 gCOD/L. SBR1 was fed by ADF regime, cycle length 6h (4 cycle per day). SBR1 feeding took place at the beginning of each cycle, for a relatively short period of about 0.5 min. No settling phase was performed (which is different from the typical SBR operation [15]), the hydraulic retention time (HRT) was equal to solid retention time (SRT), 1 d. The step of purging (withdrawn) took place immediately before the end of each cycle, also for a relatively short period (about 0.5 min) and in full aeration condition. The SBR was aerated using membrane compressors. The operative cycle was divided into the following phases: Purge (0.5 min), Reaction 1 (10 min), Feeding time (0.5 min), Reaction 2 (349 min). Through the RUN II, fermented liquid biowaste fraction was used as feed. The same organic loading rate in SBR1 was used during the entire trial, 3.5 gCOD/L. The operative cycle lengths (6h) were monitored and controlled by a software (LabVIEW™) and a hardware (cRIO, National Instruments™). During the feeding phase of each cycle the O_2 concentration decreased to low values because of high bacterial metabolic activity. The subsequent change in the slope to positive values indicated complete substrate depletion, corresponding to the length of the feast phase [14].

The SBR performance was monitored by measurement of biomass concentration as volatile suspended solids (VSS) by sampling the sludge at the end of feast phase and by measurement of PHA content (sampling the sludge both at the end of the feast phase and at the end of the cycle). The flow rate of the aerators ensured always dissolved O_2 concentration greater than 2.0 mg/L and therefore never limiting during the entire duration of the cycle. pH, OLR and Dissolved Oxigen were monitored by Hamilton® industrial probes.

Batch accumulation process

The batch tests with synthetic substrate were carried out in a dedicated batch reactor, inoculated with 35 L of biomass at the end of each cycle, withdrawn by means of the hydraulic pump from the selection reactor (SBR1). The duration of each accumulation test was of at least 4 hours. The batch reactor (B2) was equipped with aerators for the ventilation, was not thermostated and was not expected to pH control. The flow rate of the aerators ensured always dissolved O_2 concentration greater than 2.0 mg/L and therefore never limiting during the entire duration of the cycle. pH, OLR and Dissolved Oxygen were monitored by Hamilton® industrial probes. The substrate was made from an acetic acid solution concentrated. It was adopted a Substrate / Biomass ratio (S/X) higher than that of SBR1, in order to saturate the storage capacity of the biomass produced by the selection stage. The total volume of the substrate added was 5.0 L.

The batch test with biowaste fermented substrate were conducted with the same mode of batch tests with synthetic substrate. However, because the system was not equipped with a temperature control, and therefore subject to substantial increases the kinetics of consumption of the substrate in the warmest periods of the year, periodic additions of liquid fermentate were accordingly more frequent depending on the abovementioned kinetics. The most extreme cases were reached in the month of July: the accumulation tests require multiple additions of substrate up to a maximum of 30L, doubling the working volume.

Analytical methods

The reactor effluents were monitored daily per week for total solids (TS), total volatile solids (TVS), soluble chemical oxygen demand (SCOD), total Kjeldahl nitrogen (TKN) and total phosphorous (P). The process stability parameters, i.e., the pH, volatile fatty acid content and distribution, conductivity, total and partial alkalinity and ammonia nitrogen (NH4+–N), were measured daily. All the analyses were performed according to the Standard Methods for Water and Wastewater Analysis [16]. The analysis of the volatile fatty acids was conducted using a Carlo Erba™ gas chromatograph equipped with a flame ionization detector (T = 200 °C), a fused silica capillary column, Supelco NUKOL™ (15 m x 0.53 mm x 0.5 μm thickness of the film), and hydrogen was the gas carrier. The analysis was conducted by increasing the temperature from 80 °C to 200 °C (10 °C/min). The samples were filtered using a 0.45 μm filter. For PHA determination was conducted as reported from Valentino [14]. The non-polymer biomass (active biomass X_A) was calculated at the end of the feast phase (i.e. at substrate depletion) from the difference between VSS and PHA concentrations in the sample and converted into COD according to a conversion factor of 1.42 mgCOD/mgBiomass and used in the equation $X_A = (VSS - PHA) * 1.42$. The latter conversion factor was obtained by considering the generic

heterotrophic biomass formula $C_5H_7O_2N$ [17]. PHAs were also converted into COD according to the following oxidation stoichiometry: 1.67 mgCOD/mgHB monomer and 1.92 mgCOD/mgHV monomer. The PHA content of the biomass was calculated by dividing the measured PHA concentration by the biomass concentrations (both expressed as COD) [14].

Calculations

In the SBR the amount of stored PHA (ΔPHA) was calculated as the difference between the PHA concentration in the mixed liquor at the end of the feast phase (substrate depletion time) and at the beginning of the respective cycle. The specific PHA production rate was calculated as the ratio of the stored PHA and the length of the feast phase (t) per unit of non-polymer biomass (X_A) and is expressed as $rPHA = \Delta PHA / (t * X_A)$.

The storage yield during the feast phase was determined as the ratio between the amount of stored PHA (as COD) and the amount of the removed substrate (as COD) fed in the cycle, $Y_{STO} = \Delta PHA / \Delta S$. The observed yield was determined at substrate depletion as the ratio between the total biomass concentration (VSS as COD, including both PHA and non-polymer biomass) and the amount of removed substrate (as COD) as given in the following equation: $Y_{OBS} = VSS / \Delta S$.

The polymer content in the biomass was calculated (in terms of COD) at substrate depletion as the ratio of PHA concentration to the VSS concentration (the sum of non-polymer biomass and produced polymer) as given in the following equation: $\%PHA = PHA / VSS = PHA / (X_A + PHA)$. The volumetric uptake rate was calculated during the feast phase by the variation in the substrate concentration as function of elapsed time. An average volumetric substrate uptake rate was calculated for the entire feast phase by the ratio of the amount of COD fed per cycle (ΔS) and the time required for substrate depletion multiplied by the volume of the reactor as described in the equation: $\Delta S / (t * L)$.

In batch tests (B2) the volumetric specific rates (PHA storage and substrate consumption) were calculated by linear regression of the data versus time. The biomass growth was calculated from the mean nitrogen content in the biomass (10% as gN/gX_A). The growth yield (Y_{GROW}) was calculated as the ratio between the new X_A produced and the removed substrate as given in the following equation: $Y_{GROW} = \Delta X_A / \Delta S$.

The maximum polymer content in the biomass obtained during each test was also calculated based on the PHA profiles; the storage yield was calculated relative to the time when the maximum polymer content was achieved.

Result and discussion

Biowaste coming from door-to-door collection of the municipality of Treviso, pre-treated with a full scale extruder press (Tiger® HS 640) and split it into two streams, one liquid to be anaerobically digested in the full scale waste water treatment plant and a second one solid to be composted. The characteristics of the liquid fraction are described in table 1.

Table 1. Characteristics of biowaste, and pressed liquid and solid fractions

Substrate	TS g/kg biowaste	VS g/kg biowaste	TVS/TS %	COD g/kg TS	TKN g/kg TS	P g/kg TS
Liquid $_{bw}$	161±59.5	158±54.0	93.0±3	1,289±288	25±5	4.4±0.5

As for the general chemic-physical characteristics, biowaste showed an average dry matter content of 161 gTS/kg, 93% volatile solids. The COD values were typically greater than 1,200 gCOD/kgTS. The liquid phase obtained was particularly suitable for fermentation because of its total and volatile solids with a very high COD content, most of it being soluble.

The following table shows the values related to the characteristic of the fermented biowaste.

Table 2. Mean values and standard deviations of the characteristic parameters of the fermented pressed biowaste

Parameter	Value
sCOD (mg/L)	19,374 ± 5,150
VFA (mgCOD/L)	16,070 ± 3,314
VFA/COD (%)	83 ± 4
$N\text{-}NH_4^+$	593 ± 37
$P\text{-}PO_4^{3-}$	152 ± 8

By fermentation process it was possible to obtain a liquid stream corresponding to 4.05 kgCOD/d of which 23.1 kgCOD/d in the soluble fraction (SCOD) and 2.85 kg COD/d in the particulate fraction. The 83% of SCOD product is represented by volatile fatty acids, consisting mainly of acetic acid, propionic acid and butyric acid.
The COD:N:P ratio of the fermented substrate was 100:4.5:1; on the basis of these reports it was considered not necessary the addition of further nutrients in the fermented feed sent to the PHA production process. Moreover, the alkalinity presents in the fermented substrate has also excluded

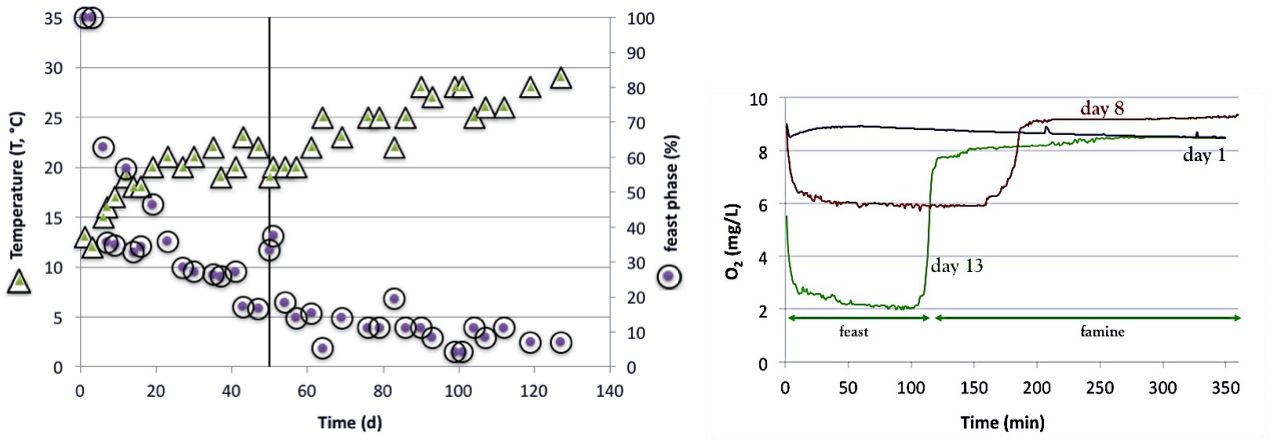
the necessity of NaOH in the medium, potentially necessary to avoid that the pH in the SBR reactor does not drop to excessively acid values.

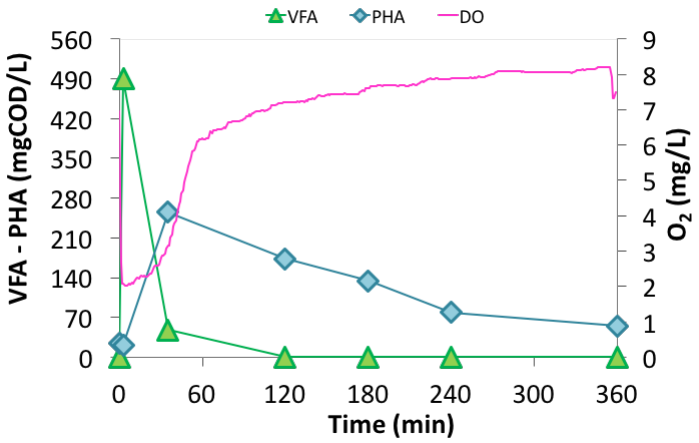
The fermented product was then subjected to a solid-liquid phase by centrifugal separation. The mass flow in low solids content was then sent to the second storage tank (S2) and then used to the sector dedicated for the production of PHA.

SBR1 efficiency of the biomass selection stage

In the initial period of operation, the OLR was reduced to ¼ for the first 10 days of operation, and for the first 50 days of the process has been used acetate as substrate feed. The operating temperatures of the reactor, by not providing a control of the temperatures, varied according to the season. Figure 2 shows the trend of the temperature measured at the end of cycle sampling. The measured T was slightly inferior to 10 ° C in the month of February and then reach values close to 30 ° C in the last experimental phase, in July. The feast phase duration assumed a continuous decrease (figure 2) demonstrating that the process operation conditions were suitable for the selection and the subsequent enrichment of the desired mixed microbial consortium. At day 50° the substrate fed was changed with fermented biowaste.

Figure 2. Temperature and feast phase during the entire trial in SBR1, reduction of the feast time during the first days of process, feast time and VFA accumulation during food waste as substrate





In the period where fermented food waste was used as substrate, the duration of the feast phase was approximately 13% (average) of the total cycle duration, value sufficiently lower than reported in literature. A suitable feast phase is when the duration is lower than 20% of the entire time cycle [14]. Time needed to allow the selection of PHA-producing microorganism. The graph underlines as the temperature is not a negligible effect on the process performance. In the period characterized by the use of acetate as feed, the feast phase was far superior compared to low values obtained using fermentation, due to lower operating temperatures, between 12 °C and 21 °C.

After day 50° until the end of the process steady state condition was achieved, which is pointed out by the constant value of qVFA during feast conditions, average value 472 $(mgVFA/gX_A * h)_{COD}$. Table 3 shows the yields of the selection biomass process.

Table 3. Yields of the SBR1 selection MMC PHA-producers step.

Parameters	Synthetic feed Acid Acetic + Digestate from AD	Fermented food waste
COD(VFA):NH_4-N:PO_4-P	100:5:1	100:4.5:1
F/M $(gVFA/gX_A)_{COD}$	0.28 ± 0.10	0.26 ± 0.09
qVFA (mgCOD/gCOD h)	246 ± 88	472 ± 72
qPHA (mgCOD/gCOD h)	168 ± 58	241 ± 39
PHA (gCOD/gCOD)	29 ± 9	24 ± 6
HV (%)	6 ± 4	16 ± 9
$Y_{PHA/VFA}$ (gCOD/gCOD)	0.61 ± 0.34	0.53 ± 0.12
$Y_{X/VFA}$ (gCOD/gCOD) Y_{OBS}	-	0.20 ± 0.04

During the period of the process with fermented food waste as substrate, the PHA accumulated in the selection reactor up to 0.66 $gCOD/gCOD_X$. Preserving efficient famine conditions, where the stored PHA is consumed, has been found to be of high significance in reaching a selected culture with a

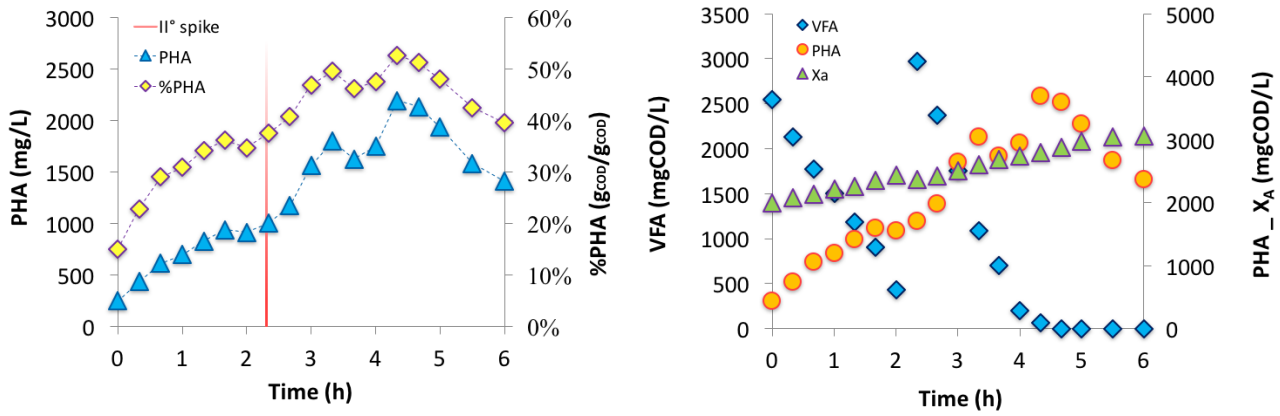
high PHA storage capacity [18]. The substrate removal efficiency of the selection step was up to 99,6% of the COD_{IN} (average value 98,8% ± 0.8%).

PHA accumulation batch step

This step of the process is necessary to increase the polymer content in the biomass by feeding the biomass again with the same type of substrate and allowing the biomass to store the substrate to its maximum content [14].

The trends of the parameters of a typical batch accumulation test performed with biomass coming from SBR 1 are reported in figure 3 (a – b).

Figure 3 a – b.



After the first spike, VFAs were rapidly consumed. Substrate consumption occurred along with residual ammonium uptake and PHA production, indicating the biomass growth and PHA storage were occurring simultaneously. Even though biomass growth remained almost constant with a slight increase ($Y_{X/VFA}$ gCOD/gCOD 0.18 ± 0.02), PHA accumulation reached value $Y_{PHA/VFA}$ gCOD/gCOD up to 0.69 with average value about 0.66 gCOD/gCOD ± 0.11.

This result was possible to obtain because of the pre-treatment of the fermented substrate which consisted of a filtration, post-centrifugation, using a filter press, for the removal of inert solids. The porosity of the filter was 0.2 μm.

The maximum capacity of biomass to store PHAs was examined. After 6 hours of operation per cycle in B2, the biomass was able to accumulate up to 45% ± 8%(gPHA/gVSS ∗ 100).

The observed yield of PHA production were around 0.41 $gCOD_{PHA}/gCOD_{VFA}$. It was a congruent result compared to literature (0.38 – 0.50 $gCOD_{PHA}/gCOD_{VFA}$) when food waste substrates are used as carbon source [19][20].

In this work the yield of active biomass per substrate consumed was significant, as it varied between 0.21 and 0.25 gCOD/gCOD.

Limiting further the presence of inert during the accumulation and selection steps would be of interest for process optimisation purposed in future research in order to maximise PHA productivity.

The biopolymers that were produced with the fermented bio-waste (VFAs) had characteristics in term of 3HB, 3HV percentage. The 3HB represents the major part of the PHA produced (in the range of 85 % to 95 %), while percentages of 3HV were closely to 8% (2% st. dev.).

The downstream process

The duration of treatment with NaClO (3 h or overnight) seems to affect slightly on the result. In fact, the extractions of longer duration could be obtained from the biomass with higher PHA content than that resulting from the shorter extractions, only in some cases. In this regard the table 4 shows the results from the accumulation and extraction tests (only a few selected examples tests), from which we can underline that the substantial increase of PHA content (obtained at the end of accumulation and by extractive treatment of biomass coming by previous accumulations days 121-127), is presumably due to the pre-treatments performed on the fermented substrate (reducing the amount of inert solids).

Table 4. Accumulation and extraction tests

Substrate	Feast phase % (SBR cycle)	COD:N:P	Extraction	Dry content extracted (g)	%PHA (g/g) end of accumulation	%PHA (g/g) end of extraction
Ferm. FW	12.0%	100:3.5:0.8	NaClO (7%) over night	11.5	36	40
Ferm. FW	11.1%	100:3.5:0.8	NaClO (7%) over night	9.5	30	63
Ferm. FW	15.0%	100:3.5:0.8	NaClO (7%) over night	-	26	63
Ferm. FW	15.0%	100:3.5:0.8	NaClO (7%) over night	70	39	73
Ferm. FW	12.5%	100:3.5:0.8	NaClO (7%) over night	4	19	47
Ferm. FW	8.3%	100:3.5:0.8	NaClO (7%) over night	11	27	51
Ferm. FW	11.1%	100:3.5:0.8	NaClO (7%) over night	11	25	50
Ferm. FW	11.1%	100:3.5:0.8	NaClO (7%) 3h	139	37	32
Ferm. FW	12.5%	100:3.5:0.8	NaClO (7%) 3h	7.4	33	39
Ferm. FW	12.5%	100:3.5:0.8	NaClO (7%) 3h	8.2	16	38
Ferm. FW	10.3%	100:3.5:0.8	NaClO (7%) 3h	5.4	34	44
Ferm. FW	7.2%	100:3.5:0.8	NaClO (7%) 3h	12	32	48
Ferm. FW	6.9%	100:3.5:0.8	NaClO (7%) over night	12	40	51
Ferm. FW	6.4%	100:3.5:0.8	NaClO (7%) 3h	25	20	63
Ferm. FW	6.4%	100:3.5:0.8	NaClO (7%) over night	49	43	60

The composition of the bio-polymer and the percentage of PHA on the material after extraction resulted with a high standard deviation about 19%, this was an indication of weakness of the accumulative step.

Conclusions

The current price of the commercial PHA, usually exceeding 5.0 €/kg, it is closely related to the monomer composition P3(HB) and is usually higher for the copolymers P3(HB-co-3HV); these values are not competitive compared to the market price of conventional polymers (<1.0 €/Kg) [21]. The economic aspect is therefore crucial for commercial diffusion of these materials and various strategies aimed at improving this aspect were first developed on a laboratory scale and have now led to the implementation of the first dedicated pilot prototypes. One of these strategies consists in the cultivation of mixed microbial cultures (MMC), instead of methods based on pure culture, and the use of organic wastes at low cost (or zero cost) used as substrates as feed for MMC culture. The final step associated with the downstream processing for the recovery of PHA from biomass are also of fundamental importance for the economy of the production process, also with respect to the quality of the material, application possibilities and its market value [22].

In the process based on the use of MMC, the PHA are synthesized as a result of the carbon processing industry in the biological treatment of waste water from activated sludge. Power dynamics aerobic conditions (ADF) are usually applied to a variety of process configurations, useful in promoting growth and / or selection of a joint consortium enriched in PHA-accumulating organisms [23].

Further improvement actions are to stabilise the concentration of VFAs controlling the fermentation, in order to obtain the biopolymer with similar HB - HV ratio after every batch of accumulation.

Purify the post-fermentation feed through the use of membranes in order to increase the purity of the polymer and obtain it in constant quantities.

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11. CONCLUSIONS

The integration of different treatments into a single facility for the conversion of urban bio-wastes into valuable bio-based products was demonstrated as a scientific feasible reality.

The first stage of the refinery chain was the food waste pre-treatment by a screw press. This approach represents a truly new and advanced pre-treatment of the biowaste that exploits low energy for pressing the substrate, obtaining a highly biodegradable stream. This flow is devoid of aggregates and inert and leads to recover much more energy of the normal pre-treatments used in the field of anaerobic digestion of the organic fraction of MSW. It is considered important to implement initiatives to support of improving the quality of biowaste treated, both in terms of collection and new paradigms for biogas plant pre-treatment configuration. Poor quality of these matrices worsens the compost that is distributed in soils and inert materials could accumulate inside the reactor allowing a reaction volume reduction and a possible risk of process failure.

Bio-waste is normally pre-treated and prepared for the anaerobic digestion process by means of steps dedicated to the inert material removal and size reduction. These steps are time and energy consuming and generally are not able to achieve high removal yields for inert materials like small pieces of plastics and heavy materials like crashed glass (e.g., sea shells or similar). To avoid this problem as first step, we have to force the collection for the best quality of biowaste as possible obtainable, for example with door-to-door collection scheme and other pre-treatment approaches, that in recent years have been developed: the one we tested is this thesis produced two streams, one semi-liquid to be anaerobically digested (with the greatest specific gas production of 0.92 $m^3/kgTVS_{fed}$ at an organic loading rate of 4.7 $kgTVS/m^3d$ in thermophilic conditions) or fermented and a second one solid to be composted. The research evaluated also the aspects related to "non-steady-state" conditions caused by organic loading rate perturbation events, in order to compare mesophilic and thermophilic process performances for a full-scale implementation. The contents of heavy metals and pathogens of fed substrate and effluent digestates were analysed in order to qualify the digestates for possible use for agronomic purposes. The contents of heavy metals and pathogens of fed substrate and effluent digestates were analysed, and results showed low levels (below End-of-Waste 2014 criteria limits) for both the parameters thus indicating the good quality of digestate and its possible use for agronomic purposes.

It was demonstrated that biowaste pressing is an alternative for treatment plants to be built or revamped. Moreover, in the bio-refinery presented the pressed juice fraction of biowaste is suitable for fermentation in order to produce volatile fatty acids and hydrogen.

The following stage was the analysis of the controlled fermentation and the double phase anaerobic digestion, in order to produce hydrogen, methane and volatile fatty acids. In this thesis the process control automation of each stage of the productive pattern were developed. The entire study was evaluated at pilot scale, where fluid dynamics properties and their effect can be considered and be directly used for an optimistic upscale.

Long-term evaluation of hydrogen VFAs and methane production in a two-phase thermophilic anaerobic digestion was discussed. It was applied the anaerobic sludge dynamic recirculation of the methanogenic-phase in order to keep the optimal hydrogenase enzymes pH in dark fermentation reactor.

This study was therefore focused on the development of a control protocol based on ammonia concentration of the recirculation sludge. In order to lay the groundwork for an automatic control of the process, models were developed to predict Ammonia levels in system. SDEP values comparable with the analytical errors standard deviations (ammonia concentration analysis) in the methanogenesis reactor make the modeling evaluation reliable and implementable in anaerobic systems that deal with this type of substrate. The algorithms presented in this thesis ensure local validity, this means that whenever the operational system changes (e.g. the type of substrate) the modeling choice must therefore be re-evaluated and calculated.

This pilot scale study shows that it is possible to obtain a stable hydrogen production by dark fermentation without physical or chemicals pretreatments when biowaste is used as sole substrate. Comparing the predictive capabilities of the models (SDEP) and the economic feasibility, the best model seems to be the one based on two predictors, conductivity and pH. A stable Biohythane production was obtained with GPR 2.78 $m^3/m^3_r d$ and SGP 0.69 $m^3/KgTVS_{fed}$. Stable volatile fatty acid production was achieved with an effluent rich on VFAs of about 18 g/L. This demonstrates that the use of electrical conductivity and pH measured on-line could be the best model option for on-line monitoring and control of this bio-refinery stage.

The succeeding evaluation dealt with the application of anaerobic co-digestion process for bio-fuels production (biomethane and biohythane) in order to cope fossil fuel used for the automotive sector. Performances in single and two-phase thermophilic anaerobic co-digestion processes were investigated and compared. The comparison of biofuel yields had the aim to ponder an integrated approach on waste and wastewater cycles for the automotive waste collection transports. The novelty of this part of the bio-refinery is a new paradigm on cycles integration between wastewater and organic waste treatment. I wanted to underline the smart opportunity to change the European paradigm for the management of water and waste cycles optimizing the energy production. I wanted to verify if thermophilic co-digestion can close the heat balance of the integrated plant (wastewater

and organic waste), and to demonstrate how the surplus of biogas, when upgraded, can cope the need of biofuels for the automotive recycling sector of the plant.

The last part of the production chain studied was dedicated to bio-polymers production. The fermentation effluent reached value in the range of 16 – 22 g/L VFAs with a fraction almost the 83% of the soluble COD concentration. After fermentation and centrifugation/filtration, the liquid part of the fermented stream was sent to polyhydroxyalkanoates production system. The strategy of the mixed microbial cultures (MMC), instead of methods based on pure culture, was feasible. Even though the use of organic wastes at "zero" costs used as substrates as feed for MMC culture, brings a fraction of inert solids which create an impurity to the final bio-polymer product (%PHA end of extraction in the range of 32 – 72 g/g). Purify the post-fermentation feed through the use of membranes in order to increase the purity of the polymer and obtain it in constant quantities might optimise the process.

Further improvement actions are to stabilise the concentration of VFAs controlling the fermentation, in order to obtain the biopolymer with similar HB - HV ratio after accumulation batch stage.

Considering the novelty of this approach, specific objectives have been achieved and the integrated and flexible Biorefinery concept demonstrated several advantages for the organic waste streams management. The integrated bio-refinery treatments considered give considerable advantages, which lead this option into the field of the 'smart' opportunities for the urban services management.