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PhD thesis

**Anaerobic Digestion of Agrifood wastes: Experimental Campaigns and ADM1- Based
Software Tool Development**

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Index

Nomenclature	4
Abstract	5
Introduction	6
Diffusion of the Anaerobic Digestion Employment.....	6
Difficulties of Anaerobic Digestion Results Prediction	14
Aim of the research	16
Experimental Campaign	17
Basic Principles of Anaerobic Digestion.....	17
Biochemical and physico-chemical processes.....	17
Parameters of influence	20
Types of Digestors	23
Laboratory instruments.....	27
Design of experiments.....	30
Beer Spent Grains energetic characterization and pre-treatment	31
Anaerobic digestion of Greengrocery Wastes at Different Particle Size and BMP estimation.....	36
Anaerobic digestion of Artichoke wastes and Olive Pomace.....	50
Results of the anaerobic digestion of artichoke wastes	54
Results of the anaerobic digestion of Olive Pomace	59
Comparisons and conclusions	63
Software Tool ADMS 2.0	68
Characteristics of the software tool	68
ADMS 2.0 Graphic User Interface	71
ADMS 2.0 Calculation core	88
ADMS 2.0 Validation.....	92
Conclusions	111
Acknowledgements	113

Nomenclature

AD	Anaerobic Digestion
toe	Tonne of oil equivalent
bcm	Billion cubic meters of natural gas
LCA	Life Cycle Assessment
EBA	European Biogas Association
IRENA	International Renewable Energy Agency
CHP	Combined heat and power
BMP	Biomethane potential
FAO	Food and Agriculture Organization
CIRDER	Interdepartmental Center for Renewable Energies Research and Dissemination
DOE	Design Of Experiment
ANOVA	Analysis of Variance
TS	Total solids
VS	Volatile solids
HRT	Hydraulic Residence Time
SRT	Solid Retention Time
OLR	Organic Load Rate
CSTR	Continuous Stirred Tank Reactor
AMOCO	Acidogenesis and Methanogenesis in Organic Compound Oxidation
ADM1	Anaerobic Digestion Model No. 1
ODE	Ordinary Differential Equation
DAE	Differential-Algebraic system of equations
ADMS2.0	Anaerobic Digestion Model Solvers v 2.0

Abstract

Nowadays the growing energy demand has brought to the definition of new challenges aimed to combine energy production processes with circular economy in order to reach social, economic and environmental sustainability. Anaerobic digestion of residual biomasses represents a possible solution to these requirements, considering its versatility and wide applicability in many different contexts. Due to its complexity, to be optimized the Anaerobic digestion process needs an in-depth knowledge of the substrate and inoculum characteristics, the functioning of the production plant and the reaction itself. In this work an assessment of the Biomethane potential (BMP) of some residual biomasses produced in Tuscany territory have been provided to improve the knowledge about the potential employment of this type of discharge for energetic purposes and, parallelly, the development of a stand-alone anaerobic digestion simulation tool with graphic user interface (GUI) in MATLAB has been carried out to enhance the technological instruments available to researchers and technicians to better study and optimize the reaction. Experimental campaigns to define BMP have been investigated by considering Tuscany territory's typical biomasses, such as greengrocery wastes (in 3 different particle sizes for the substrate), artichoke wastes and olive pomace. Results show that for greengrocery wastes, particle sizes considered do not affect BMP results, which have been calculated equal to $0.377 \pm 2\%$ NI/gSV, 0.385 NI/gSV $\pm 4\%$ and 0.379 NI/gSV $\pm 2\%$ respectively for large, medium and small particle size. Artichoke wastes (with a percentage of 17% of other green leaf vegetables) anaerobic digestion has provided a BMP value equal to $0.480 \pm 5\%$ NI/gSV, while olive pomace has reached a BMP value lower and equal to $0.136 \pm 5\%$ NI/gSV. A specific GUI and computation core in MATLAB has been developed and validated referring to benchmark results from Anaerobic Digestion Model #1 implementation. The software tool, named ADMS 2.0, constitutes a user-friendly alternative to more complex simulators such as AQUASIM, which has been not developed to fit technicians and engineers needs once involved in anaerobic digestion processes analysis and specific scenarios' output data generation. By carrying out simulations in ADMS 2.0, thanks to ad-hoc developed data import and management functions, parameters and variables can be declared by reaching around a 85% enhancement in terms of data initialization required time. Processed results can be investigated, exported and plotted by means of custom user interfaces.

Introduction

Diffusion of the Anaerobic Digestion Employment

The growing demand for energy [1], associated with the use of no-renewable fuels such as fossil fuels [2], is leading to several changes both at climate [3] and ecosystem level, with risks also for human health [4]. All over the world, directives have been approved with the aim of using energy production methods through a circular and environmentally sustainable approach [1], [2]. In 2018 the Clean Energy for all European Package was approved [3] with the main objectives, to be reached by 2030, of increasing energy supply from renewable sources by 32% and improving the energy efficiency of buildings [4]. In 2015 the Circular Economy Action Plan was also adopted in order to provide the Member States guidelines and directives aimed to reduce pressure on natural resources and create sustainable growth and jobs [5]. In May 2022, the RePower EU Plan has been launched in order to decrease both European dependence from Russia's fossil fuels and pollution related to energetic supply. This plan has three main axes of application: reduction of consumption, diversification energy supply and production of clean energy [1]. According with the EBA Statistical report 2022 *“there is a consensus that by 2030, the biogas and biomethane sectors combined can more than double production from 18.4 bcm in 2021 to approx. 35-45 bcm in 2030. By 2050, production can increase at least fivefold from today's production levels, going up to productions of 95-167 bcm.”*[2]. In addition to this, the environmental sustainability has to be accompanied by economic and social sustainability. It follows that the energy production has to be not only low-environmental impact but also convenient for the producers in an economic perspective [6], [7]. In the framework of the renewable energy employment technologies, the anaerobic digestion of organic wastes provides a meeting point between the necessity to produce energy (through the use of biogas or biomethane) and the needing to reduce the environmental footprint of the human activities [8]. Anaerobic digestion is a biochemical process in which many bacterial (and archaea) groups elaborate in syntrophic association an organic substrate, producing as ultimate step of the trophic chain mainly methane, hydrogen and carbon dioxide (this compound is called biogas) [9]. Biomethane can be extracted from biogas using a process called “upgrade”, obtaining a product characterized by a concentration higher than the 97% of methane (in this case the biogas is called “washed”). The semi-liquid secondary product of the digestion is called digestate and is rich in mineral: this characteristic makes it suitable to be used as fertilizer.

Biogas can be used in many different contexts: from cooking to transport, from heating to electricity generation. In fact, it can be used unaltered, or “washed” in order to produce electricity (using cogeneration), heat or as a fuel in vehicles. Alternatively, where possible, biomethane can be injected into grid, after been added to THT (tetrahydrothiophene) to impart a smell. If the process of biogas production is carried out with a LCA (Life Cycle Assessment) stance, paying attention to the entire life cycle of the biomasses used as substrate, the benefits extend beyond the mere production of a fuel. In fact, the greenhouse gasses usually developed during natural decomposition are not released directly in atmosphere and the production itself can be controlled in order to maximize the yielding of methane, which has the same characteristics of the natural fossil gas [3]. Adding this information to the fact that many types of organic wastes can be suitable to the biogas production, it follows that the use of manure, sludges and other biomasses the decomposition of which is considered highly pollutant can help reducing the impact of the activities responsible of the wastes themselves [4]. Also, energy crops can be used in this productive process, when their harvesting is well designed and timed. Indeed, this kind of crops can also serve the purpose of sustainability if their cultivation is designed and carried out on the basis of the concept of land use efficiency. Without reducing the complexity and scope of this concept, which can be further

detailed by consulting the publication [5], two key points have to be highlighted: energy crops can contribute to the social and environmental sustainability of biogas if cultivated from marginal lands (considering “marginal land” all the acreages where the cultivation of crops is not economically affordable) or if grown in rotation with other food crops. The potential of a marginal areas for cultivation of biomass dedicated to anaerobic digestion must always be assessed on a case-by-case basis. However, in suitable marginal areas, energy crops cultivation can contribute to curbing soil erosion and desertification, while combating the socio-economic consequences of land abandonment and marginality such as the decline and ageing of the population, the weakening of the productive fabric, the decrease in entrepreneurial initiatives and, ultimately, the lack of services offered on the territory. The implementation, instead, of a double crop approach provides the production, within one year, of both food and energy crops, ensuring benefits for the soil, as increasing of fertility, preventing erosion and nutrient leaching. Another suitable kind of biomasses for this conversion process is represented by the wastes of the agri-food market. In fact, the use of unsold crops wastes has a twofold benefit on both disposal costs and recovery energy [14], [15], promoting a circular economy approach [16], [17]. Anaerobic digestion of fruit and vegetable wastes avoids the exploitation of potentially productive land, employing only those materials that cannot be sold in the market. It must be considered that thanks to the constant growth of the population and the better eating habits, the consumption of these food products has increased considerably. Worldwide, more than 765 million tons of fruit are harvested annually. Globally, fruit and vegetables production has more than doubled since 1990, and it is expected to grow as well over the years [18]. As suggested by a study conducted by FAO in 2019, higher production goes together with a waste increase, and post-harvest loss of fruit and vegetables is globally estimated to be around 20%. Finally, many are the advantages of producing biomethane through the anaerobic digestion process derived from fruit and vegetable waste, but the recovery of unexploited energy resources and the production of low environmental impact fuels are certainly the most important. Apart from the yet mentioned types of proper biomasses for the anaerobic digestion process, many others can be found using processes of biomass characterization (which will be further detailed) and pilot scale reactors to verify the methane yield.

All these elements strongly underline the link between the economic and the environmental sustainability of a well-organized use of this technology. Moreover, the anaerobic digestion can be applied to many different backgrounds: small-scale biogas plants are used in many regions of Africa, Asia and Latino America in order to provide an overall improvement of living condition also in areas in which other energy supplies are not available, but this technology is largely used also in Countries with wide reaching and robust power system, in order to enhance the production loaded on power and gas grid. Referring to Figure 1, in 2022 Germany has produced 32'222.0 GWh using biogas technology, attesting itself as the first world producer of electricity form biogas. However, it seems interesting notice that the top 10 ranking include Countries from different areas, such as Europe, Latine America, Asia and United States. A quite similar conclusion can be reached observing data collected in Figure 2: Germany still occupies the first place with a heat generation of 12'188.00 TJ, but other countries from different mainland are mentioned in ranking.

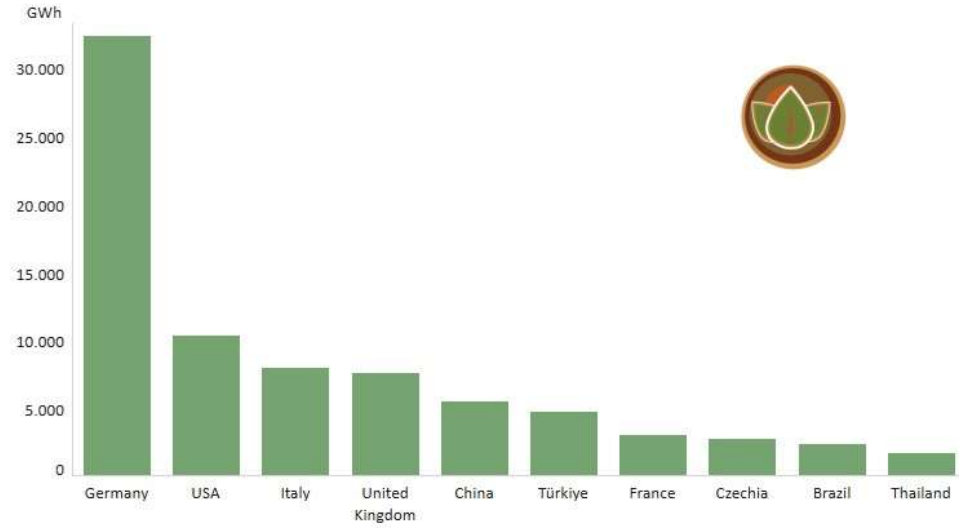


Figure 1. Top ten ranking of Countries for electricity generation from biogas in 2022 [6].

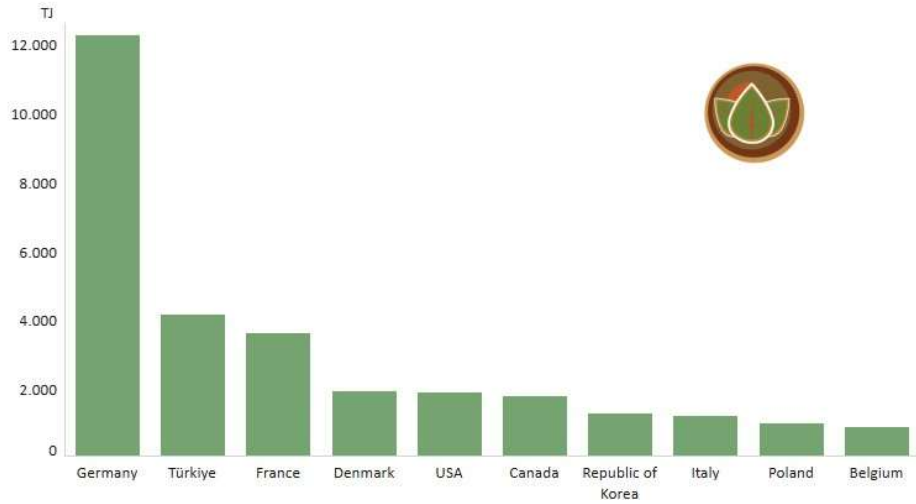
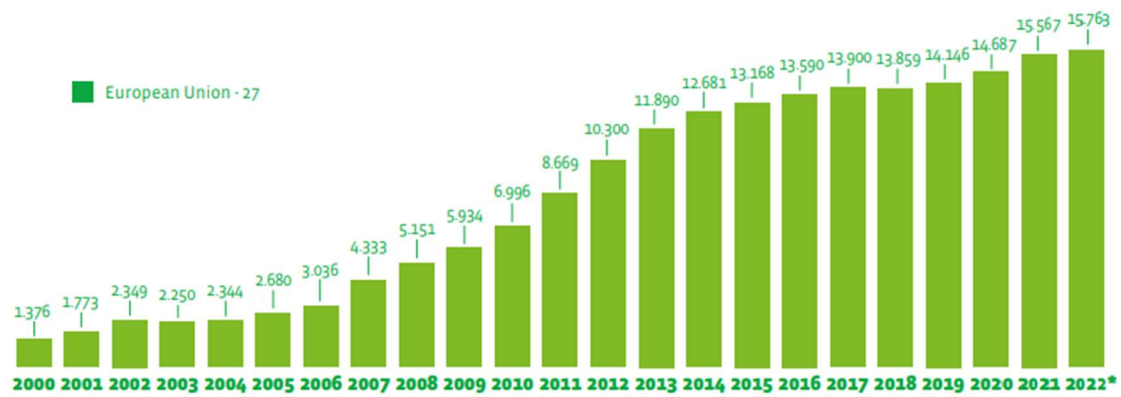


Figure 2. Top ten ranking of Countries heat generation from biogas in 2022 [6].

Focusing on the European Union situation, the primary energy production from biogas has been increased over the years, as shown in Figure 3. However, the annual growth between years 2021 and 2022 has been modest and equal to 1.3%.

Primary energy production from biogas in the European Union (27) since 2000 (in Mtoe)



Note: Methodological improvements concerning biogas production statistics in Germany have been taken into account from 2021, resulting in an increase in the level of production in this country. *Estimation. Source: Eurostat (year 2000-2020), EurObserv'ER (2021-2022)

Figure 3. Primary energy production from biogas in the European Union (27) since 2000 (Mtoe) [3].

The gross electricity production from pure biogas and from biogas blended in the grid hasn't demonstrate a strong growth, as shown in Figure 4. In fact, in 2021 the total of the produced gross electricity from biogas (both pure and blended) was equal to 59'189.9 GWh, while in 2023 the same parameter valued 58'709.9 GWh, with a slight decrease of 0.8 %. However, data demonstrates that, between 2021 and 2022, the amount of gross electricity produced by the use of pure biogas decreased of 1.2 %, while the production from biogas blended in the grid has grown of about 5%.

Gross electricity production from pure biogas and from biogas blended in the grid in the European Union in 2021 and 2022 (in GWh)*

	2021				2022			
	Electricity only plant	CHP plant	Total pure biogas	Electricity from biogas blended in the grid	Electricity only plant	CHP plant	Total pure biogas	Electricity from biogas blended in the grid
Germany	8 380.0	23 980.0	32 360.0	3 280.0	8 280.0	23 950.0	32 230.0	3 100.0
Italy	2 508.6	5 615.6	8 124.2	0.0	2 403.1	5 441.0	7 844.1	0.0
France	353.4	2 800.3	3 153.7	298.5	357.8	2 836.6	3 194.4	721.2
Czechia	37.2	2 555.6	2 592.8	0.9	37.6	2 577.6	2 615.1	1.0
Poland	0.0	1 307.3	1 307.3	0.0	0.0	1 288.3	1 288.3	0.0
Netherlands	13.3	802.0	815.3	238.1	13.0	791.0	804.0	240.0
Belgium	59.2	917.1	976.3	9.9	59.3	918.9	978.2	18.0
Spain	727.0	252.0	979.0	18.9	718.0	272.0	990.0	0.0
Denmark	1.5	611.1	612.6	334.9	1.6	572.7	574.3	333.0
Austria	557.2	44.4	601.6	14.4	549.7	43.8	593.5	14.4
Greece	80.4	376.5	456.8	0.0	68.6	449.2	517.8	0.0
Slovakia	85.0	402.0	487.0	0.0	77.5	366.5	444.0	0.0
Croatia	39.0	401.2	440.2	0.0	38.2	393.0	431.2	0.0
Hungary	52.0	243.0	295.0	4.6	55.2	257.8	313.0	4.6
Portugal	246.6	19.0	265.6	2.0	225.8	21.3	247.1	3.6
Latvia	0.0	291.9	291.9	0.0	0.0	249.6	249.6	0.0
Bulgaria	52.0	164.2	216.2	0.0	45.9	144.9	190.9	0.0
Ireland	118.7	54.0	172.7	1.3	108.4	49.3	157.8	1.3
Lithuania	0.0	156.7	156.7	0.0	0.0	158.7	158.7	0.0
Finland	145.0	184.0	329.0	8.9	26.0	95.0	121.0	0.0
Slovenia	1.5	101.1	102.6	0.0	1.4	91.2	92.6	0.0
Romania	20.6	52.3	72.9	0.0	20.6	52.3	72.9	0.0
Cyprus	0.0	59.9	59.9	0.0	0.0	57.7	57.7	0.0
Luxembourg	0.0	61.0	61.0	1.1	0.0	47.7	47.7	1.1
Sweden	0.0	12.0	12.0	10.3	0.0	30.0	30.0	10.3
Estonia	0.0	16.4	16.4	0.0	0.0	10.0	10.0	0.0
Malta	0.0	7.2	7.2	0.0	0.0	7.4	7.4	0.0
Total EU 27	13 478.2	41 487.9	54 966.1	4 223.8	13 087.7	41 173.6	54 261.4	4 448.5
<i>Note: The rank of this table is based on the cumulative biogas electricity production from biogas used pure or mixed with the natural gas network. *Estimation.</i> Source: EurObserv'ER 2023								

Figure 4. Gross electricity production from pure biogas and from biogas blended in the grid in the European Union in 2021 and 2022 in GWh [3].

A quite similar situation has been registered by the statistics related to the gross heat production in transformation sector by biogas both from pure and blended in grid biogas, as shown in Figure 5. The overall production decreased between 2021 and 2022 with 0.9%: the production from pure biogas slightly declined from 864.7 ktoe to 853.6 ktoe (-1.2%), while the amount related to biogas blended in the grid has increased of 0.3%.

Gross heat production in the transformation sector from pure biogas and from biogas blended in the grid in the European Union in 2021 and in 2022 (in ktoe)*

	2021				2022			
	Heat only plant	CHP plant	Total pure biogas	Heat from biogas blended in the grid	Heat only plant	CHP plant	Total pure biogas	Heat from biogas blended in the grid
Germany	10.7	252.5	263.2	206.6	10.7	280.4	291.2	199.2
Italy	0.1	290.8	290.9	0.0	0.1	276.0	276.1	0.0
Denmark	2.4	47.0	49.4	72.3	2.3	41.9	44.2	69.8
France	3.0	82.9	85.9	15.1	2.3	84.0	86.2	25.9
Belgium	0.0	21.4	21.4	0.2	0.0	21.5	21.5	0.2
Slovakia	0.1	17.6	17.7	0.0	0.1	20.7	20.8	0.0
Czechia	0.0	17.6	17.6	0.1	0.0	18.0	18.0	0.1
Poland	0.9	22.0	22.9	0.0	0.7	17.1	17.8	0.0
Latvia	0.2	19.3	19.6	0.0	0.2	15.2	15.4	0.0
Croatia	0.0	16.9	16.9	0.0	0.0	13.3	13.3	0.0
Netherlands	0.0	7.5	7.5	5.7	0.0	6.7	6.7	5.7
Sweden	1.5	3.8	5.3	3.3	2.3	5.9	8.2	3.3
Finland	5.8	13.4	19.2	0.4	4.8	3.5	8.3	0.4
Austria	1.2	3.6	4.8	1.1	1.1	3.2	4.3	1.1
Romania	1.8	2.9	4.6	0.0	1.8	2.9	4.6	0.0
Slovenia	0.0	3.7	3.7	0.0	0.0	4.1	4.1	0.0
Bulgaria	0.0	3.8	3.8	0.0	0.0	4.0	4.0	0.0
Hungary	0.0	2.7	2.7	0.4	0.0	3.3	3.3	0.4
Luxembourg	0.0	2.8	2.8	0.2	0.0	2.1	2.1	0.2
Lithuania	0.0	2.4	2.4	0.0	0.0	1.5	1.5	0.0
Estonia	0.3	1.3	1.6	0.0	0.2	1.0	1.3	0.0
Cyprus	0.0	0.9	0.9	0.0	0.0	0.9	0.9	0.0
Ireland	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Greece	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Spain	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Malta	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Portugal	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Total EU 27	28.0	836.7	864.7	305.3	26.5	827.0	853.6	306.3

Note: The rank of this table is based on the cumulative biogas heat production in the transformation sector from biogas used pure or mixed with the natural gas network.
*Estimation. Source: EurObserv'ER 2023

Figure 5. Gross heat production in the transformation sector from pure biogas and from biogas blended in the grid in the European Union in 2021 and 2022 (in ktoe) [3]

According to data provided by EBA, Europe currently has about 20'000 active biogas plants on its territory. EBA Statistical Report 2022 affirms that in 2021 58% of biomethane plants connected to the distribution grid, 19 % to the transport grid, 9% were off-grid and 14% made no disclosure [3]. Due to the conflict between Russia and Ukraine, Europe is making biomethane injection in grid a priority, in order to decrease the dependence from Russian fossils resources.

An interesting information that can be extrapolated by the consultation of data shown in Figure 6 concerns the origin of the wastes/biomasses used for the anaerobic digestion. In fact, as mentioned before, there are many types of biomasses which can be used in anaerobic digestion (the characteristics necessary to determine their suitability for the anaerobic fermentation will be longer elaborated further paragraphs). In Figure 6 the authors have divided biogas in “landfill biogas” produced by non-hazardous wastes storage facilities, “sewage sludge gas” from anaerobic digestion of urban and process wastes or process waste water treatment plant sludge, “thermal process biogas” by thermal gasification of solid biomass and “other biogas from anaerobic fermentation”, which includes a plethora of different substrates. This last category includes small farming anaerobic digesters, larger territorial or industrial methanation plants and plants that can manage a mixture of different feedstocks in order to obtain biogas, in a process defined co-digestion, in which different substrates are mixed in defined proportion to achieve the anaerobic digestion process.

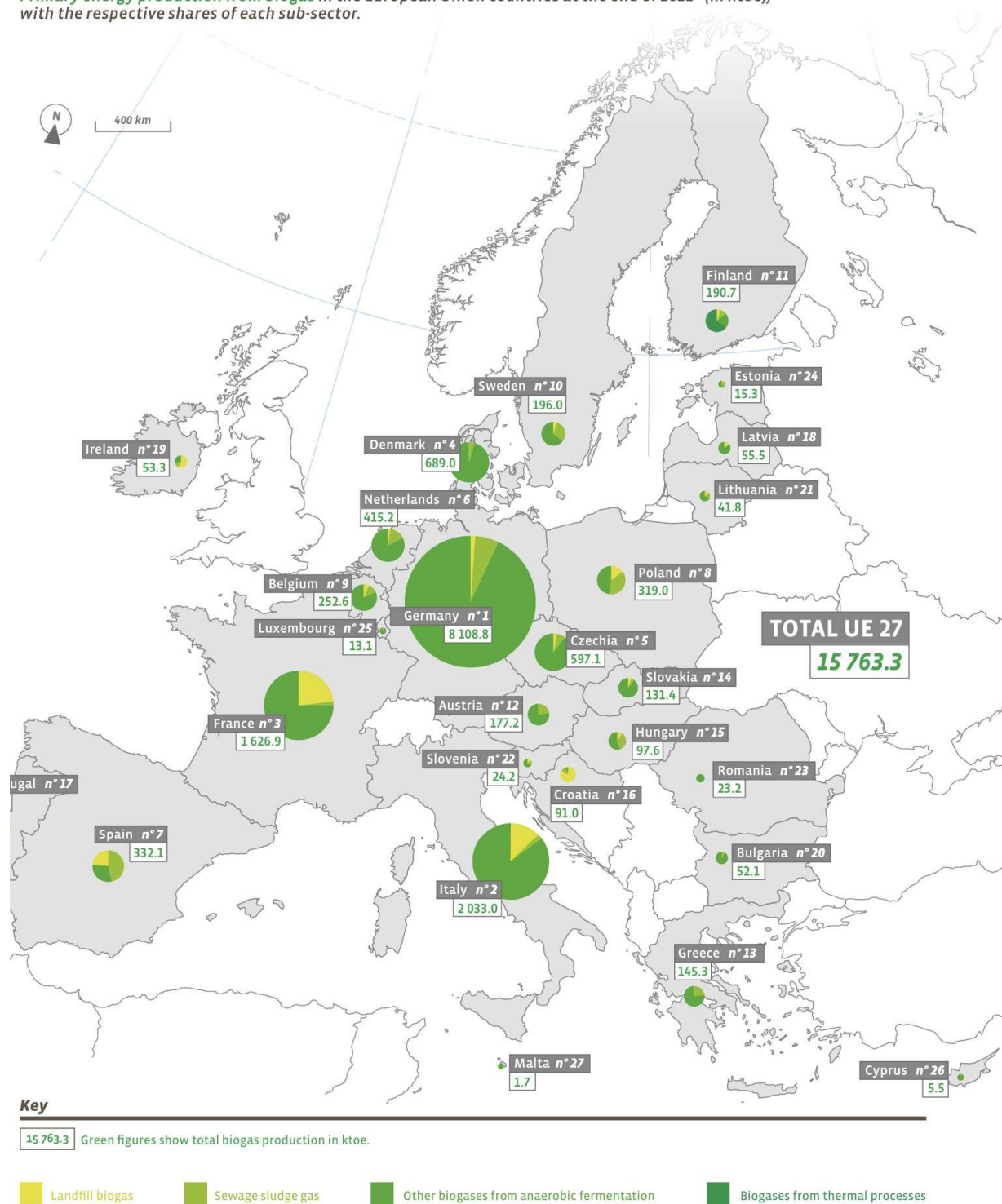
After these definitions, it appears clear that the larger portion of the produced biogas in Europe is related to the category “other biogasses”: in fact, on the total primary energy production referred to year 2022, about the 84% is referred to this category (13’287.5 ktoe on the total 15’763.3 ktoe).

2022					
	Landfill gas	Sewage sludge gas	Other biogases from anaerobic fermentation	Thermal biogas	Total
Germany	112.3	472.9	7523.6	0.0	8108.8
Italy	261.1	48.7	1715.9	7.2	2033.0
France	377.5	27.2	1222.2	0.0	1626.9
Denmark	3.5	26.0	659.5	0.0	689.0
Czechia	19.8	41.9	535.4	0.0	597.1
Netherlands	9.7	65.0	340.5	0.0	415.2
Spain	154.8	98.2	79.1	0.0	332.1
Poland	47.5	119.0	152.5	0.0	319.0
Belgium	16.6	29.0	205.5	1.4	252.6
Sweden	9.4	60.5	126.1	0.0	196.0
Finland	10.9	16.9	41.6	121.4	190.7
Austria	1.1	39.1	137.0	0.0	177.2
Greece	45.2	18.3	81.8	0.0	145.3
Slovakia	6.9	7.0	117.5	0.0	131.4
Hungary	9.0	35.0	53.6	0.0	97.6
Croatia	6.5	3.3	81.3	0.0	91.0
Portugal	62.5	8.2	4.1	0.0	74.8
Latvia	6.9	1.5	47.1	0.0	55.5
Ireland	30.1	7.8	15.3	0.0	53.3
Bulgaria	0.0	5.2	46.9	0.0	52.1
Lithuania	5.5	8.2	28.1	0.0	41.8
Slovenia	1.3	1.2	21.7	0.0	24.2
Romania	0.0	0.0	23.2	0.0	23.2
Estonia	0.9	5.5	8.9	0.0	15.3
Luxembourg	0.0	0.7	12.3	0.0	13.1
Cyprus	0.0	0.3	5.2	0.0	5.5
Malta	0.0	0.0	1.7	0.0	1.7
Total EU 27	1 198.9	1 146.8	13 287.5	130.0	15 763.3
*Estimation. Source: EurObserv'ER 2023					

Figure 6. Primary production from biogas in the European Union in 2022 (in ktoe) [3].

A visual demonstration of the importance of the biogas produced by anaerobic fermentation of other biomasses is plotted in Figure 7, in which the pie chart located on each Country shown the amount of locally produced and the biogas provenience.

Primary energy production from biogas in the European Union countries at the end of 2022* (in ktoe), with the respective shares of each sub-sector.



*Estimation. Note: When the information was not yet available, the distribution between the different types of biogas was estimated by EurObserv'ER for the year 2022 according to the distribution of the year 2021. Source: EurObserv'ER 2023

Figure 7. Primary energy production from biogas in the European Union countries at the end of 2022* (in ktoe), with the respective shares of each sub-sector [3].

Difficulties of Anaerobic Digestion Results Prediction

The information provided demonstrates the importance of the use of other types of biomasses, over urban and landfill wastes and sludges, in the anaerobic process to produce biogas and gives an overview on the variety of different feedstock which can be used in the anaerobic digestion, the characteristics of which vary in relationship with the production processes, the local availability of certain biomasses instead of others and, ultimately, the strong bond between territory and available biomasses.

In this connection, a preliminary remark has to be made: not all the organic matter is suitable for anaerobic conversion processes. In fact, organic compounds with high values of moisture ($> 30\%$) and low values of carbon on nitrogen (C/N) ratio (< 30) are usually converted using biochemical processes such as anaerobic digestion, instead biomasses with high C/N ratio (> 30) and low moisture values ($< 30\%$) are subjected to thermochemical treatments [28], [30]. Moreover, the size of solid biomasses introduced in the digester (which is the reactor where the anaerobic digestion performs) and the chemical characteristics of the organic matter give a contribution on the duration and the yield of the process. It follows that the achievement of an optimized and sustainable anaerobic digestion process is strongly related to both internal and external factors, which transversally involve multiple disciplines. First of all, the provenience of the substrates has to be tracked, in order to enhance the use of a 0 km organic matter in the digestors and to assess that the productive process doesn't involve the use of inhibitory or toxic compounds, e.g. particular antibiotics persisting in manure or pesticides in the crops. In order to carry out a sustainable process, also the timing of the production of biomasses is important: many crops are not available in every period of the year and the same is true for wastes related to agri-food sector. In addition to this, energetic characterization has to be performed, in order to have more knowledges on the humidity, the ash and volatile solid contents, the C/N ratio and other information which can be used to optimize the reaction. Also, the Biomethane Potential of the used biomasses is useful to predict the biogas yield of a certain substrate digestion, which is fundamental to correctly assess the economic benefits of the installation of plant (e.g. in a farm context) or the purchase of a certain biomass plot. Another important element of this framework is the knowledge of the suitable biomasses produced on the territory: as said before, many wastes are suitable for anaerobic digestion, but often the producers are not aware of this. It follows that the wastes are disposed without being processed in an energetic conversion process. This lack of know-how can be solved by the use of experimental campaigns aimed to detect biomasses suitable on territory and to study their properties in an anaerobic digestion stance, creating a database finalized to share them with other professionals. After these researches have been accomplished, a correct design of the digestion process is needed: the digesters have to be properly designed and the substrate has to be compatible with the bacterial pool used. During the reaction, if possible, process has to be monitored in order to assess the well prosecution. In fact, the process duration is variable and depends by many factors related to the characteristics of the substrate and the operating conditions of the plant. The wastes submitted to this energetic conversion are different (e.g. manure [10], dairy wastes [11], olive mill [12], water hyacinth [13], etc...) and their biomethane yield varies with their physico-chemical characteristics. It follows that the comprehension of the entire supply chain that ensure a circular approach to the anaerobic digestion has to be reached by the involvement of different professional, such as breeders, farmers and facilities, agronomists, biologists, chemical and process engineers and digestors managers. Also, researches can provide a fructuous support to this aim, analysing residual available biomasses and their combinations, according with the production in the time and territory, in order to explore the potential of unused wastes and draw a pathway for professionals.

Another element which could simplify research in this field is the aid of software tools employed for the calculation of mathematical models of the anaerobic digestion reactions. Nowadays, the most accurate model presented is the Anaerobic

digestion Model N.1, which was published by IWA Task Group in 2002. This model divides the processes of the AD in biochemical processes and physico-chemical processes, using a system composed by thirty-five differential equations to describe the trends of an equal number of compounds' concentrations. Moreover, the model considers also the use of a certain number of algebraic equations to define parameters related to temperature, pressure and pH. The complexity of the model requires the aid of a calculator to carry out the simulation. Nowadays, the most used software tool for this purpose is AQUASIM 2.0, which has been released in 1998 and has been not longer up dated. This software is still well functioning, but not user friendly, especially in comparison with the modern tools which are more user oriented. One of the most difficult part associated to the use of AQUASIM 2.0 is related to the implementation of the parameters, input data e and equations, because this procedure requires a deep knowledge of the model, a biochemical stance and an accurate study of the software, in addition to a large amount of time required to initialize each element needed for the ADM1 calculation. It follows that a professional figure which is not deeply involved in this fields of interest has a very hard time trying to use this software. It results the impossibility for technical workers to access the benefits of an accurate simulation of anaerobic digestion processes due to the complexity of the simulation tool. In fact, the AQUASIM 2.0 software tool has been built to be used in many different water-based systems, including also rivers, lakes and saturated soil columns. This characteristic makes this software tool very powerful and flexible to be employed by a pro user for many different simulations both in anaerobic digestion and other fields (such as sewage disposal processes simulations). Another important pro associated to the use of AQAUSIM 2.0 is the possibility to perform sensitivity analysis of the results in relationship to chosen variables. In addition to this, the software calculation core is suitable to manage stiff equations, obtaining the convergence of the results also in presence of multiple stiff differential equation.

Unfortunately, the wide applicability of AQUASIM v2.0 means that the implementation procedure for a specific mathematical model is not guided: this software tool contains pre constituted and labelled boxes to insert some variables such as reaction rate, equilibrium constants (which will be better describe in following sections), but it happen that some elements of the mathematical model are not explicitly attributable to the quantities that the software requires (for example in the definition of the links between compartments, such has the link between the liquid phase volume and the gas phase volume of the digester). For this reason, the probability of making mistakes in the implementations increases and can be related both to the lack knowhow in the biochemical field and the misunderstanding of the software tool requirements.

In addition to this, the user interface of AQUASIM 2.0 has been developed more than 20 years ago and now results extremely difficult to handle: in fact the insertion of each information contained in the mathematical model has to be implemented using one or more windows and then the equations describing the rate of each process have to be implemented manually without the possibility to access to the variables windows: it means that the exact code with which a variable was defined has to be remembered by the user.

In addition to this, the import and export of the results is difficult: it's not possible to import data about different variables and the exportation is mediated by the graphic interface.

Aim of the research

During the years, many researchers have implemented the ADM1 using MATLAB/Simulink or Python. Also IWA Task group has released some codes in order to help users in implementing the model. However, the consultation of these codes requires both the knowledge of the ADM1 model and medium skill in coding, in order to use appropriately this kind of source (e.g. the use of structure instead of matrices). In addition to this, these codes are often linked to specific simulative necessity: it follows that the equation contained in them have been modified in comparison to the ones reported in the basic version of ADM1.

This work aim to enhance the knowledges related to the exploitation of residual biomasses in Tuscia territory in anaerobic digestion, providing, for some wastes of the agrifood market, an energetic characterization and a value of BMP, obtained by anaerobic digestion processes in scale reactors in CIRDER Laboratory. Experimental campaigns have been designed using principles set out by DOE (Design of Experiment) discipline, in order to provide a stiff base to the laboratory procedures put in place. Alongside, a standalone application, called ADMS 2.0, has been studied and developed using MATLAB, in order to provide a user friendly software tool to simulate AD processes on the base of the ADM1. The validation of this tool has been carried out implementing the same simulation both in a AQUASIM 2.0 and ADMS 2.0 and evaluating the average relative error detected in each trend of each compound considered in the model.

Experimental Campaign

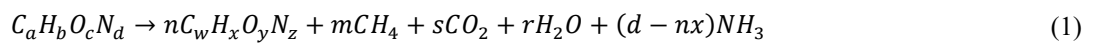
In this Chapter the laboratory campaigns carried out during the PhD course are described. The purpose of this activity is the definition of the suitability of some residual biomasses for the anaerobic digestion process. Data collected by mean of laboratory experience can guide interested researchers and technicians in the exploitation of this biomasses in the local region in which they are produced. In fact, the acknowledge of the BMP related to a compound provides a measure of the quality of the biomethane yield associated to the process, making clear the feasibility of the anaerobic digestion itself in economical and productive terms. Moreover, a low value of BMP associated to the anaerobic digestion of a certain compound should be studied in order to define if there are pre-treatments or co-digestion patterns which can be put into practice in order to facilitate the anaerobic digestion for the bacterial families involved in the anaerobic digestion process.

Basic Principles of Anaerobic Digestion

Biochemical and physico-chemical processes

Anaerobic Digestion (AD), as described before, is a biochemical process in which organic matter is degraded by mean of different archea and bacterial families, which produce, at the end of the syntrophic chain, biogas. Biogas composition is extremely variable, but mainly consisting in methane (which represent the main product, with a percentage between 55% and 75%), carbon dioxide (25-45% on the total) and other compounds [7]. Anaerobic digestion provides also a secondary product, a semi-solid compound, known as digestate, rich in minerals and suitable to be used as soil fertilizer.

As evidenced, the core of the entire process is the syntrophy of different archea and bacterial families, which mediate each stage of the anaerobic digestion exploiting some compounds and producing other intermediate reaction products. The biomass used for the digestion is often indicated as substrate and is mainly composed by carbon (C), hydrogen (H), oxygen (O) and nitrogen (N). Its molecular formula can be generically defined as $C_aH_bO_cN_d$ [8]. According to this formula, the anaerobic digestion process can be described as:



Considering:

$$s = a - nw - m \quad (2)$$

$$r = c - ny - 2s \quad (3)$$

Reaction presented in this chemical balance ranks among the reaction products, in addition to the just cited methane and carbon dioxide, also a generic organic compound, different from the initial substrate, ammonia and water. However, substrate composition influences the anaerobic digestion results, deeply: the anaerobic digestion of pure glucose cannot generate ammonia, considering the fact that nitrogen is not contained in the initial compound:



Alongside substrate characteristic, reaction products are strongly dependent by the bacterial and archea population in the reactor. In fact, the inoculum needs specific pH and temperature condition to ensure a high yield. Moreover, the presence

of certain compounds in the substrate can inhibit or even intoxicate certain bacterial families, resulting the slow down or the arrest of the process. In addition to this, the inoculum has to be suitable to digest the substrate: sometimes a certain inoculum doesn't contain the specific bacterial families needed for the digestion of, for example, fish wastes or meat protein. It follows that the results of the process are not easy predictable, because of the large amount of variables involved. Nevertheless, the observation of the methane yield and, where possible, of the compounds' trends suggests the reaction's outcomes and gives more information about the issues by which the reaction has been affected.

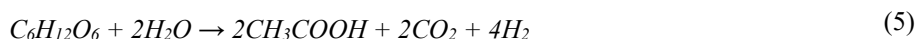
The anaerobic digestion process can be considered as composed by four stages which are consequentially related: outcomes of a stage are incomes of the next one. First stage is Hydrolysis and it is mediated mainly by bacterial families of *Bacteroides*, *Clostridium*, *Ruminococcus* and *Aerovibrio*. At this stage, the macromolecules contained in the substrate are reduced into simpler compounds (called monomers), suitable for processing from the bacterial strains of the subsequent stages. More in detail, during hydrolysis, proteins, fatty acids and sugar are produced from amino acids, lipids and carbohydrates [8]–[10]. The process of degradation is completed by hydrolytic bacteria through the use of special enzymes. These bacteria, in fact, release, near the macromolecule, digestive enzymes that trigger a particle solubilization reactions. The soluble product of the enzymatic reaction is then absorbed by bacteria [11], [12].

Finally, it is important to specify that the hydrolysis phase is accompanied by a process of disintegration (simultaneous with the hydrolysis itself), which consists in the reduction of complex compounds without biological mediation and thus contributes to the formation of substances useful for the succeeding phases, without involving bacterial families.

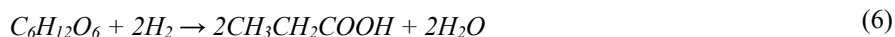
The hydrolytic phase is then replaced by the acidogenic phase. Acidogenesis (or even fermentation) is, as the name suggests, a stage in the formation of organic acids in the absence of additional donors or acceptors [13]. Like the preceding one, also this phase is mediated by more than one family, generally indicated by the name of acidogenes and mainly belonging to *Selonomomas*, *Clostridium*, *Ruminococcus*, *De-sulfovibrio*, *Syntrophomonas* and *Syntrophobacter*. During fermentation, acidogenic bacteria mainly process amino acids and monosaccharides (sugars) producing carboxylic acids [9].

The acidogenesis of monosaccharides (mainly glucose) allows the production of the following compounds:

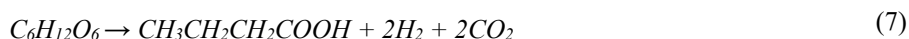
- acetate, formula CH_3COOH , obtained by reaction:



- propionate ($\text{CH}_3\text{CH}_2\text{COOH}$) resulting from the reaction between glucose and hydrogen H_2 :



- butyrate $\text{CH}_3\text{CH}_2\text{CH}_2\text{COOH}$ from the reaction:



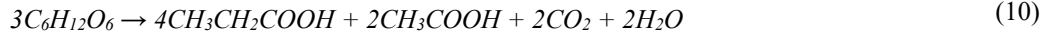
- lactate $\text{CH}_3\text{CHOHCOOH}$:



- ethanol, formula $\text{CH}_3\text{CH}_2\text{OH}$ obtained as:



In addition to this, acetate and propionate are produced simultaneously due to the following reaction:



Amino acid fermentation produces, along with the above-mentioned carboxylic acids (C2,C3,C4,C5,C6), also ammonia, carbonic anhydride, molecular hydrogen and aromatic carboxylic acids (obtained by the decalcification of phenylalanine, tryptophan and tyrosine). This production takes place through two pathways:

- Stickland oxidation-reduction: in this process different amino acids can play the role of electron acceptors or donors. Usually the electron donor loses one carbon molecule for each CO₂ molecule and forms a carboxylic acid with fewer carbon atoms than the starting amino acid. Conversely, the receptor retains its carbon atoms, forming an acid with a carbonaceous chain of the same length as that of the original amino acid. Only histidine cannot be degraded by Stickland reactions;
- the oxidation of a single amino acid in which hydrogen ions or carbon dioxide act as external acceptors of electrons.

The 90% of amino acid production takes place through oxidation-reduction [14], while only 10% of the amino acid acidogenesis is due to oxidation. This is mainly due to a deficit of external electron acceptors [15].

Fatty and carboxylic acids produced during hydrolysis and acidogenesis are then processed during the third macro-phase of the anaerobic digestion: acetogenesis. Acetogenesis involves the production of acetic acid (CH₃COOH), formic acid, carbon dioxide and bimolecular hydrogen by acetogenic bacteria. In this phase, both long-chain fatty acids (with more than 5 carbon atoms forming the carbon chain) and short-chain fatty acids are processed. Clostridium, Eubacterium and Acetogenium are the main actors of this process.

The last stage of anaerobic digestion is methanogenesis. This phase concludes the trophic chain and thus the process itself, since the methanogenesis products are not processed by other bacterial strains [8], [9]. Methane (CH₄) is produced in three ways:

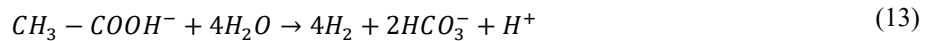
- the acetoclastic pathway, by which methane and carbonic anhydride are obtained by anaerobic dismutation of acetic acid according to a transmethylation reaction of the type:



- the hydrogenotrophic pathway. In this case methane is obtained by anaerobic oxidation of hydrogen, described by the reaction:



- the oxidation of acetate. This latter type of methanogenesis occurs by means of the following reactions:



Between the three CH₄ production pathways just described, the first one is the most widely used (it suppresses the production of about 72% of total methane), while the second one represents a marginal process for the biomethane production. The oxidation of acetate, on the other hand, is a typology of methane production that can only be observed at

temperatures above 50 °C and grows in importance as the temperature increases. Two genera utilise acetate to produce methane: *Methanosarcina* and *Methanosaeta*. The first one has a higher yield than the second one, being, however, more sensitive to changes in pH and requiring acetate concentrations greater than 10^{-3} M. *Methanosaeta*, on the other hand, operates at lower concentrations of acetate and has a lower yield. Oxidation of the acetate is mainly carried out by the strain *Syntrophobacter wolinii*. In the biologic consortium involved in anaerobic digestion, methanogen archaea are the more sensitive to changes in the physico-chemical environment in which are located, resulting in specific and restrictive operating conditions for anaerobic reactors. Based on the optimal temperature range for growing and operating, methanogens can be distinguished into:

- psychrophiles, whose temperature range is between 0 °C and 15 °C;
- mesophiles, whose temperature range is optimal between 20-40 °C, with a maximum growth rate at 35 °C;
- thermophiles, operating at temperatures between 45°C and 70°C and with a maximum growth rate at 55°C.

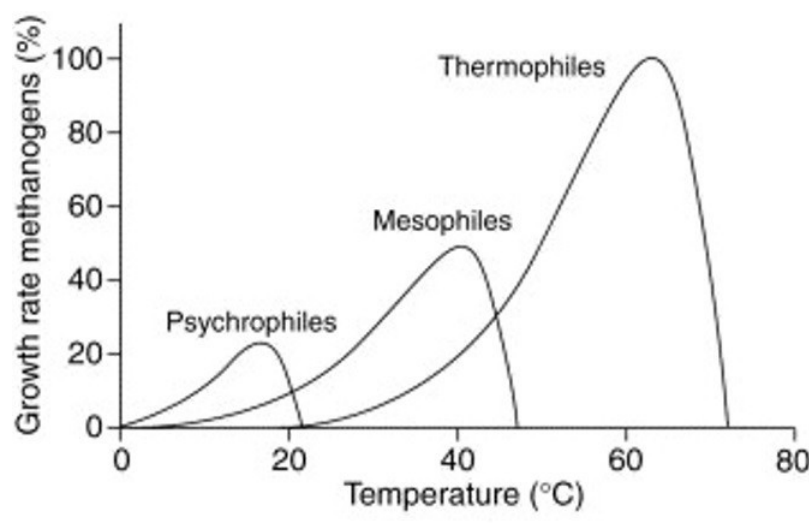


Figure 8. Relative growth rate of psychrophilic, mesophilic and thermophilic methanogens [13].

Moreover, acetogenic and hydrogenotrophic bacteria operate in syntrophy. The action of acetogens is inhibited by high concentrations of hydrogen. The action of hydrogen -utilising methanogens, which consumes hydrogen to produce methane, ensures low concentrations of H_2 , which do not interfere with the action of acetogens.

Parameters of influence

In addition to temperature, there are other parameters which strongly influence the growth and yield of the reaction, mainly due to the effects that they produce on the biological substrate. In anaerobic digestion these parameters are studied to define, optimize and control the process to obtain stable reactions whose products can be predicted and controlled. For anaerobic conversion the most important influence parameters are:

- **substrate composition:** the characterisation of the biomass in relation to total solid content (TS), volatile solids (SV), chemical oxygen demand (COD), biological oxygen demand (BOD) and C/N ratio. Total solid content describes the dry matter content of the sample and thus, in first approximation, the sum of the organic fraction and the inert fraction of the substrate. Volatile solids content consists of the dry matter fraction which is volatilized by combustion at 550 °C. The anaerobic digestion process can be classified, with reference to total

solids content in wet ($ST < 15\%$), semi-dry ($15\% < ST < 20\%$) and dry ($ST > 20\%$). The chemical oxygen demand represents the amount of oxygen consumed for the oxidation of organic matter [8], [10]. A considerable fraction of the COD may not be anaerobically degradable, for this reason the ultimate biodegradability factor is introduced to D which represents the percentage of anaerobically biodegradable components in the sample. It is very rare for a compound to have $D = 1$, whereas more often than not the total biomass can be divided into a degradable fraction D and an inert fraction ($1-D$), which is further divided into soluble aggregates (SI) and inert particulates (XI) [16], [17]. The biological demand is the quantity of oxygen consumed in five days under controlled conditions for the biological oxidation of the organic substance present in a certain sample [8]. The C/N ratio represents the comparison between the carbon and nitrogen in the sample, the optimum of this parameter lies between 16:1 and 25:1. A C/N ratio higher than these values indicates rapid nitrogen consumption and a consequent decrease in methane production. Conversely, if C/N ratio is lower than the lower than 25:1, an accumulation of ammonium occurs, which results in a rise of pH to toxic values for bacterial substrate ($pH > 8.5$) [10];

- **temperature**, which determines the bacterial families involved and the types of reactions taking place in the digester. As seen previously, at each temperature range is associated a different biological pool for methanization, whose optimum reaction rate is obtained for a very precise temperature value. When the temperature range varies, a complete substitution of bacterial flora is observed. In each range, on the other hand, the reaction rate increases with temperature, directly; however, once the optimum temperature is exceeded, this rate begins to decrease. Considering biochemical reactions, temperature values above $65\text{ }^{\circ}\text{C}$ allow to observe the production of methane (obviously by thermophilic bacteria) by oxidation of acetate. For temperature values between $50\text{ }^{\circ}\text{C}$ and $65\text{ }^{\circ}\text{C}$, acetate oxidation occurs only at low concentrations of CH_3COOH , whereas the aceticlastic pathway is widely used for higher acetate concentrations [18], [19]. At $T = 60\text{ }^{\circ}\text{C}$, the oxidation of acetate contributes to the production of 14% of total methane [18]. For $T > 65\text{ }^{\circ}\text{C}$ the oxidation of acetone is the main mechanism of production of CH_4 . The effect of temperature on thermodynamic reactions can be described by the Van't Hoff equation [16];
- **pH**, on which the life of all microorganisms involved in digestion depends. Among all bacterial groups, methanogens are the most sensitive to variations of pH. They grow in neutral environments with pH between 7 and 7.5 and are intoxicated by pH greater than 8.5. The bacteria responsible for acidogenesis and acetogenesis, on the other hand, are favoured by acid pH, with values between 5 and 5.5. The pH value in a digester is determined by the presence of carbon dioxide in the liquid medium and by the concentration of fatty acids and ammonia. The pH deviates from the optimum value due to an imbalance in the reactions constituting the process. To be able to focus on the cause of this problem, it is necessary to study the alkalinity (buffer effect) and volatile fatty acids present in the system. Alkalinity is the ability of a system to neutralize protons and is generally expressed in terms of calcium carbonate concentration. The fatty acids, represented by the general formula R-COOH , are produced during hydrolysis and acidification. The increase in acidity is a direct consequence of an increase in the substrate load to be treated, which causes the acceleration of hydrolytic and acidogenic phenomena. This results in a lowering of the pH values when the buffer capacities of the medium are lost. It follows that alkalinity and concentration of fatty acids are closely related, so in pH analysis it is useful to take into account the relationship between volatile fatty acids and alkalinity. The concentration of the first ones is

expressed in terms of acetic acid, while the alkalinity is described by the concentration of calcium carbonate. Under stable conditions the ratio of volatile fatty acids/alkalinity is in the range of 0.3, while in the presence of imbalances the numerator tends to grow while the denominator decreases to [16];

- **mixing**, since to ensure the correct development of the process it is necessary to mix the fluid in order to maintain its homogeneous characteristics, encouraging contact between the substrate and the micro-organisms and avoiding the presence of a thermal gradient. However, the degree of agitation must be carefully considered, in fact if it is too low the mixing is inadequate, while if it is too high there is a risk of separating the bacterial communities [10].

The process of anaerobic digestion may be hindered by the presence of reversible or irreversible inhibitions which slow down normal conversion reactions and metabolism of bacterial species. The chemical composition of the substrate may be the first obstacle to carry out the process correctly. A substrate rich in heavy metals, salts, ammoniacal nitrogen (NH_4^+), residues of pesticides and pharmaceuticals, substances, etc., is not suitable for bacterial cultures. However, certain metabolic processes that occur during digestion also introduce inhibitions. An example is the inhibition of the degradation mechanism of propionate. If this mechanism is slowed down, the propionate concentration increases to toxic levels (3 g/l) for bacteria. Other toxic compounds to methanogenic bacteria are salts. In fact, the growth rate of methane-generating bacteria is reduced by 50% by the presence of NaCl in concentrations ranging from 250 to 500 mM. Also, chloroform is an inhibitor of methanogenesis, but this kind of inhibition can be managed: in fact bacterial families can adapt to the presence of this substance, resuming their activity after about two weeks from the first exposure and no longer suffering the effects. Another typology of compounds responsible of inhibitory action are the long-chain fatty acids (LFCAs), which can be inhibitors at low concentrations [20]–[25]. An inhibition of this type may be irreversible and affects mainly acetoclastic methanogens. Conversely, inhibition due to weak acids and bases is reversible. The presence of these acids and bases, such as organic acids (HAc, HPr, HBu, HVa), hydrogen sulphide (SH_2) and ammonium NH_4^+ , destroys cellular homeostasis due to pH change [26]. The most sensitive organisms to this inhibition in the anaerobic digestion biological pool are acetoclastic methanogens, hydrogenotrophs and acetogens. The inhibitions due to weak acids occur at low pH values, while the ones caused by bases occur at high pH values. As the same as pH, temperature is not only an influence parameter, but it can also hinder the proper biological activities in the digester. In fact, if a methanogens family (psychrophile, mesophile or thermophiles) is subjected to a temperature not included in the above-mentioned range, the death of the entire colony occurs. Finally, inhibitions due to competition between bacterial groups can occur. For example, during the anaerobic digestion process sulphur oxidized compounds are reduced to S^{2-} by sulphate-reducing organisms. These ones, to perform the reduction, acquire electrons from the oxidation of organic acids and hydrogen H_2 , competing with hydrogenotrophic bacteria for the use of hydrogen and with acetogenic and acetoclastic bacteria for electrons and carbon. It follows that the methane production is affected by these activities and decrease. In addition, the product of the reduction (S^{2-}) reaches concentrations of about 0.002–0.003 M, which are an inhibition cause for hydrogenotrophic, acetoclastic and acetogenic bacteria. The reduction of nitrate in nitric oxides (NO_2^- , NO, N_2) is also mediated by many bacterial families, belonging to proteobacteria. The activity of these organisms competes for the use of the substrate (in particular for acetate and hydrogen) with the processes in which methanogenic bacteria are involved and leads to a decrease in the production of CH_4 . In addition, nitrogen oxides inhibit methanogenic activities [16].

Types of Digestors

Anaerobic digestion process also depends on the type of reactor used to carry out the digestion itself. In fact, different reactors require different technical measures to implement and optimize the reaction (and, eventually, the simulations). Plants for anaerobic digestion can be classified mainly by [7]

- the feeding system: continuous, semi-continuous or batch;
- the number of stages: single stage or double stage;
- the temperature at which the process takes place (referring to the temperature ranges described above for the classification of methanogens): psychrophile, mesophile, thermophile;
- fluid dynamics of the process: plug-flow, fully mixed and hybrid.

To better describe the different typologies of reactors, an introduction on management parameters has to be done.

The first parameter is the hydraulic residence time (HRT), which represents the average time spent by each fluid element in the reactor and is measured in days. Under ideal conditions, the HRT should correspond exactly to the residence time of each fluid element in the digester, but under real conditions the geometry and characteristics of the reactor suggest that the definition should be declined in average terms. Analytically this quantity can be calculated as:

$$HRT = \frac{V}{Q} \quad (15)$$

Where V stays for the reactor volume (measured in m^3) and Q describes the volumetric flow rate in m^3/d .

Another important parameter is the solids retention time (SRT), measured in days. It describes the amount of time spent in the digester by the microorganisms:

$$SRT = \frac{V \cdot X}{W} \quad (16)$$

With

- V : reactor volume in m^3 ;
- X : volatile solids concentration in the reactor [kg_{VS}/m^3];
- W : volatile substance flow rate extracted by the reactor [kg_{VS}/d].

The organic loading rate (OLR) is referred to the amount of organic material entering the reactor per unit of time and volume and is usually measured in $\frac{kg_{VS}}{m^3 \cdot d}$:

$$OLR = \frac{Q \cdot S}{V} \quad (17)$$

Where:

- Q describes the volumetric flow rate in m^3/d ;
- S defines substrate concentration in input in the reactor [kg/m^3];

- V is reactor volume in m^3 .

Other parameters are linked to the production of biogas in the reactor. To introduce these ones, the following measure are described as follows:

- produced biogas flow rate Q_{biogas} in m^3/d ;
- input flow rate Q in m^3/d ;
- substrate concentration S contained in input flow in kg_{VS}/m^3 ;
- reactor volume V in m^3 .

On the base of these definitions, the specific gas production (SGP) can be introduced. It represents the amount of biogas produced per unit of volatile solid substance in input for the reactor:

$$SGP = \frac{Q_{biogas}}{Q \cdot S} \left[\frac{m^3}{kg_{VS}} \right] \quad (18)$$

The gas production rate (GPR) describes the biogas production per unit of reactor volume:

$$GPR = \frac{Q_{biogas}}{V} \left[\frac{1}{d} \right] \quad (19)$$

Removal substrate efficiency is a percentage value which describes the amount of volatile solid substance which has been consumed during the conversion:

$$\eta = \frac{Q_{in} \cdot S_{in} - Q_{out} \cdot S_{out}}{Q_{in} \cdot S_{in}} \quad [\%] \quad (20)$$

After these introductions, the reactor typologies can be described.

The continuous stirred tank reactor (CSTR) is a type of digester characterized by a feed mode which can be continuous or semi-continuous and by the presence of a mixer which continuously maintain the agitation of the substrate. Mixing ensures homogeneous characteristics of the substrate and makes the anaerobic digestion process more productive (refer to the discussion in the previous paragraphs). The CSTR typology can then be further declined into a variant with recirculation and one without recirculation. CSTR completely mixed without recirculation is characterized by concentrations of substrate, products and biomass in the effluent equal to those observed within the reactor (Figure 9).

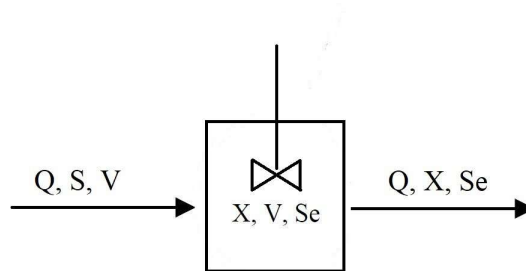


Figure 9. Scheme of a CSTR without recirculation [8].

In this case, the following equation is verified:

$$HRT = SRT \quad (21)$$

The process that takes place in this type of reactor is used to stabilize the sludge produced by sewage plants or for wet or semi-dry anaerobic digestion processes.

CSTR with recirculation has a more complex plant scheme (Figure 10), due to the presence of the recirculation of the solid fraction, which has been re-introduced into the reactor (in order to enhance the biochemical efficiency of the process) after being separated by the liquid in the proper separator. In this case $HRT \neq SRT$.

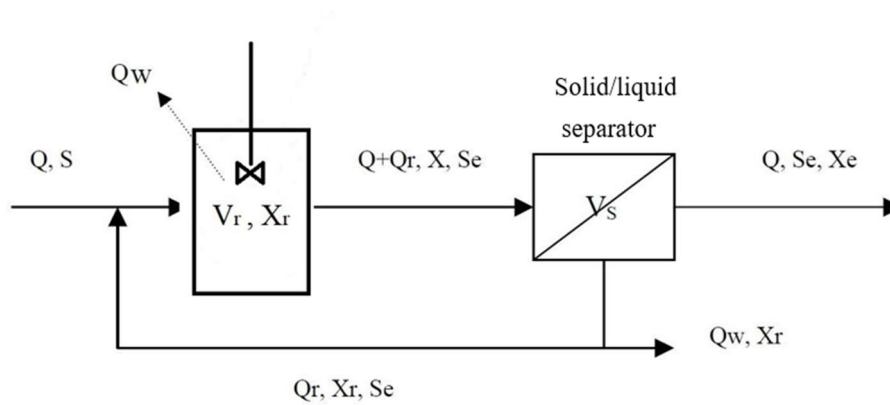


Figure 10. Scheme of a CSTR with recirculation [5].

Plug flow reactor is another type of continuous or semi-continuous flow reactors. This type of reactor is characterized by a geometry of the reactor in which one dimension predominates over the others. The substrate introduced at the digester inlet is pushed towards the outlet so that it moves following the main dimension of the reactor itself. It follows that the only possible mixing mode is the one occurring along the dimension orthogonal to the flow. This function mode determines that the substrate characteristics vary along the main dimension of the reactor. As in the previous case, this type of reactor can also be characterized by the presence of recirculation. As in the case of CSTR, recirculation is also used in installations with piston flow to introduce an active inoculum into the reactor: both the solid and liquid fraction can be reintroduced. Plant schemes of plug flow reactors are provided in Figure 11 , Figure 12 and Figure 13.

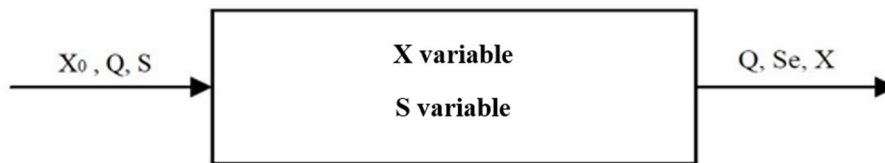


Figure 11. Plug flow reactor without recirculation [8].

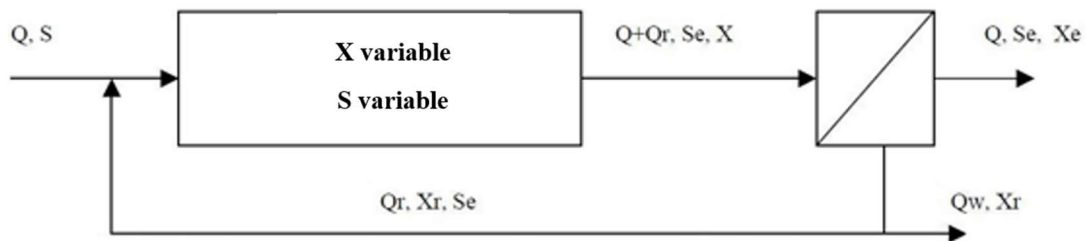


Figure 12. Plug flow reactor with solid fraction recirculation [8].

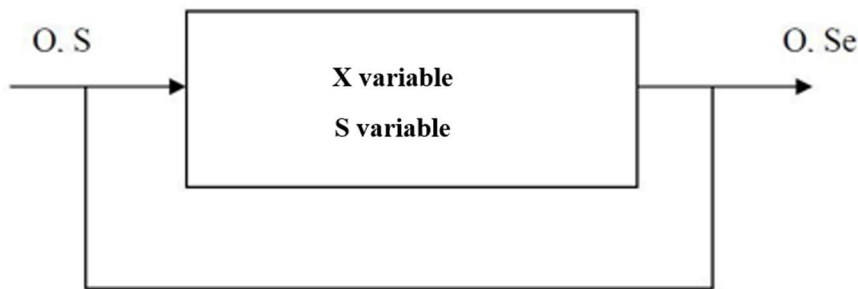


Figure 13. Plug flow reactor with both solid and liquid fraction recirculation [8].

All the above-mentioned continuous flow reactors can perform single-stage or double-stage (Figure 14). The multi-stage configuration is usually used to decouple operating conditions and times for the various stages of anaerobic digestion. In double-stage reactors, hydrolysis and acidogenesis are carried out in small reactors where a low return time is used. Acetogenesis and methanogenesis are carried out in larger reactors and for longer residence times.

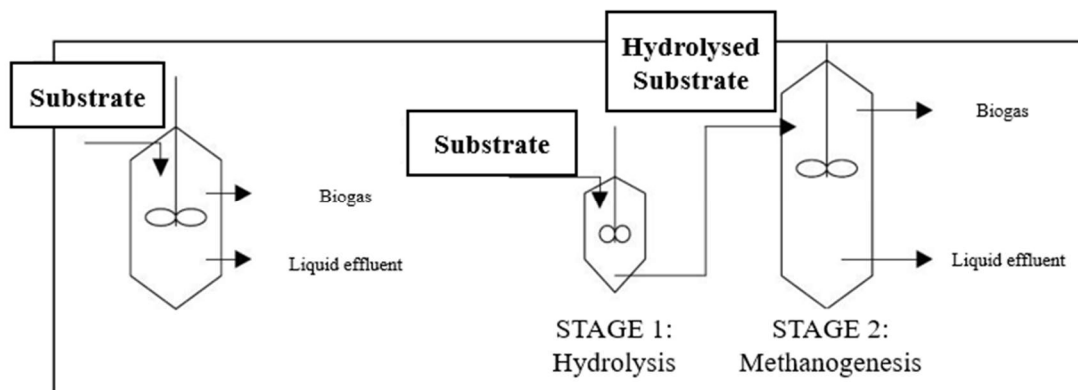


Figure 14. Plant scheme for single and double stage reactors [8].

Finally, the last reactor type is batch one, characterized by a batch-type feeding system. In this case, the substrate and the inoculum are placed in the reactor and left there until the substrate is exhausted. Whereas in CSTR the process takes place at a stable point of the growth curve of the crop, in the case of batch reactors the whole growth curve is exploited, from start to finish. This type of digester is usually equipped with an agitator to mix the substrate and ensure homogeneous characteristics.

Laboratory instruments

In this section, instruments used during experimental campaign are described. These activities have been performed in the CIRDER Laboratory of the Tuscia University (VT). This laboratory is equipped for the measurement of the moisture content, the volatile solids and ash content of biomasses by mean of the following instruments:

- analytical balance XS Excellence METTLER TOLEDO, with a readability equal to 0.1 mg and OHAUS Adventurer Pro Balance with a readability equal to 0.1 g (Figure 15);



(a) XS Excellence METTLER TOLEDO



(b) OHAUS Adventurer Pro Balance

Figure 15. Balances used in CIRDER laboratory.

The first one has been used to prepare samples for the ash content analysis and for the volatile solids content analysis. The second one has been used to prepare samples for the moisture content determination and for the loading of the pilot batch anaerobic reactors for the BMP determination tests;

- Digitalheat Drying and sterilization oven JP Selecta (Figure 16), which aim to reach and maintain constant temperatures from 40 °C to 250 °C. This instrument has been used to dry biomasses and determine the moisture content;



(a) internal view



(b) external view

Figure 16. Digitalheat Drying and sterilization oven JP Selecta.

- ME320 Muffle (



(a) internal view



(b) external view

- Figure 17), capable to reach 1100 °C and maintain the set temperature, has been used to determine volatile solids content and ash content of the biomasses analysed;



(a) internal view



(b) external view

Figure 17. ME320 Muffle.

- ceramic crucibles and lids in different dimensions to perform analysis in muffle and aluminium or steel trays to carry out analysis in the oven (Figure 18). In general, materials chosen to contain the biomasses during the tests have to respect the following requirements: be inert to the compounds they contain and not oxidize if exposed at the temperature required for tests;



(a) ceramic crucibles and lids



(b) aluminium trays

Figure 18. Container used in laboratory.

- cutting mill SM100, used to chop dried biomasses and vibratory sieve AS 200 Control with different size sieves, in order to select the particles with se maximum size required by the standard processes of biomass characterization (Figure 19);



(a) cutting mill SM100



(b) vibratory sieve AS 200 Control

Figure 19. Cutting mill and sieve to reduce dried biomass to the required particle size.

- desiccators to maintain dried samples in an moisture-free conditions (Figure 20);



Figure 20. Desiccator.

In addition to this, during the PhD experience, an instrument aimed to carry out anaerobic digestion in multiple scale batch reactors has been added, in order to determine the BMP of residual biomasses collected. This instrument is the Gas Endeavour (by BPC Instruments) [27], which is constituted mainly by three units, as shown in Figure 21. The “Unit A” is constituted by a thermostatic bath, in which fifteen 500 ml batch reactor are placed. Each reactor is constituted of a glass bottle with a plastic cap equipped with an electric agitator. The agitation can be set at different velocity and perform continuously or discontinuously, also in a reverse mode. The cap has also two holes, to provides gas input and output. The input point is usually closed by a valve, but its presence is very important in order to create an anaerobic environment at the initial time of the test and also to provide a relief valve in case of malfunction. The outlet point is linked to a filter constituted by a glass bottle, sealed with a cap equipped with two ports, filled with a solution of water and NaOH, which admits the biogas washing. The outlet port of the filter bottle is linked to the total gas measurement unit, in which a tilt mechanism provides the measure of the produced biomethane. In summary, the Biogas Endeavour admits carrying up fifteen anaerobic digestion tests at a time, because each reactor has its own filter and measurement unit. The tests share the agitation degree and the thermostatic bath temperature.

The entire plant can be remote-controlled and provides, in addition to the production trends, also the environment temperature, the temperature of the thermostatic bath and the velocity of the agitation.

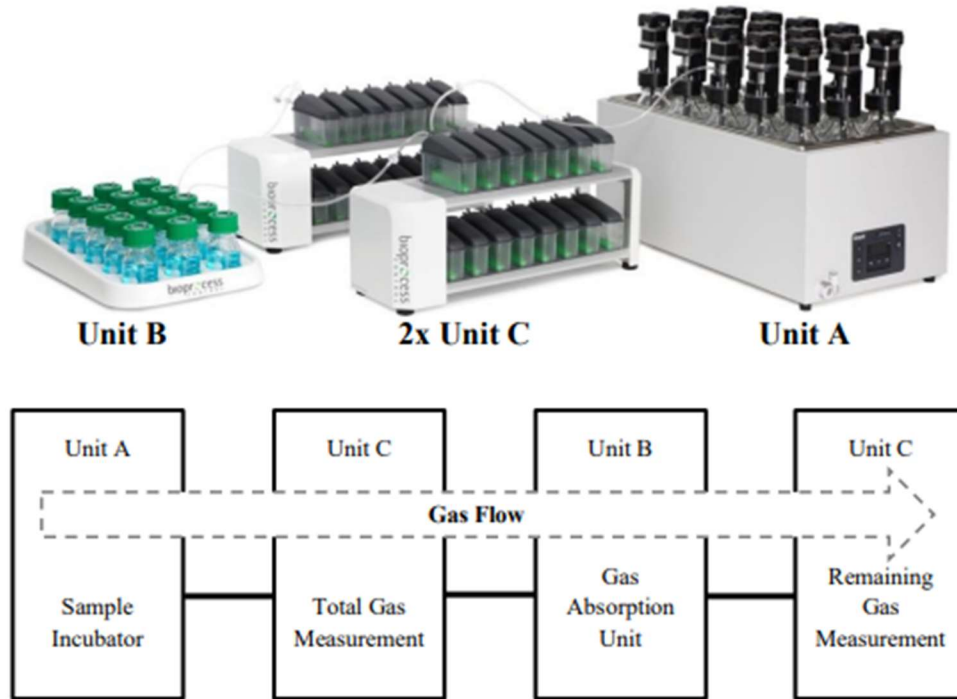


Figure 21. Biogas Endeavour by BPC Instruments [27].

Additional instruments have been used during test, such as a refrigerator for biomass storage, cutter with different meshes, knives and chopping boards for biomass manipulation. Laboratory is also equipped with a sink and a vent hood.

Design of experiments

During the experiences in laboratory, the necessity of a design of the experimental campaigns became clear. For this reason, after the first experience with brewery beer spent grains (which will be detailed in the following paragraph), the use of DOE (Design of Experiment) principles has been implemented in the laboratorial campaign and while analyzing data. Three main pillars of the DOE have been included in the sample preparation:

- **replication**, that is the repetition of the same basic experiment. It helps the experimenter to estimate the experimental error and, if sample mean has used to estimate the effect of a factor in the experiment, admits a more precise estimate of the effect;
- **randomization**, which means that the allocation of both materials and the order of runs has been casually executed. By this way, the effects of extraneous factors can be average out. In fact, during the experimental set up the experimenter can make systematic errors, which affect the results without the acknowledgement of the experimenter itself;
- **blocking**, an action used to reduce or even eliminate the nuisance factor. A block is a set of homogeneous experimental conditions. In the considered case, a batch of raw material, such as inoculum samples, can form a block. Dividing the experiences into groups that are run in each block, can reduce the nuisance factor associated to the variability between different block, such as, for examples, different inoculum.

In addition to this, guidelines proposed in [28] have been put in place to optimize the execution of the experiments. The problem has been identified before each laboratorial experience, and the response variable has been chosen. Factor, levels

and range have been also found, so that the experimental design has been selected. Then, the experiment has been run and data has been analysed. Also, the ANOVA method, which will detailed in the experience section, has been used.

Beer Spent Grains energetic characterization and pre-treatment

A first study on local residual biomasses has been focused on brewery spent grain (BSG) arising from an Italian brewery settled near Rome. In the food industry, the brewing sector holds a strategic economic position: beer is the fifth most consumed beverage in the world, with an average consumption per person of 23 litres per year. Based on the published statistics, the EU-28 beer production in the year 2013 was equal to 383,553,000 hl, with Italian production of 13,256,000 hl [29]. A huge quantity of waste material and by-products are created during this production process, such as process water, spent grains and spent yeasts. The production of just one litre of beer requires among 4 and 11 litres of water to be consumed. Furthermore, BSGs make up 85% of solid waste material of production process and 17 kg are produced for just one hectolitre of beer with a worldwide production of around 38.6 millions of tons per year [29]–[31]. For these reasons, AD of this solid waste represents an affordable option for reducing the brewery highly impactful production process. The BSG composition depends on both the different barley used species and the different operating conditions of production. BSGs are constituted mainly by lignocellulosic biomass, rich in proteins and fibres (16.8%÷25.4% of cellulose, 21.8%÷28.4% of hemicellulose and 11.9%÷27.8% of lignin). These characteristics make BSGs a suitable input material for AD applications [30]. The aim of this study is to provide an energetic characterization of BSG by evaluating moisture content (MC), ash contents (AC) and volatile solids (VS), analysing its attitude for bioconversion by means of anaerobic digestion. Furthermore, the effects of two different pre-treatments, that is the low temperature and alkaline ones have been investigated by comparing the obtained results with AC and VS values referred to raw BSG biomass, highlighting any improvement in terms digestibility once submitted to anaerobic digestion processes.

The energetic characterization of BSG biomass was conducted at CORDER laboratory at the University of Tuscia in Viterbo, Italy. The raw biomass was provided by a small brewery near Rome. UNI EN ISO 18134-1, UNI EN ISO 18134-2 and UNI EN ISO 18134-3 [32]–[34] has been used to determine the biomass moisture content. As given by authors in [35], these regulations describe the method of moisture calculation for a biofuel sample once dried within an oven. The procedure for preparing the samples can be summarized as follow: a sample of BSG is weighed and then inserted into a clean tray. Previously, also the tray's mass has been evaluated. At the end of this first phase, the sample is inserted into the tray, to obtain the overall mass of the system. The next step requires both biomass and the tray to be inserted into an oven at $105\pm 2^{\circ}\text{C}$ until a constant mass between successive weighing is achieved. Regularly, the tray with the biomass is extracted, weighed and reinserted in the oven. It is possible to assert that constant mass has been achieved once the discrepancy between two weight values is less than 0.2%. At the end of this process the biomass is totally dried, and the percentage of moisture content can be obtained using the equation suggested by the reference standard regulation [32]–[34]. In order to show more clearly the obtained results, the labels of measured values are introduced, according to the ones proposed by the mentioned regulations. The label m_1 represents the empty tray's mass, while the mass of the tray filled with wet biomass is named m_2 . The mass of the tray filled with fully dried biomass is labelled m_3 . The tray's material behaviour throughout the test has been taken into account by the use of an empty tray (of the same material of the others), which was submitted to the thermal conditions of the tester trays. This reference plate has to be weighed at the beginning and at the end of the test. The two masses obtained from these measurements are indicated with m_4 and m_5 ,

respectively. The OHAUS Adventurer Pro balance has been used for the measurement of the masses of the moisture content procedure. Once completely dried, the biomass was grinded by using a mechanical sieve in order to reduce the particle size. The suggested dimension of the particles, as reported the reference standard regulation [36], is about 1 millimetre. The UNI EN ISO 18122 [36] standard provides indications for ash content determination. Also in this case, the labels are introduced to simplify the discussion of results. It is important to notice that the labels are the same used in the moisture determination. The difference consists in the context in which these labels will be used in the exposure of the results. The requirements in terms of instrumentation are a laboratory balance with a resolution of 0.1 mg (in this case the METTLER TOLEDO XS Excellence balance was used), an electric oven (ME320 Muffle) capable to reach about 550°C in a specific given time frame and a dryer, able to prevent ambient humidity absorption by biomass. The procedure for preparing the samples can be summarized as follow: two crucibles have to be inserted into the oven for 60 minutes with a temperature of 550±10°C. At the end of this first step they have to be extracted and cooled on a heat-resistant surface for 5-10 minutes and then inserted into the dryer. Once the temperature of the crucibles is close to environmental temperature, each one has to be weighed using the laboratory balance and the obtained values has to be referred as m_1 . The next step consists in the insertion of 1 g of dried BSG in each crucible. The mass of the crucible, once filled with dried BSG, has to be determined and referred as m_2 . Subsequently, the two samples are placed in the oven, increasing the temperature uniformly until it reaches 250°C. After 60 minutes at this constant temperature, the standard requires the temperature has to be increased up to 550±10°C, exposing both crucibles at this thermal condition for the next 120 minutes. At the end of the process, the crucibles are cooled and placed into the dryer as described before. Then, each sample (crucible filled with biomass) is weighted and the measured value has to be referred as m_3 . The three different values of mass obtained, using an experimental relation, allows to determine the ash content of the BSG biomass. UNI EN ISO 18123 [37] has been used to carry on volatile solids determination. Following the previous discussion, in this section the procedure and the labels assigned to each measurement are described. The used instruments are the same previous described for the determination of the ash content: the muffle furnace ME320, capable of reaching a temperature of 900±10°C, cylindrical crucibles of inert material with their own lid and the METTLER TOLEDO XS Excellence balance with a resolution of 0.1 mg. The procedure for sample preparation requires both crucibles and lids to be placed into the oven and maintained for 7 minutes at 900±10°C. At the end of this first step, the samples have to be removed from the oven and placed on a thermo-resistant material, until room temperature has been reached, and then inserted into a dryer. The mass of each filled crucible with its lid has to be weighed and referred as m_1 . Therefore 1 gram of BSG is inserted into the crucibles and then weighed using the laboratory balance. The weight value has to be referred as m_2 . Then, the samples are placed into the oven for 7 min ± 1 s at a temperature of 900±10°C. At the end of this high temperature process, the crucibles and lids are cooled and placed into the dryer as described before. Finally, it's required to weight the samples taking note of the values, referred as m_3 . The three different values of mass obtained, using an experimental relation provided by the standard regulation [37], allow to evaluate the volatile solids content of the BSG biomass. At the end of these experimental evaluations, parameters such as moisture content, ashes and volatile solids of BSG biomass are defined.

In order to improve the BSGs aptitude to bio-conversion, maximizing the volatile solids value and so increasing overall digestibility of the biomass [38], [39], two different kinds of pre-treatment as the ones described by author in [35] have been analysed and applied to the biomass previously characterized. The first attempt to increase BSG digestibility in AD processes has required the application of a low-temperature pre-treatment. Through the combined action of water and heat, a preliminary hydrolysis is promoted. This treatment is able to break down complex organic matter into substances much easier to be digested by anaerobic bacterial action, producing biogas. The detail of the procedure is now described:

the previously BSG biomass dried and chopped into 1 mm particles dimension has been added in solution with deionized water ensuring a 1:14 ratio in grams (1 g of BSG with 14 g of deionized water). The mixture, inserted into a baker, has been placed on a magnetic stirrer with hot plate for 3 hours at a constant temperature of 80°C, ensuring a correct mixing between BSG and water. At the end of the process, the remaining water has been removed through a 250-microns sieve and the wet pre-treated biomass has been inserted into an oven for drying, according to UNI EN ISO 18134-1, UNI EN ISO 18134-2 and UNI EN ISO 18134-3 [32]–[34]. Once the biomass has been totally dried, the procedures for ash and volatile solids determination were repeated in order to identify variations on the biomass characteristics (i.e. V_d and A_d) as previously discussed. The second attempt to increase the V_d content is a chemical pre-treatment in which the complex organic matter is converted into easier substances by means of the action of sodium hydroxide NaOH, reason why it's called alkaline pre-treatment. The BSG biomass previously dried and chopped into 1 mm particles dimension has been added in solution with deionized water ensuring a 1:12 ratio in grams (1 g of BSG with 12 g of deionized water). The concentration of NaOH that needs to be added to the solution is 50% NaOH per gram of V_d . The obtained substance has been lately poured into a tray and inserted into the oven for 72 hours at 35°C. At the end of the procedure the pre-treated biomass is completely dried and ready to be used for ashes and volatile solids' determination procedures (applying the same standard used for raw BSG). For each characterization many samples have been studied. Standard deviation values are also given for each one of the average values.

In the following section all the results obtained by both the energetic characterization of BSG biomass and pre-treatments application are shown and discussed. Table 1 up to Table 7 collect raw data obtained by measurements, without any statistical analysis applied. It's important to notice that for the moisture content determination three replicates has been performed. For the volatile solid content and the ash content determination, instead, respectively two and four replicates have been carried out.

The moisture content of raw BSG has been calculated using the data referred to n.3 samples. The value of the five masses to be passed as input parameters through the formulation given by standard regulations [32]–[34] are reported by Table 1.

Table 1. Moisture content of raw BSG.

	<i>Sample #1</i>	<i>Sample #2</i>	<i>Sample #3</i>
m_1 [g]	24.90	24.90	21.70
m_2 [g]	331.20	360.90	287.90
m_3 [g]	103.00	109.80	88.70
m_4 [g]	21.70	21.70	21.70
m_5 [g]	21.60	21.60	21.60
M_{ar} [%]	74.46	74.70	74.79

The moisture content of raw BSG biomass, that is M_{ar} , is equal to $(74.65 \pm 0.14)\%$. The moisture content is quite higher than the acceptable one, pairs to 30%. This value of humidity confirms the suitability of the BSG biomass to be used in biochemical conversion processes, such as the anaerobic digestion. In fact, the suggested moisture values for anaerobic digestion are within the range of 60% and 80% [38], [40]. The determination of the ash content of raw BSG has been carried out through the analysis of n.4 samples. The obtained results are shown in Table 2.

Table 2. Ash content for raw BSG.

	<i>Sample #1</i>	<i>Sample #2</i>	<i>Sample #3</i>	<i>Sample #4</i>
m_1 [g]	33.5058	31.1597	34.0040	34.6953

m_2 [g]	34.5063	32.1590	35.0096	35.6942
m_3 [g]	33.5415	31.1950	34.0394	34.7300
A_d [%]	3.5682	3.5324	3.5203	3.4738

Ash content (as by A_d values) is equal to $(3.524 \pm 0.034)\%$ and it's obtained for each sample using the experimental equation suggested by reference standard [36]. Next step consists in identifying volatile solids characterizing raw brewery spent grains as described by UNI EN ISO 18123 [37], testing n.2 samples. Results are reported by *Table 3*.

Table 3. Volatile solids content for raw BSG.

	<i>Sample #1</i>	<i>Sample #2</i>
m_1 [g]	45.8479	46.0095
m_2 [g]	46.8519	47.0050
m_3 [g]	45.9272	46.1117
V_d [%]	92.1016	89.7338

The obtained value of the volatile solid content (V_d), that is $(90.9 \pm 1.2)\%$, highlights the suitability of BSG for anaerobic conversion processes. Once the energetic characterization of raw BSG has been carried out, the following step has been related to the evaluation of the effects of two different pre-treatments on the raw biomass.

As previously described, low-temperature pre-treatment and alkali pre-treatment has been chosen in order to evaluate changes in energetic characteristics of brewery spent grains. In this section the results of the energetic characterization of low temperature pre-treated biomass are described. As before specified, after the submission to the pre-treatment the biomass was dried to check the ashes and volatile solids contents. For the analysis of the ash content n.4 samples have been used. The results from each test are shown in *Table 4*.

Table 4. Ash content for low temperature pre- treated BSG.

	<i>Sample #1</i>	<i>Sample #2</i>	<i>Sample #3</i>	<i>Sample #4</i>
m_1 [g]	33.5079	34.0058	31.1607	34.6953
m_2 [g]	34.5099	35.0054	32.1606	35.6974
m_3 [g]	33.5435	34.0422	31.1966	34.7315
A_d [%]	3.5529	3.6415	3.5904	3.6124

The ash content of the low temperature pre-treated BSG is equal to $(3.599 \pm 0.032)\%$. In order to define the percentage of volatile solids in the low temperature pre-treated BSG biomass, two samples have been analysed. The results of the aforementioned test are given by *Table 5*.

Table 5. Volatile solids content for low temperature pre- treated BSG.

	<i>Sample #1</i>	<i>Sample #2</i>
m_1 [g]	45.8431	45.6477
m_2 [g]	46.8446	46.6500

m_3 [g]	45.9112	45.7248
V_d [%]	93.2002	92.3077

The content of volatile solids (V_d) for the low temperature pre-treated biomass is equal to $(92.7 \pm 0.4)\%$.

The second pre-treatment carried out is the alkali one. At the end of this pre-treatment the compound has been dried and used in ash content and volatile solids determination procedures. In order to define the ash percentage in this biomass, n.4 samples have been studied. The results of the test are shown in Table 6.

Table 6. Ash content for alkali pre-treated BSG.

	<i>Sample #1</i>	<i>Sample #2</i>	<i>Sample #3</i>	<i>Sample #4</i>
m_1 [g]	34.6947	34.0052	31.1605	33.5078
m_2 [g]	35.7157	35.0164	32.1649	34.5107
m_3 [g]	35.0877	34.3966	31.5484	33.8951
A_d [%]	38.4917	38.7065	38.6201	38.6180

The value of the ash content for this type of alkali pre-treated BSG biomass is pairs to $(38.609 \pm 0.077)\%$. To compute the percentage of volatile solids on dry matter for alkali pre-treated biomass, n.2 samples have been tested, and the obtained values are displayed in Table 7.

Table 7. Volatile solids content for alkali pre-treated BSG.

	<i>Sample #1</i>	<i>Sample #2</i>
m_1 [g]	45.4834	45.5241
m_2 [g]	46.4886	46.5299
m_3 [g]	45.7329	45.8016
V_d [%]	75.1791	72.4100

It follows that the volatile solids content (V_d) once the alkali pre-treatment has been applied is equal to $(73.8 \pm 1.4)\%$.

The obtained results from the performed tests, for each one of the applied pre-treatments, are summarized by Table 8, with the associated standard deviation expressed with two digits.

Table 8. Results of the characterization tests.

	<i>Raw BSG</i>	<i>Low-temperature pre-treated BSG</i>	<i>Alkali pre-treated BSG</i>
<i>Ash content [%]</i>	3.524 ± 0.034	3.599 ± 0.032	38.609 ± 0.077
<i>Volatile solids content [%]</i>	90.9 ± 1.2	92.7 ± 0.4	73.8 ± 1.4

A comparison between the ash content of raw BSG and low-temperature pre-treated BSG shows that the low-temperature pre-treatment has increased the ash percentage on dry matter of around 0.075 percentage points. The upturn of the volatile solids content, which has been improved up to +1.8% with the pre-treatment, allows to affirm that the low temperature pre-treatment really manages to raise volatile solids content through the combined action of water and heat. The available organic matter, that should be active while anaerobic digestion occurs, after this pre-treatment shows an augment, making available to bacteria a higher quantity of substance for being digested with respect to the raw BSG. The increment of ash percentage, on the contrary, denounce an increasing of inorganic matter percentage, which means a decrease of the portion of biomass available for the bacterial elaboration [16]. Nevertheless, the increasing of volatile solids content is much

greater (+1.8% of volatile solids against the +0.075% of ash) than the one associated to the ash and this is the reason why the result of the low temperature pre-treatment is considered positive.

Instead, the alkali pre-treatment influence doesn't show a bettering of the characteristics of the biomass to be submitted to AD processes. Actually, this treatment has increased the ash content of 35.085 % and has decreased the volatile solids content, with a loss of around 17.1 percentage points. These results are not in line with the most ones presented in literature. In fact, the NaOH pre-treatment usually enhance VS content [41] and VS consume [42], [43] in a percentage depending by the biomass, the NaOH ratio, the temperature and the duration of treatment. This results, united to the fact that the sum of SV and ash percentage on dry basis is major than 100%, suggest that this test has to be repeated, in order to study other data and explore the causes of these ones.

As regards the values obtained by the characterization of raw and low-temperature pre-treated biomasses, the results admit to affirm that the BSG is suitable for anaerobic conversion application:

- moisture content is included in the optimal range (between 60% and 80%), as suggested in [38], [40];
- the ash content is minor than the one associated to some biomasses already used in anaerobic digestion, such as corn silage (5% on dry matter [44]), cattle manure (16.7 % on dry matter [45]) or swine manure (17.09 % on dry matter [46]);
- the volatile solid content of BSG is high (which means that a huge percentage of the dry biomass is suitable to be elaborated by microorganisms), also in comparison to the values associated to other biomasses used for anaerobic digestion applications, such as corn silage (95% on dry matter [47]), cattle manure (83.7 % on dry matter [45]) or swine manure (82.91 % on dry matter [46]).

Anaerobic digestion of Greengrocery Wastes at Different Particle Size and BMP estimation

After the aforementioned campaign for the energetic characterization of BSG wastes, the necessity to add more information to the characterization and to provide a statistical method to analyse and compare results bring to the adoption of the DOE principles described in previous section and to the inclusion of the Biogas Endeavour to the laboratory instruments. After this introduction, other experimental campaigns have been carried out.

In particular, the experimental campaign described in this section aims to assess the biomethane production obtained by the anaerobic digestion of 0 km vegetables and fruit wastes, in order to compare it (using the BMP formulation) to other used biomasses (also from energetic cultures) and collect data about their suitability for energetic purposes. The substrate considered is produced by a local farm located in the Tuscia territory (VT, Italy) and is mainly constituted by seasonal production discharges, such as grapes, peaches, peppers and prunes. These wastes have been chosen for this investigation because these cultivations are widely diffused in Lazio region and their exploitation in energetic conversion processes should represent an alternative to produce both energy and 0 km fertilizer (from digestate) to make more economically and environmental affordable their production. The laboratorial anaerobic digestion tests have been carried out at CIRDER (Interdepartmental Center for Renewable Energies Research and Dissemination) laboratory using batch reactors. For the tests the substrate has been cut at three different dimensions in order to assess if the methane production is affected by the particle size for this specific substrate. The tests have been subjected to a laboratorial issue at day 13th: the BMP has been calculated with cumulate production associate to day 12th. The operative conditions have been restored at day 21st, but due to the lack of homogeneous condition during the seven days of laboratorial issue, the data were not

suitable for BMP calculation. For this reason their trends have been analysed in order to collect more information on the substrate behaviour after a failure. Results obtained have been investigated using ANOVA method.

The experimental campaign has been carried out at CIRDER laboratory at the University of Tuscia in Viterbo, Italy. The inoculum selected for the experimental anaerobic digestion has been taken from the main reactor of a biogas plant, which is fed with the organic fraction of agrifood wastes. The vegetable and fruit wastes used during tests have been taken by a local farmhouse that produces and sells fruit and vegetables at 0 km in the territory of Tuscia. The agricultural business provides seasonal vegetables and consequently the composition of its wastes is seasonal too. During the period in which the tests have been carried out, the originated organic wastes were mainly composed by moulded/ruined peppers, grape, prunes and peaches. In order to ensure a similar amount of each kind of biomass in each reactor, the following composition has been defined (in relationship with the total amount of vegetables provided by the farm) for the biomass mixture, expressed on the total amount of feedstock loaded in each reactor:

- 20% of grape (berries, seeds and stems);
- 24% of pepper (seeds, pulp and pedicel);
- 36% of peach (without the nub);
- 20% of prune (without the nub).

The inoculum has been pre-incubated for 15 days before starting the tests. The pre-incubation has been carried out loading inoculum in the reactors, at a temperature of 49°C and periodically mixed until the daily biomethane production falls below 50 Nm³/day. After pre-incubation, both inoculum and biomass mixture (composed as described previously) have been characterized in order to determine their moisture content and the volatile solids percentage. During the characterization, completed in two days, the unused vegetable wastes have been conserved in a fridge at -4°C until they have been prepared for the tests [48].

Energetic characterization of the biomasses (inoculum and mixture of vegetable wastes) aims to determine the moisture, the ash and the volatile solids content of the analysed organic matter. The mixture of wastes has been analysed as composed in percentages shown in the previous paragraph. At first, moisture content of the inoculum (IN) and the Vegetable and Fruit Wastes (VFW) has been determined using 3 replicates for each biomass. In order to obtain this information, each sample has been prepared using the following steps. A non-corrodible and heat resistance tray has been weighted using a laboratory balance with a resolution of 0.01 g and its mass has been labelled as “w₁”. Then a portion of wet biomass has been put into the tray and another weighing, called w₂, has been done. The tray containing the biomass sample has been inserted in an oven at a temperature of 105°C ± 2°C and the weight of the tray filled with the biomass has been periodically registered until a constant weight has been reached between two consecutive weighing, made in a time interval of more than an hour. The weight of the tray and the dried biomass has been registered and nominated w₃. The moisture content percentage on wet basis has been calculated using the equations provided by [33].

From the moisture content, the percentage of the total solid amount (TS) of the biomass has been calculated as [49]:

$$TS [\%] = 1 - MC \quad (22)$$

Once the biomass has been dried, the procedure for the determination of the ash content has been carried out. For each kind of biomass 3 replicates have been realized. For this test, each sample has been prepared according to the following

routine. A ceramic crucible has been weighted using a 0.001 g resolution laboratory balance and its mass has been collected as “ m_1 ”. Then an amount of dried biomass has been put into the crucible and the crucible filled with biomass has been weighted, in order to obtain the data called m_2 . Then the sample has been inserted in a muffle increasing the temperature uniformly until reaching $250^{\circ}\text{C} \pm 10^{\circ}\text{C}$. The muffle has been maintained at this temperature for 60 minutes and then the muffle has been set at $550^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and, once reached this temperature, the test has been carried out for two more hours before extracting the sample. Once extracted, the sample has been put into a drier and, when its temperature decreased, it has been weighted. The mass of the crucible filled with biomass ash has been labelled m_3 . The percentage of ash content (AC) on total solid is calculated referring to the formula in [36].

The percentage of volatile solid (VS) contained by the biomass (on dried basis) is derived as [50]:

$$VS [\%] = 1 - AC \quad (23)$$

The relative error associated to each value obtained by the characterization process has been calculated considering the resolution as the error of the instrument and referring to the formulas of error propagation.

Specific cutting meshes have been used in order to prepare the VFW for the tests. The wastes have been cut after being suddenly pulled out from the refrigerator in order to employ their rigidity avoiding the smashing of the pulp. Before using the cutting mesh, the biomass have been cut in slices with a constant thickness smaller than 0.5 cm and then square meshes have been used, in order to shape the biomasses squares with a maximum dimension of the diagonal. The samples defined as “Large Particle Size” (LPS) have been obtained using the cutter with a mash dimension equal to 1.2 cm, which provides a maximum dimension of particles equal to the diagonal and so equal to 1.70 cm. The “Medium Particle Size” (MPS) samples have been prepared using the 0.6 cm mesh, with a resulting maximum dimension of 0.85 cm. The “Small Particle Size” (SPS) samples have been realized blending the wastes. All the prepared samples have been weighted and portioned for each reactor when they were still cold. Reactors have been prepared when each sample has warmed up to the room temperature.

After the inoculum and the biomasses have been characterized and prepared, the anaerobic digestion tests have been arranged. The used scale plant is the Bioprocess Gas Endeavour, which has been assembled with eight 500 ml batch reactors, heated by means of a thermostatic bath. The scale plant is developed to be remotely controlled. During the loading, a headspace of 100 ml has been left and the inoculum on substrate ratio (I/S) referred to the VS content has been imposed equal to 3 [51]:

$$\frac{I}{S} = 3 \quad (24)$$

The thermostatic bath has been set at 49°C , which was the set temperature of the biogas plant in which the inoculum was collected. The mixer has been take on for 20 minutes every 70 minutes. The produced biogas outflowing from each reactor has been filtered by insufflation in a glass bottle filled with a solution of NaOH, in order to separate the carbon dioxide from biomethane. The biomethane has been measured using flow cells (one for each reactor). For each considered level (LPS, MPS, SPS) two repetitions have been made. For the blank sample, used to calculate Biomethane Potential (BMP), two repetitions have been done. The scale plant admits the user to automatically register the cumulated methane production (expressed in Nml) and the daily methane flow, measured in Nml/d. Gas production is normalised in real time by the scale plant software and measured. The tests have been carried out for 35 days.

During the 13th day of test an issue (not attributable to the Bioprocess Gas Endeavour system) happened. Due to this accident, the thermostatic bath switched off at 21°C and the reactors' agitators got turned off for eight days before the initial conditions of temperature and agitation were restored (day 21 of the experimental campaign). During the failure period the registered flow rate was about equal to zero [52]. Data extracted from day 21 to day 35 are not suitable to determine the BMP of the biomasses, but can be elaborated in order to study the different answers between samples (if present).

Data collected from the anaerobic digestion test have been elaborated using the following steps. First of all, for each level investigated a curve composed by the mean value and the standard deviation of each data has been provided. Both mean and standard deviation have been referred to each day and they are composed using the data of the repetition made for each size particle level. Then, another analysis has been made separating data in two different sets: the first one referred to days 0-12 and the second one composed by data collected goes from day 21 up to day 35. The first ensemble has been studied in order to ensure the suitability of data for the BMP calculation and to define the differences and the similarities linked to the biomethane production of the three levels of biomass size studied during the tests. The second set of data is not suitable to be used for BMP determination, due to the drop in temperature which has affected the tests during days from 13 to 20. Anyway, these data provide information about the behaviour of each substrate in relationship to a critical situation verified in the reactor.

By first, the first set of data has been studied in order to assess that the biomethane production registered in the last 3 days of test has a daily growth minor than 1%. This information has been obtained using the following formula applied to each curve of the substrate [53] :

$$V\% = \frac{V_{daily,i}}{V_{cumulated,i}} \cdot 100 \quad (25)$$

where “i” indicates the i-th day and V% indicates the percentage variation of daily methane production referred on the cumulate produced volume. Also the analysis of relative standard deviation has been provided in order to complete the determination of the framework in which the comparison has been made.

The BMP has been calculated at 12th day using the following equation for each investigated level [50]:

$$BMP_{12,a} \left[\frac{Nl}{g_{SV}} \right] = \frac{(\bar{V}_{sub,12,a} - \bar{V}_{in,12})}{VS_{sub,wet basis}} \quad (26)$$

where:

- $\bar{V}_{sub,12,a}$ is the mean of the cumulated volume at 12th day of the substrate with the particle size associated to the specific level a. This data is expressed in [Nl];
- $\bar{V}_{in,12}$ is the mean of the cumulated volume at 12th day of the blank reactors, measured in [Nl]; ;
- VS_{sub} is the amount of volatile solids of substrate located in the reactor, expressed in [g_{sv}].

The comparison between the biomethane yield produced by using the three different particle size has been carried out using the cumulate production registered at day 12th and the one at day 35th. In fact, the first set of data (the cumulative volume production of each reactor at time “12 days”) describes the production obtained at optimal conditions of

temperature and mixing rate, while the second set of data (the cumulative production at the end of the test) takes into account the behaviour of each test condition after the temperature and mixing issue.

The comparison has been conducted using the ANOVA method and calculating the variance between and within the levels of samples. The number of used levels during the experiment is described using “a” and is equal to 3:

- level 1: large size particles;
- level 2: medium size particle;
- level 3: small size particle.

The number of repetition is labelled “n” and is equal to 2. It follows that the number of samples is equal to:

$$N = a \cdot n = 6 \quad (27)$$

The ANOVA (ANAlisis Of VAriance) consists in using statistic tools in order to reject or no reject a null hypothesis called H_0 , under the normally assumption [28]. The null hypothesis is that the means of all treatments are equal:

$$H_0: \mu_1 = \mu_2 = \mu_3 \quad (28)$$

The alternative hypothesis is that:

$$H_1: \mu_k \neq \mu_i \text{ for at least one pair } (k, i)$$

The mean square associated to the variance between different treatments is calculated as [28]:

$$MS_B = \frac{SS_B}{v_1} = \frac{n \cdot \sum_{k=1}^a (\bar{x}_k - \bar{x})^2}{a - 1} \quad (29)$$

The mean square describing the error within levels is defined using the formula [28]:

$$MS_W = \frac{SS_W}{v_2} = \frac{\sum_{k=1}^a \sum_{i=1}^n (x_{ki} - \bar{x}_k)^2}{N - a} \quad (30)$$

Where the acronym SS stays for Sum of Square, the v defines the degrees of freedom, $\bar{x}$ is the grand average of all the observations considered by the ANOVA analysis and $\bar{x}_k$ is the average of the observations under the k-th level [28]:

$$\bar{x} = \frac{\sum_{k=1}^a \sum_{i=1}^n x_{ki}}{N} \quad (31)$$

$$\bar{x}_k = \frac{\sum_{i=1}^n x_{ki}}{n} \quad (32)$$

Using these values it is possible to define the test statistic F for the hypothesis of no differences in treatments means [28]:

$$F_0 = \frac{MS_B}{MS_W} \quad (33)$$

Assuming a confidence value $\alpha = 0.05$, it's possible to compare F_0 with the value obtained by the tables of percentage points of the F Distribution and reject or not reject the null hypothesis.

As described before, total solid content and ash content have been determined in order to define the amount of substrate and inoculum needed for the anaerobic digestion process. In Table 9 data collected during the tests for the determination of moisture content (with the relative error associated to each measurement) are shown.

Table 9. Moisture content of the inoculum and the substrate.

	<i>Sample 1</i>	<i>Sample 2</i>	<i>Sample 3</i>
<i>Inoculum</i>	(88.9±0.04)%	(89.1±0.04)%	(89.1±0.04)%
<i>Vegetable and fruit wastes</i>	(87.7±0.04)%	(86.6±0.04)%	(87.5±0.04)%

Table 10 contains results of the ash content determination of inoculum and substrate for each replicate, with the associated relative error of the measurement.

Table 10. Ash content on dry basis of inoculum and substrate.

	<i>Sample 1</i>	<i>Sample 2</i>	<i>Sample 3</i>
<i>Inoculum</i>	(29.4±0.4)%	(29.4±0.3)%	(29.5±0.4)%
<i>Vegetable and fruit wastes</i>	(12.6±0.6)%	(11.6±0.6)%	(13.0 ±0.6)%

In Table 11 the obtained characteristic investigated for the used biomasses are summarized. Data collected in this table describe the mean and the average measurement error of each property.

Table 11. Energetic characteristics of inoculum and substrate.

	<i>Total solid [%]</i>	<i>Moisture content [%]</i>	<i>Ash content on dry basis [%]</i>	<i>Volatile solid content on dry basis</i>
<i>Inoculum</i>	(11.0±0.04)%	(89.0±0.04)%	(29.5±0.4)%	(70.5±0.4)%
<i>Vegetable and fruit wastes</i>	(12.7±0.04)%	(87.3±0.04)%	(12.4 ±0.6)%	(87.6±0.6)%

The moisture content of the VFW is in the range of the food wastes' moisture content [54]. In particular, vegetable and fruit wastes are characterized by a moisture between 88% and 94% and the obtained results for the VFW characterized are consistent with this range [55]. The volatile solid content of VFW is low in comparison to the values associated to other vegetable biomasses used for anaerobic digestion applications, such as corn silage (95% on dry matter [47]), but it's higher than the one associated to cattle manure (83.7 % on dry matter [45]) or swine manure (82.9 % on dry matter [46]).

In order to load with an I/S ratio (based on volatile solid content) equal to 3 each reactor, the value of the volatile solid content on wet basis has been calculated:

$$VS_{IN,wet\ basis} = (7.7 \pm 0.6)\%$$

$$VS_{VFW,wet\ basis} = (11.1 \pm 1.0)\%$$

Using these data, it is possible to calculate the amount of inoculum and substrate needed to load the reactors to obtain a I/S ratio equal to 3. Each reactor has been loaded using (325±1) g of inoculum and (75±1) g of fruit and vegetable wastes, composed as described before. Knowing this information is also possible define the amount of volatile solids referred to the substrate contained in each batch reactor:

$$VS_{sub} = 8.3 \text{ g} \pm 0.64 \%$$

After the 35 days of test, the trends of biomethane produced have been collected in Figure 22.

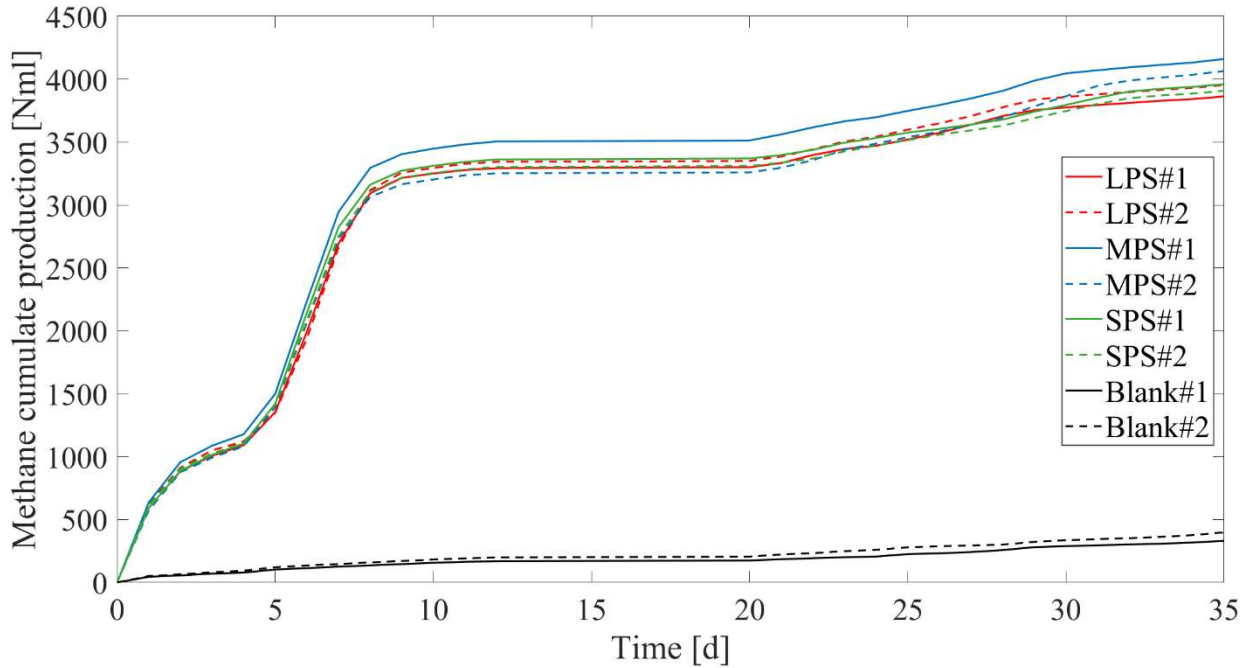


Figure 22. Cumulate methane production.

According to the information provided by Figure 22, it's possible to notice that the biomethane production associated to the substrate is about seven times greater than the production of the blank test. In order to detect the differences between each studied level, a plot containing the mean and the standard deviation of each group of data is provided in Figure 23.

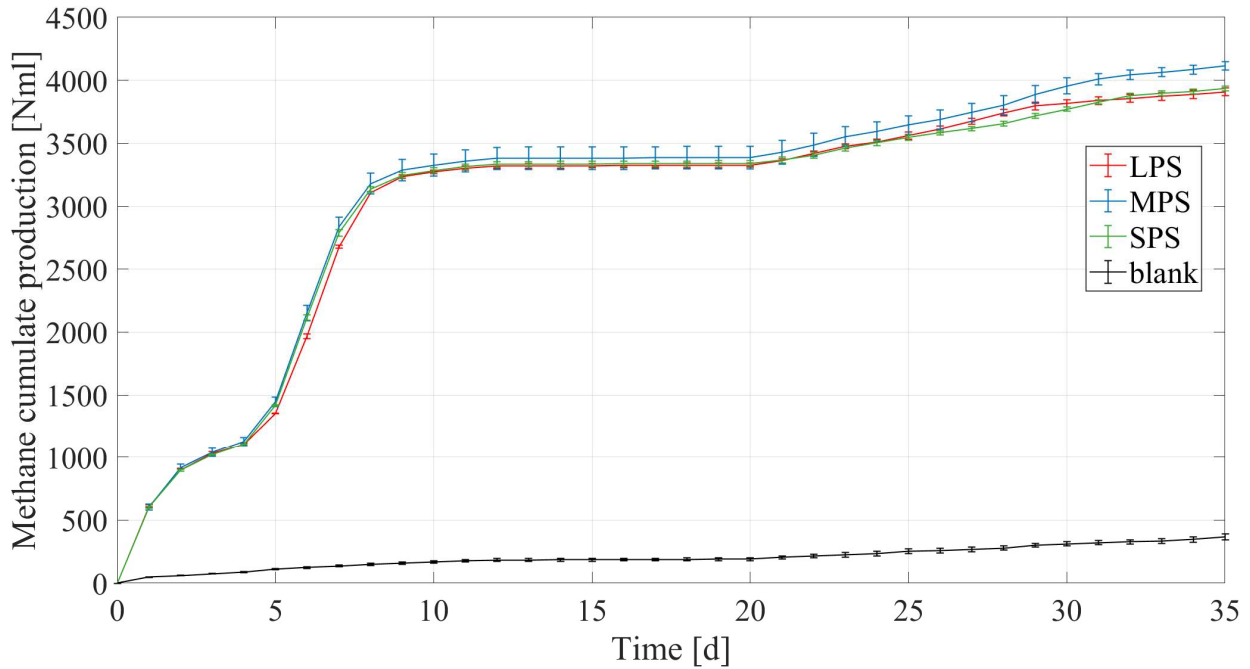


Figure 23. Average curves of the cumulate biomethane production for each experiment's level.

The trend associated to the medium size particle substrate reaches the highest cumulative production during the whole test duration. It is also the curve with the highest values of standard deviation associated to each point: this information

can be observed also graphically. The small size particle level follows the trend of the MPS curve until day 7, then departs until reaching the values of cumulate production associated to the large size particle test at day 9. These last two curves are quite overlapped until day 25, then the LPS curve registers a major daily production compared to the SPS curve. Between days 31 and 32, the SPS production trend surpasses the LPS one, obtaining a final cumulative biomethane production higher than the one associated to the use of large size particles substrate. The issue happened between days 13 and 21 is evidenced by the fact that all the four curves presented during this period are parallel to the x axis for the same amount of time and without any slope variation.

Daily biomethane production as registered between days 0 to 12 is described in Figure 24.

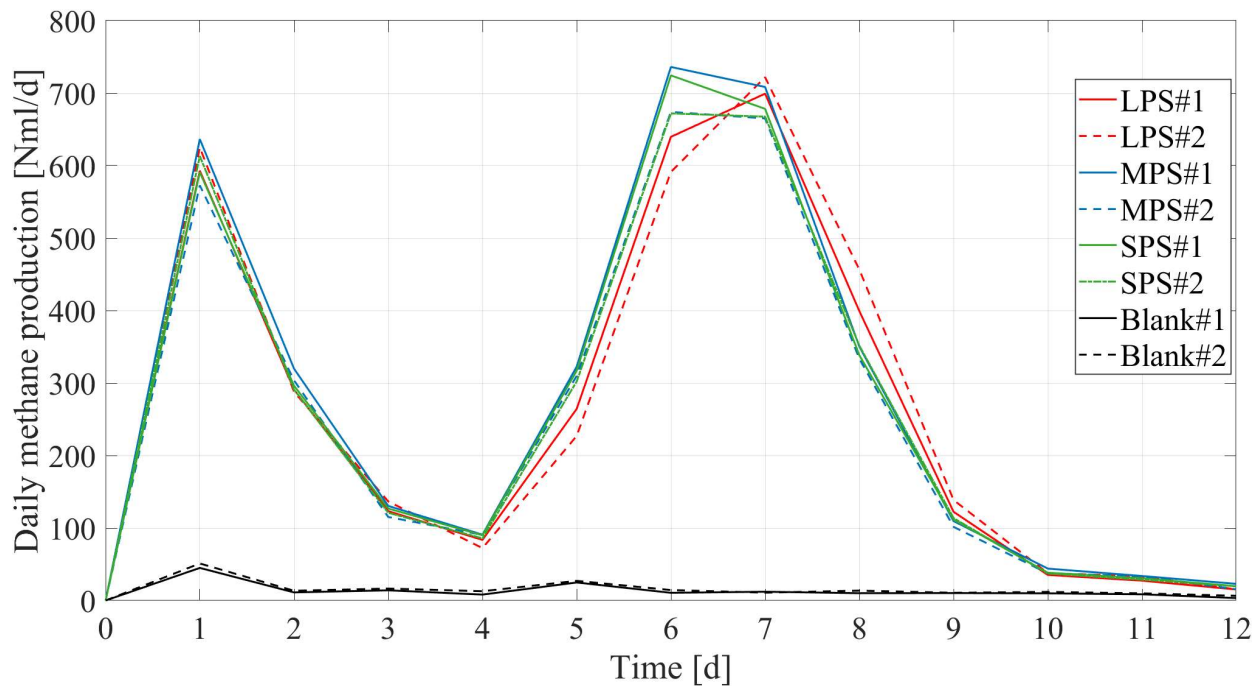


Figure 24. Daily methane production registered between days 0 to 12.

In order to analyse the trends, the average curves with the associated standard deviation have been plotted in Figure 25. It shows that for the first three days no differences have been detected between the different levels of the tests. At 4th day the methane flow rate produced by the LPS test is lower (about -14%) than the ones associated to the other two levels of the experiment. The MPS and SPS curves are overlapped until day 6, when they reach the maximum daily production in this time interval (equal to 705 NmL/d and 698 NmL/d, respectively). The trends of the daily production associated to MPS and SPS treatments are similar for the studied time interval, also considering the changes of the slope of each segment of the curve. The curve of the LPS substrate presents a trend equal to the ones associated to the other test levels, but characterized by a maximum flow rate shifted by one day in comparison to the ones of the other two curves. In fact, observing the LPS trend, it's evident that the daily production for days 4,5 and 6 is lower than the one associated to MPS and SPS experiments, but at day 7 the MPS and SPS curves decreases, while the LPS one reaches its peak (710 NmL/d). After day 7, the LPS flow rate is characterized by higher values in comparison to the production of the MSP and SPS samples. However, the three curves have a decreasing trend and reach the overlapping at day ten. This difference in daily production trend suggest that the dimension of the substrate particles influences the rate of substrate elaboration by biomasses groups. In fact the daily biomethane production of the LSP substrate is quantitatively equal (the differences between the LS and the MS peaks' values is equal to 0.7 %) to the one associated to the other two test levels, but seems to be slower, with a delay of nearly a day.

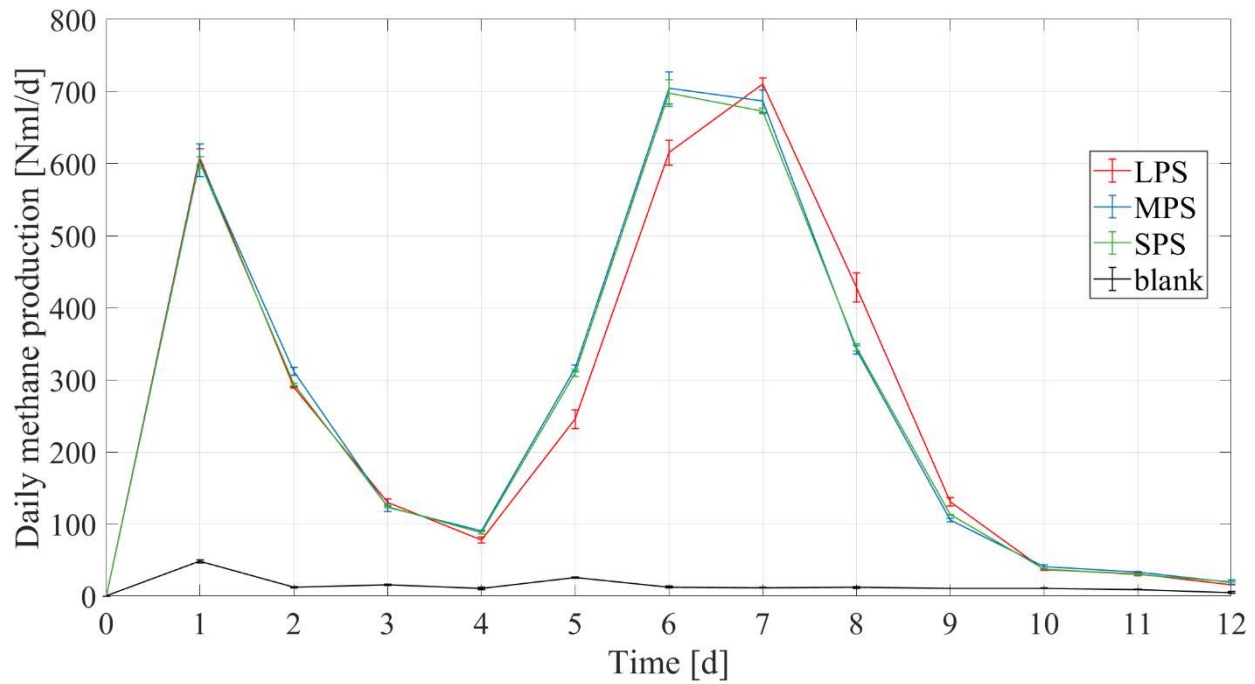


Figure 25. Daily biomethane production (from day 0 to day 12) expressed using average values and standard deviation.

In order to define the framework in which the BMP has been estimated, the percentage variation of the daily production during the three last days (in relationship to the laboratorial issue) of tests has been calculated and plotted in Figure 26. The variation value for the last three days of test has to be lower than 1% [53] in order to assess that anaerobic conversion process has been completed. In this case, at the 10th day all the curves present an increasing of methane production in the range between 1.5% and 1%. From day 11, the variation curves have values minor than 1% and at day 12 the trend is still decreasing and under the 1% for all the six samples. Despite the variation value collected at day 10, the variation trends and values suggest that the methane production has been stabilized at day 12. For this reason, data collected has been considered useful to calculate the BMP value associated to each substrate treatment.

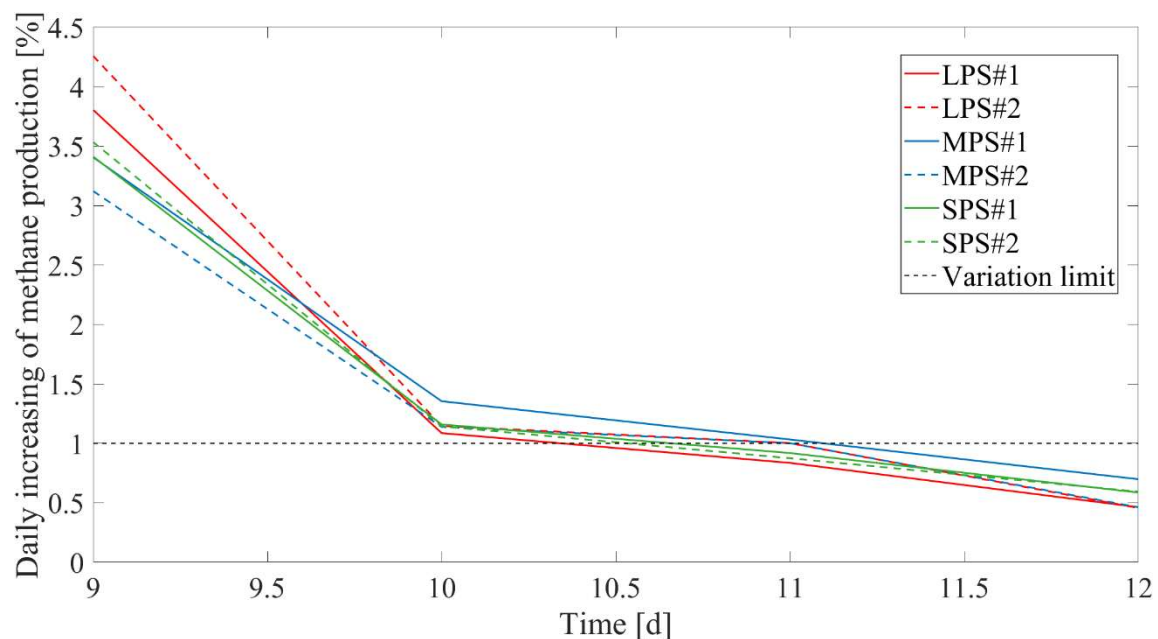


Figure 26. Daily increasing of methane production.

Also the relative standard deviation (RSTD) has been studied, in order to compare the standard deviation that affect each group of data collected. The results are shown in Figure 27 and have been obtained dividing the standard deviation calculated for each couple of points (associated to a single timestep) for the average of the two values. The maximum relative standard deviation is associated to the blank group of samples. The value of the blank's RSTD is in the range between 9% and 13%. This value should be considered not acceptable for the BMP calculation, but it should be justified considering the combined effect of the inoculum composition and the low reactor capacity. In fact, the inoculum has not been filtered before the use and its composition was not homogeneous: the liquid part is mixed with suspended solids. The used reactor volume is lower than 400 ml: the effects of the heterogeneity of the inoculum are emphasized by the small volume of the sample. The relative standard deviation associated to the samples containing the substrate is around 5%. In these samples, the effect of the not homogeneity of the inoculum is mitigated by the presence of the substrate, to which is associated a major biomethane production and so an higher value of the average at the denominator of the RSTD formula.

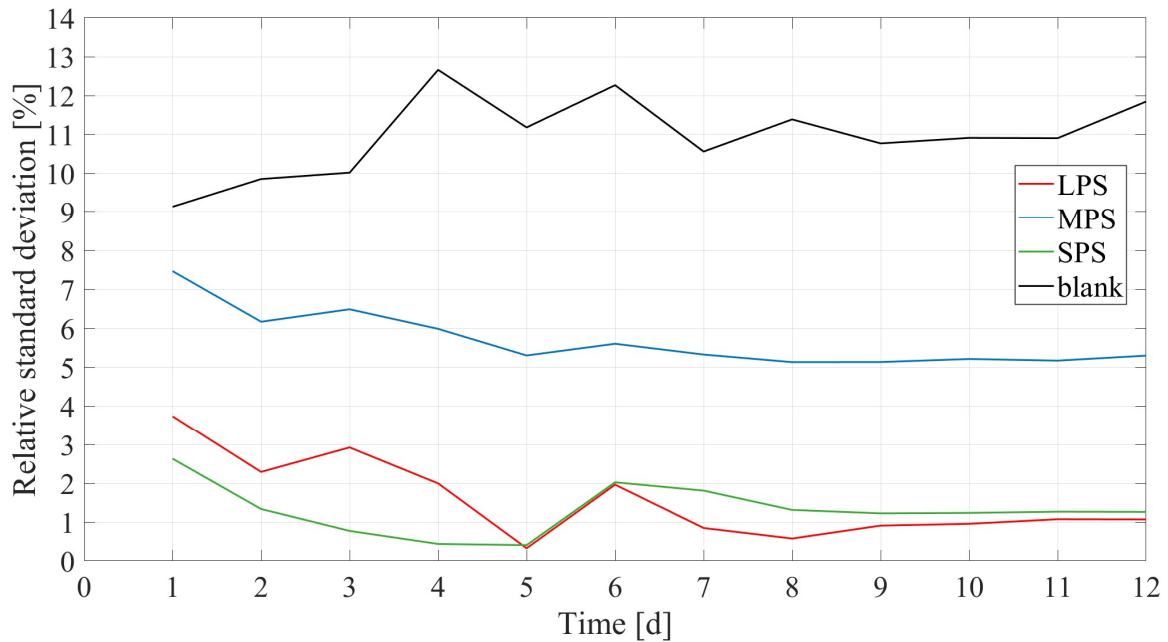


Figure 27. Relative standard deviation trends.

In Figure 28 the methane yield is presented. The ultimate points of these trends (associated to day 12) are the BMP of the three particle size treatment on the substrate. The error bars are not reported in the plot in order to make it more readable.

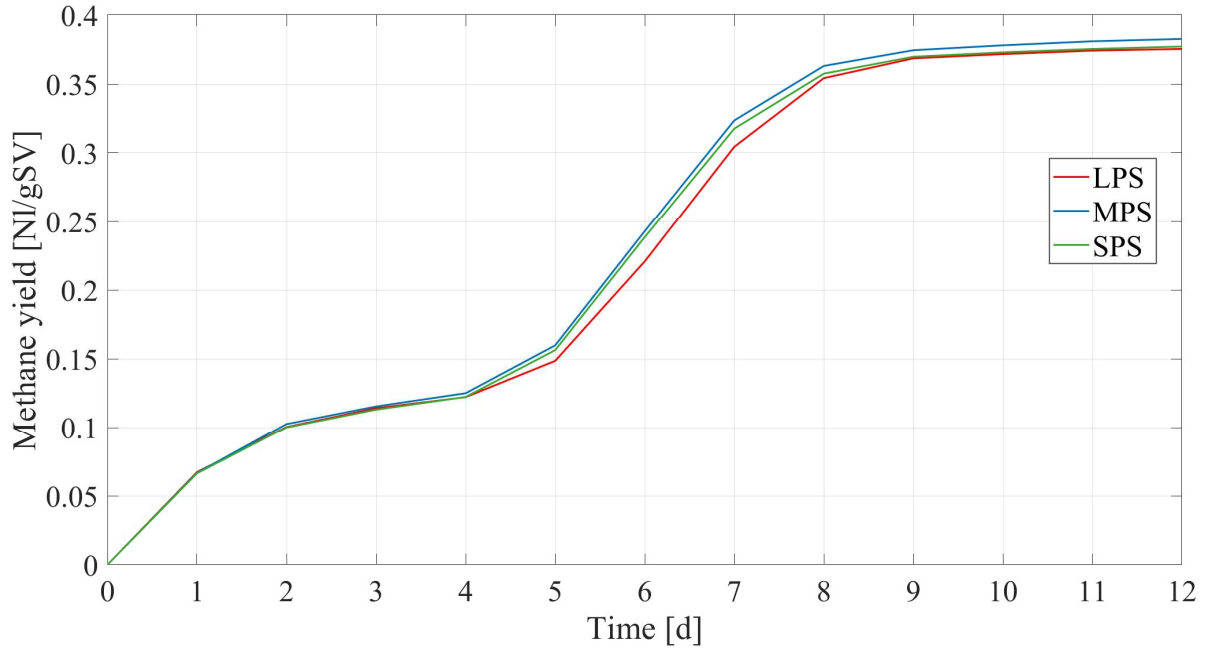


Figure 28. Methane yield associated to the production during days 0 to 12.

The obtained BMP values are equal to:

$$BMP_{LPS} = 0.377 \frac{Nl}{g_{SV}} \pm 2\%$$

$$BMP_{MPS} = 0.385 \frac{Nl}{g_{SV}} \pm 4\%$$

$$BMP_{SPS} = 0.379 \frac{Nl}{g_{SV}} \pm 2\%$$

The calculated values show that the MPS substrate has given the highest BMP result but the relative error associated to this value is bigger than the difference between BMP_{MPS} and BMP_{LPS} or BMP_{SPS} . In order to define if there is a statistical difference between the levels studied, the ANOVA method has been applied. The ANOVA test has been conducted on the cumulate methane production obtained from each test reactor (with substrate) at day 12. Raw data used are collected in Table 12, without reporting the error.

Table 12. Data for the ANOVA on accumulated biomethane production at day 12.

Level	Replication#1 [Nml]	Replication#2 [Nml]	$\bar{x}_k$ [Nml]
LPS	3293	3343	3318.0
MPS	3505	3252	3378.5
SPS	3362	3303	3332.5

The grand average associated to this set of data is equal to:

$$\bar{x} = 3343 \text{ Nml}$$

The results of the ANOVA analysis are shown in Table 13.

Table 13. Analysis of Variance for the cumulate biomethane production at day 12.

<i>Source of Variation</i>	<i>Sum of squares</i>	<i>Degrees of freedom</i>	<i>Mean Square</i>	<i>F₀</i>
<i>Particle size (between levels)</i>	3991	2	1995.5	0.171
<i>Error (within levels)</i>	34995	3	11665.0	
<i>Total</i>	38986	5		

As noticed during the study of the differences between the three obtained BMP values, the differences between treatments are smaller than the ones within the same level, as shown also by the difference between the value of the mean square associated to the error and the one associated to the particle size. Under these conditions, it's possible to affirm that the mean value of cumulated production of the three particle size substrates are equal. In order to assess this affirmation, the comparison between F_0 and the $F_{0.05,2,3}$ obtained by the F Distribution tables, has been carried out:

$$F_{0.05,2,3} = 9.55$$

if $F_{0.05,2,3} > F_0$ H_0 cannot be rejected

$$9.55 > 0.171$$

The null hypothesis cannot be rejected because the F_0 ratio is minor than the $F_{0.05,2,3}$, it follows that the means of the three studied groups are statistically equal and the chosen particle size doesn't influenced the biomethane production in the conditions in which the tests have been conducted.

Considering the three BMP values statistically equals (due to the fact that the cumulate production with which they have been calculated is considered equal), the value of the BMP obtained by the anaerobic digestion of this biomass is:

$$BMP_{VFW} = 0.380 \frac{Nl}{g_{VS}} \pm 4\%$$

Referring to the collected data, VFW are suitable for anaerobic digestion processes in substitution or in co-digestion with other biomasses. It follows the possibility to employ this specific kind of wastes for the production of biomethane, which can be used as biofuel or in a cogeneration process to produce both heat and electricity also for farms interested in decreasing the environmental and economic impact of their production. The use of vegetable and fruit wastes is also a 0 km alternative for region in which this wastes is largely produced, as the Tuscany territory. Another interesting application of the collected results concerns the pre-treatment to apply to the waste before digestion: considering the fact that the size of the wastes has not affected the biomethane yield (for the particle size range studied), it is not necessary a fine chopping of this kind of substrate before loading the reactor, a particle size with an average dimension of 1.7 cm produces, according to the data collected, the same quantity of biomethane of a finer chopped substrate.

The second set of data presented is the one registered after the lack of heating and mixing of the plant. The biomethane production observed during this period (days 21 to 35) is not suitable to calculate a value of the BMP, but provides information about the response of each reactor system to a critical situation. Figure 29 shows the daily production trend registered after the laboratory issue. It's possible to notice that the value of flow rate is minor than 100 Nml/d for all the curves. Nevertheless, the daily production is higher than the one collected during the days 10-12. It could be justified considering that for the days in which the mixing system was off, the biomethane production continued but the lack of agitation caused the generation of suspended dry stores on the reactors: the consequence is that methane gas has been trapped into the liquid phase of the reactor. When agitation has been restored, the biogas bubbles has been gradually

released depending to the breaks of solid clusters of substrate. As consequence, the daily production registered is higher than the previous one (day 12) because it's the result of the accumulated production of more than one day.

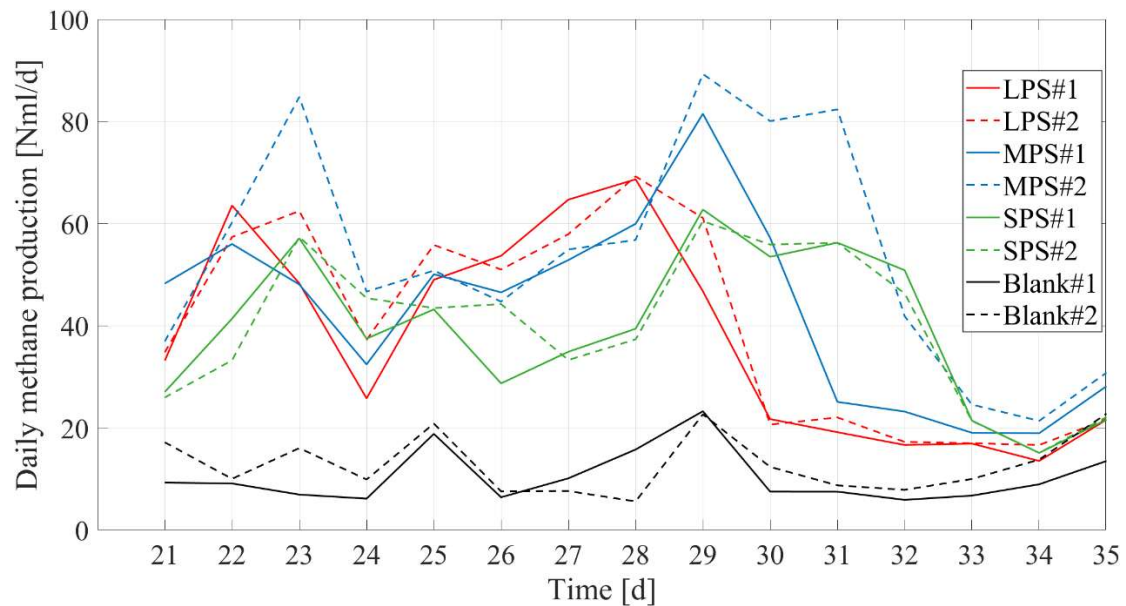


Figure 29. Biomethane flow rate collecting during days 21 to 35.

This result interpretation is also confirmed by the fact that the variability of the daily production between curves referred to the same level is higher than the one observed during days 1-12, as clear by a comparison between Figure 25 and Figure 30. The standard deviation associated to the second set of data is graphically more important than the one observed during the analysis of the first set.

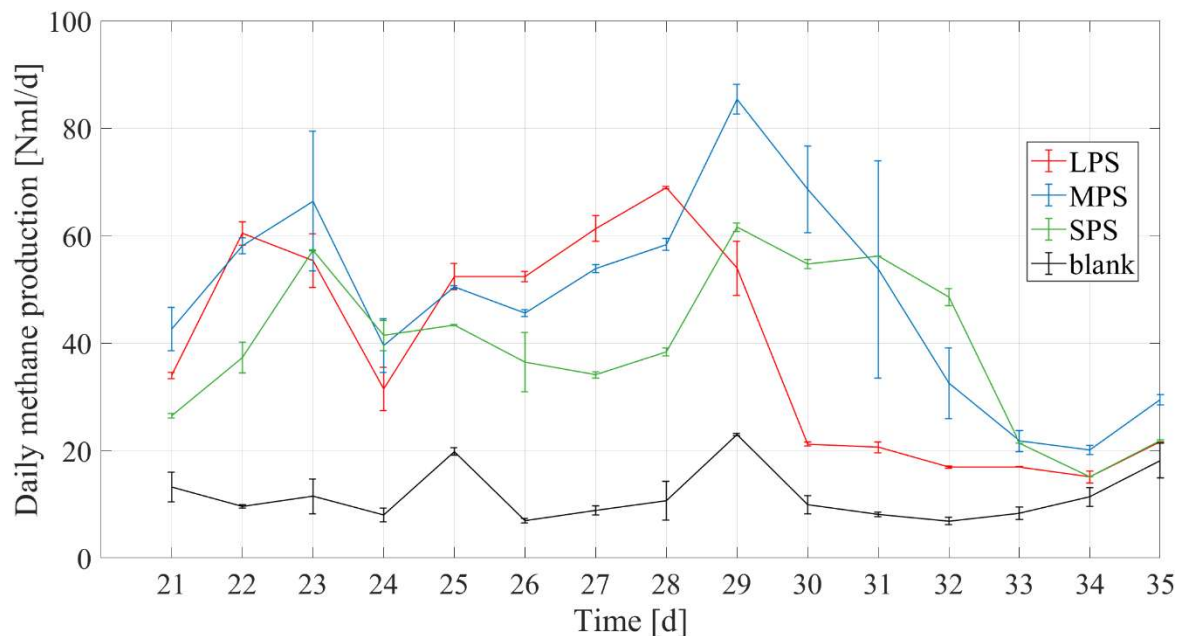


Figure 30. Daily biomethane production (from day 21 to day 35) expressed using average values and standard deviation.

It's also possible to study the plot of the percentage variation during days 7 to 35 (Figure 31), in order to appreciate the trend of the percentage variation of the biomethane production. As shown in detail in Figure 26, the trend of the flow rate for the days between 7 and 12 is decrescent. During the days of the laboratorial issue, the implant collected null flow

rates, so the production is low and constant and the variation is nearly equal to 0%. Then, at day 21 the variation starts to increase, remaining between 1% and 2% with fluctuation that doesn't make the trend decrescent. Around day 30 the fluctuations start to stabilize and the trend of variation return decrescent.

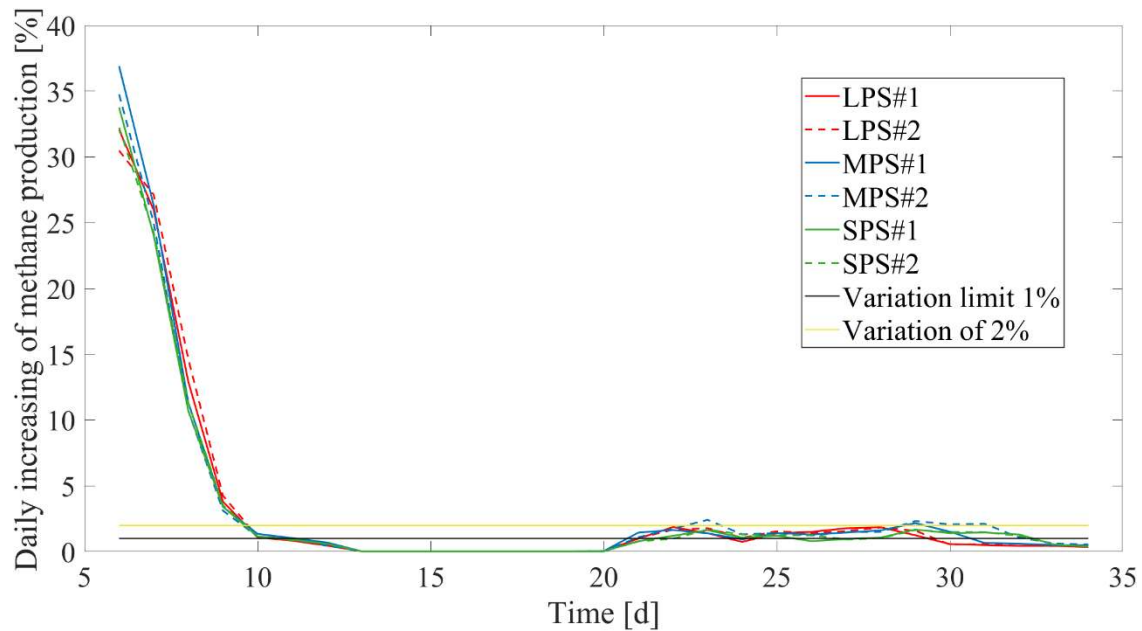


Figure 31. Percentage variation of daily production of biomethane between days 7 to 35.

The biomethane yield calculated on these data is reported in **Figure 32**. Unless the BMP data obtained by this second part of the test are not acceptable, due to the change of the test condition, the curves show a more remarkable difference between the methane yield obtained by the three different levels of the experiment if this data is confronted with the one reported in **Figure 28**.

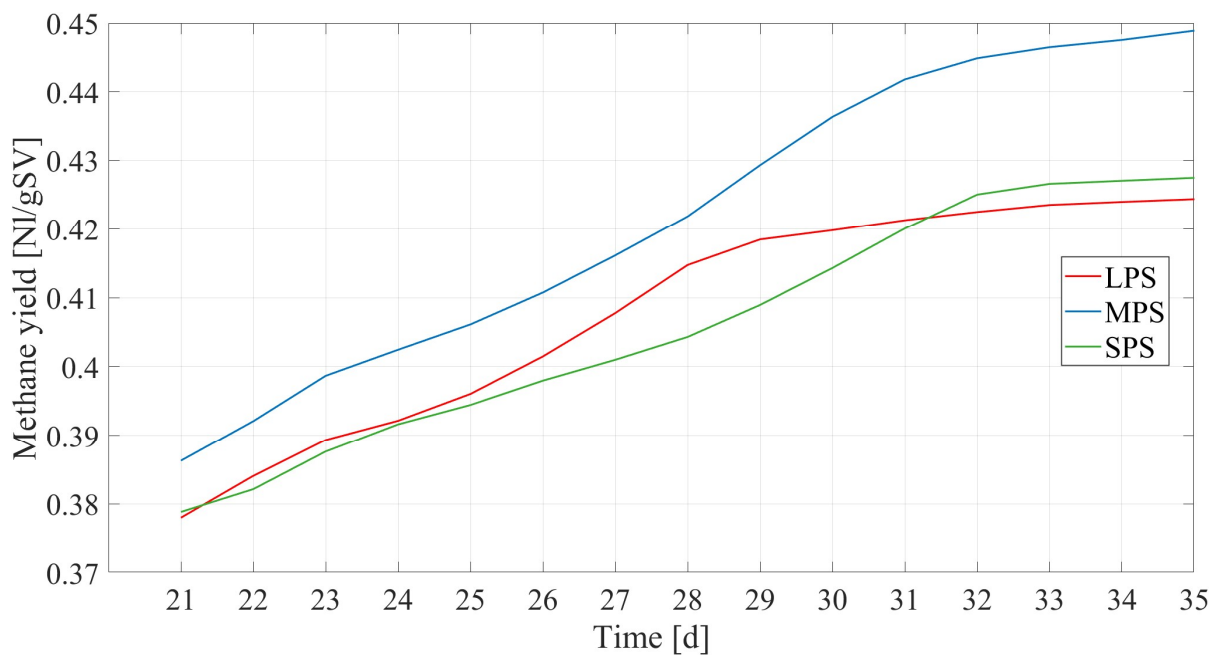


Figure 32. Methane yield collected during days 21 to 35.

For this reason the ANOVA analysis (Table 15) has been applied to the cumulate production detected at day 35 (Table 14). The grand average in this case is equal to 3985 Nml.

Table 14. Data for the ANOVA on accumulated biomethane production at day 35..

<i>Level</i>	<i>Replication#1 [Nml]</i>	<i>Replication#2 [Nml]</i>	$\bar{x}_k$ [Nml]
<i>LPS</i>	3863	3953	3908.0
<i>MPS</i>	4161	4066	4113.5
<i>SPS</i>	3961	3908	3934.5

Table 15. Analysis of Variance for the cumulate biomethane production at day 35.

<i>Source of Variation</i>	<i>Sum of squares</i>	<i>Degrees of freedom</i>	<i>Mean Square</i>	F_0
<i>Particle size</i> <i>(between levels)</i>	49982	2	24991.5	7.52
<i>Error</i> <i>(within levels)</i>	9967	3	3322.3	
<i>Total</i>	59949	5		

Also in this case the null hypothesis cannot be rejected. Nevertheless, the F_0 associated to these data is higher than the one studied for the cumulated production at day 12 and this value confirms the graphical data observed in Figure 32: the differences between the means associated to each level at day 35 are major the ones associated to day 12.

Anaerobic digestion of Artichoke wastes and Olive Pomace

According to the pathway defined before, aimed to the description of the characteristic of agri-food wastes produced in Tuscia territory in an energetic exploitation stance, artichokes' wastes and olive pomace have been collected and study separately. Artichokes are a very diffuse vegetable in Lazio region, which is the fifth product in Italy. In particular, in Tuscia territory the cultivation of the Roman artichoke is practiced. The variety studied and described in this paragraph is the autumnal one, which is produced between October and November. Olive pomace is residual production of the olive oil production process. Olive pomace is mainly composed by the pulp, husks and fragment of stones of the olive. This residual biomass is largely produced during the period of the olive harvesting, between October and December. Artichoke wastes have been taken by a small farmer which produce and sell at 0 km its products in Viterbo. The wastes of the sales activity (then indicated as "artichoke wastes") has been collected and their composition can be described as follow:

- 50% artichoke leaves;
- 33% artichoke stalks;
- 17% other vegetables and artichoke discharge.



Figure 33 Raw artichoke wastes

Olive pomace has been produced in an olive mill in Vetralla (Viterbo, Italy) from the squeezing of olive of the Caninese cultivar.

These wastes have been collected and submitted, separately, to energetic characterization in CIRDER laboratory.

During the characterization process, the unused biomass has been preserved in a fridge at 4°C of temperature, slowing down decomposition phenomena. After obtained the characterization results, the biomasses have been prepared for the anaerobic digestion tests: artichoke wastes have been left at room temperature and chopped before the insertion in the reactor (Figure 34), while olive pomace has been used in its raw size (Figure 35). The reduction of particle size for artichoke wastes has been mandatory, since the dimension of some elements (e.g. the stalks) was incompatible with the dimension of the reactor.



Figure 34. Dimension of artichoke wastes.



Figure 35. Dimension of olive pomace.

The used inoculum is from the same digester which have provided the inoculum for the greengrocery wastes described in the previous paragraph, but the characterization tests have been repeated in order to provide updated information. In fact, during the period elapsed between the two experimental campaigns, that is the one with fruit wastes and the current one with artichoke, the feedstock used to feed the digester has changed. It follows that is non-trivial the fact that the inoculum properties may have been changed. Also in this case, inoculum has been preincubated for 15 days, until its daily production has fallen under 50 Nml, before starting characterization analysis and anaerobic digestion.

As said before, the experimental campaigns have been carried out at the same time but separately: interaction between artichoke wastes and olive pomace has not been investigated. The subscript “AR” indicates artichoke, while the subscript “OP” is referred to olive pomace in the following discussion.

To obtain the energetic characterization, each sample has been prepared using the following steps. A non-corrodible and heat resistance tray has been weighted using a laboratory balance with a resolution of 0.01 g and its mass has been labelled as “w₁”. Then a portion of wet biomass has been put into the tray and another weighing, called w₂, has been done. The tray containing the biomass sample has been inserted in an oven at a temperature of 105°C ± 2°C and the weight of the tray filled with the biomass has been periodically registered until a constant weight has been reached between two consecutive weighing, made in a time interval of more than an hour. The weight of the tray and the dried biomass has been registered and nominated w₃. The moisture content percentage on wet basis has been calculated using the equations provided by [34].

From the moisture content, the percentage of the total solid amount (TS) of the biomass has been calculated as [49]:

$$TS [\%] = 1 - MC \quad (34)$$

Once the biomass has been dried, the procedure for the determination of the ash content has been carried out. For each kind of biomass nr. 3 replicates have been realized. For this test, each sample has been prepared according to the following routine. A ceramic crucible has been weighted using a 0.001 g resolution laboratory balance and its mass has been collected as “m₁”. Then, an amount of dried biomass has been put into the crucible and the crucible filled with biomass has been weighted, in order to obtain the data called m₂. Then the sample has been inserted in a muffle increasing the temperature uniformly until reaching 250°C ± 10°C. The muffle has been maintained at this temperature for 60 minutes

and then the muffle has been set at $550^{\circ}\text{C} \pm 10^{\circ}\text{C}$ and once reached this temperature the test has been carried out for two more hours before extracting the sample. Once extracted, the sample has been put into a drier and, when its temperature decreased, it has been weighted. The mass of the crucible filled with biomass ash has been labelled m_3 . The percentage of ash content (AC) on total solid is calculated referring to the formula in [36].

The percentage of volatile solid (VS) contained by the biomass (on dried basis) is derived as [50]:

$$VS [\%] = 1 - AC \quad (35)$$

The relative error associated to each value obtained by the characterization process has been calculated considering the resolution as the error of the instrument and referring to the formulas of error propagation

After the inoculum and the biomasses have been characterized and prepared, the anaerobic digestion tests have been arranged. During the loading, a headspace of 100 ml has been left and the inoculum on substrate ratio (I/S) referred to the VS content has been imposed equal to 3.5 both for artichokes' samples and olive pomace samples:

$$\frac{I}{S} = 3.5 \quad (36)$$

The thermostatic bath has been set at 49°C , which was the set temperature of the biogas plant in which the inoculum was collected. The mixer has been maintained on for 30 minutes every 120 minutes. Three repetitions have been done for artichoke wastes, three for the olive pomace and two for the blank samples. The scale plant admits the user to automatically register the cumulated methane production (expressed in Nml) and the daily methane flow, measured in Nml/d. Gas production is normalised in real time by the scale plant software and measured. The tests have been carried out for 52 days.

Results obtained by the characterization have been collected in Table 16, Table 17 and Table 18.

Table 16. Moisture content of the inoculum and the substrates (artichoke and olive pomace wastes).

	<i>Sample 1</i>	<i>Sample 2</i>	<i>Sample 3</i>
<i>Inoculum</i>	$(92.4 \pm 0.04)\%$	$(92.6 \pm 0.04)\%$	$(92.6 \pm 0.04)\%$
<i>Artichoke wastes</i>	$(91.2 \pm 0.1)\%$	$(92.0 \pm 0.1)\%$	$(91.9 \pm 0.1)\%$
<i>Olive Pomace</i>	$(42.6 \pm 0.2)\%$	$(43.0 \pm 0.2)\%$	$(42.7 \pm 0.2)\%$

According to data shown in Table 16, artichoke wastes have high moisture content, while olive pomace presents a lower moisture content, due to the squeezing process which characterize its production.

Table 17. Ash content on dry basis of inoculum and substrates (artichoke and olive pomace wastes).

	<i>Sample 1</i>	<i>Sample 2</i>	<i>Sample 3</i>
<i>Inoculum</i>	$(32.7 \pm 0.8)\%$	$(32.3 \pm 0.8)\%$	$(32.5 \pm 0.8)\%$
<i>Artichoke wastes</i>	$(18.4 \pm 0.8)\%$	$(19.0 \pm 0.8)\%$	$(20.9 \pm 0.7)\%$
<i>Olive Pomace</i>	$(5.6 \pm 2.5)\%$	$(4.5 \pm 3.0)\%$	$(5.8 \pm 2.4)\%$

Table 18. Energetic characteristics of inoculum and substrates (artichoke and olive pomace wastes).

	<i>Total solid [%]</i>	<i>Moisture content [%]</i>	<i>Ash content on dry basis [%]</i>	<i>Volatile solid content on dry basis</i>
<i>Inoculum</i>	(7.4 ± 0.04)%	(92.6 ± 0.04)%	(32.5 ± 0.8)%	(67.5 ± 0.8)%
<i>Artichoke wastes</i>	(8.3 ± 0.1)%	(91.7 ± 0.1)%	(19.4 ± 0.8)%	(80.6 ± 0.8)%
<i>Olive Pomace</i>	(57.2 ± 0.2)%	(42.8 ± 0.2)%	(5.4 ± 2.7)%	(94.6 ± 2.7)%

To load with an I/S ratio (based on volatile solid content) equal to 3.5 for each reactor, the value of the volatile solid content on wet basis has been calculated:

$$\begin{aligned}
 VS_{IN,wet\ basis} &= (5.0 \pm 0.8)\% \\
 VS_{AR,wet\ basis} &= (6.7 \pm 1.0)\% \\
 VS_{OP,wet\ basis} &= (54.1 \pm 1.0)\%
 \end{aligned}$$

Using these data, it is possible to calculate the amount of inoculum and substrate needed to load the reactors to obtain a I/S ratio equal to 3.5. Each reactor for artichoke test has been loaded using (329 ± 1) g of inoculum and (71 ± 1) g of artichoke wastes, while each reactor for the olive pomace test has been loaded with (390 ± 1) g of inoculum and (10 ± 1) g of olive pomace. Knowing this information, it is also possible to define the amount of volatile solids referred to the substrate contained in each batch reactor:

$$\begin{aligned}
 VS_{sub,AR} &= 4.7\ g \pm 2.9\ \% \\
 VS_{sub,OP} &= 5.4\ g \pm 1\ \%
 \end{aligned}$$

Once all the needed parameters have been calculated, the anaerobic digestion tests have been carried out.

Results of the anaerobic digestion of artichoke wastes

Artichoke wastes cumulate methane production is shown in Figure 36. The trends show a rapid growth until day 5, then the slope of the curves became less steep. It's also notable the fact that the sample 2 has a step of growth at day 12, while the other two samples shown a quite similar step shifted at day 17 and 18, respectively for sample #3 and sample #1. After day 16, in which the three samples cumulate production is overlapped and equal to 2241.9 Nml, the three trends present a cumulative production clearly distinguishable and at day 52 the first sample has reached a cumulate production equal to 3111.3 Nml, while the second sample has a cumulate production lower for about 5 percentage points, equivalent to 2793.5 Nml. The third sample is placed between these two results, with 2942.8 Nml.

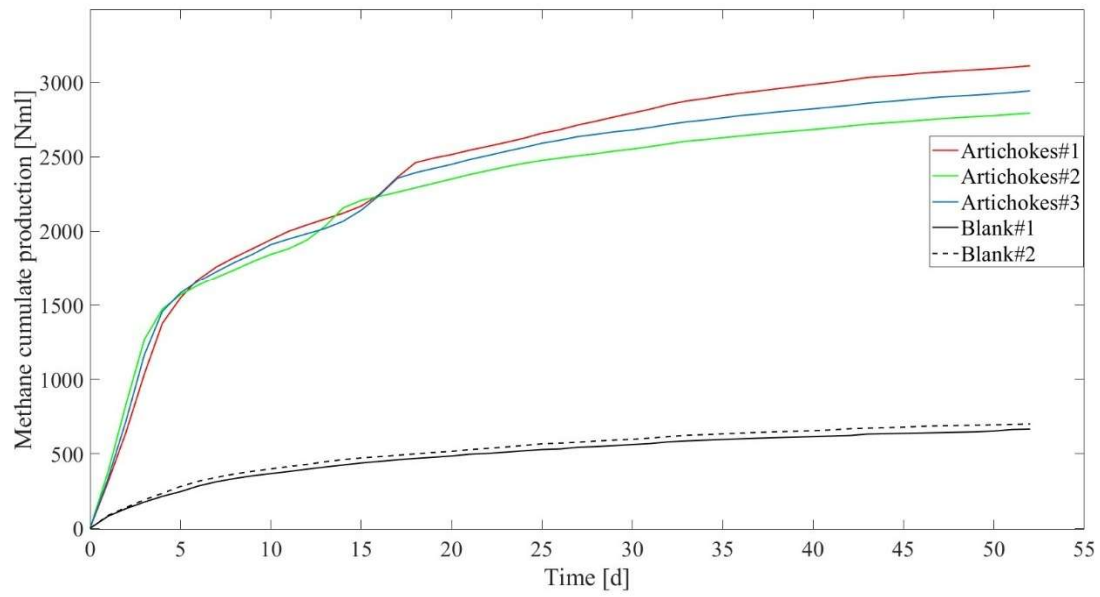


Figure 36. Methane cumulate production of artichoke wastes.

Using the data collected from the three samples, mean cumulative production and associated standard deviation has been calculated and plotted in Figure 37.

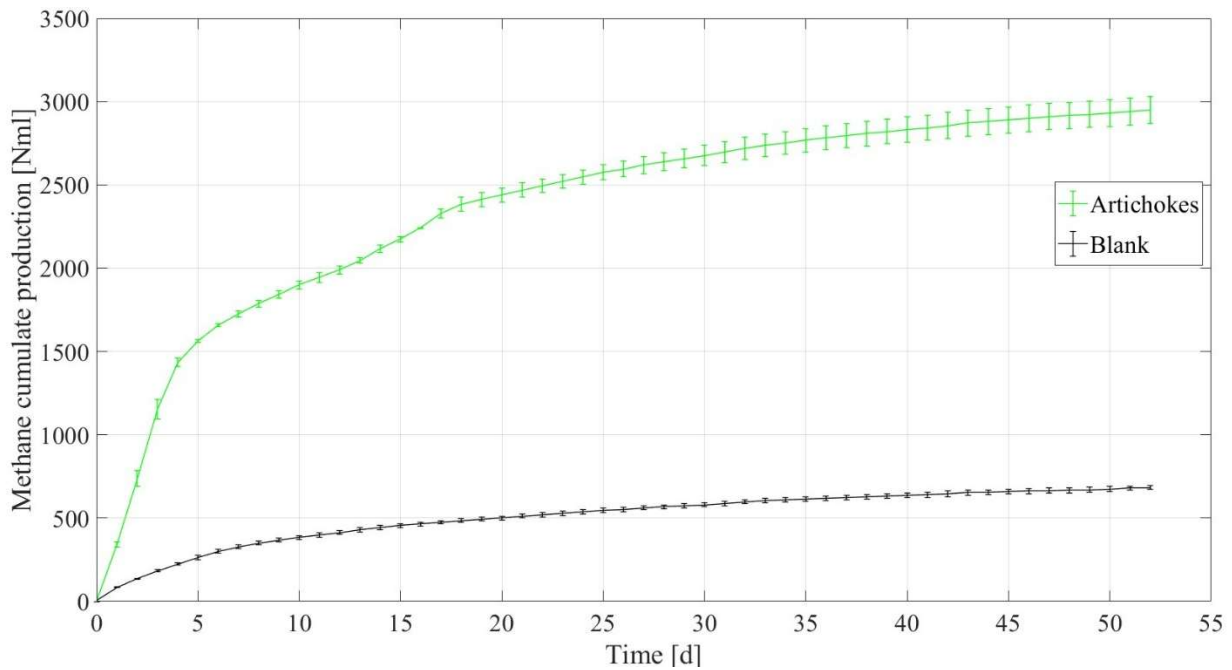


Figure 37. Average curves of the cumulative biomethane production for artichoke wastes and blank samples.

As previously observed, the standard deviation associated to the results of the experimentation between days 0 to 20 is lower than 100 Nml (with the only exception of the value associate to day 3, which reach about 116 Nml). Then, after day 20, standard deviation grows until reach a value of 158 Nml. Trend followed by standard deviation can be also observed by the study of the relative standard deviation (RSDV) in Figure 38.

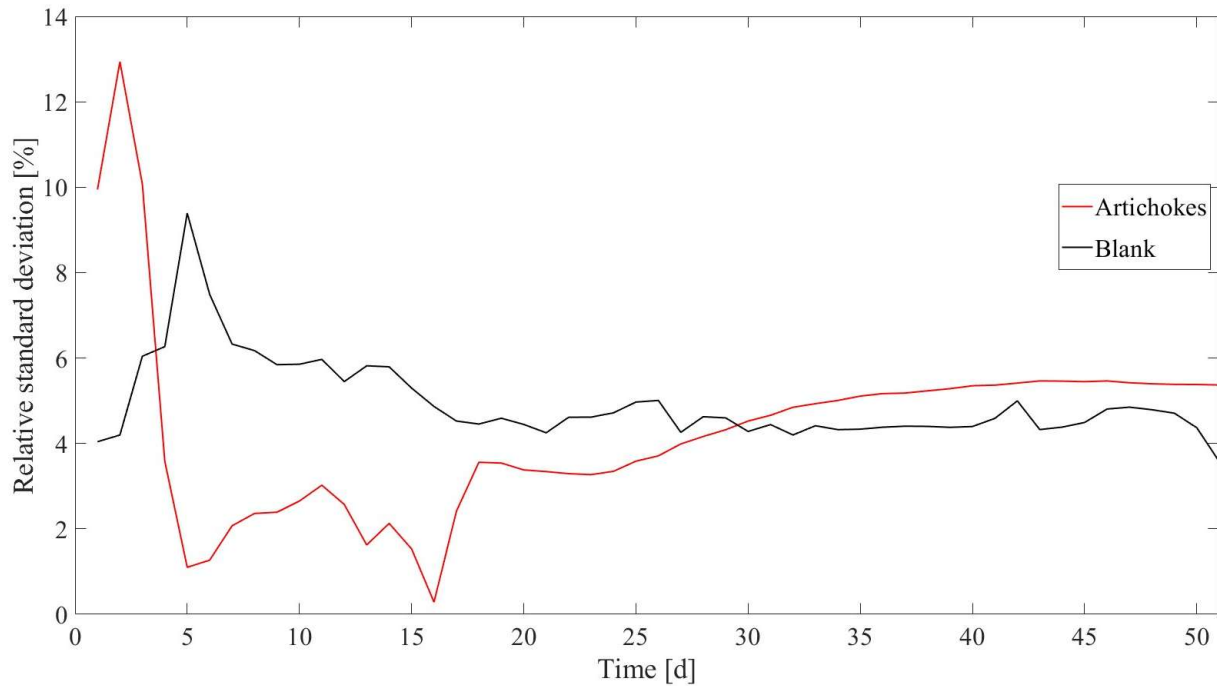


Figure 38. Relative standard deviation trend associated to methane production of artichoke wastes and blank samples.

Relative standard deviation should be minor than 6% [56] in order to calculate BMP and, except for some spot values related to days 2 and 3 for artichoke samples and days 5 to 6 for blank samples, relative standard deviation remains under the indicated value. It is interesting notice that blank samples results are characterized by relative standard deviation level higher than the ones associated to artichoke. This information, coupled to the one just discussed in the analysis of greengrocery wastes' anaerobic digestion, supports the hypothesis that the inoculum used, which presents different size particles in its composition, can affect the results because of the small quantity used in the reactors. In fact, reactors with an inner volume of 500 ml admit the use of a small amount of inoculum and a small amount of biomass, as seen before in the sample preparation section. It follows that the presence of inhomogeneous particle size dimension probably makes a certain difference between different samples results. This difference is detected numerically by the standard deviation information.

Also the daily biomethane production can be studied using Figure 39. As seen in the cumulate methane production analysis, two peaks are detected in the biomethane production: the first one, happened between day 0 and 5, is the most noticeable. In this interval of time, in fact, the three samples reach a production higher than 350 Nml. There are, as seen before, differences in this production: sample #2 has produced 458.6 Nml at day 2, while sample #3 has reached 437.5 Nml at day 3 and, at the same time, sample #1 has achieved its maximum production with a value of 385.3 Nml, with a difference of about 15 percentage points in comparison to the peak associated to sample #2. Qualitatively speaking, however, the daily production trends shown by the three samples are consistent and agree with the maximum production in the first three days.

The second peak, which is characterized by a lower value of the daily production, happen between day 14 and 17. Sample #2 reaches this second peak at day 14, with a value of 123.0 Nml, while samples #1 and #3 achieve the same daily production value equal to 114.6 Nml at day 17. After this second event, daily production decrease under value of 50 Nml.

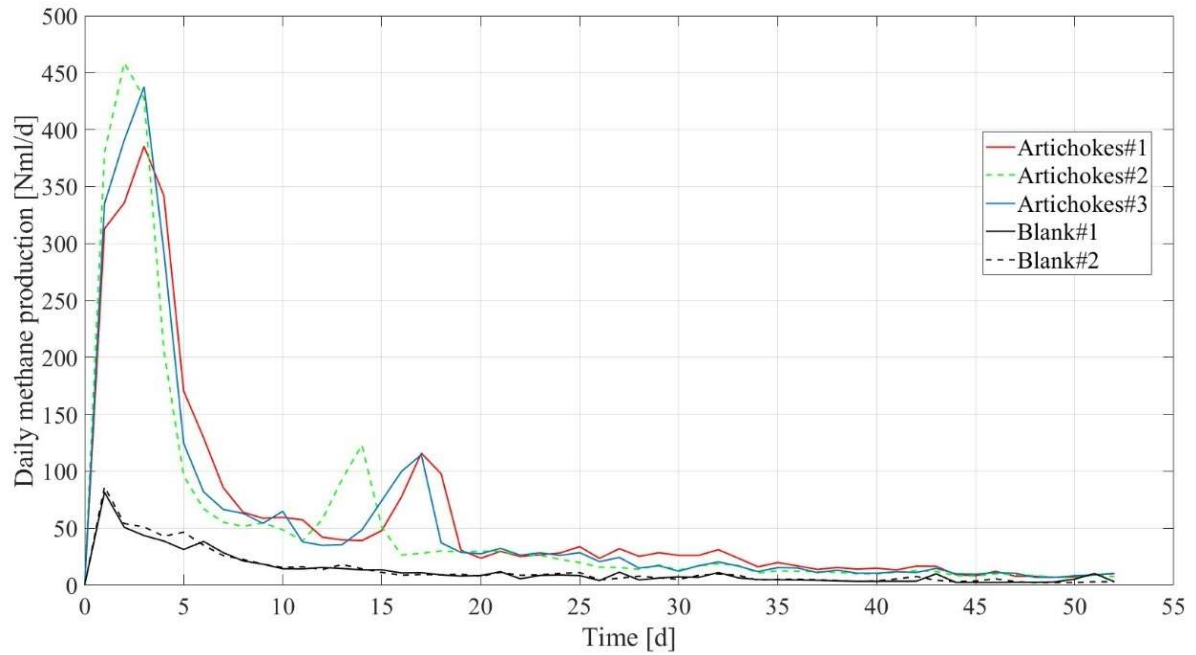


Figure 39. Daily methane production of artichoke wastes.

In Figure 40, the standard deviation associated to the daily biomethane production is described. As seen in the previous graph, higher values of standard deviation are associated to days between 2 and 5 and days between days 13 and 18, when the peak of production happens on each sample curve.

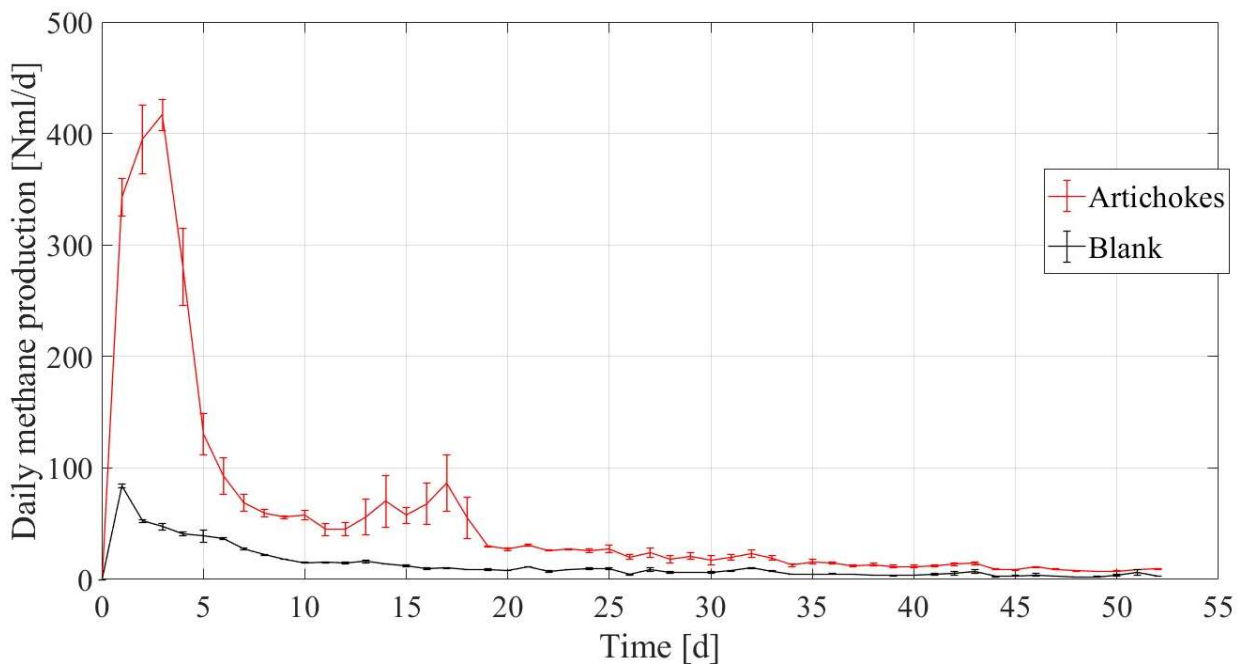


Figure 40. Daily biomethane production expressed using average values and standard deviation.

To define the BMP value, the daily production variation has to be lower than 1% between consecutive days. The daily production has been calculated using formula (25) and is plotted in Figure 41. Sample #1 has reached variation values under 1% after day 34, sample #2 after day 25 and sample #3 after day 42.

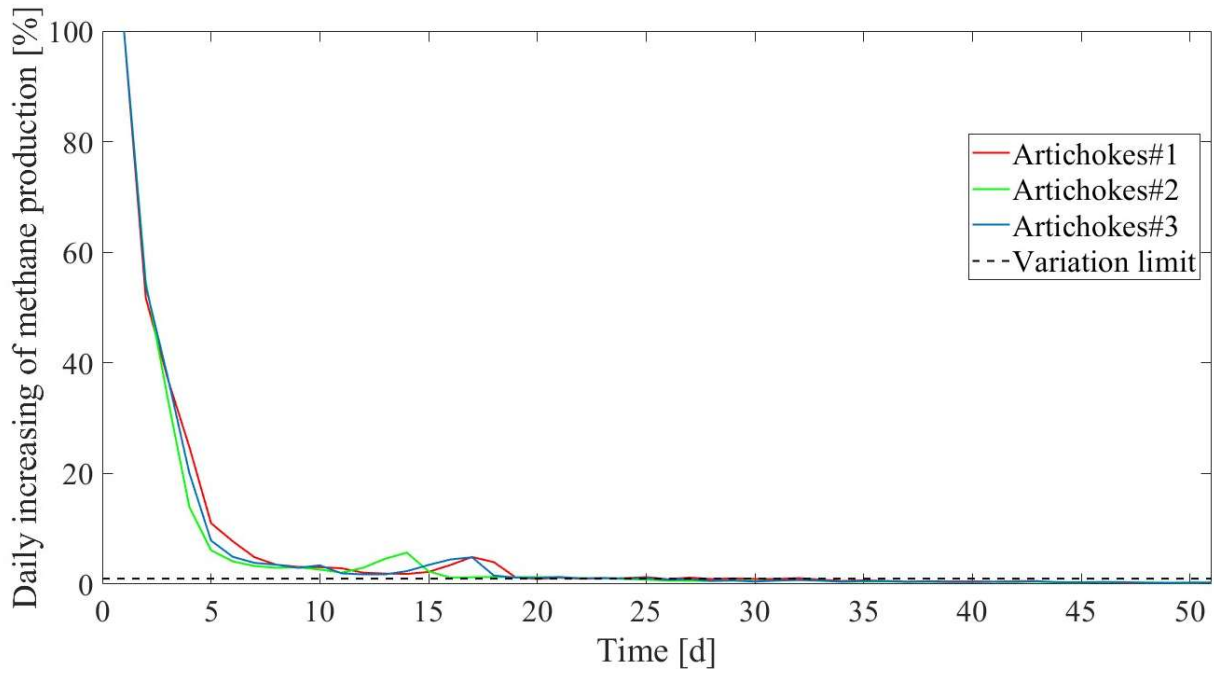


Figure 41. Daily increasing of methane production.

For this reason, the value of BMP has been calculated at day 50, when methane production has been stabilized. The equation (26) has been used to calculate the methane yield, as shown in Figure 42. The error bars are not reported in the plot to make it more readable.

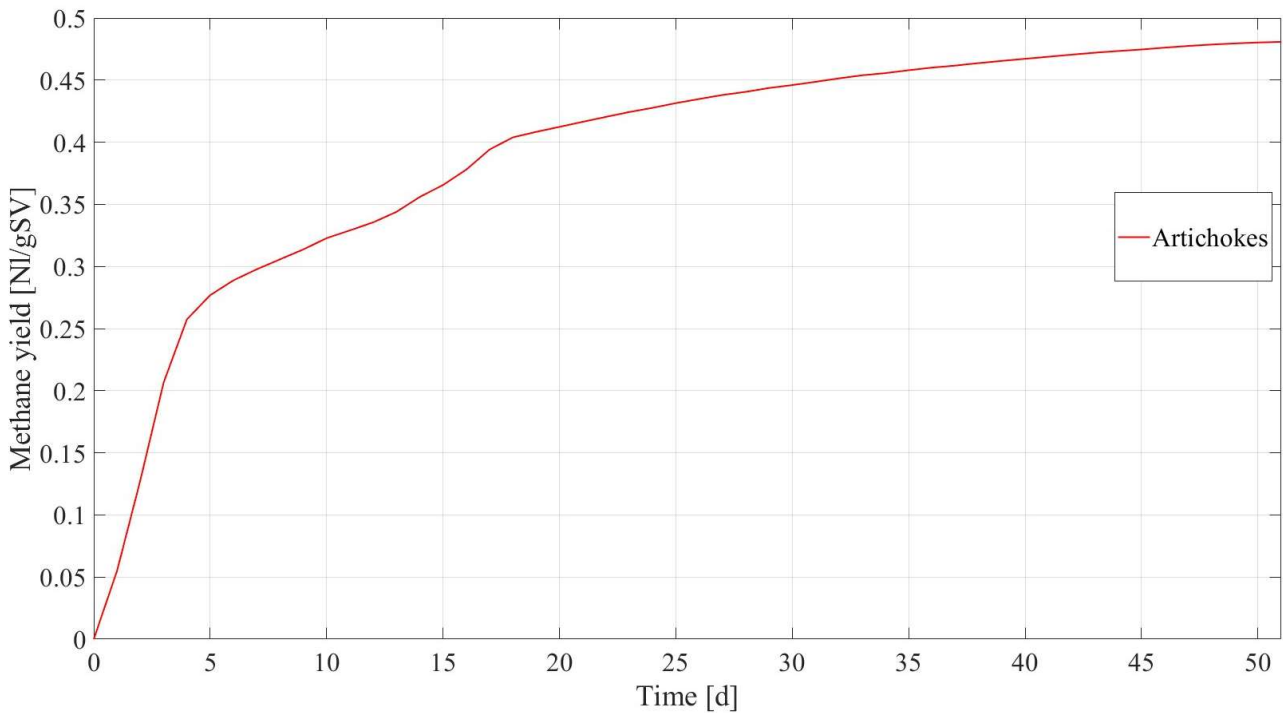


Figure 42. Methane yield associated to the anaerobic digestion of artichoke wastes.

Biomethane potential for artichoke wastes measured at day 50 is equal to:

$$BMP_{artichoke\ wastes} = 0.480 \frac{NL}{g_{sv}} \pm 5\%$$

Results of the anaerobic digestion of Olive Pomace

In this paragraph results related to the anaerobic digestion of olive pomace are discussed.

Olive pomace cumulative production is described in Figure 43 and results of digestion show that sample #2 has had a cumulative production higher than the other two samples. At day 52, in fact, sample #2 is characterized by a cumulative production equal to 2015.6 Nml, while samples #1 and #3 have reached 1693.9 Nml and 1675 Nml respectively. The percentage difference between value related to sample #2 and values associated to the other two samples is about 20%. Also the blank samples have trends quantitative different each in relationship to the other: blank #1 at day 52 is equal to 9069 Nml, while blank #2 has a cumulate value of 1152.2 Nml.

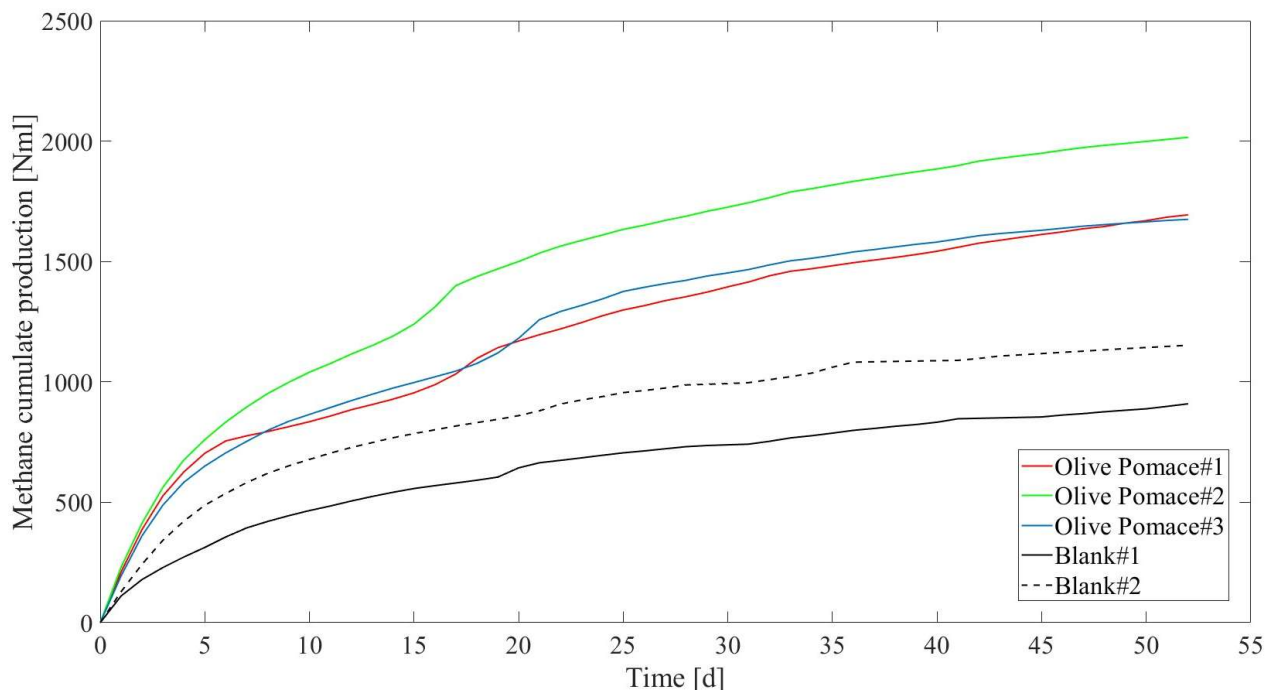


Figure 43. Cumulate methane production from olive pomace.

These differences are more noticeable consulting the graph in Figure 44, in which the representation of standard deviation is included. Standard deviation associated to the olive pomace samples is low in days between 1 to 8: in fact, at day 8 the standard deviation is equal to 10% of the mean cumulative production of that day. In the following days the standard deviation grows until reach the value of 208.4 Nml associated to the mean value of 1203.7 Nml produced at day 18 and the decreases, stabilizing its value around 190 Nml.

A similar trend of the standard deviation is associated to the mean cumulative production of blank samples, which reach a standard deviation of 200 Nml at day 37, then the standard deviation decreases around 170 Nml and 185 Nml.

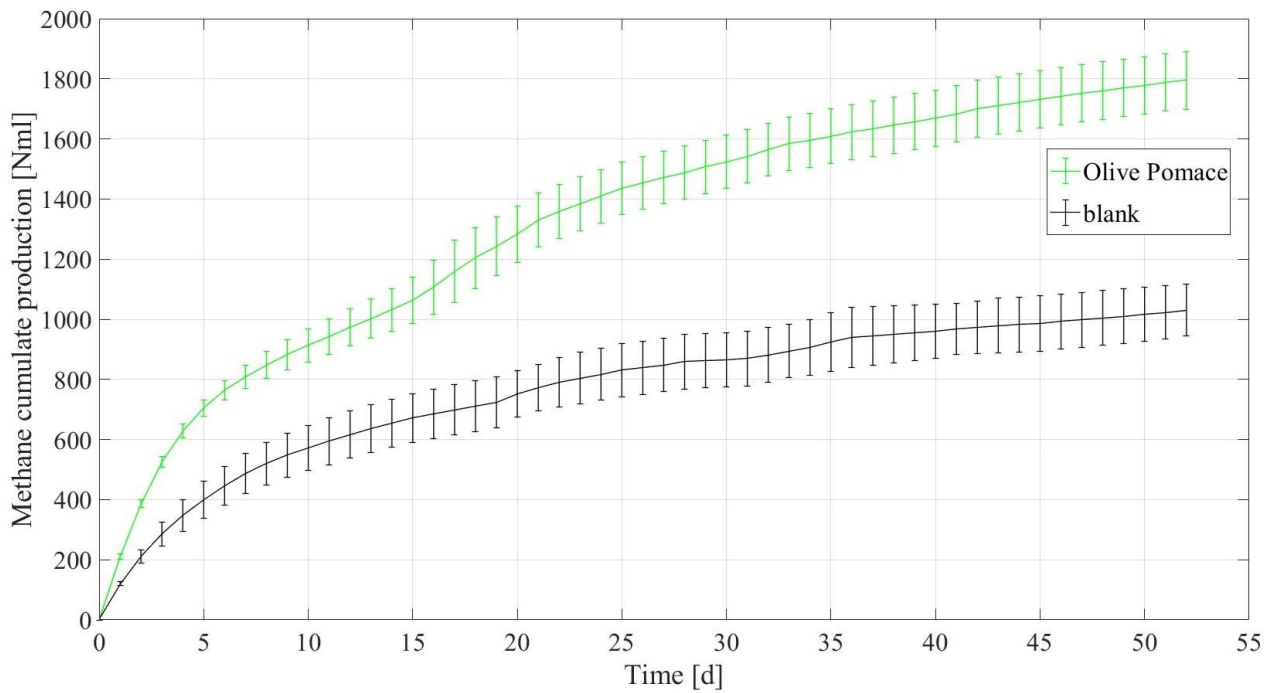


Figure 44. Average curves of the cumulate biomethane production by olive pomace.

The percentage value of the standard deviation on the mean cumulative production value can be observed using the plot Figure 45, which represents the relative standard deviation. It's possible to notice that value acquired during the experiment are affected by a relative standard deviation higher than the limit value of 6%. In particular, blank samples present relative standard deviation that reach also 30%, while olive pomace samples have a peak value at day 18, as seen before, and then a stabilization around 10-11 percentage points.

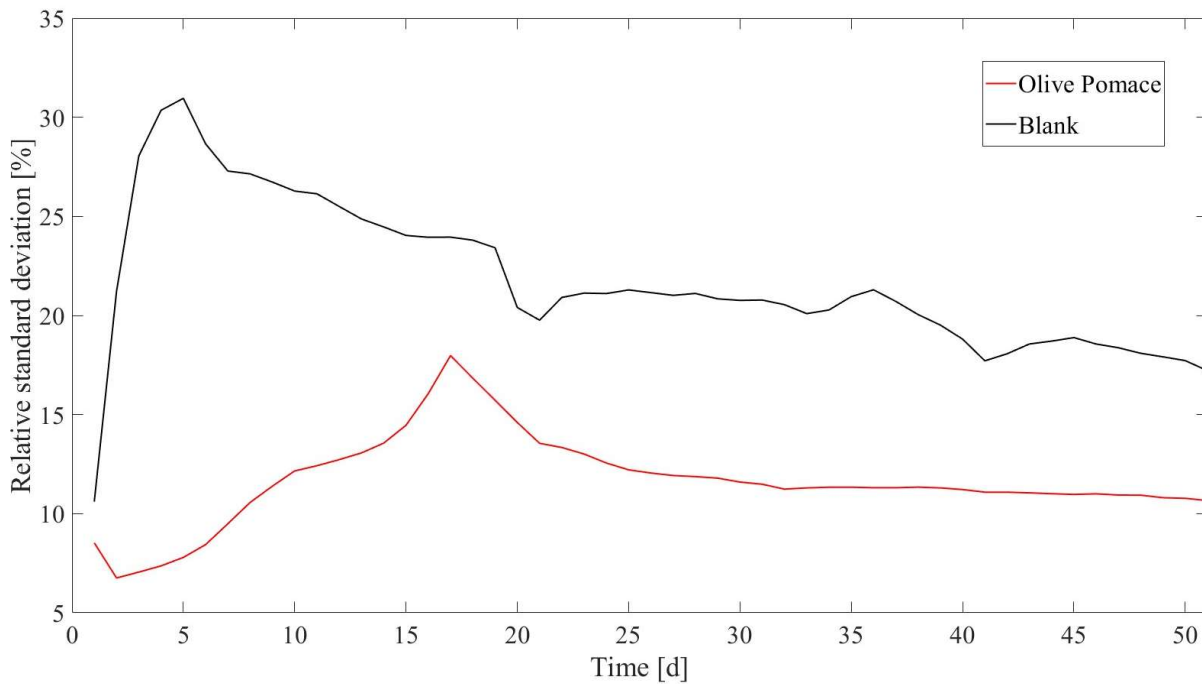


Figure 45. Relative standard deviation trends (olive pomace).

Considering the daily production associated to olive pomace samples, it's possible to notice that the qualitative trends of the three samples are quite similar: the highest production peak is reached before day 5, than the production sudden

decrease until reach a secondary peak between days 15 and 25. After this second peak, the production doesn't show any further significant peak and decrease under 50 Nml per day. An interesting thing is represented by the fact that also blank samples present the same trend, which suggest that the reason of these peaks is not related to the biomass, but rather to external factor which can affect the reaction.

In fact, the origin of the inoculum used (both in blank samples and in biomass loaded reactor) is the same and the anaerobic digestion of artichokes (and their blank samples) and olive pomace (and their blank samples). Also anaerobic digestion tests on artichoke wastes and olive pomace has carried out in the same time period, in different reactor but using the same thermal bath and the same mixing rate. Blank samples associated to artichoke digestion tests do not present the peaks described for blank of olive pomace, as testified by trends in Figure 37 and Figure 39.

As said before, this situation suggests the presence of an unknown external factor which has affect the tests related to olive pomace.

A positive consideration is that the peaks affect both biomass samples and blank samples: since the BMP calculation requires the difference between mean of production of biomass samples and blank samples, the effect of the noise can be partially reduced.

The first peak on daily production is reached by the three samples at day 1. Sample #1 reaches 210 Nml, sample #2 is associated to a daily production of 230.55 Nml and sample #3 produces 194 Nml of biogas. Then, at day 17 sample #2 reaches its second peak with a production of 88.7 Nml, while sample #1 obtained 64.2 Nml at its secondary maximum production and sample #3 reaches 77,2 Nml at day 21. As affirmed before, the qualitative trends are consistent, but the peaks are reached in different days for the three samples considered.

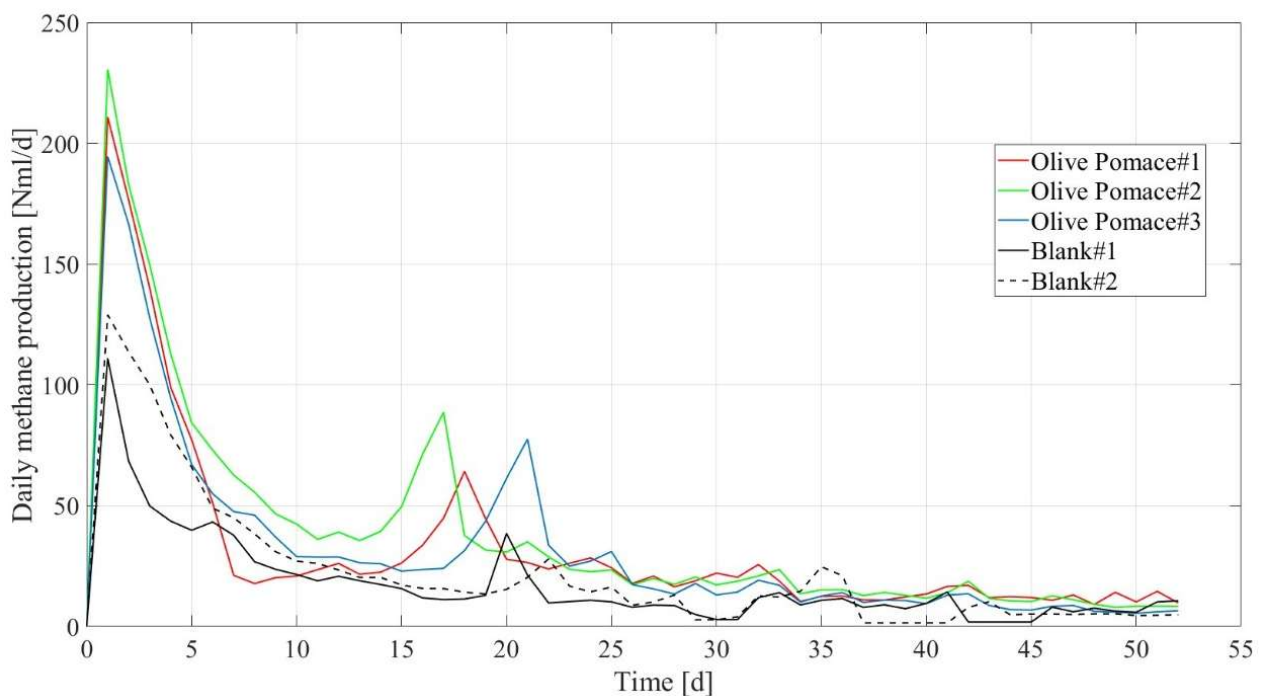


Figure 46. Daily methane production registered (olive pomace).

Figure 47 represents the mean daily production and its standard deviation: as expected, higher values of standard deviation are associated to time intervals where the peaks happen.

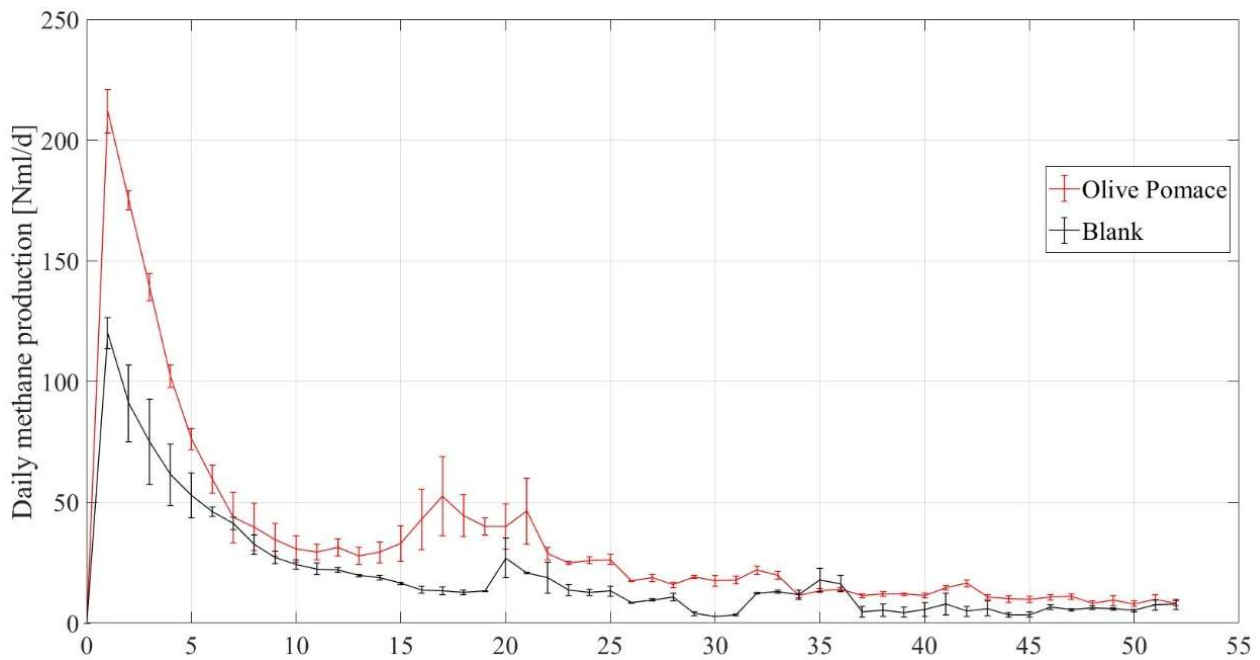


Figure 47. Daily biomethane production (olive pomace) expressed using average values and standard deviation.

In order to assess the daily increasing of methane production, Figure 48 has been generated. It's possible to see that after day 35 all the samples are characterized by a percentage variation between two daily productions (calculated with equation (25)) lower than 1%.

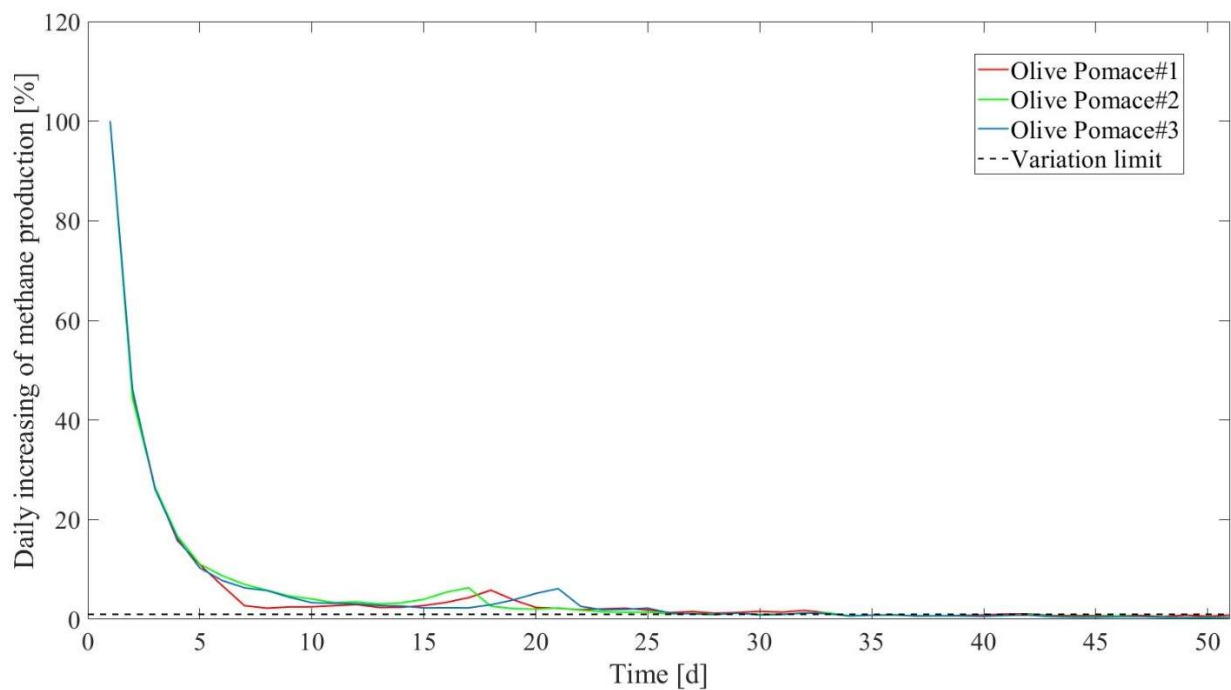


Figure 48. Daily increasing of methane production for anaerobic digestion of olive pomace.

The value of BMP has been calculated at day 50. The equation (26) has been used to calculate the methane yield, as shown in Figure 49. The error bars are not reported in the plot in order to make it more readable.

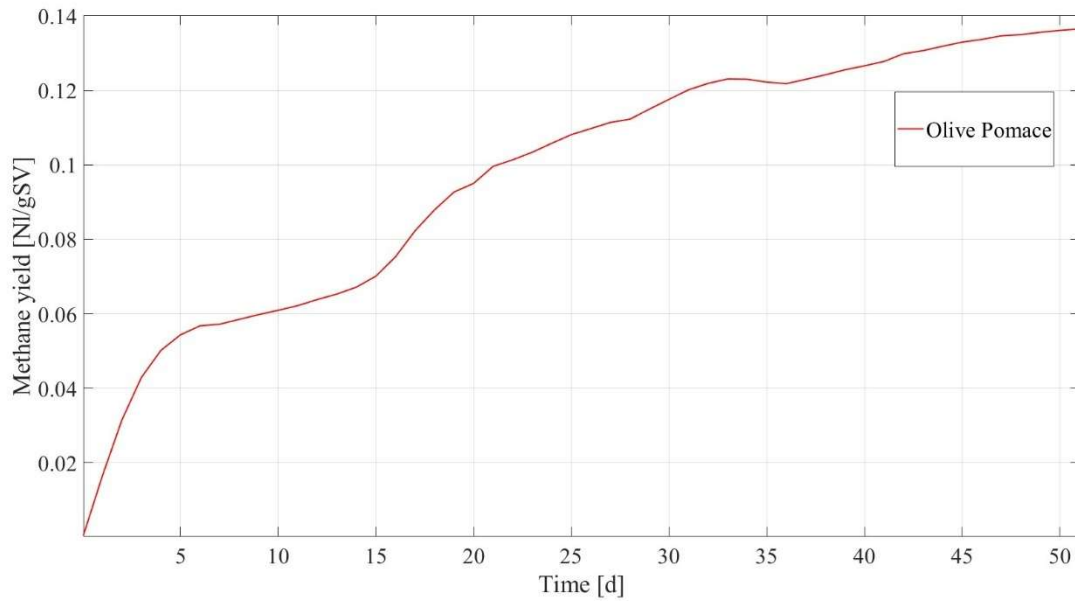


Figure 49. Methane yield associated to the production from olive pomace.

Biomethane potential for olive pomace measured at day 50 is equal to:

$$BMP_{olive\ pomace} = 0.136 \frac{NL}{g_{SV}} \pm 5\%$$

Comparisons and conclusions

Investigations about the energetic potential of residual biomasses in Tuscia territory has bring to the consideration that small factories and farmers own wastes suitable for anaerobic digestion conversion, which are different for different periods of the year, according to the natural production of harvesting.

In this chapter, different types of wastes have been analysed. Brewery spent grains has been submitted to characterization analysis and the results demonstrate their suitability for biochemical energetic conversion processes.

Anyway, the lack of a scale anaerobic digester and a design of experiment brings to the adoption of a anaerobic digestion instrument, composed by 15 scale reactors.

With this instrument and a study of DOE methods, new experimental campaigns have been carried out, favouring the study of agrifood wastes, in order to assess the biomethane potential and their energetic characteristics.

Looking for 0km farmer and sellers, at different times of the year, wastes associated to the production of different food have been collected.

During the summer greengrocery wastes have been analysed: their composition was mostly linked to fruit wastes and their biomethane yield stabilized before 20 days of tests (daily production variation lower than 1% at day 12). In addition to the BMP associated to anaerobic digestion of greengrocery wastes, also the influence of the particle size of the biomass has been analysed using ANOVA method. Results have shown that the particle size used during test have not influenced the methane yield produced during anaerobic digestion.

In the autumnal season, artichoke and vegetable wastes and olive pomace have been subjected to energetic characterization and anaerobic digestion tests. Both the biomasses have shown characteristics suitable with anaerobic digestion energetic conversion process and their BMP have been assessed. Artichoke wastes have reached a variability of their production under 1% after day 42, while olive pomace has reached this variation after 35 days of reaction.

Laboratorial failures occurred during test on fruit and vegetable wastes, while the set of data related to olive pomace show the influence of an external and unknown factor which has probably caused a peak on the daily biomethane production both in samples and in blank reactors.

In addition to this laboratory failure, other one happened and have made unusable data from other tests. The cause has been mainly attributed to two elements:

- cold air currents under 15 °C in some period of the day in the laboratory, which has caused the malfunctioning of the measurement cells, as also indicated by the provider of the plant;
- power failure for periods longer than the UPS coverage, which has caused lack of power in the implant mixture system.

Both these causes are mentioned in this section to provide a practical information about the problems which can affect the experimental campaign. It's important to underline that the problems are not imputable to BPC Instruments (Gas Endeavour).

In Table 19 the results obtained by the energetic characterization of wastes from 0km agrifood facilities have been collected.

Table 19. Comparison table of the results of biomass characterization.

	<i>Total solid [%]</i>	<i>Moisture content [%]</i>	<i>Ash content on dry basis [%]</i>	<i>Volatile solid content on dry basis</i>
<i>Inoculum (I period)</i>	$(11.0 \pm 0.04)\%$	$(89.0 \pm 0.04)\%$	$(29.5 \pm 0.4)\%$	$(70.5 \pm 0.4)\%$
<i>Vegetable and fruit wastes</i>	$(12.7 \pm 0.04)\%$	$(87.3 \pm 0.04)\%$	$(12.4 \pm 0.6)\%$	$(87.6 \pm 0.6)\%$
<i>Inoculum (II period)</i>	$(7.4 \pm 0.04)\%$	$(92.6 \pm 0.04)\%$	$(32.5 \pm 0.8)\%$	$(67.5 \pm 0.8)\%$
<i>Artichoke wastes</i>	$(8.3 \pm 0.1)\%$	$(91.7 \pm 0.1)\%$	$(19.4 \pm 0.8)\%$	$(80.6 \pm 0.8)\%$
<i>Olive Pomace</i>	$(57.2 \pm 0.2)\%$	$(42.8 \pm 0.2)\%$	$(5.4 \pm 2.7)\%$	$(94.6 \pm 2.7)\%$

Consulting the above-mentioned table, it's possible to assess that artichoke wastes have the higher moisture content detected, followed by fruit and vegetable wastes and olive pomace. Consulting the ash content, instead, the lower value (on dry basis) is linked to olive pomace, followed by vegetable and fruit wastes and then artichoke wastes.

Consulting [57] food wastes considered in Italy have a proximate moisture content between 66% and 95%, so the moisture content detected for this greengrocery wastes (fruit and vegetables), equal to about 89%, is consistent. In addition to this, grapes' moisture content namely ranges between 70% and 80% [58], [59], while the moisture content associated to peaches is about 87% [60], the one related to prunes is about 89% [61] and peppers have moisture content higher than 90% [62]. It follows that the value obtain during moisture determination is aligned with expected value.

Measured artichoke moisture content is higher than the one proposed in [63] and also its ash content is higher than the one detected in [63]. Probably, the fact that in the used biomass the 83% was composed by artichoke wastes influenced the results: there is a 17% of studied biomass which is not related to artichoke properties.

Moisture content associated to olive pomace is in line with the range proposed by [64] and also the ash content seems to be coherent with data detected by other researchers [65].

It's also interesting to notice that the inoculum, described in Table 19 with the name "I period" and "II period" is from the same anaerobic digestion plant, but collected in two different periods. The inoculum properties change: moisture content increases of about 4.0% and volatile content decrease of about 4.2%. This is probably linked to the fact that, during the time interval elapsed between the measures, feedstock of the anaerobic digester from which the inoculum is originated, changes.

Also a comparison between methane yield can be studied to detect characteristics of anaerobic digestion of residual biomasses considered.

Consulting Figure 50, the methane yield associated to the experiences (for fruit and vegetable wastes the data set considered is the one associated to the period before failure) can be observed and compared. Greengrocery wastes are characterized by a steep growth, which reach a variability percentage of the daily biomethane production after about 10 days. It suggests that this type of wastes can be quickly processed by anaerobic digestion pool, probably because it is rich in sugar and that in summer season its decomposition is favoured by external high temperatures (in Tusciana region, summer season is characterized by $>40^{\circ}\text{C}$ temperature peaks). It follows that the discharge has started a valuable decomposition process before starting anaerobic digestion and probably this event makes the biomass more easily attackable by archaeobacterial pool in anaerobic reactor.

At day 12, artichoke wastes are characterized by a methane yield lower than the one associated to greengrocery wastes. However this second type of biomass presents a slower growth of its daily production: in fact the variability of daily production decrease under 1% about between days 35 and 40. On the other hand the methane yield at day 50 is higher than the one associated to greengrocery wastes at day 12, which suggest that artichoke wastes can be elaborated more slowly than fruit wastes, but their yield can be also higher.

At least, olive pomace presents the slower growth of biomethane yield and the lower values of BMP. This biomass is rich in fat and this characteristic probably slows down and limits biomethane production.

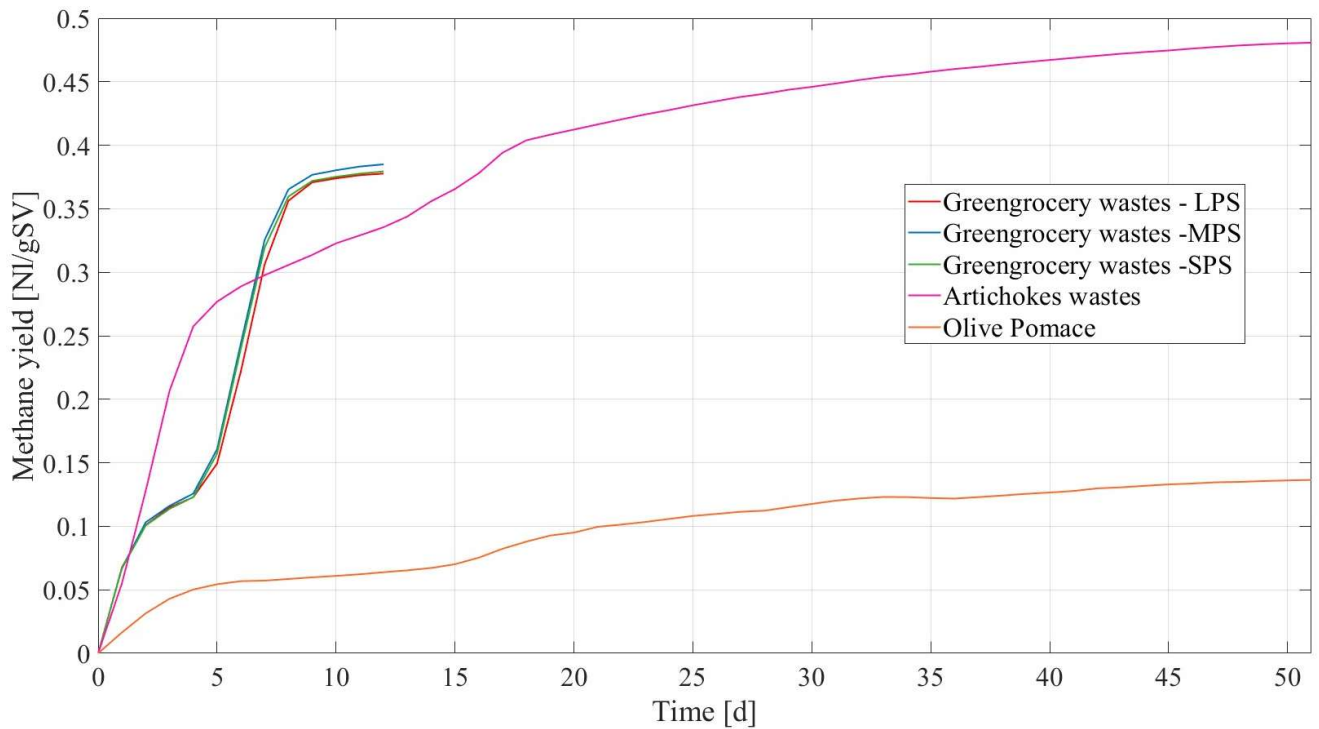


Figure 50. Results obtained during experimental campaigns.

Table 20. BMP comparison between investigated vegetables.

	<i>BMP [NL/gsv]</i>	<i>Cut off day</i>
<i>Greengrocery wastes- LPS</i>	$0.377 \pm 2\%$	12
<i>Greengrocery wastes- MPS</i>	$0.385 \pm 4\%$	12
<i>Greengrocery wastes- SPS</i>	$0.379 \pm 2\%$	12
<i>Artichoke wastes</i>	$0.480 \pm 5\%$	50
<i>Olive Pomace</i>	$0.136 \pm 5\%$	50

Greengrocery wastes BMP is within the range of the BMP of fruit wastes [66], while the biomethane potential associate to Artichoke wastes (in which a 17% of other vegetables is considered) is higher (about +15%) than the one observed by other researchers [67]. This difference can be explained both with the variability of the biomass characteristics and the presence of other vegetables in considered samples. Also olive pomace biomethane potential is comparable with the results obtained by other experimenter [68]. Comparing obtained BMP values with the ones associated to more used biomasses [69], [70], it's clear that these residual biomass are competitive.

In conclusion, studied agri-food wastes produced in Tuscia territory not only are suitable for anaerobic conversion, but the associate biomethane potentials detected are comparable with the ones of the used biomasses in anaerobic digestion. It follows that the design of micro-plants for anaerobic digestion could be considered to ensure a source of energy from a circular economy behaviour and make economically advantageous the 0km production of food.

Further investigation could involve:

- study of a wider range of residual biomasses produced in Tuscia territory;
- study of pretreatment on biomasses to improve methane yield from anaerobic digestion;
- study of the I/S ratio, particle size and mixing rate influence on biomethane yield;

- biochemical characterization of inoculum and biomasses;
- creation of a unified procedure and database to collect information acquired by experimental campaigns.

Software Tool ADMS 2.0

As aforementioned in the Introduction chapter, beside the experimental campaigns, the development of a software tool for the simulation in virtual environment of anaerobic digestion processes has been carried out. The aim of this second part of the PhD course consists in providing a software tool which can be used to predict anaerobic digestion biochemical and physico-chemical processes, in order to assess biomethane production, but also detect possible inhibition of the process studying the trends of the intermediate products of the reaction.

Characteristics of the software tool

The users of the software are mainly identify in researchers and technicians coming from different disciplines, such as biologist, agronomists, engineers and other professional figures which can have an interest in including anaerobic digestion in their field of interest. It follows that the characteristics that this software tool has to show are :

- 1 user friendly: in order to allow the user to approach the simulation focusing on input and output information and not on how to communicate these values to the software;
- 2 complete: results of the virtual experiment have to consider the main processes and phasis of the anaerobic digestion for the purpose to admit the investigation in different ways, not only aimed to the prediction of the biomethane production, but also of the other secondary compounds which can be object of interest in a determined field, such as hydrogen production or elaboration of a specific compound in the substrate;
- 3 widely interfaceable: input values required by the simulation tool are probably collected using instruments which provide a digital version of the acquired data. Moreover, data calculated by the simulations could be submitted to further analysis or give as input information in other software tools. For this reason, it's important that the software can manage import and export of files in a widely diffuse format, like, for example, .xml;
- 4 customizable: the user should be able to modify or add equations to the mathematical model, in order to make wider its application field. This requirement is particularly important in the research field, because admit the researchers to validate hypothesis on correlations between data sets and mathematical formulations of processes;
- 5 clear section for input data: considering the complexity of the mathematical models for the anaerobic digestion description, the input section of the software has to guide the user to compile the fields with section and tags which can orient the user;
- 6 results expressed by plots: the results obtained by the simulation has to be consultable by the use of graphic representations, in addition to the compilation of analytical tables. This can give an immediate feedback on the trend of the reaction products.

The principles described above have been applied in a practical way by choosing as software instrument MATLAB and considering, as mathematical model, the ADM1 (Anaerobic Digestion Model No 1) developed by IWA Task Group in 2002.

The choice to use MATLAB to develop the simulation tool was made by comparing this one with Python. Python is a high level, general-purpose programming language, which is accompanied by a large number of libraries, in order to make it suitable for a wide range of applications. Python is also open source and allows the user to create stand-alone application. MATLAB, instead, is a computing platform, largely employed in scientific and engineering fields. MATLAB is not open source. MATLAB and Python can interface and so be used in an integrated way.

Using Python requires to have basis of programming and assessed skills in order to select the correct and suitable libraries to carry out an eventual customization. MATLAB, instead, has guided interfaces in order to make the user experience easier: it follows that a non-expert informatic user has more possibilities to reach his goals using MATLAB than employing Python, despite this last one is more diffuse. This choice answers both the first and the fourth necessities above itemized: a guided platform is user friendly and customizable, without required an in-depth study on the software tool. The decision to use MATLAB also does not penalize the need to make the developed software tool suitable for interfacing with other calculation tools, having input and export data sections.

The ADM1 model has been preferred to the AMOCO model because of its completeness. In fact, the AMOCO model masterfully combines modelling needs with exemplification for greater usability, mathematically describing only the acidogenesis and methanogenesis phases. It follows that the hydrolysis, disintegration and acetogenesis are not explicitly described. The AMOCO incorporates also electrochemical equilibria in order to include the alkalinity, which has to play a central role in the related monitoring and control strategy of a treatment plant.

The ADM1 instead is more complex to be implemented and used in monitoring plants, but it is also more complete, investigating all the macro-phases of the anaerobic digestion process and including also physico-chemical processes such as:

- liquid-liquid processes: ion association/dissociation ;
- liquid-gas processes : liquid-gas transfer.

The liquid -solid processes and solids precipitations are not included in the model. According to the second point of the item, the use of the ADM1 as mathematical model on which the software tool is based admit the use of the software tool for simulation which are not only oriented to the prediction of biomethane production, but also can offer an overview on the other intermediate and final products of the reaction.

Summarizing the main characteristics of the software tool, which will now be referred to as ADMS 2.0 (Anaerobic Digestion Model Simulator 2.0):

- ADMS 2.0 is a stand-alone application with a graphic interface designed to be user-friendly and to guide the implementation:
- ADMS 2.0 is totally developed using MATLAB;
- the software tool has a calculation core based on the ADM1.

ADMS2.0 validation has been carried out using the set of data provided by [71]: the input data and values of parameters and variables used for the simulation are reported together with the results of a steady-state simulation at day 200.

Despite the presence, in [71], of the results of simulation for all the biochemical and chemical compounds modelled in ADM1, trends for each species are not provided. For this reason, a simulation with the same input values has been implemented also using the AQUASIM 2.0 software tool, in order to compare the trends obtained by this instrument with the ones acquired by running the simulation through ADMS 2.0. Results are compared by the use of the relative percentage error.

AQUASIM 2.0 is a software tool developed in 1998 which is, still now, largely used to simulate processes in aquatic environment, both for laboratorial, plant and natural events. Despite its “age”, this software tool is very flexible and this admits its application in many different contexts. In fact, it is structured in a way that allows the user to implement a wide

range of reactions aimed to describe chemical, physical and biochemical processes by using different types of variables. Also the volumes on which calculations can be implemented are customizable and therefore, overall, the software is one of the most versatile and easily usable tools available for the simulation of anaerobic digestion processes.

The strength of AQUASIM 2.0 is its flexibility, that allows the user to explore different mathematical models, but the weaknesses are related to the fact that it is not updated in comparison to the nowadays available software tool for multiphysic simulation (such as COMSOL Multiphysics or ANSYS). In fact the interface design is not very user friendly, the import and export data sections do not provide integration with excel or similar, all variables and equations that are part of a model must be entered manually: it follows that the user has to build a strong knowledge both in relationship to the mathematical elements constituting the model and the AQUASIM 2.0 functioning before successfully implementing the simulation. Considering the fact that ADM1 requires more than 100 values in input to calculate a system of 35 differential equations, related to a number of algebraic equations to calculate some parameters and equilibrium processes, the implementation of the model in AQUASIM 2.0, especially considering the lack of guide in the software interface, is a very time-consuming job.

ADMS2.0 aims to maintain the flexibility of AQUASIM 2.0, adding a functional import/export data section and an interface that guides the user to insert required values without implementing manually the entire equation set. However, in order to keep intact the possibility of customizing the mathematical model with which calculation is made, an advanced setting button is available to access directly the code at the base of the ADMS 2.0.

Finally an overview on the AQUASIM 2.0 structure is provided in order to detect the main differences between the implementation with ADMS 2.0 and AQUASIM 2.0 itself.

AQUASIM 2.0 implementation structure is composed by four macro sections: variables, processes, compartments and links.

In the *variables* section the user has to implement elements to which a numerical value can be associated. There are differences between variables:

- *state variables* describe properties the meaning of which can be obtained indirectly by the process in which they are involved;
- *program variables*, which represent independent variables, parameters or solution to differential-algebraic systems of equations;
- *constant variables* are used to describe single measured quantities characterized by an associated standard deviation value ;
- *formula variables* are aimed to allow the user to build functional relations as algebraic expressions using previously defined variables;
- *probe variables* describe the value of another variable evaluated at a given location;
- *real list variables* are used to provide measured data or to formulate dependencies on other variables with the aid of interpolation data pairs;
- *variable list variables* are used to interpolate between other variables at given values of an arbitrary argument.

In the section *Processes* the user can insert *dynamic processes* and *equilibrium processes*. The first ones are characterized by a process rate which has to be inserted manually by the user, while the second ones are implemented by mean of an equation: the reaction rate associated to them is, in fact, considered equal to zero.

The environment in which the reaction takes place is described by the choice and setting of Compartments. AQUASIM 2.0 make available six different types of compartment: mixed reactor, biofilm reactor, advective-diffusive reactor, saturated soil column, river section and lake. As said before, the software allows great flexibility, that admits to range in a plethora of different environments, not limited to anaerobic digestion only.

Finally, in order to admit the connection between created compartments, *Links* can be used. *Advective links* allow the user to describe water flows and substance transport between compartments, while *diffusive links* model boundary layers or membranes between compartments which can be diffusively penetrated by certain substances.

AQUASIM 2.0 has some panels to set the simulation parameters, such as initial time, timestep and other settings necessary for the simulation calculation. This software can provide also sensitivity analysis.

Results obtained by the simulation process can be exported as .list files and also plotted in AQUASIM 2.0 interface.

This description make clear the characteristics of AQUASIM 2.0 both in terms of strength and weakness, and also suggests the amount of time and knowledges necessary to simulate an anaerobic digestion process by mean of this software. All the initial data and the equation of the chosen mathematical model, in this case the ADM1, have to be inserted manually and the details of the variables themselves have to be known in order to select the correct label under which collect them in AQUASIM 2.0. Mistakes on these choices can affect the correct calculation of the simulation.

ADMS 2.0 Graphic User Interface

As affirmed in previous sections, the graphical user interface developed using has been based on the parameters division proposed by the ADM1 itself in [16].

In fact the model introduces a nomenclature on which the software is aligned, in order to facilitate the user in implementing the simulation Table 21.

Table 21. Stoichiometric and equilibrium coefficients and constants, kinetic parameters and rates, dynamic state and algebraic variables used in ADM1 [16].

<i>Symbol</i>	<i>Description</i>	<i>Units</i>
C_i	Carbon content of component i	KmoleC kgCOD ⁻¹
N_i	Nitrogen content of component i	KmoleC kgCOD ⁻¹
$v_{i,j}$	Rate coefficients for component i on processs j	Nominally kgCOD m ⁻³
$f_{product,substrate}$	Yield (catabolism only) of product on substrate	Nominally kgCOD m ⁻³
H_{gas}	Gas law constant (equal to K_H^{-1})	bar M ⁻¹
$K_{a,acid}$	Acid base equilibrium coefficient	M
K_H	Henry's law coefficient	M bar ⁻¹
pK_a	$-\log_{10} K_a$	
R	Gas law constant ($8.314 \cdot 10^{-2}$)	Bas M ⁻¹ K ⁻¹

ΔG	Free energy	J.mole ⁻¹
$k_{A/Bi}$	Acid base kinetic parameter	M d ⁻¹
k_{dec}	First order decay rate	d ⁻¹
$I_{inhibitor,process}$	Inhibition function	d ⁻¹
$k_{process}$	First order parameter	d ⁻¹
$k_L a$	Gas-liquid transfer coefficient	d ⁻¹
$K_{I,inhibit,substrate}$	50%inhibitory concentration	kgCOD m ⁻³
$k_{m,process}$	Monod maximum specific uptake rate	kgCOD_S kgCOD_X ⁻¹ d ⁻¹
$K_{S,process}$	Half saturation value	kgCOD_S m ⁻³
ρ_j	Kinetic rate of process	kgCOD_S m ⁻³ d ⁻¹
$Y_{substrate}$	Yield of biomass on substrate	kgCOD_X kgCOD_S ⁻¹
μ_{max}	Monod maximum specific growth rate	d ⁻¹
pH	$-\log_{10}[H^+]$	
$p_{gas,i}$	Pressure of gas -i	bar
P_{gas}	Total gas pressure	bar
S_i	Soluble component -i	kgCODm ⁻³
$t_{res,X}$	Extended retention of solids	d
T	Temperature	K
V	Volume	m ⁻³
X_i	Particulate component -i	kgCODm ⁻³

Table 22. Compounds considered in ADM1.

<i>Variable</i>	<i>Unit</i>	<i>Description</i>
S _{su}	kgCOD/m ³	Sugar
S _{aa}	kgCOD/m ³	Amino acids
S _{fa}	kgCOD/m ³	Fatty acids
S _{va}	kgCOD/m ³	Valerate
S _{bu}	kgCOD/m ³	Butyrate
S _{pro}	kgCOD/m ³	Propionate
S _{ac}	kgCOD/m ³	Acetate
S _{h2}	kgCOD/m ³	Hydrogen
S _{ch4}	kgCOD/m ³	Methane
S _{IC}	kmole C/m ³	Inorganic carbon
S _{IN}	kmole N/m ³	Inorganic nitrogen
S _I	kgCOD/m ³	Soluble inerts
X _C	kgCOD/m ³	Composites
X _{ch}	kgCOD/m ³	Carbohydrates
X _{pr}	kgCOD/m ³	Proteins
X _{li}	kgCOD/m ³	Lipids
X _{su}	kgCOD/m ³	Biomass-sugar

X_{aa}	kgCOD/m^3	Biomass- amino acids
X_{fa}	kgCOD/m^3	Biomass- fatty acids
X_{c4}	kgCOD/m^3	Biomass-valerate and butyrate
X_{pro}	kgCOD/m^3	Biomass-propionate
X_{ac}	kgCOD/m^3	Biomass-acetate
X_{h2}	kgCOD/m^3	Biomass-hydrogen
X_I	kgCOD/m^3	Particulate inerts
S_{cat}	kmole/m^3	Cations
S_{an}	kmole/m^3	Anions
S_{va-}	kgCOD/m^3	Valerate ion
S_{bu-}	kgCOD/m^3	Butyrate ion
S_{pro-}	kgCOD/m^3	Propionate ion
S_{ac-}	kgCOD/m^3	Acetate ion
S_{hco3-}	kmole C/m^3	Hydrogen carbonate ion
S_{nh3}	kmole N/m^3	Ammonia
$S_{gas,h2}$	kgCOD/m^3	Hydrogen (gas phase)
$S_{gas,ch4}$	kgCOD/m^3	Methane (gas phase)
$S_{gas,co2}$	kmole C/m^3	Carbone dioxide (gas phase)

As seen in

Table 22, ADM1 describe the trends of the concentration of 35 chemical and biochemical compounds. The processes of growth and decrease of each compound concentration are defined by mean of the generic equations for the water phase:

$$\frac{dS_i}{dt} = \frac{Q_{ad}}{V_{ad,liq}} \cdot (S_{i,in} - S_i) + \sum_{j=1}^{19} v_{i,j} \cdot \rho_j + \text{transport termos; where } i = 1 \dots 12 \quad (37)$$

$$\frac{dX_i}{dt} = \frac{Q_{ad}}{V_{ad,liq}} \cdot (X_{i,in} - X_i) + \sum_{j=1}^{19} v_{i,j} \cdot \rho_j + \text{transport termos; where } i = 13 \dots 24 \quad (38)$$

Petersen matrices (Table 23 and Table 24) summarize the equations required to calculate the terms used in the sum, i.e. rate coefficient and reaction rates.

Table 23. Biochemical rate coefficients and kinetic equations for soluble components [72].

j	Component	Process →												Rate (ρ , kg COD·m ⁻³ ·d ⁻¹)
		1	2	3	4	5	6	7	8	9	10	11	12	
		S_{su}	S_{sa}	S_a	S_{va}	S_{bu}	S_{pro}	S_{ac}	S_{ac}	S_{gh}	S_G	S_{in}	S_i	
1	Disintegration												f_{dis}	$k_{dis} X_{su}$
2	Hydrolysis Carbohydrates	1												$k_{hyd,cd} X_{su}$
3	Hydrolysis of Proteins		1											$k_{hyd,pr} X_{su}$
4	Hydrolysis of Lipids	$1-f_{hyd}$		$f_{a,il}$										$k_{hyd,l} X_{su}$
5	Uptake of Sugars	-1				$(1-Y_{su}) f_{bu,ss}$	$(1-Y_{su}) f_{pro,ss}$	$(1-Y_{su}) f_{ac,ss}$	$(1-Y_{su}) f_{gh,ss}$		$-\sum_{i=1}^{n-1} C_{Y,i} V_{i-24}$	$-(Y_{su}) N_{bac}$		$k_{m,su} \frac{S_{su}}{K_S + S_{su}} X_{su} I_1$
6	Uptake of Amino Acids		-1		$(1-Y_{su}) f_{va,ss}$	$(1-Y_{su}) f_{bu,ss}$	$(1-Y_{su}) f_{pro,ss}$	$(1-Y_{su}) f_{ac,ss}$	$(1-Y_{su}) f_{gh,ss}$		$-\sum_{i=1}^{n-1} C_{Y,i} V_{i-24}$	$N_{su} - (Y_{su}) N_{bac}$		$k_{m,sa} \frac{S_{sa}}{K_S + S_{sa}} X_{sa} I_1$
7	Uptake of LCFA			-1				$(1-Y_{su}) 0.7$	$(1-Y_{su}) 0.3$			$-(Y_{su}) N_{bac}$		$k_{m,sa} \frac{S_{sa}}{K_S + S_{sa}} X_{sa} I_2$
8	Uptake of Valerate				-1		$(1-Y_{su}) 0.54$	$(1-Y_{su}) 0.31$	$(1-Y_{su}) 0.15$			$-(Y_{su}) N_{bac}$		$k_{m,sa} \frac{S_{sa}}{K_S + S_{sa}} X_{sa} \frac{1}{1 + S_{su}/S_{su}} I_2$
9	Uptake of Butyrate					-1		$(1-Y_{su}) 0.8$	$(1-Y_{su}) 0.2$			$-(Y_{su}) N_{bac}$		$k_{m,sa} \frac{S_{sa}}{K_S + S_{sa}} X_{sa} \frac{1}{1 + S_{su}/S_{su}} I_2$
10	Uptake of Propionate						-1	$(1-Y_{su}) 0.57$	$(1-Y_{su}) 0.43$		$-\sum_{i=1}^{n-1} C_{Y,i} V_{i-24}$	$-(Y_{su}) N_{bac}$		$k_{m,pr} \frac{S_{pro}}{K_S + S_{pro}} X_{pro} I_2$
11	Uptake of Acetate							-1		$(1-Y_{su})$	$-\sum_{i=1}^{n-1} C_{Y,i} V_{i-24}$	$-(Y_{su}) N_{bac}$		$k_{m,ac} \frac{S_{ac}}{K_S + S_{ac}} X_{ac} I_3$
12	Uptake of Hydrogen								-1	$(1-Y_{su})$	$-\sum_{i=1}^{n-1} C_{Y,i} V_{i-24}$	$-(Y_{su}) N_{bac}$		$k_{m,h_2} \frac{S_{h_2}}{K_S + S_{h_2}} X_{h_2} I_1$
13	Decay of X_{su}													$k_{dec,su} X_{su}$
14	Decay of X_{sa}													$k_{dec,sa} X_{sa}$
15	Decay of X_{va}													$k_{dec,va} X_{va}$
16	Decay of X_{bu}													$k_{dec,bu} X_{bu}$
17	Decay of X_{pro}													$k_{dec,pro} X_{pro}$
18	Decay of X_{ac}													$k_{dec,ac} X_{ac}$
19	Decay of X_{h_2}													$k_{dec,h_2} X_{h_2}$
Inhibition factors (3.7): $I_1 = I_{pH} I_{N_{in}}$ $I_2 = I_{pH} I_{N_{in}} I_{h_2}$ $I_3 = I_{pH} I_{N_{in}} I_{NH_3} X_{ac}$														

Table 24. Biochemical rate coefficients and kinetic equations for particulate components [72].

Component	Process	13	14	15	16	17	18	19	20	21	22	23	24	Rate (ρ , kg COD.m ⁻³ .d ⁻¹)
1	Disintegration	X_d	X_{dp}	X_{er}	X_d	X_{dp}	X_{er}	X_d	X_{dp}	X_{er}	X_d	X_{dp}	X_{er}	$k_{d1} X_c$
2	Hydrolysis Carbohydrates	-1	$f_{ch,sc}$	$f_{er,sc}$	$f_{ch,sc}$	$f_{er,sc}$	$f_{ch,sc}$	$f_{er,sc}$	$f_{ch,sc}$	$f_{er,sc}$	$f_{ch,sc}$	$f_{er,sc}$	$f_{ch,sc}$	$k_{hyd,1} X_{c1}$
3	Hydrolysis Proteins		-1	-1	-1									$k_{hyd,2} X_{c2}$
4	Hydrolysis Lipids				-1									$k_{hyd,3} X_{c3}$
5	Uptake of Sugars					Y_{su}								$k_{m,10} \frac{S_{su}}{K_S + S} X_{c1} I_1$
6	Uptake of Amino Acids						Y_{aa}							$k_{m,10} \frac{S_{aa}}{K_S + S} X_{c1} I_1$
7	Uptake of LCFA							Y_{lc}						$k_{m,10} \frac{S_{lc}}{K_S + S} X_{c1} I_1$
8	Uptake of Valerate								Y_{va}					$k_{m,10} \frac{S_{va}}{K_S + S} X_{c1} I_1$
9	Uptake of Butyrate								Y_{bu}					$k_{m,10} \frac{S_{bu}}{K_S + S} X_{c1} I_1$
10	Uptake of Propionate									Y_{pr}				$k_{m,10} \frac{S_{pr}}{K_S + S} X_{c1} I_1$
11	Uptake of Acetate										Y_{ac}			$k_{m,10} \frac{S_{ac}}{K_S + S} X_{c1} I_1$
12	Uptake of Hydrogen											Y_{h2}		$k_{m,10} \frac{S_{h2}}{K_S + S} X_{c1} I_1$
13	Decay of X_{su}	1				-1								$k_{dec,su} X_{su}$
14	Decay of X_{aa}	1					-1							$k_{dec,aa} X_{aa}$
15	Decay of X_{lc}	1						-1						$k_{dec,lc} X_{lc}$
16	Decay of X_{va}	1							-1					$k_{dec,va} X_{va}$
17	Decay of X_{bu}	1								-1				$k_{dec,bu} X_{bu}$
18	Decay of X_{pr}	1									-1			$k_{dec,pr} X_{pr}$
19	Decay of X_{ac}	1										-1		$k_{dec,ac} X_{ac}$
													-1	$k_{dec,h2} X_{h2}$
														Inhibition factors (3.7): $I_1 = I_{pH} I_{N,lim}$ $I_2 = I_{pH} I_{N,lim} I_{h_2}$ $I_3 = I_{pH} I_{N,lim} I_{h_2} I_{h_3}$

The equations describing ion state (six ion species are considered) can be generically summarized as:

$$\frac{dS_{ion}}{dt} = -\rho_{ACID/BASE} \quad (39)$$

Gas phase equation consists of three differential equations which describes hydrogen, carbon dioxide and methane gas concentration:

$$\frac{dS_{gas,h2}}{dt} = -\frac{S_{gas,h2} \cdot Q_{gas}}{V_{ad,gas}} + \rho_{T,8} \cdot \frac{V_{ad,liq}}{V_{ad,gas}} \quad (40)$$

$$\frac{dS_{gas,ch4}}{dt} = -\frac{S_{gas,ch4} \cdot Q_{gas}}{V_{ad,gas}} + \rho_{T,9} \cdot \frac{V_{ad,liq}}{V_{ad,gas}} \quad (41)$$

$$\frac{dS_{gas,co2}}{dt} = -\frac{S_{gas,co2} \cdot Q_{gas}}{V_{ad,gas}} + \rho_{T,10} \cdot \frac{V_{ad,liq}}{V_{ad,gas}} \quad (42)$$

All the equations above collected, implies the use of parameters and algebraic equation in order to determine the variable involved: process inhibitions, pH variation, thermodynamic conditions reaction rates and coefficients for some differential equations are calculated by mean of a number of parameters combined in algebraic equations of which the formulation is collected in [71].

Considering the type and the quantities of the variables/parameters used to model anaerobic digestion according with ADM1, the implementation in a software tool can be difficult and chaotic. For these reasons, the graphic user interface developed presents a main panel, in which macro-areas are detected, considering it as the “main menu” of the tool as shown by Figure 51.



Figure 51. Main menu of the software tool.

Initial concentrations button allows the user to access to an area in which the initial concentration of soluble and particles compounds can be initialized. Considering that the required initial concentration refers to nr. 26 parameters, the panels dedicated to this initialization are two: one for soluble compounds and the other for particles one. The selection of each panel is made by an intermediate window which appears after clicking “Initial concentration” button as by Figure 52.

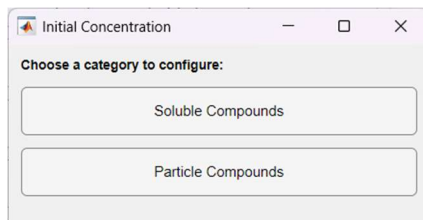


Figure 52. Intermediate window for specific compound initial concentration assignment.

Both soluble compounds and particle compounds windows (Figure 53 and Figure 54, respectively) can be accessed, also simultaneously, allowing the initial parameters specification. Button related features will be explained in a specific section.

State no.	Variable name	Description	Value	Unit
1	1.0000 S_{su,i}	Sugar initial concentration	0.0100	kg_{COD}/m^3
2	2.0000 S_{aa,i}	Aminoacids initial concentration	0.0010	kg_{COD}/m^3
3	3.0000 S_{fa,i}	Fatty acids initial concentration	0.0010	kg_{COD}/m^3
4	4.0000 S_{va,i}	Valerate initial concentration	0.0010	kg_{COD}/m^3
5	5.0000 S_{bu,i}	Butyrate initial concentration	0.0010	kg_{COD}/m^3
6	6.0000 S_{pro,i}	Proponiate initial concentration	0.0010	kg_{COD}/m^3
7	7.0000 S_{ac,i}	Acetate initial concentration	0.0010	kg_{COD}/m^3
8	8.0000 S_{h2,i}	Hydrogen initial concentration	0.0000	kg_{COD}/m^3
9	9.0000 S_{ch4,i}	Methane initial concentration	0.0000	kg_{COD}/m^3
10	10.0000 S_{IC,i}	Inorganic carbon initial concentration	0.0400	kmole C/m^3
11	11.0000 S_{IN,i}	Inorganic nitrogen initial concentrati...	0.0100	kmole N/m^3
12	12.0000 S_{I,i}	Inerts initial concentration	0.0200	kg_{COD}/m^3
13	25.0000 S_{cat,i}	Cations initial concentration	0.0400	kmole/m^3
14	26.0000 S_{an,i}	Anions initial concentration	0.0200	kmole/m^3

Figure 53. Initial soluble compounds concentration window.

State no.	Variable name	Description	Value	Unit
1	13.0000 X_{C,i}	Composites initial concentration	2.0000	kg_{(COD)}/m^3
2	14.0000 X_{ch,i}	Carbohydrates initial concentration	5.0000	kg_{(COD)}/m^3
3	15.0000 X_{pr,i}	Proteins initial concentration	20.0000	kg_{(COD)}/m^3
4	16.0000 X_{li,i}	Lipids initial concentration	5.0000	kg_{(COD)}/m^3
5	17.0000 X_{su,i}	Sugar degraders initial concentration	0.0000	kg_{(COD)}/m^3
6	18.0000 X_{aa,i}	Aminoacids degraders initial concentration	0.0100	kg_{(COD)}/m^3
7	19.0000 X_{fa,i}	LCFA degraders initial concentration	0.0100	kg_{(COD)}/m^3
8	20.0000 X_{c4,i}	Valerate and Butyrate degraders initial concentration	0.0100	kg_{(COD)}/m^3
9	21.0000 X_{pro,i}	Propionate degraders initial concentration	0.0100	kg_{(COD)}/m^3
10	22.0000 X_{ac,i}	Acetate degraders initial concentration	0.0100	kg_{(COD)}/m^3
11	23.0000 X_{h2,i}	Hydrogen degraders initial concentration	0.0100	kg_{(COD)}/m^3
12	24.0000 X_{li,i}	Particulate inerts initial concentration	25.0000	kg_{(COD)}/m^3

Save Initialized Data Import Data Export Data Done

Figure 54. Initial particulate compounds concentration window.

The following section is related to the “Reactor Parameters”: in this section the user has to insert the parameters which describes the reactor such as liquid volume, head volume, temperature, pression, inlet and outlet flow as shown in Figure 55.

State no.	Variable name	Description	Value	Unit
1	-	V_{ad,liq}	3400	m^3
2	-	V_{ad,gas}	300	m^3
3	-	Q_{ad}	170	m^3/d
4	-	T_{op}	308.1500	K
5	-	T_{base}	298.1500	K
6	-	P_{atm}	1.0130	bar
7	-	k_p	50000	m^3/(d*bar)
8	-	K_{La}	200	1/d
9	-	pH	7.5000	1

Save Initialized Data Import Data Export Data Done

Figure 55. Reactor parameters window.

“Stoichiometric and Biochemical Parameters” button allows the access to an intermediate selection window shown by Figure 56, as well as for initial concentrations.

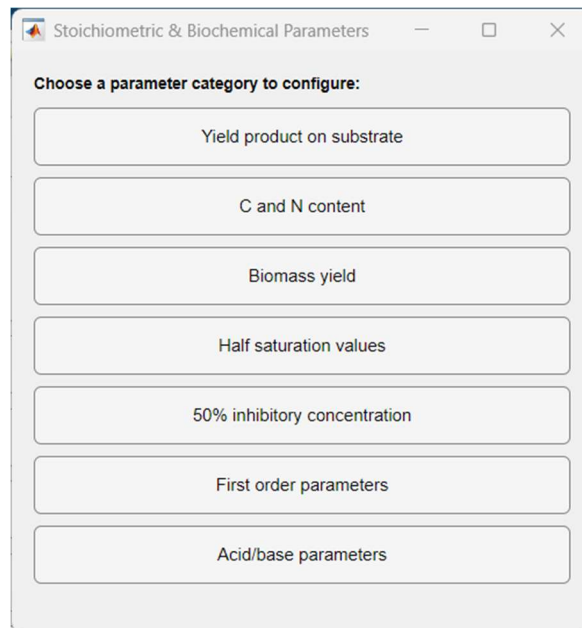


Figure 56. Stoichiometric and Biochemicals parameters selection window.

In this case, user is enabled to open one or more initialization tables between the followings:

- product yield on substrate (Figure 57);
- C and N content to define carbon content of component i and nitrogen content of component i (Figure 58);
- biomass yield (Figure 59);
- half saturation values (Figure 60);
- 50% inhibitory concentration (Figure 61);
- first order parameters to define first order decay rates (Figure 61);
- acid/base parameters to define acid base kinetic parameters and acid-base equilibrium coefficients (Figure 63).

State no.	Variable name	Description	Value	Unit
1	f_(Sl,x)	Fraction of soluble inerts in composite material (Xc)	0.1000	-
2	f_(Xl,x)	Fraction of particulate inerts in composite material (Xc)	0.2000	-
3	f_(ch,x)	Fraction of carbohydrates in composite material (Xc)	0.2000	-
4	f_(pr,x)	Fraction of proteins in composite material (Xc)	0.2000	-
5	f_(li,x)	Fraction of lipids in composite material (Xc)	0.3000	-
6	f_(fa,li)	Fraction of long-chain fatty acids in lipids	0.9500	-
7	f_(h2,su)	Fraction of hydrogen from sugar degradation	0.1900	-
8	f_(bu,su)	Fraction of butyrate from sugar degradation	0.1300	-
9	f_(pro,su)	Fraction of propionate from sugar degradation	0.2700	-
10	f_(ac,su)	Fraction of acetate from sugar degradation	0.4100	-
11	f_(h2,aa)	Fraction of hydrogen from amino acid degradation	0.0600	-
12	f_(va,aa)	Fraction of valerate from amino acid degradation	0.2300	-
13	f_(bu,aa)	Fraction of butyrate from amino acid degradation	0.2600	-
14	f_(pro,aa)	Fraction of propionate from amino acid degradation	0.0500	-
15	f_(ac,aa)	Fraction of acetate from amino acid degradation	0.4000	-

Figure 57. Yield product on substrate window.

State no.	Variable name	Description	Value	Unit
1	N_(xc)	Nitrogen content of composite material (Xc)	0.0027	kmole_N / kg_(COD)
2	N_l	Inorganic nitrogen content	0.0043	kmole_N / kg_(COD)
3	N_(aa)	Nitrogen content of amino acids	0.0070	kmole_N / kg_(COD)
4	C_(xc)	Carbon content of composite material (Xc)	0.0279	kmole_C / kg_(COD)
5	C_(Sl,x)	Soluble inert fraction of composite material	0.0300	kmole_C / kg_(COD)
6	C_(ch,x)	Carbohydrate fraction of composite mate...	0.0313	kmole_C / kg_(COD)
7	C_(pr)	Protein content	0.0300	kmole_C / kg_(COD)
8	C_(li)	Lipid content	0.0220	kmole_C / kg_(COD)
9	C_(Xl)	Inert particulate organic matter content	0.0300	kmole_C / kg_(COD)
10	C_(su)	Sugar content	0.0313	kmole_C / kg_(COD)
11	C_(aa)	Amino acid content	0.0300	kmole_C / kg_(COD)
12	C_(fa)	Long-chain fatty acid content	0.0217	kmole_C / kg_(COD)
13	N_(bac)	Nitrogen content of biomass	0.0057	kmole_N / kg_(COD)
14	C_(bu)	Butyrate content	0.0250	kmole_C / kg_(COD)
15	C_(pro)	Propionate content	0.0268	kmole_C / kg_(COD)
16	C_(ac)	Acetate content	0.0313	kmole_C / kg_(COD)
17	C_(bac)	Biomass content	0.0313	kmole_C / kg_(COD)
18	C_(va)	Valerate content	0.0240	kmole_C / kg_(COD)
19	C_(ch4)	Methane content	0.0156	kmole_C / kg_(COD)

Figure 58. C and N content window.

Biomass Yields

	State no.	Variable name	Description	Value	Unit
1	-	Y _(su)	Yield of sugar fermentation (saccharolytic fermenters)	0.1000	kmole _{(((COD)_x)} / kg _{(((COD)_s)}
2	-	Y _(aa)	Yield of amino acid degradation (amino acid degrad...	0.0800	kmole _{(((COD)_x)} / kg _{(((COD)_s)}
3	-	Y _(fa)	Yield of long-chain fatty acid degradation (LCFA degr...	0.0600	kmole _{(((COD)_x)} / kg _{(((COD)_s)}
4	-	Y _(c4)	Yield of short-chain fatty acid degradation (butyrate, ...	0.0600	kmole _{(((COD)_x)} / kg _{(((COD)_s)}
5	-	Y _(pro)	Yield of propionate degradation (propionate degraders)	0.0400	kmole _{(((COD)_x)} / kg _{(((COD)_s)}
6	-	Y _(ac)	Yield of acetate degradation (acetoclastic methanog...	0.0500	kmole _{(((COD)_x)} / kg _{(((COD)_s)}
7	-	Y _(h2)	Yield of hydrogen degradation (hydrogenotrophic me...	0.0600	kmole _{(((COD)_x)} / kg _{(((COD)_s)}

Save Initialized Data Import Data Export Data Done

Figure 59. Biomass yield window.

Half Saturation Values

	State no.	Variable name	Description	Value	Unit
1	-	K _(S.in)	Half-saturation constant for inerts (non-biodegradabl...	0.0001	kg _{_(COD)} /m ³
2	-	K _(S.su)	Half-saturation constant for sugars	0.5000	kg _{_(COD)} /m ³
3	-	K _(S.aa)	Half-saturation constant for amino acids	0.3000	kg _{_(COD)} /m ³
4	-	K _(S.fa)	Half-saturation constant for long-chain fatty acids (L...	0.4000	kg _{_(COD)} /m ³
5	-	K _(S.c4)	Half-saturation constant for short-chain fatty acids (C4)	0.2000	kg _{_(COD)} /m ³
6	-	K _(S.pro)	Half-saturation constant for propionate	0.1000	kg _{_(COD)} /m ³
7	-	K _(S.ac)	Half-saturation constant for acetate	0.1500	kg _{_(COD)} /m ³
8	-	K _(S.h2)	Half-saturation constant for hydrogen	0.0000	kg _{_(COD)} /m ³

Save Initialized Data Import Data Export Data Done

Figure 60. Half saturation values window.

50% Inhibitory Concentrations

	State no.	Variable name	Description	Value	Unit
1	-	K _(I,h2,fa)	50% inhibitory concentration of LCFA on acetoclastic...	0.0000	kg _{_(COD)} /m ³
2	-	K _(I,h2,c4)	50% inhibitory concentration of H ₂ on acetoclastic m...	0.0000	kg _{_(COD)} /m ³
3	-	K _(I,h2,pro)	50% inhibitory concentration of H ₂ on hydrogenotrop...	0.0000	kg _{_(COD)} /m ³
4	-	K _(I,NH3)	Inhibition constant for free ammonia (NH3)	0.0018	M

Save Initialized Data Import Data Export Data Done

Figure 61. 50% inhibitory concentration.

State no.	Variable name	Description	Value	Unit
1	k _(dis)	Disintegration rate constant	0.5000	1/d
2	k _(hyd, ch)	Hydrolysis rate constant of carbohydrates	10.0000	1/d
3	k _(hyd, pr)	Hydrolysis rate constant of proteins	10.0000	1/d
4	k _(hyd, li)	Hydrolysis rate constant of lipids	10.0000	1/d
5	k _(m, su)	Maximum uptake rate of sugars (saccharolytic ferme...	30.0000	1/d
6	k _(m, aa)	Maximum uptake rate of amino acids	50.0000	1/d
7	k _(m, fa)	Maximum uptake rate of long-chain fatty acids	6.0000	1/d
8	k _(m, c4)	Maximum uptake rate of valerate and butyrate (C4)	20.0000	1/d
9	k _(m, pro)	Maximum uptake rate of propionate	13.0000	1/d
10	k _(m, ac)	Maximum uptake rate of acetate	8.0000	1/d
11	k _(m, h2)	Maximum uptake rate of hydrogen	35.0000	1/d
12	k _(dec, Xsu)	Decay rate of sugar degraders (Xsu)	0.0200	1/d
13	k _(dec, Xaa)	Decay rate of amino acid degraders (Xaa)	0.0200	1/d
14	k _(dec, Xfa)	Decay rate of LCFA degraders (Xfa)	0.0200	1/d
15	k _(dec, Xc4)	Decay rate of valerate/butyrate degraders (Xc4)	0.0200	1/d
16	k _(dec, Xpro)	Decay rate of propionate degraders (Xpro)	0.0200	1/d
17	k _(dec, Xac)	Decay rate of acetate degraders (Xac)	0.0200	1/d

Figure 62. First order parameters.

State no.	Variable name	Description	Value	Unit
1	K _(a, va)	Acid dissociation constant of valeric acid	1.3804e-05	M
2	K _(a, bu)	Acid dissociation constant of butyric acid	1.5136e-05	M
3	K _(a, pro)	Acid dissociation constant of propionic acid	1.3183e-05	M
4	K _(a, ac)	Acid dissociation constant of acetic acid	1.7378e-05	M
5	k _(A, Bva)	Henry's law constant for valeric acid	1.0000e+10	1/(M*d)
6	k _(A, Bbu)	Henry's law constant for butyric acid	1.0000e+10	1/(M*d)
7	k _(A, Bpro)	Henry's law constant for propionic acid	1.0000e+10	1/(M*d)
8	k _(A, Bac)	Henry's law constant for acetic acid	1.0000e+10	1/(M*d)
9	k _(A, Bco2)	Henry's law constant for carbon dioxide (CO ₂)	1.0000e+10	1/(M*d)
10	k _(A, BIN)	Henry's law constant for ammonia (NH ₃)	1.0000e+10	1/(M*d)

Figure 63. Acid/base parameters.

Simulation's pH parameters settings as by Figure 64 – including the equation to be considered pH value and pH inhibitions while computing species concentration is varying over time – could be accessed by means of the main menu.

Equation Method: Batstone equation

State no.	Variable name	Description	Value	Unit
1	pH_([UL,aa)	Upper pH inhibition limit for amino acid degradation	5.5000	-
2	pH_([LL,aa)	Lower pH inhibition limit for amino acid degradation	4.0000	-
3	pH_([UL,ac)	Upper pH inhibition limit for acetic acid degradation	7.0000	-
4	pH_([LL,ac)	Lower pH inhibition limit for acetic acid degradation	6.0000	-
5	pH_([UL,h2)	Upper pH inhibition limit for hydrogen degradation	6.0000	-
6	pH_([LL,h2)	Lower pH inhibition limit for hydrogen degradation	5.0000	-

Save Initialized Data Import Data Export Data Done

Figure 64. pH parameters settings.

General simulation settings as in Figure 65 – such as initial and final time, timestep, solving equation method, accuracy and both absolute tolerance and relative tolerances – could be accessed by the main menu (Figure 65).

ODE Method: ode15s

State no.	Variable name	Description	Value	Unit
1	t_0	Initial time	0	d
2	t_f	Final time	20.0000	d
3	t_step	Timestep	1.0000	d
4	abs_tol	Absolute tolerance	0.0000	<as_computed_variable>
5	rel_tol	Relative tolerance	0.0000	<as_computed_variable>

Save initialized data Import Data Export Data Done

Figure 65. Simulations settings.

Once initialized each simulation scenario parameter, user could run the computation procedure by means of the “COMPUTE NOW!” button. Resulting data can be accessed by the results window, which provides multiple categories of computed values, as by Figure 66.

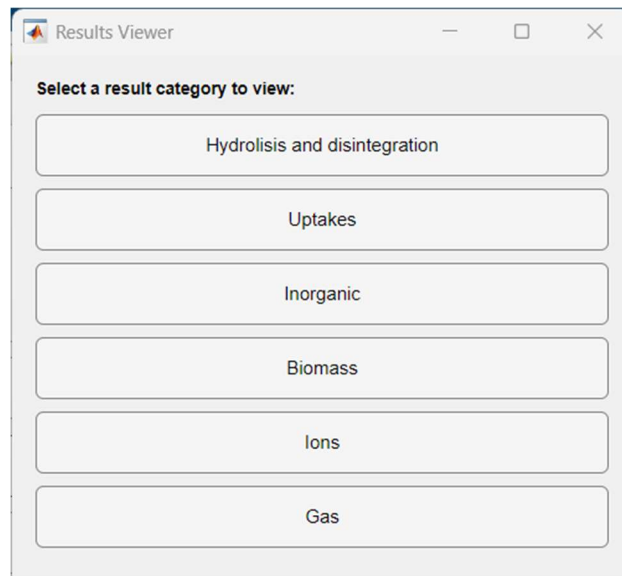


Figure 66. Results intermediate window.

The user could select a specific or even multiple categories of results to be shown by the desired category of result windows. Each graph has been set to allow a customization about trends to be plotted. In fact, referring to the options section the user should selected which trend to be plotted within every category context, customizing both axis limits and providing embedded graphs function (export as specific image format, data brush, data point tags to show coordinates, pan and zoom features, reset to default zoom and axis limits values). Legends are updated in real-time while user defines which trend needs to be plotted. The export data feature, that can be called by pressing the “Export to Excel” button, will be referred only to the currently selected trend flags.

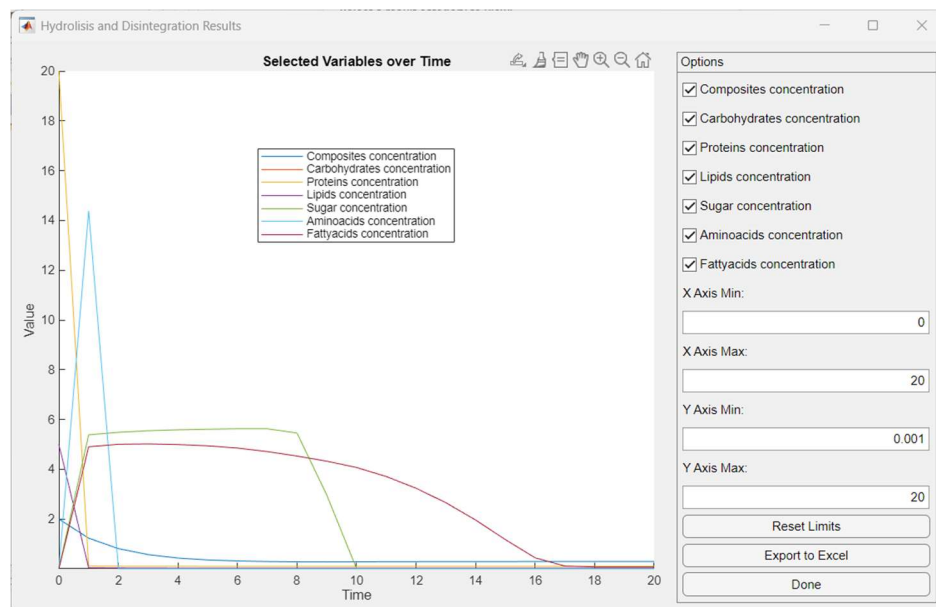


Figure 67. Resulting hydrolysis and disintegration trends for species window.

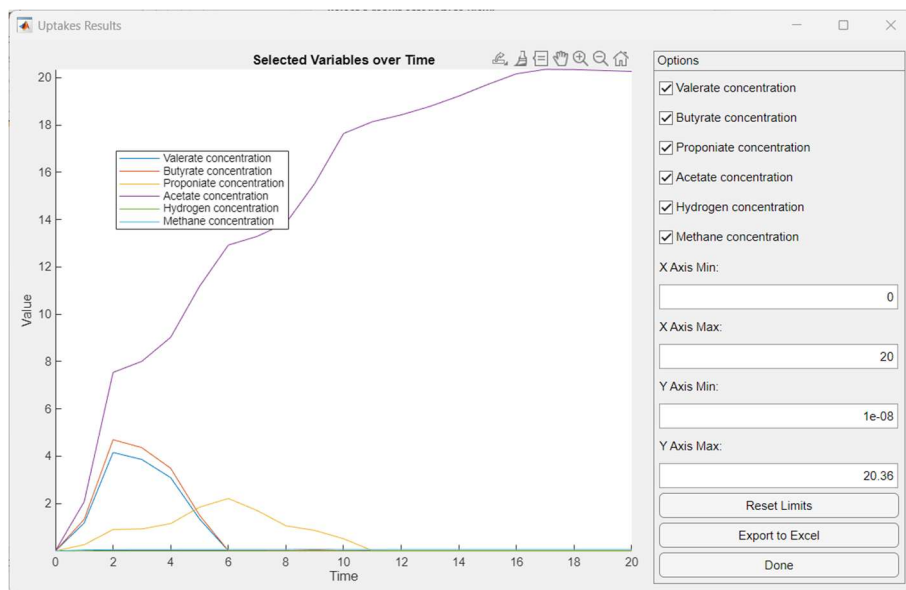


Figure 68. Resultings uptakes trends window.

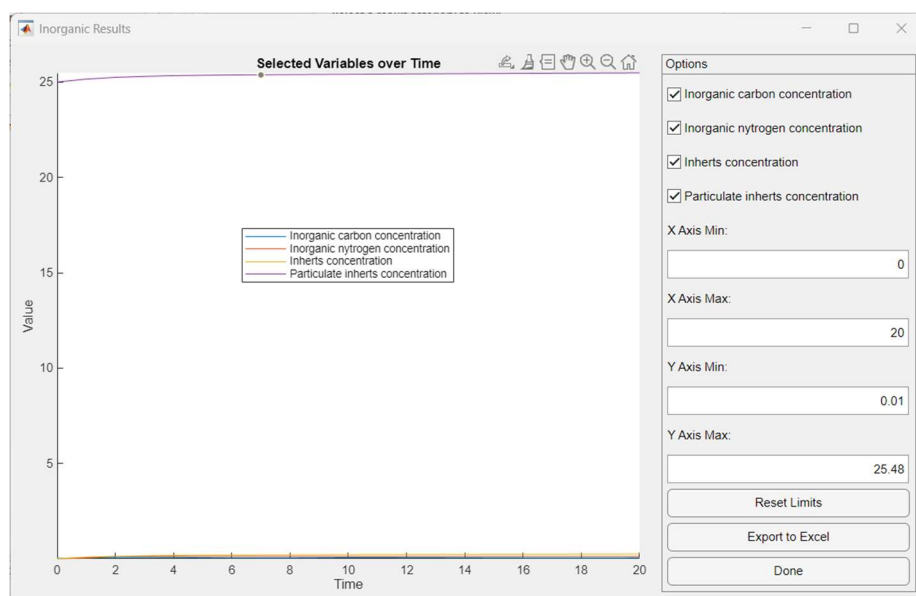


Figure 69. Resulting inorganic compounds trends window.

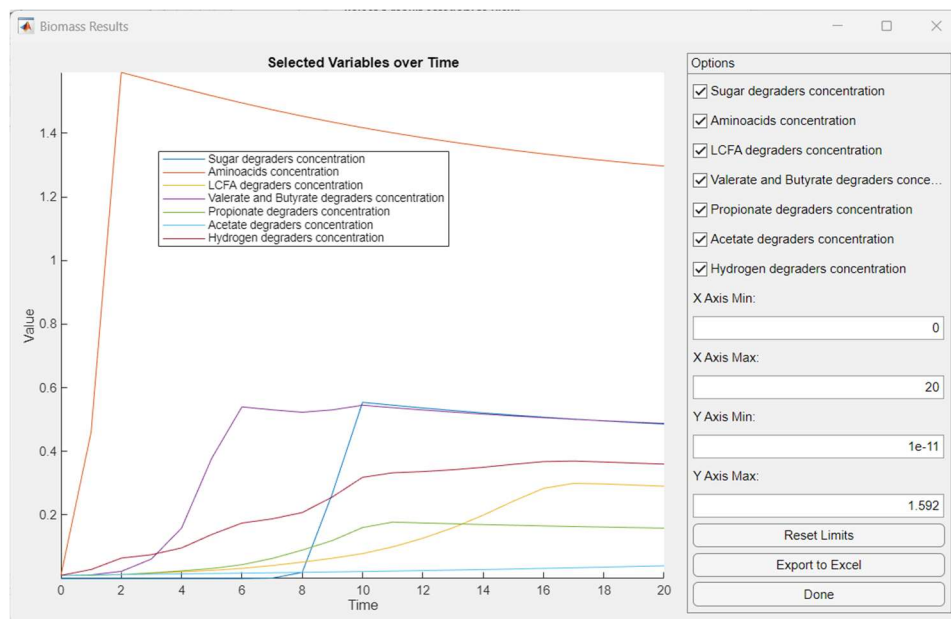


Figure 70. Resulting biomass trends window.

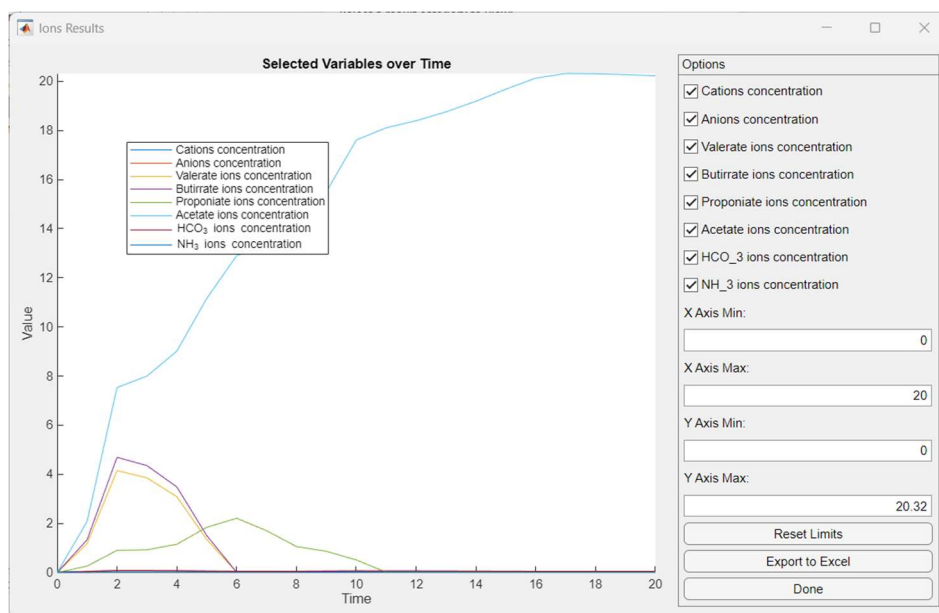


Figure 71. Resulting ions concentration trends window.

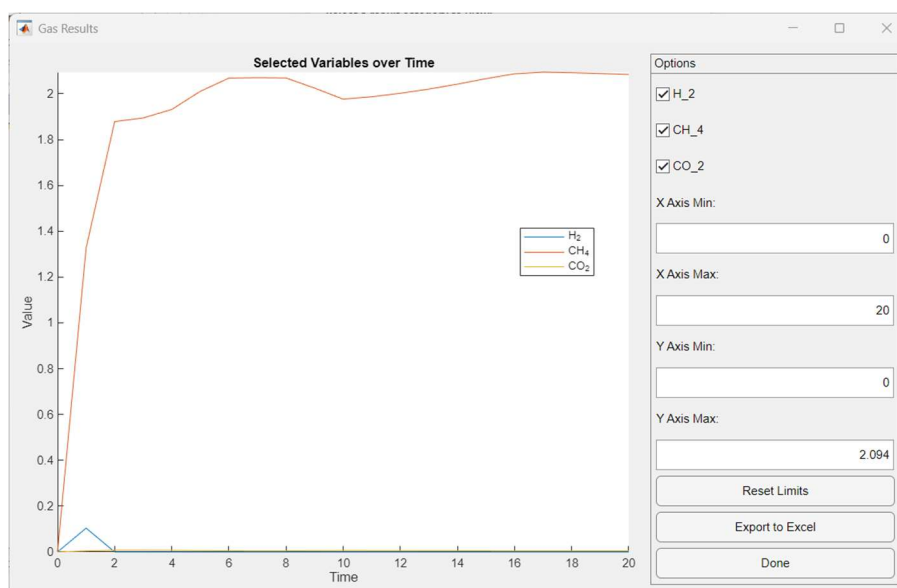


Figure 72. Resulting gas concentration trends window.

Finally, the button “Advanced setting” on the main menu provides some information about the access to the source code of both GUI and simulation computing core.

As shown in this digression, the graphic interface follows the nomenclature given by ADM1 (e.g. for “State no.” assignment to variables), also organizing data in sections to enhance user experience while initializing simulation scenario data.

Button related custom features

The following common set of functions has been considered for each parameter initialization window:

- data saving function, called once pressing the “Save Initialized Data” button. It allows the user to save all table current data to be restored once opened a new window instance. To ensure an easy users’ data initialization experience, the Save Initialized Data” button changes in colour to highlight that one or more of table’s rows has been modified and data should be lost if not saved before closing the current window. Default data is initialized once opening the GUI by a specific file;
- import data function, called once pressing the “Import Data” button. It allows the user to import data from a specific Excel file (.xlsx). depending on the specific table which has to be imported by the .xlsx file, the containing sheet must be called:
 - “IC - Soluble Compounds” for simulation’s Initial Soluble Compounds window;
 - “IC - Particulate Compounds” for simulation’s Initial Particulate Compounds window;
 - “Reactor Parameters” for simulation’s Reactor Parameters window;
 - “SBP-Yield product on substrate” for simulation’s Yield Product on substrate window (accessed by main menu’s *Stoichiometric and Biochemicals Parameters* button);

- “SBP-C and N contents” for simulation’s C and N content window (accessed by main menu’s *Stoichiometric and Biochemicals Parameters* button);
 - “SBP-Biomass yields” for simulation’s Biomass yield window (accessed by main menu’s *Stoichiometric and Biochemicals Parameters* button);
 - “SBP-Half saturation values” for simulation’s Half saturation values window (accessed by main menu’s *Stoichiometric and Biochemicals Parameters* button);
 - “SBP-50% inhibitory concentr.” for simulation’s 50% inhibitory concentration window (accessed by main menu’s *Stoichiometric and Biochemicals Parameters* button);
 - “SBP-First order parameters” for simulation’s First order parameters window (accessed by main menu’s *Stoichiometric and Biochemicals Parameters* button);
 - “SBP-Acid & Base parameters” for simulation’s Acid/base parameters window (accessed by main menu’s *Stoichiometric and Biochemicals Parameters* button);
 - “pH parameters” for simulation’s pH parameters window (accessed by main menu’s *pH paramters* button);
 - “Settings” for simulation’s settings window (accessed by main menu’s *Settings* button).
- export data function, called once pressing the “Save Initialized Data” button. All current table initialized data is exported to a specific sheet (called as the window data comes from) on an .xlsx file, that can be named by the user;
 - close current window function, called once pressing the “Done” button.

To facilitate the simulation parameters pre-initialization once the user is intended to use the import data function, a specific template file is also provided (data_to_import.xlsx). Every sheet is called to fit the software requirements while reading and importing data from the .xlsx file by one of the windows. Only current window data will be imported to overwrite current table data once Import Data button is pressed and the Excel file is selected.

ADMS 2.0 Calculation core

In this section, the structure of the calculation core used in “COMPUTE NOW!” function is described.

First of all, the values inserted in the previous panels are organized in arrays which can be used during the running of the simulation. The use of different arrays instead of a singular matrix or a structure has been preferred in a stance of customization of the calculation core by the end user. A person with an education not oriented to the informatic fields can easily modify single arrays and control them without acquiring high programming knowledges. In this way data are collected in the following arrays:

- “conc_0” vector contains the initial concentration of compounds ordinated by mean of their state number from 1 to 26;

- Reactor parameters are collected in an array nominated “reactor”, in which the inserted parameters are ordinated in the same way as the one required by the “Reactor parameter” window. If the pH value inserted by the user is equal to “0” the concentration of ion hydrogen (S_{H^+}) is calculated as described in [71]:

$$S_{H^+} = -\frac{\Theta}{2} + \frac{1}{2} \cdot \sqrt{\Theta^2 + 4K_W} \quad (43)$$

$$\Theta = S_{cat^+} + S_{IN} - S_{nh3} - S_{hco3^-} - \frac{S_{ac^-}}{64} - \frac{S_{pro^-}}{112} - \frac{S_{bu^-}}{160} - \frac{S_{va^-}}{208} - S_{an^-} \quad (44)$$

If the pH value is inserted and different from 0, ADMS 2.0 doesn't calculate the trend of concentration of ion hydrogen as seen in the previous equations, but considers this value as a constant and, once calculated a value of concentration of ion hydrogen associated to the pH inserted by the user, maintains this value for the whole simulation.

- “f” vector is composed by the values of the yields of product on substrate in the same order of the ones required in the “Yield product on substrate” window in the “Stoichiometric & Biochemical Parameters” section ;
- “CN” vector is composed by the values of carbon and nitrogen content of component i in the same order of the ones required in the “C and N content” window in the “Stoichiometric & Biochemical Parameters” section ;
- “Y” vector collects the yields of biomass on substrate in the same order of the ones required in the “Biomass Yield” window in the “Stoichiometric & Biochemical Parameters” section ;
- “KS” vector is composed by half saturation values in the same order of the ones required in the “Half saturation values” window in the “Stoichiometric & Biochemical Parameters” section ;
- “KI” vector collects the 50% inhibitory concentration in the same order of the ones required in the “50% inhibitory concentration” window in the “Stoichiometric & Biochemical Parameters” section ;
- “k” vector contains first order parameters, Monod maximum specific uptake rates and first order decay rates in the same order of the ones required in the “First order parameters” window in the “Stoichiometric & Biochemical Parameters” section ;
- “K_AB” vector contains acid base kinetic parameters and acid base equilibrium coefficients in the same order of the ones required in the “Acid/Base Parameters” window in the “Stoichiometric & Biochemical Parameters” section ;
- “pH_L” arrays ids composed by the values of lower and upper limits to calculate pH inhibition associated to amino acids, acetate and hydrogen. Using the dropdown menu, it is possible to change the function used to calculate pH inhibition. As seen in the previous section, three options are available:

1. “Batstone equation”, is discontinuous and in a stiff system it can cause numerical instabilities. The function proposed in [71] is described in the following equations:

$$I_{pH,aa} = \begin{cases} \exp\left(-3\left(\frac{pH-pH_{UL,aa}}{pH_{UL,aa}-pH_{LL,aa}}\right)^2\right) & : pH < pH_{UL,aa} \\ 1 & : pH > pH_{UL,aa} \end{cases} \quad (45)$$

$$I_{pH,ac} = \begin{cases} \exp\left(-3\left(\frac{pH-pH_{UL,ac}}{pH_{UL,ac}-pH_{LL,ac}}\right)^2\right) & : pH < pH_{UL,ac} \\ 1 & : pH > pH_{UL,ac} \end{cases} \quad (46)$$

$$I_{pH,aa} = \begin{cases} \exp\left(-3\left(\frac{pH-pH_{UL,h2}}{pH_{UL,h2}-pH_{LL,h2}}\right)^2\right) & : pH < pH_{UL,h2} \\ 1 & : pH > pH_{UL,h2} \end{cases} \quad (47)$$

2. “Hyperbolic tangent equation” is a continuous function describing pH inhibition [71]:

$$I_{pH,aa} = \frac{1}{2}(1 + \tanh(11 \cdot \phi)) \quad \text{with } \phi = \frac{pH - \frac{pH_{LL,aa} + pH_{UL,aa}}{2}}{\frac{pH_{LL,aa} + pH_{UL,aa}}{2}} \quad (48)$$

$$I_{pH,ac} = \frac{1}{2}(1 + \tanh(22 \cdot \phi)) \quad \text{with } \phi = \frac{pH - \frac{pH_{LL,ac} + pH_{UL,ac}}{2}}{\frac{pH_{LL,ac} + pH_{UL,ac}}{2}} \quad (49)$$

$$I_{pH,h2} = \frac{1}{2}(1 + \tanh(22 \cdot \phi)) \quad \text{with } \phi = \frac{pH - \frac{pH_{LL,h2} + pH_{UL,h2}}{2}}{\frac{pH_{LL,h2} + pH_{UL,h2}}{2}} \quad (50)$$

3. “Siegrist Equation” is another formulation of the pH inhibition function [71]:

$$I_{pH,aa} = \frac{K_{pH}^{n_{aa}}}{S_{H^+}^{n_{aa}} + K_{pH}^{n_{aa}}} \quad \text{with } K_{pH} = 10^{-\frac{pH_{LL,aa} + pH_{UL,aa}}{2}} \quad \text{and } n_{aa} = \frac{3}{pH_{UL,aa} - pH_{LL,aa}} \quad (51)$$

$$I_{pH,ac} = \frac{K_{pH}^{n_{ac}}}{S_{H^+}^{n_{ac}} + K_{pH}^{n_{ac}}} \quad \text{with } K_{pH} = 10^{-\frac{pH_{LL,ac} + pH_{UL,ac}}{2}} \quad \text{and } n_{ac} = \frac{3}{pH_{UL,ac} - pH_{LL,ac}} \quad (52)$$

$$I_{pH,h2} = \frac{K_{pH}^{n_{h2}}}{S_{H^+}^{n_{h2}} + K_{pH}^{n_{h2}}} \quad \text{with } K_{pH} = 10^{-\frac{pH_{LL,h2} + pH_{UL,h2}}{2}} \quad \text{and } n_{h2} = \frac{3}{pH_{UL,h2} - pH_{LL,h2}} \quad (53)$$

- the setting parameters are used to build the simulation setting: time vector, nominated “time” is a vector containing a number of days from 0 to the day described by the user, with a number of steps selected by the user. Also the tolerances are organized in a vector called “options”, which contains Relative Tolerance, Absolute Tolerance and the NonZero condition, with which the concentration vector calculated doesn’t admit negative results to solve the system. In the “Settings” panel, a dropdown menu admit the user to choice the ordinary differential equation (ODE) solver between four options. Before describing the options, an overview on MATLAB ODEs is provided. The ODE solving feature is provided by MATLAB and is the acronym of Ordinary Differential Equation. There are many types of ODE functions and each one is designed to fit and solve specific computational requirements or issues by means of different numerical methods. The appropriate syntax for the ODEs is reported by [73], [74]:

$$[t,y]= \text{ode}(\text{odefun}, \text{tspan}, y0, \text{options})$$

where:

- “odefun” defines the function to solve, in which the ordinary equation system is contained. In this calculation core it is called “System”;
- “tspan” defines the time array over which the system needs to be solved. In the ADMS 2.0 is “time” vector;

- “y0” is the array of initial conditions, referring to the concentrations of the simulated components. In this case, the “conc_0” vector is the vector used to initialize the initial concentration in the ODE function;
- “options” contains information about the tolerances and non -zero condition;
- “y” is the array of the integration results, which, in the ADMS 2.0 is called “S”.

The implemented ODE solvers in ADMS 2.0 are indicated as follow, with a description obtained by the MATLAB website itself [75]:

1. ode45 : solves nonstiff differential equations, with a medium accuracy, using an algorithm based on an explicit Runge-Kutta (4,5) formula, the Dormand-Price pair. It is a single-step solver [72], [76], [77];
2. ode23 : solves nonstiff differential equations, with a low accuracy, using an algorithm based on an explicit Runge-Kutta (2,3) formula, pair Bogacki and Shampine. It is a single-step solver [72], [78], [79];
3. ode23tb : solves stiff differential equations, with a low accuracy, using an algorithm based on an implicit Runge-Kutta (2,3) formula with trapezoidal rule step as its first stage and a backward differentiation formula of order two as its second stage. By construction, the same iteration matrix is used in evaluating both stages. Like ode23s and ode23t, this solver may be more efficient than ode15s for problems with crude tolerances [80]–[82];
4. ode15s: solves stiff differential equation and DAEs with a low to medium accuracy. It is a variable-step, variable-order (VSVO) solver based on the numerical differentiation formulas (NDFs) of orders 1 to 5. Optionally, it can use the backward differentiation formulas (BDFs, also known as Gear's method) that
5. are usually less efficient. Like ode113, ode15s is a multistep solver. Use ode15s if ode45 fails or is very inefficient and you suspect that the problem is stiff, or when solving a differential-algebraic equation (DAE) [72], [83], [84].

Once described the vectors in which data inserted are collected, an overview on the functioning of “System” function is provided.

“System” function is called in the ODE and contains the differential equation system described by the ADM1, plus the equations for pH calculation and pH inhibition discussed before. This function is defined as:

$$[S] = \text{System}(\text{time}, \text{conc}, \text{conc_0}, f, \text{CN}, Y, \text{KS}, \text{KI}, k, K_AB, \text{pH_L}, \text{reactor})$$

Where:

- S is the matrix containing the system results, which has 35 columns (one for each compound included in the simulation) and a number of row equal to the time step set;
- “conc” represents the vector of the value of the concentration of each compound at a time different from 0;
- the other arrays inserted in the System function are the ones described above.

In the “System” function, each element of each array is named according to the name given in ADM1: this helps the user to customize the tool in an orderly and conscious way.

After each element has been named, the differential equation system is built according to the equation described in [71]. DAE solvers are not implemented in this version of the tool. Parameters which can be calculate with DAE equation, such

as the pH equation above mentioned, inhibitions, other parameters required (such as the one linked to pressure and temperature) are calculated during the execution of the System function. The vector S, in output from “System”, contains the value of the derivative of first order in relation to the time of concentration of each compound having state number from 1 to 35, as described in the following equation.

$$S_{System} = \begin{pmatrix} \frac{dS_{su}}{dt} \\ \frac{dS_{aa}}{dt} \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \cdot \\ \frac{dS_{gas,CH}}{dt} \\ \frac{dS_{gas,CO2}}{dt} \end{pmatrix}$$

This result vector is then used by ODE solver to obtain the value of the concentration of each time step.

Once the System has been calculated, results are plotted by the use of the selection panel described in the previous section.

ADMS 2.0 Validation

In this section a comparison between the results obtained by implementing the same simulation in ADMS 2.0 and in AQUASIM are provided. In addition to this, the results obtained by both the software tools are related to the ones reported in [71].

First of all, the initial parameter e concentrations used to simulate are listed in Table 25.

Table 25. Values implemented for the simulation.

State no.	Variable name	Description	Value	Unit
1	S _{su,i}	Sugar initial concentration	1,00E-02	kgCOD / m ³
2	S _{aa,i}	Aminoacids initial concentration	1,00E-03	kgCOD / m ³
3	S _{fa,i}	Fatty acids initial concentration	1,00E-03	kgCOD / m ³
4	S _{va,i}	Valerate initial concentration	1,00E-03	kgCOD / m ³
5	S _{bu,i}	Butyrate initial concentration	1,00E-03	kgCOD / m ³
6	S _{pro,i}	Proponiate initial concentration	1,00E-03	kgCOD / m ³
7	S _{ac,i}	Acetate initial concentration	1,00E-03	kgCOD / m ³
8	S _{h2,i}	Hydrogen initial concentration	1,00E-08	kgCOD / m ³
9	S _{ch4,i}	Methane initial concentration	1,00E-05	kgCOD / m ³
10	S _{IC,i}	Inorganic carbon initial concentration	4,00E-02	kmoleC / m ³
11	S _{IN,i}	Inorganic nitrogen initial concentration	1,00E-02	kmoleN / m ³
12	S _{I,i}	Inherits initial concentration	2,00E-02	kgCOD / m ³
25	S _{cat,i}	Cations initial concentration	4,00E-02	kmole / m ³
26	S _{an,i}	Anions initial concentration	2,00E-02	kmole / m ³
13	X _{C,I}	Composites initial concentration	2,00E+00	kgCOD / m ³
14	X _{ch,i}	Carbohydrates initial concentration	5,00E+00	kgCOD / m ³
15	X _{pr,i}	Proteins initial concentration	2,00E+01	kgCOD / m ³

16	$X_{li,i}$	Lipids initial concentration	5,00E+00	kg _{COD} / m ³
17	$X_{su,i}$	Sugar degraders initial concentration	0,00E+00	kg _{COD} / m ³
18	$X_{aa,i}$	Aminoacids initial concentration	1,00E-02	kg _{COD} / m ³
19	$X_{fa,i}$	LCFA degraders initial concentration	1,00E-02	kg _{COD} / m ³
20	$X_{c4,i}$	Valerate and Butyrate degraders initial concentration	1,00E-02	kg _{COD} / m ³
21	$X_{pro,i}$	Propionate degraders initial concentration	1,00E-02	kg _{COD} / m ³
22	$X_{ac,i}$	Acetate degraders initial concentration	1,00E-02	kg _{COD} / m ³
23	$X_{h2,i}$	Hydrogen degraders initial concentration	1,00E-02	kg _{COD} / m ³
24	$X_{li,i}$	Particulate inerts initial concentration	2,50E+01	kg _{COD} / m ³
-	$V_{ad,liq}$	Reactor liquid volume	3,40E+03	m ³
-	$V_{ad,gas}$	Reactor gas volume	3,00E+02	m ³
-	Q_{ad}	Liquid flowrate	1,70E+02	m ³ / d
-	T_{op}	Operative temperature	3,08E+02	K
-	T_{base}	Base temperature	2,98E+02	K
-	P_{atm}	Atmospheric pressure	1,01E+00	bar
-	k_p	Mass transfer coefficient for liquid/gas interface	5,00E+04	m ³ /(d*bar)
-	k_{La}	Global mass transfer coefficient for liquid/gas interface	2,00E+02	1/d
-	pH	pH value (set 0 if to be computed according with ADM1)	7,50E+00	l
-	t_0	Initial time	0,00E+00	d
-	t_f	Final time	2,00E+01	d
-	t_{step}	Timestep	1,00E+00	d
-	Abs tol	Absolute tolerance	1,00E-12	(*)
-	Rel tol	Relative tolerance	1,00E-10	(*)
-	Ode type	Type of ODE function	ode23tb	-
-	f_{SI,x_c}	Fraction of soluble inerts in composite material (Xc)	1,00E-01	-
-	f_{XI,x_c}	Fraction of particulate inerts in composite material (Xc)	2,00E-01	-
-	f_{ch,x_c}	Fraction of carbohydrates in composite material (Xc)	2,00E-01	-
-	f_{pr,x_c}	Fraction of proteins in composite material (Xc)	2,00E-01	-
-	f_{li,x_c}	Fraction of lipids in composite material (Xc)	3,00E-01	-
-	$f_{fa,li}$	Fraction of long-chain fatty acids in lipids	9,50E-01	-
-	$f_{h2,su}$	Fraction of hydrogen from sugar degradation	1,90E-01	-
-	$f_{bu,su}$	Fraction of butyrate from sugar degradation	1,30E-01	-
-	$f_{pro,su}$	Fraction of propionate from sugar degradation	2,70E-01	-
-	$f_{ac,su}$	Fraction of acetate from sugar degradation	4,10E-01	-
-	$f_{h2,aa}$	Fraction of hydrogen from amino acid degradation	6,00E-02	-
-	$f_{va,aa}$	Fraction of valerate from amino acid degradation	2,30E-01	-
-	$f_{bu,aa}$	Fraction of butyrate from amino acid degradation	2,60E-01	-
-	$f_{pro,aa}$	Fraction of propionate from amino acid degradation	5,00E-02	-
-	$f_{ac,aa}$	Fraction of acetate from amino acid degradation	4,00E-01	-
-	N_{xc}	Nitrogen content of composite material (Xc)	2,69E-03	kmolen / kg _{COD}
-	N_I	Inorganic nitrogen content	4,29E-03	kmolen / kg _{COD}
-	N_{aa}	Nitrogen content of amino acids	7,00E-03	kmolen / kg _{COD}
-	C_{xc}	Carbon content of composite material (Xc)	2,79E-02	kmole _C / kg _{COD}
-	C_{SI,x_c}	Soluble inert fraction of composite material (Xc)	3,00E-02	kmole _C / kg _{COD}

-	$C_{ch,xc}$	Carbohydrate fraction of composite material (Xc)	3,13E-02	kmole _c / kg _{COD}
-	C_{pr}	Protein content	3,00E-02	kmole _c / kg _{COD}
-	C_{li}	Lipid content	2,20E-02	kmole _c / kg _{COD}
-	C_{XI}	Inert particulate organic matter (XI) content	3,00E-02	kmole _c / kg _{COD}
-	C_{su}	Sugar content	3,13E-02	kmole _c / kg _{COD}
-	C_{aa}	Amino acid content	3,00E-02	kmole _c / kg _{COD}
-	C_{fa}	Long-chain fatty acid content	2,17E-02	kmole _c / kg _{COD}
-	N_{bac}	Nitrogen content of biomass	5,71E-03	kmole _N / kg _{COD}
-	C_{bu}	Butyrate content	2,50E-02	kmole _c / kg _{COD}
-	C_{pro}	Propionate content	2,68E-02	kmole _c / kg _{COD}
-	C_{ac}	Acetate content	3,13E-02	kmole _c / kg _{COD}
-	C_{bac}	Biomass content	3,13E-02	kmole _c / kg _{COD}
-	C_{va}	Valerate content	2,40E-02	kmole _c / kg _{COD}
-	C_{ch4}	Methane content	1,56E-02	kmole _c / kg _{COD}
-	Y_{su}	Yield of sugar fermentation (saccharolytic fermenters)	1,00E-01	kmole _{CODx} / kg _{CODs}
-	Y_{aa}	Yield of amino acid degradation (amino acid degraders)	8,00E-02	kmole _{CODx} / kg _{CODs}
-	Y_{fa}	Yield of long-chain fatty acid degradation (LCFA degraders)	6,00E-02	kmole _{CODx} / kg _{CODs}
-	Y_{c4}	Yield of short-chain fatty acid degradation (butyrate, valerate)	6,00E-02	kmole _{CODx} / kg _{CODs}
-	Y_{pro}	Yield of propionate degradation (propionate degraders)	4,00E-02	kmole _{CODx} / kg _{CODs}
-	Y_{ac}	Yield of acetate degradation (acetoclastic methanogens)	5,00E-02	kmole _{CODx} / kg _{CODs}
-	$K_{S,in}$	Half-saturation constant for inerts (non-biodegradable soluble organics)	1,00E-04	kg _{COD} / m ³
-	$K_{S,su}$	Half-saturation constant for sugars	5,00E-01	kg _{COD} / m ³
-	$K_{S,aa}$	Half-saturation constant for amino acids	3,00E-01	kg _{COD} / m ³
-	$K_{S,fa}$	Half-saturation constant for long-chain fatty acids (LCFA)	4,00E-01	kg _{COD} / m ³
-	$K_{S,c4}$	Half-saturation constant for short-chain fatty acids (C4: butyrate, valerate)	2,00E-01	kg _{COD} / m ³
-	$K_{S,pro}$	Half-saturation constant for propionate	1,00E-01	kg _{COD} / m ³
-	$K_{S,ac}$	Half-saturation constant for acetate	1,50E-01	kg _{COD} / m ³
-	$K_{S,h2}$	Half-saturation constant for hydrogen	7,00E-06	kg _{COD} / m ³
-	$K_{I,h2,fa}$	Inhibition constant for hydrogen on long-chain fatty acid degraders	5,00E-06	kg _{COD} / m ³
-	$K_{I,h2,c4}$	Inhibition constant for hydrogen on valerate and butyrate degraders (C4)	1,00E-05	kg _{COD} / m ³
-	$K_{I,h2,pro}$	Inhibition constant for hydrogen on propionate degraders	3,50E-06	kg _{COD} / m ³
-	$K_{I,NH3}$	Inhibition constant for free ammonia (NH ₃)	1,80E-03	M
-	k_{dis}	Disintegration rate constant	5,00E-01	1 / d
-	$k_{hyd,ch}$	Hydrolysis rate constant of carbohydrates	1,00E+01	1 / d
-	$k_{hyd,pr}$	Hydrolysis rate constant of proteins	1,00E+01	1 / d
-	$k_{hyd,li}$	Hydrolysis rate constant of lipids	1,00E+01	1 / d
-	$k_{m,su}$	Maximum uptake rate of sugars (saccharolytic fermenters)	3,00E+01	1 / d
-	$k_{m,aa}$	Maximum uptake rate of amino acids	5,00E+01	1 / d
-	$k_{m,fa}$	Maximum uptake rate of long-chain fatty acids	6,00E+00	1 / d
-	$k_{m,c4}$	Maximum uptake rate of valerate and butyrate (C4)	2,00E+01	1 / d
-	$k_{m,pro}$	Maximum uptake rate of propionate	1,30E+01	1 / d

-	$k_{m,ac}$	Maximum uptake rate of acetate	8,00E+00	1 / d
-	$k_{m,h2}$	Maximum uptake rate of hydrogen	3,50E+01	1 / d
-	$k_{dec,Xsu}$	Decay rate of sugar degraders (Xsu)	2,00E-02	1 / d
-	$k_{dec,Xaa}$	Decay rate of amino acid degraders (Xaa)	2,00E-02	1 / d
-	$k_{dec,Xfa}$	Decay rate of LCFA degraders (Xfa)	2,00E-02	1 / d
-	$k_{dec,Xc4}$	Decay rate of valerate/butyrate degraders (Xc4)	2,00E-02	1 / d
-	$k_{dec,Xpro}$	Decay rate of propionate degraders (Xpro)	2,00E-02	1 / d
-	$k_{dec,Xac}$	Decay rate of acetoclastic methanogens (Xac)	2,00E-02	1 / d
-	$k_{dec,Xh2}$	Decay rate of hydrogenotrophic methanogens (Xh2)	2,00E-02	1 / d
-	$K_{a,va}$	Acid dissociation constant of valeric acid	1,38E-05	M
-	$K_{a,bu}$	Acid dissociation constant of butyric acid	1,51E-05	M
-	$K_{a,pro}$	Acid dissociation constant of propionic acid	1,32E-05	M
-	$K_{a,ac}$	Acid dissociation constant of acetic acid	1,74E-05	M
-	$k_{A,Bva}$	Henry's law constant for valeric acid	1,00E+10	1 / (M * d)
-	$k_{A,Bbu}$	Henry's law constant for butyric acid	1,00E+10	1 / (M * d)
-	$k_{A,Bpro}$	Henry's law constant for propionic acid	1,00E+10	1 / (M * d)
-	$k_{A,Bac}$	Henry's law constant for acetic acid	1,00E+10	1 / (M * d)
-	$k_{A,Bco2}$	Henry's law constant for carbon dioxide (CO ₂)	1,00E+10	1 / (M * d)
-	$k_{A,BIN}$	Henry's law constant for ammonia (NH ₃)	1,00E+10	1 / (M * d)

(*) unit is the same of the computed variables

Simulation has been carried out considering a time from zero to 200 days, with a timestep of 1 day. For the ADMS2.0 implementation, ODE15s has been chosen as ode solver. Both AQUASIM and ADMS2.0 have been set to use Siegrist functions for inhibition. Considering the fact that [71], in chapter 7 reports that “*Note that the resulting pH in the AD is about 7.5, i.e the pH inhibition functions are not really active in this test case*”, the simulations carried out to validate the software in relationship to the benchmark data provided by [71] has been set with a constant pH value, equal to 7.465537769904638 in order to not activate pH functions with a value consistent to the one reported in the benchmark results. ADMS 2.0 relative tolerance has been set at 1.10^{-10} and the absolute tolerance has been implemented as 1.10^{-12} .

Results obtained are collected in Table 26 , with an assessment of the percentage error calculated as:

$$\varepsilon_{software\ tool} [\%] = \frac{|S_{benchmark} - S_{software\ tool}|}{S_{benchmark}} \cdot 100 \quad (54)$$

Considering data reported in Table 26, it possible to notice that both the software tools provide results with a percentage error lower than 5% (which is consider the maximum acceptable error). In particular the relative error associated to ADMS 2.0 simulation is lower than the one associated to AQUASIM results, except for the compounds S_{ch4} , S_{IC} , X_{ch} , X_{li} , X_{fa} , X_{ac} , S_{hco3-} and gas species. In particular the relative error reaches over 2% value in the ADMS 2.0 results associated to S_{IC} , which then affects also S_{hco3-} and $S_{gas,co2}$. The consistency of the error for these three parameters seems to have the same cause, which has not detected in the code, considering the fact that the equation proposed by [9], [71] have been implemented avoiding modifications in the calculation core. AQUASIM 2.0 requires a different implementation of the equations, based also on the volumes identified and the links between these volumes: it's possible that the differences in these formulations have caused a difference in the calculation of the above mentioned compounds.

Anyway, as seen before, for the remaining compounds the error associate to the use of ADMS2.0 is consistently lower than 1%, which admits to affirm that the software tool has been successfully validated. The overall error (calculated as the mean of relative errors contained in Table 26) associated to the time 200 days is equal to :

$$\varepsilon_{overall\ AQUASIM\ 2.0} = 0.04\ \%$$

$$\varepsilon_{overall\ ADM\ 2.0} = 0.22\ \%$$

As expected, the overall error associated to AQUASIM 2.0 simulation is lower than a factor of ten in comparison to the overall error obtained by ADMS2.0.

Table 26. Results and comparison of AQUASIM and ADMS 2.0 Simulation.

<i>Variable</i>	<i>Unit</i>	<i>Benchmark Parameters</i>	<i>Results AQUASIM 2.0</i>	<i>Results ADMS 2.0</i>	$\varepsilon_{AQUASIM}\ [\%]$	$\varepsilon_{ADMS2.0}\ [\%]$
S _{su}	kgCOD/m ³	0.011955	0.011950	0.0119548	4.0 E-02	3.0 E-04
S _{aa}	kgCOD/m ³	0.005315	0.005315	0.0053147	4.9 E-03	9.4 E-05
S _{fa}	kgCOD/m ³	0.098621	0.098620	0.0986219	1.4 E-03	4.9 E-04
S _{va}	kgCOD/m ³	0.011625	0.011620	0.0116245	4.3 E-02	4.4 E-03
S _{bu}	kgCOD/m ³	0.013251	0.013250	0.0132502	5.5 E-03	4.4 E-03
S _{pro}	kgCOD/m ³	0.015784	0.015780	0.0157836	2.3 E-02	1.6 E-04
S _{ac}	kgCOD/m ³	0.197630	0.197600	0.1976133	1.5 E-02	8.3 E-03
S _{h2}	kgCOD/m ³	0.000000	0.000000	0.0000002	1.9 E-02	4.1 E-05
S _{ch4}	kgCOD/m ³	0.055089	0.054940	0.0554186	2.7 E-01	6.0 E-01
S _{IC}	kmole C/m ³	0.152678	0.152400	0.1494846	1.8 E-01	2.1 E+00
S _{IN}	kmole N/m ³	0.130230	0.130200	0.1302289	2.3 E-02	7.1 E-04
S _I	kgCOD/m ³	0.328698	0.328700	0.3286989	7.1 E-04	3.8 E-04
X _C	kgCOD/m ³	0.308698	0.308700	0.3087035	7.6 E-04	1.9 E-03
X _{ch}	kgCOD/m ³	0.027947	0.027950	0.0279473	9.9 E-03	2.1 E-04
X _{pr}	kgCOD/m ³	0.102574	0.102600	0.1025742	2.5 E-02	5.7 E-05
X _{li}	kgCOD/m ³	0.029483	0.029480	0.0294908	1.0 E-02	2.6 E-02
X _{su}	kgCOD/m ³	0.420166	0.420200	0.4201736	8.1 E-03	1.8 E-03
X _{aa}	kgCOD/m ³	1.179172	1.179000	1.1791736	1.5 E-02	1.5 E-04
X _{fa}	kgCOD/m ³	0.243035	0.243000	0.2430995	1.5 E-02	2.6E-02
X _{c4}	kgCOD/m ³	0.431921	0.431900	0.4319223	4.9 E-03	2.8 E-04
X _{pro}	kgCOD/m ³	0.137306	0.137300	0.1373068	4.3 E-03	6.6 E-04
X _{ac}	kgCOD/m ³	0.760563	0.760600	0.7606232	4.9 E-03	8.0 E-03
X _{h2}	kgCOD/m ³	0.317023	0.317000	0.3170427	7.2 E-03	6.2 E-03
X _I	kgCOD/m ³	25.617395	25.620000	25.6173978	1.0 E-02	1.0 E-05
S _{cat}	kmole/m ³	0.040000	0.040000	0.0400000	0.0 E+00	0.0 E+00
S _{an}	kmole/m ³	0.020000	0.020000	0.0200000	0.0 E+00	0.0 E+00
S _{va-}	kgCOD/m ³	0.011596	0.011600	0.0115957	3.2 E-02	4.4 E-03
S _{bu-}	kgCOD/m ³	0.013221	0.013220	0.0132202	6.2 E-03	4.4 E-03
S _{pro-}	kgCOD/m ³	0.015743	0.015740	0.0157428	1.8 E-02	1.6 E-04
S _{ac-}	kgCOD/m ³	0.197241	0.197200	0.1972248	2.1 E-02	8.3 E-03
S _{hco3-}	kmole C/m ³	0.142777	0.142500	0.1397913	1.9 E-01	2.1 E+00
S _{nh3}	kmole N/m ³	0.004091	0.004090	0.0040909	2.3 E-02	7.0 E-04
S _{gas,h2}	kgCOD/m ³	0.000010	0.000010	0.0000103	9.8 E-03	1.2 E-01

$S_{\text{gas, ch4}}$	kgCOD/m^3	1.625607	1.621000	1.6366771	2.8 E-01	6.8 E-01
$S_{\text{gas, co2}}$	kmole C/m^3	0.014151	0.014130	0.0138551	1.5 E-01	2.1 E+00

Considering that the data provides in [71] are the ones associated to the last timestep of the simulation, in order to assess the quality of the simulation provided by ADMS 2.0 tool, data provided by simulation with AQUASIM 2.0 have been used to plot a comparison between the trends of concentration obtained from the two software tools.

In order to graphically describe the consistency of the results provided by each software tool, trends obtained by ADMS 2.0 are plotted together with the ones obtained by AQUASIM 2.0. The first ones are plotted with markers while the second ones are characterized by a continuous line. Each species has its own colour in a single graph, in order to facilitate the comparison.

Disintegration, hydrolysis and uptakes processes are described respectively in Figure 73 ,Figure 75 and Figure 76. It's possible to assess that the trends calculated by ADMS 2.0 are quite similar to the ones obtained by AQUASIM 2.0: in fact the marker are overlapping the lines describing AQUASIM 2.0 results. In order to appreciate the results related to hydrolysis and disintegration processes , a plot after day 3 has been inserted in Figure 74: after this time concentrations of the simulated compounds has the same order of magnitude and the graph admits to have an in depth sight to compare the results.

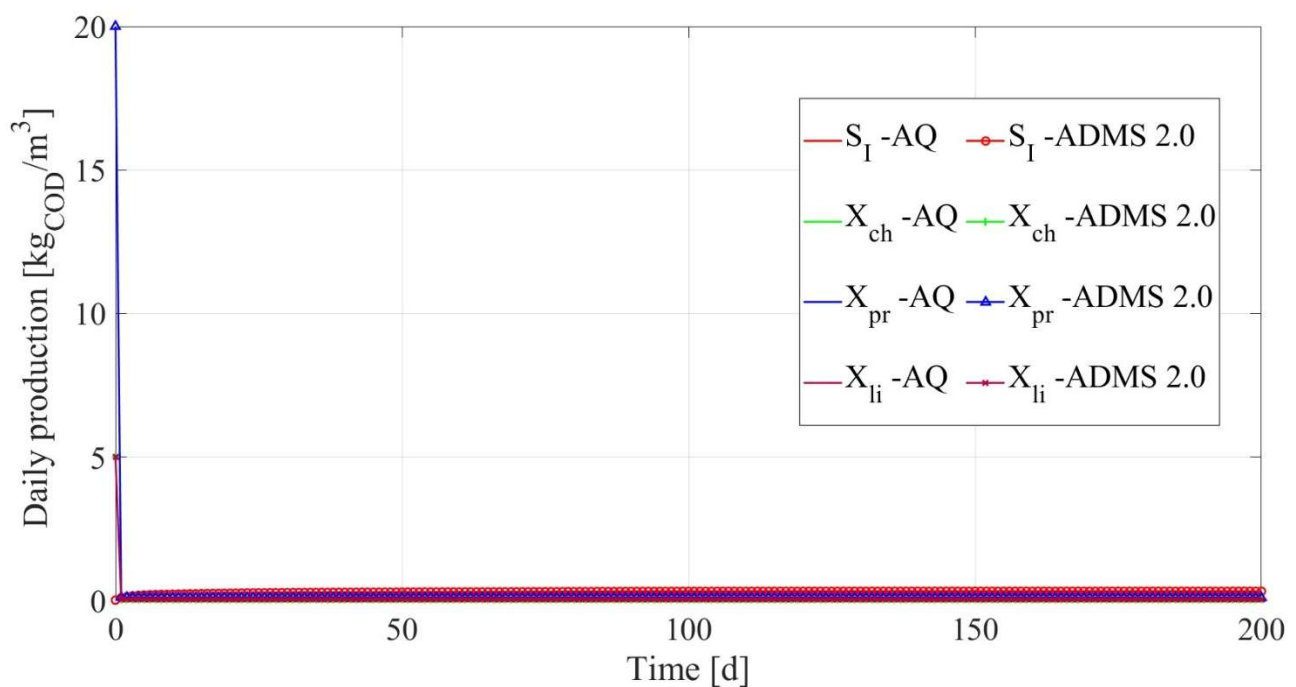


Figure 73. Simulated concentration trends for disintegration and hydrolysis processes (simulation carried out at constant pH).

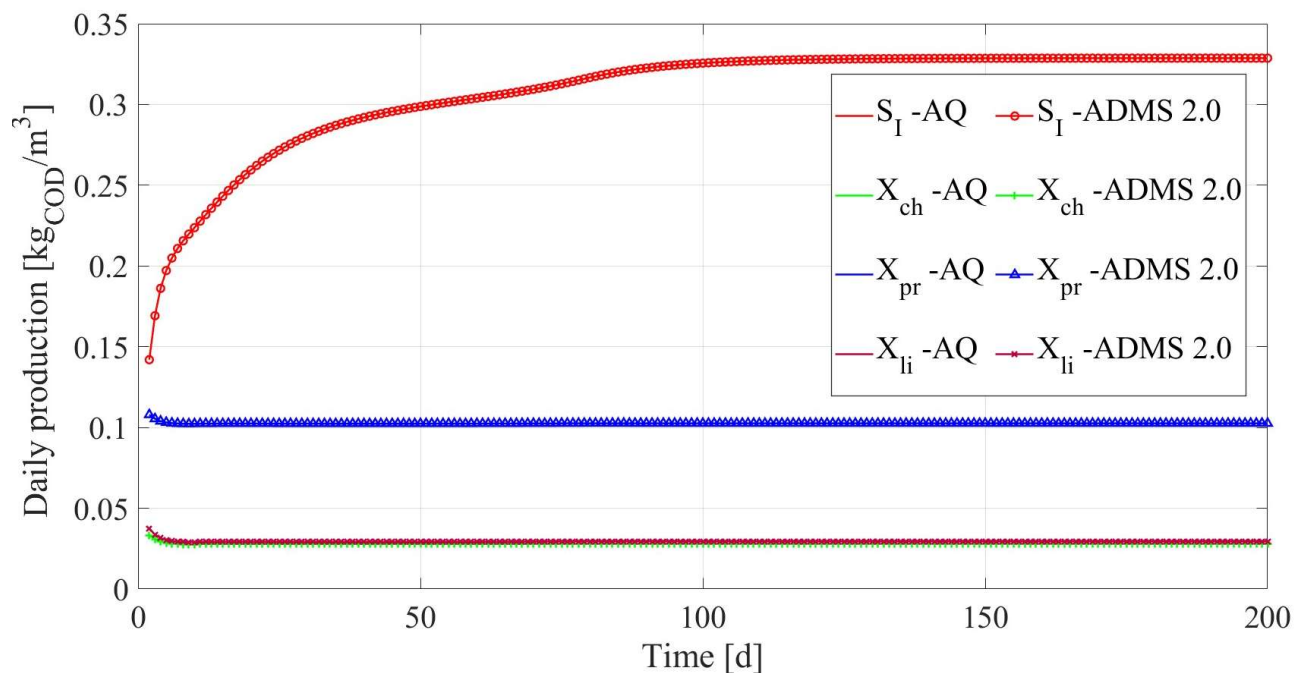


Figure 74. Simulated concentration trends for disintegration and hydrolysis processes from day 3 to 200 (simulation carried out at constant pH).

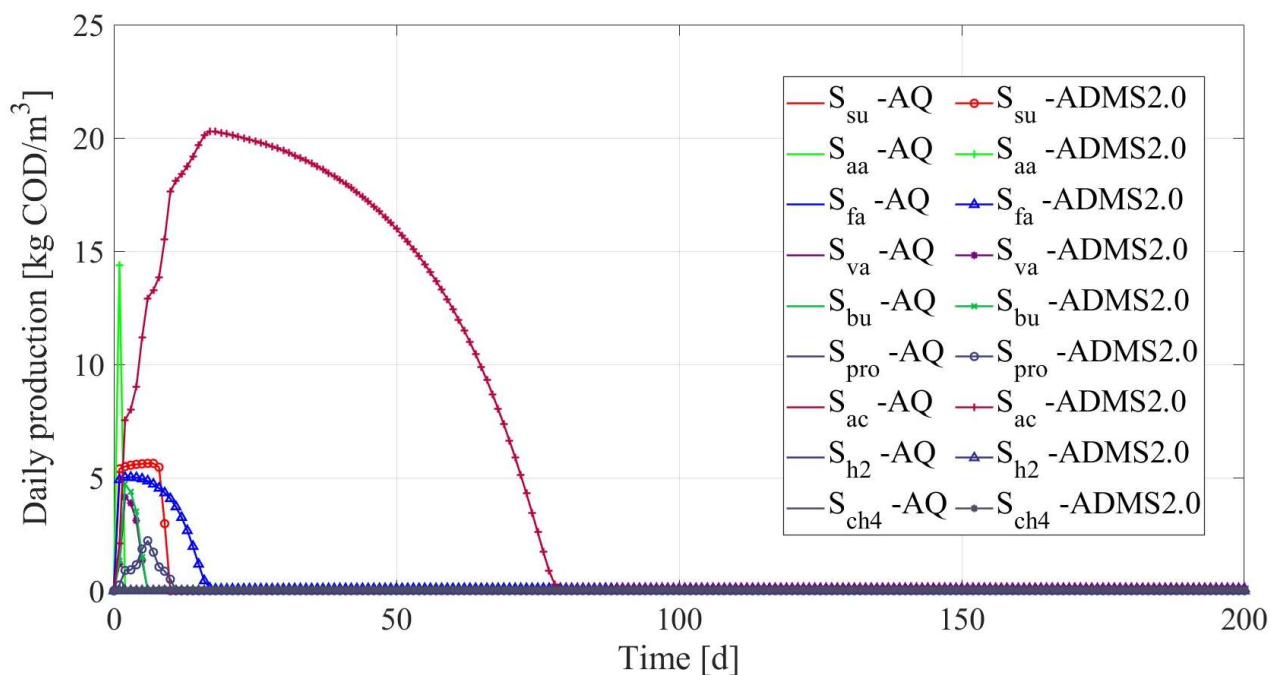


Figure 75. Simulated concentration trends for uptakes processes (simulation carried out at constant pH).

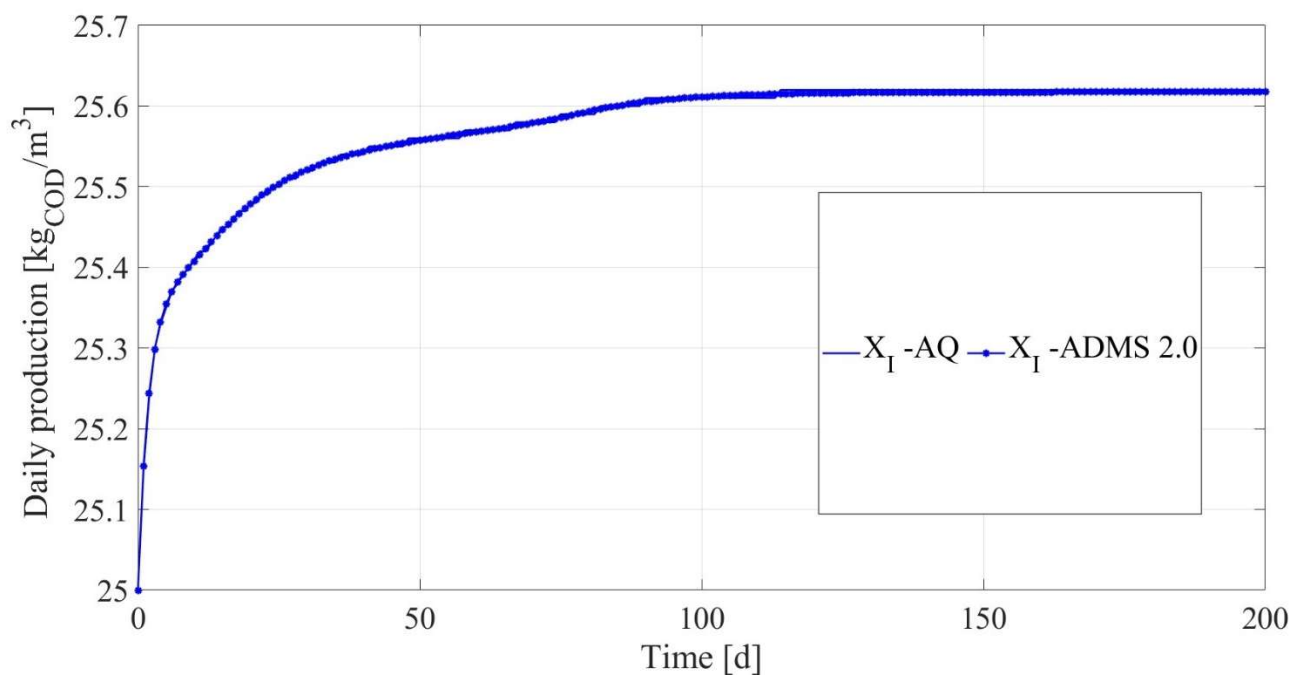


Figure 76. Simulated concentration trend for particulate inert (simulation carried out at constant pH).

Also decay process of biomasses have been simulated and plotted: in Figure 77 the trend obtained by ADMS 2.0 and AQUASIM 2.0 are described. As seen before, the trends, including the position of peaks, are overlapped.

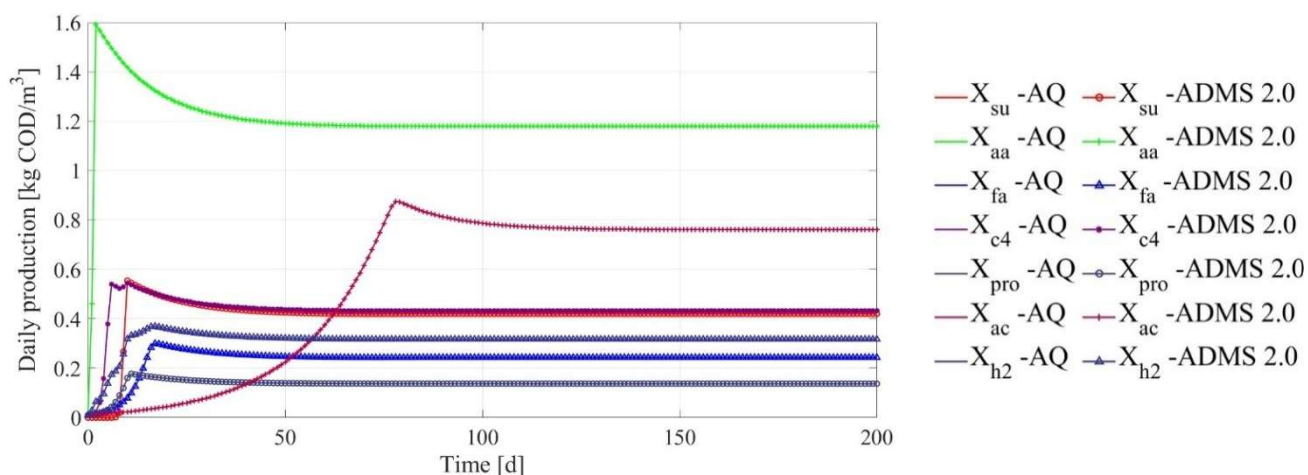


Figure 77. Simulated concentration trends for decay processes (simulation carried out at constant pH).

Figure 55 describe the trends of ion compounds produced during the anaerobic digestion process: also in this case, the results provided by the simulations with the two software tools describe the same concentration trends.

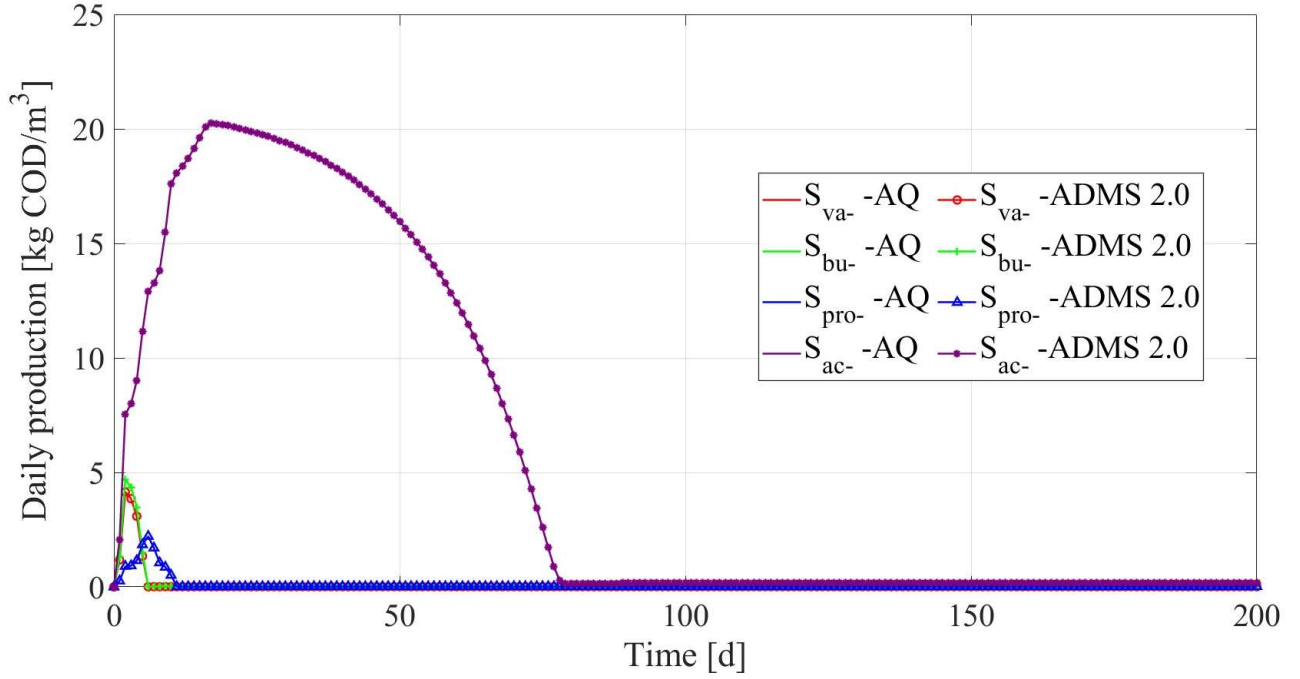


Figure 78. Simulated concentration trends for ion compounds (simulation carried out at constant pH).

A difference between the trends described by AQUASIM 2.0 and ADMS 2.0 can be detected studying the results related to inorganic carbon and nitrogen compounds. The first ones, in particular, affects also the production of methane and carbon dioxide gasses, which present a detachment between curves provided by ADMS 2.0 and AQUASIM 2.0. Figure 79 describes the trends of inorganic carbon and HCO_3^- : ADMS 2.0 provides values lower than the one calculated by AQUASIM 2.0. As mentioned before, this difference could be caused by the propagation of a numerical error during the calculation. In addition to this, the description of the carbon dioxide in liquid phase has been initialized as a DAE equation in ADMS 2.0 calculation core, as suggested by [71]:

$$S_{CO2} = S_{IC} - S_{HCO_3^-} \quad (55)$$

In AQUASIM 2.0, the description of this compound has been carried out building a differential equation, in order to optimize the description of the liquid gas transfer in the appropriate link. This difference in the calculation of the compound concentration could have lead to this discrepancy in results. The value of the detachment is equal to about 2.5%. Also Figure 80 shows a detachment between results related to inorganic nitrogen compounds, which can be considered as an effect of a propagation of a calculation error.

As said before, also methane concentration in gas phase presents an underestimation by ADMS 2.0 in relation to the results provided by AQUASIM 2.0 (Figure 81), but this detachment is weaker than the one associated to carbon dioxide gas, plotted in Figure 81.

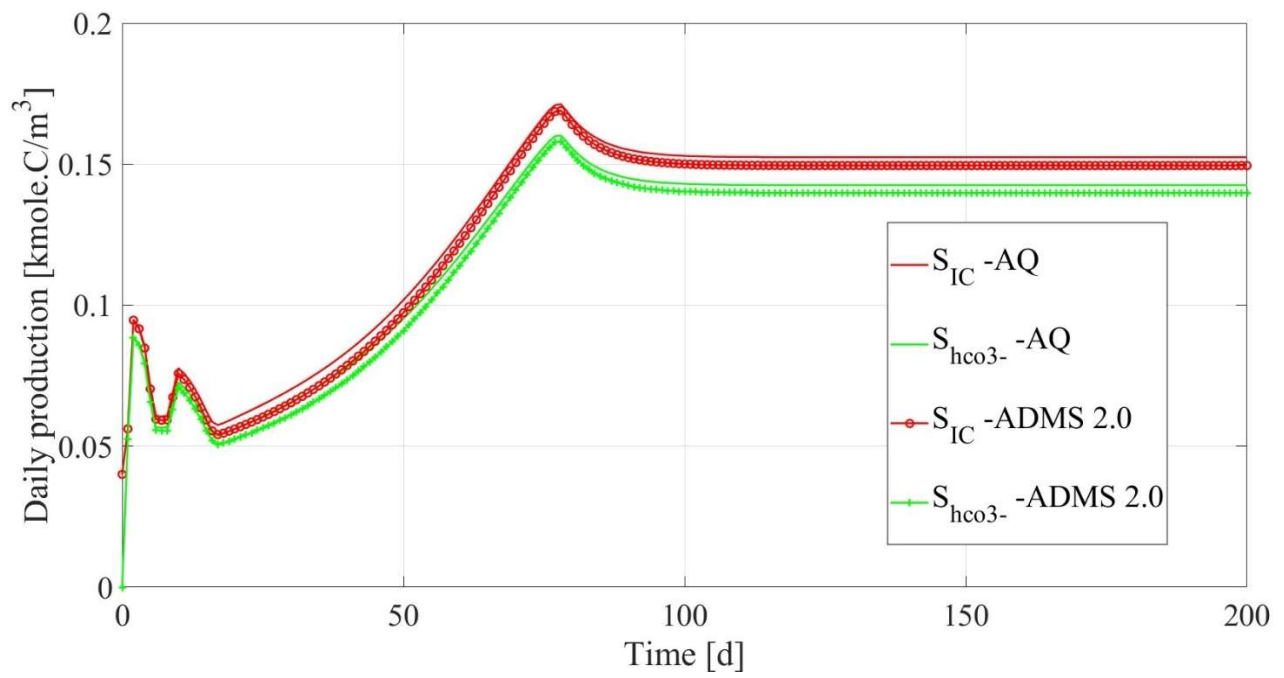


Figure 79. Simulated concentration trends for inorganic carbon and HCO_3^- ion (simulation carried out at constant pH).

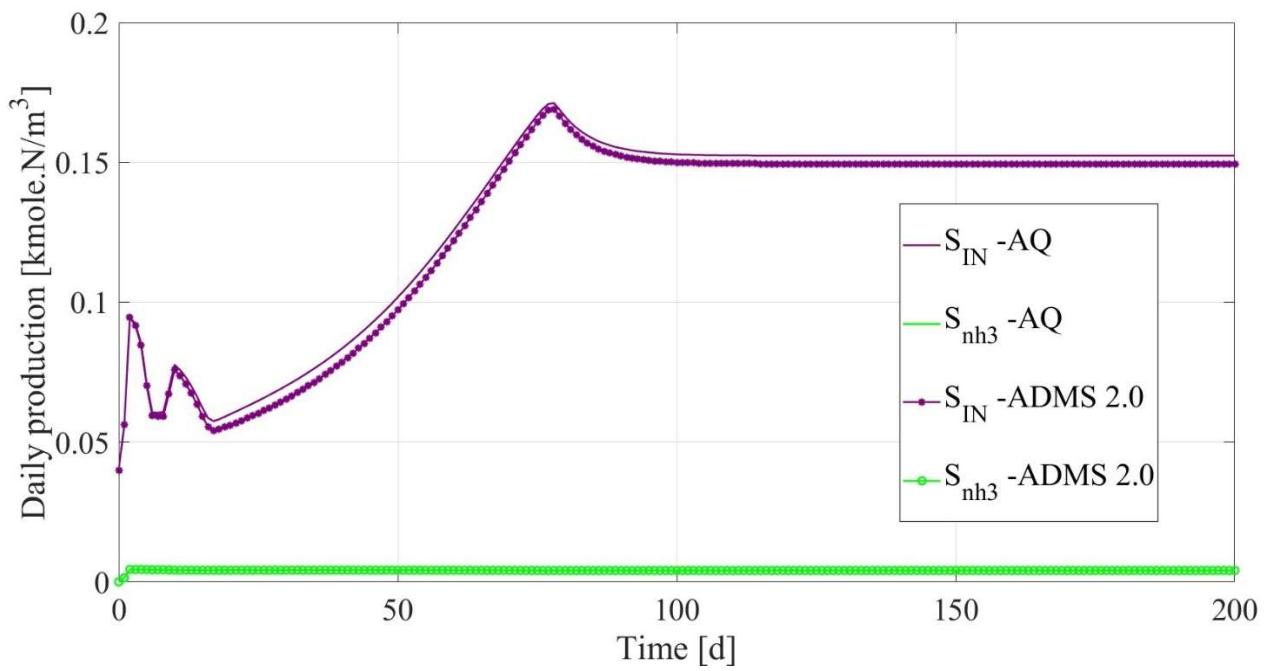


Figure 80. Simulated concentration trends for inorganic nitrogen and NH_3 (simulation carried out at constant pH).

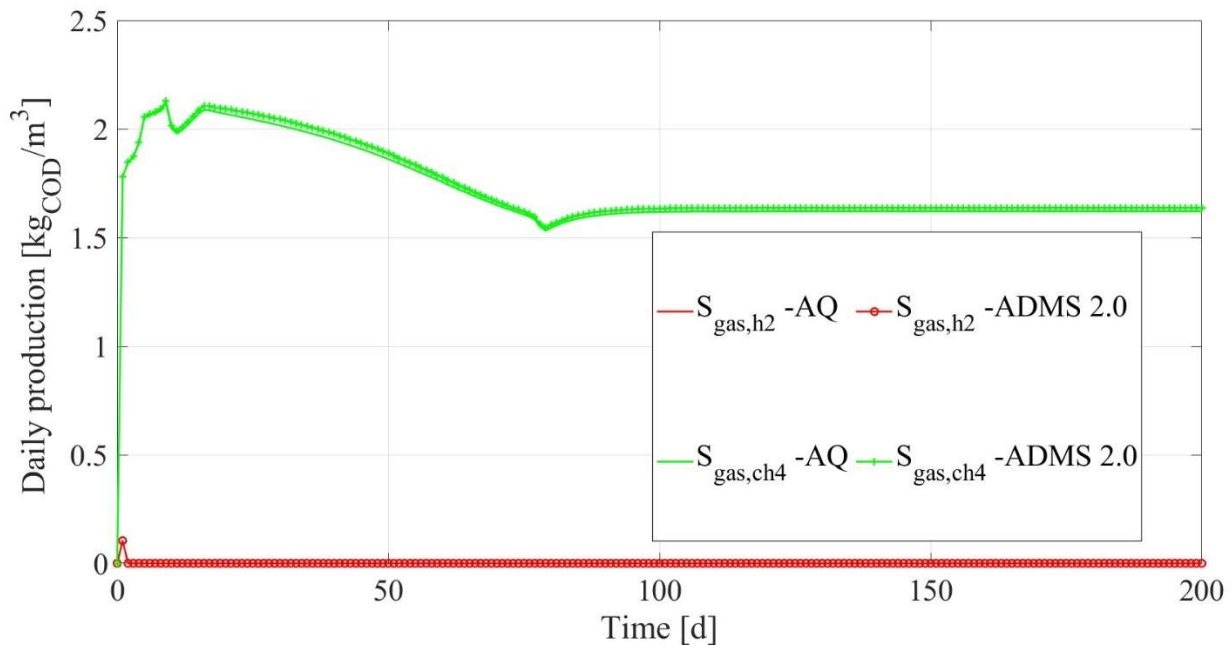


Figure 81. Simulated concentration trends for hydrogen and methane concentration in gas phase (simulation carried out at constant pH).

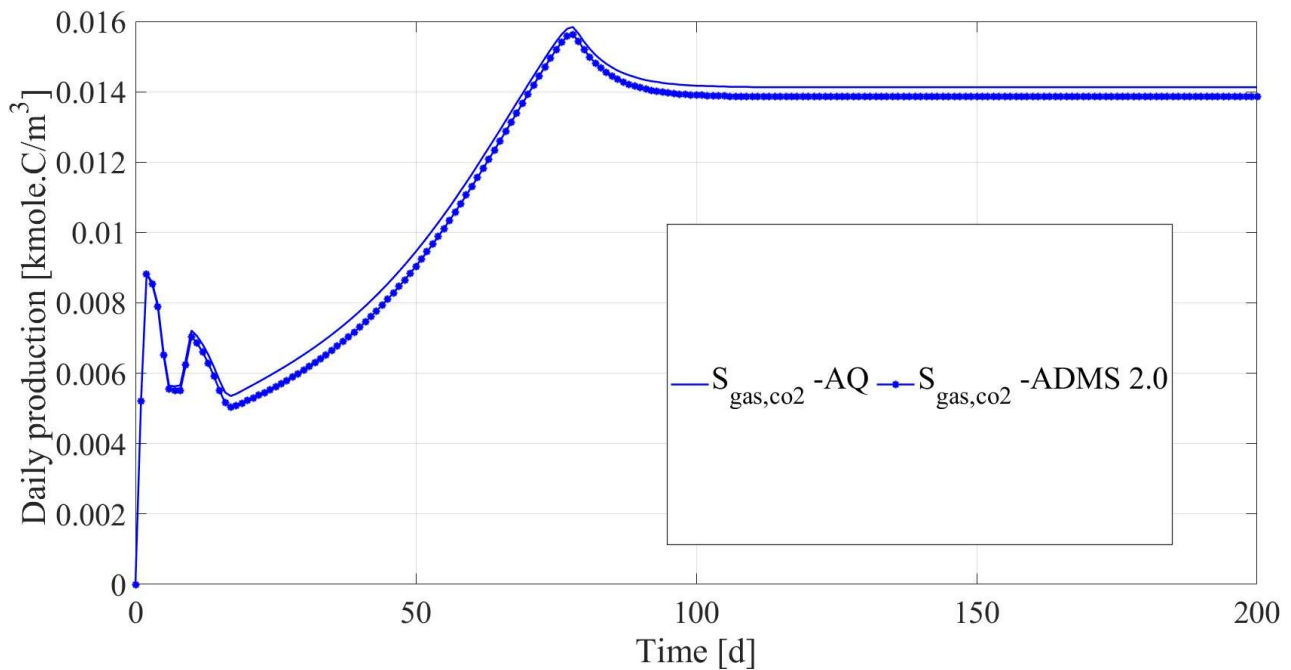


Figure 82. Simulated concentration trends for carbon dioxide in gas phase (simulation carried out at constant pH).

The overlapping of almost all concentration trends provided by the two software tools gives a graphical proof of the quality of the calculation core of the ADMS 2.0 software tool. Anyway, a numerical information about the consistency of the results has been defined in order to provide overall descriptive parameter to assess the detachment between the curves. This parameter is calculated as the mean error that affect each chemical compound and its calculation consists in two step: for the i compound, the calculation of the relative error at each timestep and then the calculation of the mean associated to these relative errors, in order to obtain a synthetic index of deviation between the curves. The relative error is calculated against the data obtained from simulation in AQUASIM 2.0, which, when compared with benchmark data at time 200, provided more accurate data than ADMS 2.0. In Table 27 values of mean error for each compound are

described. This values are relevant (higher than 1%) only for the aforementioned elements related to the inorganic carbon, in which the error is equal to about 2.7 %.

Table 27 Mean relative error (calculated between AQUASIM 2.0 and ADMS 2.0 results) associated to each simulated compound, simulation at constant pH.

<i>Variable</i>	$\bar{\epsilon}_{constant\ pH} [\%]$
S _{su}	2.8 E-02
S _{aa}	5.3 E-03
S _{fa}	4.8 E-03
S _{va}	2.5 E-02
S _{bu}	1.5 E-02
S _{pro}	1.9 E-02
S _{ac}	5.3 E-02
S _{h2}	1.3 E-02
S _{ch4}	9.1 E-01
S _{IC}	2.7 E+00
S _{IN}	1.8 E-02
S _I	8.0 E-03
X _C	7.5 E-03
X _{ch}	8.1E-03
X _{pr}	2.1 E-02
X _{li}	2.9 E-02
X _{su}	1.7 E-02
X _{aa}	2.0 E-02
X _{fa}	2.5 E-02
X _{c4}	6.2 E-03
X _{pro}	1.4 E-02
X _{ac}	1.9 E-02
X _{h2}	1.1 E-02
X _I	1.1 E-02
S _{cat}	0.0 E+00
S _{an}	0.0 E+00
S _{va-}	2.8 E-02
S _{bu-}	1.5 E-02
S _{pro-}	1.7 E-02
S _{ac-}	5.4 E-02
S _{hco3-}	2.7 E+00
S _{nh3}	1.6 E-02
S _{gas,h2}	1.5 E-01
S _{gas,ch4}	1.0 E+00
S _{gas,co2}	2.7 E+00

In order to provide a single parameter to describe the quality of the numerical results obtained by the ADMS 2.0 calculation core, an overall mean relative error has been calculated and indicated with the symbol $\bar{\epsilon}_{constant\ pH}$. It represents the mean of the error listed in Table 27 and its value is:

$$\bar{E}_{constant\ pH} = 0.31\ \%$$

As expected the overall estimation of the relative error which affects the ADMS 2.0 simulation results is lower than 1%, demonstrating the substantial accuracy of the software tool for the scenario implented.

Another comparison between results provided by AQUASIM 2.0 and ADMS2.0 has been carried out in order to asses the functioning of the software tool also for active pH inhibition functions. In the “reactor parameter” section, box “pH” has been set equal to 0 and the concentration of ion S_{H+} has been calculated by the software for each timestep. The input values used for the simulation are equal to the ones used for the pH constant simulation yet studied.

Results at non constant pH presents the same characteristics of the ones refererred to constant pH in terms over overlapping of trend between AQUASIMA 2.0 and ADMS2.0 calculation.

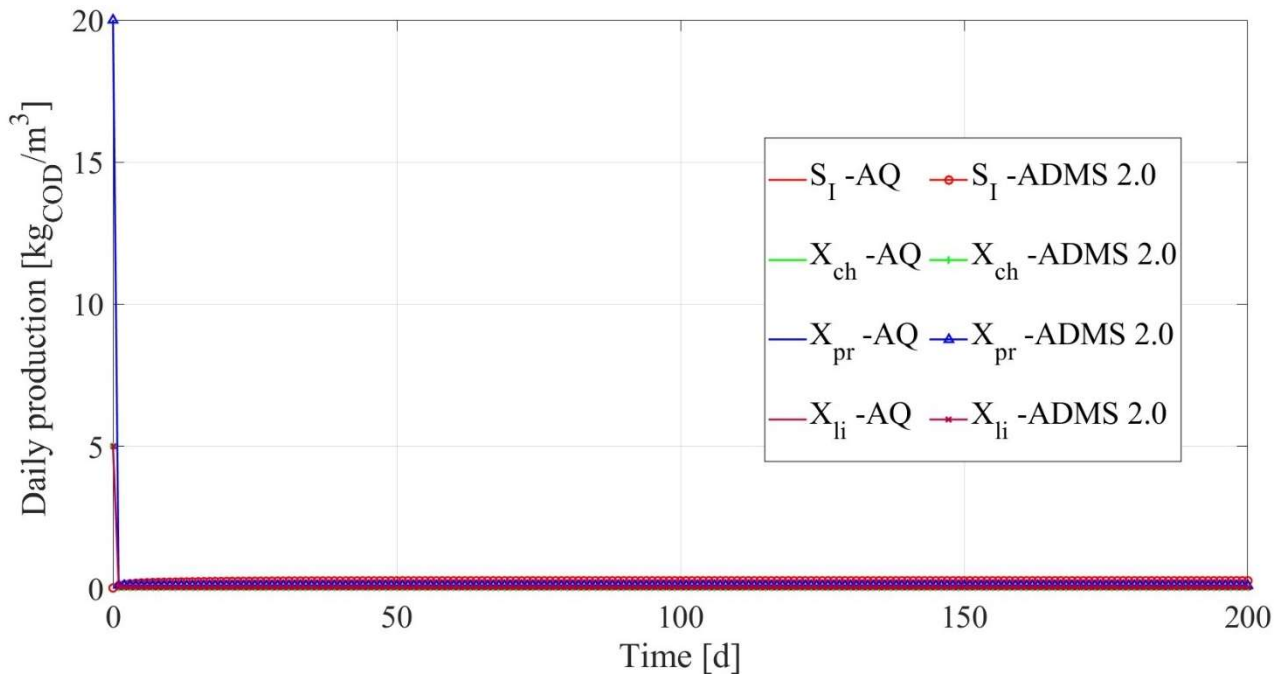


Figure 83. Simulated concentration trends for disintegration and hydrolysis processes (simulation carried out with pH calculation).

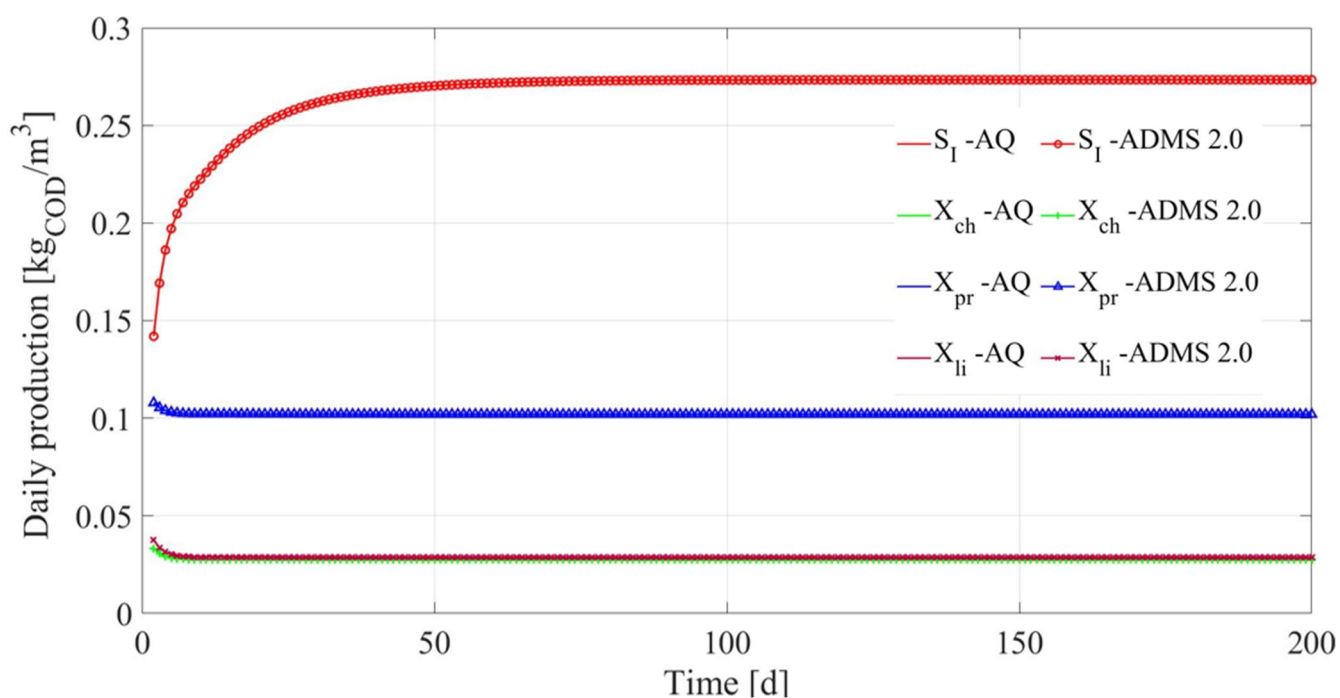


Figure 84. Simulated concentration trends for disintegration and hydrolysis processes from day 3 to 200 (simulation carried out with pH calculation).

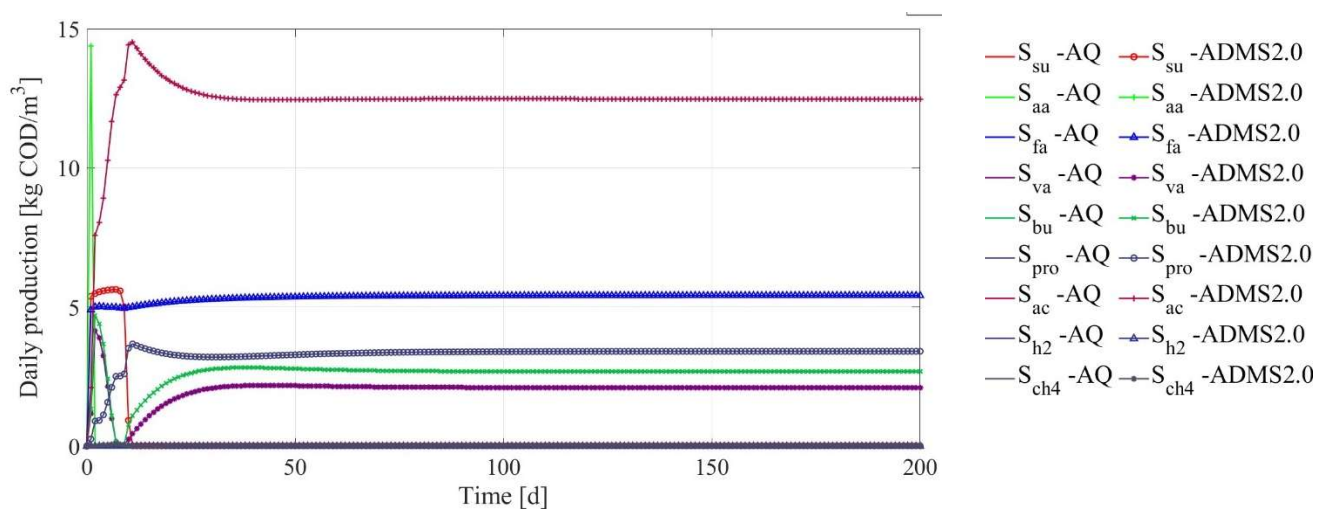


Figure 85. Simulated concentration trends for uptakes processes (simulation carried out with pH calculation).

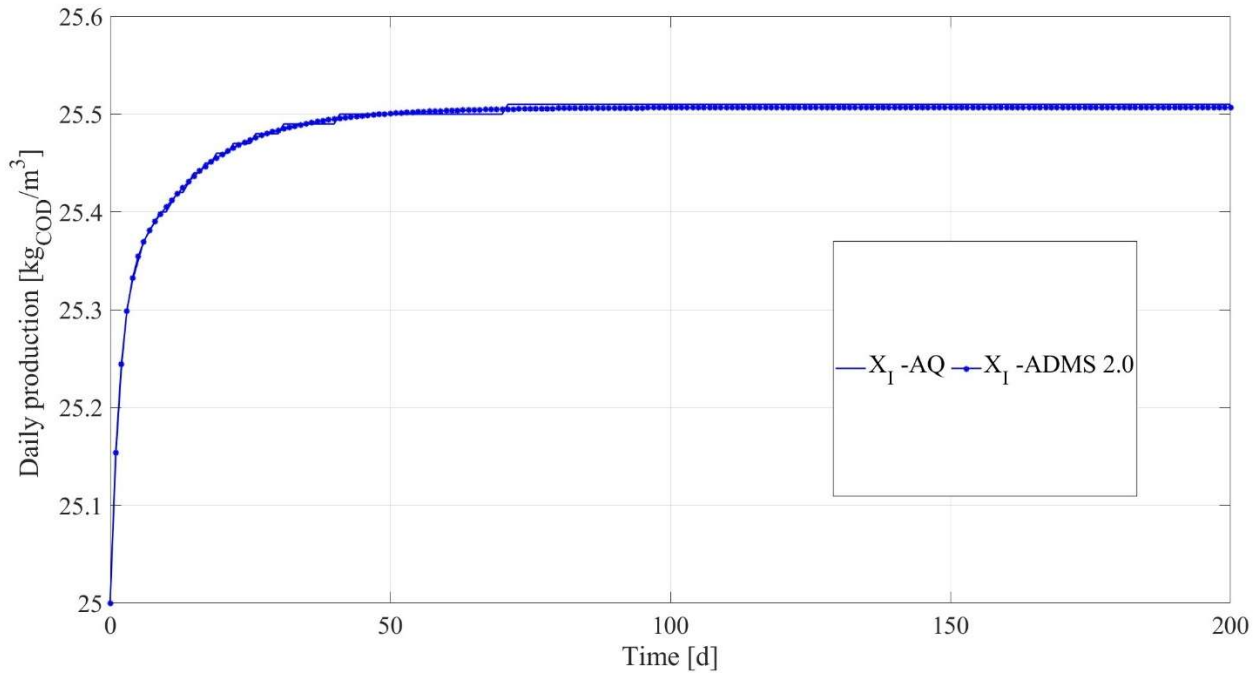


Figure 86. Simulated concentration trend for particulate inert (simulation carried out at constant pH).

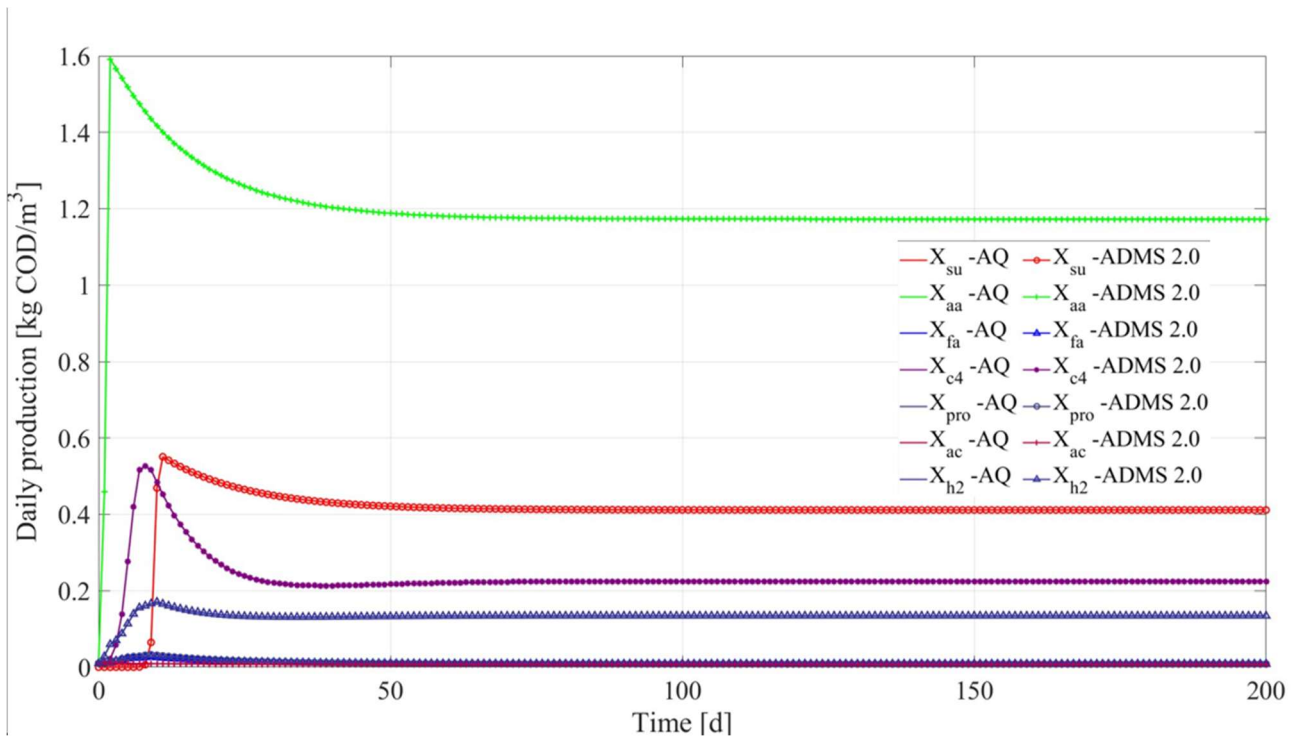


Figure 87. Simulated concentration trends for decay processes (simulation carried out with pH calculation).

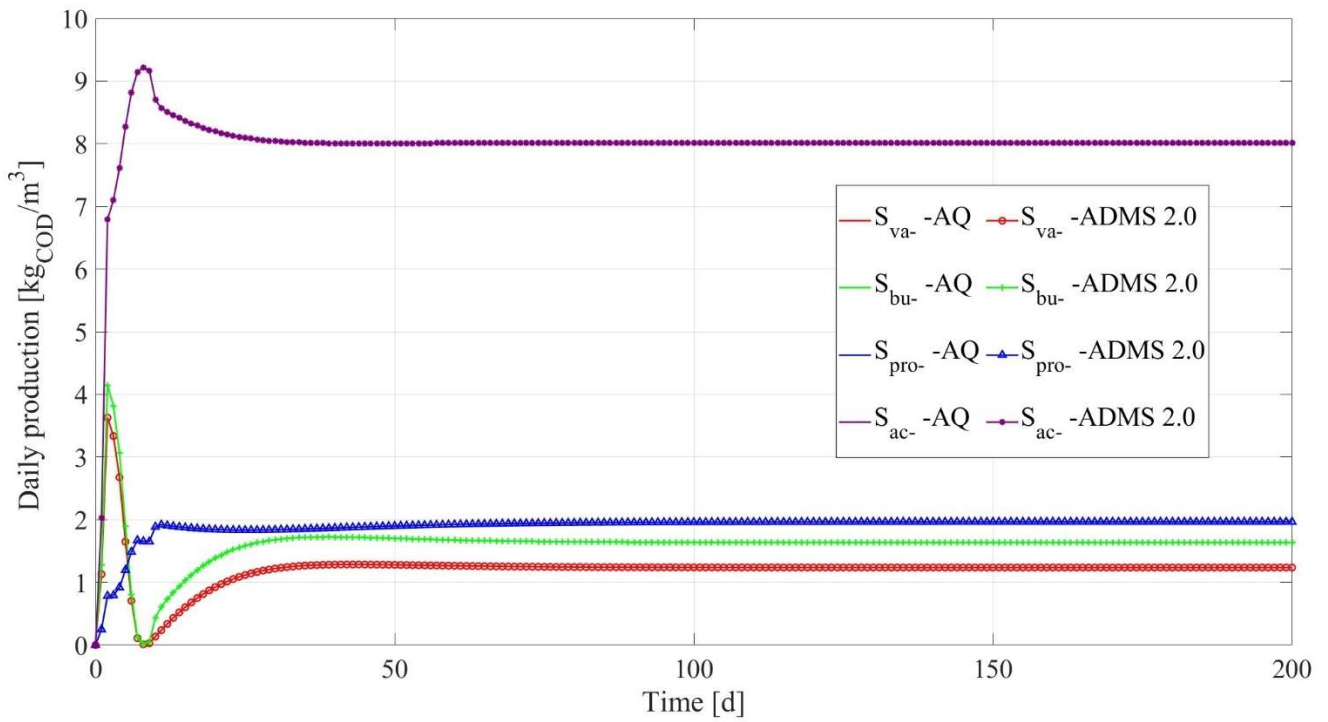


Figure 88. Simulated concentration trends for ion compounds (simulation carried out with pH calculation).

Also in this case, a detachments between values related to inorganic carbon compounds are detected, as shown in Figure 89, Figure 91 and Figure 92.

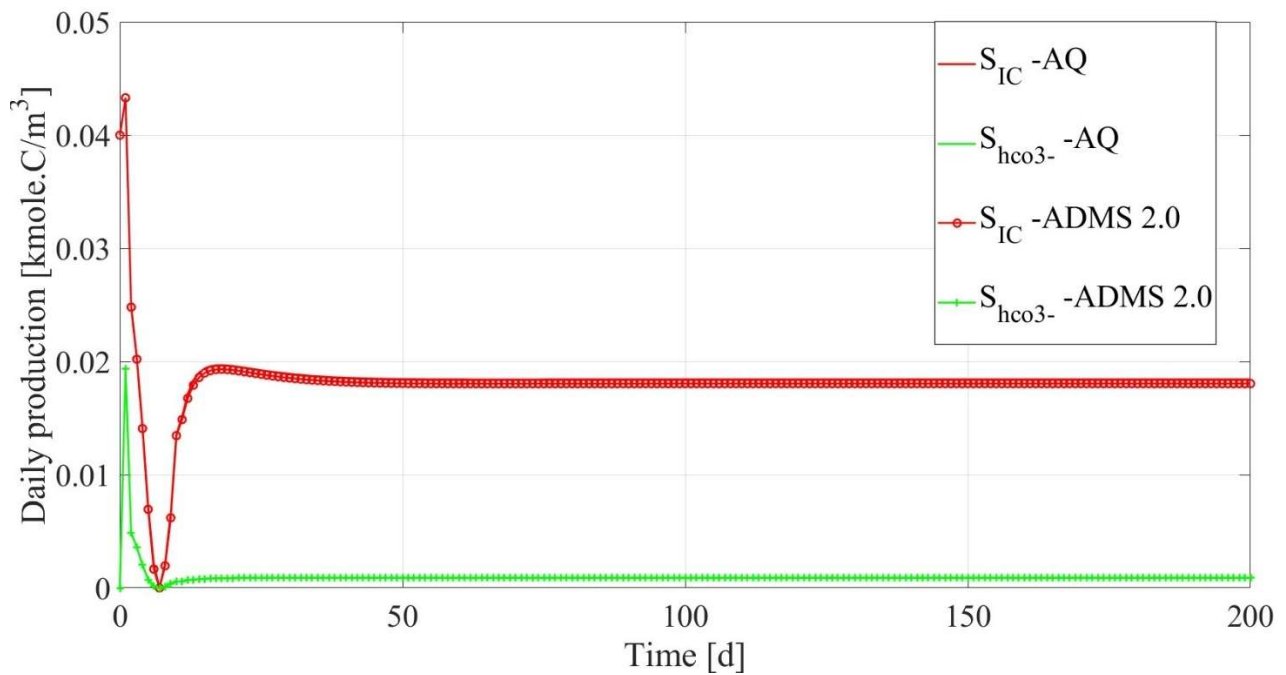


Figure 89. Simulated concentration trends for inorganic carbon and HCO_3^- ion (simulation carried out with pH calculation).

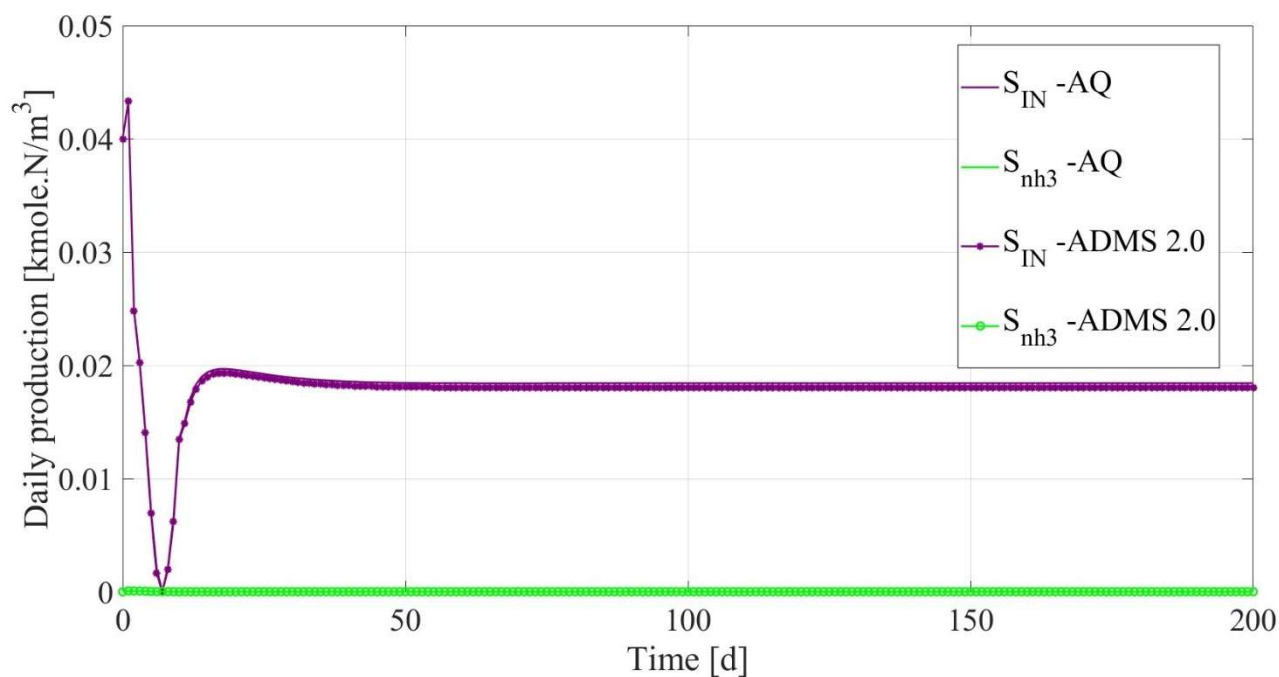


Figure 90. Simulated concentration trends for inorganic nitrogen and NH_3 (simulation carried out with pH calculation).

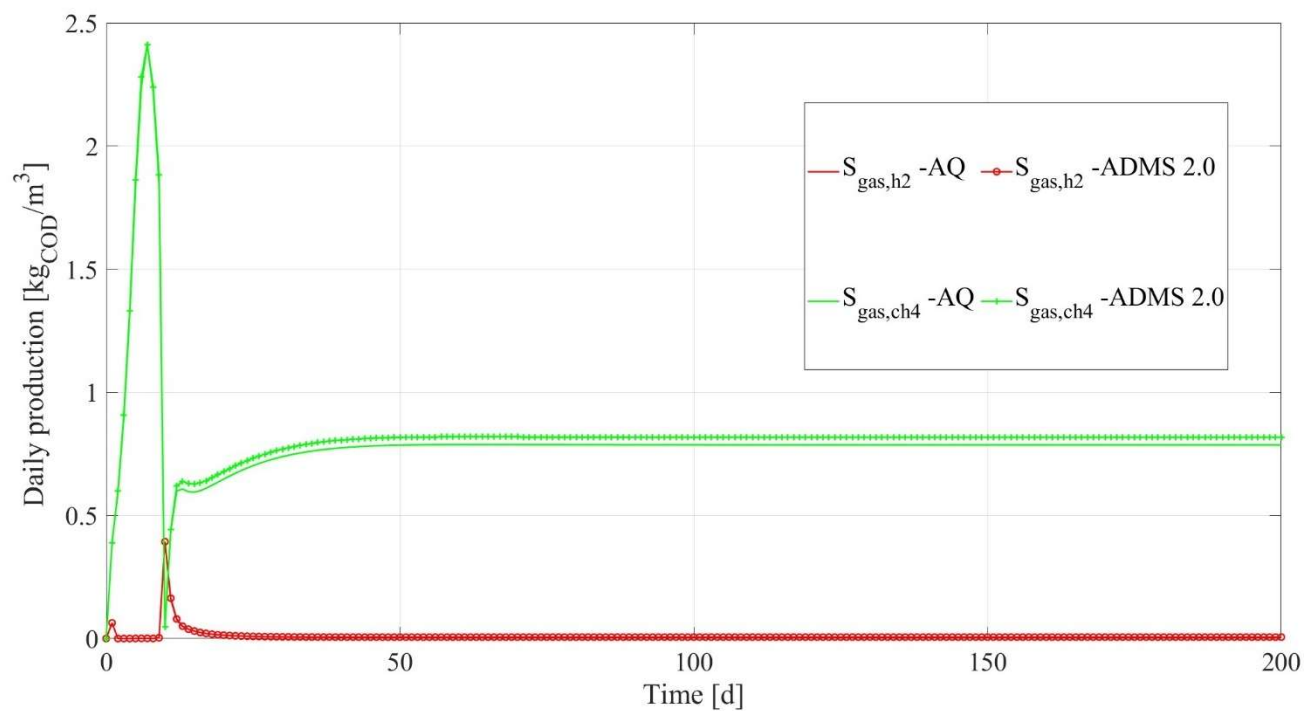


Figure 91. Simulated concentration trends for hydrogen and methane concentration in gas phase (simulation carried out with pH calculation).

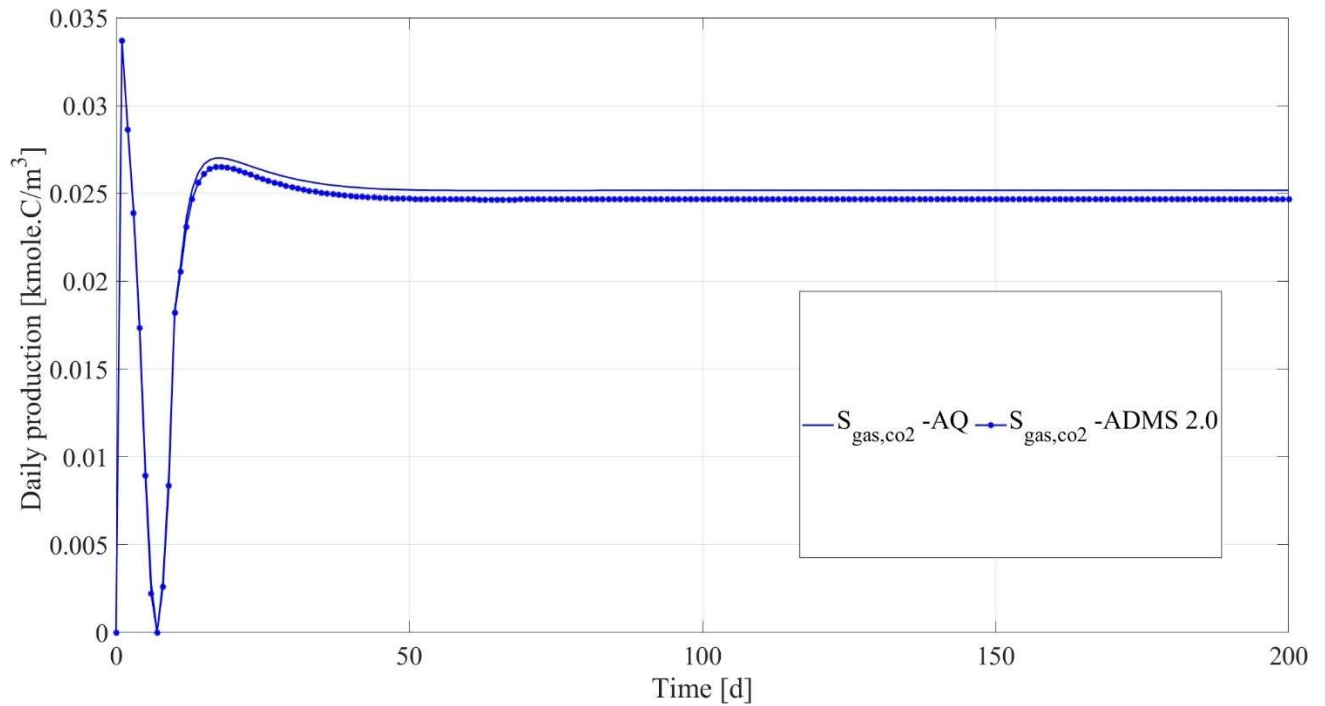


Figure 92. Simulated concentration trends for carbon dioxide in gas phase (simulation carried out with pH calculation).

Considering data collected in Table 28, it's possible to notice that the differences between AQUASIM 2.0 and ADMS 2.0 results are increased in comparison to the ones detected in the pH constant simulation. In fact the mean error associated to inorganic carbon, HCO_3^- ion and carbon dioxide is equal to about 2.6%, but the error associated to liquid phase and gas phase methane is equal to about 3.9%. This evidence suggests that the discrepancy between data provided by the calculation of the two software tools is linked to calculation errors which increase adding sources of instability to the model, as, in this case, the activation of pH inhibitions.

Table 28. Mean relative error (calculated between AQUASIM 2.0 and ADMS 2.0 results) associated to each simulated compound, simulation with pH calculation.

<i>Variable</i>	$\bar{\epsilon}_{\text{calculated pH}} [\%]$
S_{su}	1.5E-02
S_{aa}	4.9E-03
S_{fa}	2.0E-02
S_{va}	9.6E-02
S_{bu}	9.9E-02
S_{pro}	2.9E-02
S_{ac}	2.2E-02
S_{h2}	1.1E-01
S_{ch4}	3.8E+00
S_{IC}	2.6E+00
S_{IN}	1.9E-02
S_{I}	1.1E-02
X_{C}	9.8E-03
X_{ch}	9.9E-03
X_{pr}	2.5E-02
X_{li}	1.8E-02
X_{su}	1.2E-02

X _{aa}	1.8E-02
X _{fa}	1.8E-02
X _{c4}	7.9E-02
X _{pro}	2.3E-02
X _{ac}	3.9E-03
X _{h2}	5.6E-02
X _I	1.2E-02
S _{cat}	0.0E+00
S _{an}	0.0E+00
S _{va-}	1.1E-01
S _{bu-}	1.1E-01
S _{pro-}	2.8E-02
S _{ac-}	2.1E-02
S _{hco3-}	2.6E+00
S _{nh3}	1.7E-02
S _{gas,h2}	2.1E-01
S _{gas,ch4}	3.9E+00
S _{gas,co2}	2.6E+00

As seen before, also in this case an overall mean error is provided:

$$\overline{E}_{pH \text{ calculation}} = 0.48 \%$$

As expected by the consultation of Table 28, the value of the error is higher than the one calculated for the condition of constant pH. Anyway the percentage value detected is lower than 1%, confirming the accuracy of the simulation tool results.

Finally, a comparison between the time required to implement input data in ADMS 2.0 and AQUASIM 2.0 is provided. Considering the fact that AQUASIM 2.0 doesn't admit the import of data from excel files directly, it is clear that the ADMS 2.0 import section represents an improvement for the user.

However, considering an implementation without the import from excel, input data can be inserted in ADMS2.0 in about 15 minutes for an entry level user who has the data. AQUASIM 2.0 requires at least 1 hour and a half to implement the entire ADM1, also for an advanced user.

The fact that an entry level user can easily implement the input data in ADMS 2.0 GUI represents the core novelty of this proposal, in addition to the assurance that the validation of procedure has been conducted by comparing data with reference benchmarks results: in fact calculation core developed in Python or MATLAB have been yet developed, but the GUI entry level user-oriented has been not released yet.

Conclusions

The work presented in this document has been aimed to improve knowledge and technological tools dedicated to the anaerobic digestion process diffusion. In fact this conversion process admits to elaborate residual biomasses while producing compounds with an energetic value, such as biomethane. In addition to this, the anaerobic digestion of foodwastes represents an application of circular economy principles and promotes economic, social and environmental sustainable practices such as the recover of discharges, the limitation of greenhouse gasses release in atmosphere, the possibility to provide energy also in absence of a power grid, almost in every inhabited place and the use of a plethora of different biomasses and biomass combinations. The versatility of the anaerobic digestion process for energy production is associated to a strong complexity of the physico-chemical and bio-chemical processes which characterize the reaction: it follows that an optimized process can be carried out only knowing the potential of the employed substrate, the characteristics of the inoculum and the calibrated functioning of the production plant.

In this scenario, this research has been finalized in detecting and applying, after an accurate choice of the laboratorial instruments, a easy but reliable procedure to measure the biomethane potential of agrifood wastes produces in Tuscia territory, considering also the seasonality of the available biomasses. Biomethane potential determination tests have been carried out, associated also to an energetic characterization of biomasses submitted to anaerobic digestion, using scale batch reactors and preparing samples according to DOE principles. At first, brewery spent grains have been submitted to energetic characterization and pretreatments, in order to define their suitability to the anaerobic digestion process. Results, which demonstrate the compatibility of this residual biomass to the anaerobic digestion conversion, have lead to the comprehension of the necessity to adding to the chain of characterization also the biomethane potential determination and the application of a standardized procedure to carry out tests minimizing errors. After this experience, energetic characterization and BMP determination tests have been carried out on fruit and vegetables wastes, artichoke wastes and olive pomace. All these biomasses have been collected by 0-km farmers operating on Tuscia territory. Results of anaerobic conversion demonstrate that these residual biomasses available in Tuscia territory are suitable for anaerobic digestion and represent a source comparable to other ones yet used in other territories, as confirmed by literature data.

In addition to this, the development of a software tool with a user friendly graphical interface for the simulation of anaerobic digestion processes has been carried out. The tool named ADMS 2.0 has been developed as a stand alone application by using MATLAB computation core. The calculation core has been implemented referring to Anaerobic Digestion Model N.1, the differential equations of which are solved by mean of ode functions. ADMS 2.0 software tool has been developed to be user friendly, complete, widely interfaceable and customizable. The software has been validated using two methods: the same input data set has been implemented both in ADMS 2.0 and in AQUASIM 2.0 (which is a widely used software tool for anaerobic digestion processes simulation). Trends obtained by the simulations have been compared and an overall estimation of the relative error afflicting ADMS2.0 data has been calculated. Data obtained have been also compared with benchmark results provided by [71] and an overall relative error has been calculated. In addition to this, a simulation with pH inhibition equation activated has been carried out. The overall relative errors calculated by all the comparison described are lower than 1%: it follows that the software is validated. The implementation carried out through ADMS 2.0 is more guided and fast than the one associated to AQUASIM 2.0. Import and export of data and results allow the user to reduce the time spent on simulation preparation and data processing, consistently. ADMS 2.0 guides the user to the compilation of the model, providing guidelines to the comprehension of the mathematical model itself. On the other hand, AQUASIM 2.0 is more stable in its calculation processes and admits the user to carry out

sensitivity analysis (without taking into account, obviously, that it is not a software exclusively dedicated to the simulation of anaerobic digestion processes).

Due to the stiffness of the differential equation system proposed by ADM1, results can consistently change on the base of the ODE solver used and the implementation of the equations describing pH inhibition. For this reason, according to [71], ADMS 2.0 allows the user to choose the ODE solver and the pH inhibition function which optimize the simulation results.

Further development of this work include:

- the energetic characterization of more agri-food wastes produced in Tuscia territory, and BMP determination associated to their employment in anaerobic digestion with a calendarization of their availability during the year;
- the evaluation of effects on BMP value of the co-digestion of different agri-wastes biomasses;
- the enhancing of the characterization chain aimed to analyse also biochemical and chemical composition of agrifood and biomasses, in order to obtain values useful to simulate the products of the anaerobic digestion by mean of ADM1 model;
- the constitution of a standardized database in which collect all the data about the agri-food residual biomasses, organized in sheets suitable to be inserted in the ADMS 2.0 software tool;
- the distribution of the ADMS 2.0, which is a beta-test, to interested laboratories/research centers to detect the weaknesses and the elements which can be improved;
- the addition, in the ADMS 2.0 calculation core, of DAE formulations of the system and sensitivity analysis;
- the development of an “add-on” to enhance the integration of ADMS 2.0 with COMSOL Multiphysics.

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List of figures

Figure 1. Top ten ranking of Countries for electricity generation from biogas in 2022 [6].	8
Figure 2. Top ten ranking of Countries heat generation from biogas in 2022 [6].	8
Figure 3. Primary energy production from biogas in the European Union (27) since 2000 (Mtoe) [3].	9
Figure 4. Gross electricity production from pure biogas and from biogas blended in the grid in the European Union in 2021 and 2022 in GWh [3].	10
Figure 5. Gross heat production in the transformation sector from pure biogas and from biogas blended in the grid in the European Union in 2021 and 2022 (in ktoe) [3].	11
Figure 6. Primary production from biogas in the European Union in 2022 (in ktoe) [3].	12
Figure 7. Primary energy production from biogas in the European Union countries at the end of 2022* (in ktoe), with the respective shares of each sub-sector [3].	13
Figure 8. Relative growth rate of psychrophilic, mesophilic and thermophilic methanogens [13].	20
Figure 9. Scheme of a CSTR without recirculation [8].	24
Figure 10. Scheme of a CSTR with recirculation [5].	25
Figure 11. Plug flow reactor without recirculation [8].	25
Figure 12. Plug flow reactor with solid fraction recirculation [8].	26
Figure 13. Plug flow reactor with both solid and liquid fraction recirculation [8].	26
Figure 14. Plant scheme for single and double stage reactors [8].	26
Figure 15. Balances used in CIRDER laboratory.	27
Figure 16. Digitalheat Drying and sterilization oven JP Selecta.	27
Figure 17. ME320 Muffle.	28
Figure 18. Container used in laboratory.	28
Figure 19. Cutting mill and sieve to reduce dried biomass to the required particle size.	29
Figure 20. Desiccator.	29
Figure 21. Biogas Endeavour by BPC Instruments [27].	30
Figure 22. Cumulate methane production.	42
Figure 23. Average curves of the cumulate biomethane production for each experiment's level.	42
Figure 24. Daily methane production registered between days 0 to 12.	43
Figure 25. Daily biomethane production (from day 0 to day 12) expressed using average values and standard deviation.	44
Figure 26. Daily increasing of methane production.	44
Figure 27. Relative standard deviation trends.	45
Figure 28. Methane yield associated to the production during days 0 to 12.	46
Figure 29. Biomethane flow rate collecting during days 21 to 35.	48
Figure 30. Daily biomethane production (from day 21 to day 35) expressed using average values and standard deviation.	48
Figure 31. Percentage variation of daily production of biomethane between days 7 to 35.	49
Figure 32. Methane yield collected during days 21 to 35.	49
Figure 33. Raw artichoke wastes.	51
Figure 34. Dimension of artichoke wastes.	51
Figure 35. Dimension of olive pomace.	52
Figure 36. Methane cumulate production of artichoke wastes.	55
Figure 37. Average curves of the cumulative biomethane production for artichoke wastes and blank samples.	55
Figure 38. Relative standard deviation trend associated to methane production of artichoke wastes and blank samples.	56
Figure 39. Daily methane production of artichoke wastes.	57
Figure 40. Daily biomethane production expressed using average values and standard deviation.	57
Figure 41. Daily increasing of methane production.	58

Figure 42. Methane yield associated to the anaerobic digestion of artichoke wastes.....	58
Figure 43. Cumulate methane production from olive pomace.	59
Figure 44. Average curves of the cumulate biomethane production by olive pomace.	60
Figure 45. Relative standard deviation trends (olive pomace).	60
Figure 46. Daily methane production registered (olive pomace).	61
Figure 47. Daily biomethane production (olive pomace) expressed using average values and standard deviation.	62
Figure 48. Daily increasing of methane production for anaerobic digestion of olive pomace.....	62
Figure 49. Methane yield associated to the production from olive pomace.....	63
Figure 50. Results obtained during experimental campaigns.....	66
Figure 51. Main menu of the software tool.	76
Figure 52. Intermediate window for specific compound initial concentration assignment.....	77
Figure 53. Initial soluble compounds concentration window.....	77
Figure 54. Initial particulate compounds concentration window.....	78
Figure 55. Reactor parameters window.....	78
Figure 56. Stoichiometris and Biochemicals parameters selection window.....	79
Figure 57. Yield product on substrate window.....	80
Figure 58. C and N content window.....	80
Figure 59. Biomass yield window.....	81
Figure 60. Half saturation values window.....	81
Figure 61. 50% inhibitory concentration.....	81
Figure 62. First order parameters.....	82
Figure 63. Acid/base parameters.....	82
Figure 64. pH parameters settings.....	83
Figure 65. Simulations settings.....	83
Figure 66. Results intermediate window.....	84
Figure 67. Resulting hydrolysis and disintegration trends for species window.....	84
Figure 68. Resultings uptakes trends window.....	85
Figure 69. Resulting inorganic compounds trends window.....	85
Figure 70. Resulting biomass trends window.....	86
Figure 71. Resulting ions concentration trends window.....	86
Figure 72. Resulting gas concentration trends window.....	87
Figure 73. Simulated concentration trends for disintegration and hydrolysis processes (simulation carried out at constant pH).....	97
Figure 74. Simulated concentration trends for disintegration and hydrolysis processes from day 3 to 200 (simulation carried out at constant pH).	98
Figure 75. Simulated concentration trends for uptakes processes (simulation carried out at constant pH). ...	98
Figure 76. Simulated concentration trend for particulate inert (simulation carried out at constant pH).	99
Figure 77. Simulated concentration trends for decay processes (simulation carried out at constant pH).	99
Figure 78. Simulated concentration trends for ion compounds (simulation carried out at constant pH).	100
Figure 79. Simulated concentration trends for inorganic carbon and HCO_3^- ion (simulation carried out at constant pH).....	101
Figure 80. Simulated concentration trends for inorganic nitrogen and NH_3 (simulation carried out at constant pH).....	101
Figure 81. Simulated concentration trends for hydrogen and methane concentration in gas phase (simulation carried out at constant pH).....	102
Figure 82. Simulated concentration trends for carbon dioxide in gas phase (simulation carried out at constant pH).....	102
Figure 83. Simulated concentration trends for disintegration and hydrolysis processes (simulation carried out with pH calculation).	104
Figure 84. Simulated concentration trends for disintegration and hydrolysis processes from day 3 to 200 (simulation carried out with pH calculation).....	105

Figure 85. Simulated concentration trends for uptakes processes (simulation carried out with pH calculation).	105
Figure 86. Simulated concentration trend for particulate inert (simulation carried out at constant pH).	106
Figure 87. Simulated concentration trends for decay processes (simulation carried out with pH calculation).	106
Figure 88. Simulated concentration trends for ion compounds (simulation carried out with pH calculation).	107
Figure 89. Simulated concentration trends for inorganic carbon and HCO_3^- ion (simulation carried out with pH calculation).	107
Figure 90. Simulated concentration trends for inorganic nitrogen and NH_3 (simulation carried out with pH calculation).	108
Figure 91. Simulated concentration trends for hydrogen and methane concentration in gas phase (simulation carried out with pH calculation).	108
Figure 92. Simulated concentration trends for carbon dioxide in gas phase (simulation carried out with pH calculation).	109

List of table

Table 1. Moisture content of raw BSG.....	33
Table 2. Ash content for raw BSG.....	33
Table 3. Volatile solids content for raw BSG.....	34
Table 4. Ash content for low temperature pre- treated BSG.....	34
Table 5. Volatile solids content for low temperature pre- treated BSG.	34
Table 6. Ash content for alkali pre-treated BSG.....	35
Table 7. Volatile solids content for alkali pre-treated BSG.	35
Table 8. Results of the characterization tests.....	35
Table 9. Moisture content of the inoculum and the substrate.	41
Table 10. Ash content on dry basis of inoculum and substrate.....	41
Table 11. Energetic characteristics of inoculum and substrate.	41
Table 12. Data for the ANOVA on accumulated biomethane production at day 12.	46
Table 13. Analysis of Variance for the cumulate biomethane production at day 12.....	46
Table 14. Data for the ANOVA on accumulated biomethane production at day 35..	50
Table 15. Analysis of Variance for the cumulate biomethane production at day 35.....	50
Table 16. Moisture content of the inoculum and the substrates (artichoke and olive pomace wastes).	53
Table 17. Ash content on dry basis of inoculum and substrates (artichoke and olive pomace wastes).	53
Table 18. Energetic characteristics of inoculum and substrates (artichoke and olive pomace wastes).	53
Table 19. Comparison table of the results of biomass characterization.....	64
Table 20. BMP comparison between investigated vegetables.....	66
Table 21. Stoichiometric and equilibrium coefficients and constants, kinetic parameters and rates, dynamic state and algebraic variables used in ADM1 [16].....	71
Table 22. Compounds considered in ADM1.....	72
Table 23. Biochemical rate coefficients and kinetic equations for soluble components [72].....	74
Table 24. Biochemical rate coefficients and kinetic equations for particulate components [72].	75
Table 25. Values implemented for the simulation.....	92
Table 26. Results and comparison of AQUASIM and ADMS 2.0 Simulation.	96
Table 27 Mean relative error (calculated between AQUASIM 2.0 and ADMS 2.0 results) associated to each simulated compound, simulation at constant pH.....	103
Table 28. Mean relative error (calculated between AQUASIM 2.0 and ADMS 2.0 results) associated to each simulated compound , simulation with pH calculation.	109

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