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Master's degree in Environmental and Land Engineering – Environmental Protection

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**Reuse of agro-food industry
wastewater for fertirrigation:
laboratory investigation on the role of
soil as a natural filter**

Candidate:

Moraglio Noemi

Supervisors:

Tosco Tiziana Anna Elisabetta

Granetto Monica

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ABSTRACT

During the last years, problems in the availability of water increased due to climate change and irresponsible use of this resource. The depletion of water sources has become a major problem in the society since it may cause diseases, death, and reduction in agricultural production. This study aims to investigate an innovative approach for sustainable water resource management, namely Soil Aquifer Treatment (SAT), which is a specific case of Managed Aquifer Recharge (MAR) exploiting the possible reuse of wastewater for irrigation purposes. One of the industrial sectors that use the largest quantities of water in their production processes and therefore produces a great amount of wastewater is represented by the agro-food industry. In this thesis a lab-scale study was performed to assess the main processes occurring when wastewater originated in two food production plants when such water is put in contact with soil or sand, thus simulating its use for irrigation. Batch experiments with different duration and agitation conditions and column leaching tests were performed using representative wastewater of dairy industry and jam production industry collected from specific sites. The analyses included measurements of pH, Total Organic Carbon (TOC), Total Nitrogen (TN), Electrical Conductivity (EC), turbidity, Redox Potential (ORP) to assess the effectiveness of different types of substrates in the degradation of dissolved organic matter (DOM). Analyses provided insights into chemical changes during the treatment process, which need to be further investigated. The results highlighted that both batch tests and column leaching tests proved effective in decreasing the TOC present in the effluent over time. This study contributes to understanding the potential of MAR and SAT as tools for sustainable water resource management, providing a preliminary scientific basis for implementing these practices in specific environmental contexts.

1. Introduction

1.1. Water resources

Nowadays, water is recognized as being one of the fundamental factors for the development of regions and life. The availability of water plays a major role in various fields, such as agriculture, industrial development and, the most important, health and food providing for people.

The lack of water is one of the leading causes of diseases, death, reduction in agricultural production, while a constant increasing in the food and water demand has been registered due to a rapid population growth. Moreover, the increasing of temperature due to climate changes, caused by the increasing of greenhouse gases in the atmosphere, will cause a further depletion of this resource and decline in agricultural yield.

One of the most important sources of fresh water is groundwater, which most of the times provide potable water for the cities and industries near which they are. The overexploitation of groundwater in the past few decades has caused a lowering in the groundwater levels and has led to severe environmental harm, including groundwater salinization (intrusion of salt water), particularly threatening coastal aquifers [1], [2]. The continuous stress on groundwater has been worsened by the contamination of the water with pollutants and contaminant due to anthropogenic activities, such as nitrate, emerging pollutants (pharmaceuticals, personal care products), heavy metals, phosphorus, pathogens, herbicides, human wastes.

In non-arid regions the floods and rainy seasons can contribute to reduce the depletion of groundwater, and the water can be stored during these periods to be reused, when necessary; on the other hand, in many arid and semi-arid regions such as African countries, it is necessary to find strategies that can be applied even if there is a constant lack of water resources. It is fundamental to understand the water storage balance of each aquifer system, analysing the inputs (natural recharge, direct or indirect recharge, managed recharge) and the outputs (natural discharge, anthropogenic extraction).

There are considerable examples of water conservation practiced by old civilization to fulfil the agriculture and domestic needs worldwide: indeed, it is cheaper and easier to save and conserve water than treating non-potable water and/or wastewater. A good example can be found in the Inca population: as it is possible to observe in Machu Picchu site, they built an efficient water storage (which was collected during the flood season) and transportation system, which exploited the slope of the area and helped the distribution of water for both agricultural and domestic needs.

Nowadays, countries and organizations are developing different methods of water storage and aquifer recharge, even though there are some difficulties to deal with:

- The countries have varying hydrological conditions; therefore, it is hard to adapt the same policies and strategies everywhere.
- Studies need investments and few industries are financing the projects, though recently there has been an increasing in the awareness of the effects of climate changes.
- Many poor and developing countries, which mostly need water, cannot afford the costs related to the building of proper infrastructures and, moreover, do not have the proper technologies to collect the data from the studies done on the sites.
- The selected sites should not be near a groundwater drinking community or near the place where the groundwater is flowing into the river.

One of the most important methods for the recharge of the groundwater is the managed aquifer recharge (MAR).

1.2. Managed Aquifer Recharge (MAR)

The managed aquifer recharge is a dynamic system which intentionally introduces water into an aquifer. It encompasses various approaches designed to redirect, convey, store, seep, and replenish excess surface water into aquifers in wet periods, with the intention of recovering it during dry periods or for environmental advantages. This technique is very useful to prevent depletion of groundwater in arid and semi-arid areas, where it is quite difficult to have a natural recharge of the aquifer due to the lack of precipitations. The objectives of this technique include:

- Storage of excess water during the wet period for later use in dry periods (mainly in arid/semi-arid regions).
- The introduction of a water treatment barrier (improving water quality for future specific use).
- The creation of a hydraulic barrier that prevents seawater intrusion.
- Flood control [3].

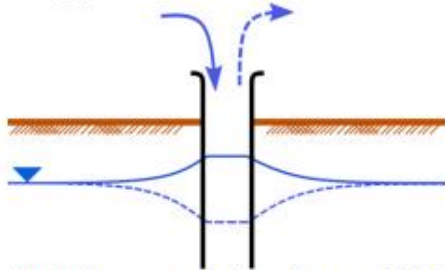
Different water sources can be utilized, basing the choice on the infrastructure and system goals: stream water, stormwater, desalinated seawater, treated wastewater. In the latter case, the system is named SAT (Soil Aquifer Treatment) and has a double function: it both helps the recharge of aquifers, and a high degree of upgrading of the wastewater can be achieved by allowing the effluent to infiltrate into the soil and move down to the groundwater.

In the artificial replenish of aquifers it is necessary to control some factors such as porosity, permeability, hydraulic conductivity, transmissivity, and the stratigraphical arrangement. Usually, the program of MAR consists in four phases [4]:

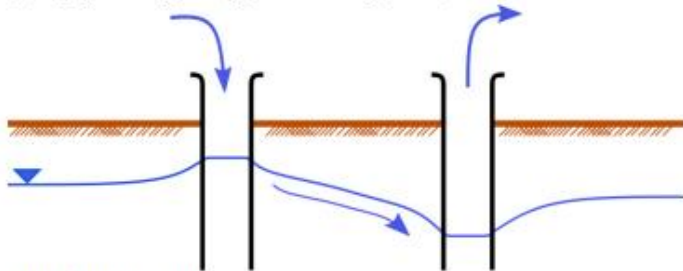
1. Feasibility studies: it is a detailed evaluation of the entire project in which studies about the parameters of the area and the water are conducted.
2. Analytical and testing: these are based on the results of the first phase. In this second part the test programs are usually designed using different existing facilities.
3. Designing and operation: depending on the first and second phase, a design of the recharge structure is made. This is a fundamental part of the entire process because if the system is not well designed the operation of the recharge structure will stop in few days. One of the most important problems is related to the clogging of infiltration systems: it causes water logging problems, that can result in several environmental hazards such as soil salinity and loss of vegetation covers or irrigated area.
4. Project implementation: it usually consists of full-scale program parameters that include sites.

There is a considerable amount of literature concerning the different methods to achieve the objective of an effective MAR, which are summarised in Figure 1.

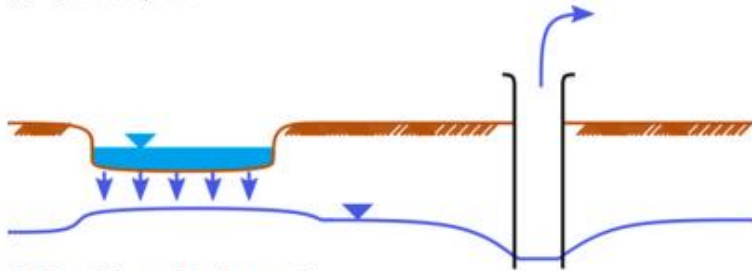
(A) Aquifer storage and recovery (ASR)



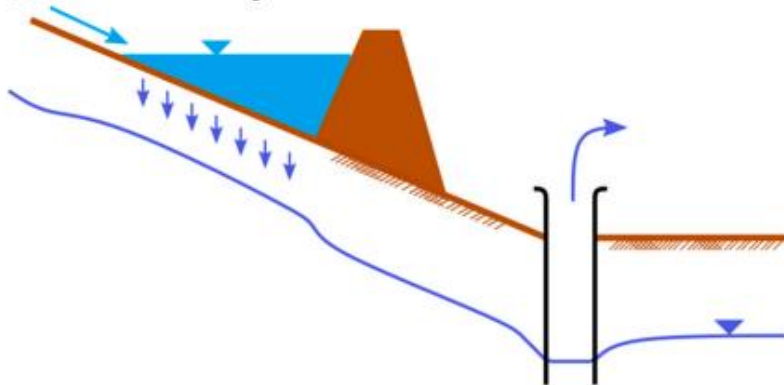
(B) Aquifer storage, transfer, and recovery (ASTR)



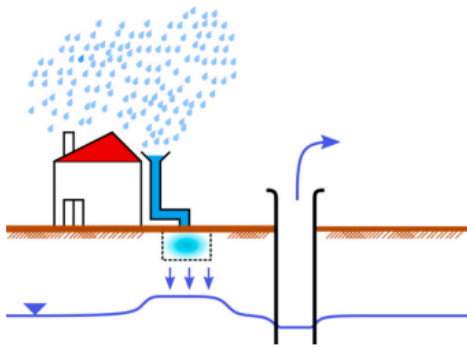
(C) Infiltration ponds



(D) Percolation tank/recharge weir



(E) Rainwater harvesting



(F) Soil aquifer treatment (SAT)

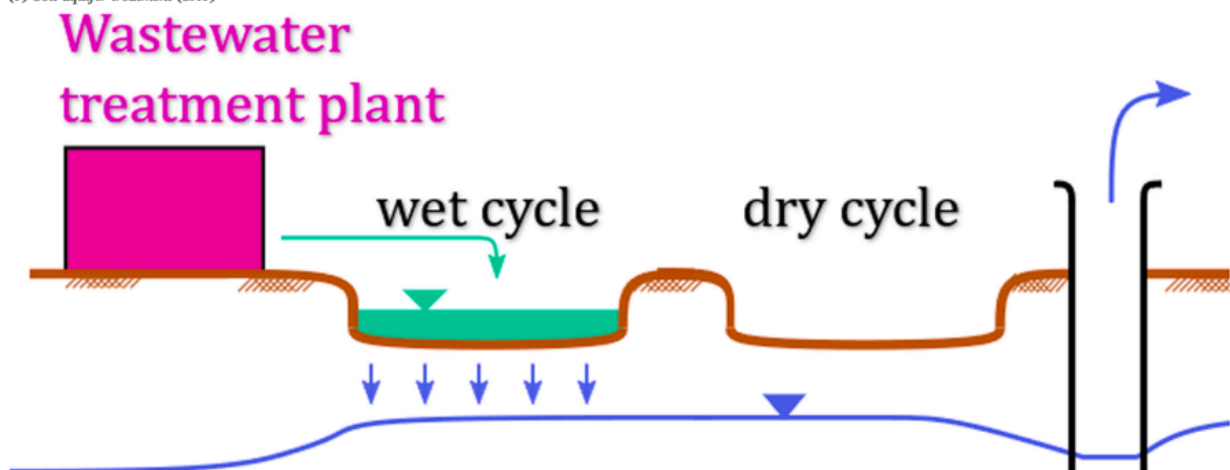


Figure 1: Schematic of managed aquifer recharge (MAR) methods. [3]

The aquifer storage and recovery (ASR) consist in injecting water into a well with the purpose of storing it and then recovery the water from the same well (useful when the storage is the main goal).

The aquifer storage, transfer, and recovery (ASTR) are similar to the previous one, except for the fact that the water is recovered from a different well: this helps an additional water purification in the aquifer thanks to the extension of the residence time.

In infiltration ponds the surface water is diverted into basins outside the watercourse, allowing water to percolate through the vadose zone to the underneath unconfined aquifer, and in the end, it is recovered from a designated well.

In the case D it is possible to observe that there is a dam built on a seasonal stream which is used to retain floodwater to let it percolate through the soil, enhancing unconfined aquifer storage, and it is ultimately recovered from a designated well located down valley.

In the second-to-last case the roof run-off is conveyed into a well or a sand/gravel-filled cell to let the water percolate to the underlying aquifer and it is collected in a designated well.

One of the most interesting and studied system is the SAT, which will be discussed in the following paragraph.

A recent review of the literature on this topic [5] done in 2019, found that from the introduction of MAR technique in the 1960s, there has been a global increase in the groundwater recharge of about 5% per year. Indeed, in 1965 the total MAR capacity was around 1 km³ per year, instead of in 2015 in which the MAR capacity increased until 10 km³. The two country leaders in MAR are India and USA in which, in 2015, there was a total MAR volume of 3070 Mm³ and 2598 Mm³, respectively.

The constant increase in the application of MAR, registered during the past years thanks to amassed research, improved operating practices, reliability, and public awareness, has helped the spread of MAR all over the world. Other countries in which MAR is practiced are reported in Table 1.

Table 1: Countries practicing MAR in 2015. [5]

Country	Volume of MAR in 2015 [Mm³]
Australia	410
Italy	461
Israel	134
Germany	870
Spain	380

Despite this substantial increase, to date the authors estimate that this technique makes up less than 3% of groundwater extraction in countries in which MAR is operated, or around 1% of global groundwater extraction: therefore, MAR is not sufficiently spread to satisfy the global rate of groundwater depletion.

1.3. Soil Aquifer Treatment (SAT)

In this unique technology the treated wastewater (TWW) is periodically introduced into infiltration basins to enhance the removal of nutrients and pathogens as it traverses the vadose zone. Subsequently, it is retrieved through wells after residing in the unconfined aquifer.

The most important feature of this treatment is its double function of both recharge the aquifer and improving the wastewater effluent to high quality recharged effluent through soil passage [3].

In the conventional SAT configuration, the treated effluent discharged from the wastewater treatment plant (WWTP) undergoes intermittent release, involving cycles of flooding and drying, to infiltrate through specific infiltration basins. SAT stands out from many managed aquifer recharge (MAR) methods due to its distinctive features: the intermittent operational pattern and the focus on water quality. Two of the key points of a well-designed SAT are the right set of periods and duration of flooding/drying cycles (usually the SAT is a year-round operation) and constant infiltration rates. During the process, the TWW percolates through the vadose zone and it is simultaneously subjected to contaminant removal: several mechanisms take place, such as physical filtration, biodegradation, adsorption, chemical precipitation, ion exchange and dilution. All these processes are facilitated in the vadose zone thanks to the high presence of oxygen, necessary for the biological treatments [6]. Following a residence time spanning from a few months to a year, encompassing both the unsaturated and saturated zones, the effluent is retrieved through recovery wells from the underlying unconfined aquifer. The prolonged residence time contributes to the effective elimination of harmful pathogens, disinfecting the effluent efficiently. Chemical, physical and biological parameters are effectively reduced, such as suspended solids (SS), chemical and biological oxygen demand (COD and BOD), ammonia, phenols, organic nitrogen, nitrite, phosphorus, iron and turbidity [6], and denitrification takes place. Ultimately, the reclaimed water is suitable for diverse applications, including groundwater recharge, agricultural and landscape irrigation, recreational use, aquaculture, and industrial purposes.

While doing a SAT there are some parameters that must be monitored, (i.e., the concentration of pathogens) to confirm that constituents' levels are acceptable, since the most critical objective is the protection of public health and the prevention of environmental contamination.

According to EPA, there are some compulsory primary and secondary treatments that must be done prior to SAT for the recharge of non-potable aquifers, while for indirect potable reuse treatments such as chlorination and disinfection are required [7]. Post treatments are usually carried out.

An operating scheme of the SAT is reported in Figure 2:

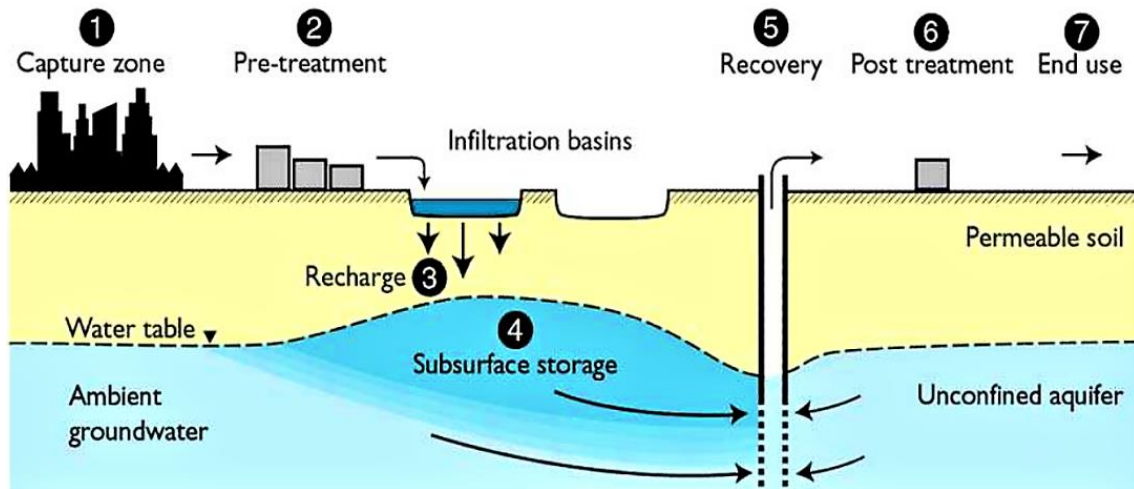


Figure 2: Scheme of working phases of SAT. [8]

One of the main concerns related to SAT is the deterioration of hydraulic properties and infiltration rates in the soil: tillage is periodically done during the dry cycle; it breaks the surface crusts and vegetation and allows the aeration of the upper soil layer by removing the clogging layer caused by biological and physical deposition.

Examples of SAT in the world can be found in Israel ([9], [10]), Australia ([11]), South Africa ([12]), Belgium ([13]), and USA ([9], [14]).

In Table 2 some of the main parameters that must be controlled in SAT are reported; they will be discussed afterwards (0): especially the infiltration rate, the recharge capacity, the typology of effluent (primary or secondary, namely the output of a primary treatment) and the wet/dry ratio are detailed.

Table 2: SAT sites in the world [3].

Country	Location	Hydraulic loading rate (m/year)	Recharge capacity (Mm ³ /year)	Inlet effluent	Wet/ dry ratio	Post -treatment
Israel	Dan region	73-150	115-125	Secondary	0.5	Chlorination or NF
Australia	Alice Springs, Northern Territory		0.6 – 1.3	Secondary	0.33	
South Africa	Atlantis		2.75	Secondary		Ion exchange+ chlorination
Belgium	Torrelee/ St. Andre		1.95	Advanced treated effluent		Aeration +RSF+UV
USA	Boulder, Colorado	30.3-48.5		Secondary	0.03	
	Ft. Devens, Massachusetts	28.8		Primary	0.14	
	Hollister, California	15.2		Primary	0.07	
	Fresno, California	13.4				
	Phoenix, Arizona	60.6-100	14.6 – 21.9	Secondary	0.75	
	Scottsdale, Arizona					
	Vineland, New Jersey	21.2	11.7-39.5	Primary	0.2	
	Brooking, South Dakota	12.2				
	Calumet, Michigan	35			0.14	
	Darlington, South Carolina	28				
	Lake George, New York	58				
	Orange County, Florida	119			0.08	
	West Yellowstone, Montana	168				

According to the values shown in Table 2, the loading rate ranges 12.2 to 168 m/year, while the recharge capacity ranges from 0.6 to 125 Mm³/year. Most of the plants use secondary effluent, and the wet/dry ratio is always lower than 0.75, with a minimum value of 0.03. In some plants, post treatments are needed to comply with the prescriptions of the law.

Israel was among the first to have a SAT plant: the Dan Region consists in a WWTP, SAT system, and the distribution system which started to work in 1986. As shown in Table 2, it is one of the biggest plants for SAT in the world. In Table 3 it is possible to see the quantities of wastewater, recharged effluent, and reclaimed water in the period 1987-2000, while in Table 4 it is possible to observe the efficiency of the removal of parameters in 2000 [6].

Table 3: Quantities of wastewater, recharged effluent, and reclaimed water (Mm³/year). [6]

Year	Raw sewage	MBTP effluent recharged	Reclaimed water
1987	38.5	36.5	19.3
1988	81.3	68.0	25.8
1989	95.7	67.1	21.3
1990	91.3	76.6	74.5
1991	88.8	78.8	85.0
1992	84.0	75.2	76.1
1993	86.3	73.3	81.0
1994	91.7	78.9	93.5
1995	92.6	72.7	96.6
1996	102.3	89.6	109.5
1997	111.5	102.8	112.1
1998	112.3	103.3	112.3
1999	115.4	108.7	133.6
2000	122.0	117.3	122.3
Total	1,313.7	1,148.8	1,162.9

Table 4: Year 2000 effluent parameters for the SAT system in Israel [6]

Parameter	Units	Before SAT	After SAT	Removal (%)
SS (105°C)	mg/L	9	0	100
pH		7.99	8.00	–
Alkalinity, as CaCO ₃	mg/L	297	337	–
BOD	mg/L	8	<0.5	>94
BOD _f	mg/L	2	<0.5	>75
COD	mg/L	53	5.70	89
COD _f	mg/L	41	6	86
DOC	mg/L	12	2	83
UV ₂₅₄ absorbance		221	56	75
NH ₄ -N	mg/L	6.97	<0.02	>99
TKN	mg/L	9.6	0.3	97.0
TKN _f	mg/L	8.7	0.33	96.0
NO ₃ -N	mg/L	0.28	9.34	
NO ₂ -N	mg/L	1.17	0.05	96.0
TP	mg/L	2.2	0.05	98.0
Detergents	mg/L	0.280	<0.100	>64
Phenol	µg/L	0.006	<0.001	>83
Trace elements				
Ca	µg/L	0.20	0.20	
Pd	µg/L	<3	<3	
Se	µg/L	<1	<1	
Cr	µg/L	4	3	>25
Cu	µg/L	<3	<3	
Fe	µg/L	88	15	83
Mn	µg/L	32	409	
B	µg/L	470	570	
Co	µg/L	<3	<3	
Ni	µg/L	11	6	45
Mo	µg/L	<4	<3	25
F	µg/L	540	130.0	76
Total bacteria	No./ml	327,000	192	3.2 logs
TC	MPN/100 ml	219,000	0	–5.3 logs
FC	MPN/100 ml	33,000	0	–4.5 logs
<i>S. faecalis</i>	MPN/100 ml	5,000	0	–3.7 logs
SAR		4.4	4.2	
Sodium	mg/L	181	185	
Potassium	mg/L	18	24	

1.3.1. System performance

In 2007, Harun did some general studies on the efficiency of SAT systems [15], which turned out to be fundamental for further researches. His main conclusions, which may be applied to any kind of system, can be summarized as follows:

- Since the removal of contaminants on SAT is case specific, it is not possible to establish a unique kinetic model.
- In oxic conditions, around the first 1.5m of soil, a great removal of 50% and 80% for DOC and ammonia, respectively, occurs.
- Since the main mechanism of DOC removal is biodegradation, its elimination is sustainable.
- DOC removal is more influenced by travel times than travel distances.
- The typical influent Dissolved Organic Carbon (DOC) for primary, secondary, and tertiary SAT systems ranged from 24-35 mg/L, 10-15 mg/L, and 5-12 mg/L, respectively. The typical effluent DOC for primary, secondary, and tertiary SAT systems was in the range of 14-18 mg/L, 2-8 mg/L, and 2-10 mg/L, respectively.
- Emerging pollutants, which are hard to be removed, were found to be persistent during the SAT of a secondary effluent while they were removed during SAT of tertiary effluent: it is possible to assume that contaminants persistent in one SAT system can be removed to another.
- It is not recommended the removal of phosphorus (done by adsorption) with this technique since the condition of breakthrough easily occurs.
- It is necessary to reduce the nutrient load from the wastewater just in case of direct potable reuse; otherwise, in case of unrestricted irrigation this must not be considered during the design of the SAT system.
- The highest DOC removal efficiency was found in sandy soil. Therefore, compared to other soil types, this soil is the best one.
- Generally, at travel distance lower than 10m, the bacteria and virus removal was higher than 99%.

Some of the parameters that influence the performance of the system and the different zones of the SAT are listed in Table 5.

Table 5: Process/parameter in different SAT zones. [10]

Process/parameter	Infiltration interface	Soil percolation	Groundwater transport
Treatment mechanism	Filtration, biodegradation	Biodegradation, adsorption	Biodegradation, adsorption, dilution
Transport	Saturated	Unsaturated	Saturated
Residence time	Minutes	Hours to days	Months to years
Travel distance	Centimetres	3-30 m	Variable
Mixing	No	No	Yes (regional GW)
Oxygen supply	Recharge water	Unsaturated zone	Regional GW
Biodegradable organic carbon availability	Excess	Excess/Limiting	Limiting
Redox conditions	Aerobic	Aerobic to anoxic	Anoxic to aerobic

The characteristic parameters of the SAT have been described in a general way. Henceforth, the parameters of interest will be detailed in the following paragraphs.

1.3.1.1. Infiltration rate

The most important factor the yield of a SAT treatment depends on is the infiltration rate: it influences the final water quality, together with the retention time.

Studies [10] show that, considering a vadose zone depth and a wastewater quality, if the infiltration rate decreases the retention time for the biological treatment will increase, while, on the other hand, if the infiltration rate increases the rest period increases (one day of flooding, 2-4 days of rest) and more oxygen can penetrate the vadose zone, helping the removal of biodegradable organic matter and reducing the anaerobic conditions in the aquifer, preventing manganese dissolution.

Further experiments were carried out during the years:

- According to Sperlich et al. [16], high concentration of biopolymers in the secondary effluents can be effectively removed using the upper soil layers as biofilter. Large filtration areas are required for this process, since the natural biofiltration processes work better at low filtration rates (<0.05 m/h or <1.2 m/d): to reduce the needed area, the systems can work at higher filtration rates (0.1-0.5 m/h or 2.4-1.2 m/d).
- In 2009 a group of researchers from Germany [17] studied the treated wastewater, showing that high filtration rates, such as the ones of rapid sand filters (>5 m/h) cannot effectively remove DOC or biopolymers due to the short retention times.

The factors that influence infiltration rate and SAT are [10]:

- Soil type and permeability
- Surface clogging material
- Pond depth
- Duration of wetting/drying cycles.

1.3.1.2. Residence time/travel distance

Directly depending on the infiltration rate, the residence time is the main parameter governing the effluent quality for all primary, secondary and tertiary effluents [18].

Some studies [18] have performed statistical analysis to establish the removals of DOC according to different residence times and travel distances during the SAT of primary, secondary and tertiary effluents. The residence times and travel distances are linked to the horizontal residence time in the aquifer and to the distance of the recovery well from the infiltration point: indeed, the higher residence times are related to the horizontal travel velocities and not to the vertical travel in the vadose zone.

1.3.1.3. Hydraulic loading rate (HLR)

This parameter is linked to the infiltration rate: if it increases, more effluents are infiltrated, and therefore there will be more time to introduce oxygen in the soil. On the other hand, a major amount of infiltrated organic matter may compromise the bioactivity in the upper layers. Still the longer relaxation time enabling the oxygen introduction is a dominant mechanism [10].

1.3.2. Advantages and disadvantages of SAT

Some work was carried out in several years ago, establishing the main advantages and disadvantages of this technology [10].

Benefits include microbiological (bacteria and viruses), nutrient (ammonia, phosphorus, and carbon) and DOC removal, as well as an efficient removal of micropollutants.

The main disadvantages of SAT are the deterioration of the infiltration rate after years of use (which causes a decrease in the recharge of the groundwater) and the dissolution of manganese due to anoxic conditions. In addition, this technique requires large areas, and care is required to the clogging of the infiltration interface and the changing of pH caused by algae photosynthesis in soil. The latter one can cause the precipitation of carbonate, gypsum, phosphorus, and other chemicals in the soil which is one of the causes of the clogging below the infiltration area.

However, this process offers more advantages if compared to other MAR technologies:

1. Improvement in the physical, chemical and microbial quality of recharged water through vadose zone filtration (efficient removal of organics, nutrients, heavy metals, microorganisms, and micropollutants) [19].
2. The substitution or support of other treatment processes reducing the overall cost of wastewater treatment and water reuse.
3. Protection of coastal aquifers against the seawater intrusion.
4. Promotion of water recycling and water reuse [9].
5. The constant recharge of water over a harvesting of water only during wet periods in other MAR techniques.

1.3.3. Agricultural Soil Aquifer Treatment (Ag-SAT)

A big problem related to the feasibility of SAT projects is the lack of infiltration sites near urban or industrial areas, where the wastewater is produced. Thus, the use of agricultural lands during their fallow period could be a solution, as shown in Figure 3. There is no evidence of installations that use this configuration, hence this possibility must be explored and studied.

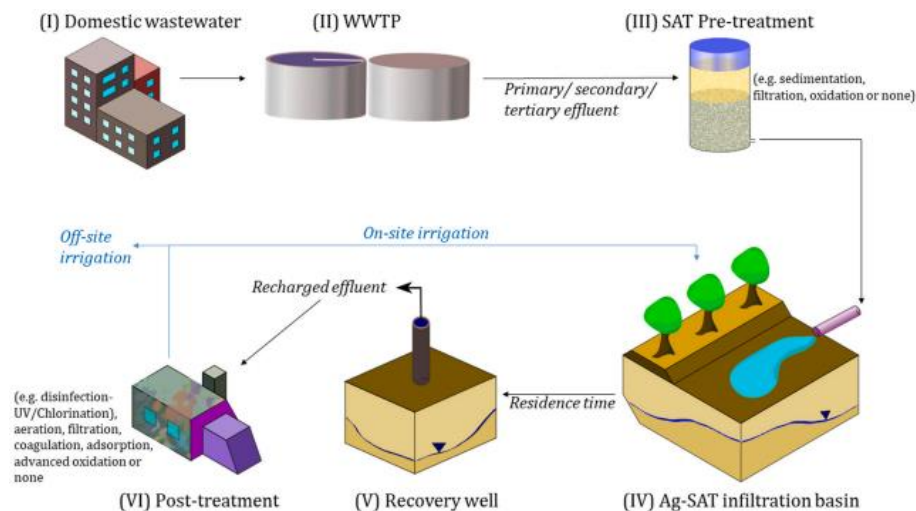


Figure 3: Scheme of working phases of Ag-SAT [3]

The dissolved organic matter (DOM) contained in the wastewater may cause environmental issues: if there is a lack of nutrients in the soil, the input of high levels of DOM can lead to the removal of nutrients already present in the soil by microorganism, which use the nutrients to degrade DOM, thus removing them from the plants. In surface water bodies, an excess of organic matter can cause a removal of dissolved oxygen by microorganisms capable of degrading DOM, causing eutrophication. Moreover, although the content and quality of DOM in groundwater is often poorly investigated, it can be a precursor for the formation of compounds harmful to human health. Finally, the organic matter has impacts on the organoleptic characteristic of water and on the performance of wastewater treatment plants, in which the DOM reacts with disinfection agents and reaction by-products are formed.

One of the main pathways for dissolved organic matter to enter surface water bodies and groundwater is through surface recharge by infiltration through partially saturated soil or through exchanges that may occur between water bodies and surface water.

1.3.4. Advantages and disadvantages of Ag-SAT

As already said, one of the main problems related to the execution of Ag-SAT projects is related to the site selection, since the application of this technology is often hindered by the lack of infiltration sites in highly sought-after land (as the wastewater source is mostly urban) [20]. Another danger related to the use of these effluents is the risk of crop contamination through root uptake of pharmaceutical compounds found in TWW; sandy soils, which are the foundation for SAT basins, present a greater risk for pharmaceutical compound accumulation. In addition, it is necessary to avoid the buildup of a clogging layer over time (caused by the repeated use of TWW) that will reduce infiltration capabilities and create longer waterlogged conditions for crops, which is a major risk [21]. Another drawback is the development of hydrophobicity, which adversely affects agricultural production by creating an uneven distribution of water and nutrients in crop root zone, and causes reduced infiltration rates that might lead to enhanced runoff and erosion across the soil surface. [22]

However, some benefits can be listed for this technique, which may contribute to its diffusion, even though a project using TWW infiltration as a means of aquifer recharge in an on-farm setting has not yet been undertaken or even explored:

- Decreased stress on freshwater sources.
- Increased water security and availability of irrigation water from recharged TWW.
- Recycling and amendment of soils with organic matter and other nutrients [21].
- Improved soil properties, such as soil texture and fertility [23].

1.4. Legislation

1.4.1. International legislation

The current legislation about the managed aquifer recharge and the soil aquifer treatment is different worldwide.

In 2015, the UN drafted the SDGs (Sustainable Development Goals) in which it was highlighted the necessity to treat and reuse the wastewater to achieve an adequate availability of water for the community.

The norm ISO 16075-1:2015 contains the guidelines for the development and the execution of projects whose aim is the reuse of wastewater to irrigate, indicating the steps for the planning of the operations. This norm has been updated in 2020 (ISO 16075-1:2020), introducing a list of the parameters to be monitored, including the agronomic parameters (nutrients, salinity factors and heavy metal concentration) and presence of pathogens.

The norm ISO 20426:2018 has been introduced as a guideline for the monitoring and management of the risks that may derive from pathogens contained in the wastewater when these are treated, stored, or transported.

The WHO guidelines [24] are divided in four volumes, each of which focuses on a different aspect related to treatment and reuse in agriculture of wastewater and water containing biological wastes. The second volume lists guidelines to promote practices related to the safe use and minimum health objectives of wastewater industrial, greywater and blackwater. Three vulnerable groups are identified: agricultural workers, the members of communities where wastewater is used in agricultural settings, and end consumers. In this volume is also introduced the health impact assessment of new projects related to the treatment and use of wastewater.

The Australian Water Reuse Guidelines [8] were written as a reference for the reuse and recycling of wastewater, greywater and stormwater. The compilation of the standards occurred in two stages: in the first, a comprehensive set of guidelines was established for managing the risks health and environmental risks related to wastewater [25], in the second they were extended to aspects specifics. These include controlled recharge of aquifers, for which there is special attention to issues related to the protection of aquifers and the quality of recovered water in recharge projects using all water sources, including wastewater.

1.4.2. National legislation

The Italian legislation about the reuse of wastewater in MAR or SAT is quite recent and it is evolving according to the EU directives. Nowadays it is allowed the reuse of wastewater for the protection of water resources, under environmentally safe conditions, avoiding alterations to ecosystems, soil, and crops, as well as sanitation and health risks to the exposed population.

The Ministerial Decree n.185, 2003 explains the technical guidelines for the reuse of wastewater: the reuse of domestic, urban, and industrial wastewater, regulated according to the uses and the resulting quality requirements and aimed at the protection of water resources from both a quantitative and qualitative point of view, must take place under conditions of environmental, hygienic, and sanitary safety.

Requirements about the quality of the wastewater meant for the reuse are specified (Table 6).

Table 6: Parameters listed in the DM 185, 2003

	Parameter	Unit of measurement	Limit value
Chemico-physical parameters	pH	-	6-9,5
	SAR	-	10
	Coarse particles	-	Absent
	TSS	mg/L	10
	BOD ₅	mgO ₂ /L	20
	COD	mgO ₂ /L	100
	P _{TOT}	mgP/L	2
	N _{TOT}	mgN/L	15
	NH ₄ ⁺	mgNH ₄ /L	2
	Electric conductivity	μS/cm	3000
	Al	mg/L	1
	As	mg/L	0,02
	Ba	mg/L	10
	Be	mg/L	0,1
	Bo	mg/L	1
	Cd	mg/L	0,005
	Co	mg/L	0,05
	Cr _{TOT}	mg/L	0,1
	Cromo VI	mg/L	0,005
	Fe	mg/L	2
	Mn	mg/L	0,2
	Hg	mg/L	0,001
	Ni	mg/L	0,2

	Parameter	Unit of measurement	Limit value
	Pb	mg/L	0,1
	Cu	mg/L	1
	Se	mg/L	0,01
	Sn	mg/L	3
	Tl	mg/L	0,001
	V	mg/L	0,1
	Zn	mg/L	0,5
	Total cyanides (as CN)	mg/L	0,05
	Sulphides	mgH ₂ S/L	0,5
	Sulphites	mgSO ₃ /L	0,5
	Sulphates	mgSO ₄ /L	500
	Active Chlorine	mg/L	0,2
	Chlorides	mgCl/L	250
	Fluorides	mgF/L	1,5
	Fat & animal/vegetal oils	mg/L	10
	Mineral oils	mg/L	0,05
	Total phenols	mg/L	0,1
	Pentachlorophenol	mg/L	0,003
	Total aldehydes	mg/L	0,5
	Tetrachloroethylene, trichloroethylene (sum of concentrations of specific parameters)	mg/L	0,01
	Total chlorinated solvents	mg/L	0,04
	Trihalomethanes (sum of concentrations)	mg/L	0,03
	Total aromatic organic solvents	mg/L	0,01
	Benzene	mg/L	0,001
	Benzo(a)pyrene	mg/L	0,00001
	Total nitrogenous organic solvents	mg/L	0,01
	Total surfactants	mg/L	0,5
	Chlorinated pesticides (each)	mg/L	0,0001
	Phosphorus pesticides (each)	mg/L	0,0001
	Other pesticides (total)	mg/L	0,5
Microbiological parameters	Escherichia Coli	UFC/100mL	10 (80% of the samples) 100 max puntual value
	Salmonella		Absent

The D. Lgs. 152/2006 contains specific indications related to the discharge of wastewater onto the soil, into the surface layers of the subsoil and into aquifers, thus providing prescriptions and limit that are to be considered applicable in cases in which the wastewater is used for a controlled recharge of the aquifer.

The art. 101 of the Decree states that, to pursue the quality objectives of water bodies, the discharges must comply with the emission limit values set out in Annex V of Part Three of the Decree. The Regions have the right to define different emission limit values if they are not less restrictive than those set out in Annex V of Part Three. In the Table 3 of the Annex V are listed the limits for the discharge of the wastewater in surface water, while in Table 4 of the Annex are listed the limits for the discharge of the wastewater on the soil.

In the art. 103 of the Decree the discharge on the soil and in the surface layers of the subsoils are prohibited, with some exceptions (e.g. wastewater for which it is evident the impossibility of discharge in the surface water bodies).

In the art. 104 of the Decree the direct discharge into the subsoil and groundwater is prohibited. However, in some specific circumstances, it is allowed the reuse of wastewater for irrigation purposes.

The latest law concerning the reuse of wastewater in Italy is “*Decreto Siccità*” (Law 68/23), which contains the urgent provisions to face with the water scarcity in Italy. Thanks to this law, a simplified procedure has been introduced for the release of the authorization regarding the reuse of wastewater for irrigation purposes.

In conclusion, starting from the last century Italy initiated a procedure for the implementation of the European directives, which are still developing. The reference law is the D. Lgs. 152/06, which aims to reach the qualitative and quantitative objectives for the water and determine the criteria that must be adopted to promote the reuse possibilities. The reuse of wastewater for irrigation purposes has been discussed in the Law 68/2023 and is allowed, according to the current Italian legislation.

The aim of this study is to evaluate, by means of batch adsorption and column percolation tests performed in laboratory, the role of different soil types in the removal of organic carbon from an agro-industrial wastewater with a high DOM content that can be used for irrigation processes (wastewater from the washing of tanks used for the milking milk, production of blueberry jam and production of pepper jam).

2. Materials and methods

2.1. Materials

Different types of wastewaters with high DOM content have been used in this study:

- Washing water from milking plants.
- Washing water from blueberries and peppers jam production plants.

2.1.1. Washing water from milking plants

A sample of full cream milk was collected from a dairy barn and both pasteurized and sterilized to simulate the heat treatment stage that takes place in the milk processing industry.

The milk was then diluted by a factor of 1:50 to achieve an effluent with an initial TOC of 600 ± 30 ppm.

2.1.2. Washing water from blueberries and peppers jam production plants

Different samples from the jam production plant *Agricoop Pecetto* have been analysed.

Samples deriving from the boiling water for the treatment of blueberries and from the washing water used to clean the tanks in which the blueberries are contained have been centrifuged and sterilized. Both the samples have been characterized, but further experiments were done only on the boiling water since it contained more DOM. Half of the samples have been stored in the fridge for a week, while the other samples have been stored in the freezer to evaluate which is the best storage method. The samples were then diluted by a factor of 1:10 to achieve effluents with an initial TOC of 415 ± 30 ppm for the sample from the freezer and 405 ± 30 ppm for the sample stored in the fridge.

Samples deriving from the boiling water for the treatment of peppers have been centrifuged and diluted by a factor of 1:5 to achieve an effluent with an initial TOC of 2000 ± 50 ppm.

All the dilutions have been done to obtain an effluent comparable to the one which may be obtained from washing production facilities.

2.1.3. Substrates

Different materials have been used as substrates for both the batch and the column tests: sand Dorsilit 5, sand Dorsilit 8 and soil Lufa 2.2. Their main characteristics are reported in the following paragraphs.

2.1.3.1. Sand – Dorsilit nr. 5

This sand has been used, thanks to the dimensions of its grains, as a filter at the top and at the bottom of the columns.

Table 7: Dorsilit nr. 5 sand characteristics [26].

SAND CHARACTERISTICS	
Grain dimension (mm)	1-2.2
Bulk density (g/cm ³)	2.7
Apparent density (g/cm ³)	1.5
SiO ₂ %m/m	98

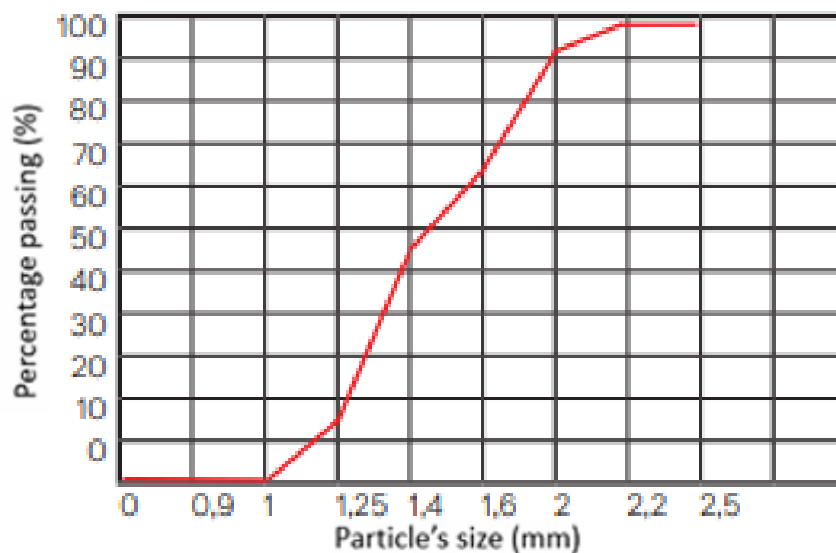


Figure 4: Granulometric curve of Dorsilit nr. 5 sand [26].

2.1.3.2. Sand – Dorsilit nr. 8

The chosen sand is characterized by a high siliceous content, as reported in Table 8.

Table 8: Dorsilit nr. 8 sand characteristics [27].

SAND CHARACTERISTICS	
Grain dimension (mm)	0.3-0.8
Bulk density (g/cm ³)	2.65
Apparent density (g/cm ³)	1.4-1.5
SiO ₂ %m/m	97.9
Fe ₂ O ₃ %m/m	0.02
Al ₂ O ₃ %m/m	0.47
TiO ₂ %m/m	0.03

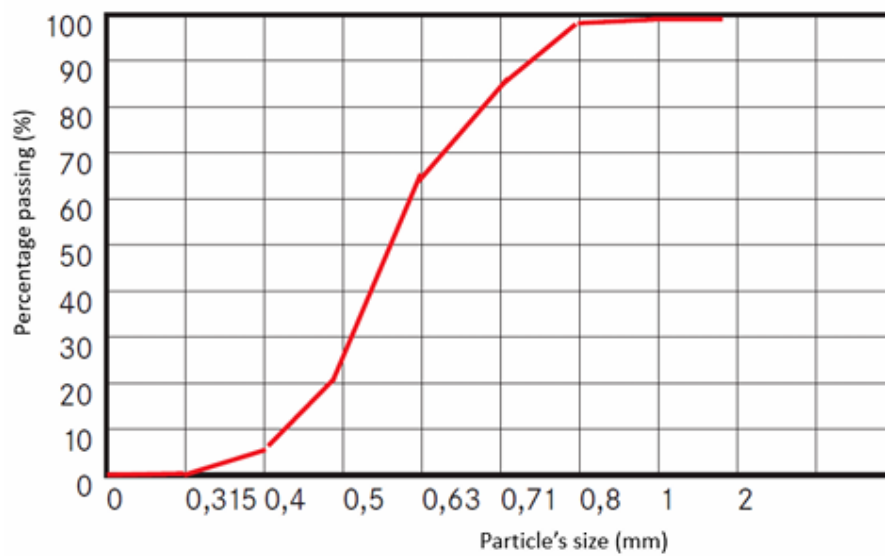


Figure 5: Granulometric curve of Dorsilit nr. 8 sand [27].

2.1.3.3. Soil – Lufa 2.2

The chosen soil is a standard one, the Lufa 2.2, whose characteristics are listed in Table 9.

Table 9: Lufa 2.2 soil characteristics [28].

Soil characteristics		
Parameter	Method	Value
Organic carbon [%C]	VDLUFA MB 1, A 4.1.3.1:2016-7; DIN EN 15936	1.82 ± 0.48
Nitrogen [%]	DIN EN 16168: 2012-11	0.19 ± 0.05
pH value (0.01 M CaCl ₂)	VDLUFA MB 1, A5.1.1:2016-7	5.60 ± 0.29
Mean pH-value (derivated from proton activity)		5.53
Cation exchange capacity (meq/100g)	DIN 19684:1973-1	9.54 ± 1.36
Particle size distribution (mm) according to USDA (%)		
<0.002	DIN ISO 11277: 2002-08 (mod.)	9.7 ± 1.2
0.002 - 0.05		16.3 ± 1.5
0.05 - 2.0		9 74.0 ± 2.2
Soil type USDA	Soil survey staff 1999, Soil taxonomy, 2nd edition	sandy loam
Maximum water holding capacity DIN (g/100g)	DIN EN ISO 14240- 2:2011-09	48.9 ± 5.6
Weight per volume (g/1000ml)	VDLUFA MB 1, A13.2.1:1991	1150 ± 36

2.2. Methods

The abatement of organic load was initially investigated in batch for all the samples and then in flow by means of column leaching studies just for the washing water from milking plants.

2.2.1. Effluent characterization

All the effluents, prior to testing the samples, have been characterized in terms of TOC (TOC-L analyser equipped with an autosampler ASI-L – Shimadzu, Japan) and pH (DeltaOhm HD 2105, equipped with a combined glass electrode) at different concentrations.

The working phases of the TOC-L analyser can be described as follows: at the beginning of the process the sample is put into a combustion furnace, where it is exposed to purified air and undergoes combustion at 680°C with the contribution of a platinum catalyst. During this step, the sample decomposes, transforming into carbon dioxide. The resulting carbon dioxide is then cooled, dehumidified, and detected using NDIR (Non-Dispersive Infrared) technology. The concentration of TC (total carbon) in the sample is determined by comparing it to a calibration curve formula. Additionally, a further step involves subjecting the oxidized sample to the sparging process, converting the IC (inorganic carbon) in the sample into carbon dioxide, which is then detected by the NDIR. The IC concentration is measured in this manner. Subsequently, the TOC (total organic carbon) concentration is calculated by subtracting the IC concentration from the initially obtained TC concentration [29].

2.2.1.1. *Characterization of the milk and washing water from milking plant*

The milk and washing water used for the study have been taken from a cow milking plant located in Cavallerleone (CN, Italy) on Wednesday, 3rd of May 2023 and analysed the following day.

After the treatments, all the samples have been diluted at different concentrations to detect the TOC and the pH, to have a better characterization of the input effluent. According to the results of the characterization, pasteurized milk was used for the tests.

2.2.1.1.1. *Characterization of the milk*

After measuring the pH of the milk (pH= 6.56), the sample has been centrifuged (Centrifuge A 6-50 MAX.: 9500 rpm/10050 xg MAX.: 6x50g, shown in Figure 6) for 10 minutes at 8000rpm to detect the formation of milk cream and sediment: this is a crucial step, done to avoid possible future clogging situation [10] in the column tests due to the presence of suspended particles in the effluent.



Figure 6: Samples in the centrifuge.

As it is possible to see in Figure 7 it was observed the formation of a superficial layer of milk cream and the sedimentation of a part of the material which was removed.

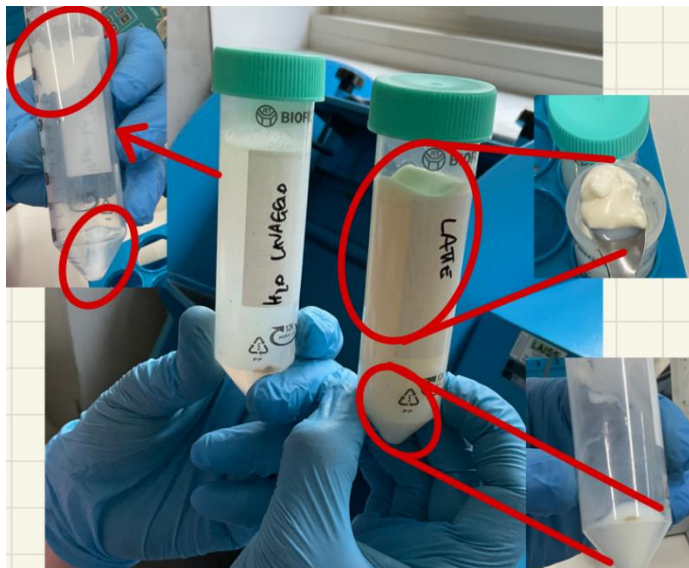


Figure 7: On the left: residues of washing water found after the centrifugation; On the right: residues of milk found after the centrifugation.

The pH measured after the centrifugation and the removal of the milk cream and sedimented solids was equal to 6.78.

A sample of non-centrifuged milk has been pasteurized (heated at 71.5°C for 15 seconds) and then cooled in cold water and ice. The measured pH was 6.55.



Figure 8: Procedure for the pasteurization of the milk.

A portion of the pasteurized milk has been centrifuged, measuring a pH of 6.55. The formation of milk cream and sedimentation solids was detected, despite the thermal process done on the sample.

A further sample of non-centrifuged milk has been sterilized (heated at 135°C for 10 seconds) and then cooled in cold water and ice. The measured pH was 6.57. As it is possible to observe in Figure 9, it was observed the formation of a layer at the bottom of the pot in which the milk was sterilized.



Figure 9: On the left: sterilization process of the milk; On the right: layer formed at the bottom of the pot.

Subsequently, a portion of the sterilized milk has been centrifuged, measuring a pH of 6.57. The formation of a lower quantity of milk cream and sedimentation solids with respect to the other samples (milk and pasteurized milk) was detected: this may be attributed to the layer formed at the bottom of the pot during the sterilization process, which was removed before the centrifugation.

2.2.1.1.2. Characterization of the washing water from water production plant

The same treatments have been done on the washing water (initial pH=7.04): a portion has been pasteurized and centrifuged (pH=7.93), another portion has been sterilized and centrifuged (pH=8.5). The centrifugation pointed out that no cream nor sedimentation layer formed, suggesting a lower concentration of solids in the specimen compared to the milk sample.

2.2.1.2. Characterization of the effluents from jam production plant

Two different effluents from the jam production plant *Agricoopecetto* have been analysed: blueberry and peppers.

2.2.1.2.1. Characterization of the effluent from blueberry jam production

There were two different types of effluent: washing water of the tanks used for the treatment of the blueberry and residues of the boiling water used for the cooking of the blueberry.

A part of the effluents has been centrifuged to eliminate the sedimented fraction (which was found to be lower than the one detected in the samples containing milk).

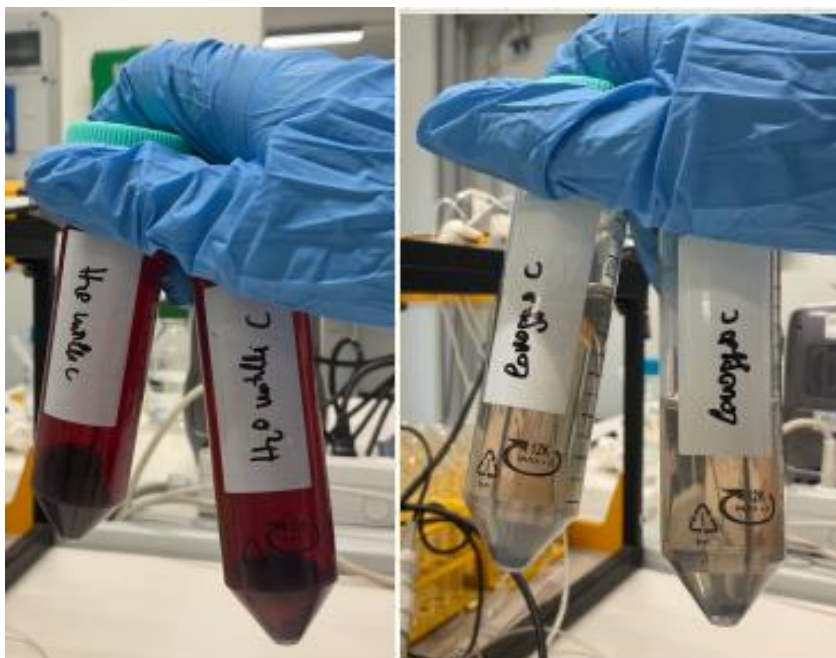


Figure 10: Residues of the boiling water (left) and washing water (right) from the blueberry jam production plant after the centrifugation.

Different samples have been prepared by diluting the effluents (both centrifuged and non-centrifuged) in deionized water at different concentrations, measuring the pH and the absorption spectrum (AnalytikJena Specord S600 single-beam spectrophotometer, equipped with Hellma quartz cuvettes with an optical path of 1 cm), and further analysing the TOC. The spectrophotometer determines the intensity of the electromagnetic light absorbed, transmitted, or reflected by a solution. Each compound interacts in a unique way with specific wavelengths of light, absorbing energy in certain spectral ranges, including visible and ultraviolet, giving an information about the quantity and the dimensions of the suspended particles contained in the sample.

In the end, a fraction of the effluent has been put in the fridge while the other part has been frozen for a week to decelerate the degradation and analyse which is the best conservation method.

2.2.1.2.2. Characterization of the washing water from pepper jam production

The boiling water from pepper jam production has not been centrifuged since there was not a residual or suspended material, it has been diluted to reach a proper concentration.

2.2.2. Batch tests

For the batch tests, multiple samples have been prepared to investigate the degradation of the organic matter when using different substrates.

The tests were carried out both in spring (May), summer (July) and winter (January) to investigate the influence of the temperature on the degradation.

The so-obtained effluents were placed in contact with a siliceous sand (Dorsilit8 – Dorfner, put in a sodium hydroxide solution 100mM for one night and then sonicated and washed twice with both tap water and deionized water) and a sandy-loam soil (standard soil Lufa2.2 - Lufa Speyer, organic content 1.7%, pH=6.2, measured after 50 hours) in a solid:liquid ratio of 1:25 (2g of sand/soil in 50ml of effluent). Some milk mixed with deionized water, boiling water from blueberries jam and pepper jam production, and deionized water-treated soil samples were prepared as a blank. All the samples have been wrapped up using aluminium film to prevent the influence of the sunlight.

2.2.2.1. *Spring batch test*

The tests done in May were carried out under agitation conditions.

The effluents used for this test are a dilution of deionized water and centrifuged milk (1:50) and a dilution of deionized water and centrifuged boiling water from blueberry jam production stored in freezer and fridge for one week (1:10). The mixed samples were centrifuged again before starting the test.



Figure 11: Samples in the orbital stirrer.

Samples were periodically taken from the different series (after 1h, 24h, 48h, 120h) and centrifuged for 10 min at 8000rpm to remove the fine fraction of soil remaining in suspension. The recovered supernatant was analysed in terms of pH, total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC), and trends from the initial value were evaluated.

The samples were then filtered to further analyse and identify the groups of compounds able to be detected by fluorescence as time of degradation changed: the machinery used is the Cary Eclipse spectrofluorometer – Agilent, in which emission and excitation range both 200-600nm, 5nm of resolution. The spectrofluorometer consists of an electromagnetic radiation source, a first excitation wavelength selection monochromator, the sample chamber, a second monochromator for selecting a wavelength of the emission spectrum, and a system for the detection and quantification of the output radiation. The sample is excited by a monochromatic light source, of a selected wavelength, and the fluorescence signal emitted by the sample is processed by the output monochromator (or optical filter system) that selects the band and wavelength of the light that will reach the photosensitive element of the system, which will

quantify it. In this way, the fluorescence spectrum of the examined sample is reconstructed. Very frequently in these devices the reading of the emitted light is done at a 90° angle to the excitation direction, so that the excitation radiation overlaps as little as possible with the fluorescence signal. Moreover, to evaluate quantitatively the changes over time, the absorption spectrum has been detected for each of the samples.

2.2.2.2. Summer batch test

In the test carried out in July the degradation of different effluents was analysed under agitation conditions.

The effluents used for this test are a dilution of deionized water and centrifuged milk (1:50) and a dilution of deionized water and centrifuged boiling water from blueberry jam production (1:10). The samples were centrifuged again before starting the test.

Samples were periodically taken from the different series (after 1h, 24h, 48h, 96h, 168h, 240h, 1008h, total duration of the test: 42 days) and centrifuged for 10 min at 8000rpm to remove the fine fraction of soil remaining in suspension. The same analyses done on the effluent of the spring batch test (0) have been done on the recovered supernatant, and trends from the initial value were evaluated.

2.2.2.3. Winter batch test

In the test carried out in January the degradation of different effluents was analysed under both agitation and non-agitation conditions (to detect the influence of the agitation).

2.2.2.3.1. Non-agitation conditions

The effluents used for this test are a dilution of deionized water and centrifuged pasteurized milk (1:50) and a dilution of deionized water and boiling water from pepper jam production (1:5). The samples were centrifuged again before starting the test.

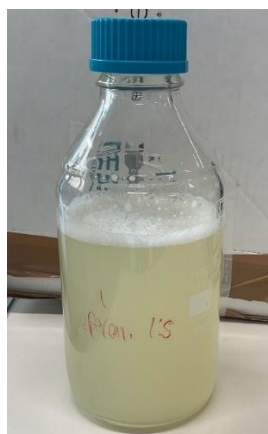


Figure 12: Blank of boiling water of pepper jam production and deionized water (1:5)

Samples were periodically taken from the different series (after 24h, 48h, 72h, 144h, 168h, 192h, 240h, total duration of the test: 10 days) and centrifuged for 10 min at 8000rpm to remove the fine fraction of soil remaining in suspension. The recovered supernatant was analysed in terms of pH, total nitrogen (TN), total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC), and trends from the initial value were evaluated.

During this test the BOD₅ has been evaluated using a VELP Respirometric Sensor system (Figure 13). This equipment measures a difference in pressure: the cap contains a KOH pads, which absorb the carbon dioxide generated during the degradation; the difference in pressure is measured and subsequently converted in substrate consumption, which is plotted on the software “VELP RESPIROsoft” thanks to the transmission of the data operated by a wireless databox. Some soil improvers have been added to the samples to obtain a better understanding of the degradation processes.



Figure 13: Respirometric sensor System [30].

2.2.2.3.2. Agitation conditions

The effluent used for this test is a dilution of deionized water and centrifuged pasteurized milk (1:50). The sample was centrifuged again before starting the test.



Figure 14: Samples in the orbital stirrer.

Samples were periodically taken from the different series (after 24h, 48h, 72h, 168h, 192h, 240h, total duration of the test: 10 days) and centrifuged for 10 min at 8000rpm to remove the fine fraction of soil remaining in suspension. The recovered supernatant was analysed in terms of pH, total nitrogen (TN), total organic carbon (TOC), total carbon (TC) and inorganic carbon (IC), and trends from the initial value were evaluated.

In Table 10, the differences between the batch tests carried out during the project have been schematized:

Table 10: Scheme of the characteristics of the batch tests.

Test	Effluent	Duration (days)	Sampling intervals (h)	Parameters monitored	Agitation conditions
Spring	Milk (1:50), boiling water from blueberry jam production (stored in fridge and freezer 1:10)	5	1, 24, 48, 120	pH, TC, TOC, IC	Yes
Summer	Milk (1:50), boiling water from blueberry jam production (stored in freezer 1:10)	42	1, 24, 48, 96, 168, 240, 1008	pH, TC, TOC, IC	Yes
Winter (I)	Milk (1:50), boiling water from pepper jam production (1:5)	10	24, 48, 72, 144, 168, 240	pH, TN, TC, TOC, IC, BOD ₅	No
Winter (II)	Milk (1:50)	10	24, 48, 72, 168, 192, 240	pH, TN, TC, TOC, IC	Yes

2.2.3. Column leaching tests

The abatement of the organic load of the effluents was initially investigated in batch and then in flow by means of column leaching studies: liquid and solid samples have been investigated in two separated tests, carried out in August 2023 and January 2024. In the first test a known flux has injected for a fixed period, while in the second test there has been an alternance of wetting (24h) and drying (72h) periods.

2.2.3.1. Continuous column leaching test

For the leaching test two Plexiglas columns have been filled with sand (Dorsillit8) and soil (Lufa 2.2.), respectively. The inner diameter of the columns is 4cm, while they have been filled with the substrates for a total length of 13.5cm: 1cm of gravel at the top (Dorsilit 5), which allows to stabilize the input flow and does not interact with the effluent, and 12.5cm of sand/soil (which correspond to 274g of sand and 204.2g of soil). A filter has been put at the bottom of the columns to avoid the clogging of the output hole. The columns were saturated with a flow of water from the bottom to the top, and the saturated porosity was determined by injecting a tracer (KBr 3mM) with a flow rate of 15.2 cm/h (according to previous studies [10]). The effluent (solution of deionized water with pasteurized and centrifuged milk 1:50) was injected into the column at the same rate for 5 pore volumes (PVs), followed by a flushing with deionized water for the same number of PVs. The flow exiting the columns was analysed each 60s in terms of absorbance (as shown in Figure 15) and sampled each 10 minutes using fraction collectors for TOC, TC, and IC analysis.

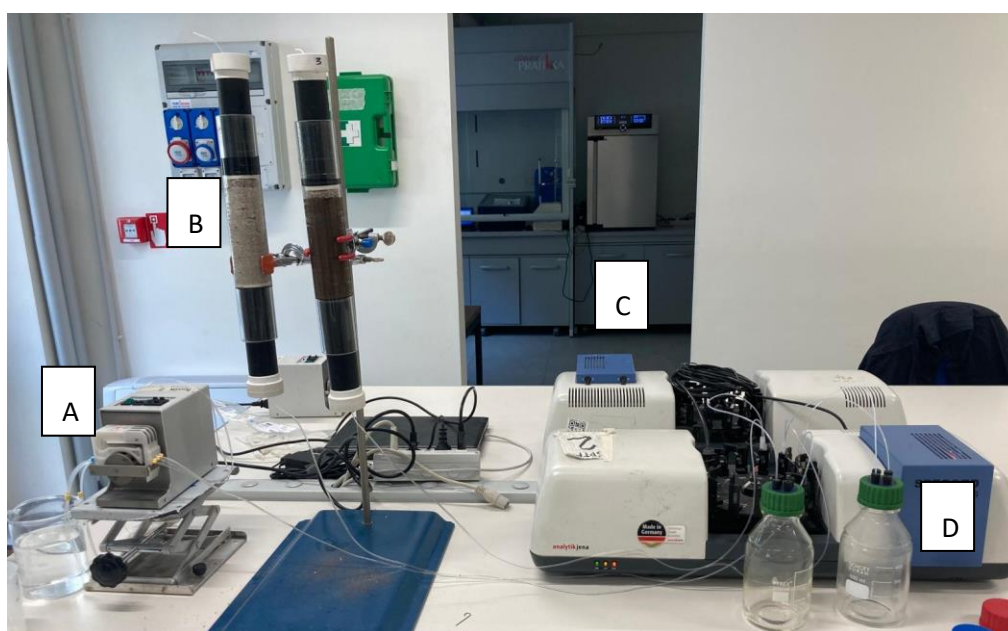


Figure 15: Organization of the column setup. A: peristaltic pump; B: Columns (left: sand, right: soil); C: Spectrophotometer; D: Bottles for sampling

2.2.3.2. Intermittent column leaching test

For the leaching test carried out in January, a plastic column has been filled with soil (Lufa 2.2.). The inner diameter of the column is 4cm, and it has been filled with the substrate for a total length of 16.5 cm: 2cm of gravel (Dorsilit 5) both at the top and the bottom of the column, which allows to stabilize the input flow, does not interact with the effluent, and avoids the clogging of the tube, and 12,5cm of soil (which corresponds to 211.645g). The setup is shown in Figure 16.



Figure 16: Organization of the column setup: it is organized as the test done in summer, without the spectrophotometer.

The columns were saturated with a flow of water from bottom to top with a flow rate of 8.34 cm/h, to have 2l of effluent injected in 24 hours.

The effluent (a solution of deionized water with pasteurized and centrifuged milk 1:50) was injected into the column at the same rate from the top to the bottom, following the procedure described by Moshe et al. in 2021 [31]: 3 cycles of flooding for 24 hours, followed by a drying period of 3 days, for a total duration of the test of 12 days. The flow exiting the columns was sampled periodically and analysed in terms of pH, Total dissolved Solids, electric conductivity, ORP, FNU (using the multiparametric probe shown in Figure 17) absorbance, TOC, TC, and IC. The TOC analysis has been carried out by diluting 5ml of the output

sample in 25ml of deionized water, since the solid content was too high and might have caused problems to the TOC-analyser.



Figure 17: Multiparametric probe used for the analysis of the leachate.

After the last flooding, the column was let drain under gravity for three days and subsequently it was unpacked: 14 samples have been taken, two constituted by the gravel at the top and at the bottom of the column, while the soil samples has been taken and weighed every centimetre of the column (except for the last sample, which comprised the last 1.5cm of the column) as shown in Figure 18.

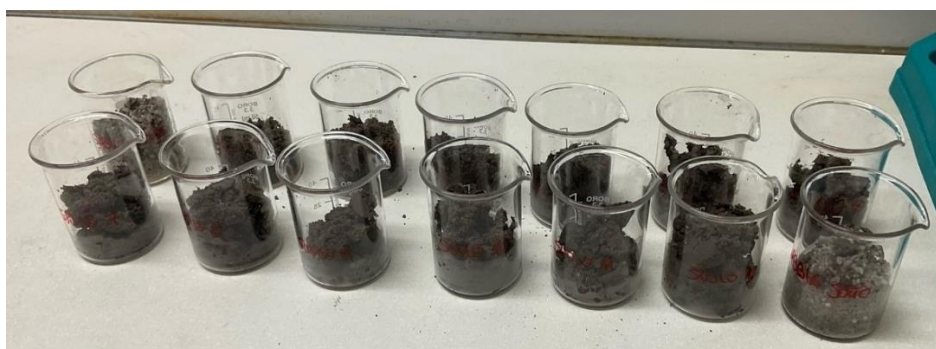


Figure 18: Samples taken from the unpacking of the column.

The solid samples have further dried for two days and then their total organic carbon content (solid and liquid) has been analysed using the Shimadzu's TOC-L analyser, equipped with an SSM-5000A sampling system.

3. Results and comment

3.1. Effluent characterization

3.1.1. Characterization of the milk and washing water from milking plant

3.1.1.1. Characterization of the milk

The initial TOC of the milk was 31500 ppm, which after a dilution 1:50 reached a value of 600 ± 30 ppm. This result confirms previous findings [32] in which it has been analysed a sample of sheep cheese from a dairy industry, finding its total organic carbon equal to 32060 ppm at the beginning of the test.

The calibration line has been found by calculating the TOC at different concentrations of both pasteurized and sterilized milk in deionized water, in centrifuged (C) and non-centrifuged (NC) samples. The results are reported in Figure 19.

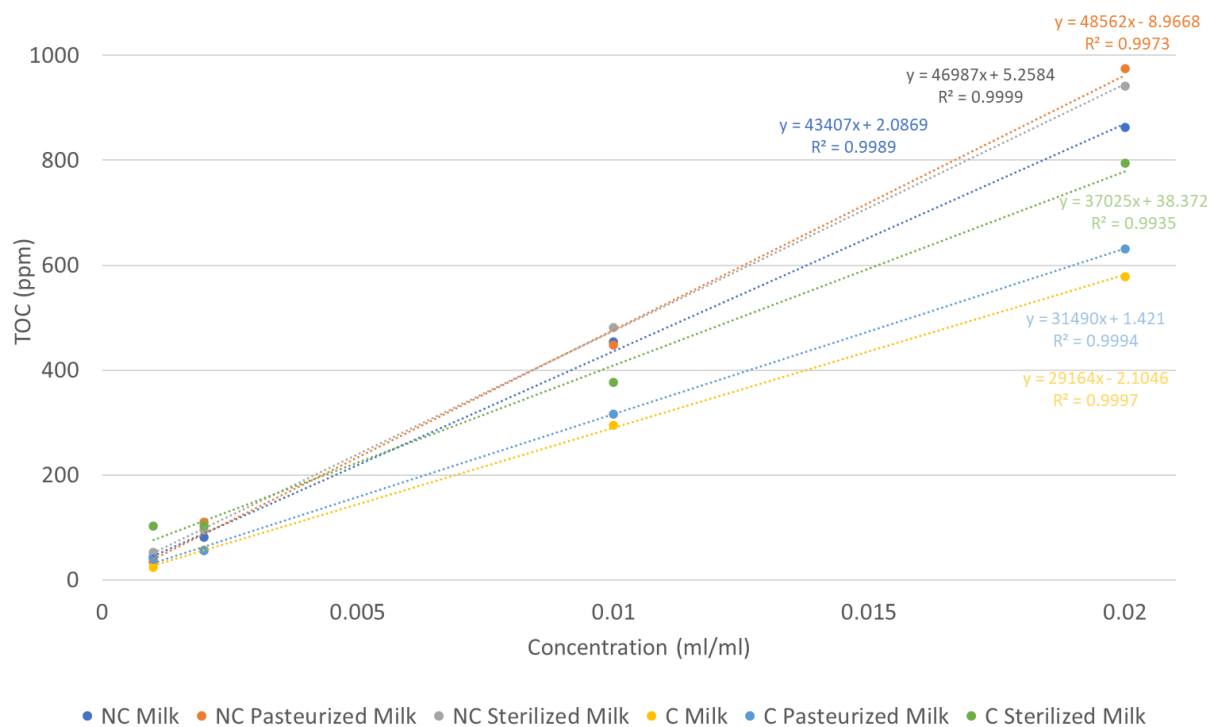


Figure 19: Calibration line of the TOC of the milk samples. C: centrifuged; NC: non-centrifuged.

As it is possible to notice in Figure 19, the TOC of the centrifuged milk results in a lower value with respect to the ones of the sterilized centrifuged milk: in the sterilizing process, a fraction of the liquid evaporated leading to a more concentrated milk, with a higher TOC value. Generally, the non-centrifuged samples had higher values of TOC compared to the centrifuged ones: this outcome is largely in line with expectations, since the milk cream which created after the centrifuge had been removed from the samples before the TOC analysis.

In the calibration line (Figure 19) it is possible to notice that there had been errors in the dilution of the milk at lower concentrations (0.001 ml/ml), probably due to the density of the milk, which had led to difficulties in the picking up of the samples and causing a trend that does not reflect the general trend of the other samples: for this reason, the analysis have been carried out again the day after, starting from a concentration of 0.01 ml/ml of milk in deionized water, and diluting this samples to reach lower concentrations. The results, showed in Figure 20, had demonstrated the error in the dilution.

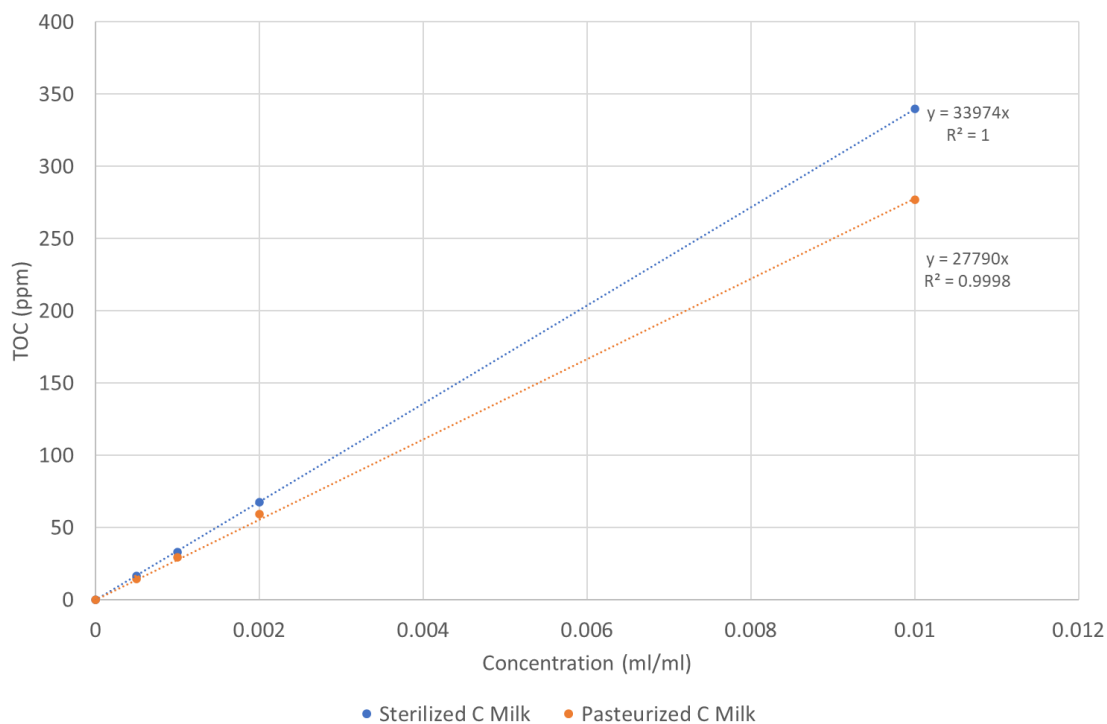


Figure 20: Calibration line of the TOC at lower concentrations.

The trend of TN at different concentrations is represented in Figure 21. The value of R^2 suggests that the data well fits with a linear interpolation.

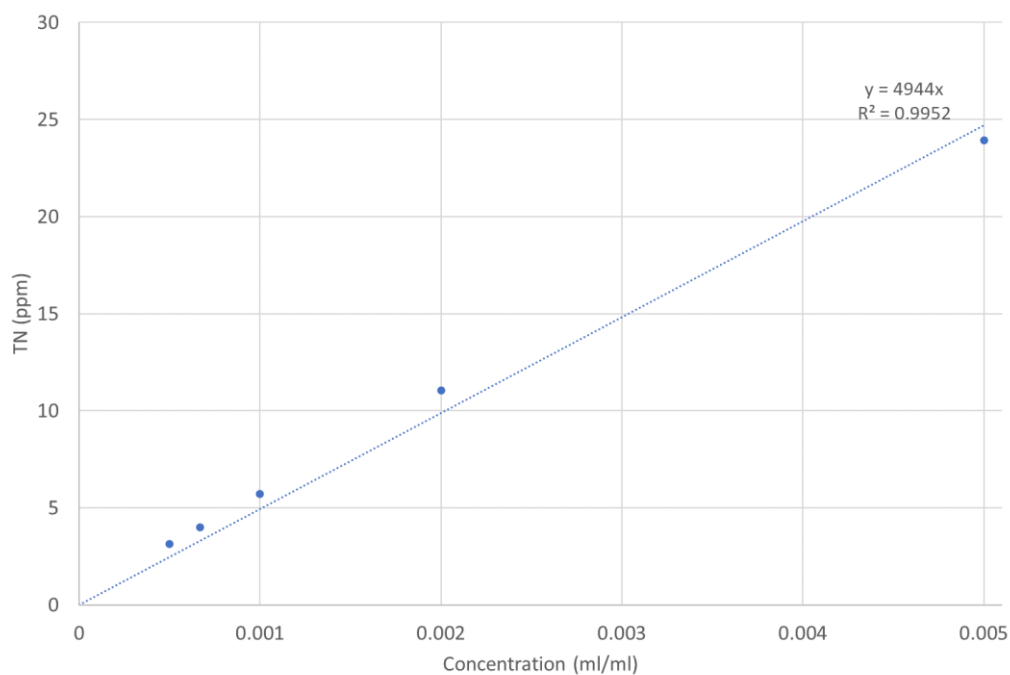


Figure 21: Calibration line of the TN of the milk samples.

For all the samples, the pH has been evaluated at different concentrations: the results are listed below (Figure 22).

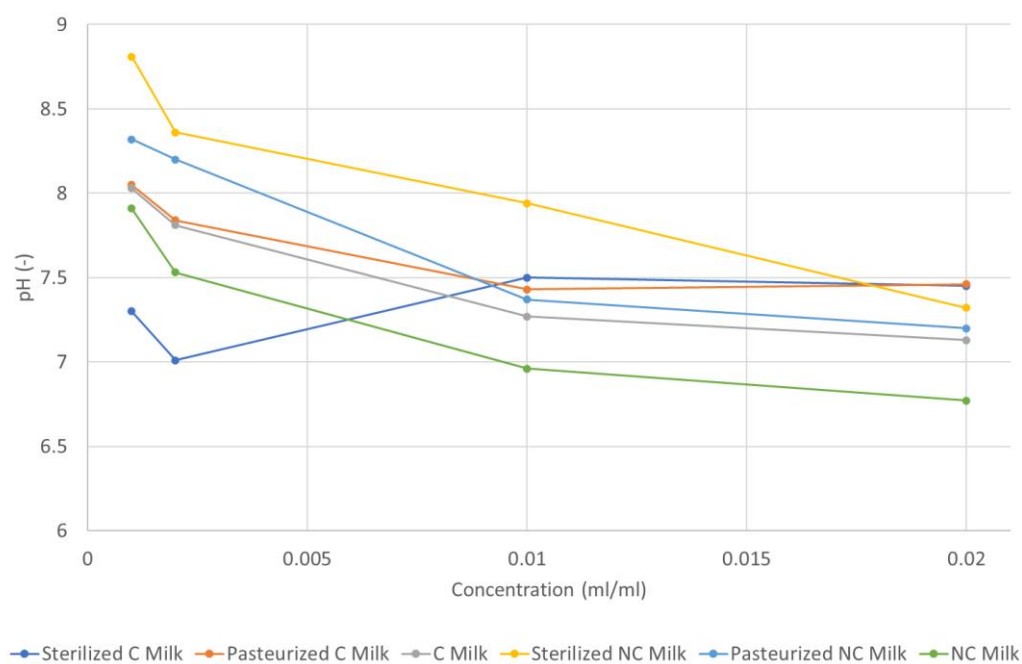


Figure 22: Trend of pH at different concentrations. C: centrifuged; NC: non-centrifuged.

It is possible to notice a generalized decrease of the value of the pH as the concentration increases: in the sterilized centrifuged milk this behaviour cannot be observed, probably due to errors in the measurements, caused by the instrument not being stabilised as these were the first measurements to be taken.

3.1.1.2. Characterization of the washing water

The analysis carried out on the washing water of the milking plant suggested a lower concentration of organic carbon, as it is possible to see in Figure 23: for this reason, further analysis had been carried out on dilution of milk and deionized water, not considering anymore the washing water.

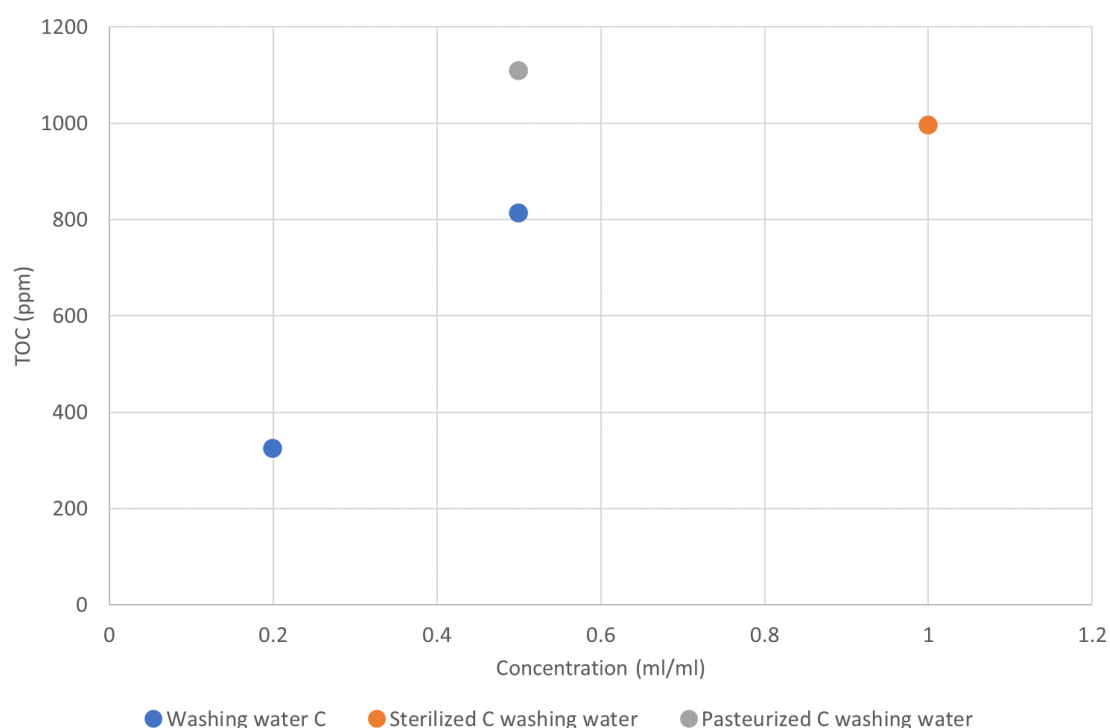


Figure 23: TOC of the washing water.

3.1.2. Characterization of the effluents from jam production plant

3.1.2.1. Characterization of the effluent from blueberry jam production plant

There calibration lines of the samples collected in the blueberry jam production plant are represented in Figure 24, in which the concentration on the x-axis indicates the dilution ratio: the TOC of the non-centrifuged washing water and boiling water show a similar trend, as well as the TOC of the centrifuged washing water and boiling water.

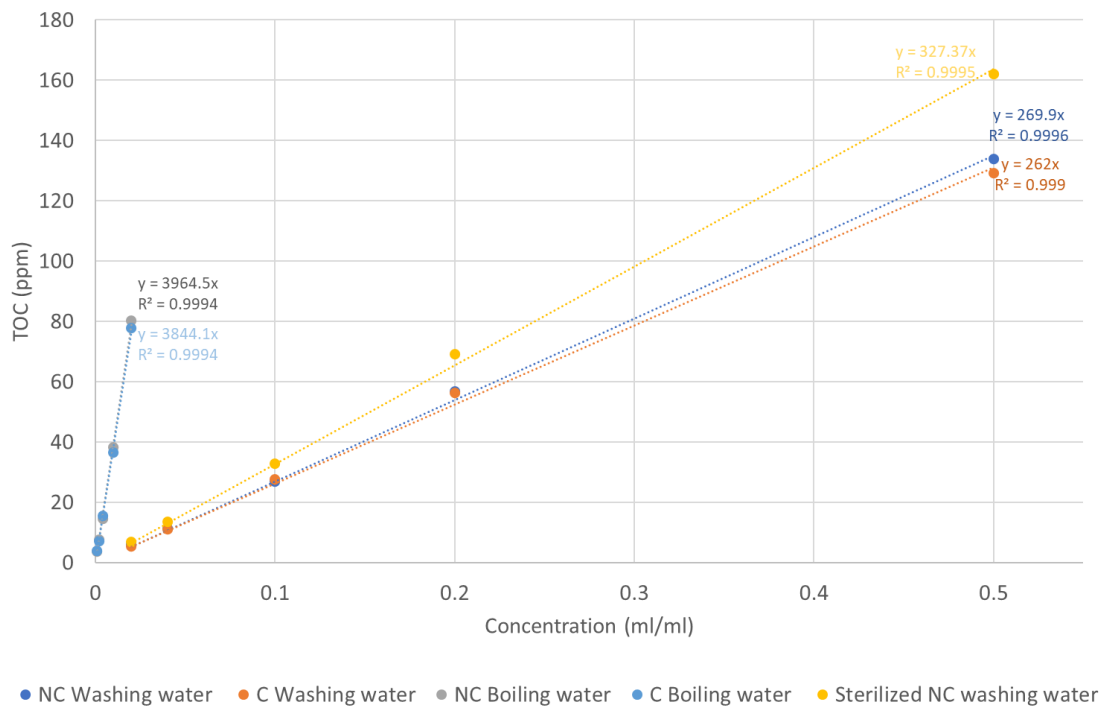


Figure 24: Calibration line of the TOC of the blueberry samples. C: centrifuged; NC: non-centrifuged.

For all the samples, the pH has been evaluated at different concentrations: the results are listed below (Figure 25).

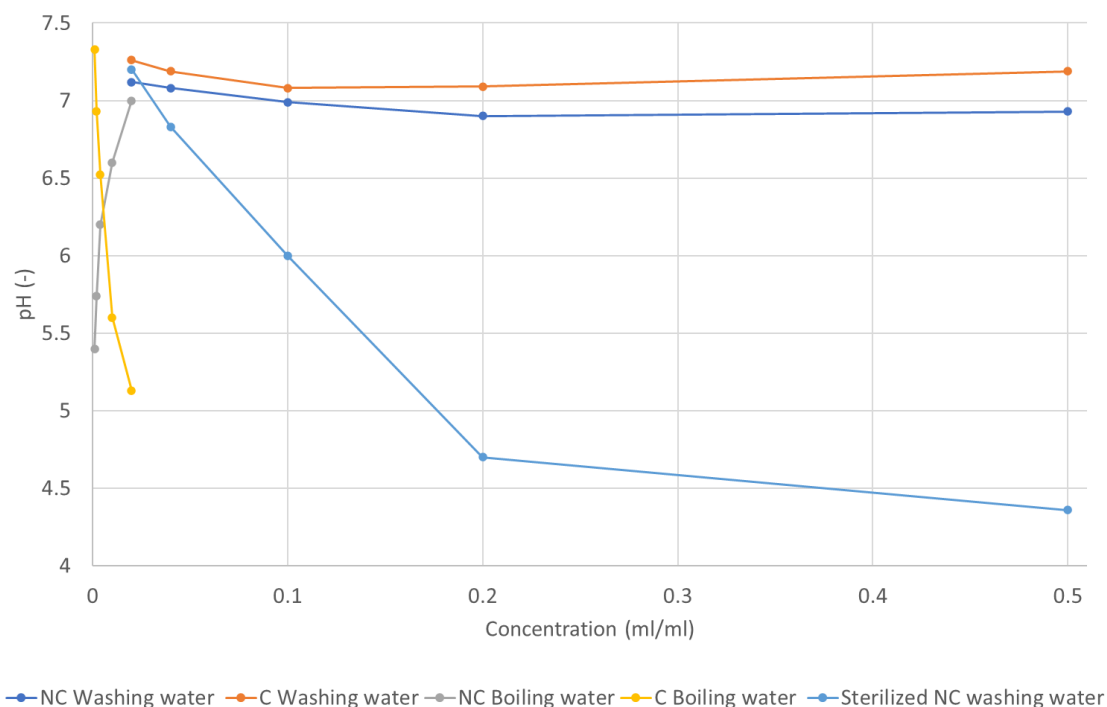


Figure 25: Trend of pH at different concentrations for different types of samples. C: centrifuged; NC: non-centrifuged.

A general decrease in the value of pH is registered as the concentration increases: the only sample with an opposite behaviour is the non-centrifuged boiling water, in which the pH increases according to the increasing of the concentration. Two groups are identified: the first one is constituted by the samples of boiling water, which was diluted at lower concentrations, while the second is composed by the washing water samples, which have been diluted at higher concentrations.

The TOC analysis on the samples stored in freezer and fridge was evaluated after one week. The results are reported in Figure 26.

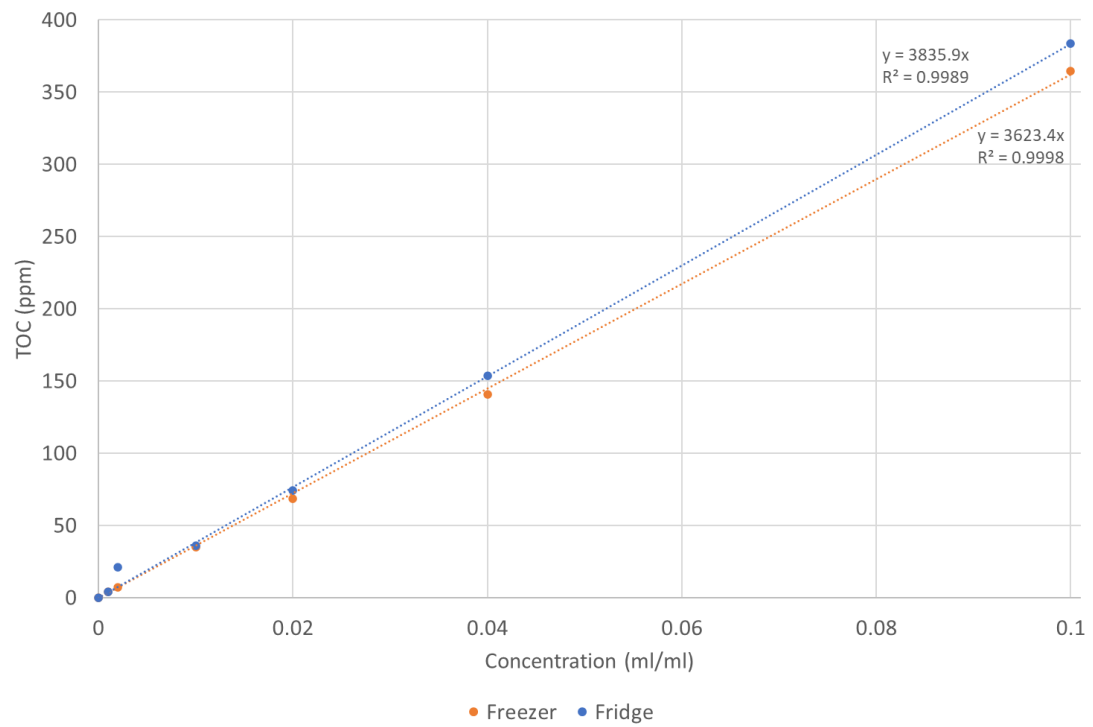


Figure 26: Trend of TOC of stored boiling water in freezer and fridge.

3.1.2.2. Characterization of the washing water from pepper jam production plant

There calibration line of both the TOC and the TN of the samples collected in the pepper jam production plant are represented in Figure 27 and Figure 28.

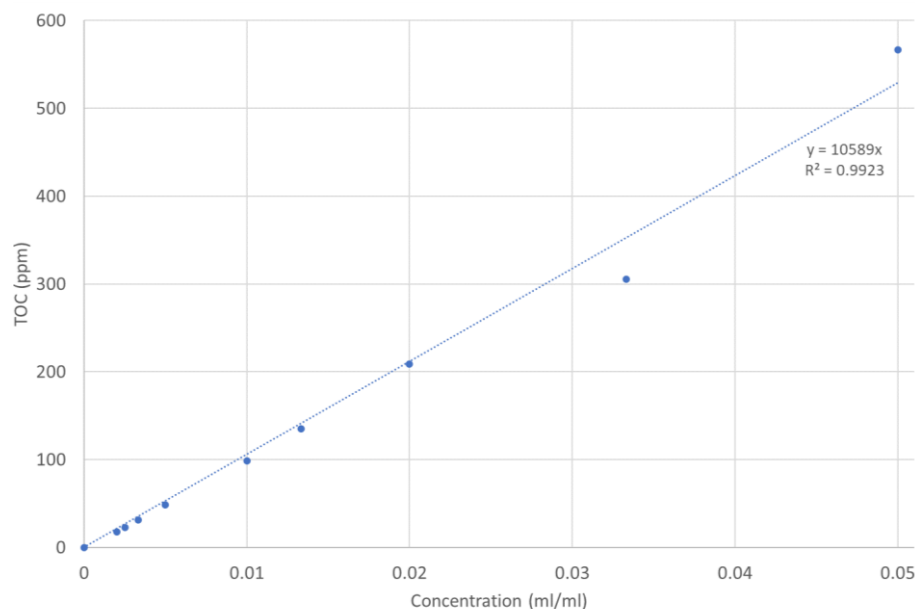


Figure 27: Calibration line of the TOC of the pepper samples.

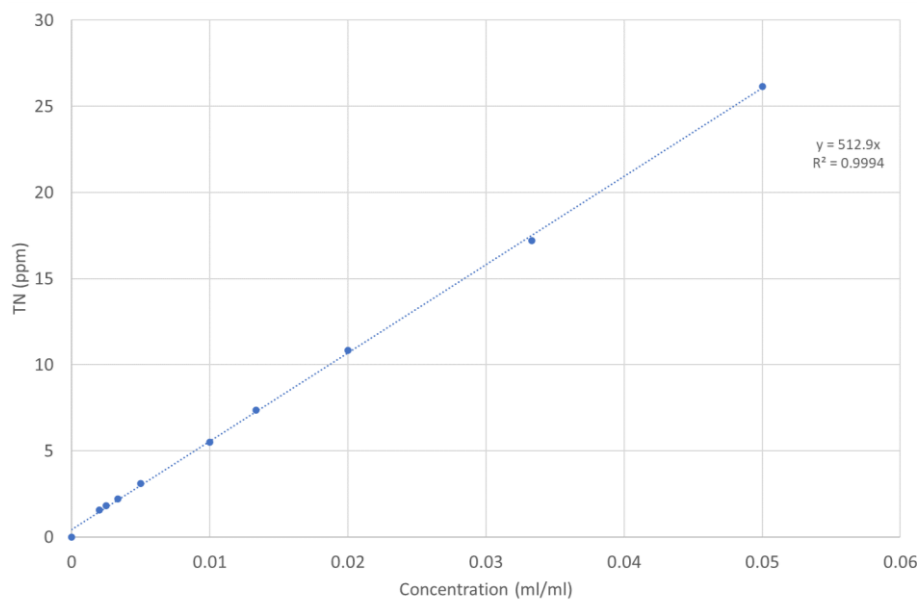


Figure 28: Calibration line of the TN of the pepper samples.

Generally, the trend-line better fits with the results corresponding to the lower concentrations: however, the R^2 value suggests that all the data well fit with a linear interpolation.

3.2. Batch tests

The results of the analysis carried out in the tests are reported in the following paragraphs.

3.2.1. Spring

3.2.1.1. Milk

3.2.1.1.1. TOC and pH analyses results

During the degradation of both pasteurized and sterilized centrifuged milk, a solution with 1:50 dilution has been used, to simulate the possible wastewater of a milking plant. The measures of TOC and pH during time are reported in Figure 29, Figure 30, Figure 31, respectively.

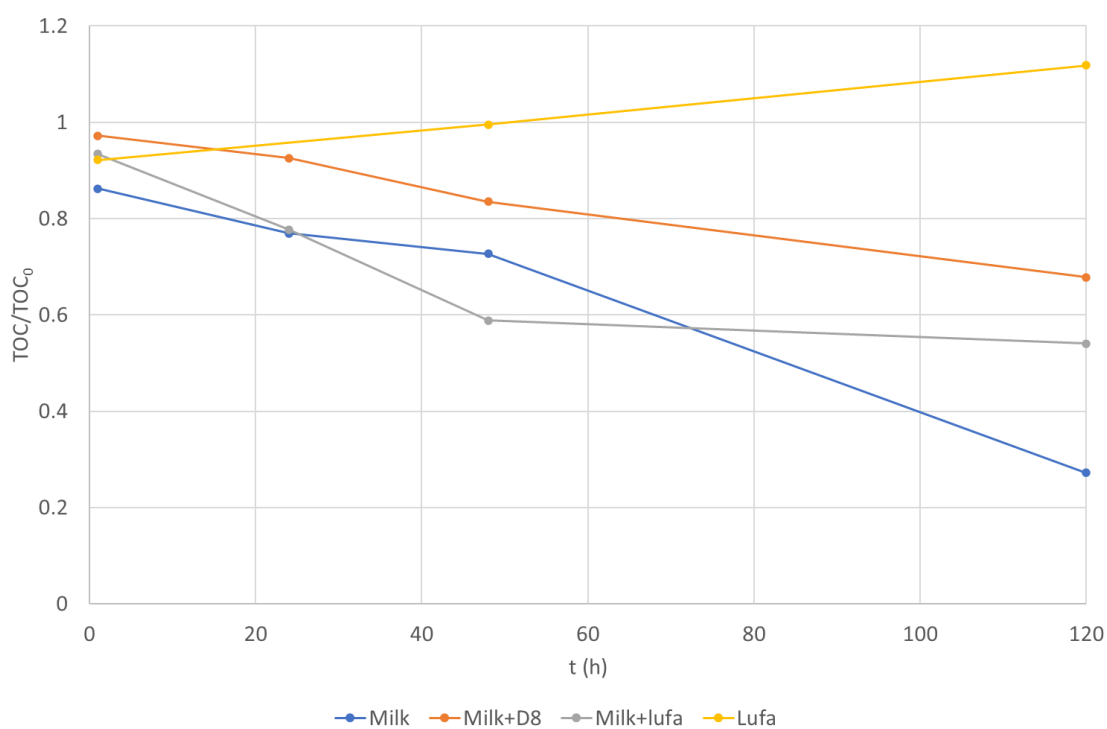


Figure 29: Degradation of pasteurized milk over time.

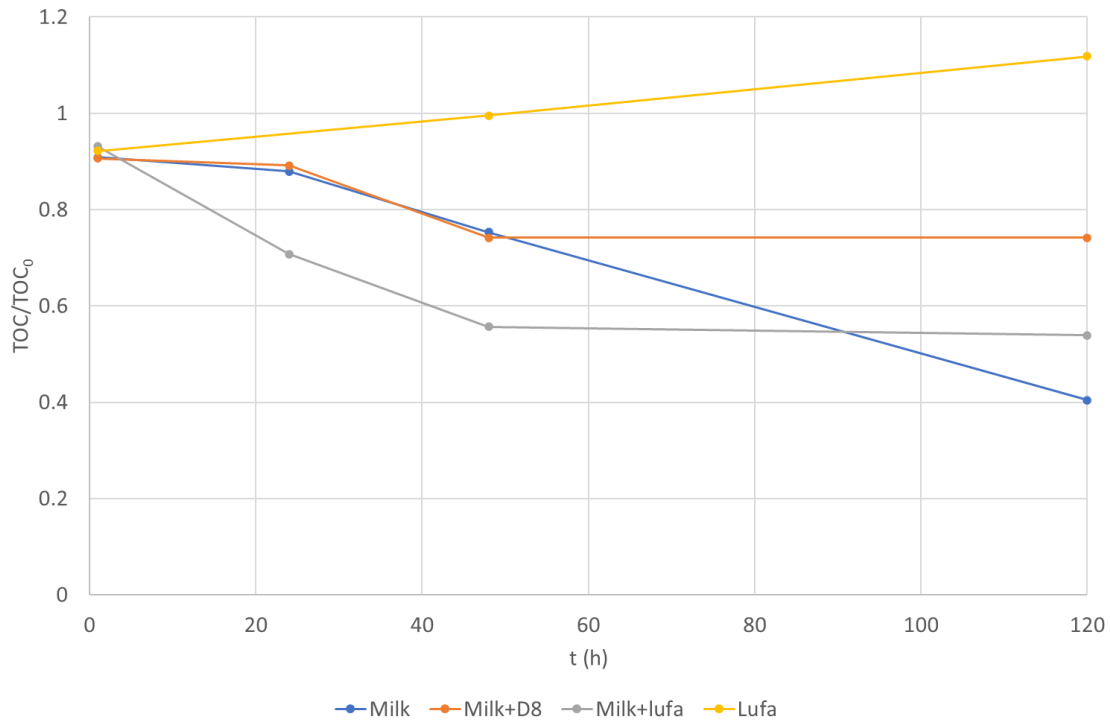


Figure 30: Degradation of sterilized milk over time.

The trends registered show a general decrease in the TOC content, except for the soil which increased the organic carbon content, probably due to the microbial population already present in the sample, which could grow thanks to the metals naturally contained in the soil. The samples containing milk mixed with soil seemed to be the faster in the degradation at the beginning of the test, while they registered a slowing down in the final part of the test. The pure milk registered an increase in the degradation velocity as time increased for both the typologies of milk. The degradation of the milk mixed with sand was the slower trend for both the types of milk, appearing to reach an asymptote for long time-degradation. Further longer lasting tests were conducted to investigate the behaviour of the samples and better understand the degradation of the milk.

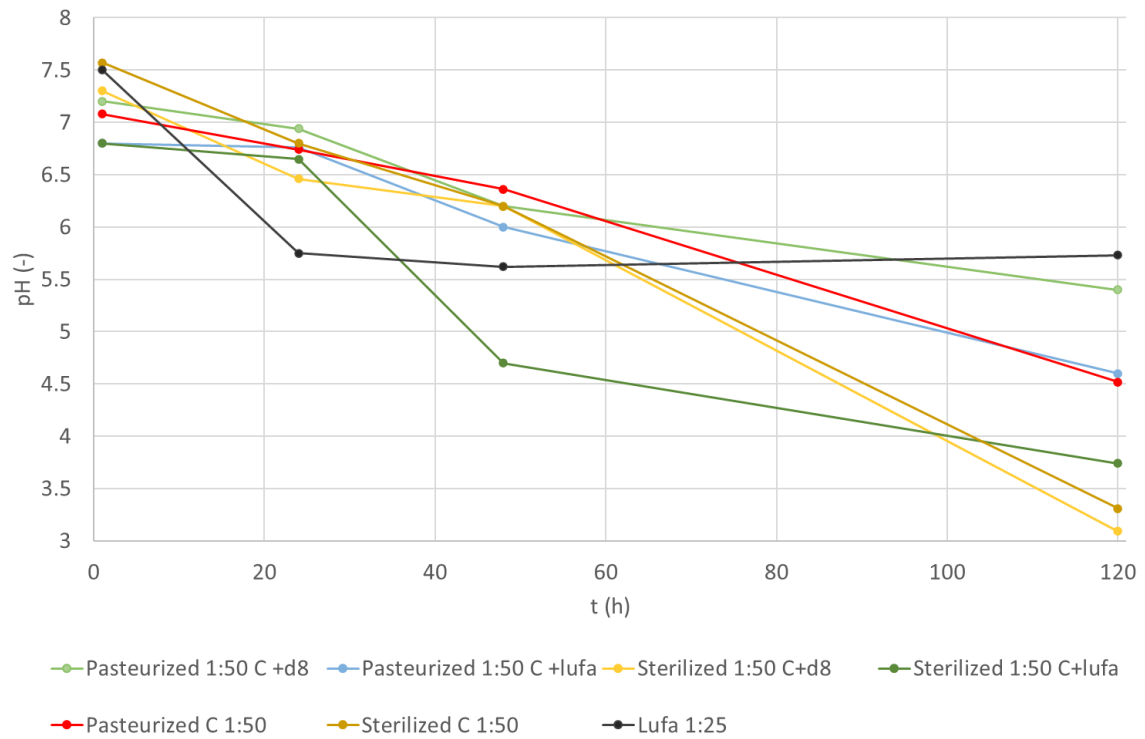


Figure 31: pH values over time registered during the degradation-milk.

In Figure 32, the percentage variations of the pH registered in the milk samples are listed.

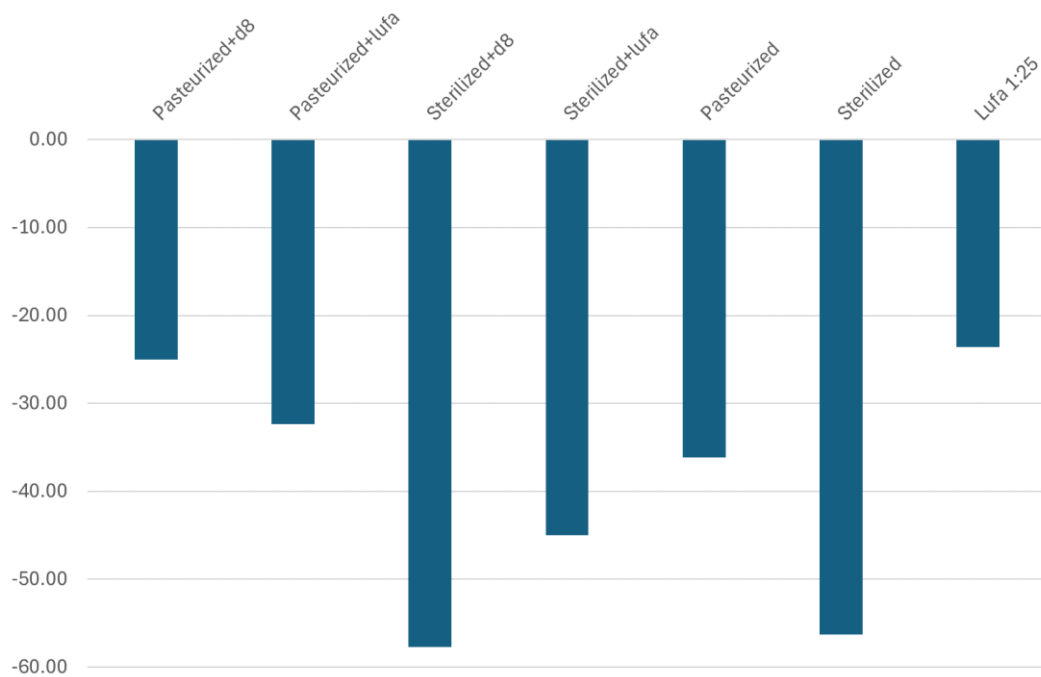


Figure 32: Percentage variations of the pH in the milk samples.

Generally, a decrease in the pH values was registered from the first to the last sampling: the sterilized samples showed a greater acidification of the system. Both the substrate showed a deceleration in the acidification of the system with respect to the pure milk samples, probably due to a buffering action of the substrates which tended to constrain the acidification of the environment. The trend of the pH registered in the pasteurized milk mixed with sand has a different path with respect to the others.

3.2.1.1.2. Spectrophotometer analyses results

The trend of changing in the absorbance has been evaluated using a spectrophotometer in the samples in which there was diluted milk mixed with soil (since the values of absorbance registered at the beginning were higher than 1, the samples have been further diluted by a factor of 4).

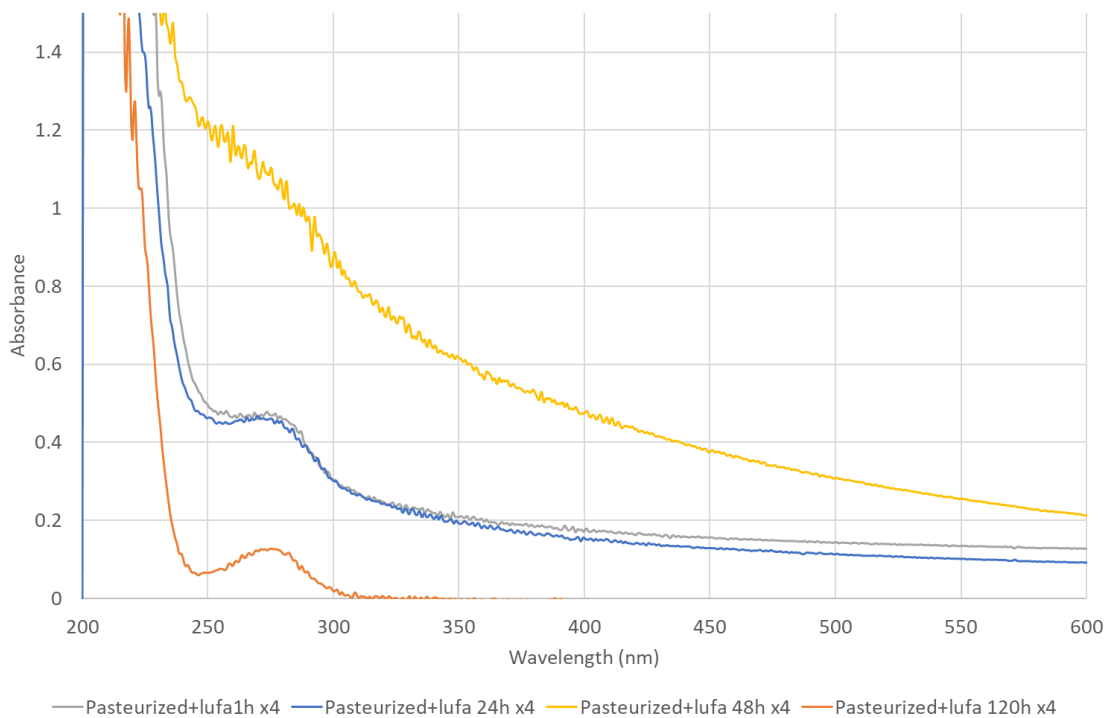


Figure 33: Trend of the absorbance in the degradation of pasteurized milk mixed with soil.

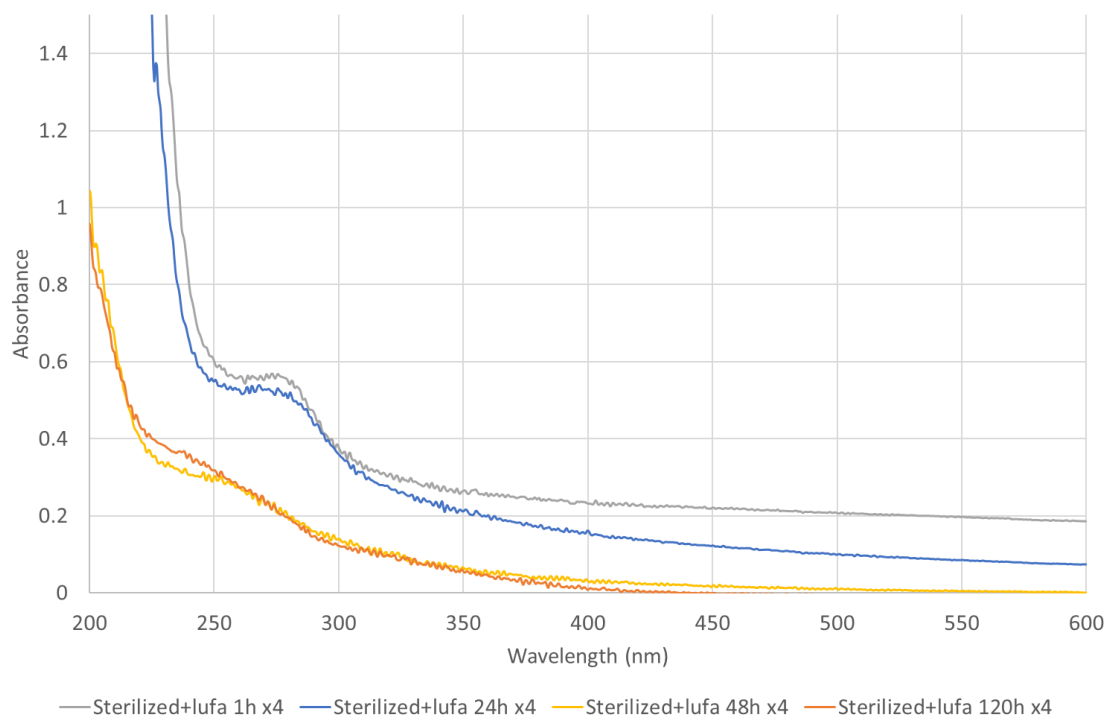


Figure 34: Trend of the absorbance in the degradation of sterilized milk mixed with soil.

According to the graphs shown in Figure 33 and Figure 34, the interval of wavelengths between 250 and 300 nm has been investigated: in this range, a characteristic behaviour of the milk has been highlighted, which increases its absorbance resulting in a peak value around 275nm. The variation of the wavelength associated to the peak value has been investigated to detect the possible formation of new substances during the degradation, and further analysed with the spectrofluorometer.

Table 11: Wavelengths associated with the peak of absorbance in the investigated interval for the pasteurized milk mixed with soil.

Time (h)	Wavelength (nm)	Peak of absorbance
1	273.5	0.478783
24	269.5	0.462532
48	272	1.118515
120	275	0.127574

Table 12: Wavelengths associated with the peak of absorbance in the investigated interval for the sterilized milk mixed with soil.

Time (h)	Wavelength (nm)	Peak of absorbance
1	273	0.568599
24	275.5	0.531554
48	270	0.238467
120	270	0.239148

As it is possible to observe in Table 11 and Table 12, the wavelength associated with the peak value of absorbance tends to remain in a small range (around 270nm): it suggests that no great modification in the substances present in the samples had occurred.

3.2.1.1.3. Spectrofluorometer analyses results

The samples containing the sterilized milk, after being filtered, have been analysed using a spectrofluorometer (range of excitation and emission: 260-600 nm, slit equal to 5 nm) and following the PARAFAC method, which facilitates manipulation of the represented object by replacing it with orthogonal or diagonal matrixes, that are often easier to work with. PARAFAC was developed as a generalization of the factor analysis model for two-way data, where each factor represents a pattern due to a distinct influence on the data [33].

There were two components for the milk samples, with a similar profile, shown in Figure 35:

1. For the first component, the maximum excitation wavelength was around 230/280nm, while the maximum emission wavelength was 350nm.
2. For the second component, the maximum excitation wavelength was around 230/285nm, while the maximum emission wavelength was 360nm.

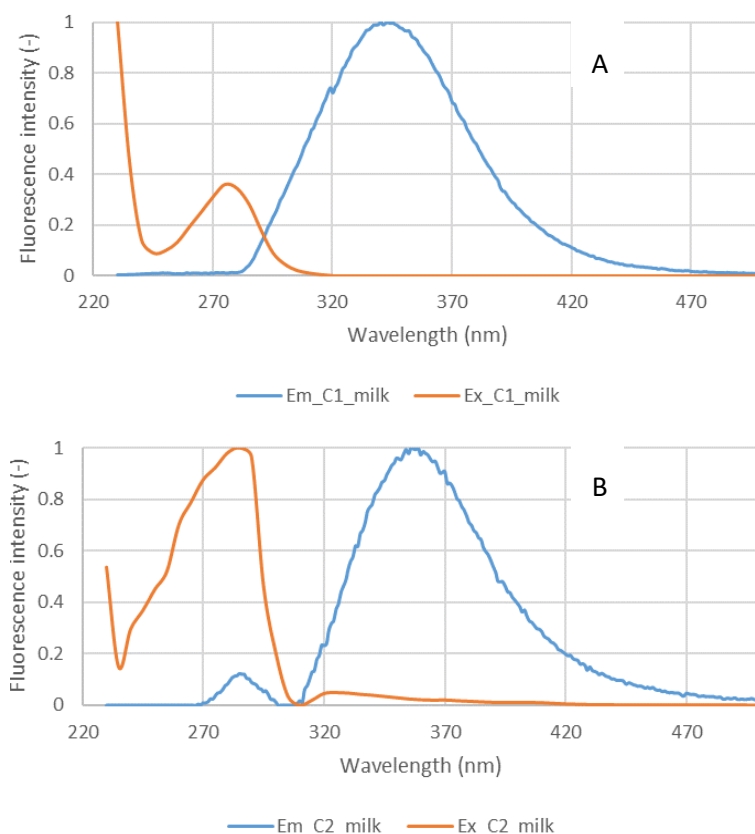


Figure 35: Principal components of the pasteurized milk. A: component 1; B: component 2

A review of the literature on this topic ([34], [35]) found that the second component can be linked to the presence of tryptophan-based peptides and compounds, i.e. proteins.

There were three principal components for the pure soil samples, reported in Figure 36:

1. For the first component, the maximum excitation wavelength was 235/320nm, while the maximum emission wavelength was 420nm.
2. For the second component, the maximum excitation wavelength was 245/340nm, while the maximum emission wavelength was 490nm.
3. For the third component, the maximum excitation wavelength was 220/285nm, while the maximum emission wavelength was 430nm.

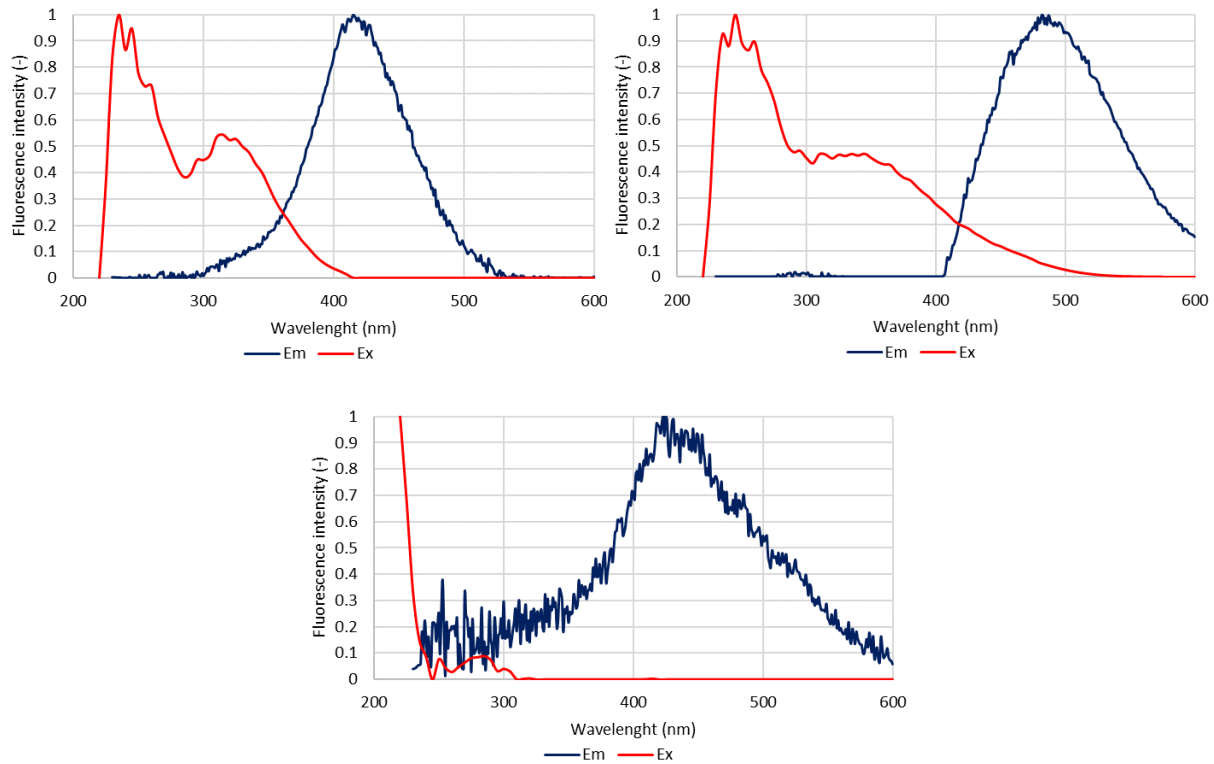


Figure 36: Principal (three) components of the soil.

The first component is attributable to A-type humic substances (which contain humina), the second component to C-type humic substances (which contain fulvic acids) and the third to C-type humic substances and protein compounds. The humic substances are identified with different letters (A, C) according to their properties and the values of their main parameters.

Figure 37 shows the fractions of the different components in the soil samples at different dilutions, from 1:5 to 1:100. Increasing the dilution factor, the fraction of component 1 decreased, the fraction of component 3 increased slightly, while component 2 remained almost unchanged.

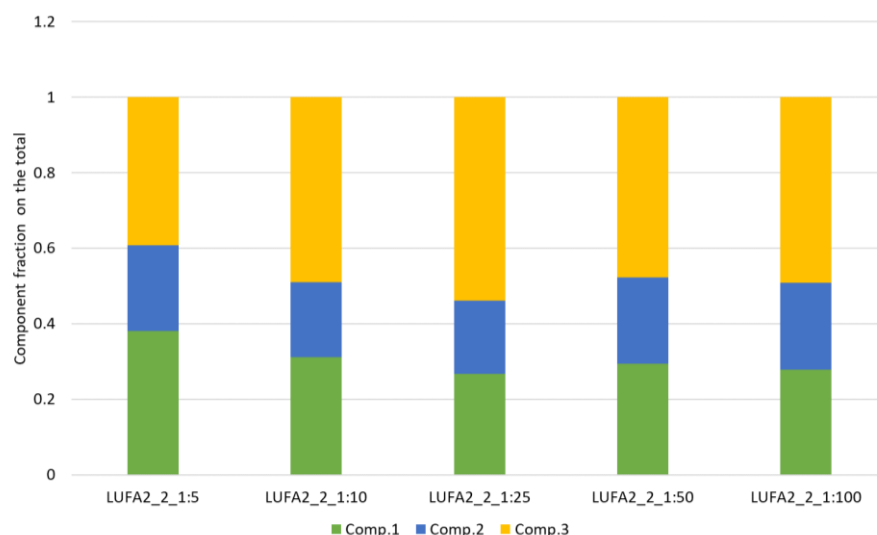


Figure 37: Variation of the fraction of different components in the soil at different dilutions.

Comparing the pattern of the milk components with those of the soil it is possible to obtain the pattern shown in Figure 38. It can be clearly seen that the emission peaks of milk are quite distinct from those of soil.

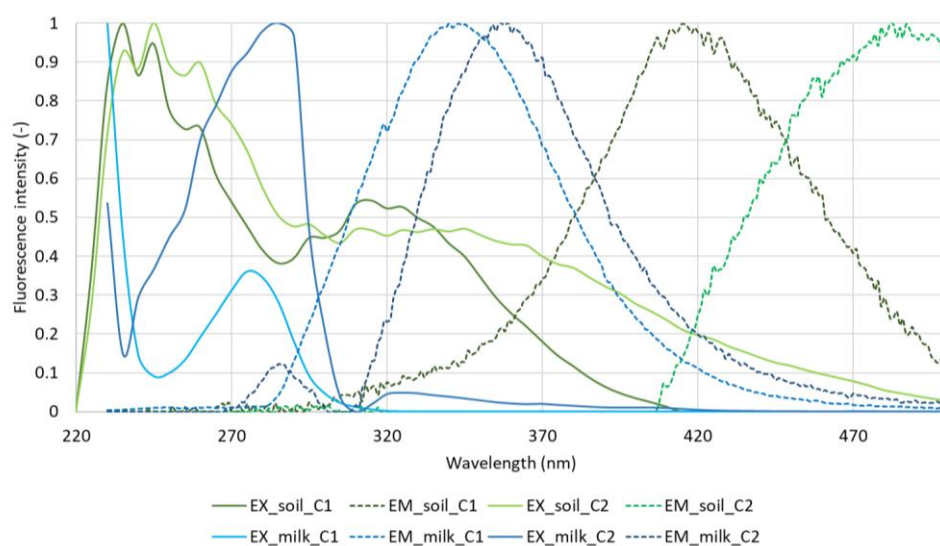


Figure 38: Comparison between excitation and emission patterns of the components of soil and milk.

In Table 13, the maximum excitation and emission wavelengths for milk and soil have been summarized.

Table 13: Maximum excitation and emission wavelength for different components of soil-only and milk-only samples.

	Ex max (nm)	Em max (nm)
Milk-C1	225-280	350
Milk-C2	220-280	360
Soil-C1	235-320	410
Soil-C2	235-340	490
Soil-C3	220-285	430

For sterilized milk samples in contact with D8 sand or LUFA soil, five main components have been determined. The maximum excitation and emission wavelength for each component are listed in Table 14, together with an indication of the similarity of the pattern of the component that had been obtained for pure-milk or pure-soil.

Table 14: Maximum excitation and emission wavelength for the different components of milk samples in contact with soil and sand.

	Ex max (nm)	Em max (nm)	Similar compound
Component 1	230-280	340	Milk-C1
Component 2	235-270-410	460	-
Component 3	225-280	350	Milk-C1
Component 4	220-280	360	Milk-C2
Component 5	230-315	405	Soil-C1
Component 6	225-275	310	tyrosine+microbial humic substances

As it can be noticed in Table 14, it has been possible to identify all the samples with a similar compound, except for the component 2. The component 6 appeared in these analyses and not in the one of the soil-only, confirming that organic matter biodegradation phenomena are involved.

In Figure 39 it is represented the composition of the different components in terms of percentage abundance for the degradation of milk in sand, compared to the ones of the milk-only.

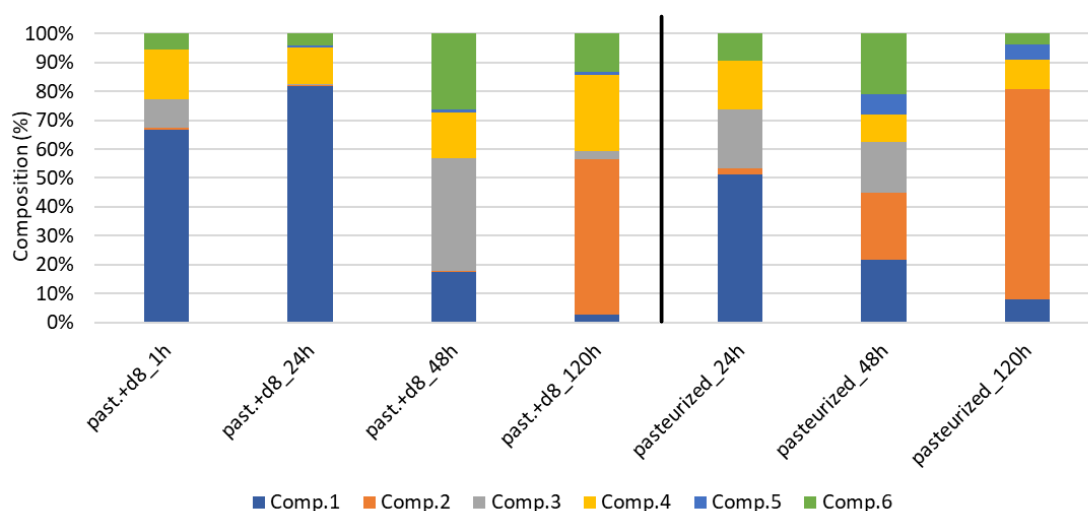


Figure 39: Composition of the different samples in terms of relative abundance of the different components for sand degradation.

It can be noticed that after 24h the components 1-3-4, characteristic of milk-only, were still the predominant ones, while a hint of component 2 and 6 could be seen. After 48h, component 2 increased to 22% and component 5 also appeared, characteristic of the soil, but more generally of humic substances: this is a sign of the degradation in progress which was taking place. Component 2 after 120h represented 73% of the sample, while the components 1-3-4 (characteristic of milk-only) together made up less than 20% of the sample. For the milk samples with the sand, after 1h almost the entire sample consisted only of the components 1,3,4 characteristic of milk, with a small percentage, less than 5% of component 6. After 24h the situation was almost unchanged with component 1 and 4 constituting the entire sample. After 48h there was a significant growth of component 6 and only in the last sample, after 120h, there was 53% of the total constituted by component 2. Component 3 and 4, being very similar each other, may sometimes have been swapped. Component 5 of the humic substances, characteristic of the soil, was not present in sand samples. The results found during the TOC analyses (3.2.1.1.1) identified significantly different parameter trends for milk-only and for samples in which sand was present, which appeared to inhibit the degradation process. The non-appearance of component 5 in the samples with sand may confirm this.

The same results are represented in Figure 40, in which the composition of the different components is reported in terms of percentage abundance for the degradation of milk in soil, compared to the ones of the milk-only. The soil-only samples showed almost no change in the components with a slight increase in component 6, while component 1 decreased. For the milk and soil samples, after 1h the main components

were 1-3-4, those characteristics of milk, whereas after 24h the component 6, tyrosine, came up with 35%. Component 5 also appeared and increased, characteristic of the soil, while components 1-3-4 were reduced. For the milk-only, component 3 practically disappeared after 48h, the contribution of component 1 remained at 48h and component 4 was present at both 48h and 120h. The tyrosine component increased with increasing time, a sign that degradation had taken place; its increase was greater than in soil-only samples.

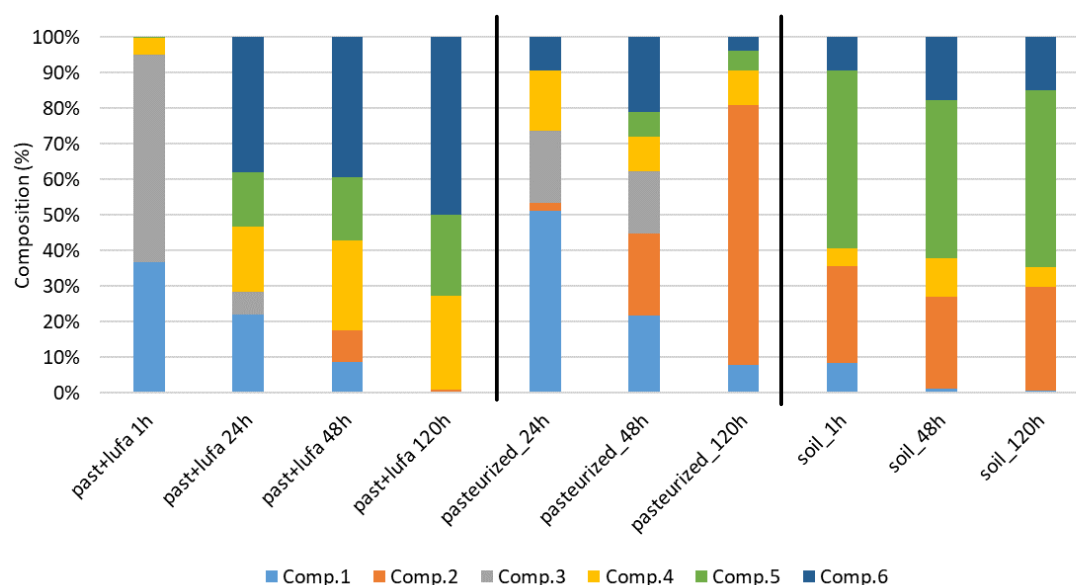


Figure 40: Composition of the different samples in terms of relative abundance of the different components for soil degradation.

3.2.1.2. Blueberry jam

3.2.1.2.1. TOC and pH analyses results

During the degradation of centrifuged blueberry boiling water from both fridge and freezer storage, a solution with 1:10 dilution has been used, to simulate the possible wastewater of a jam production plant resulting from washing operations of the production machines. The measures of TOC and pH during time are reported in Figure 41, Figure 42, and Figure 43, respectively.

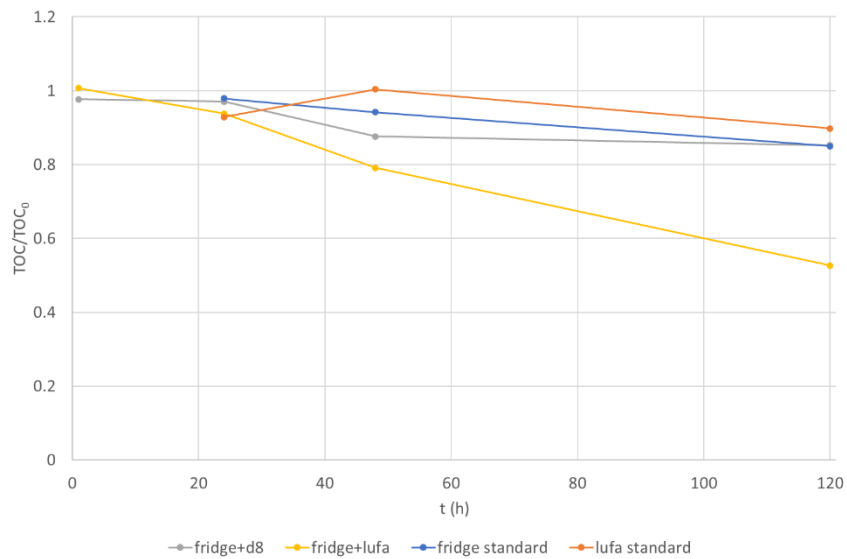


Figure 41: Degradation of the boiling water stored in fridge over time.

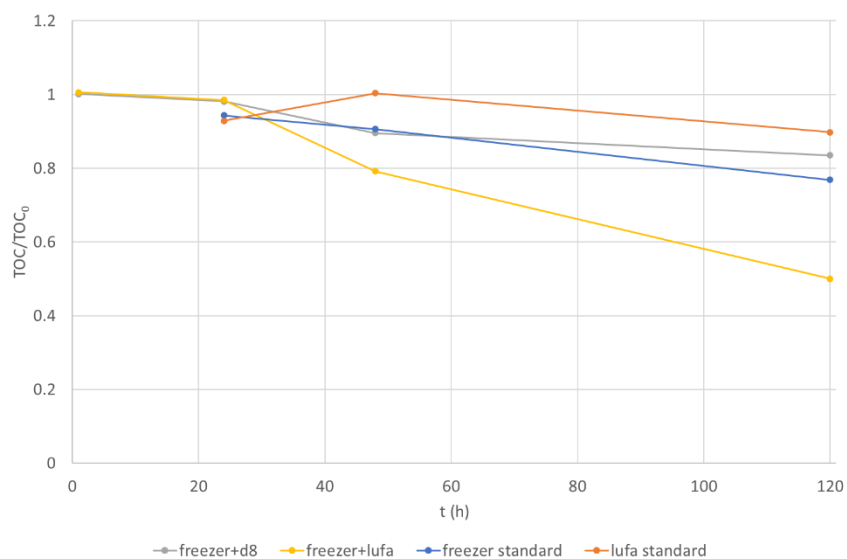


Figure 42: Degradation of the boiling water stored in freezer over time.

The trend of the degradation is quite similar for both the typologies of samples: it is possible to choose indistinctly one storage modality for the future. The substrate which led to the higher degradation is the soil combined with the wastewater, halving the organic carbon content after 5 days.

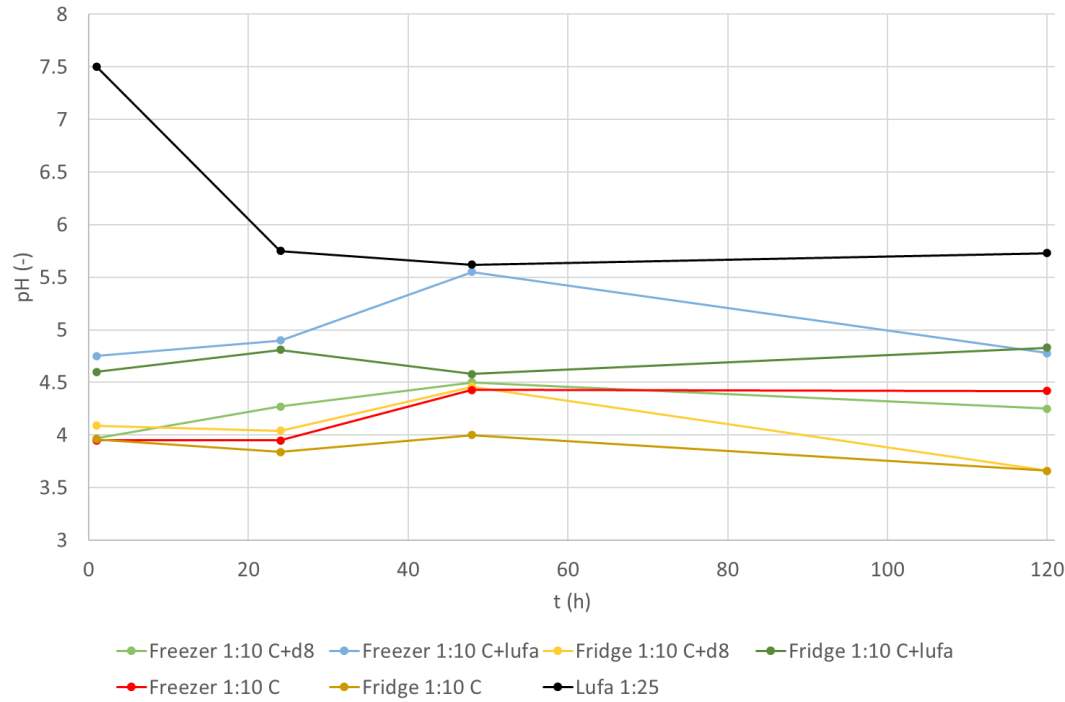


Figure 43: pH values over time registered during the degradation- blueberry jam.

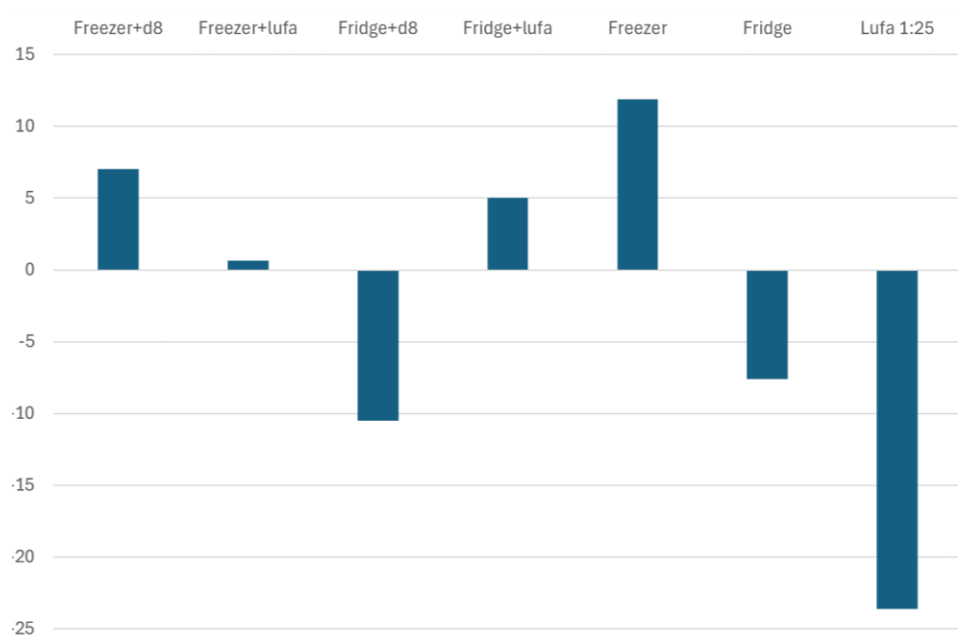


Figure 44: Percentage variations of the pH in the blueberry samples.

As showed in Figure 44, different trends were registered:

- The samples in which the substrate was the sand had different behaviours, having an increasing of 7% for the wastewater stored in the freezer, while the one stored in the fridge decreased of 10%.
- The samples in which the substrate was the soil increased a bit their pH value.
- The pure samples had an increase in the freezer-stored water, while the fridge-stored water and the pure soil decreased their values.

3.2.2. Summer

This test lasted for 42 days, during which the degradation has been analysed in terms of TOC, IC, and pH changes.

3.2.2.1. Milk

According to the results shown in the previous paragraph, for this test pasteurized milk has been used, since it is the most used in the dairy industry and there were no significant differences compared to the sterilized milk parameters. The calibration line of the pasteurized milk used for this test can be assumed the one used for the tests carried out in spring.

The results of the TOC, IC and pH measures can be seen in Figure 45, Figure 46, and Figure 47, respectively.

The trends of the TOC were quite different from each other, as shown in Figure 45. The TOC of the milk-only (effluent) showed a rapid degradation in the first 7 days, reaching a TOC/TOC_0 value of 0.6 in 48h. The degradation rate decreased for longer times, reaching $\text{TOC}/\text{TOC}_0 = 0.4$ after 10 days and 0.15 after 42 days. A similar trend in the first days of degradation could be observed for the effluent mixed with the standard soil, for which a decrease of 60% was registered in the first 48h, and then a modest increase for longer times with a stabilisation to $\text{TOC}/\text{TOC}_0 \approx 0.55$. The discrepancy with the trend obtained for the effluent-only can be attributed to an initial contribution to the degradation process carried out by the bacteria present in the soil in the first 24h, with subsequent acclimatisation and growth of the microbial population (and consequently of the TOC). On the other hand, the behaviour of the effluent in the presence of siliceous sand is different, whereby the degradation is slower, reaching a TOC/TOC_0 value of 0.6 in the first 10 days and then substantially maintaining this value, probably due to the absence of significant bacterial colonies in the sand sample and the generally more unfavourable environment for bacterial proliferation. The TOC of the effluent mixed with soil increased after 8 days, probably due to the passage from an aerobic

system to anaerobic conditions: new micro-organisms grew in the system, contributing to increase the presence of organic carbon; however, the system ended up with new equilibrium conditions, leading to the reach of an asymptote in the graph.

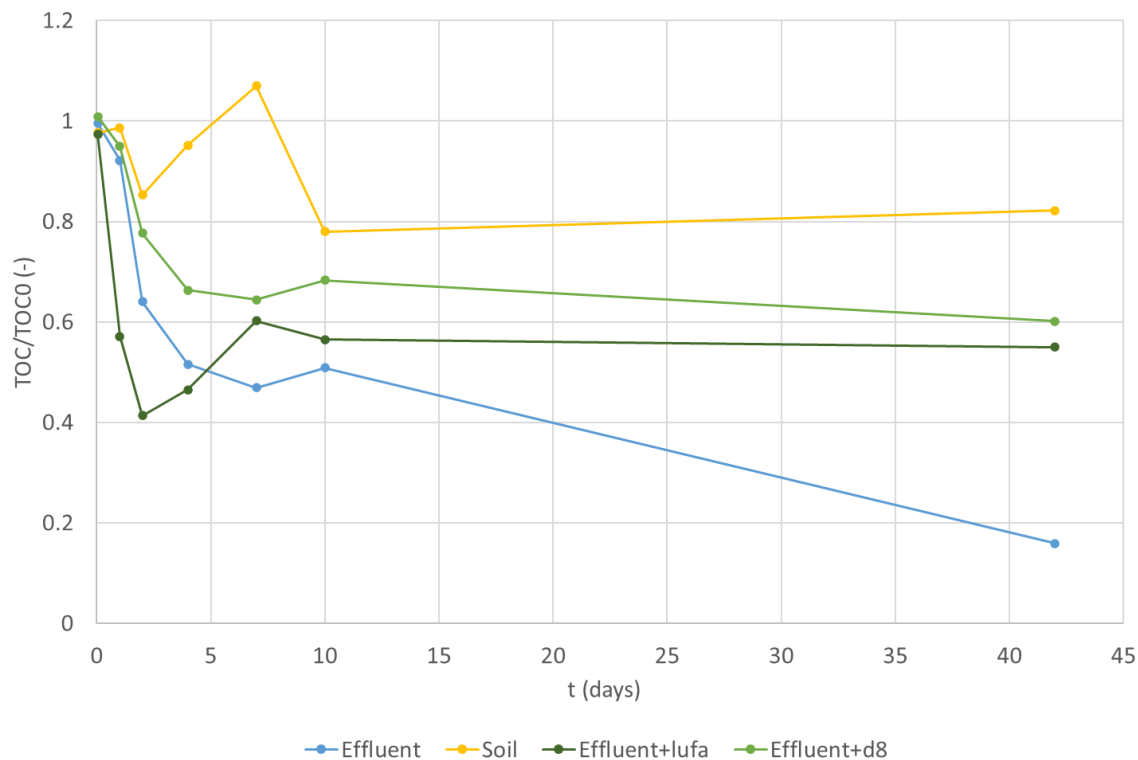


Figure 45: Degradation of milk over time (TOC)

These assumptions seemed to be confirmed by the results of the IC analyses, showed in Figure 46: as the TOC content decreases, the IC content increases (except for the soil-only sample), indicating that the microbial population is producing carbon dioxide and the carbohydrates contained in the milk are degrading. This theory also explains the difference between the values registered for the IC of the milk-only and the ones of the milk mixed with soil: the first one is lower than the latter, since in the soil are naturally present metals which helps the degradation of carbohydrates and the growth of bacteria [36].

Another possible reason which can cause the registered trend of IC is in the treatment of the samples: before evaluating their TOC content, they were centrifuged, and the solid phase was removed. The solid phase contained in the samples in which the effluent was mixed with the soil was higher than the solid phase removed from the milk-only samples, and this may have caused a higher removal of inorganic carbon for the first typology of samples.

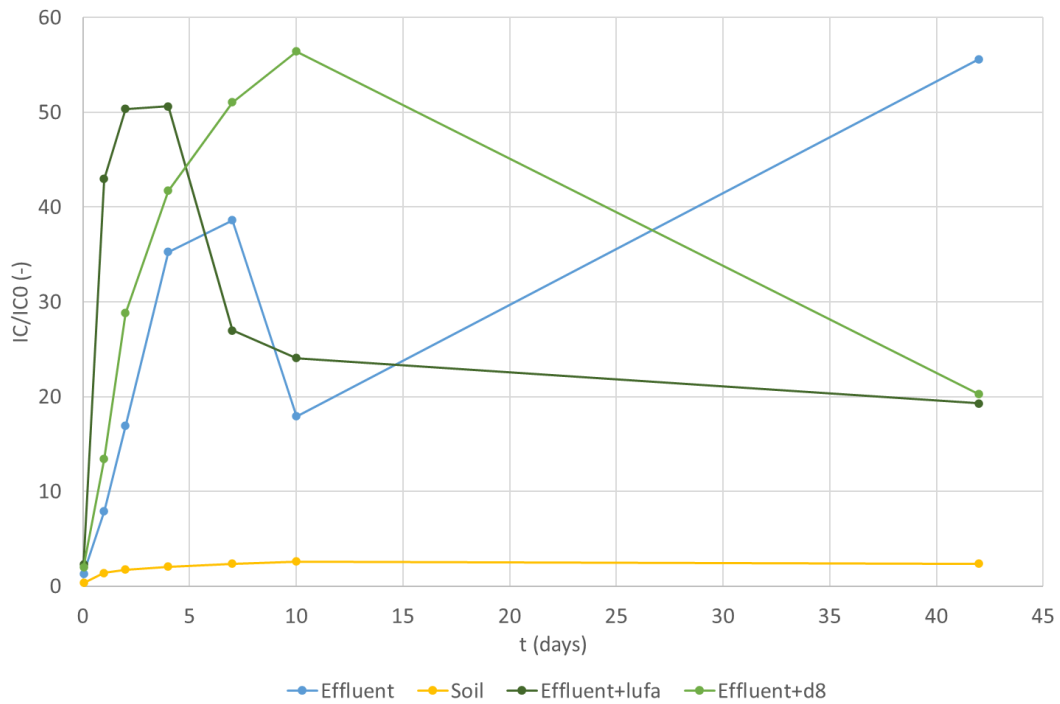


Figure 46: Degradation of milk over time (IC)

The trend of the pH showed a significant acidification of the systems, of which the percentage decrease in the 42 days range is reported in Figure 48.

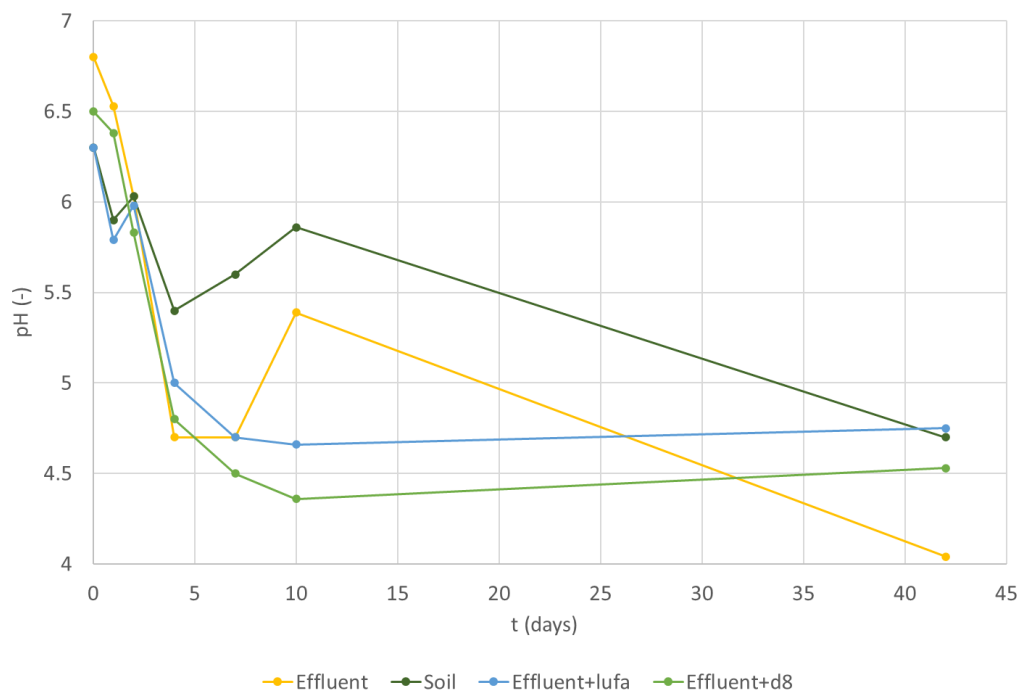


Figure 47: pH values over time registered during the degradation-milk.

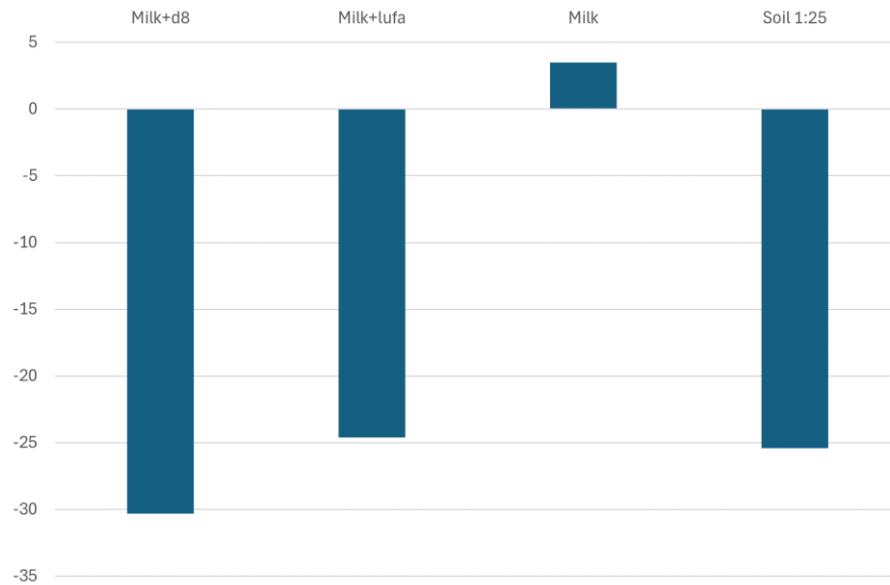


Figure 48: Percentage variations of the pH in the milk samples.

The pH decreased probably due to the liberation of acids from the degradation of fats, which naturally occurs and was enhanced by the high temperatures registered in July (up to 35°C). The percentage variation of decrease registered for the milk mixed with soil was lower than the one registered for milk mixed with sand probably due to the “buffering” action of the soil, which tended to decrease the acidification of the system.

Recent literature reviews ([37], [38]) showed that an acidification in the environment contributes to a worst degradation of the milk, explaining the trends observed in the TOC analyses (Figure 45) if linked with the pH trends (Figure 47):

- In the first period of the degradation, in which the pH decreased but did not reach high acid conditions, the degradation ratio was high.
- When the pH further decreased, the TOC started to increase because the degradation stopped.
- After 8 days anaerobic conditions substituted aerobic conditions increasing the degradation ratio and the pH.
- In the last days the degradation ratio was almost constant, and the pH decreased, probably due to the reach of an equilibrium system.

3.2.2.2. Blueberry jam

According to the results of previous analyses (3.2.1.2.1), for this test it was used boiling water from the blueberry jam production plant stored in freezer.

The results of the TOC, IC and pH measures can be seen in Figure 49, Figure 50, and Figure 51, respectively.

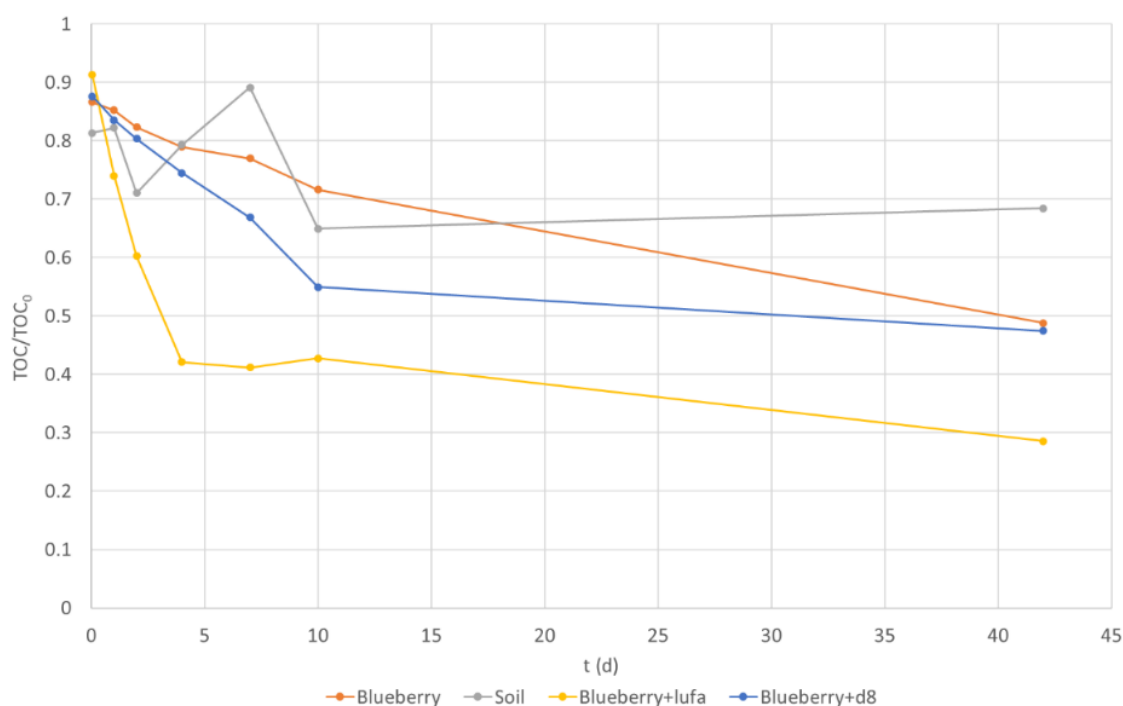


Figure 49: Degradation of blueberry jam over time (TOC)

As it is possible to notice in Figure 49, there is a general decrease in the TOC content for all the samples. The presence of a substrate (soil or sand) seemed to generally help the degradation. However, the trend for the sample containing blueberry jam mixed with soil showed a greater degradation over time with respect to the sample containing blueberry jam and sand. For the blueberry jam-only and blueberry mixed with sand the final degradation was around 50%, while for the sample in which the soil was present the degradation reached 70%.

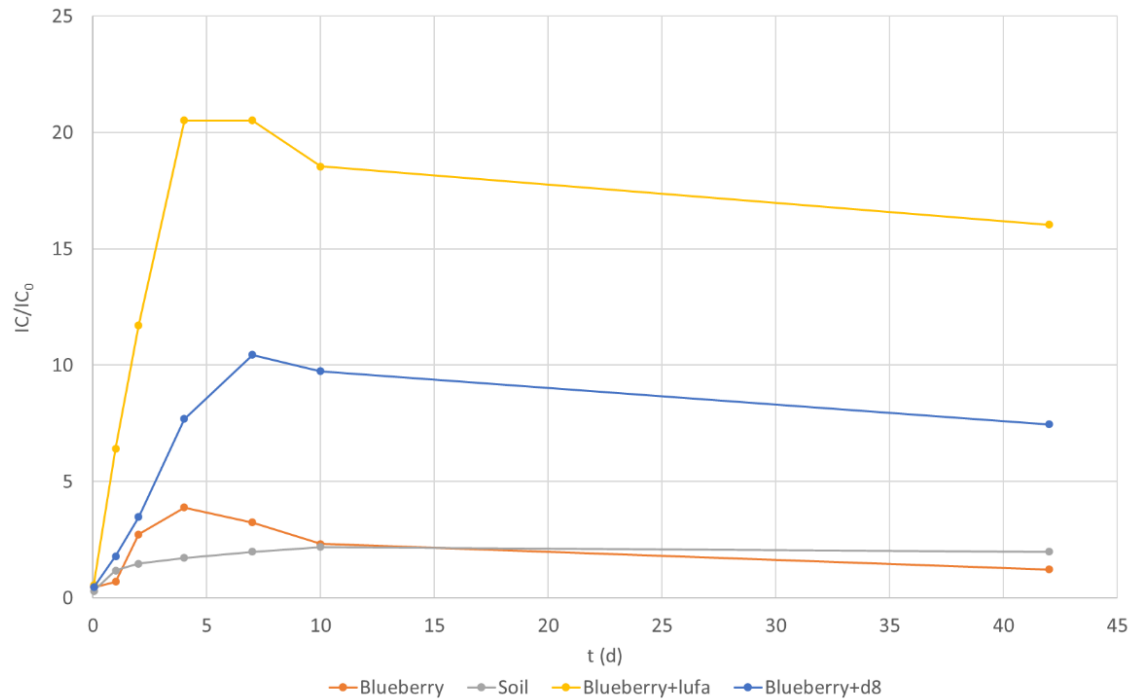


Figure 50: Degradation of blueberry jam over time (IC)

The trends of the IC reported in Figure 50 show a similar pattern for all the samples, in which three phases can be identified:

1. A general increase in the presence of IC for the first days.
2. The reach of a peak, in the 4th/5th day of the test.
3. A decrease in the values registered until the end.

The patterns reflect what happened in the sample: when there was an increase in the IC, there had been the production of carbon dioxide by the action of microorganisms; in the milk mixed with soil, the increasing was higher than what happened in the sand, because in the soil there were already naturally present some microorganisms at the beginning of the test, while in the sand it was necessary to wait for their development.

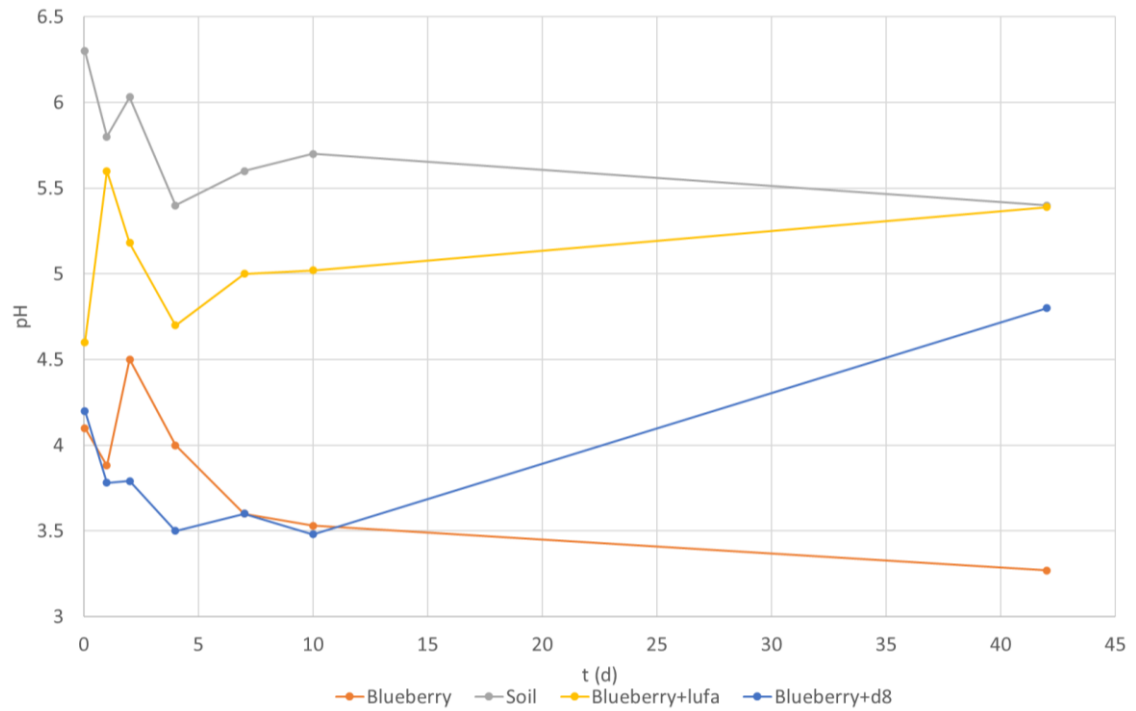


Figure 51: pH values over time registered during the degradation-blueberry jam.

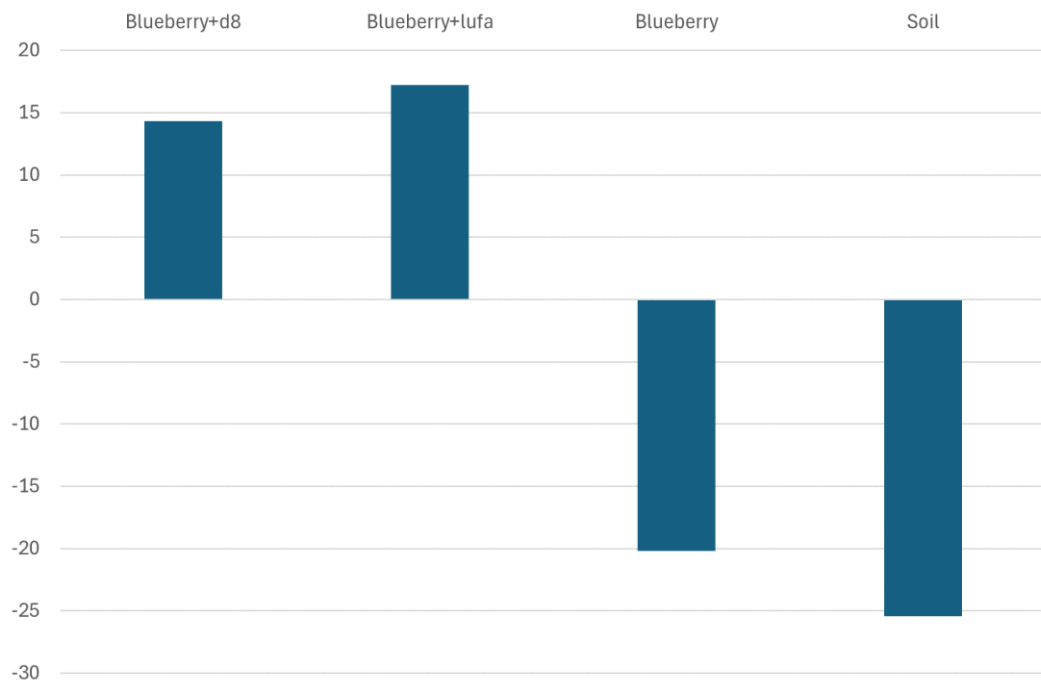


Figure 52: Percentage variations of the pH in the blueberry samples.

The trend of pH represented in Figure 51 shows different behaviours for the different samples:

- In the blueberry-only sample there was an acidification of the environment.
- The blueberry samples mixed with a substrate increased the pH in the end, turning in a positive variation.

As reported by Howard et al. [39], the storage of blueberry samples likely caused the degradation of anthocyanins, which released acids, causing an acidification of the environment. At lower pH (≈ 3.5) the degradation of this molecules tends to stop, and this justifies the trend showed in the graph for the blueberry-only sample. Probably, as happened in the milk degradation, the soil acted as a “buffer”, slowing the acidification of the system. The same consideration may be done on the sample in which the sand was the substrate.

3.2.3. Winter

These tests lasted for 10 days, during which the degradation has been analysed in terms of TOC, TN, and pH changes.

3.2.3.1. Non-agitation conditions (NA)

3.2.3.1.1 Milk

The result of the BOD₅ measure is 1202 mg/l. The results of the TOC, IC, TN and pH measures can be seen in Figure 53, Figure 54, Figure 55, and Figure 56, respectively.

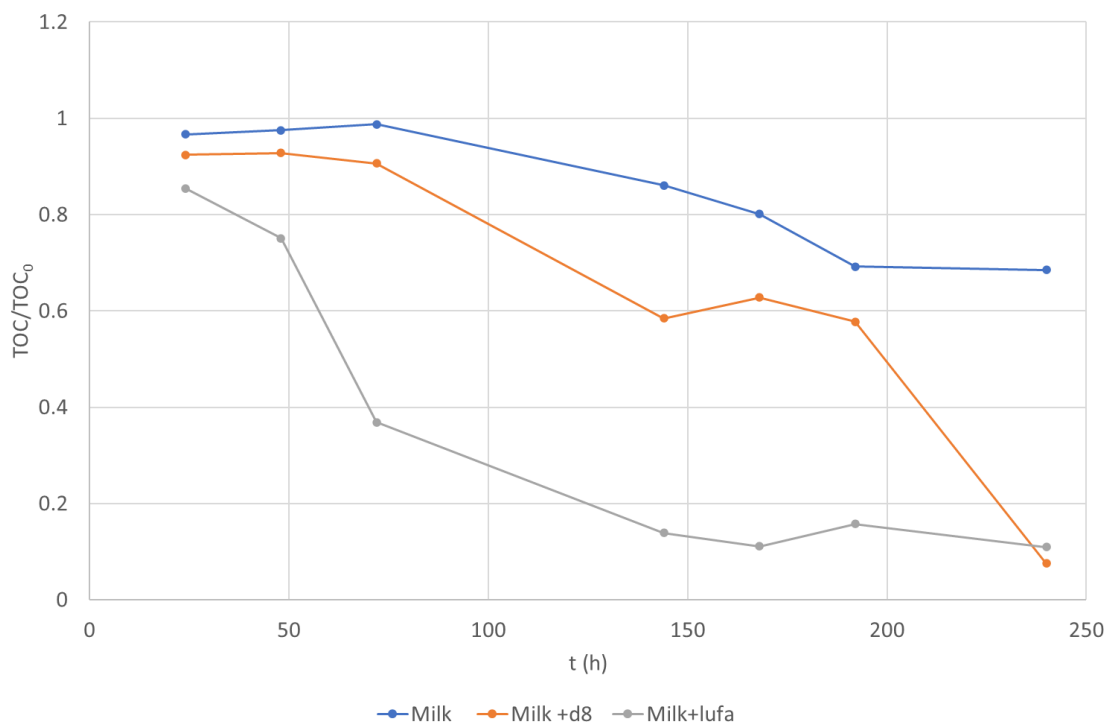


Figure 53: Degradation of milk over time (TOC)

As it is possible to see in Figure 53, the trends of the degradation were quite different:

- The milk-only samples reached a degradation of 30% in 10 days, showing a degradation velocity almost constant over time, reaching an asymptote in the last part of the test.
- The milk mixed with sand samples reached a degradation of almost 90%, showing different degradation velocities over time. In the first two days it seemed that the degradation was not

happening, instead, starting from the end of the second day, the degradation velocity increased, until the seventh day; then there was a decrease in the degradation velocity, probably due to the instauration of anaerobic conditions and the subsequent change in the microbial population present in the substrate. In the last part of the test, the degradation velocity increased leading to a large degradation of the organic carbon present in the sample.

- The milk mixed with soil samples reached a degradation of almost 90%, like the ones of the sand, despite the differences in the paths. The soil showed in fact a major degradation rate in the first three days of the degradation, decreasing it until the seventh day of the test, and then remaining almost constant. This trend may be explained by the change in the substrate conditions, which passed from aerobic to anaerobic (as it had happened in the test done in summer, 2.2.2.2).

These assumptions seemed to be confirmed by the results of the IC analyses, showed in Figure 54: as the TOC content decreases, the IC content increases, indicating that the microbial population is producing carbon dioxide, which is contained in the liquid sample, and the carbohydrates contained in the milk are degrading. This theory also explains the difference between the values registered for the IC of the milk-only and the ones of the milk mixed with soil, as already said in 2.2.2.2.

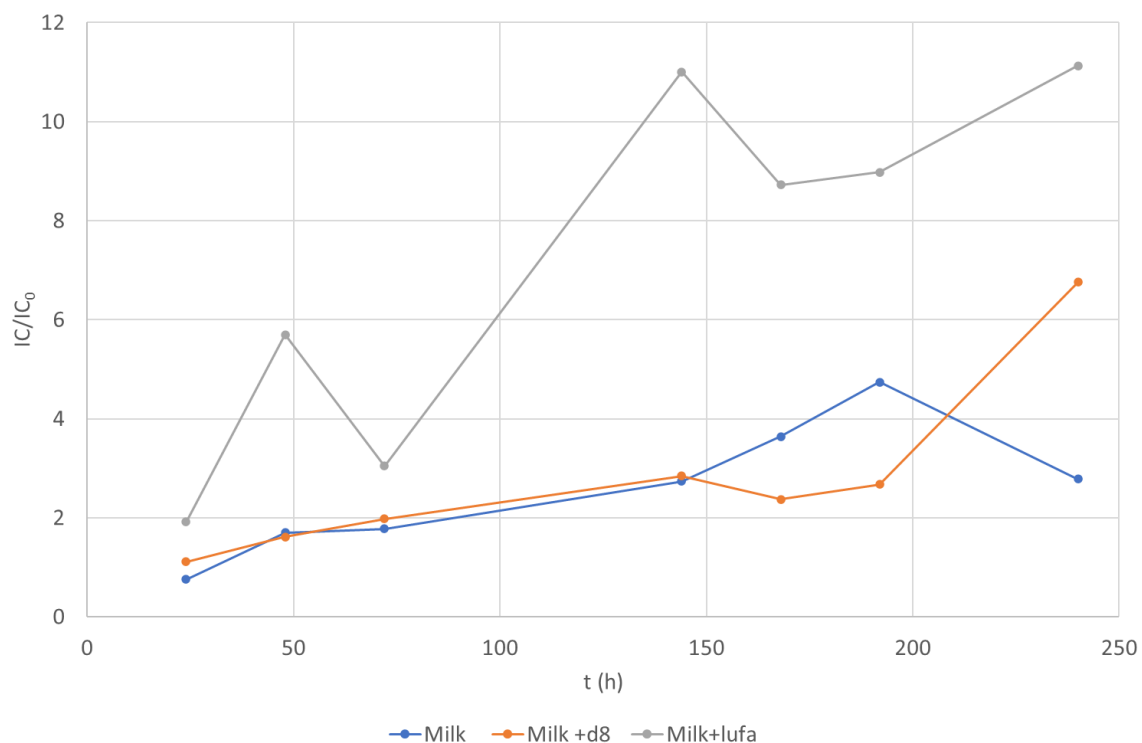


Figure 54: Degradation of milk over time (IC)

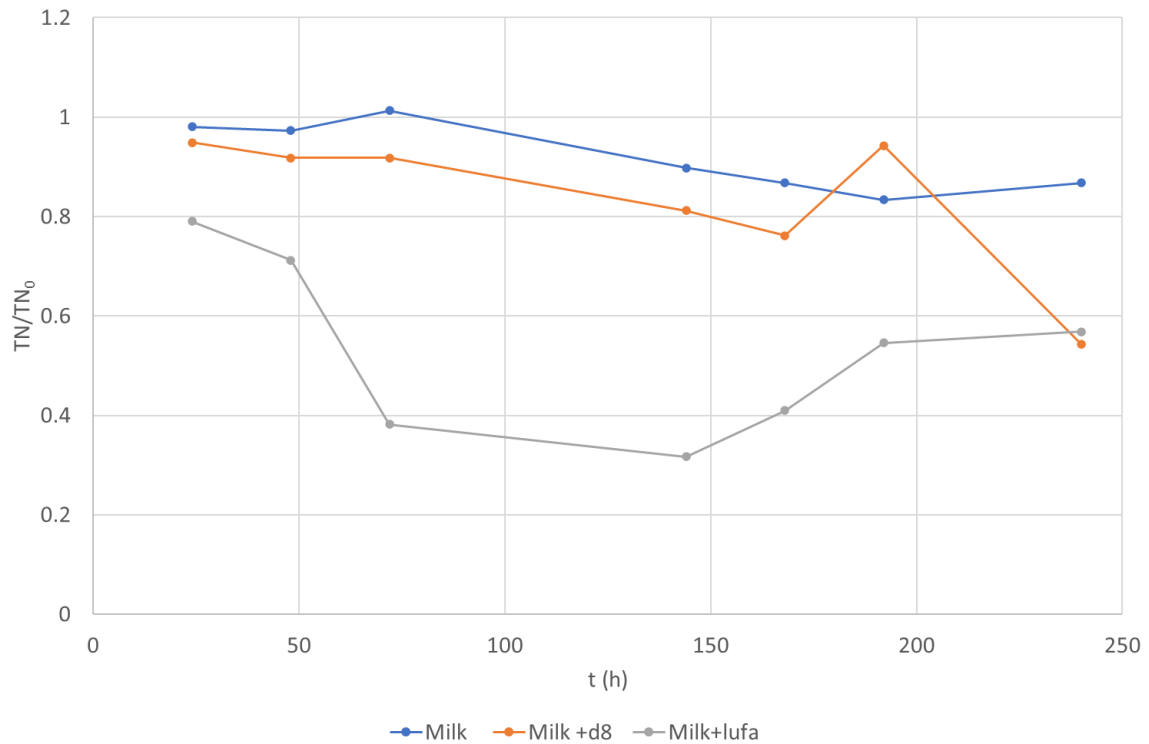


Figure 55: Degradation of milk over time (TN)

The trends of the total nitrogen content can be discussed by looking at Figure 55:

- For the milk-only samples there was a general decrease in the TN content, reaching a 10% of degradation.
- For the milk mixed with sand samples there was a decrease in the TN content in the first seven days, showing a degradation of the organic matter present in the sample. After, it seemed to happen an increase in the TN content, which corresponded to the stop in the degradation of TOC analysed in the previous paragraph, probably due to the instauration of anaerobic conditions which inhibited the activity of the microorganisms (and led to a non-consumption of nitrogen). After the restoration of an equilibrium in the substrate, the TN started to decrease again, reaching a total degradation of almost 50%.
- As it happened in the study of the TOC, the trend of the degradation in the milk mixed with soil samples showed a major slope, suggesting a greater velocity in the first three days. After, it seemed to be inhibited and the TN content increased, probably due to the instauration of anaerobic conditions which required a change in the microbial population responsible for the degradation of the organic matter, not using the nitrogen for their microbiological activity.

Additionally, the increasing in the TN content corresponded to an increase in the pH values, which caused the release of ammonia and proteins.

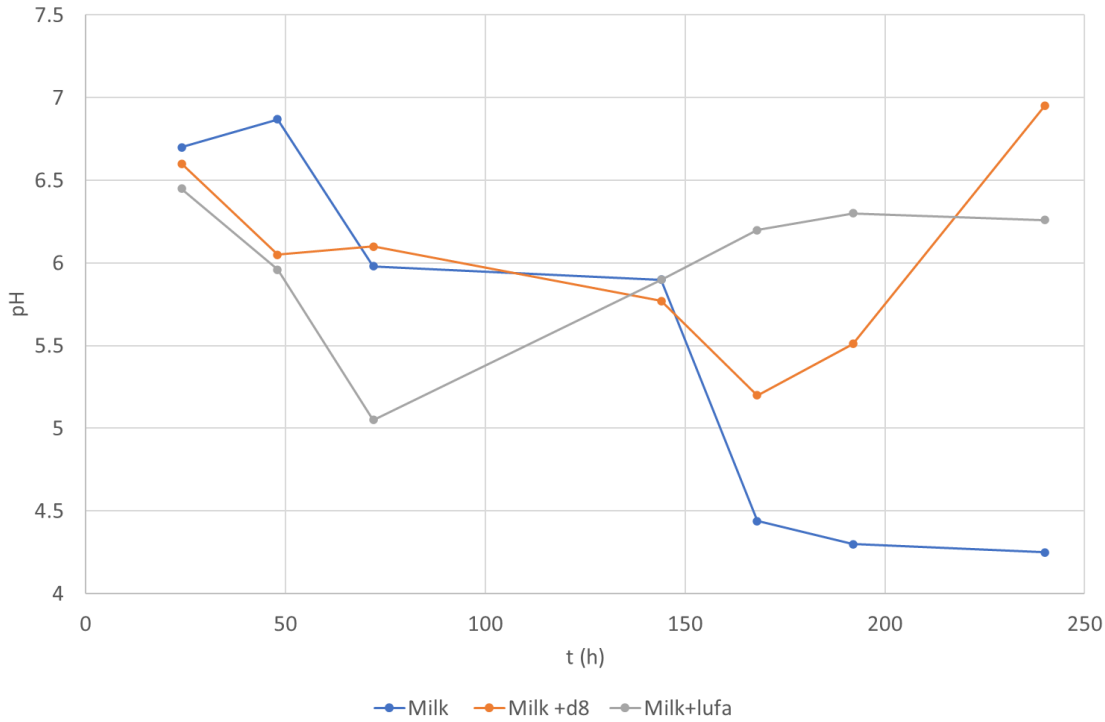


Figure 56: pH values over time registered during the degradation-milk.

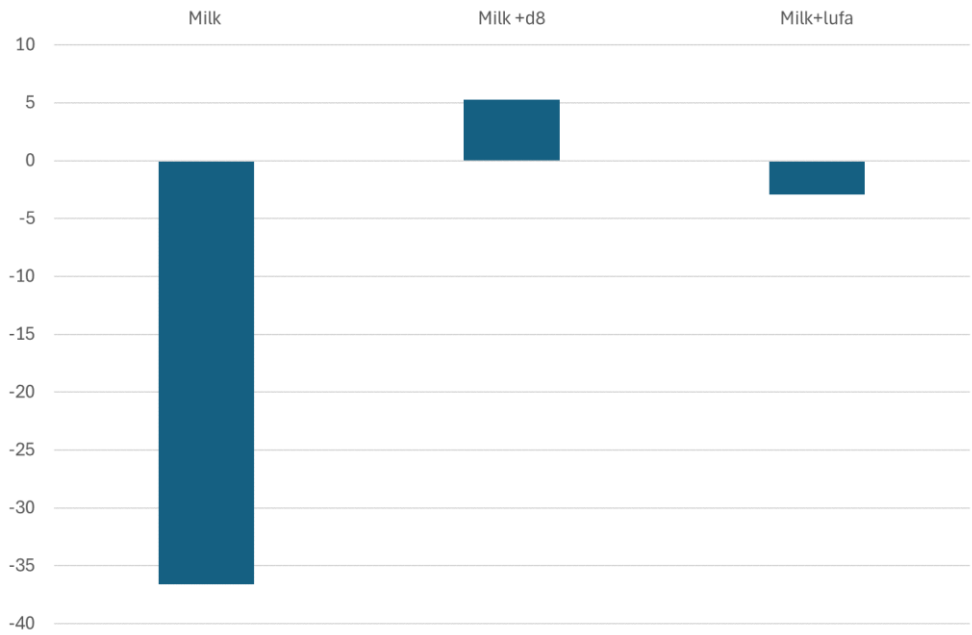


Figure 57: Percentage variations of the pH in the milk samples.

The pH trends were quite different, as it is possible to notice in Figure 56:

- For the milk-only samples there was a great acidification, which explains the inhibition of the degradation in the last days and the general lower decrease percentage with respect to the other samples; the acid environment, in fact, does not allow the development of an adequate microbial population for the degradation [38].
- For the milk mixed with sand samples there was an acidification in the first days, followed by a basification which led to a neutral final pH, showing a total percentage variation of 5%. This trend justifies the increase in the degradation velocity in the last days of the test since a basic environment helped the development of an adequate microbial population.
- The trend of the degradation in the milk mixed with soil samples showed an initial acidification of the substrate, followed by a basification: this last condition could have been reached thanks to the “buffer action” of the soil, as already explained.

3.2.3.1.2 Pepper

The result of the BOD₅ measure is 170 mg/l. The results of the TOC, IC, TN and pH measures can be seen in Figure 58, Figure 59, Figure 60, and Figure 61, respectively.

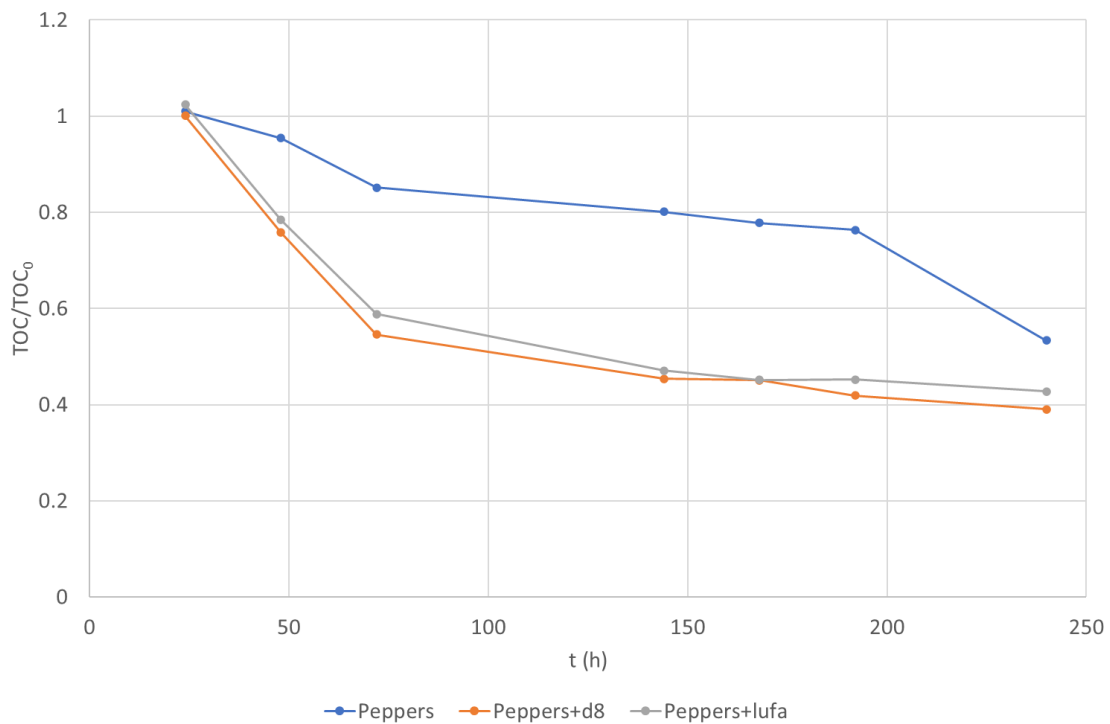


Figure 58: Degradation of pepper jam over time (TOC).

As it is possible to see in Figure 58, the trends of the degradation show a reduction in the organic carbon present in the samples of almost 60%:

- The pepper-only samples had a constant degradation rate for most of the test, that had increased in the last three days.
- The milk mixed with sand and milk mixed with soil samples showed an almost equal trend, which had a great degradation in the first days and then seemed to stabilize probably due to the strong acidification of the substrate (Figure 61).

Unfortunately, it was not possible to find previous studies in literature to compare the results achieved in this test.



Figure 59: Degradation of pepper jam over time (IC).

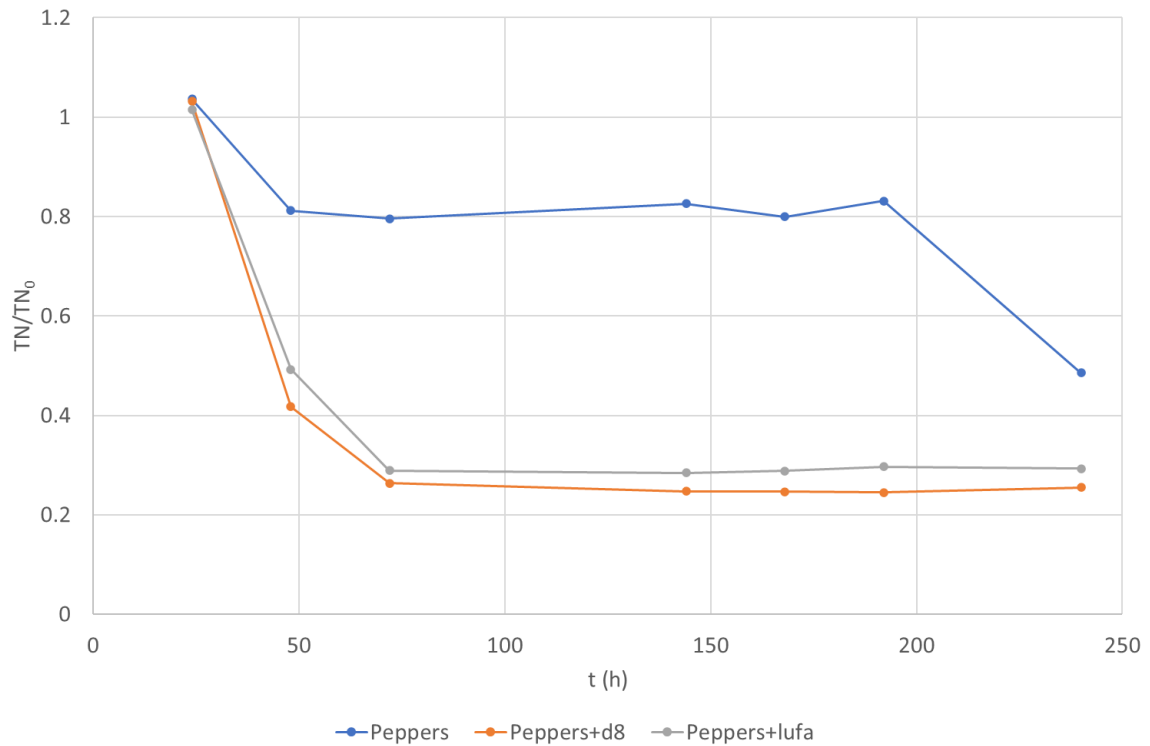


Figure 60: Degradation of pepper jam over time (TN).

In Figure 59 it is represented the trend of the IC:

- For the peppers-only samples, there was a general increase, with a higher rate in the last days.
- For the peppers mixed with sand samples, there was a general increase with a lower rate with respect to the peppers-only.
- For the peppers mixed with soil samples, the IC content increased rapidly in the first day, and then reached an asymptote.

The trend represented in Figure 60 showed a general decrease in the TN content.

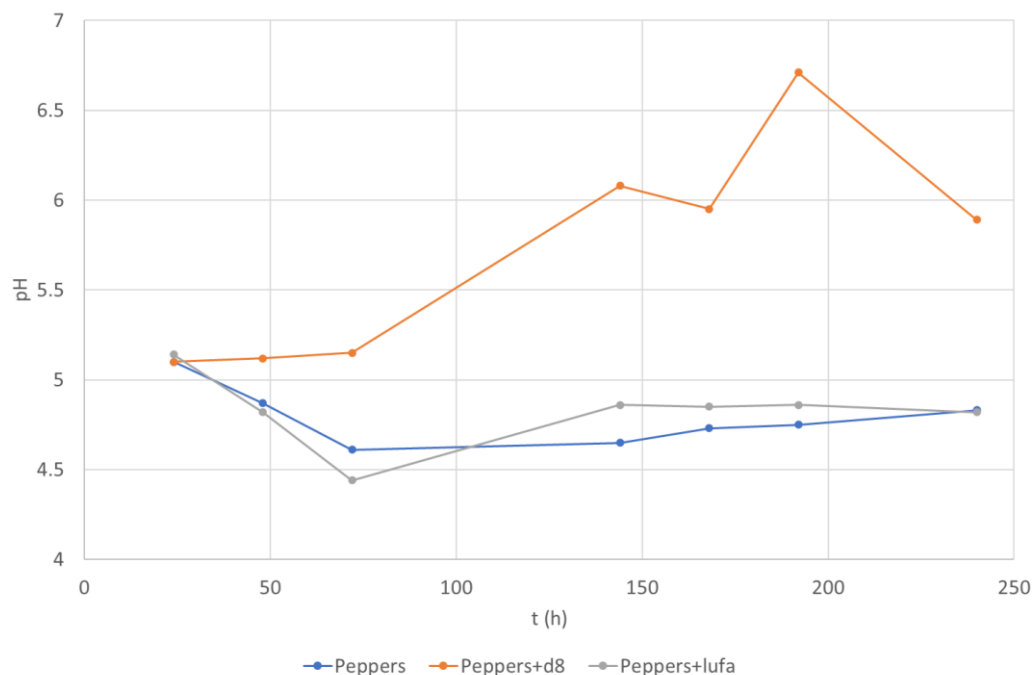


Figure 61: pH values over time registered during the degradation-pepper jam.

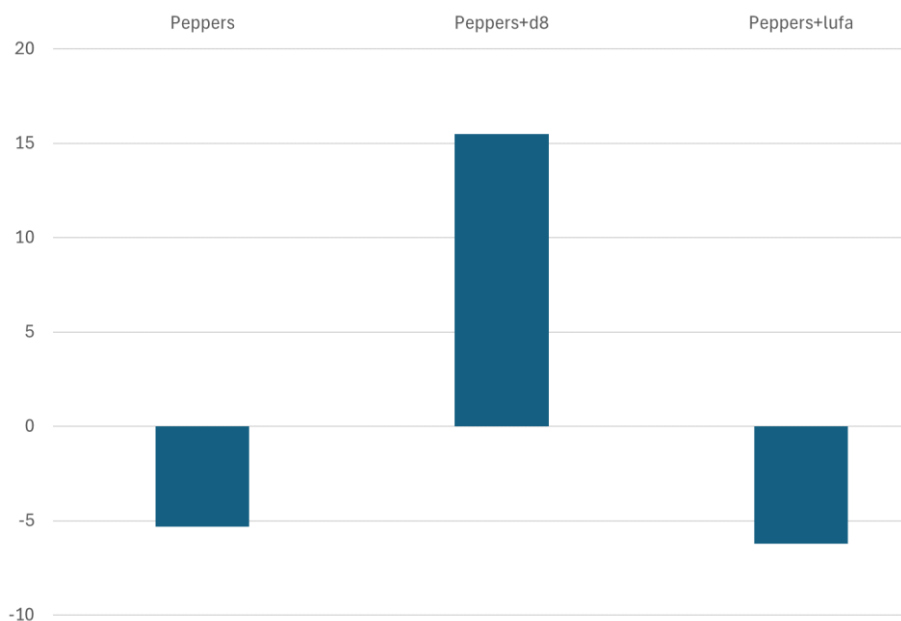


Figure 62: Percentage variations of the pH in the pepper samples.

As shown in Figure 62, the peppers-only and the peppers mixed with soil samples registered a total percentage decrease of 5.3 and 6.2, respectively, showing a starting acidification of the system, followed by a basification. The samples in which there were peppers mixed with sand registered a great basification, with a total percentage increase of 15.5%. Further studies are needed to explain these results.

3.2.3.2. Agitation conditions (A)

The results of the TOC, IC, TN and pH measures can be seen in Figure 63, Figure 64, Figure 65, and Figure 66, respectively.

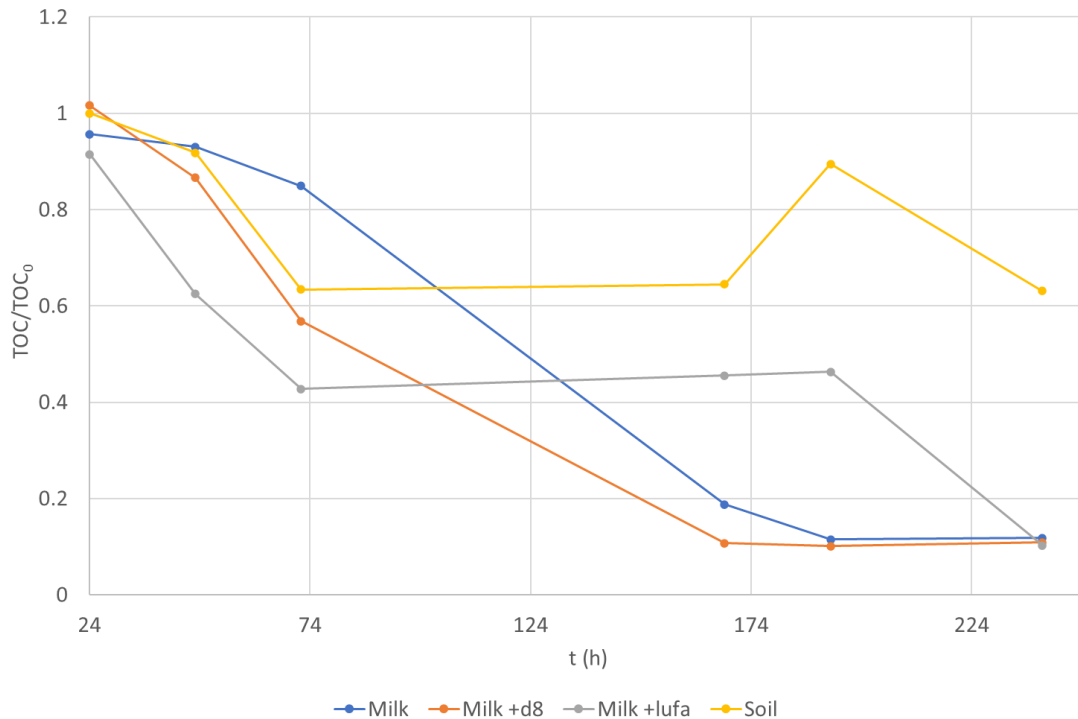


Figure 63: Degradation of milk over time (TOC).

As it is possible to see in Figure 63, the trends of the degradation were quite different, but reached the same degradation of 90%:

- The milk-only samples showed a degradation velocity almost constant over time, reaching an asymptote in the last part of the test.
- The milk mixed with sand samples showed different degradation velocities over time. In the first six days it seemed that the degradation was faster, instead, starting from the sixth day, the degradation velocity decreased, until the end, reaching an asymptote. This last condition happened probably due to the instauration of anaerobic conditions and the subsequent change in the microbial population present in the substrate. However, it seemed to be less efficient if compared to the soil at the beginning of the test because the microbial population developed later.

- The milk mixed with soil samples reached a degradation of almost 90%, like the ones of the sand and the milk-only, despite the differences in the paths. The soil showed in fact a major degradation rate in the first three days of the degradation, as it had happened in the batch tests done in non-agitation conditions, and then remaining almost constant until the ninth day, after which it started to decrease again. This trend may be explained by the change in the substrate conditions, which passed from aerobic to anaerobic (as it had happened in the test done in summer, 2.2.2.2), and then, thanks to the agitation conditions, developed a new adequate microbial population which started again the degradation.

These assumptions seemed to be confirmed by the results of the IC analyses, showed in Figure 64: as the TOC content decreases, the IC content increases, indicating that the microbial population is producing carbon dioxide and the carbohydrates contained in the milk are degrading. According to what has already been said in 2.2.2.2, this theory also explains the difference between the values registered for the IC of the milk-only and the ones of the milk mixed with soil.

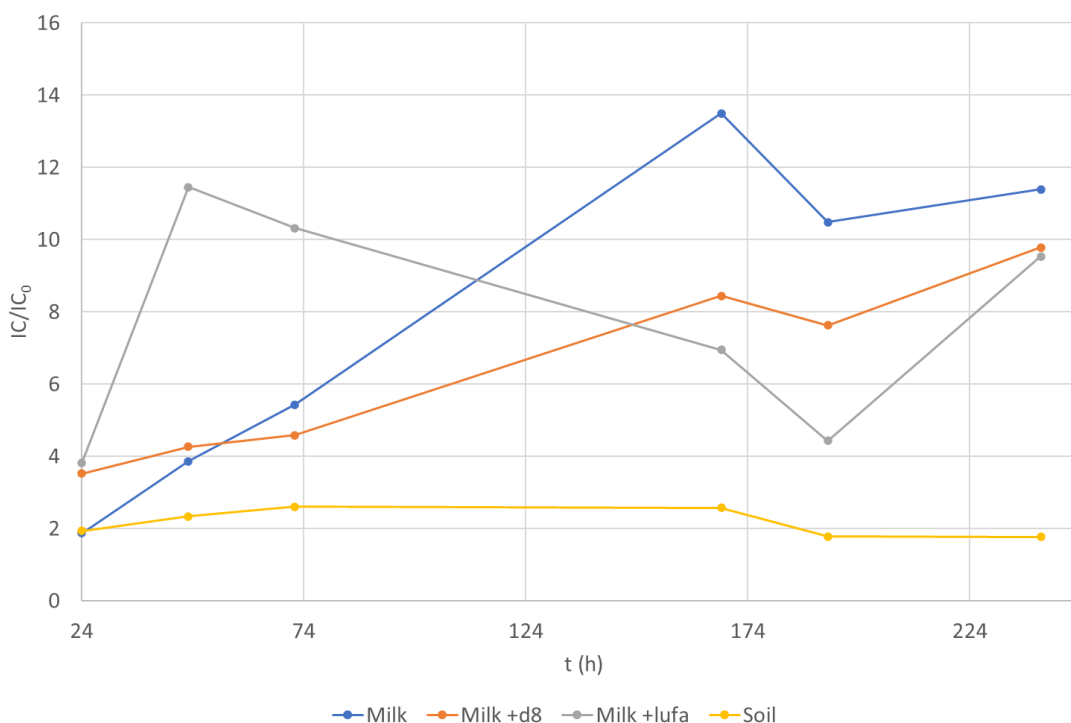


Figure 64: Degradation of milk over time (IC).

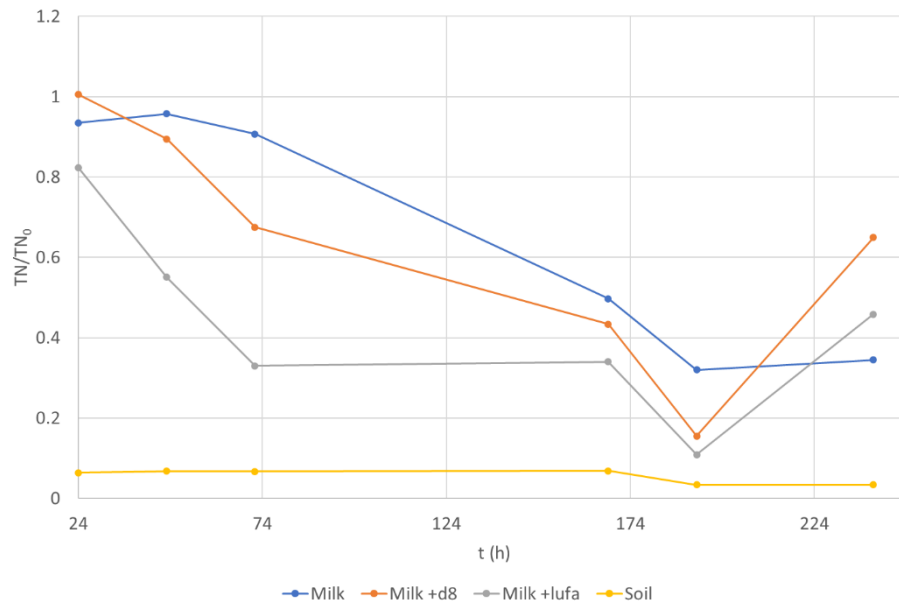


Figure 65: Degradation of milk over time (TN).

The trends of the total nitrogen content can be discussed by looking at Figure 65: all the samples registered a decrease of 60% after seven days, despite the different degradation velocities. After, there had been registered an increase of the slope of the degradation, followed by a large increase in the TN content for the milk mixed with sand and soil, and the reach of an asymptote for the milk-only. Probably, this last increase was caused by the instauration of anaerobic conditions which inhibited the activity of the microorganisms (and led to a non-consumption of nitrogen).

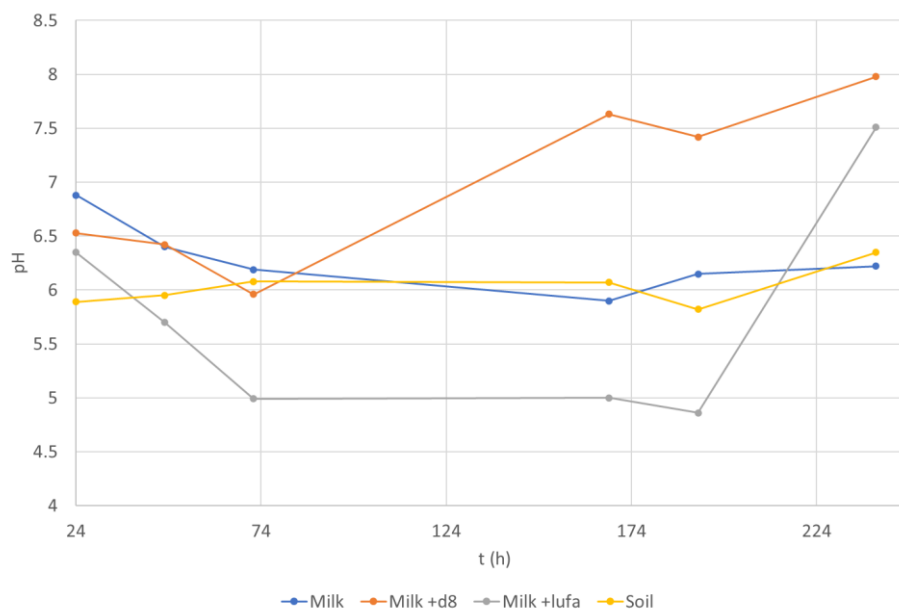


Figure 66: pH values over time registered during the degradation-milk.

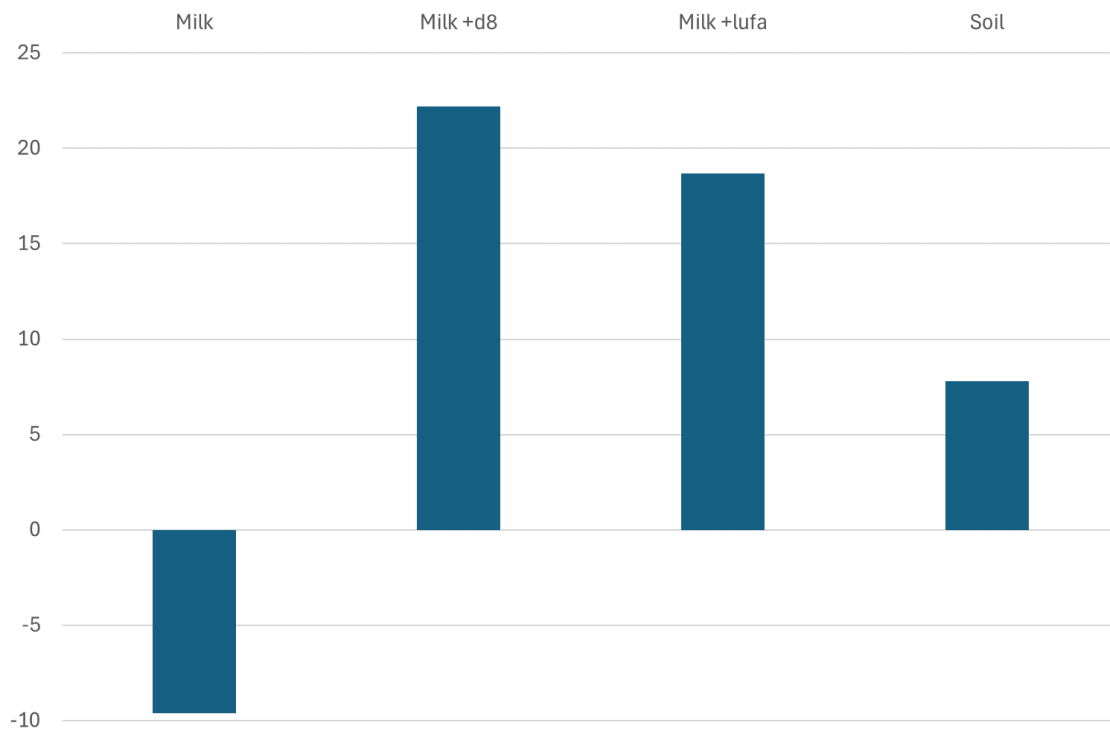


Figure 67: Percentage variations of the pH in the milk samples.

The pH trends were quite different, as it is possible to notice in Figure 66:

- For the milk-only samples there was a total decrease in the value of the pH corresponding to almost 10%.
- For the other substrates, there was a total increase in the registered value of pH of approximately 20%. However, the trends shown in Figure 66 identify different paths: despite a similar decrease in the first three days, there was an increase in the milk mixed with sand samples until the seventh day, and a constant value in the milk mixed with soil. Subsequently, a similar decrease followed by a great increase was registered, leading to a final pH ≈ 7.5 .

3.3. Considerations

According to the results of the batch tests showed in the last paragraphs, the degradation efficiency (in terms of TOC/TOC_0) reached in the different typologies of sample after 24 hours and 10 days have been compared in Figure 68 and Figure 69, respectively.

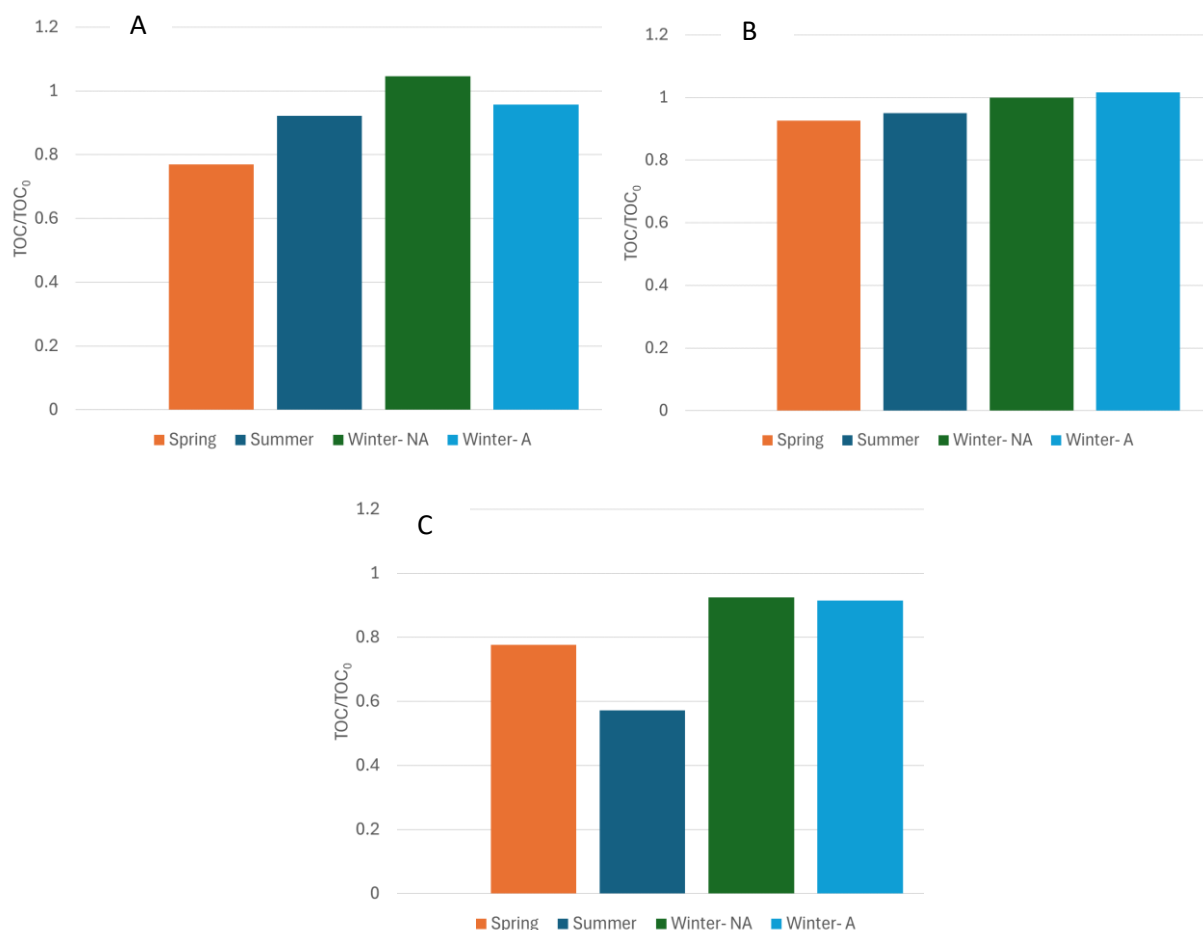


Figure 68: Total organic carbon remaining in the sample (%) after 24h. A: milk; B: milk+sand; C: milk+soil.

In Figure 68 A it is possible to notice that, after 24h, the best degradation for the milk-only was achieved in the spring test, while the worst was during the winter test, in the non-agitation conditions. Figure 68 B shows that the best degradation for the milk mixed with sand was achieved, again, in the spring test. In Figure 68 C, finally, it is clear that for the milk mixed with soil the best degradation conditions are the ones of the summer test, while the worst are the one of the winter tests, both agitation and non-agitation condition. However, the highest degradation was reached in the milk mixed with soil samples.

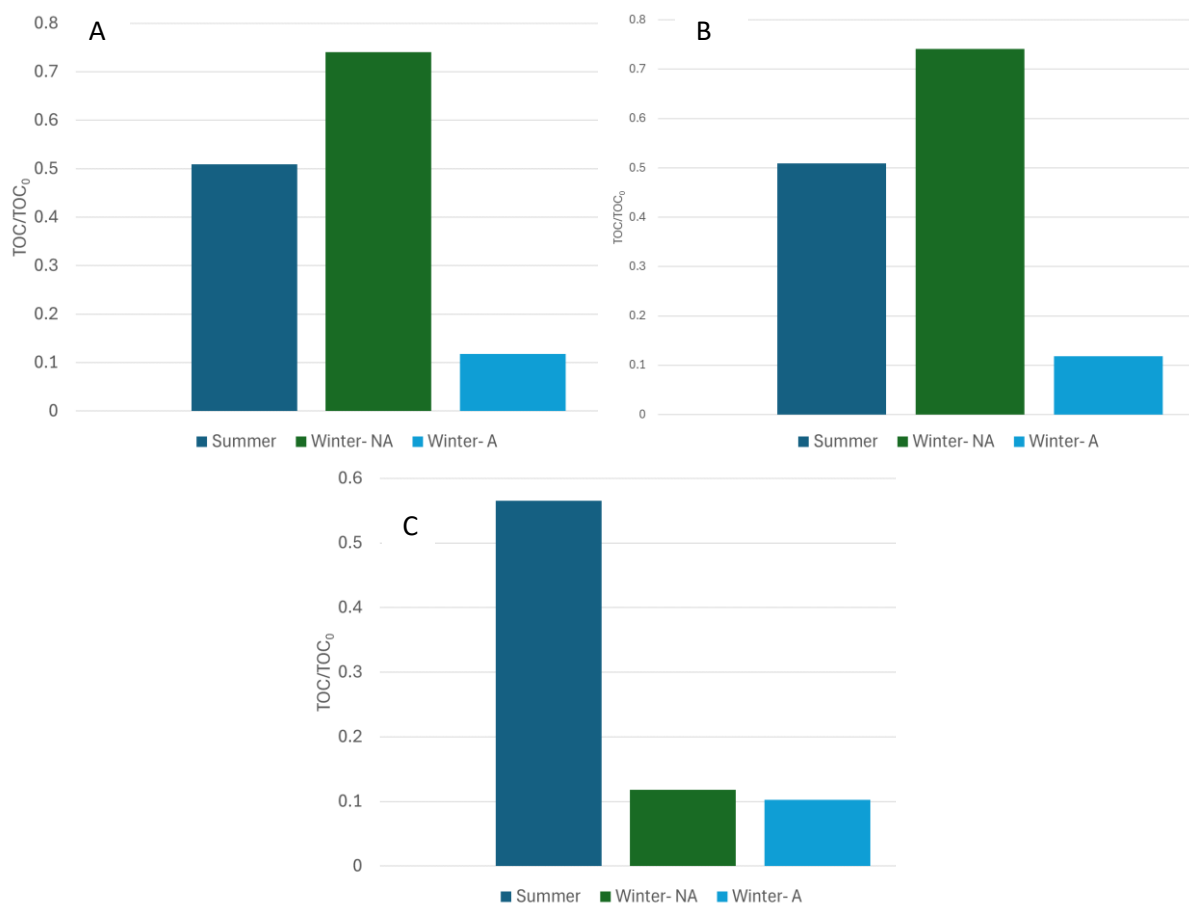


Figure 69: Total organic carbon remaining in the sample (%) after 10 days. A: milk; B: milk+sand; C: milk+soil

In Figure 69 A it is possible to notice that, after 24h, the best degradation for the milk-only samples was achieved in the winter test, in the agitation conditions ($\approx 90\%$). Figure 69 B shows that the best degradation for the milk mixed with sand was achieved, again, in the winter test, but for this sample in non-agitation conditions. In Figure 69 C, finally, for the milk mixed with soil the best degradation conditions are the ones of the winter test, both agitation and non-agitation conditions, while the worst are the one of the summer tests.

In conclusion, it seems that, for the batch tests, after 10 days the highest degradation of total organic carbon present in the samples had been reached in winter, both in non-agitation and agitation conditions, despite the results showed that, after 24 hours, they were the worst. The type of substrate, in addition, seemed to not affect significantly the degradation rate, since for the three different typologies of samples the final content of total organic carbon was about 1% of the TOC₀, while after 24 hours it seemed that in the soil samples there was a higher degradation. The lower degradation rate registered in winter may be attributed to a higher acidification of the substrate, which led to unfavourable conditions, and higher temperatures which may have decreased the activity of the enzymes involved in the degradation [40].

3.4. Column leaching tests

The results of the analyses carried out in the percolation tests are reported in the following paragraphs.

3.4.1. Continuous column leaching test

The results of the effluent leaching tests in sand and soil columns are shown in terms of column outlet concentrations normalised to inlet concentrations.

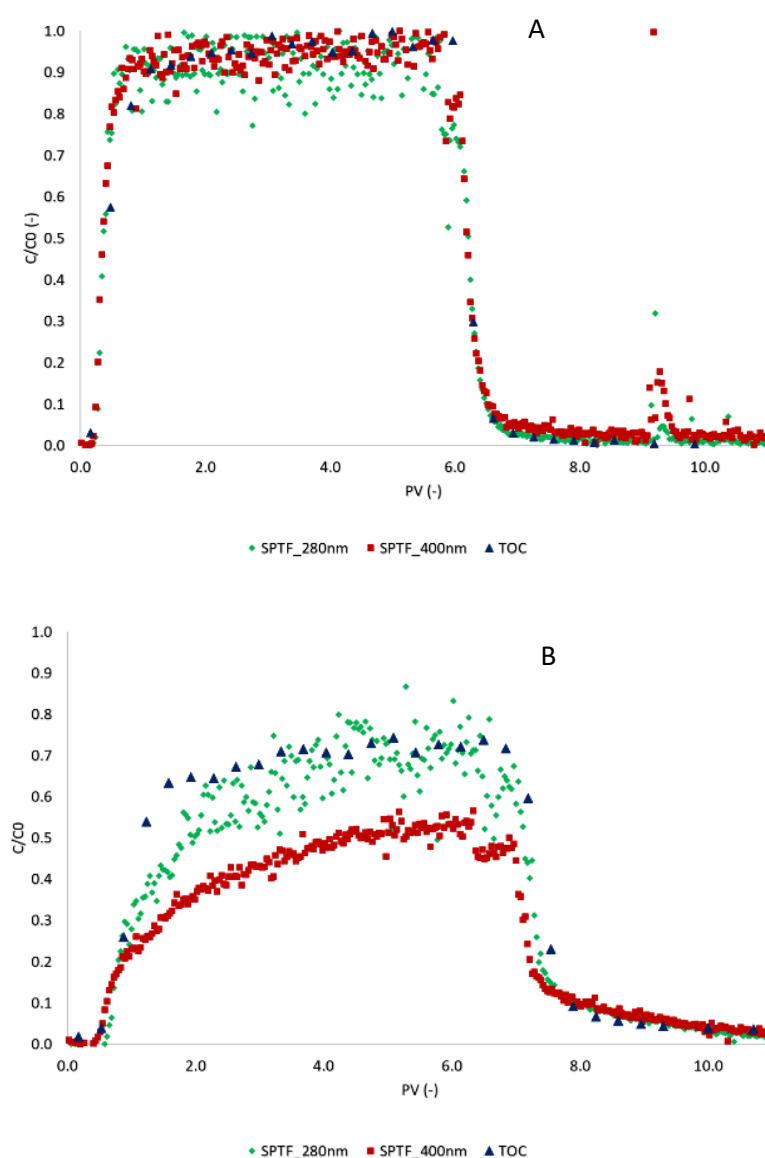


Figure 70: Wastewater breakthrough curve obtained with absorbance measurements by spectrophotometer at two different wavelengths (in green and red) and with TOC (in blue) in silica sand (A) and in standard soil (B).

The pore volume values (PV) measured in both the columns were quite comparable each other, and equal to 30 minutes. The injection of milk mixed with deionized water lasted for 5 PVs, which is a shorter time than the duration of the batch tests, but it was more representative of a meteorological event from which the percolation of the effluent in the soil may occur. The flushing has been done for 5 PVs using deionized water.

The absorbance measured at 400 nm wavelength was given by the colloidal organic fraction of the effluent, while the one registered at 280 nm was attributable to the cumulative effect of the response of the colloidal and dissolved fractions, thus more like the measurement provided by TOC analysis. In silica sand, the trends of the three breakthrough curves were essentially overlapping, indicating that the colloidal and dissolved fractions of the effluent exhibited the same mechanism of interaction with the sand. The C/C_0 values presented an increasing trend in the first 2 PVs, without significant lag, and then settled around values close to unity, an indication that all the input effluent had been recovered at the outlet.

The behaviour was different in standard soil, where the colloidal fraction (response at 400 nm) reached lower C/C_0 values (about 0.5 after 5 PVs of effluent injection) than the ones measured at 280 nm and TOC (about 0.7 after 5 PV). The colloidal fraction was more retained by the soil than the dissolved fraction, which was more mobile. This result can be explained by the different particle size of silica sand (d_{50} equal to 0.45mm) compared to that of silty sand (d_{50} equal to 0.085mm), and by the different organic content of the soil, close to 0 for sand and 1.7% for standard soil. Furthermore, the trend of the different breakthrough curves denoted a marked interaction of the effluent with the soil, with a slow rise in values during injection and the failure to reach C/C_0 values of 1. While sand had not been effective in breaking down the organic load of the effluent, the soil had been able to halve the concentrations already for very short injection and residence times in the column, which were shorter than the half-life recorded in the batch. According to the mass balance, the percentage of fractions in the outlet effluent at different wavelengths corresponded to 81.9% for the colloidal and the dissolved particles together, and 68% for the only colloidal fraction. The removal mechanisms probably are attributable to a combination of mechanical removal (of the colloidal fraction), chemical-physical removal (by adsorption of the dissolved fraction) and biological removal (by the bacteria present in the soil).

3.4.2. Intermittent column leaching test

According to the results exposed in the previous paragraph, the substrate used for this test is constituted by soil.

The results of each parameter registered during the percolation test done in January are summarized in Figure 71.

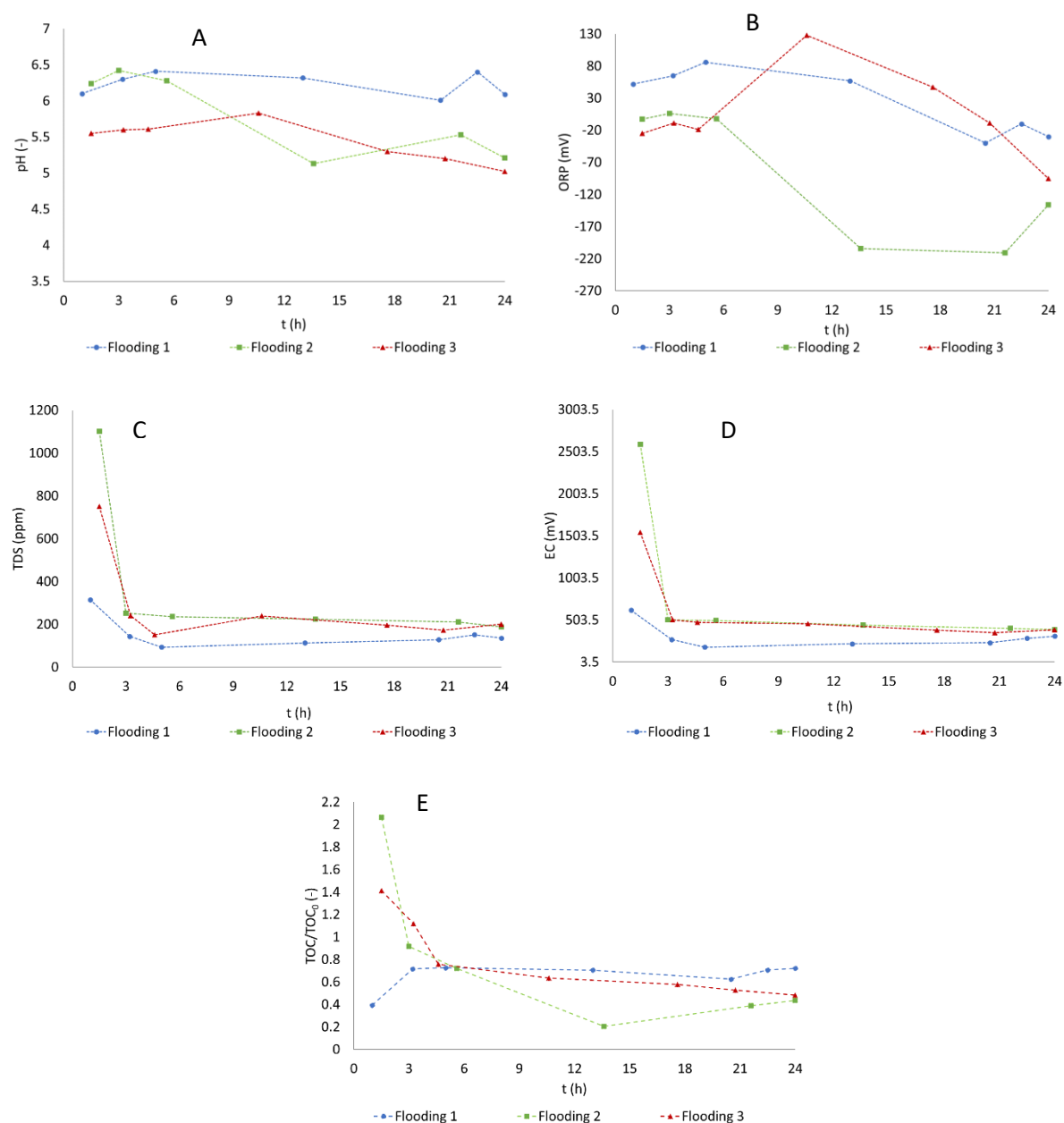


Figure 71: Trends of the parameters monitored in the floodings- multiparametric probe. A: pH; B: redox potential; C: total dissolved solids; D: electric conductivity; E: degradation of the organic carbon.

The values reported in Figure 71 A, E correlate favourably with Kleve and Clark [38], and further support the idea of the pH conditioning the degradation of the effluent in the soil: in the periods in which the pH decreased the degradation efficiency decreased, since the enzymatic activity was inhibited and the total balance of biomass energy utilisation efficiency decreased. The main reason causing the decrease of the pH may be the liberation of acids deriving from the degradation of the fats; the soil acted like a buffer agent, reducing the acidification of the milk: this explains the higher values of pH registered for this effluent with respect to the ones measured in the milk-only samples during the batch tests (2.2.2).

As indicated by Larsen et al. [41], the evidence we found (Figure 71 B) pointed to an influence of the ORP on the microbial population present in the effluents:

- First flooding: at the beginning of the test, the effluent was mainly constituted by particles contained in the soil; after a while, also particles linked to the milk components in an aerobic environment started to appear, leading to a decrease in the ORP values, until negative values, causing the instauration of anaerobic conditions.
- Second flooding: the test started with a positive ORP, higher than the last one registered in the first flooding; this may have been caused by characteristics of the injected effluent, which was composed by a new sample of milk and had caused aerobic conditions. After a while, anaerobic conditions were restored again and the ORP values were negative.
- Third flooding: aerobic conditions seemed to not have been restored, registering negative values since the first measurement of the samples.

In conclusion, the degradation efficiency had been influenced by many parameters and had different trends in the different periods of the floodings:

- In the first flooding, the degradation of the organic carbon seemed to not appear since the effluent was composed of soil and milk particles.
- In the second flooding, at the beginning the carbon content seemed to increase since the substrate was composed mainly of anaerobic microorganisms, instead the effluent needed aerobic microorganisms to be degraded: for this reason the first values of the second flooding, reported in Figure 71 D, is higher than the last one of the first flooding. When the anaerobic conditions have been restored, the carbon content decreased.
- In the third flooding, it could be identified the same mechanism of the previous flooding: at the beginning there were aerobic conditions which became anaerobic after a while. In the last part of the flooding, the pH had become more acid, and this caused a decrease in the degradation efficiency, since the degradation had been inhibited as already explained.

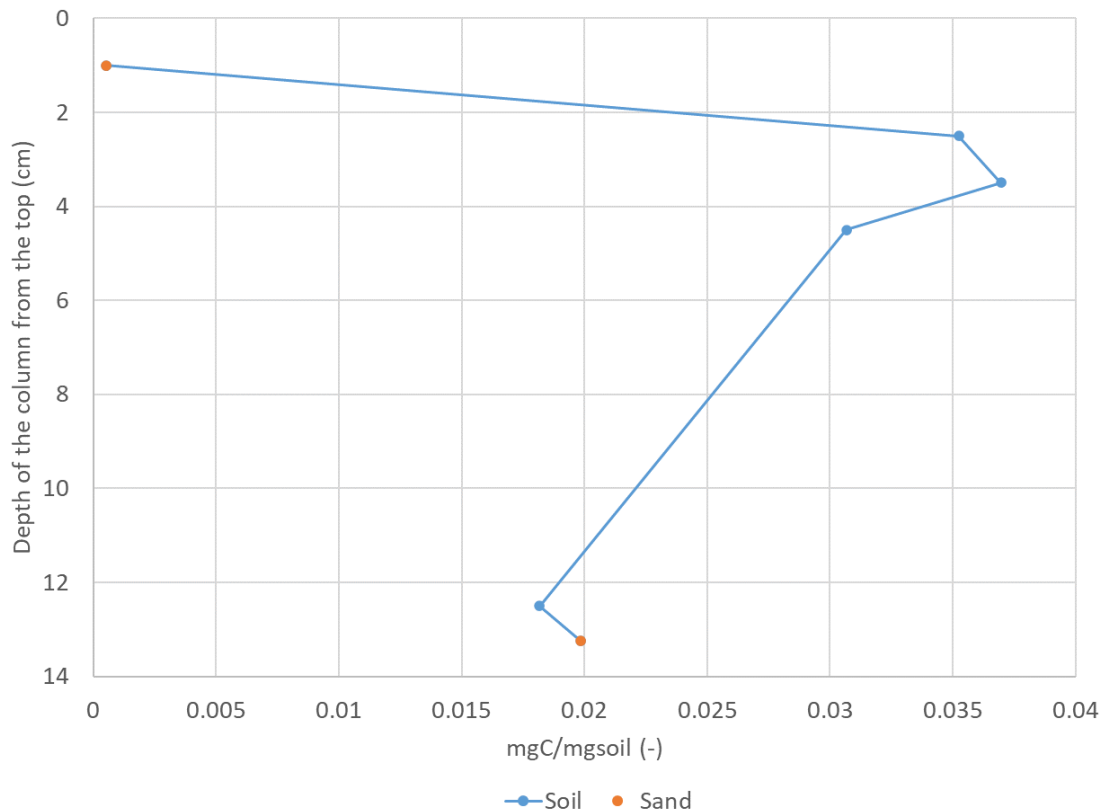


Figure 72: Results of the solid TOC analyses done on the substrate present in the column at the end of the test.

A final investigation on the carbon content of the substrate of the column at the end of the percolation test has been done, of which the results are represented in Figure 72. As it is possible to see, most of the organic carbon was blocked in the first centimetres of the column, while in the last part of it the carbon content was very low. This is probably caused by the physical nature of the colloidal particles present in the milk, which could not pass through the soil. The sand layers, put at the top and at the bottom of the column, did not retain it, confirming the previous hypothesis of the soil better degrading and acting as a filter if compared to the sand.

4. Design of a large-scale laboratory set-up

According to the results of the tests, the main parameters that affect the phenomena and must be monitored have been identified: pH, total organic carbon content, electric conductivity, redox potential. However, the results obtained are not representative of the complex mechanisms that happen in the soil and of what could occur in a real SAT system. For this reason, a pilot-scale laboratory set-up has been designed to better understand the degradation mechanisms and better simulate the action of the water on the soil during a percolation process.

The system, represented in Figure 73, will be composed of a steel cylinder, having the following characteristics:

- Length: 1m; External diameter: 219mm; Thickness: 4mm; Total weight: 26kg/m.
- Three ground support feet, height: 150 mm.
- A central outlet hole with a diameter of 40 mm; it can be reduced in size with an adapter.
- A ring at the top of the column protruding 20 mm and 20 mm high to possibly isolate the 'air' part.

At each of the 4 different heights (125, 375, 625 and 875 mm from the base) 4 different sensors will be located, consisting of: 1 sensor of humidity, 1 of pH, 1 of redox potential and 1 Rihzon sampler. Each of the parameter that will be monitored has demonstrated to somehow affect the degradation processes: the humidity is one of the main parameters for the life of microorganisms in the soil, the acidification of the environment seemed to slow the action of the microorganisms, the redox potential indicated aerobic/anaerobic conditions and gave an idea of the typology of microorganisms present in the sample, and in the end the total dissolved solids content must be monitored according to the law.

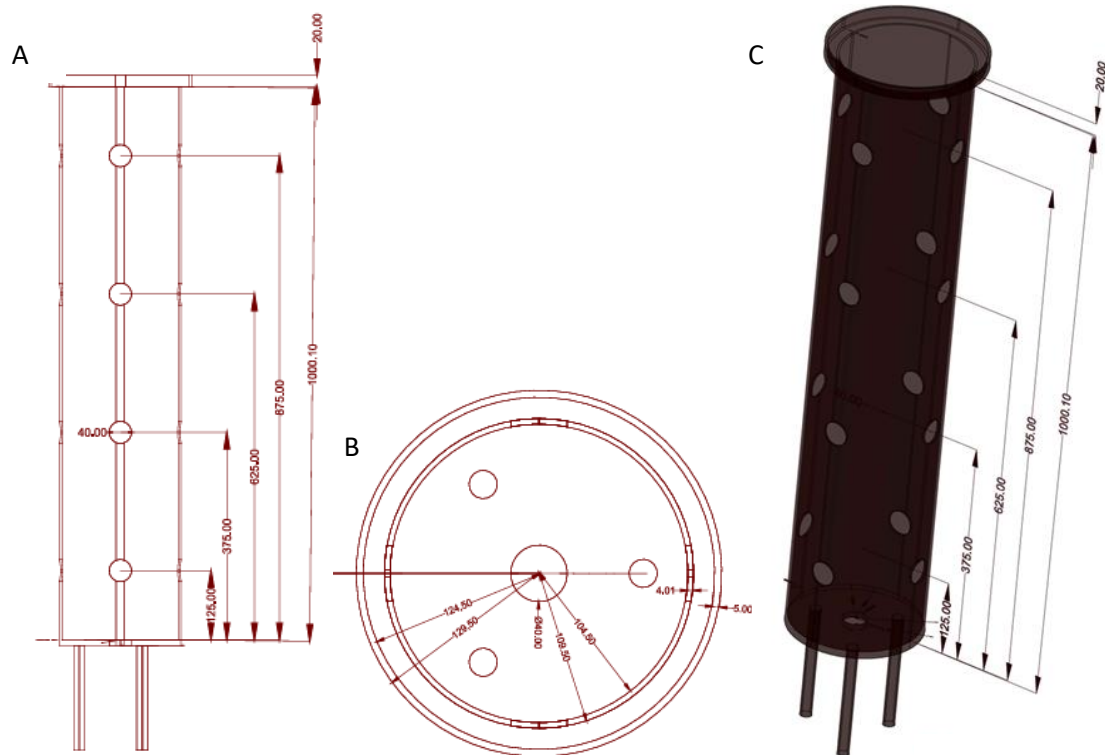


Figure 73: A: frontal view of the system, B: plant view of the system, C: axonometric view of the system.

The diameters of the probes are different and range from 30 mm for the humidity probes, to 5 mm for the pH and redox probes to 2.5 mm for the samplers. However, in the scheme of the system all the holes have been preferred to be represented with a diameter of 40mm, even for the smaller probes. This could give the advantage of being able to interchange probes with each other in the future.

5. Conclusion

The results of this study may help the development of a large-scale laboratory set-up which can then be transformed in a SAT system, having to main advantages:

- The reuse and upgrading of wastewater.
- The fight against water scarcity and the depletion of groundwater thanks to the use of the treated effluents for irrigation purposes.

The abatement of the organic load of different agro-food industry effluents was studied in the laboratory by means of batch and column percolation tests.

The milk effluent, alone or in contact with a sand or standard soil, is subjected to degradation mechanisms that lower its organic load by about 50% within the first 48h in the presence of a loamy soil. In summer, after 10 days the abatement of the organic carbon reached the 50% in effluent-only samples, and lower values for the samples in which a substrate was added. On the other hand, in winter, at the end of the test, the organic load is lowered by 90% using both the substrate and independently from the agitation conditions. So, the best results have been achieved in winter, probably due to the better temperature conditions which avoided an excessive acidification of the samples.

The blueberry effluent, when used in the spring test, after 5 days lowered its organic load by 60% if put in contact with soil, while the sand did not provide a higher abatement than the one registered in the effluent only ($\approx 20\%$). The same wastewater, in the summer test, had similar result for the degradation in soil after 10 days, while the other two types of samples increased their efficiency up to $\approx 40\%$.

The boiling water deriving from the pepper jam production processes registered a decrease in the organic content of 60% in both the substrates, while the effluent alone reduced its TOC of 50%. The total nitrogen content decreased for both milk and pepper effluents, when measured in the winter tests.

These results demonstrate that this technology, if properly used, is effective for different types of effluents.

A fair abatement of the organic load is also recorded in column percolation tests conducted with the same soil and for shorter contact times. In the presence of only sand, the abatement is absent. A further investigation has been conducted in a percolation test, in which the flooding-drying cycle used for irrigation in agriculture has been simulated, to better understand the possible consequences of the continuous use of dairy industrial effluents on the soil. The degradation of the organic carbon has turned in its almost-completely removal, conversely to the trends registered for other parameters such as pH, ORP: the measured values do not comply with the prescriptions of the Italian and European legislation.

The evidence from this study suggests that the degradations mechanisms of agro-food industry effluents are quite various, and further studies are necessary to point out all the details: the influence of the pH and redox potential has turned out to be quite strong and affecting most of the degradations happened in the tests. However, despite the problems related to the law prescriptions, these results may be a good starting point to develop additional possibilities of substrates combination, test conditions and longer contact times with the substrates.

Moreover, the development of a laboratory setup would help to simulate the mechanisms of the degradation and investigate the trends related to the different parameters monitored having less interference between the column wall and the sample.

A further collaboration with a microbiological laboratory would help to figure out the microorganisms involved in the processes, their growth rate and the conditions that affect it.

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