DEMOWARE

Deliverable D1.5

Innovative schemes for water reuse in the agricultural sector

The project "Innovation Demonstration for a Competitive and Innovative European Water Reuse Sector" (DEMOWARE) has received funding from the European Union's 7th Framework Programme for research, technological development and demonstration, theme ENV.2013.WATER INNO&DEMO-1 (Water innovation demonstration projects) under grant agreement no 619040

Deliverable Title	D1.5 Recommendations on water reuse in the agricultural sector
Related Work Package:	WP1: Demonstrating innovative treatment processes and reuse scheme operation
Deliverable lead:	Fundació CTM Centre Tecnològic
Author(s):	Xavier Martínez (CTM), Carme Bosch (CTM), Marta Terré (IRTA), Carme Biel (IRTA), Pompilio Vergine (IRSA), Alfieri Pollice (IRSA).
Contact for queries	CTM: Xavier Martínez Fundació CTM Centre Tecnològic Plaça Ciència, 2 08243 Manresa, Barcelona T +34 39 877 73 73 E xavier.martinez@ctm.com.es
Dissemination level:	Public
Due submission date:	30/09/2016 (M33)
Actual submission:	21/11/2016
Grant Agreement Number:	619040
Instrument:	FP7-ENV-2013-WATER-INNO-DEMO
Start date of the project:	01.01.2014
Duration of the project:	36 months
Website:	www.demoware.eu
Abstract	This report includes results from the task 1.4 developed within the FP7 DEMOWARE project: Innovative water reuse schemes in the agricultural sector, including two case studies in two different demonstration sites: Torre Marimon in Spain and Capitanata in Italy.

Versioning and Contribution History

Version	Date	Modified by	Modification reason
v.01	05/07/2016	Carme Bosch	Initial draft
v.02	15/09/2016	Marta Terré, Pompilio Vergine, Alfieri Pollice, Carme Biel.	Complete first draft
v.03	30/09/2016	Xavier Martínez	Revision first draft
v.04	17/10/2016	All authors	Revised draft
v.05	21/11/2016	All authors	Integration of comments from external reviewer

Table of contents

List of figures

List of tables

Glossary

a	Annual
ADG	Average daily gain
AOP	Advanced oxidation processes
AR	Antibiotic resistance
ARB	Antibiotic resistant bacteria
ARG	Antibiotic resistant genes
BOD	Biological oxygen demand
BW	Body weight
CEC	Contaminants of emerging concern
CFU	Colony-forming units
COD	Chemical oxygen demand
d	day
D	UV dose
DAF	Dissolved air flotation
DM	Dry matter
DNA	Deoxyribonucleic acid
DW	Drinking water
EU	European Union
GC	Genome copy
GW	Groundwater
ha	hectare
LED	Light-emitting diode
LMH	Transmembrane flux
MBR	Membrane bioreactor
mo	months
MPN	Most probable number
MR	Milk replacer
n	Number of samples
n.a.	not analysed
n.d.	not detected
NTU	Nephelometric turbidity unit
PCR	Polymerase chain reaction
PDT	Pressure decay test
PFU	Plaque-forming units
ppm	parts per million
RNA	Ribonucleic acid

RW	Reclaimed wastewater
SAR	Sodium adsorption ratio
SEM	Standard error of the mean
SFD-MBR	Self forming dynamic membrane bioreactor
SW	Secondary treated wastewater
TDS	Total dissolved solids
TIC	Total inorganic carbon
TMP	Transmembrane pressure
TOC	Total organic carbon
TSS	Total suspended solids
TW	Tertiary treated wastewater
UF	Ultrafiltration
UV	Ultraviolet
WW	Wastewater
WWTP	Wastewater treatment plant
µS	Microsiemens

Executive Summary

Agriculture is the most water-demanding sector in Europe and worldwide. In Europe, southern countries affected by occasional episodes of water scarcity and droughts, are those with the highest water withdrawals for the agricultural sector (>40%). The use of reclaimed wastewater represents a promising alternative source of water.

The present report demonstrates the technical and economic feasibility of innovative water reuse schemes applied in the agricultural sector: a) reclamation of agro-food industrial effluents for food-crops irrigation by a treatment at full-scale consisting of conventional secondary treatment plus a tertiary process with sand filtration, membrane ultrafiltration and UV disinfection, performed in Capitanata, Italy, and b) reclamation of municipal WW effluents for water use in livestock production, by a treatment at pilot-scale consisting of membrane ultrafiltration and UV disinfection, performed in Torre Marimon, Spain.

In general, both tertiary treatments based on *onsite* systems, achieved water qualities that fulfil legal limits established for water reuse in irrigation and livestock production (to the authors' knowledge this last only exists in legislation from Australia). However, static water storage used in both demonstration studies led to bacterial re-growth in the reservoirs and occasionally compromise water quality.

Concretely for the demonstration irrigation study in Capitanata, some critical points of the treatment scheme were identified during the study, such as difficulties in eliminating oil, which may have an effect on the productivity of the membranes. The introduction of an additional process consisting, for instance, in a dissolved air flotation to improve oil removal, was strongly recommended. In addition, for an irrigation water reuse application, it was crucial to follow water quality parameters such as sodium, calcium, magnesium and nutrients (N, P), that may have an important effect on crop performance. In Capitanata, no salt accumulation was observed in soils when using reclaimed wastewater.

In Torre Marimon, two different types of experiments were performed using reclaimed wastewater. *In vitro* experiments on intestinal bovine cells showed that ultrafiltration technology contributed very positively to the overall cell performance (low expression of cell damage, inflammation and apoptesis markers). This substantial enhancement may be associated to the reduction of microbiological load plus the elimination of toxic compounds attached to the suspended particles. In contrast, waters with chlorination treatments showed greater gene expressions and one may think that this was caused by toxic disinfection by-products even though these were not determined in the present study. *In vivo* experiments showed that animals fed with reclaimed wastewater had lower feed consumptions and weight gains, and reclaimed wastewater was less preferred by animals compared to tap drinking water. A reason for this lower palatability may be the higher content of dissolved salts in reclaimed wastewater. However, not severe health effects were detected in these animals.

Both water reuse applications resulted in technically feasible solutions, ensuring the elimination of microbial and chemical hazards, with minimum negative impacts on animal health and performance, human health, and on soil and irrigated crops performance. The estimated cost for reclaimed wastewater, including investment and operating costs, ranged from 0.18 to 0.61 €/m^3. Both demonstration reuse applications are economically feasible considering that potential water shortages could affect the normal operation of a farm raising animals and compromise crop production.

1 Introduction

1.1 Water management background in the agricultural sector

The agricultural sector accounts for around 70% of water used worldwide (FAO, Food and Agriculture Organization of the United Nations 2016). The importance of agricultural water withdrawal is dependent on climate and the place of agriculture in the economy, i.e., in Africa and Asia the agricultural sector represents more than 80% and Europe just over 20% of water withdrawal, and among European countries, Spain has over 60% in contrast to Germany with only the 2.5% of water withdrawal for the agricultural sector. In addition, agriculture is the most inefficient user of water and at the same time contributes to water pollution from excess nutrients, pesticides and other pollutants. All these factors not only negatively impact the environment in many regions, but also affect the viability of the agricultural sector itself and that of other water users.

Agricultural regions have been subject to extensive and increasing water constraints in recent years. Water scarcity and droughts, which are increasingly frequent and widespread across Europe, have become a major challenge. At least 11% of the European population and 17% of its territory have been affected by water scarcity to date (Alcalde-Sanz and Gawlik 2014). Drought occurrence has increased not only in southern and central Europe, but also in northern and eastern Europe. Global climate change is already exacerbating these problems, with projections indicating significant and widespread impacts over the medium to long term. Water scarcity is reported in nearly all river basin districts in the Mediterranean area. This trend is expected to continue. Projections reveal that agricultural production will have to rely on much less freshwater resources than before. Climate change is predicted to increase fluctuations in precipitation and surface water supplies and reduce snow packs and glaciers. Extreme weather events, like droughts and floods, are also expected to be more frequent. Coupled with these changes, farmers in many regions will face increasing competition from non-agricultural users due to rising urban population density and water demands from the energy and industry sectors.

Water quality is also likely to deteriorate in many regions, due to the growth of polluting activities, salination caused by rising sea levels and the abovementioned water supply changes. These water challenges are expected to strongly impact agriculture – a highly water-dependent sector – undermining the productivity of rain-fed and irrigated crop and livestock activities in many regions. These changes could in turn further impact markets, trade and broader food security.

This trend is encouraged by the fact that farmers in most countries do not pay for the full cost of the water they use. Intensive groundwater pumping for irrigation depletes aquifers and can generate negative environmental externalities, leading to significant economic impacts on the sector and beyond. In addition, agriculture remains a major source of water pollution: agricultural fertiliser run-off, pesticide use and livestock effluents all contribute to the pollution of surface freshwaters and groundwater.

1.2 Motivations and benefits of water reuse in the agricultural sector

The increasing water scarcity and water pollution control efforts in many countries have made treated wastewater (WW), mainly from urban sources, a suitable economic means of augmenting the existing water supply, especially when compared to expensive alternatives such as desalination or the development of new water sources involving dams and reservoirs. Water reuse makes it possible to close the water cycle at a point closer to cities by producing “new water” from WW and reducing WW discharge to the environment.

Treated WW can be used for various non-potable purposes. The dominant applications for the use of treated WW (also referred to as reclaimed wastewater or recycled water) include agricultural irrigation, landscape irrigation, industrial reuse, and groundwater recharge. More recent and rapidly growing applications are for various urban uses, recreational, and environmental uses, and indirect and direct potable reuse. Within the agricultural sector, the most popular application of the use of reclaimed wastewater is the irrigation of food crops, pastures, and industrial non-food crops. Other applications for the agriculture sector, less widespread but more innovative could be aquaculture, silviculture and livestock production.

The use within the agricultural sector of reclaimed water from urban, agricultural, and industrial sources provides economic, social, and environmental benefits, including:

- Increase water availability
- Obtain integrated and sustainable use of water resources
- Reduce over-abstraction of surface and groundwater
- Reduce energy consumption and treatment costs compared to using deep groundwater resources or desalination
- Reduce nutrient loads to receiving waters
- Increase agricultural production
- Reduce application of fertilisers
- Increase employment and local economy

1.3 Brief overview of current situation and legal framework summary

The pressures on freshwater resources have raised awareness and encouraged the use of alternative water sources as a strategic option to supplement water supplies and protect natural resources in Europe. The Commission is nowadays taking a series of actions to promote the reuse of treated WWs, as stated in COM (2015)615: “Closing the loop – An EU action plan for the circular economy”, including establish common quality criteria for water reuse, creating a global water market, and setting-up specific networks such as the European Innovation Partnerships on Water and Agricultural Productivity and Sustainability, and the Water supply and sanitation Technology Platform (WssTP).

The potential for replacing freshwater by water reuse in Europe is very high. This is estimated to vary between 1-17% in European countries, with even higher levels of potential reuse on local and regional scales (i.e, the highest potentials in Israel, Malta, Cyprus and Spain) (Hochstrat, R., et al. 2006). More than 200 water reuse projects have been implemented in the EU to date, with an estimated volume of over 900 Mm^3/a, including industrial, urban and agricultural applications. In southern Europe, water reuse projects in the agricultural sector are mainly for crops irrigation (Angelakis, A.N. and Gikas, P. 2014). Spain is the leading European country reusing a volume of 347 Mm^3/a of water followed by Italy with 233 Mm^3/a. In Europe, there are no available data about the amount of reclaimed water used for agricultural irrigation (the most common agricultural application), but the estimation is 44% in southern Europe and 16% in northern Europe. In contrast, California in USA uses half of its reclaimed water for agriculture and the rest for landscape irrigation and other uses and Florida uses for agriculture 12% of the reclaimed water produced and 19% for golf courses (Parsons, LR, et al. 2010). Many countries worldwide utilize reclaimed water for irrigation, usually treated municipal WW. In this regards, Australia has become one of the most active countries in promoting water reuse and the agricultural irrigation represents the 82% of their total reclaimed water supply. Other countries situated in arid climates, such as Israel, Jordan, Kuwait, Tunisia, and the United Arab Emirates use reclaimed water for irrigate extensive crop fields. Israel recycles the 72% of the municipal WW produced, mainly for irrigation purposes (Asano, T., et al. 2007).

The need to ensure health and environment safety implies the development of guidelines and regulations for the safe use of reclaimed WW. In Europe, there are no regulations on water reuse at the European Union level. However, several member states and regions in Europe have produced their own legislative frameworks, regulations, or guidelines for some water reuse applications. At European level, guidelines establishing the minimum quality requirements for water reuse in agricultural irrigation and aquifer recharge are currently under development and will be available beginning 2017.

Despite these recent progresses to regulate water reuse at European and national levels, water reuse applications addressed in the existing guidelines are still very narrow. These are mainly focused on crops irrigation, aquifer recharge, and landscape irrigation, and usually only consider urban WW as the targeted source. Reuse applications including industrial and agricultural sources, and applications for the industrial or livestock production sectors are hardly regulated within EU-countries. Other non EU-countries, such as Canada, Australia, and some states from the USA, have issued regulations and guidelines including more applications and potential sources (i.e, Guidelines for Environmental Management, GEM: Use of Reclaimed Water in Australia) (EPA Victoria 2003).

European and worldwide regulations, guidelines and standards addressing the water reuse issue in the agricultural sector are summarised in Table 1-1.

Table 1-1 Standards on water reuse – Europe and Worldwide; adopted from (Asano, T., et al. 2007) and own review

Country	Standard / Guideline	Issuing organization
Cyprus	Law 106 (I) 2002 Water and Soil pollution control and associated regulations KDP 772/2003, KDP 269/2005	Ministry of Agriculture, Natural resources and Environment Water development Department (Wastewater and reuse Division)
France	JORF num.0153, 4 July 2014 Order of 2014, related to the use of water from treated urban wastewater for irrigation of crops and green areas	Ministry of Public Health Ministry of Agriculture, Food and Fisheries Ministry of Ecology, Energy and Sustainability
Greece	CMD No 145116 Measures, limits and procedures for reuse of treated wastewater	Ministry of Environment Energy and Climate Change
Italy	DM 185/2003 Technical measures for reuse of wastewater	Ministry of Environment Ministry of Agriculture, Ministry of Public Health
Portugal	NP 4434 2005 Reuse of reclaimed urban water for irrigation	Portuguese Institute for Quality
Spain	RD 1620/2007 The legal framework for the reuse of treated wastewater	Ministry of Environment Ministry of Agriculture, Food and Fisheries, Ministry of Health
Australia – Victoria	Guidelines for Environmental Management (GEM): Use of Reclaimed Water (EPA publication 464.2) GEM: Dual Pipe Water Recycling Schemes – Health and Environmental Risk Management (EPA publication 1015). Guide for the completion of a Recycled Water Quality Management Plan – For Class A water recycling schemes Guidelines for validating treatment processes for pathogen reduction: Supporting Class A recycled water schemes in Victoria	EPA Victoria
USA – California	Groundwater Replenishment with Recycled Water – June 26, 2013 draft regulations Title 17 of the California Code of Regulations – for cross connections Title 22 – Water Recycling Criteria	EPA California
China	China National Reclaimed Water Quality Standard; China National Standard GB/T 18920-2002, GB/T 19923-2005, GB/T 18921-2002, GB 20922-2007 and GB/T 19772-2005.	
Israel	Ministry of Health regulation (2005)	Unrestricted agricultural irrigation use. Based on the California Title 22 standards,

Country	Standard / Guideline	Issuing organization
		very restrictive. Methods of treatment and setback distances are included.
South Africa	Policies: The latest revision of the Water Services Act of 1997 relating to grey-water and treated effluent (DWAF, 2001) The latest revision of the National Water Act of 1998, 37(1) (DWAF, 2004a) relating to irrigation of any land with waste or water containing waste generated through any industrial activity or by water works	Regulation: Government Gazette No. 9225, Regulation 991: Requirements for the purification of wastewater or effluent (EAF, 1984) Guidelines: The South African Guide for the Permissible Utilization and Disposal of Treated Effluent (DNHPD, 1978) The South African Water Quality Guidelines (DWAF, 1996)
European Union	Development of minimum quality requirements at EU level for water reuse in agricultural irrigation and aquifer recharge	Joint Research Centre (under development)
Global	Guidelines for the safe use of wastewater, excreta and greywater, 2006 Volume 1: Policy and regulatory aspects Volume 2: Wastewater use in agriculture Volume 3: Wastewater and excreta use in aquaculture Volume 4: Excreta and greywater use in agriculture	World Health Organization, WHO
Global	Guidelines for municipal wastewater reuse in the Mediterranean region, 2005 Development of performance indicators for the operation and maintenance of wastewater treatment plants and wastewater reuse, 2011	United Nations Environment Programme, UNEP
Global	Proceedings on the UN-Water project "Safe use of wastewater in agriculture", 2013.	United Nations Water Decade Programme on Capacity Development, UNW-DPC
Global	ISO 16075-3:2015 Guidelines for treated wastewater use for irrigation projects	International Organization for Standardization. ISO/TC 282, *Water reuse*, Subcommittee SC 1, *Treated wastewater use for irrigation*

1.4 Main barriers to overcome

Despite the water reuse applications for the agricultural sector already developed in many countries, a number of barriers still prevent the widespread implementation of water reuse throughout Europe and on a global scale. These barriers will have to be overcome if WW reuse strategies are to be adopted on a larger and more effective scale than at present, developing the huge eco-innovation potential in terms of technologies and services related to water recycling in the agricultural sector. Barriers and drawbacks can be technical or non-technical including institutional, economic and social aspects. These main barriers are the following:

- Mismatch between demand and availability of water across both temporal and spatial scales
- Inconsistent and unreliable methods for identifying and optimising appropriate WW treatment technologies for reuse applications, which are able to balance the competing demands of sustainable processes
- Lack of appropriate infrastructure for water collection, treatment and distribution

- Difficulties in specifying and selecting effective monitoring techniques and technologies for the whole system
- Significant challenges in reliably assessing the environmental and public health risk/benefit of water reuse across a range of geographical scales (i.e., public health concerns about food products irrigated or livestock fed with reclaimed water, impacts on soils and groundwater).
- Lack of harmonisation in the regulatory framework to manage health and environmental risks related to water reuse, and thus a lack of confidence in the health and environmental safety of water reuse practices.
- Inconsistent or inadequate water reuse regulations/guidelines, which lead to delays and misjudgements
- Low levels of public and government enthusiasm for water reuse
- Limited institutional capacity to formulate and institutionalise recycling and reuse measures
- Poorly developed business models for water reuse schemes, and markets for reclaimed water
- Lack of financial incentives for reuse schemes

From a technical standpoint, water reuse is a logical part of the overall water supply and resource management solution. Technology is now available in the developed countries to produce water for any use, including both conventional and more advanced technologies such MBR applications, combination of different membrane filtration and disinfection techniques. Nevertheless, technically feasible water reuse projects often are not implemented due to institutional, economic, and organisational barriers, or poor public perception and education. These non-technical barriers are a limitation to the expansion of water reuse planning.

There are at least two broad barriers to plan water reuse projects: 1) limited institutional capacity to formulate and institutionalise enabling legislation and to subsequently conduct adequate enforcement and monitoring of water reuse activities, and 2) lack of expertise in health and environmental risk assessment and mitigation.

Additional barriers include public perceptions that may drive fear of the dangers of, for instance, consuming food irrigated with reclaimed water. Public outreach programmes to build awareness and involve community members in planning could change public resistance to water reuse.

Long-term economic viability also represents an important barrier to water reuse. Reclaimed wastewater is often priced just below the consumer cost of drinking water to make it more attractive to potential users, but this may also affect the ability to recover costs (Jimenez and Asano, T. 2008). Distortion in the market for drinking water supply complicates the pricing of reclaimed water, as does the lack of accounting for externalities, including water scarcity and social, financial, and environmental burdens of effluent disposal in the environment (Hochstrat, R., et al. 2006). In the agricultural sector, additionally, many farmers do not pay or pay less than the real water cost. Another concern for the economic viability of most of the reuse projects is the lack of appropriate infrastructure for the collection, storage and distribution, and if exists, this may lead to more expensive investment costs. The best solutions to reduce the need of storage and distribution infrastructures are: the implementation of onsite systems, with minimum distance between water source and reuse destination, and use WW sources with not much variable water supply and destinations with water demand as constant as possible.

Fragmentation of responsibilities between different governmental authorities in charge of different parts of the water cycle is another impediment that must be overcome before water reuse projects can go forward. In many regions, the authority over the water supply sector resides in an entirely different organisation than that responsible for WW management. This separation of powers leads to long periods of inaction, disagreements, and negotiations that make the resulting water reuse project far more costly and complex than necessary.

The agriculture is the highest water demand sector globally and in southern Europe. It is therefore a primary objective to expand the water reuse to agriculture, but issues such as storage, distribution and risk management as well as financing must be addressed. From an agronomic point of view, the main limitation in using treated WW for irrigation arises from its quality. Treated WW contains higher concentrations of inorganic suspended and dissolved materials (total soluble salts, sodium, chloride, boron, and heavy metals), which can damage soil and irrigated crops. Dissolved salts are not removed by conventional WW treatment technologies and appropriate good management, agronomic, and irrigation practices are intended to be used to avoid or minimize potential negative impacts (Parsons, LR, et al. 2010). Plants differ in their sensitivity to salt levels, thus the salinity of reclaimed water should be measured to select appropriate crops and/or irrigation rates. High levels of sodium can be toxic for plants through both root uptake and accumulation in plant leaves if sprinkler irrigation is used. The specific concentration of sodium in irrigation water that is considered to be toxic will vary depending on plant species and the type of irrigation system (Ayers and Westcot, DW 1985). The Sodium Adsorption Ratio (SAR), which measures the ratio of sodium over other ions, is used to evaluate the potential effect of irrigation water on soil structure, depending on sodium, calcium and magnesium concentrations. Although boron and chloride are necessary at low levels for plant growth, at high concentrations can cause toxicity problems. From 1 to 2 mg/L of boron and over 100 mg/L of chloride may be damaging for leaves of sensitive plant species. Free chlorine from disinfection can also damage plants at high concentrations (>5mg/L).

Another concern related to the water quality of reclaimed wastewater for the agricultural sector is the presence of contaminants of emerging concern (CECs), including biocides, hormones, pharmaceuticals, personal care products, etc. and their transformation products, which can overstep the barriers represented by low-pressure filtration and some UV treatments (Huang, et al. 2016). Although the impact on environment and human health is still poorly investigated, CECs can represent a danger for human health and environment even at very low concentrations. At the moment, CECs are not included in the lists of parameters that WW reuse regulations require to monitor, although the guidelines establishing minimum quality requirements at EU level currently under development consider the monitoring of performance indicators for CECs (see Table 1-1). Their presence in WWTPs effluents has been widely demonstrated and studies on the related environmental and health effects in case of WW discharge or reutilization are being carried out. First experiments under simulated conditions showed CECs accumulating in soil and groundwater or even reaching the food chain through plant uptake (Wu, C, et al. 2012). Experimentations at field scale still lack, and this does not allow for a pragmatic evaluation of the risks associated with the consumption of crops irrigated with treated WW.

Moreover, the diffusion of antibiotic resistance (AR) is entailing a reduced capacity to control pathogens in treated WW. The presence of antibiotic resistant bacteria (ARB), antibiotic resistant genes (ARG), and some pathogens that showed a particular resistance to conventional disinfection systems has been observed in WWTPs effluents, even after tertiary treatments (Amador, et al. 2015). Therefore, the use of the commonly adopted fecal indicators such as *Escherichia coli* and *Salmonella*, could be not sufficient to assess health risks associated to WW reuse in agriculture. ARB and ARGs are normally present in natural ecosystems, but they are enriched under anthropogenic pressures. The presence of contaminants such as antibiotics, metals or biocides seems to select for ARB and to promote the transfer of ARG between bacteria and, potentially, to pathogens (Gullberg, et al. 2014). WWTPs have been identified as a hotspot of AR spread in the environment (Rizzo, et al. 2013) but, at present, few contrasting studies investigated the fate of ARG and ARB in the agricultural reuse of WW (Ben Said, et al. 2015).

Innovations on reuse of treated WWs should consider also these important emergent aspects for the safety of the public health and environment. It is widely accepted that none of the approaches for the assessment of water quality is suitable to assess all the microbiological and chemical contaminants. However, there exist bioanalytical tools intended to examine the presence of complex mixtures of contaminants based on their biological action. The abovementioned guidelines for water reuse in agricultural irrigation at EU level, currently under development, selects a list of *in vitro* bioassays for the assessment of the reclaimed water safety. Innovative methods combining chemical methods for specific compounds and effect-directed bioassays are needed to improve non-target screening and identification of unknown transformation products in water, soils and crops, added to national policies and regulations.

1.5 Water reuse scheme possibilities in the agricultural sector

The choice of a water reuse scheme will be governed by the WW source and the targeted reuse application. The characteristics of reclaimed WW clearly depend on its source and final destination, and the treatment scheme must be chosen consequently. Table 1-2 shows the three elements to consider when selecting a water reuse scheme: WW source, reclamation treatment and application use.

Table 1-2 Water reuse scheme possibilities for agricultural applications

Wastewater source	Reclamation treatment	Application use
• Municipal • Industry • Agricultural	• Primary + Secondary (conventional treatment) • Primary + Secondary + Desinfection • Primary + Secondary + Tertiary + Desinfection	• Irrigation of food crops to be eaten raw • Irrigation of processed food crops • Irrigation of non-food crops • Livestock drinking • Cleaning of livestock facilities

In principle, the *WW source* can have a municipal, industrial or agricultural origin, and its initial water quality will vary based on this feature. Water reclamation and reuse from municipal WW is more well-established than the water reuse from industrial effluents because water quality from industrial processes is more complex. Additionally, each potential application has unique requirements related to water quality, volume of water required, rate of use, and time of use (continuous, intermittent, or seasonal).

The *reclamation treatment* has to ensure that water quality requirements are met consistently. Usually, in selecting the appropriate treatment systems for water reuse applications, the provision of multiple barriers is an important consideration. This is based on the principle of establishing a series of barriers to prevent the passage of pathogens and harmful organic and inorganic contaminants into the water system. Different technologies are usually utilized for WW treatment and water reuse applications, which provide various levels of treatment: primary (e.g., removal of suspended solids by clarification), secondary (e.g., removal of biodegradable organic matter by an activated sludge process), tertiary (e.g., removal of residual suspended solids by membrane filtration), and disinfection (e.g., inactivation of pathogens by chlorination)(Table 1-2).

The technologies available for reclaiming municipal WW developed considerably during the last century. A conventional WWTP designed for discharging the effluent into a waterbody generally consists of primary treatments (screening, oil removal, settling) and secondary treatment (activated sludge process followed by secondary sedimentation). With respect to this scheme, different technologies or additional treatments should be applied in order to comply with the limits for reuse in irrigation or other agricultural application uses. The effluent of a conventional municipal WWTP that treats mainly domestic WW would require rather small additional treatments (also called tertiary treatment), mainly focused to eliminate microbiological contamination and suspended solids. On the contrary, industrial effluents may contain higher concentrations of contaminants that are difficult to remove, that's the reason why most of the implemented water reuse projects use municipal WW as water source.

The most common treatment scheme adopted to reclaim municipal WW for irrigation is based on coagulation-flocculation, sedimentation, sand filtration and chlorination. The simplicity of this scheme made it one of the most used in several countries, so that real applications have been already operating for several years. This is the case of the large-scale crops irrigation system managed by AquaSoil in Fasano (Apulia, Italy, recently upgraded). The main drawbacks of this scheme are the large space requirements and the risk of relevant loss of effluent quality in case of problems with the secondary treatment, such as incomplete nitrification and poor sludge settleability in the secondary sedimentation.

The surface filtration processes, used as tertiary treatment or integrated with the activated sludge process (Membrane BioReactor, MBR), allow to reduce the space requirements, with respect of the conventional treatments, and also provide effluents with higher quality in terms of suspended solids, organic substances and pathogens (Lateef SJ, Soh BZ and Kimura K 2013). The use of membranes with different pore size, materials and configurations may lead to permeates of different quality, and the choice depends on the final destination desired and on the limits defined by the local reuse regulations. Ultrafiltration membranes (pore size between 0.01 and 1 µm) produce effluents that, even without any additional disinfection process, comply with the reuse regulations into force in most of the countries (Lopez A, et al. 2010). Membrane processes are able to reject spores and helminth eggs, which are hardly inactivated by disinfection processes. However, a mild post-disinfection is usually performed for coping with a possible membrane failure and with possible contamination and bacterial regrowth in the storage tanks. The main drawbacks of membrane systems are investment and operating costs. An evolution towards filtration systems having lower costs consists in the Self Forming Dynamic Membrane BioReactor (SFD-MBR). The key of this system is the self-formation of a biological layer on a support of inert material. This layer has a higher filtering capability than the support itself, so it constitutes the membrane that operates the filtration (Rezvani, Mehrnia and Poostchi 2014). However, this kind of membrane filtration does not allow for the removal of microbiological contamination, so it should necessarily be followed by a disinfection process.

As regards the disinfection, for effluents characterized by low turbidity, UV radiation represents a valid alternative to chlorination and other chemical processes. An example is the reuse of the effluent of Be'er Sheva WWTP (Israel), which is managed by Kolchei Hanegev Ltd. The ability of UV rays to damage bacteria DNA is well recognized (Hassen A., et al. 2000). Other advantages of the UV system are the low space requirements and the absence of harmful by-products, which on the contrary are generated by chemical processes. The main drawback is the short-term pathogen inactivation provided by the UV systems, since a relevant bacterial re-growth is known to occur after the radiation. To avoid this phenomenon, UV disinfection can be applied in line with irrigation. An evolution of this technology towards low energy demanding system is represented by the development of UV LED (Nelson, KY, et al. 2013). Another concern of this scheme is the ineffectiveness of UV radiation to inactivate protozoan cysts, helminth eggs and certain viruses. To avoid the presence of this kind of microbiological contamination the treatment should additionally include a filtration process.

Extensive natural systems (ponds, lagoons and constructed wetlands) have been also proposed as alternative to the intensive ones. Natural systems reproduce, under engineered conditions, the processes that naturally occur in the environment (Garcia, J., et al. 2010). An example of large-scale project of indirect effluent reuse that includes constructed wetlands as tertiary treatment is the Can Cabanyes in Granollers (Barcelona, Spain). Natural systems have very low operating costs. However, the high space requirements limit their application.

The most common *water reuse applications* in the agricultural sector are for the irrigation of food, fodder, fiber, flower and seed crops, and silviculture. Required water quality will depend on whether the crop is intended for human or animal consumption, or the food is to be eaten raw or otherwise is processed. As part of the agricultural sector, water reuse possibilities for the livestock production sector have been considered for livestock drinking and cleaning farming facilities. Whereas the irrigation of pastures with recycled water is widely practiced around the world, reuse water for livestock drinking is rarely employed. To our knowledge, in Europe reclaimed wastewater is not used for livestock drinking purposes. However, in Australia and Canada reclaimed wastewater is used for direct consumption by livestock after a treatment intended for pathogen reduction including helminth removal (i.e, either pondage detention or an approved method of filtration).

There is a lack of variety in the already implemented water reuse projects for agriculture as regards the overall scheme. Usually the WW source is municipal and the application use is for irrigation. There is the need to undertake research to answer technical, environmental, food safety and economic feasibility of implementing new schemes, such as using industrial effluents as WW source since these might be closer to the point of use, for other application uses as farming cleaning, animal refrigeration, or livestock and drinking, favour the implementation of onsite systems and match water source supply and destination demand.

2 Objectives

The main objective of this report is to demonstrate the technical and economic feasibility of innovative water reuse schemes applied in the agricultural sector. These water reuse schemes are innovative from the point of view of the wastewater source considered for reclamation and the reuse application, by using state-of-art treatment technologies. The activities involved in this report were developed in the framework of the DEMOWARE project and were intended to demonstrate the feasibility, in terms of risk management, water quality, and water treatment efficiency, of using reclaimed WW for the following applications in the agricultural sector:

- Demonstration study in Capitanata, Italy. Reclamation of agro-food industrial effluents for food-crops irrigation at full-scale by a treatment consisting of primary and secondary conventional treatment plus a tertiary process with sand filtration, membrane ultrafiltration and UV disinfection.
- Demonstration study in Torre Marimon, Spain. Reclamation of municipal WW for water use in livestock production (drinking and cleaning) at pilot scale, by a tertiary treatment consisting of membrane ultrafiltration and UV disinfection.

Table 2-1 Water reuse schemes in the proposed case studies of Capitanata and Torre Marimon

Case study	
Capitanata	
Torre Marimon	

Each case study includes the selection of the treatment scheme, water quality criteria, water monitoring, on-site preventive measures, and economic evaluation. Both case studies are based on onsite systems, with a maximum of 2-km distance between water source and destination. The novelty of the first scheme is the use of an industrial effluent for reclamation. Industrial WW is not usually a WW source considered due to the presence of compounds that are more toxic and chemical hazards in general. The innovation of the second scheme is the application use, for livestock production considering using reclaimed wastewater for livestock drinking. To date and to the authors' knowledge, the use of reclaimed WW for livestock drinking has only been regulated in Australia. Water reuse for livestock production has been identified as a promising reuse application due to its constant water demand throughout the year, along with other advantages.

3 Case study water reuse in the agro-food industry in Capitanata

3.1 Introduction and objectives

The food and drink industry is the European Union's biggest manufacturing sector in terms of jobs and added-value (Food Drink Europe 2015). In particular, the economy of Southern Europe countries is strongly based on irrigated agriculture. Crop irrigation and food and drink processing require a considerable amount of water. On average, water requirements for the agro-industrial sector account for 33% of the total water demand in Europe but this percentage can reach values of about 80% in Southern Europe (EEA Report No 1/2012). Apulia (Southern Italy) is one of the European regions most affected by water shortage (Scognamiglio 2005, Xiloyannis, Montanaro and Sofo 2002). In order to fulfil the high water requirements for the agro-industrial sector, due to dry weather conditions and absence of natural surface water resources, GW resources in Apulia were overexploited during the past decades, causing a progressive GW salinization (Polemio 2016, Tulipano and Fidelibus 2002). The use of non-conventional water sources, such as reclaimed water, may be helpful to mitigate water stress, support the agriculture sector and protect GW.

Many studies have been performed to evaluate the suitability of treated municipal WW as alternative water source for crops irrigation. Pilot studies and full-scale installations demonstrated the existence of several technologies capable of treating municipal WW and produce reclaimed effluents that comply with limits for reuse in irrigation (Bixio, De Koning, et al., 2006, Norton-Brandão, Scherrenberg and van Lier 2013, Lopez, Pollice, et al., 2006). In particular, membrane filtration followed by disinfection has been appointed as a reliable and effective technology for this purpose (Vergine, Saliba, et al., 2015, Pollice, Lopez, et al., 2004). However, the feasibility of an urban WW reuse strategy may be limited by the distance between the WWTP and the final users. Moreover, the safety of reclaimed municipal WW in agriculture has been recently questioned, since it may cause the dispersion of chemical micropollutants and antibiotic resistance into the environment (see section 1.4). On the contrary, there is a lack of knowledge about the reuse of reclaimed industrial effluents for irrigation. This is mainly due to the potential presence of compounds toxic for the crops or for the microbial community involved in the WW biological reclamation process. The characteristics of industrial effluents strongly depend on the particular operation of the manufacturing process, therefore their degree of contamination may vary considerably among different industrial sectors and, for the same sector, along the year. These factors make the reuse of industrial effluents in irrigation a challenge. The recovery of water consumed by food and drink processing industries would be a significant help for reducing water shortage. Moreover, in some cases vegetable processing companies also grow the crops, so they control the industrial processes that generate WW, the treatment plant and the irrigation network. This represents a relevant advantage for the success of these reuse project. First of all, the quality of the reclaimed water resulting of the industrial processes is well known. Moreover, custom tailored reuse practices could be realized, aiming, for instance, at recovering the type and amount of nutrients required for the specific crops cultivated. Compared with urban WW reuse, costs related to pumping and pipes from the WWTP to the site of consumption are smaller. Finally, one of the main barriers, which is the lack of acceptance by farmers, becomes less relevant.

The main objective of this demonstration activity is to assess the suitability of reusing treated agro-industrial WW effluents for the irrigation of vegetable crops. Several technologies were evaluated in order to provide different water sources to fit the quality required for the crops. The variability of source water quality and its effect on the performance of the reuse treatment scheme and on the reclaimed water quality were investigated. Costs and water saving opportunities of different water reuse schemes within the agro-industrial sector were evaluated. The fate of microbiological contamination was also studied. Finally, effects on crops productivity and possible salt accumulation on soil were considered in order to provide a comprehensive review of opportunities and drawbacks involved in this type of reuse practices.

3.2 Site description and reuse application

The demonstration activity has taken place within the company Fiordelisi s.r.l., an agro-food industry located in Apulia (Southern Italy), whose business includes growing, processing, packaging, and marketing preserved ready-to-eat horticultures (tomato, eggplant, zucchini, pepper, broccoli, etc.).

All the water used by the company Fiordelisi, for both cultivating and processing the vegetables, is pumped from GW. The company has limited water availability for both processing purposes (about 15 m^3/h) and irrigation (about 70 m^3/h). The company produces on average about 80,000 m^3/a of WW (based on the data measured during the year 2015), which is treated through a WWTP owned and managed by Fiordelisi itself. Before 2012, the WWTP was composed only of primary and secondary treatments and the treated WW was discharged. Recently, due to a growing industrial production, water consumption for both processing and irrigation needs significantly increased. The latter reached, during the warm season, a value close to the maximum flow rate available for irrigation. Therefore, in 2012 the company decided to reuse part of the reclaimed WW for irrigating its own fields and so reducing GW demand. For this purpose, a full-scale tertiary treatment was commissioned and open field tests were planned.

3.2.1 Industrial process

Fiordelisi produces mainly two types of products: oil preserves and dried vegetables. Their production processes are described by the diagrams in Figure 3.1 and Figure 3.2.

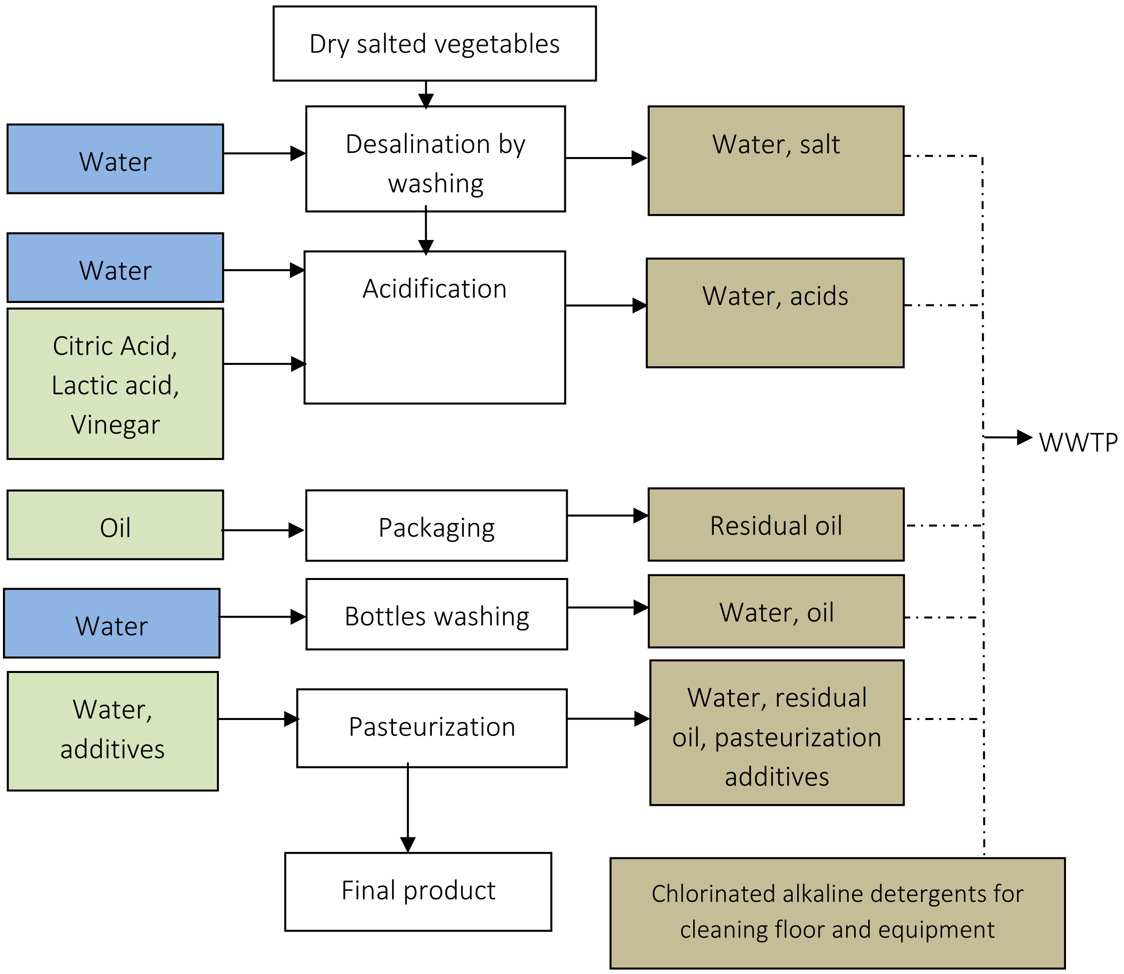


Figure 3.1 Flow diagram of the production process of acidified vegetable preserves.

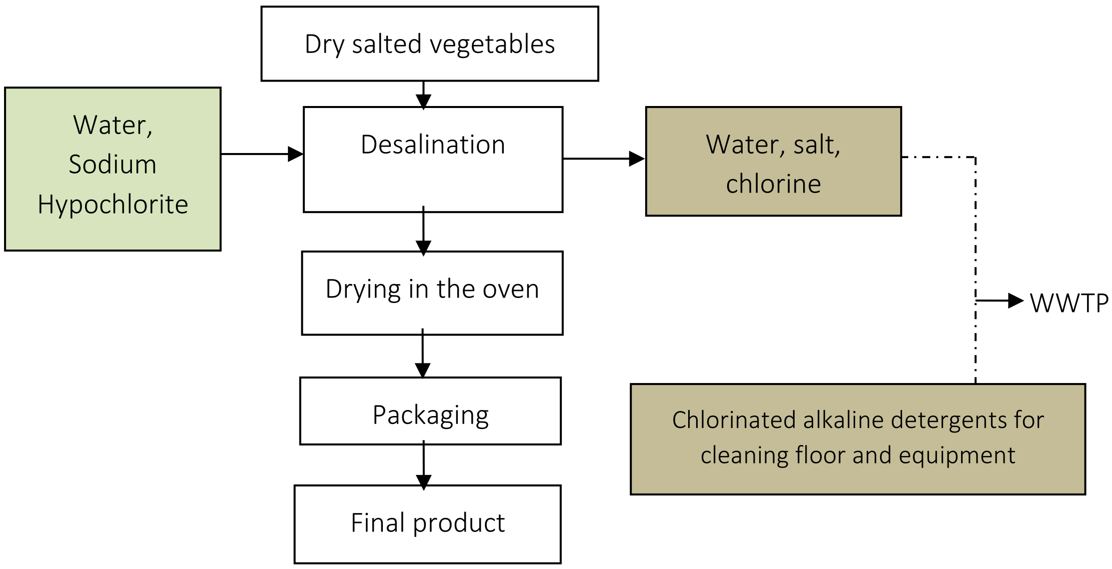


Figure 3.2 Flow diagram flow of the production process of dried vegetables.

There are four steps of the production process that generate WW streams: washing, acidification, packaging, and pasteurization. The flow rates and the qualitative characteristics of these streams differ considerably. Washing and pasteurization are the most water consuming steps. Salt is present mainly in the washing stream, oil in the packaging one. However, all the steps may contain significant organic pollution, which is difficult to predict, since it depends on the type of vegetables processed and on the specific conditions applied (temperature, duration, amount of acids and additives used).

3.2.2 Wastewater treatment plant

The WWTP, reported in Figure 3.3 and Figure 3.4, is composed of three main steps:

- Primary treatments: screening (0.5 mm); deoiling, performed by gravity separation; equalization, in a tank of 270 m^3; pH adjustment, by dosing sodium hydroxide.
- Secondary treatments: activated sludge process, composed by anoxic (130 m^3) and aerobic (520 m^3) phases, with aeration provided through fine bubble diffusers; secondary sedimentation, chemically assisted by adding aluminium polychloride.
- Tertiary treatments: sand filtration, membrane ultrafiltration (8 modules, with a nominal surface of 60 m^2 each), and UV radiation (6 mercury-vapour lamps, 200 W each).

The tertiary treatment is a full-scale plant, designed for the average flow rate treated by the primary and secondary processes. The modules used are the Kristal 600ER (Hyflux), composed of hollow fibre membranes made of specially formulated polyethersulfone (PES). They had a nominal pore size of 0.05 µm and worked in outside-in mode. Membranes were pressurised to drive filtration through the fibres in cross-flow recirculation mode, as shown in Figure 3.5. The feed water entered the bottom of the module and the filtered water (permeate) was channelled to the top and sent into a collection tank. A reject port allowed for recirculation of the retentate into the feed tank.

The filtration unit was operated at an influent pressure that ranged between 2.0 and 2.5 bar. Part of this pressure was necessary for the filtration through the sand filter (1.0-2.0 bar), so that the residual influent pressure to membranes was between 0.5 and 1.5 bar (outlet pressure close to 0).

Periodical backwashings were performed with the ultrafiltrated membrane permeate for both the sand filter (15 min duration every 8 h of operation) and the ultrafiltration (30 sec duration every 45 min). Membranes were also cleaned chemically (with soda and sodium hypochlorite) approximately every 10 days, according to the procedures suggested by the manufacturer (during 45 min, clean water at 40°C plus soda up to pH=11; during the next 15 min, addition of 100 mg NaClO/L). The simplified scheme of the filtration unit is shown in Figure 3.6.

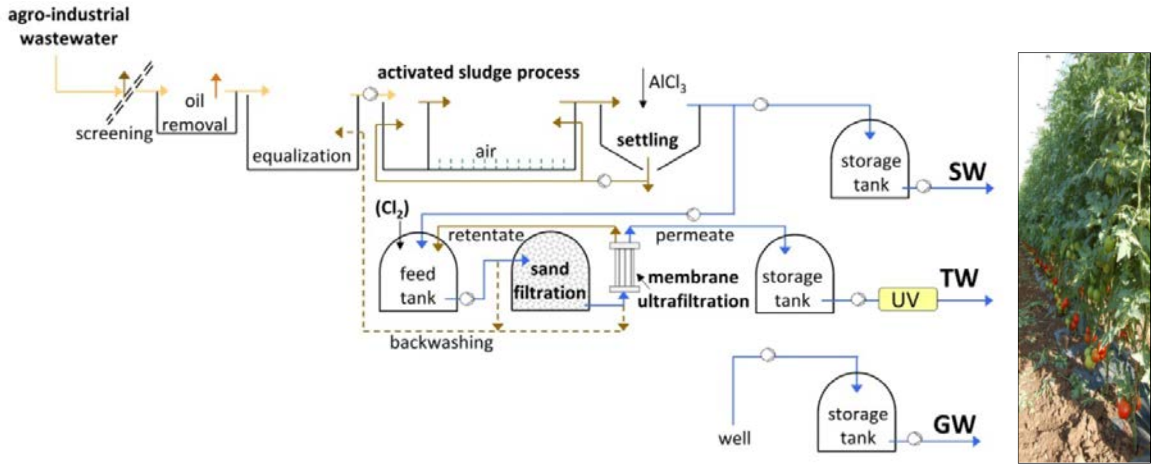


Figure 3.3 Configuration of the WWTP operated at Fiordelisi company and identification of the water sources used for irrigation.
The effluent of the settling phase, after the storage, is called SW (secondary treated wastewater), the UV outlet TW (tertiary treated wastewater), the well water GW (groundwater).

Figure 3.4 Pictures of WWTP and test field at Fiordelisi company.

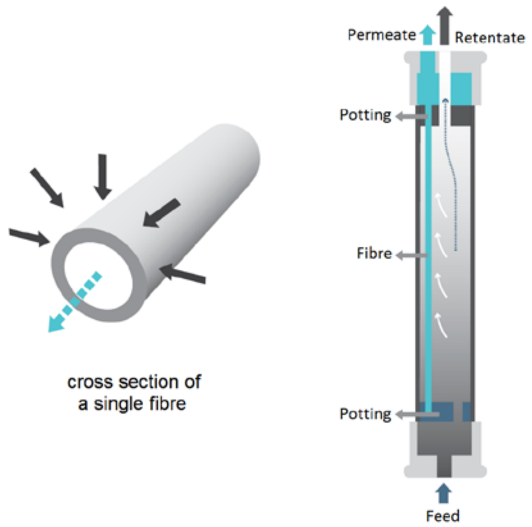


Figure 3.5 Scheme of operation of the Kristal 600ER (Hyflux) membranes.

3.2.3 Field tests for reuse in irrigation

Field tests consist on WWTP monitoring, irrigation tests, and assessment of the chemical and microbiological quality of the water used for irrigation, of the irrigated soil and of the crops, along with agronomic evaluations (crop yield, etc.). Irrigation tests were performed in an open field of about 5000 m^2, located in an agricultural area within the premises of Fiordelisi company (see Figure 3.4). Three types of water were used for irrigating the field, two reclaimed WWs with different degrees of treatment and the water source conventionally used by Fiordelisi: secondary treated wastewater (SW), tertiary treated wastewater (TW), and groundwater (GW), respectively. Field test was arranged following a latin square design. Before being used for irrigation, the three water sources were stored in a 10 m^3 tank for a period between 1 and 7 days, depending on the irrigation needs. For the TW, on-demand UV disinfection was operated in-line with irrigation. Considering the flow rate used during irrigation, the UV dose applied ranged between 110 and 160 Wh/m^3. Tomato and broccoli were cultivated, in close succession, under a structure covered with an anti-hail net. Irrigation was performed when the available soil moisture was depleted to the threshold value of 40%. A drip irrigation method was used with the drip lines placed under a black plastic mulching film. Fertilization, pest and weed control were performed according to local management practices.

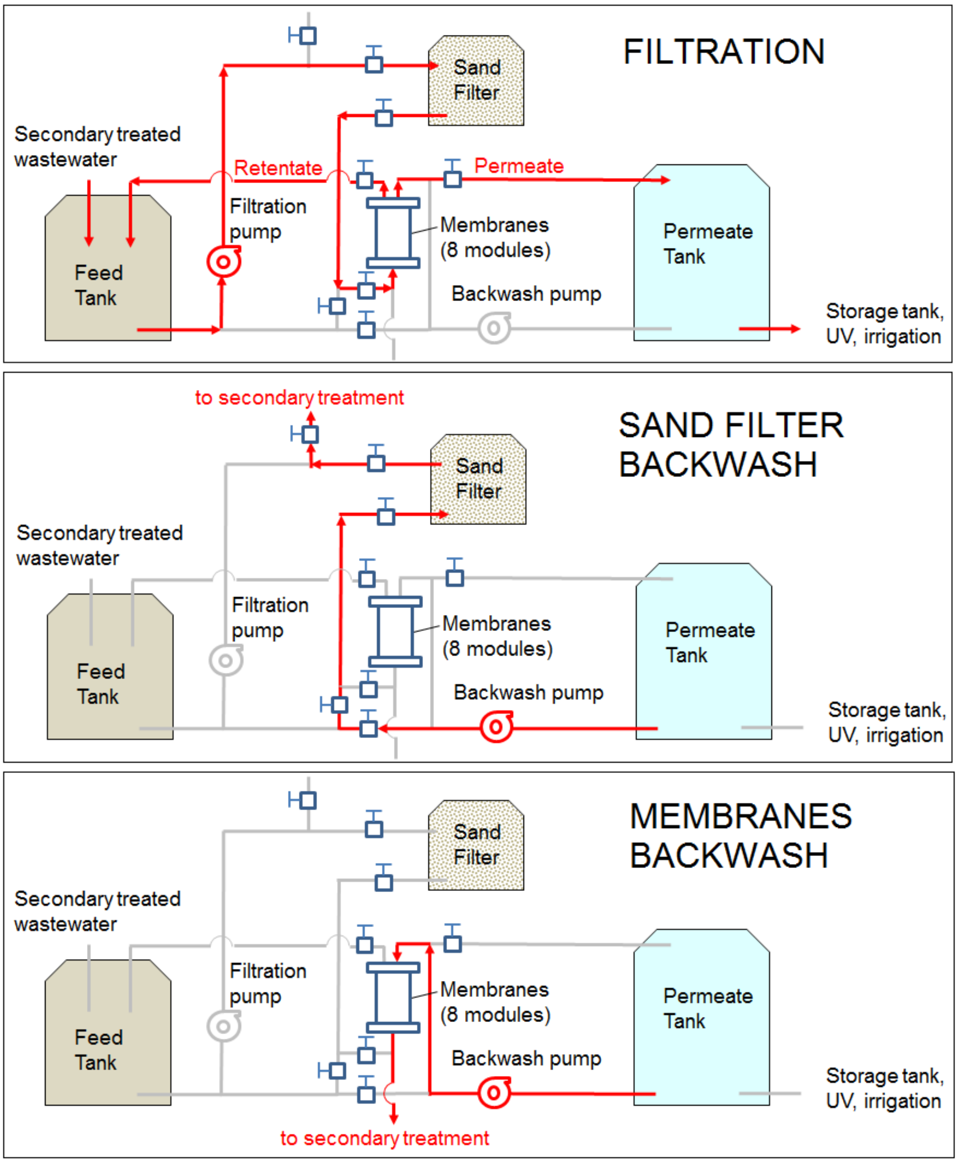


Figure 3.6 Simplified scheme of the filtration unit.
The pipes and the pumps working during each operation mode are highlighted in red.

3.3 Raw wastewater characteristics

The WW produced by the company Fiordelisi is mainly composed of process water (vegetables washing, processing and cleaning of premises) and water from the bottles washing line. However, there is also a relevant component (between 5 and 10% of the total) coming from the toilets. The WW has been characterized monthly and the average values of the main parameters during the years 2014 and 2015 are summarized in Table 3-1. This agro-industrial WW had low pH, high salinity, and a relevant presence of surfactants and organic substances. The type and the amount of vegetables processed vary considerably along the year, as is seen by the charts displayed from Figure 3.7 to Figure 3.10. Occasionally, the pollution load of the raw WW was much higher than the average value shown in Table 3-1. Under these conditions, the WWTP resulted undersized, meaning that the secondary settled effluent (SW) had high concentrations of COD and suspended solids. This would have affected significantly the tertiary treatment operability, requiring frequent chemical cleanings and reducing the long-term productivity of both sand filter and ultrafiltration membranes. To avoid this, the operation of the filtration unit was interrupted when the turbidity of the SW increased considerably (appreciated by naked eye). This occurred four times during the years 2014 and 2015, with a duration that lasted between one and three weeks.

At the end of 2015, the company added new industrial processes, which caused a relevant increase of the flow rate (by 30%), COD, electrical conductivity, and surfactants between January and March 2016 (from Figure 3.7 to Figure 3.10). During this period the quality of the SW was very low (COD>100 mg/L, TSS>50 mg/L), so the tertiary treatment was not operated. The main cause of the great additional organic and inorganic load was identified (highly salted vegetable preserving solutions) and, since April 2016, this effluent is sent to a specialised company to be treated as oil residue. This allowed to recover the WWTP functioning. However, the values of electrical conductivity observed in April and May 2016 (Figure 3.9) indicate that other modifications in the industrial processes were contributing to the average increase of this parameter.

Table 3-1 Average values of the agro-industrial WW composition during the years 2014-2015. (n=26)

Parameter	Units	Average value
Suspended solids	mg/L	234.0 ± 42.7
COD	mg O_2/L	1168 ± 663
Total nitrogen	mg N/L	32.9 ± 17.8
Anionic surfactants	mg/L	31.9 ± 13.5
Phosphate	mg PO_4^{-2}/L	5.9 ± 4.3
pH	-	5.5 ± 1.0
Temperature	°C	31.4 ± 0.8
Electrical conductivity	mS/cm	2.9 ± 0.9
E. coli	MPN/100mL	1.4 E+07 ± 1.4 E+07(*)
Total oil	mg/L	36.5 ± 26.4(**)

(*)Median 8.7 E+06 MPN/100mL; first quartile 4.7 E+06 MPN/100mL; third quartile 1.7 E+07 MPN/100mL.

(**) In the influent to the activated sludge process, i.e. after the oil removal step (n=3).

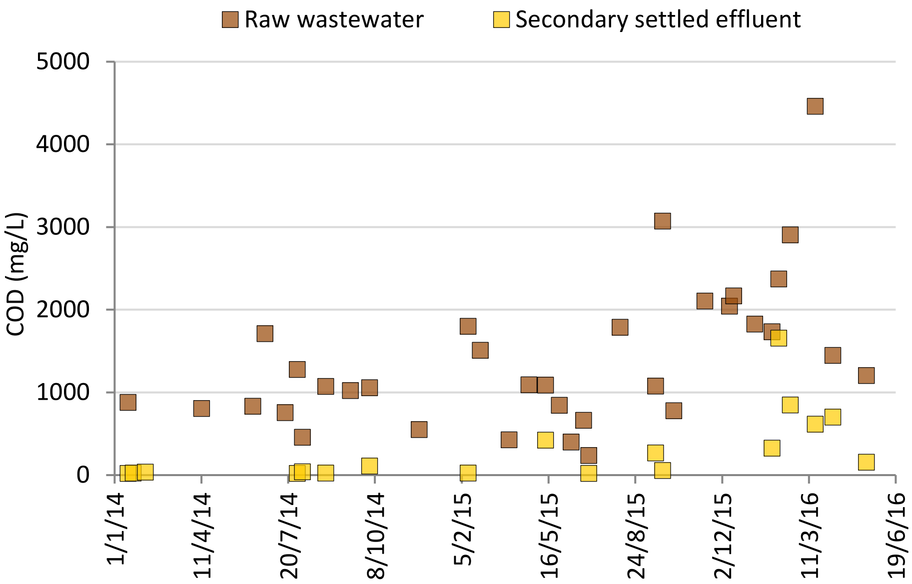


Figure 3.7 Evolution of COD in raw WW and secondary effluent (SW) over time.

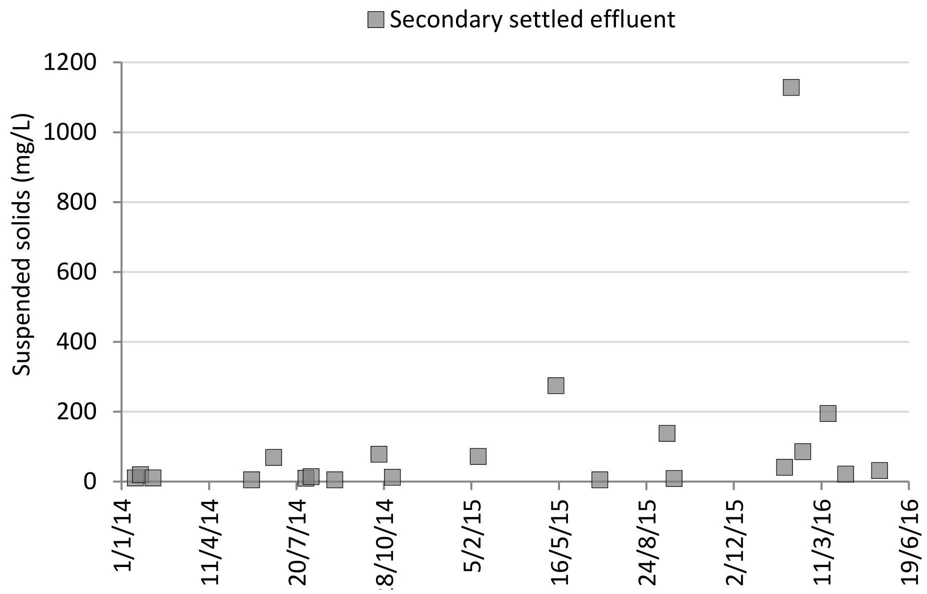


Figure 3.8 Evolution of suspended solids in the secondary effluent (SW) over time.

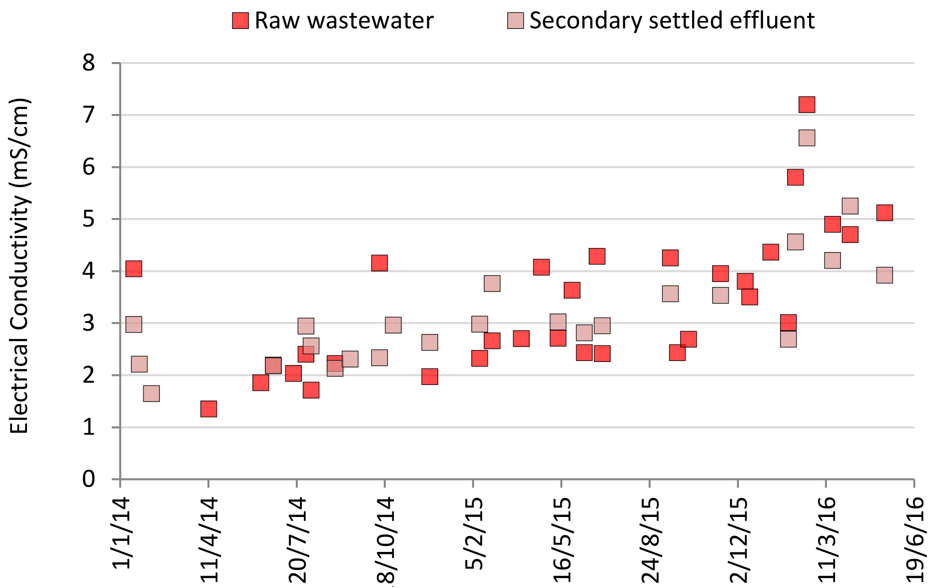


Figure 3.9 Evolution of the electrical conductivity in raw WW and secondary effluent (SW) over time.

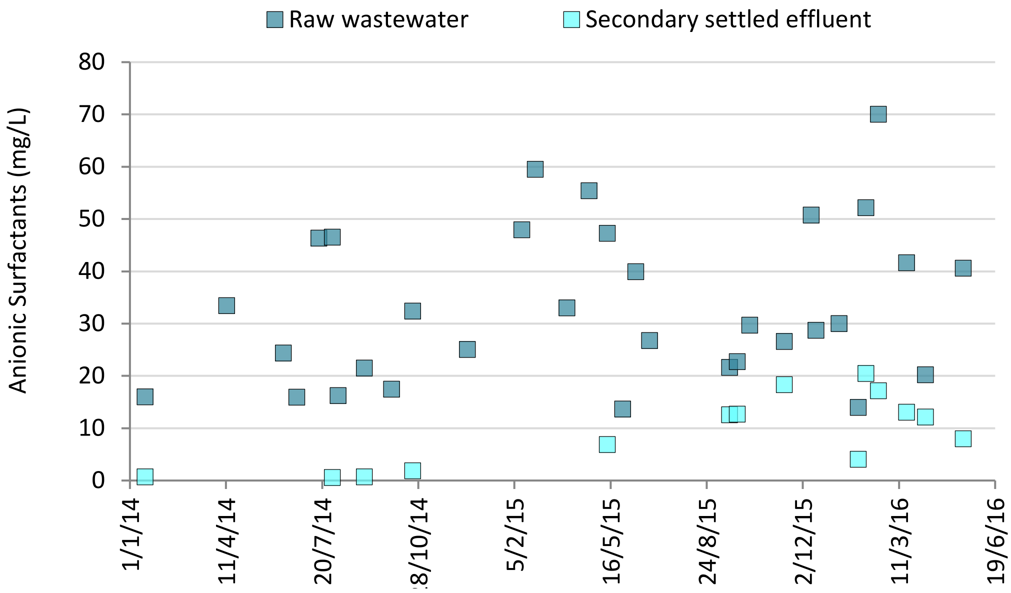


Figure 3.10 Evolution of anionic surfactants in raw WW and secondary effluent (SW) over time.

3.4 Tertiary treatment performance

Initially, the results of the first monitoring campaigns, performed at the beginning of 2014, indicated the presence of suspended solids (up to 19 mg/L) and faecal contamination (up to 1000 MPN/100mL of *Escherichia coli*) in the membranes permeate, suggesting a possible leakage. Membrane integrity was then evaluated by a pressure decay test (PDT) (ASTM 2003), whose procedure consists of the following steps:

1. Stop filtration.
2. Close all valves except the recirculation, reject discharge and permeate valves.
3. Wait for 15 min for the air pressure in the system to be released and then close all valves except the permeate valves.
4. Open air valve and introduce 1 bar of air into the modules. If the feed pressure gauge shows that the air pressure in the module is not able to reach 1 bar, check for air leakages in the pipes.
5. When the feed pressure gauge reaches 1 bar, stop the air flow and close-off the air valve.
6. Monitor for 10 min. If, after 10 min, the feed pressure gauge shows a significant drop in pressure (more than 0.1 bar drop) or if it is observed that there are a lot of air bubbles in the permeate pipe, a leak has probably occurred and leak arrests must be done.

The results of the PDT, shown in Table 3-2, indicate that 6 modules out of 8 presented relevant leakages. These modules were isolated and, in order to fulfil irrigation needs, the membrane system was operated with the remaining two modules having no relevant leakages.

Then, in October 2014, new modules were installed and operated during approximately 14 months under an influent pressure of 0.8-1.2 bar (2.0-2.5 bar was the influent pressure to the upstream sand filter). During these 14 months, the average membrane productivity was 4.2±1.2 m^3/h, corresponding to a flux of 8.8±2.5 L/m^2/h, which is much lower than the manufacturer indication for a secondary effluent (50 L/m^2/h). The data describing membrane feed characteristics and the evolution of the membrane flux over time were shared with the manufacturer, which indicated as the most probable cause of the low productivity the relatively high content of oil (about 2 mg/L) in the membrane feed. Considering the high content of grease and oil in this type of agro-industrial WW, in order to have a good productivity of the ultrafiltration, the oil removal step should be addressed through a more effective process. Deoiling of the raw WW is actually performed by gravity separation using a small tank (30 m^3) and no other deoiling processes are currently applied. A Dissolved Air Flotation (DAF) applied to the secondary settled effluent would be a good system for removing both suspended solids and oil (with performances ranging between 50% and 90% for both parameters, respectively), therefore it could substitute the sand filter currently used.

Table 3-2 Results of the PDT (Pressure Decay Test) performed on membrane modules.

Examined module	Initial pressure (bar)	Pressure after 10 min (bar)	Pressure drop	Evaluation
None (piping leaks check)	1.0	~1.0	<<0.1	Piping leaks negligible
Module 1	1.0	0.95-1.0	<0.1	Negligible leaks
Module 2	1.0	0.90-0.95	<0.1	Negligible leaks
Module 3	1.0	0.75	>0.1	Relevant leaks
Module 4	1.0	0.75-0.80	>0.1	Relevant leaks
Module 5	0.95(*)	0.65-0.70	>0.1	Relevant leaks
Module 6	0.90(*)	0.2	>>0.1	Severe leaks
Module 7	0.95(*)	0.2	>>0.1	Severe leaks
Module 8	1.0	0.3	>>0.1	Severe leaks

(*) The air pressure in the module was not able to reach 1 bar.

The tertiary treatments removed completely *E. coli*, so providing up to 5 log removal, and also partially remove COD (71%), suspended solids (66%), and surfactants (82%), as can be seen in Table 3-3, where the average characteristics of SW and TW are reported in comparison with GW and legislated limits for water reuse. Small, but not statistically significant, reductions of nitrogen and phosphates were also observed, of about 29 and 80%, respectively.

Table 3-3 Average values of the reuse-related parameters in the three sources of water used.
Sources were used for irrigating the test field during the 14 months of operation of the new membrane modules (October 2014-December 2015) (n=16).

Parameter	SW	TW	GW	Local limits for reuse(*)
pH (-)	6.6±0.5	7.5±0.4	7.5±0.3	6-9.5
Electrical Conductivity (mS/cm)	2.7±0.6	2.9±0.5	0.8±02	3
TSS (mg/L)	48.7±73.6(**)	16.4±12.7	4.2±2.9	10
COD (mg/L)	89.7±126.5(**)	26.3±21.2	<15	100
Total Nitrogen (mg N/L)	4.5±5.3	3.2±2.3	24.7±3.8	35
Phosphates (mg P/L)	0.5±0.6	0.1±0.2	<0.1	10
Potassium (mg K/L)	64.2±9.3	60.3±4.1	11.6±1.5	-
E. coli (MPN/100mL)	$3{\cdot}10^5{\pm}7{\cdot}10^5$	1.2±3.1	0	10(***)
Salmonella (-)	Absent	Absent	Absent	Absent
Anionic surfactants (mg/L)	6.8±7.0(**)	1.2±0.9	0.4±0.0	0.5
Sodium Adsorption Ratio (-)	10.7±8.1	11.2±8.0	1.3±0.6	10

(*) The standard limits for reuse in agriculture established by the regional regulation (Regione Puglia 2012) coincide with those indicated by the national legislation (Legislative Decree n. 152/2006) / (**) Variations within the industrial processes resulted in a high variability of the wastewater characteristics. Peaks of concentration were only partially mitigated by the secondary treatments (see paragraph 3.3) / (***) For 80% of samples. Max value = 100 MPN/100mL.

The operability of the ultrafiltration membrane was significantly affected by the characteristics of the influent to the filtration unit. Increasing the concentration of suspended solids in feed water reduced membrane productivity. Indeed, as shown in Figure 3.11, higher TSS concentrations caused bigger drops in the permeate flow, so increasing chemical cleaning requirements. In the first two months of operation of the new membranes (October-December 2014), the chemical cleaning frequency varied between once every three days and once every twelve days, depending on water feed quality.

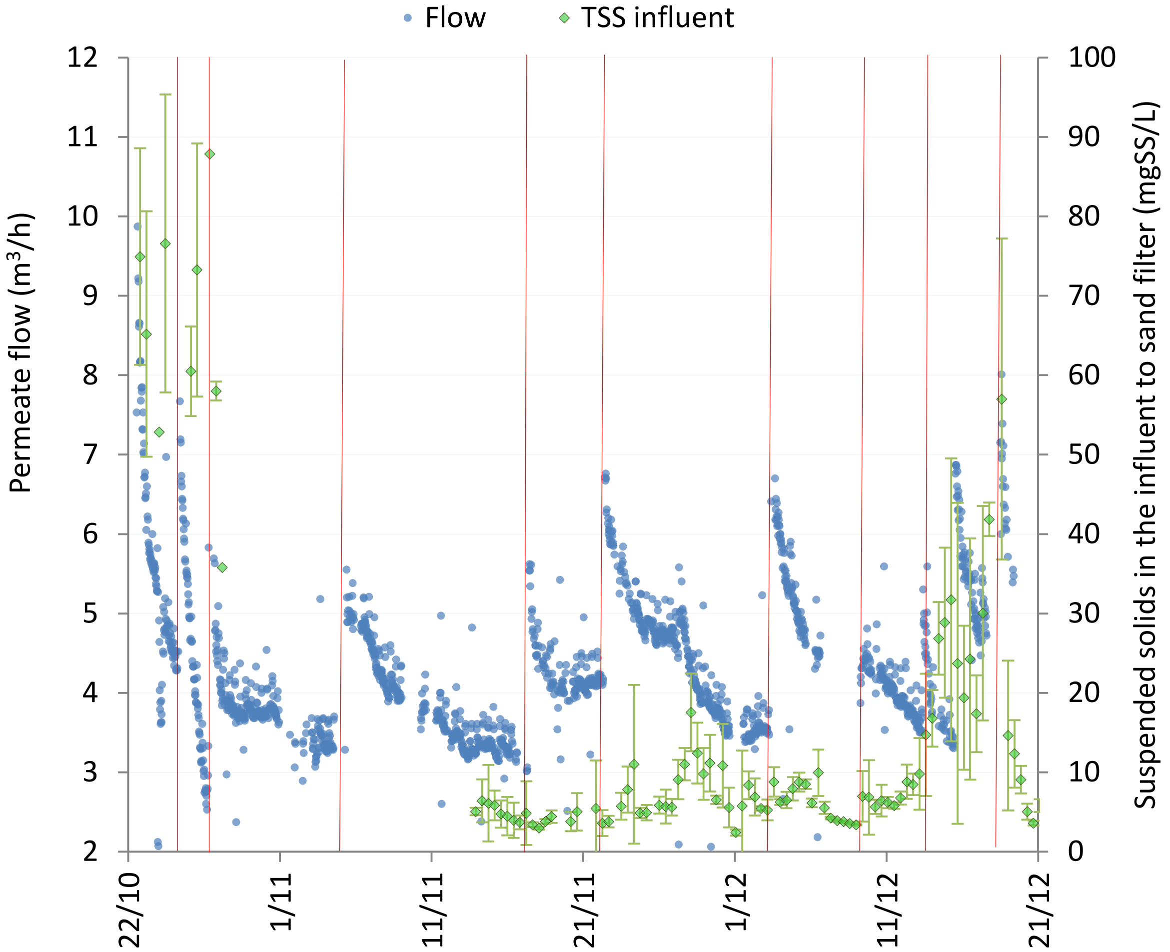


Figure 3.11 Influence of the characteristics of the feed water to the filtration unit on its productivity.
Evolution of TSS in the influent and of the permeate flow rate during the first two months of operation of the new membranes (October-December 2014). Red vertical lines indicate chemical cleanings.

The box-plots of the concentrations of suspended solids and *E. coli* in the outlet of each section of the tertiary treatment during the 14 months of operation of the new membrane modules (October 2014-December 2014), are reported in Figure 3.12. New modules still showed a residual presence of *E. coli* and suspended solids in the membrane effluent. A careful inspection of the P&I of the filtration unit revealed the possibility of a slight contamination of the membranes during backwashing/cleaning operations, which could be avoided by modifying the piping of the filtration unit. However, the final UV disinfection, performed in line with irrigation, effectively removed the residual faecal contamination, as shown in Figure 3.12.

It is also important to highlight that two phenomena were observed in the tank where the tertiary treated WW was stored: (i) an increase of suspended solids concentration (up to 100%); (ii) a relevant die-off of the faecal indicator *E. coli*. Suspended solids increase can be associated to a bacterial re-growth, as shown by the results of the prokaryotic abundance, measured through Flow Cytometry analysis, which are displayed in Figure 3.13. The considerable extent of both bacterial re-growth and *E. coli* die-off was caused by the particular type of storage performed, which was static, meaning that when the tank was full, the membrane permeate was sent directly to discharge without passing through the tank, and this may have favoured bacterial re-growth.

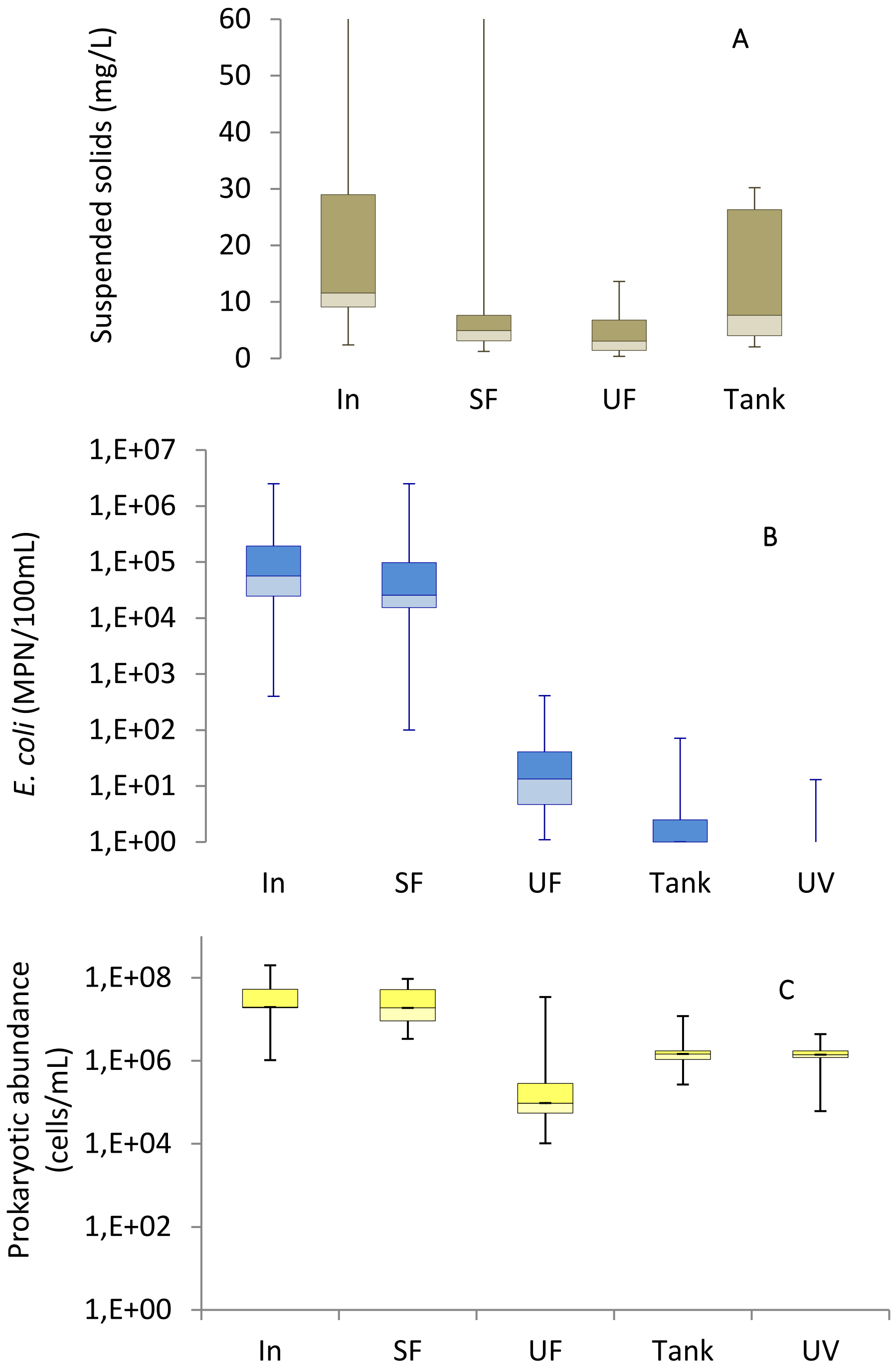


Figure 3.12 Average performance of the tertiary treatment during the period 20/10/2014-31/12/2015.
Box-plot of the concentrations of suspended solids (A), *E. coli* (B), and prokaryotic abundance (C).
Sampling points: influent to the sand filter (In), sand filter outlet (SF), ultrafiltration membranes outlet (UF), storage tank (Tank), after UV disinfection (UV).

Flow Cytometry analysis was used in order to evaluate the effectiveness of the UV disinfection on bacteria different from the indicator *E. coli*. The total abundance of prokaryotes and protozoa was measured in four pairs of samples of the inlet and outlet of the disinfection system. Then the analyses were repeated after incubating the sample in small vials for 24h and the fold increase determined for each sample. The average results, reported in Figure 3.13, showed that the UV radiation reduced the re-growth of both prokaryotes and protozoa in the first 24 h after the treatment. More details on this flow cytometry methodology, which could be a useful instrument to estimate the inactivation caused by UV, are provided in Demoware Deliverable D2.5 “Applicability of flow cytometry and qPCR methods to assess and control microbial contamination”.

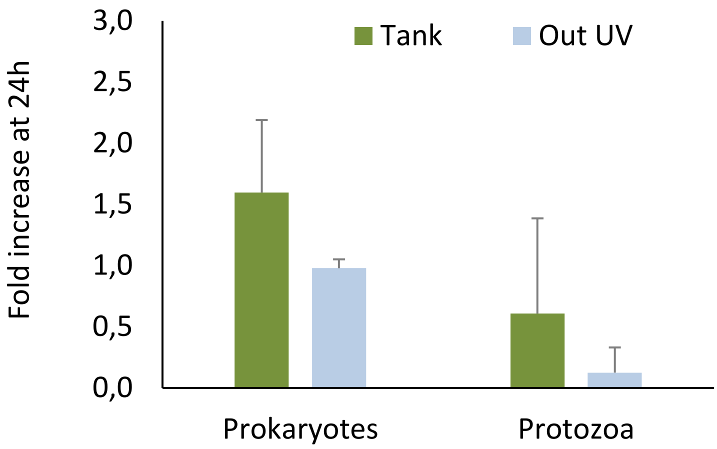


Figure 3.13 Influence of UV disinfection on bacterial re-growth.
Fold increase of the abundance of prokaryotes and protozoa after 24 h of incubation.
Samples: storage tank (Tank), after UV disinfection (UV).

3.5 Evaluation of several water sources for food crops irrigation

The average characteristics of the three types of water used for irrigation are reported in Table 3-3, together with the local limits for reuse. For the SW, the main non-compliances were *E.coli*, TSS, and surfactants. SAR and electrical conductivity were often close or slightly above the limits. After the substitution of the membranes in October 2014, the tertiary treatment allowed to comply with the limits related to *E.coli*. During the first 18 months of field experiment (until December 2015), the content of surfactants and SAR in the TW complied with the limits for reuse, as well. Then a sharp change in the raw WW characteristics occurred, as mentioned in section 3.3. This affected the quality of the TW, since neither the conventional WWTP nor the tertiary treatment were designed for a WW with such a high content of salt and surfactants. The solution in this case consisted in sending the extra volumes of highly polluted WW (collected separately) to external alternative treatments, and can be considered a good short-term strategy under these conditions. However, the upgrade of the entire WWTP would be necessary in order to cope with the increasing industrial production. The average TSS concentration in the TW was above the limit of 10 mg/L only because of the bacterial re-growth in the tanks. This suggests that, in order to comply with this limit, for large-scale applications, a static storage of the membrane effluent should be avoided. The membrane permeate should continuously pass through the tank before being discharged.

In terms of fertilization potential, all the irrigation water sources had a very low content of phosphorus and a relevant content of nitrogen and potassium (Table 3-3). As regards these three elements, differences between SW and TW can be considered negligible. The concentration of nitrogen was much lower in treated WWs than in GW. This result can be explained by two factors that are typical of the agro-industrial sector. The first is the over-fertilization of soil, which causes the presence of nitrogen in GW. This is common in intensive farming areas and represents a serious environmental issue that needs to be tackled

(Bouraouia and Grizzetti 2014). The second is the very high COD/N ratio in the raw WW, typical in agro-industrial effluents, which is the reason of the low content of nitrogen in treated WW. Indeed, even if vegetable processing and toilets generated WW with high concentrations of nitrogen (and phosphorus, as well) (Table 3-1), most of these elements would have been removed during the activated sludge process to fulfil the metabolic needs of the microorganisms responsible for the biodegradation of organic substances. On the contrary, the requirement of potassium for microbial metabolism is very low, so its removal within the WWTP is negligible.

Fertilization practices commonly adopted by Fiordelisi consist in providing about 200 kg N/ha, 250 kg P/ha, and 150 kg K/ha for the cultivation of tomato and about 100 kg N/ha, 150 kg P/ha, and 70 kg K/ha for the cultivation of broccoli. Fertilization schedule starts with the supply of a solid fertilizer, composed of nitrogen and phosphorus (in both organic and inorganic forms), before transplanting. Then, along the cultivation, a weekly fertirrigation is performed with ammonium nitrate (substituted by ammonium sulphate in the last month of the cultivation), ammonium phosphate, and potassium nitrate (substituted by potassium sulphate in the last month of the cultivation). The overall irrigation volumes supplied during the cultivation periods were approximately 1000 and 5000 m^3/ha for broccoli and tomato, respectively. The amounts of nutrients provided through irrigation were calculated by multiplying the overall irrigation volumes by the concentrations of nutrients (reported in Table 3-3) and were displayed in Figure 3.14. These were in addition to the nutrients supplied through fertilization. The high irrigation requirements for the cultivation of tomato allowed for a relevant recovery of nitrogen and potassium from both water sources, indicating that it is possible to reduce the amount of these nutrients that needs to be supplied through chemical fertilization. In particular, with respect to the local fertilization practices, the irrigation with well water would cover for about 60% of the nitrogen requirements, while the irrigation with treated WW would cover for about 150% of potassium requirements.

As a consequence of the different irrigation volumes applied for tomato and broccoli, the characteristics of the irrigation water had no significant influence on the productivity of broccoli, whereas the higher content of nitrates in GW slightly enhanced (by about 6%) tomato productivity, as shown in Figure 3.15. Previous studies reported that supplying nitrates through the irrigation water has positive effects on crops yield, generating up to 50% increase (Vergine, Lonigro, et al. 2016).

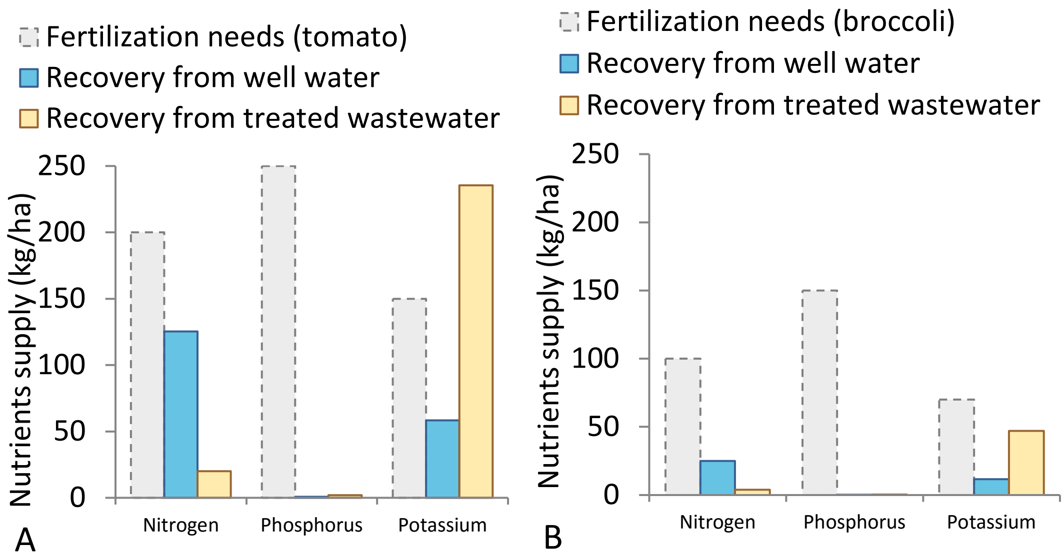


Figure 3.14 Nutrient recovery from the well water and from the secondary treated wastewater for tomato (A) and broccoli (B).
Differences in terms of nutrients between secondary and tertiary treated wastewater were negligible.

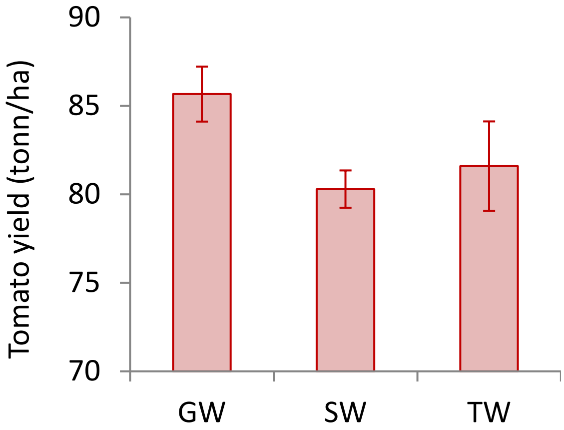


Figure 3.15 Average productivity of the tomato fields irrigated with the three different water sources.

In terms of faecal contamination, the three types of water differed considerably. Compared with GW and TW, the SW, which was non-disinfected, was characterised by a considerably higher content of *E. coli*. This caused a significant presence of *E. coli* in the soil, but it did not result in any relevant contamination of plants or fruit (Figure 3.16). *E.coli* was never detected in fruits and only once in plants, which had been cultivated in a plot irrigated with conventional water (GW).

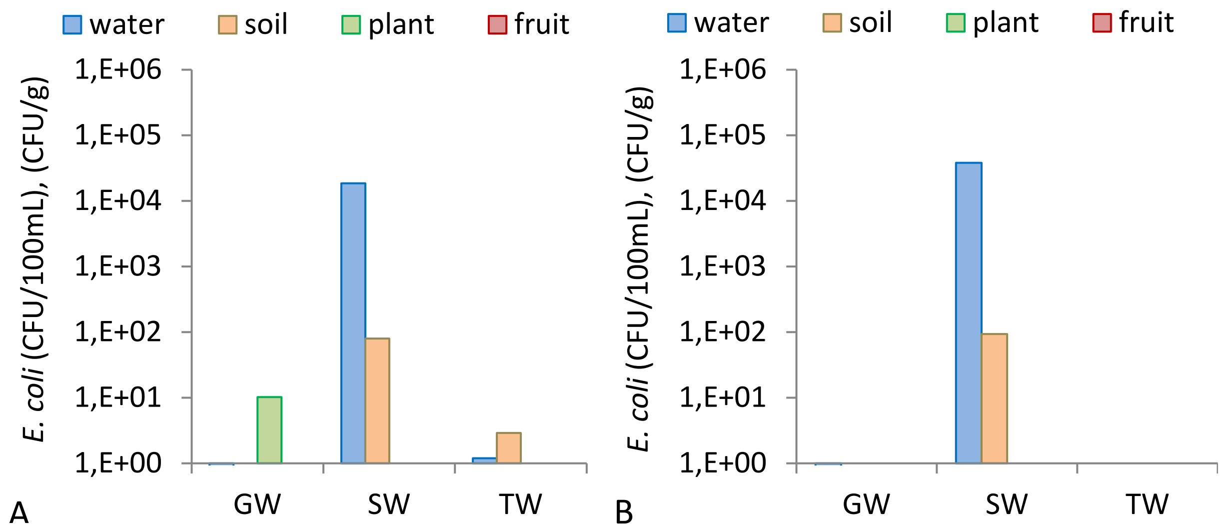


Figure 3.16 Presence of the indicator *E. coli* in the different irrigation water sources and in the corresponding irrigated soils and crops.
Average values observed during the cultivation of tomato (A) and broccoli (B).

Faecal coliforms were also analysed in the different irrigation water sources and in the corresponding irrigated soils and crops. Results, displayed in Figure 3.17, confirm that the SW had a considerably higher faecal pollution than the other two water sources (on average, from two to three orders of magnitude more). However, irrigated soils and crops had similar content of faecal coliforms (differences were lower than one order of magnitude).

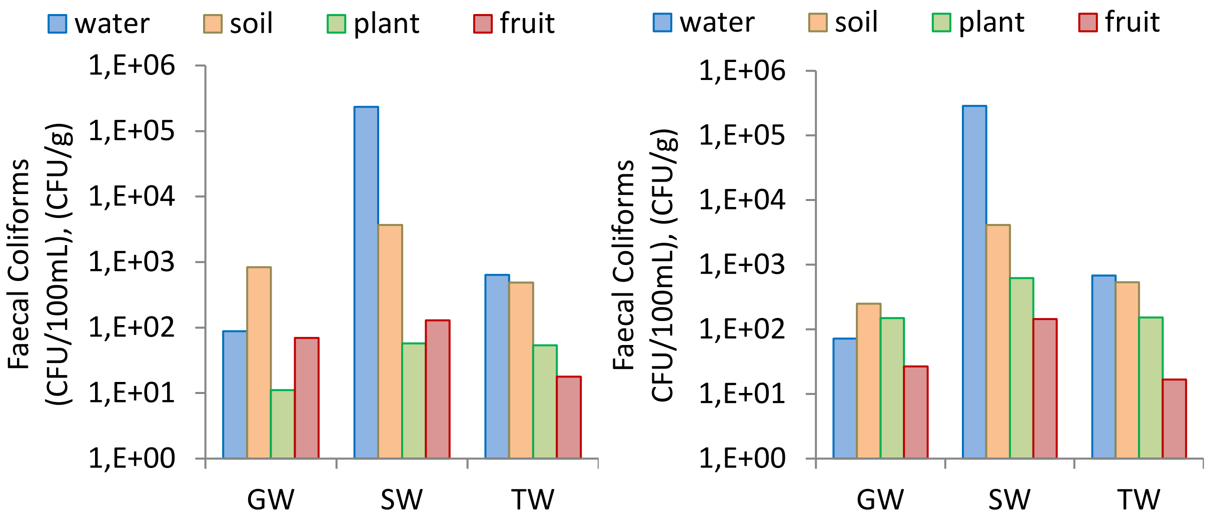
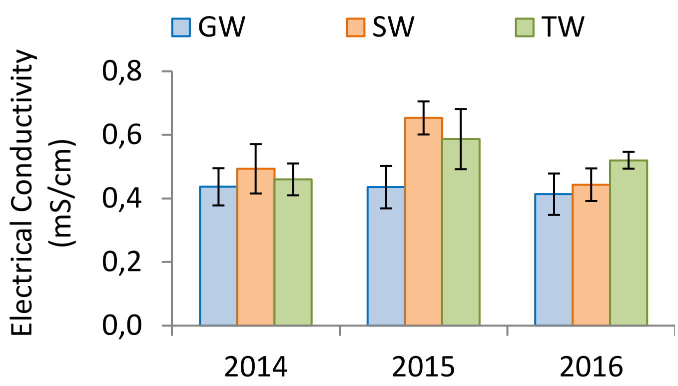


Figure 3.17 Presence of Faecal Coliforms in the different irrigation water sources and in the corresponding irrigated soils and crops.

Average values observed during the cultivation of tomato (A) and broccoli (B).

Findings shown in Figure 3.16 and Figure 3.17 are in agreement with other studies (Cirelli, et al. 2012, Palese, et al. 2009) and indicate that irrigating with reclaimed WW having a residual presence of faecal contamination does not imply a relevant contamination of the crops. Moreover, the presence of *E. coli* on plants irrigated with well water (having no *E. coli*) suggests that, under the conditions applied in the present field study, the effect of possible external sources of contamination was comparable (or even higher) to the effect of contamination related to the irrigation water. Previous studies also highlighted the importance of the external environment, typically wildlife, as source of faecal contamination (Langholz and Jay-Russell, Potential role of wildlife in pathogenic contamination of fresh produce 2013, Forslund, Ensink, et al., Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater 2012, Vergine, Saliba, et al., Fate of the fecal indicator Escherichia coli in irrigation with partially treated wastewater 2015).

The electrical conductivity, pH, and percentage of organic matter in the soil irrigated with the three different water sources were monitored along the experimental period. The values measured in 2014, 2015 and 2016 (Figure 3.17 and Figure 3.18) indicate that 2 years of irrigation with reclaimed WW had no significant effects on soil characteristics. In particular, no relevant salt accumulation was observed in the soil.



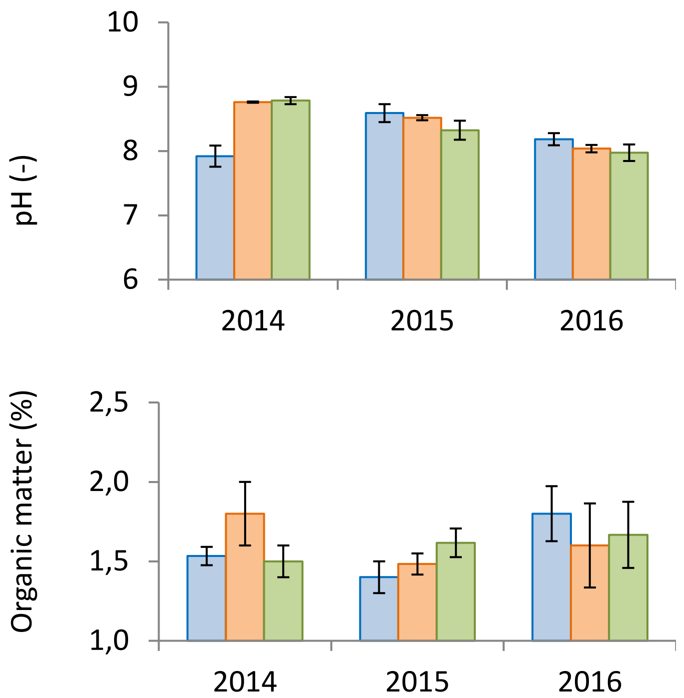


Figure 3.18 Evolution of the characteristics of the soil (first 30 cm) irrigated with three different water sources during the experimental period.

These results are in agreement with the findings of Morugán-Coronado and co-authors (2011), which observed a temporary effect of the irrigation with salty treated WW on soil electrical conductivity, but no salt accumulation after two years of field tests. However, it is not possible to exclude that relevant durable long-term effects could be observed.

3.6 Economic evaluation

The cost of treating the secondary effluent to produce a tertiary treated WW suitable for irrigation was determined considering the actual investment cost of the tertiary treatment equipment, the power consumption for the filtration unit and the UV system, and the labour cost. The membrane productivity was assumed to be constant and equal to the average value measured during the first 14 months of operation (4.2 m^3/h).

The power consumption of the filtration unit, which included the sand filter and the ultrafiltration membranes, was measured by a counter. Considering the results related to the first 14 months of operation of the new modules installed in October 2014, the energy requirements of the filtration unit were 0.87 kWh/m^3 of treated water. An additional 0.68 kWh/m^3 was estimated for the treatment of the backwashing water, which was sent back to the biological process. Considering also energy consumption for UV radiation, the overall energy requirements of the tertiary treatment was 1.68 kWh/m^3 (Table 3-4). This corresponds to about 0.20 €/m^3 (considering a price of 0.12 €/kWh).

Table 3-4 Energy requirements of the tertiary treatment.

Operation	Energy consumption (kWh/m^3)
Filtration (sand filter plus membranes)	0.86
Sand filter backwashing	0.57
Membranes backwashing	0.11

Operation	Energy consumption (kWh/m^3)
Membrane chemical cleaning	0.01
UV radiation	0.13

The investment cost of the equipment was 125,000 € (Table 3-5). This included pumps, piping, 8 membrane modules, whose price was 2,500 € each, and 6 UV lamps, whose price can be estimated as 150 € each. Considering a lifespan of 7 years for membranes and UV lamps and a lifespan of 20 years for the rest of the equipment, the investment cost per unit of treated water resulted in 0.23 €/m^3.

Table 3-5 Investment costs of the tertiary treatment.

Equipment	Capital cost (€)	Life span expected (y)
Sand filter, piping and pumps of the filtration unit	95,000	20
Ultrafiltration membranes (8 modules)	20,000	7
UV system (lamps excluded)	9,100	20
UV lamps (6)	900	7

An additional cost of labour of one hour per day was considered, as well. This corresponds to approximately 0.18 €/m^3. The cost of the reagents used for the periodical membrane chemical cleaning was lower than 0.01 €/m^3, so it was neglected.

The costs for fertilization is about 800 €/ha for tomato cultivation and 400 €/ha for broccoli cultivation. Fertilization costs can be divided as follows: 40% for potassium, 30% for nitrogen, and 30% for phosphorus. Considering the percentage of nutrients recovered (Figure 3.14), the saving of money for reducing the fertilization during tomato cultivation can be estimated as 240 €/ha and 280 €/ha for GW and TW, respectively. For broccoli, the corresponding savings would be about half of the values related to tomato. Since there are no relevant differences between the two water sources, this saving was not considered.

Therefore, the overall cost of the tertiary treatment resulted in 0.61 €/m^3 (0.23 and 0.38 €/m^3 for capital and operational costs, respectively). Fiordelisi does not pay any tax for discharge neither for the use of the conventional source of water used for irrigation. The only cost avoided by using reclaimed WW for irrigation is related to pumping from groundwater, which is approximately 0.1 €/m^3, considerably lower than the estimated tertiary treatment cost.

Even though this was a full-scale installation, the size of the plant can be considered quite small (12 m^3/h expected in the design, 4.2 m^3/h actually treated) and probably still optimizable (by substituting the sand filter with a DAF, for instance). Therefore, reasonably the treatment cost would decrease significantly for larger scale optimized plants.

3.7 Conclusions

Two years of monitoring of the WWTP operation at full-scale allowed for the identification of the main critical points of the treatment scheme used by the company Fiordelisi. The conventional WWTP scheme removed only partially the high content of oil present in the raw agro-industrial WW, so causing a low membrane productivity. Specific processes (Dissolved Air Flotation, for instance) should be introduced upstream in order to increase the productivity and consequently reduce the operating costs. In terms of quality, the tertiary treated WW complied with the limits for reuse in agriculture, except for suspended solids, due to a considerable re-growth in the reservoir. A different strategy should be used for storing the water (no static storage). The monitoring campaign showed also that changes in the industrial process that may not be considered relevant from the industrial point of view can considerably affect the quality of the reclaimed WW, as observed for the parameters SAR and surfactants in the last semester of the field tests.

The overall cost of the tertiary treatment added for the purpose of reusing the WW for irrigation was estimated as 0.61 €/ m^3. This value is higher than the price of the water actually paid by the company for the conventional source of water (well). However, the availability of the well water is limited and during part of the year (summer) the maximum volume of water available for pumping is easily reached. The additional water available from WW reclamation would decrease the water stress for crops cultivation.

In terms of nutrient recovery, the reuse of treated WW provided a considerable contribution as potassium and also a small, but still relevant, contribution as nitrogen. However, the conventional water sources had a higher content of nitrogen, due to previous over-fertilization in the area. For this reason, no yield enhancement was observed due to reuse of reclaimed WW.

After 2 years of irrigation with reclaimed WW, no relevant salt accumulation in the soil was observed.

4 Reclaimed wastewater for livestock production in Torre Marimon

4.1 Introduction and objectives

It is estimated that the livestock production sector consumes 8% of the global water supply, with most of that water being used for intensive production (Schlink, Nguyen and Viljoen 2010). Within the livestock production sector, water reuse possibilities span from irrigation of pastures and fodder to cleaning of farming facilities and livestock drinking. Since the irrigation of crops has been addressed already in the other case study (Chapter 3), the present case study will focus on the remaining water uses of the sector, such as cleaning of farming facilities and livestock drinking.

The main objectives of this demonstration activity are, a) compile information about current water management strategies applied in several livestock farms in terms of water consumption, water sources, water consuming activities, and b) to assess the susceptibility of using reclaimed water from a WWTP for livestock production in a farm raising calves.

Current water management strategies and water reuse possibilities, including water quality requirements and the legal framework, will be reported. There are different productivity systems to raise animals (intensive, semi-intensive, pasture, organic, etc.), what makes difficult to include all types of farms in the present report. We will focus on farms with an intensive system because are the ones with more complexity.

Reclaimed wastewater (RW) is a potentially valuable resource for the agricultural sector. However, as it has been mentioned in the introduction, it does not exist either European legislation or quality standards for the use of reclaimed water for livestock production. Thus, for this demonstration study, EPA Victoria's Guidelines and other scientific studies were considered when planning the reclamation water strategy to reuse the secondary effluent from the WWTP in Caldes de Montbui (Barcelona, Spain), which included a biological and settling secondary treatment, with additional treatment processes consisting of filtration and disinfection (EPA Victoria 2003, Schlink, Nguyen and Viljoen 2010). This reclaimed water will be used for livestock drinking purposes by dairy calves during the pre-weaning period (from 5 to 47 d of age) and for cleaning farming facilities in Torre Marimon (Caldes de Montbui, Barcelona, Spain).

In the case of DW, livestock need water of similar quality to that required by humans to ensure animal health and performance. However, these water quality requirements are poorly researched. The provision of DW aims at ensuring a good acceptability, no negative effects on animal performance (e.g., reduced milk production, weight loss, reduced feed consumption), as well as ensure water free of toxic compounds which could lead to animal toxicity problems and contaminate animal products intended for human consumption. This demonstration study will assess the susceptibility in terms of safety and acceptability of the RW achieved by the proposed treatment scheme. In the other hand, this demonstration will also assess the susceptibility of the RW for cleaning purposes of the farming facilities.

The specific objectives of the present demonstration study in Torre Marimon, in addition to the identification of current water management strategies in the farming sector, are the following:

- To evaluate cytotoxicity and inflammation markers of intestinal cells in contact with reclaimed water (section 4.8.1.)
- To evaluate short-term effects on performance, health, and metabolism of offering reclaimed water to dairy calves from 5 to 47 d of age (section 4.8.2.)
- To determine if calves show a preference for tap water versus reclaimed water (section 4.8.2.).
- To evaluate cleaning efficiency of using reclaimed water for cleaning livestock facilities (section 4.9.).

4.2 Examples of current water management strategies in livestock production

Several livestock farms in Spain were visited in order to compile information about their current water management strategies: water consumption, water entrance points, and water consuming activities. Farms raising different types of animals were visited: dairy, sheep, goat, beef, pig and poultry. We visited at least one of each type of farm and we determined their water management strategies. In some cases, water consumption was measured with water meters installed at the entrance of the farm, in others the amount of water used was estimated by measuring the time of cleaning the milking room and the hose flow, and in others water consumption was estimated by considering the physiological characteristics of the animals and reported water intakes. These water management strategies are described in the following sections.

4.2.1 Dairy farms

Four different dairy farms were visited, three in Girona Province (La Coromina, SAT Sant Mer and Mas Candell) and one in Huesca Province (Granja San José). They have among 100 to 2,500 lactating dairy cows, and their water management strategies are shown in Figure 4.1, Figure 4.2, Figure 4.3 and Figure 4.4 for La Coromina, SAT Sant Mer, Mas Candell and San José, respectively.

La Coromina farm is located in Joanetes (Girona, Catalonia), and has 380 cows, from which 215 are lactating. Figure 4.1 shows its water management strategy. This farm uses mainly water from the public net, arrives at high pressure and they need to regulate this pressure for drinking and cleaning the milk equipment. Furthermore, the water for cleaning the milk equipment is subjected to a softening treatment and heating. The rest is used under high pressure for cleaning the milking parlour and others. More than 70% of the water is used for drinking purposes, and the rest for cleaning different equipment. After using the water, it is transformed into milk, urine, faeces, and the rest turns into dirty water with a high organic load. WW goes to the manure pit, and it will be thrown to the fields as an organic fertiliser.

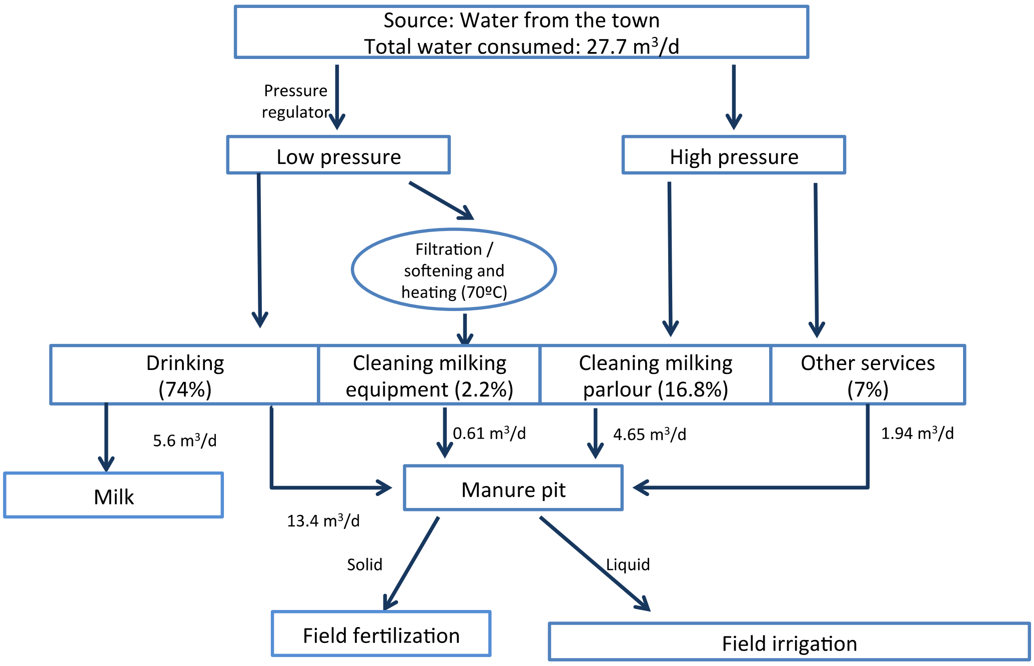


Figure 4.1 Water management strategy in La Coromina farm
In brackets the percentages of water used in the different farm applications

SAT Sant Mer farm is located in Sant Esteve de Guialbes (Girona, Catalonia), and they have 850 cows, from which 700 are lactating. Figure 4.2 presents its water management strategy. In this farm, groundwater is subjected to a chlorination treatment before its use. Similarly to La Coromina, 70% of the water is used for drinking purposes and the rest for cleaning several equipments or cooling the cows at summer time. Finally, water is transformed to milk, faeces, urine and dirty water with a high organic matter load. In this farm, the liquid fraction from the manure is separated and reused for yard flushing. The solid fraction is reused for cubicle bedding and the rest as organic fertiliser.

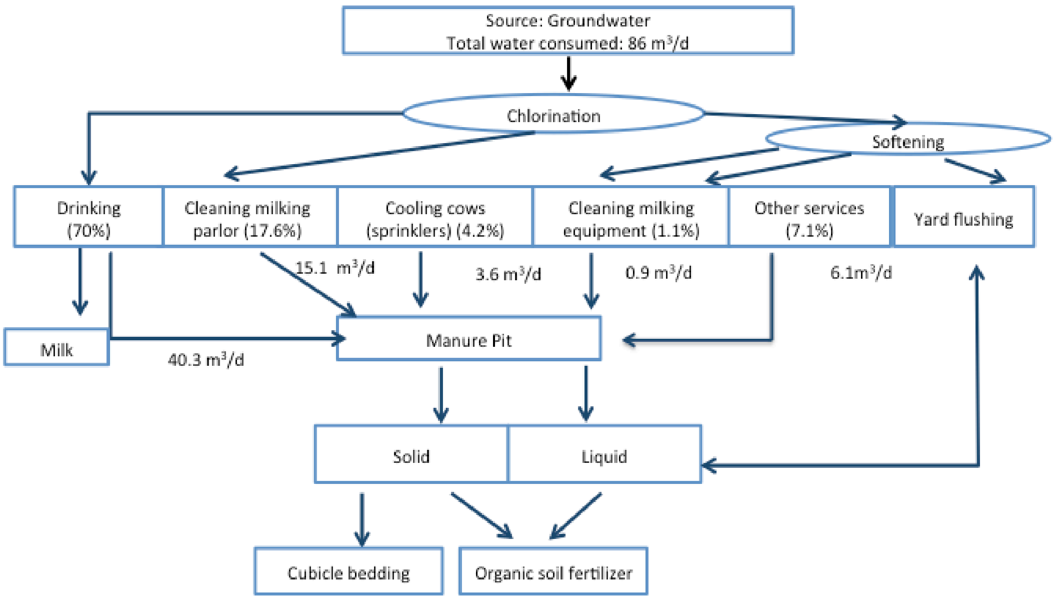


Figure 4.2 Water management strategy in SAT Sant Mer farm

Mas Candell farm is located in Camós (Girona, Catalonia), has 175 cows, from which 100 are lactating. In this case, water percentages used in each application could not be determined, but the overall water management strategy in this farm is presented in Figure 4.3. They use groundwater, and they need to do filtration and disinfection treatments before using it for drinking or cleaning purposes.

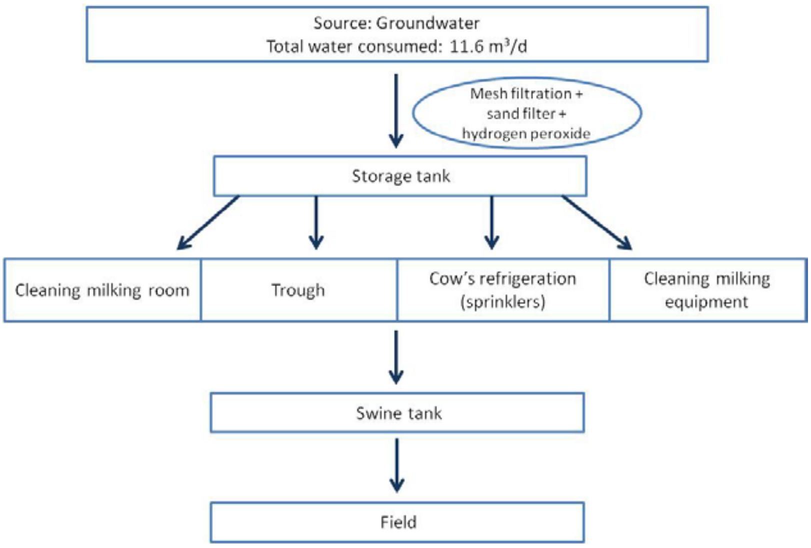


Figure 4.3 Water management strategy in Mas Candell farm

San José farm is located in Tamarite de Llitera (Huesca, Aragón), and they have 2,500 lactating cows. In this case, water percentages used in each application could not be determined, but the overall water management strategy is presented in Figure 4.4. They get the water from a dam, and the water used for drinking and cleaning the milking equipment is subjected to flocculation and chlorination treatments before use.

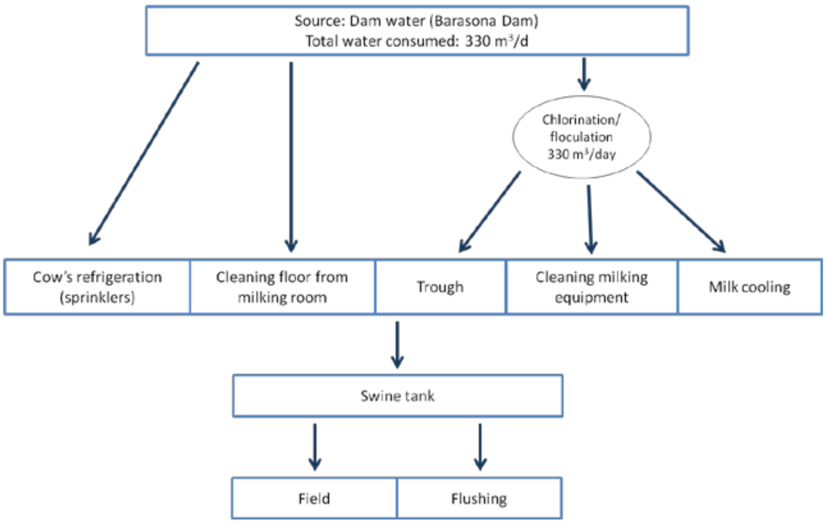


Figure 4.4 Water management strategy in San José farm

In summary, it could be concluded that the three main water uses in a dairy farm are drinking (70-75%), cleaning farming facilities and equipment (17-20%), and cooling the cows during summer (4%). Daily water consumptions in the four farms ranged from 66 to 165 L/animal, and the average use efficiency is approximately of 4 L of water to produce 1 kg of milk.

4.2.2 Beef farms

Different beef production farming activities can be considered. Some farms raise beef cattle (females) to produce beef calves (for meat production). In these farms, new-born calves are with the mothers during 6 months. Then, they are weaned and moved to barns where they are finished in an intensive feeding management (confined in pens and fed mainly with concentrate and straw). However, there are also farms that receive young male calves from the dairy industry (Holstein breed), in which calves are raised from one week old until they achieve the slaughter body weight (usually in less than 1 year).

Within the present project, we visited *Can Pau* farm located in Montgai, Lleida Province. This farm is characterised by raising Holstein bulls from the dairy industry. They have the capacity to allocate 900 young calves and 1,000 fattening bulls. Figure 4.5 shows its water management strategy.

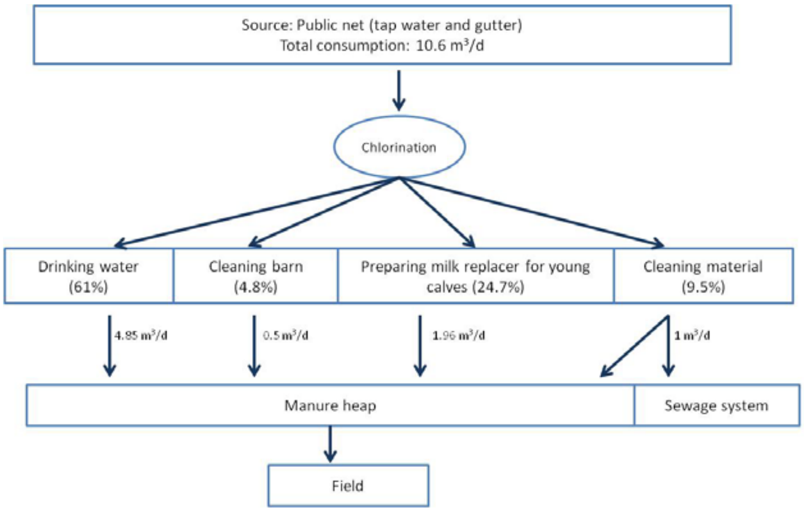


Figure 4.5 Water management strategy in Can Pau farm

As indicated in Figure 4.5, water uses can be divided in two main applications: water for drinking purposes and water for cleaning. In the literature is considered that young calves consume around 10 L/d (including the water needed to prepare milk replacer), and growing bulls on average 25 L/d. These amounts are an average used to estimate the amount of water used in Can Pau, since water requirements depend on body weight, type of feeding, and environmental conditions. Similarly to dairy farms, the most water demanding use is for livestock drinking (85% of the total water consumption).

Regarding their WW management, it should be mentioned that this mostly goes to the manure heap in form of urine and faeces, and the water used for cleaning goes to either the sewage system or the manure heap depending on the organic load.

4.2.3 Sheep and goat farms

Sheep and goat husbandry may be intended for either meat or milk production. In the present project, we report one strategy of each type of farms, a meat sheep farm located in Torre Marimon, Caldes de Montbui and a dairy goat farm located in Tona, both in Barcelona Province.

It should be mentioned that there exist a huge variety of sheep meat production systems. Roughly, these are extensive systems, based on pastures, and mainly located outdoors, or intensive systems, based on grain supplementation and most of the time located indoors. In contrast to an intensive system, the use of water in an extensive system is low because animals usually drink water from natural sources.

Torre Marimon is located in Caldes de Montbui, Barcelona Province and they host a meat sheep farm based on an intensive system. The overall water management strategy is presented in Figure 4.6, although water percentages used in each application could not be determined.

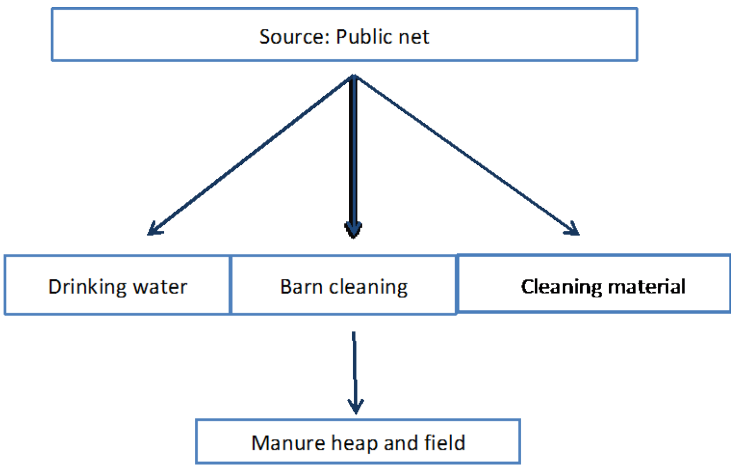


Figure 4.6 Water management strategy in Torre Marimon - Caldes de Montbui farm

El Garet is located in Tona, Barcelona Province, and hosts an intensive dairy goat farm. Figure 4.7 presents its water management strategy.

The amount of water consumed for goat drinking depends on the physiological state of the animal and body weight. The breed of this farm was Murciona-Granadina with a female adult body weight of 40-55 kg. During lactation, goats drink between 5-6 L/d, and pregnant goats 2-3 L/d. Considering that El Garet owns 300 lactating goats and 220 pregnant/young goats, the daily consumption of water for drinking in this farm is about 2000 L/d. In general, dairy goat water needs for drinking related to DM intake and milk yield are: 2-3 L/Kg DM intake plus 1 L/kg milk yield. Besides, water is needed for cleaning the milking machine (150 L/d), cleaning the milking tank (300 L/week), and cleaning the floor and walls of the milking parlour (350

L/week). As it can be observed in Figure 4.7, water uses can be divided in two main applications: drinking and cleaning, being the drinking use the most demanding (83% of the total water consumption).

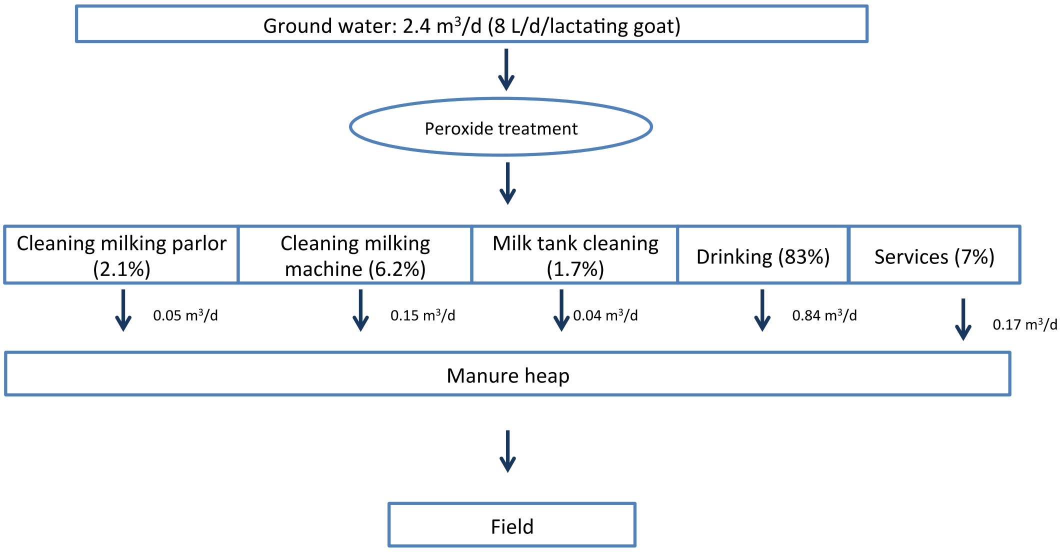


Figure 4.7 Water management strategy in El Garet farm

4.2.4 Pig farms

There are different kinds of pig farms depending on the type of animals the farmer is raising: sow or fattening farms. In a sow farm, females are raised to produce piglets. In a fattening farm, piglets are raised from 20 kg until slaughter body weight (around 100 kg of BW). In these farms pigs are in the fattening unit for a period of 13-15 weeks. In the following example we describe the case of a fattening farm.

Alfés farm is located in Lleida Province. Figure 4.8 presents the water management strategy in the farm.

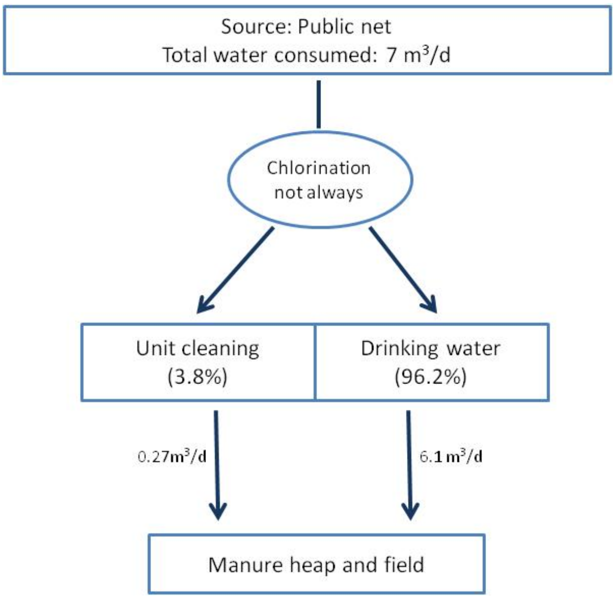


Figure 4.8 Water management strategy in Alfés farm

In this case, water intake was variable depending on the season. During the winter, 1,100 pigs consumed 670 m^3 (609 L/pig/fattening period), in contrast to the summer time, in which water consumption was 966 m^3 (878 L/pig/fattening period).

On the other hand, the cleaning consists on removing the faeces of the pens with water at high-pressure with disinfectants (in Alfés farm a machine consuming 30 L/min is used, and they spend 15 h to clean the entire barn). Then, 27,000 L of water were used to clean 1,036 m^2 of farm (26 L/m^2). The cleaning is done once all the animals are sent to the slaughterhouse (all-in all-out system). For this type of farm, again, livestock drinking is the most demanding use (>90% of the total water consumption).

4.2.5 Poultry farms

There are three types of poultry farms: those dedicated to grow chicken for meat production, those dedicated to raise laying hens to produce eggs, and those dedicated to reproduction and selection of poultry lines. In this report, the water cycle of a meat production farm is described. In a poultry meat production farm, the animals arrive with 1 day old and they are confined in a poultry unit until they reach the final BW with 30-35 days of life. Once the animals leave the unit, the entire barn is cleaned and disinfected waiting for the next batch of animals.

Roger farm is located in Empuriabrava, Girona Province, and it has the capacity for 20,000 chicken. Figure 4.9 presents its water management strategy. Similarly to the other water cycles, more than 80% of the water is used for drinking purposes and the rest for cleaning.

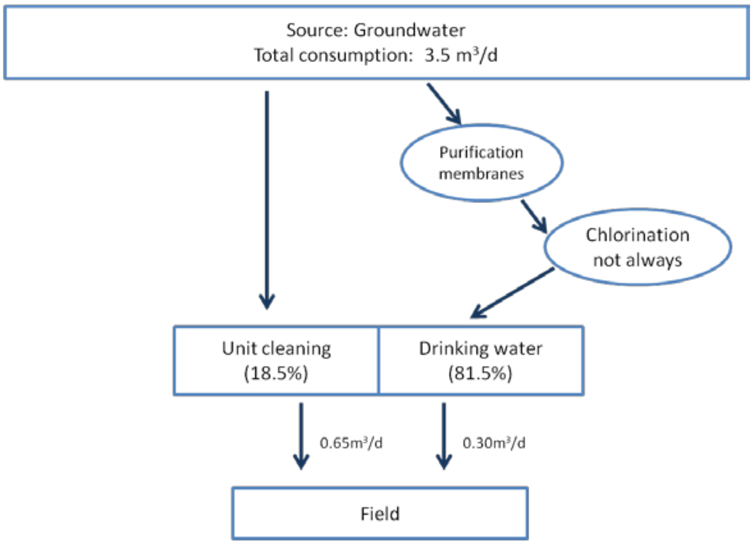


Figure 4.9 Water management strategy in Roger farm

Generally, in all livestock animals there are differences in water intake between summer and winter months. Figure 4.10 describes the evolution of individual DW in the chicken barn at four different periods of the year. From this figure, it can be concluded that approximately for a fattening cycle of 30 days, 4 L of DW per animal are needed. On the other hand, cleaning consists on a first physical removal of the litter, with a subsequent cleaning with high-pressure water and disinfectants. In our example, 19,132 L of water were used to clean 1,200 m^2 of farm (16 L/m^2).

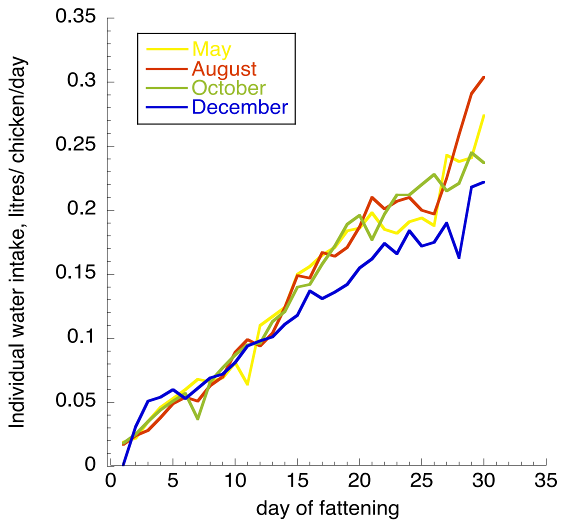


Figure 4.10 Evolution of daily water intake (l water/kg BW) throughout the fattening cycle of chickens
Data corresponds to four different periods of the year (May, August, October and December) in a poultry farm located in Empuriabrava (Girona, Spain).

Within the livestock sector, drinking is the use that requires the greatest amount of water. Water used for drinking purposes represents the 90% of the total water consumption in fattening pigs and broiler farms, 85% in beef farms, and 70-75% in dairy farms. The remaining other water uses, accounting for 10-30% of the total water consumption, are: cleaning of barns and yards, animals' refrigeration, milk cooling and cleaning of dairy machinery.

4.2.6 Water source quality

The livestock production sector currently uses different water sources and qualities. Four out of the eight visited farms use potable water from the public net, three use groundwater and one uses water from a dam. Depending on the source, water is treated, usually disinfected, to ensure good water quality. Among the farms visited, ground- and surface water are disinfected by chlorination or hydrogen peroxide before use, to guarantee that water meets quality criteria. Sometimes water is also softened.

In San José, they use water from a dam. Then water used for drinking purposes and for cleaning the milking equipment is chlorinated. Chlorinated water is analysed periodically in order to guarantee that water is potable. Roger and El Garet farms use disinfected groundwater. Table 4-1 shows the water physicochemical and biological characterization of water sources used in San José, Roger and El Garet.

Table 4-1 Water characterization in San José, Roger and El Garet farms

Parameter	Unit	San José (n=3)	Roger	El Garet
pH	upH	7.8±0.3	7.8	7.4
Conductivity	mS/cm	0.2±0.05	0.6	1.8
Turbidity	NTU	2.3±1.5	1.1	5.1
Ammonium	mg/L	0.01±0.0	-	-
Nitrate	mg/L	6±3	-	-
Nitrite	mg/L	0.02±0.01	-	-
N Kjeldhal	mg N/L	-	<1	<1
E. coli	cfu/100 mL	0	0	<5

Parameter	Unit	San José (n=3)	Roger	El Garet
Aerobes 22ºC	cfu/100 mL	0	-	-
C. perfringens	cfu/100 mL	0	-	-
P. aeruginosa	cfu/100 mL	n.d.	-	-
Enterococcus	cfu/100 mL	0	-	-
Coliforms	cfu/100 mL	0	24	<5

4.3 Water quality for livestock production

In this section, water quality requirements for the cleaning of farming facilities and livestock drinking, the most water demanding uses in farming activities as shown in 4.2, will be addressed. Water for livestock drinking and for cleaning the milking machinery and tanks requires high quality standards, but water for cleaning parlours could be of less quality.

4.3.1 Water quality for livestock drinking

There are no specific legal requirements concerning quality of livestock DW. In principle, livestock DW should be clean and not harmful for animals or indirectly affecting food safety. Some guidelines exist in few countries defining water requirements for livestock. Some examples are:

- Livestock water quality: a field guide for cattle, horse, poultry and swine in Canada, 2009 (Olkowski 2009)
- National water quality management strategy in Australia, 2000 (Chapter 4.3 for livestock drinking water) (Australian Government Dept. of the Environment 2000)
- Water quality guideline. Volume 5, agricultural use: livestock watering, South Africa, 1996 (Department of Water Affairs and Forestry 1996)
- Sustainable water for livestock, a project done in United Kingdom, 2010 (Department of Environment, Food and Rural Affairs 2010)
- Water requirements for livestock production: a global perspective, 2010 (Schlink, Nguyen and Viljoen 2010)

However, there is a lack of studies evaluating the impact of different compounds on livestock health or its bioaccumulation in animal tissues. In general, it is recommended to use water of similar quality to that required by humans. However, water quality requirements are poorly researched and usually defined by the presence or absence of certain substances, by taste, smell, turbidity and electrical conductivity. The health and productivity of livestock are affected by the quantities of various substances ingested as feed and as water. Accordingly, the amount of certain substances that can be present without harm in DW will depend in part on the amount of the same substances that are present in the feed, in addition to a number of other factors, which include the daily water requirements and the species, age and the physiological condition of the animals. In addition to the direct effects on the animal health, certain substances may contaminate animal products to the point where they will not be acceptable for human consumption. The variability of the factors that influence the acceptability of DW for livestock must be considered when using the following water quality criteria. Although the criteria provide a general guide to the quality of water that will be acceptable for most livestock, there may be cases where water of different quality than that indicated by the criteria will be required or accepted because of the nature, age or condition of species being raised or because of special rearing conditions or feed components. In such cases, or where the quality of an individual supply is in doubt, the quality should be assessed in relation to the specific uses.

The provision of adequate supplies of good quality DW for livestock is of major concern to farmers all over the world. The quantity of water required by livestock depends upon many factors including:

- Type of stock
- Environmental factors
- Water quality

- Type of feed
- Animal's physiological conditions

Factors typically considered in water quality evaluation include odour and taste (organoleptic properties), physical and chemical properties, presence of toxic compounds, concentrations of macro- and micro-mineral elements, and microbial contamination. Excess concentrations of some of these factors may have direct effects on the acceptability (palatability) of DW; whereas, others may affect the animal's digestive and physiological functions once consumed and absorbed or affect the animal performance as milk production or weight gain.

The main contaminants found in livestock DW are the following:

1. Total dissolved solids (TDS): high amounts of TDS generally are considered an unwanted characteristic, which is a measure of the total inorganic salts dissolved in water.

Table 4-2 provides guidelines for the use of waters containing varying amount of TDS in different classes of animals. Just a few controlled studies reported the effects of salinity on milk production, with conflicting conclusions. (Jaster, Schuh and Wegner 1978) found that milk production decreased when cows consumed water containing 2,500 ppm of NaCl added to tap water. (Challis, Zeinstra and Anderson 1987) found a trend for decreased milk yield when cows were given water with 4,300 ppm TDS during hot weather. However, (Bahman, Rooket and Topps 1993) reported that water with 3,500 ppm TDS did not affect milk production. (Solomon, et al. 1995) reported similar results to those of (Jaster, Schuh and Wegner 1978). It should be noted that all of these studies were carried out in semiarid, hot climates. No studies were found with lactating dairy cattle that tested the effects of TDS in cool weather or temperate climates.

In poultry, an increase in the salinity of water by adding NaCl increased the presence of defective shells in eggs (Zhang, Moreng and Balnave 1991, Pourreza, Nili and Edriss 2000), and decreased the hatchability of fertile eggs (Zhang, Moreng and Balnave 1991). In contrast, piglets offered a DW with more than 4,000 mg/L of TDS increased its water intake, feed intake and performance (Maenz, D. D., Patience, J.F. and Wolynetz, M.S. 1994).

Table 4-2 Guidelines for total dissolved solids
Adapted from the Canadian livestock quality water guide (Olkowski 2009)

Animal	Recommended TDS mg/L	Maximum	Tolerance Limits
Sheep	5,000	5,000-10,000	10,000-13,000
Beef cattle	4,000	4,000-5,000	5,000-10,000
Dairy cattle	2,500	2,500-4,000	4,000-7,000
Horses	4,000	4,000-6,000	6,000-7,000
Pigs	4,000	4,000-6,000	6,000-8,000
Poultry	2,000	2,000-3,000	3,000-4,000

2. Sulphur and sulphate: sulphur present as hydrogen sulphide (H_2S), imparting the rotten egg smell, is believed to affect water intake. High levels of sulphur can be more easily tolerated by monogastric than ruminants. In guidelines, it is cited 1,000 mg/L of sulphate as safe, but in ruminants it might cause health problems and this value should be reviewed. The main problem associated with an excess of sulphur is poliencephalomalacia, and levels 0.45% of dietary sulphur (including contents in DW and feed) caused this health problem in cattle (Kul, et al. 2006). Also, lower levels such as 0.2% of dietary sulphur may have a negative impact on performance (Zinn, et al. 1997).

3. Iron: besides the anions sulphate and chloride, iron in DW is probably the most frequent and important anti-quality consideration for dairy cattle. Whereas iron deficiency in adult cattle is rare because of abundant iron (Fe^{+3}, ferric iron) in feedstuffs, excessive total iron intake can be a problem because its interaction with essential nutrients such as Co, Cu, Mn, Se and Zn. Iron concentrations in DW greater than 0.3 ppm are considered a risk for human health, and are a concern for dairy cattle health and performance. However,

(Genther, O.N. and Beede, B.K. 2013) studied the preference and drinking behaviour of dairy cows and they tolerated concentrations of Fe up to 4 mg/L.

4. Manganese: this micromineral element is often considered along with iron when addressing water quality. However, specific information of the effects of manganese on dairy cattle is limited. In general, a concentration greater than 0.05 ppm is thought to affect water intake because of the off-taste it imparts.

5. Nitrate/nitrite: ruminant livestock is more susceptible to nitrate levels than monogastric livestock (pigs and poultry) because the rumen capacity to reduce nitrate to nitrite, in contrast to monogastric that rapidly eliminate nitrate in urine. The risk of nitrite is its capacity to convert hemoglobin to metahemoglobin leading a tissue deprivation of oxygen. Threshold concentrations of over 20 ppm N-NO_3^- should be of concern (Table 4-3). An excess of nitrate/nitrite in all species may cause production and reproduction performance impairment.

Table 4-3 Guidelines for nitrate concentration in drinking water for livestock.
Adapted and modified from (NRC, Nutrients and Toxic Substances in Water for Livestock and Poultry 1974)

N-Nitrate (mg/L)	Guidelines
0-10	Safe for consumption by ruminants
10-20	Generally safe in balanced diets with low nitrate feeds
20-40	Could be harmful if consumed over long periods of time
40-100	Cattle at risk and possible death losses
Over 101	Unsafe: possible death losses and should not be used as a source of water

6. Microorganisms in water: sources of recycled water can contain pathogenic microorganisms, and thus, pose a risk to the health of livestock. Generally, pathogenic microorganisms are bacteria, protozoa, viruses and helminths. However, many human pathogens are not of significant concern for livestock health. Anyway, there are some exceptions to this. For instance, the eggs of helminthic parasites (i.e., *Taenia saginata* and *Taenia solium*) can cause parasitic cysts in cattle and has the potential to affect human health (meat consumption as vector). *Mycobacterium paratuberculosis*, a bacterial pathogen, can infect cattle and cause the bovine Johne's disease. Other less severe illnesses can occur, the most common waterborne disease in animals is gastroenteritis following ingestion of pathogens (i.e., rotavirus, coronavirus, *Escherichia coli*, etc.), with symptoms such as diarrhea. The use of coliform counts is used as an indicator of fecal contamination, but this is not directly related to pathogenic bacteria.

7. Other chemicals in water: recycled water sources can contain a wide array of chemicals including inorganic and organic chemicals, pesticides, potential endocrine disruptors, pharmaceuticals and disinfection by-products. Some of these chemicals have been identified to have the potential to alter the normal endocrine function of humans and wildlife. Not much research has been conducted to assess the impacts of these chemicals on livestock health. Therefore, a potential human health impact would come from the consumption of meat or milk with high contents of these chemicals, which some of them have the potential for bioaccumulation in fat and tissues.

4.3.2 Water quality for cleaning of farming facilities

As mentioned before, water is mainly used for drinking purposes in livestock production. If there are no reference values for the quality of livestock DW, there is still less information for the quality of water needed for cleaning purposes. Cleaning of farming facilities includes cleaning of barns and yards, but also cleaning of milking equipment and feeders. Water quality for cleaning farm surfaces, which will not be in contact with human food, it is not critical. In fact, some dairy farm yards are cleaned with the liquid fraction of manure. However, water quality requirements for cleaning surfaces that will be in contact with human commodities (i.e., milk) have to achieve the potable water standards, to avoid foodborne risks.

The use of recycled water for cleaning purposes is possible, but some aspects have to be considered before its use:

- evaluate the risk of microbial contamination: consider the risk of biofilm formation with its consequences of algae growth, toxin production, and tubes clogging. Furthermore, if disinfectants are not used when cleaning, there is the risk of bacteria contamination on the cleaning surfaces
- evaluate the risk of pipe sealing due to its high salinity content

4.4 Legal framework concerning water reuse for livestock production

To our knowledge, the only country that specifically regulated the use of reclaimed water for livestock production is Australia. Countries in UE like Spain have regulated the use of reclaimed for some uses grouped in five categories: urban, agricultural, industrial, recreational and environmental, by the Royal Decree 1620/2007 (Boletín Oficial del Estado 2007). This explicitly prohibits the use of RW for human consumption, but RW for livestock consumption is not mentioned.

Australian legislation (State Government Victoria)

The Livestock Disease Control Act 1994, the Australian legislation concerning control of diseases, prohibits the use of water from sewerage systems for cattle production unless it has been treated to an acceptable standard. The use of reclaimed water must comply with Environment Protection Agency (EPA) guidelines (EPA Victoria 2003), otherwise formal EPA licensing of the reuse site is required.

Four classes of reclaimed water (A, B, C and D, Table 4-4) are recognised in *EPA Victoria's Guidelines for Environmental Management (GEM): Use of Reclaimed Water* (EPA Victoria 2003), based on a combination of prescribed treatment processes and microbiological criteria (primarily bacteria) for the treated water. The treatment grade affects the appropriateness of the water for use in livestock production and the necessary management controls (Table 4-5). In addition, reclaimed water intended for use with cattle must have had a treatment to remove helminth parasites. Such treatment may be based on lagooning for a period of 25 days or longer or an approved method of filtration such as sand or membrane filtration. Therefore, reclaimed water for use by cattle must have received effective helminth removal at the treatment plant, with water also having a minimum of secondary treatment and disinfection.

- CLASS A (including helminth treatment): may be used without restriction in cattle production
- CLASS B (including helminth treatment): may be safely used with observation of some withholding periods (Table 4-5).
- CLASS C (including helminth treatment): may be safely used with observation of some brief withholding periods (Table 4-5).
- CLASS D (not including helminth treatment): not recommended for use in livestock production.

Table 4-4 Classes of reclaimed water and corresponding standards for biological treatment and pathogen reduction in the Australian legislation.
Modified from (EPA Victoria 2003)

Water Class	Water quality objectives	Treatment processes
A	<10 E. Coli org/100 mL Turbidity < 2 NTU <10/5 mg/L BOD/SS pH 6-9 1 mg/L Cl_2 residual (or equivalent disinfection)	Tertiary and pathogen reduction with sufficient log reductions to achieve: < 10 *E. coli*/100 mL, < 1 helminth/L, < 1 protozoan/50 L, < 1 virus/50 L.
B	<100 E. Coli org/100 mL pH 6-9 <20/30 mg/L BOD/SS	Secondary and pathogen (including helminth reduction for cattle grazing) reduction

Water Class	Water quality objectives	Treatment processes
C	<1,000 E. Coli org/100 mL pH 6-9 <20/30 mg/L BOD/SS	Secondary and pathogen (including helminth reduction for cattle grazing use schemes) reduction
D	<10,000 E. Coli org/100 mL pH 6-9 <20/30 mg/L BOD/SS	Secondary – no pathogen reduction

Table 4-5 Opportunities for reclaimed water usage in the Australian legislation
Recommended use of differing classes of reclaimed water in cattle production

Type of water usage	Class of water (with helminth treatment)		
	Class A	Class B	Class C
Livestock drinking water	✔	✔	✘
Dairy shed washdown	✔	✔[1]	✘
Pasture or fodder crop application – lactating dairy cattle	✔	✔[2]	✔[3]
Pasture or fodder crop application – non-lactating cattle	✔	✔	✔[2]
Hay production for use on farm by cattle	✔	✔	✔
Pasture ensilage- Use on farm by cattle	✔	✔	✔[4]
Sale of fodder or crops	✔[5]	✔[5]	✔[5]

✔use is recommended (subject to comments, if any, below)

✘ not to be used for this purpose

1 but not for milking machinery
2 restrict access for 4 hours or until pasture/fodder crop is dry
3 restrict access to lactating cattle for 5 days after application
4 wait for 4 hours or until dry before ensiling
5 product to be labelled or sold with instruction 'fodder not for consumption by pigs'

NOTE: If the reclaimed water includes inputs from saleyard or slaughterhouse waste, additional measures to these should be undertaken to ensure that young cattle (under 12 months of age) are not exposed to the reclaimed water to minimise the risk of infection with Johne's disease.

United States

Generally in the United States, reclaimed water is not utilised for direct consumption by livestock; however de facto reuse often occurs. In this case, there exist guides to acceptable water quality for livestock consumption ((FAO 1985) (Raisbeck 2011), Table 4-6). Guidelines values are based on amounts of constituents normally found in surface and groundwater and are not necessarily the limits of animal tolerance. Additional sources of these substances may need to be considered along with DW, such as additional animal intake of these substances through feedstuffs.

Table 4-6 shows reference concentrations for evaluating water quality for livestock drinking.

Canada

Equally to United States, Canada has not regulated reuse practices for livestock production. However, for general farmstead uses of water, including drinking, other household uses, and handling of produce and milk, it is recommended that water of a quality meeting the "Drinking Water Objectives“. In general, raw waters should be free of impurities that are offensive to sight, smell, and taste. At point of use, they should be free of significant concentrations of substances and organisms harmful to public health and detrimental to the market value of agricultural products. Ontario and New Brunswick have their own water quality considerations for farming uses. In the same manner, Canada developed Water Quality Guidelines for the Protection of Agricultural Water Uses with recommended concentration limits of contaminants in livestock water.

Table 4-6 shows reference concentrations set by the ministries of Ontario and New Brunswick in Canada for evaluating water quality for livestock drinking.

Table 4-6 Guidelines for livestock drinking water in Canada and United States

Quality criteria	Recommended concentration[1](mg/L)	Upper limit guideline livestock[2](mg/L)	Limiting threshold livestock[3](mg/L)	Maximum Acceptable Drinking Water Parameters[4](mg/L)	Upper limit guideline livestock[5] (mg/L)
Aluminium	5	0.5	5	5	5
Arsenic	0.2	0.05	0.2	0.025	0.025
Beryllium	0.1	-	-	0.1	0.1
Boron	5	5	5	5	5
Cadmium	0.05	0.005	0.05	0.08	0.08
Chromium	1	0.1	1	0.05	0.05
Cobalt	1	1	1	1	1
Cooper	0.5	1	0.5	1.0 (cattle)	
Iron		0.2			
Fluorine	2.0	2	2	2	
Lead	0.1	0.015	0.1	0.1	0.1
Manganese	0.05	0.05	-	-	
Mercury	0.01	0.01	0.01	0.003	0.003
Molybdenum	0.3		-	0.5	0.5
Nickel		0.25	1	1	1
NO_3-N + NO_2-N	100	-	100	100	100
NO_2-N	10	-	10	10	10
Calcium		-	-	1000	1000
Sulphate		-		1000	1000
Radionuclides		-	meeting DW objectives		
Selenium	0.05	0.05	0.05	0.05	0.05
Uranium				0.2	0.2
Vanadium	0.1	0.1	0.1	0.1	0.1
Zinc	24	5	25	50	50
Salinity (TDS)		-	3000	3000	3000
Toxic algae		-	No heavy growth	-	
Aldicarb					0.011
Aldrin		-	0.001	-	
Atrazine					0.005
Bromacil					1.1
Bromoxynil					0.011
Chlordane		-	0.003	-	0.007
Chlorpyrifos					0.024
DDT		-	0.05	-	
1,2-Dichloroethane					0.005

1 (FAO 1985), and updated with (Raisbeck 2011)

2 (NRC, Nutrients and Toxic Substances in Water for Livestock and Poultry 1974); (NRC 1980); (EPA 1997)

3 (Ontario Ministry of the Environment 1984)

4 (Government of New Brunswick 2014)

5 (Canadian Council of Ministers of the Environment 1999)

Dieldrin		-	0.001	-	
Endrin		-	0.0005	-	
Heptachlor		-	0.0001	-	
Heptachlor epoxide		-	0.0001	-	
Lindane		-	0.005	-	0.004
Methoxychlor		-	1	-	
Trichloroethene					0.050
Toxaphene		-	0.005	-	
Carbamate and organo-phosphorus pesticides		-	0.1	-	

4.5 Site and treatment description

4.5.1 Site description

The demonstration study was performed by the Ruminant Production Department at Torre Marimon, Caldes de Montbui, Barcelona province (Figure 4.11). This research group is formed by 5 researchers (3 in Animal Science, and 2 in Biotechnology), 4 technicians (3 in Animal Science, and 1 in Biotechnology), and several PhD students. The research group has a biomolecular laboratory (Figure 4.12), equipped with a safety cabinet and incubators, and a barn, in which a maximum of 78 calves can be raised individually from 1 to 70 days old with an additional room to prepare the milk replacer (Figure 4.13). Torre Marimon is approximately at 500 m from the WWTP of Caldes de Montbui, and water from the secondary effluent can be transported easily to IRTA's facilities, in where the reclamation water treatment scheme was installed. Animal studies done in Torre Marimon facilities are performed according to recommendations of the Animal Care Committee of IRTA. Calves are bought in the commercial market, and raised in the research facilities until 70-90 days old, when animals are re-introduced again to the commercial market if there is not any health risk after the research done.

Figure 4.11 View of Torre Marimon

Figure 4.12 Biomolecular laboratory of the Ruminant Production Department of IRTA in Torre Marimon

Figure 4.13 General view of the calf barn in Torre Marimon

4.5.2 Secondary effluent wastewater characteristics

WW intended for reclamation is the effluent from the secondary treatment of the WWTP in Caldes de Montbui. This WWTP collects the WW effluents from the municipality, mainly including domestic WW but also industrial effluents. The water treatment in WWTP Caldes de Montbui includes a physicochemical primary treatment and biological and settling secondary treatments. This WW effluent has been characterized several times before and during the implementation of the treatment scheme. Water composition is shown in Table 4-7.

Table 4-7 Summary of the secondary treated WW composition from WWTP Caldes de Montbui
Data from years 2015-2016

Parameter	Units	Average ± deviation	n
TSS	mg/L	3.6 ± 4.8	7
Turbidity	NTU	3.4 ± 3.6	8
TOC	mgC/L	5.7 ± 0.6	5
BOD_5	mgO_2/l	7.2 ± 7.3	3
pH	upH	7.9 ± 0.5	8
Conductivity	µS/cm	1396 ± 188	8
TIC	mgC/L	47 ± 1.6	3
TDS	mg/L	919 ± 115	2
Chloride	mg/L	226 ± 46	3
Sulphate	mg/L	60 ± 13	3

Parameter	Units	Average ± deviation	n
Bromide	mg/L	0.5 ± 0.0	2
Nitrate	mg/L	16 ± 7.8	4
Nitrite	mg/L	0.8 ± 1.3	3
Phosphate	mg/L	7.0 ± 4.9	4
Ca^{2+}	mg/L	70 ± 0.8	2
Mg^{+}	mg/L	20 ± 1.6	2
Na^{+}	mg/L	182 ± 2.5	2
K^{+}	mg/L	20 ± 0.0	2
NH_4^{+}	mg/L	0.5 ± 0.8	4
Total Mn	µg/L	9.5 ± 3.5	2
Total Fe	µg/L	74 ± 19	2
Aerobic counts	CFU/mL	5.23E+05 ± 4.35E+05	5
Total coliforms	CFU/mL	30 ± 47	5
E. Coli	CFU/mL	2 ± 5	5
Clostridium perfringens	CFU/mL	n.d.	4
Giardia spp.			
Cryptosporidium spp.	oocysts/L	<8	4
Bovine polyomaviruses[6]	GC/L	n.d.	4
Human adenoviruses[7]	GC/L	7.0E+05 ± 7.4E+05	4
Somatic coliphages	PFU/mL	8 ± 10	4
Helminth eggs	egg/10L	<1	3
Taenia spp. eggs	egg/10L	<1	3

This secondary effluent from the WWTP Caldes de Montbui is characterised by low content of biodegradable organic carbon and suspended solids, low to moderate turbidity, slightly high in salt content and with a considerable load of microbiological contamination of faecal origin. This characterization fulfils water quality standards for an effluent after biological and settling secondary treatments.

4.5.3 Selection of water treatment technology

Water quality requirements for livestock production identified previously in this report (section 4.3.1), helped identifying the rationale of the reclamation treatment having into account feed WW characteristics. The water treatment technology should pursue the following objectives:

- Reduction of the microbiological load, in terms of pathogenic organisms for animals such as viruses, helminth eggs, some spore-forming bacteria, protozoa, among other pathogens.
- Reduction of water turbidity

EPA Victoria's Guidelines for Environment Management (GEM) establishes four classes of reclaimed water (A, B, C and D), based on a combination of prescribed treatment processes and microbiological criteria (Table 4-4). The treatment grade affects the appropriateness of the water for use in livestock production and the necessary management controls. Water class A may be safely used without restriction in cattle production and water class B may be safely used with observation of some brief withholding periods (Table 4-5). Both water classes must have had a treatment to remove helminth parasites, such as lagooning, sand

[6] analysed in the influent WW of WWTP Caldes de Montbui, before the primary treatment

[7] idem above

or membrane filtration. With these premises, the demonstration case study here proposed the following 2-step tertiary treatment: membrane filtration followed by UV disinfection.

Technical specifications of each water treatment proposed are detailed in the following sections.

4.5.3.1 Membrane filtration

The chosen membrane filtration process is an ultrafiltration (UF). UF membranes have pores in the range 2-100 nm, and need a considerable working pressure (1-5 bar). Rejection in UF is determined mainly by the size and shape of the solutes relative to the pore size in the membrane and where the transport of solvent (permeability) is directly proportional to the applied pressure. Components with a size above the pore size are retained by a sieving mechanism. The rationale of this UF treatment step is the elimination of suspended solids, colloids, part of the microbiological load (bacteria, protozoa and viruses), helminth eggs and spores from water.

For the implementation of the pilot treatment plant, a HyperFlux tubular module was used (Berghof Membrane Technology GmbH, Eningen, Germany). Table 4-8 describes the manufacturer' specifications for the module used.

Table 4-8 UF membrane specifications from the manufacturer

Supplier, module model, membrane model	Berghof, MO P1U(1m)_I8, 66.03 I8
Configuration	Cross-flow
Module material	PVC-U, resin
Membrane material	PVDF
Molecular weight cut-off (nm)	30
Number of channels	13
Inner tubes diameter (mm)	8
Module length (cm)	100
pH tolerance	2-10
Maximum module pressure (bar)	6
Maximum membrane pressure (bar)	8
Maximum module temperature (ºC)	40
Maximum membrane temperature (ºC)	60
Membrane filtration area (m^2)	0.3267
Filtration transversal area (cm^2)	6.53
Design flux ($l/m^2{\cdot}h$)	>750

4.5.3.2 Ultraviolet disinfection

The rationale of a disinfection treatment is the reduction of active microorganisms in water. There are several disinfection technologies mainly based on either chemical or physical mechanisms. Disinfection technologies include chlorination, ozonation, membrane filtration, UV radiation, among others. In the present demonstration study, the chosen disinfection treatment was UV because has been shown that is an effective bactericidal and virucidal agent and, in contrast to chlorination, does not form toxic disinfection by-products (Hijnen, W.A.M., Beerendonk, E.F. and Medema, G.J. 2006). It has been reported that some reproductive problems in farm animals may be associated with adverse effects of disinfection by-products.

The effectiveness of UV disinfection is based on the UV dose to which the microorganisms are exposed. The UV dose D, in mJ/cm^2, is defined as the UV intensity, in mW/cm^2, times the exposure time, in seconds. The recommended D for reclaimed water systems, concretely for a membrane filtration effluent, is of 80 mJ/cm^2 (Asano, T., et al. 2007). This selected D is intended to provide 4 log of poliovirus inactivation with a factor of safety of about 2.

For the implementation of the pilot treatment plant in Torre Marimon, a STERILUX MINI-1000 was used (CEASA, Castellví de Rosanes, Barcelona). Table 4-9 describes the manufacturer' specifications for the UV module used.

Table 4-9 UV specifications from the manufacturer

Supplier, UV model	CEASA, Sterilux Mini-1000 230V
Power consumption (W)	23
Lamp voltage (V)	2 x 51
Efficiency (%)	99
Power lamp (W)	TUV 21
Temperature (ºC)	5-30
Pressure (bar)	1-6
Lamp length (mm)	302
Lamp diameter (mm)	16
Water flow (L/h)	1000
UV Dose (mJ/cm^2)	25

Water flow will be regulated at 300 L/h in order to get a targeted D of 80 mJ/cm^2, following recommendations of Asano and collaborators, by using the UV equipment specified in Table 4-9.

4.5.4 Installation, operation and maintenance of the water treatment demonstration plant

The demonstration pilot is installed in two different sites within the Torre Marimon complex. The membrane filtration module is installed close to the experiment field plots in Torre Marimon, and the UV disinfection module next to the farm (Torre Marimon 1 and 2, respectively, Figure 4.14).

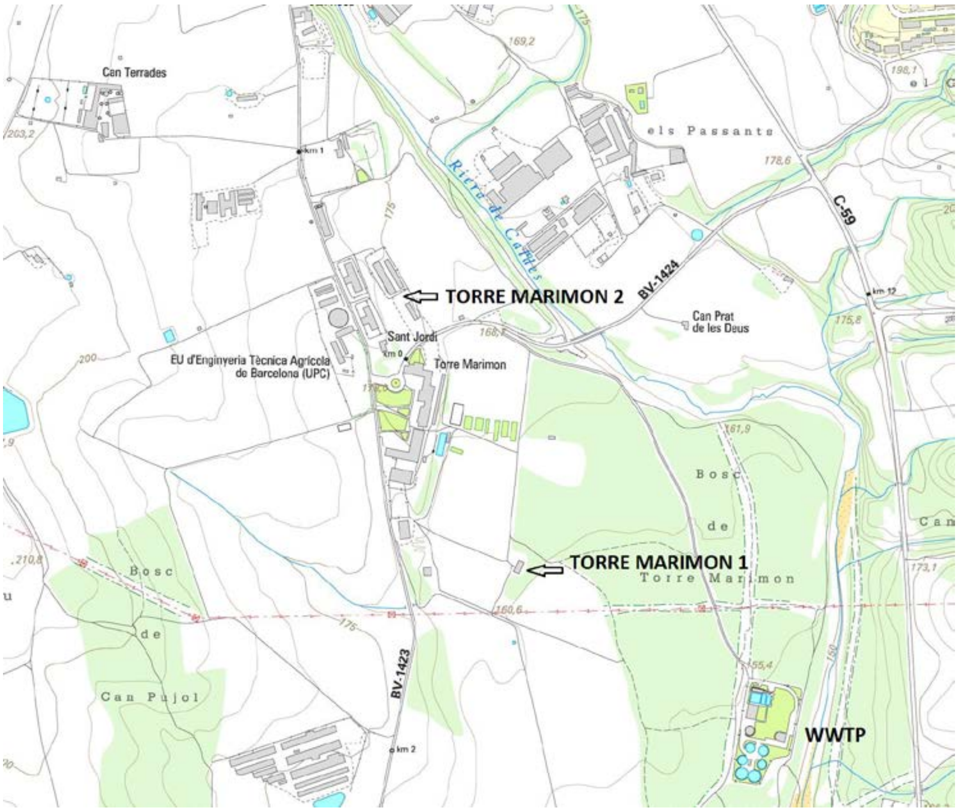


Figure 4.14 Map of Torre Marimon facilities and WWTP in Caldes de Montbui

The schematic diagram of the overall set-up for the treatment pilot plant is shown in Figure 4.15.

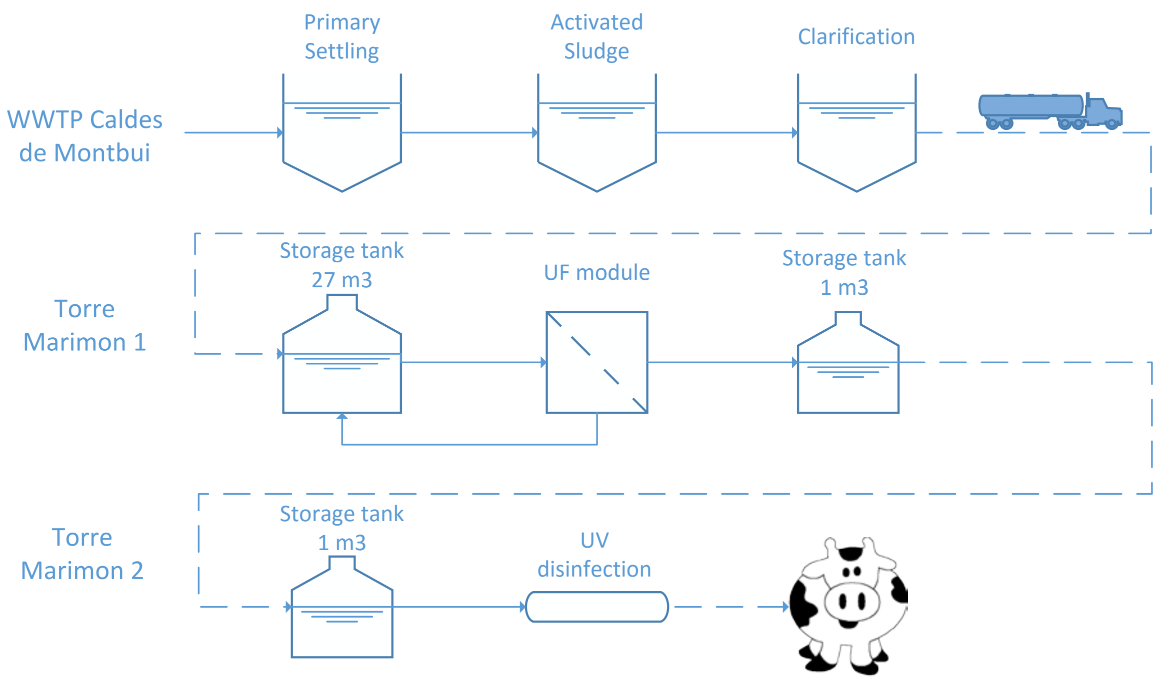


Figure 4.15 Schematic diagram of the water treatment pilot in Torre Marimon

The effluent resulting from the secondary treatment in the WWTP in Caldes de Montbui is transported by a tanker truck to a 27 m^3 closed tank in the facilities of Torre Marimon. The UF module is attached to this tank and operates in crossflow mode during the necessary daily period to have around 300L/d of permeate. All piping is made of plastics (PVC) black tubing to avoid algae growth. To avoid the heating of the raw water pump, the system works intermittently and starts/stops automatically following a set program. The installation of the UF consists of some programmable logic controllers with programs and security sensors (i.e., water level). In the input and output of the UF module there is a manometer to check the working pressure and pressure drop during filtration. Permeate water is diverted to a black storage tank of 1000L (Figure 4.15). UF and UV modules are separated by half km distance (Figure 4.14). Every approximately three days, the filled tank of 1000 L is exchanged with the one that feeds the UV module. The UV module operates daily around one hour at a flow rate of 300L/h in order to get around 200-300 L/d of reclaimed water, which are the water requirements for the preparation of milk replacers and drinking for 10 calves (number of animals foreseen in the in vivo experiment (section 4.8.2) (Figure 4.15).

Both UF and UV modules are operated according to the operating conditions specified in their respective operating manuals (Table 4-8 and Table 4-9, respectively). Cleaning and preservation instructions are followed for the maintenance of each module. Briefly, after the installation of the UF module, the storage solution was washed from the module with tap water first and then with the target water influent. When not in use, the membrane module is filled with a storage solution consisting in distilled water with sodium metabisulfite 1% in solution, and kept closed. During periods of short term storage (less than 2 weeks), the membrane is first washed with chlorinated water (0.2% hypochlorite in solution) and then rinsed with tap water until neutral pH (pH<8). The system is then filled with tap water (pH = 8.0) and kept closed to prevent drying. In the present demonstration study, the UF unit is disconnected and transported to the laboratory when cleaning. Operation and performance of a UF membrane is greatly influenced by raw water quality variations. Turbidity as well as TOC of the raw water are water quality parameters of major importance that drive operation mode and membrane flux. Therefore, since the present demonstration was working in crossflow mode, to prevent membrane fouling the raw water tank was refilled with new water when water level is below the 50% of tank's capacity. The permeate flux is monitored on a daily basis to adjust the working program and notice about membrane fouling.

Before the installation, the UV module was cleaned with a commercial solution following the manufacturer' instructions. Before operation the lamp needed 1 min to heat up. The first 2 L of disinfected water were always discarded. The water flux was checked on a daily basis to ensure that water receives the desired UV dose D.

Figure 4.16 UF module installation in Torre Marimon 1

Figure 4.17 UV module installation in Torre Marimon 2

4.6 Hazard identification and reference indicators

Untreated sewage potentially contains a range of agents that may pose risks to the health of livestock, including chemicals and pathogenic microorganisms. Regarding chemical hazards, similar water quality to that required by humans should be provided to livestock. A potential hazard for human health may come from the consumption of meat or milk with high contents of toxic chemicals, which some of them have the potential to be bioaccumulated in the animal products. Publications differ significantly on the guidelines for upper limit concentrations for livestock, due, in part, to the limited amount of information available on the impact of toxic substances in water on the animals (Table 4-6). Throughout the duration of the demonstration study in Torre Marimon, a wide variety of chemical substances (including soluble salts, metals and organic substances) were determined in the reclaimed water and compared to reported thresholds in DW guidelines for humans and livestock (EPA 1997, Government of New Brunswick 2014, NRC, Mineral Tolerance of Domestic Animals 1980, Canadian Council of Ministers of the Environment 1999).

Regarding microbial hazards, many human pathogens are not of significant concern for livestock health. There are some exceptions to this, such as the eggs of the helminthic parasites *Taenia saginata* and *Taenia solium*, which may be present in sewage and other source waters contaminated with human faeces. Both tapeworms may cause severe diseases to animals but also to humans consuming contaminated meat. Cattle exposed to eggs of *T. Saginata* may develop parasitic cysts of beef measles. Eggs of *T. Solium* can infect pigs, causing cysticercus, and cause a severe neurological disease in humans. In the Australian guidelines for Water Reuse (EPA Victoria 2003), the use of reclaimed water is prohibited for pig drinking and fodder. Another concern for livestock health is related to Johne's disease, caused by the pathogenic bacteria *Mycobacterium paratuberculosis*, which may be present in waste containing animal faeces such as those derived from abattoirs or livestock saleyards. Bovine's Johne's disease is a fatal wasting disease of cattle and animals with less than 12 months of age are more susceptible to this infection. As a preventive measure, Australian guidelines ban the use of recycled water for cattle under 12 months if the source of water contains WW from abattoirs or saleyards (Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council and the Australian Health Ministers' Conference 2006).

Another pathogen of emerging concern, linked to faecal-oral transmission in animals and with potential to infect humans through ingestion of contaminated meat, is the hepatitis E virus (HEV). A considerable amount of recent scientific reports indicate that HEV infection may be a porcine zoonosis, as pigs are commonly infected, in developed countries (Dalton, H.R., et al. 2008, Pavio, Meng, X-J. and Doceul, V. 2015). This case of zoonosis jointly with *T. Solium* lead to dismiss the possibility to perform the present demonstration study with pigs.

Potential pathogens for animals include viruses (e.g., animal-specific enteric viruses), bacteria (e.g., *E. Coli, Salmonella spp., Campylobacter spp.*), protozoa (e.g., Giardia and Cryptosporidium species) and helminths (e.g., eggs of Taenia tapeworms). Table 4-10 lists all microbial hazards considered in the present demonstration study and the indicators analysed throughout the duration of the experimental demonstration.

Table 4-10 Microbial hazard identification and reference indicators

Hazard	Indicators
Viruses	Bovine polyomaviruses as indicator of bovine fecal contamination Human adenoviruses as indicator of the UV disinfection performance Somatic coliphages
Bacteria	Total coliforms *E. Coli* *Clostridium perfringens*
Protozoa	*Giardia* spp. *Cryptosporidium spp.*
Parasitic helminths	Helminth eggs

Hazard	Indicators
	Taenia spp. Eggs

4.7 Validation of the treatment performance and reclaimed water quality

4.7.1 Initial validation of the water treatment

Before the installation of the water treatment in Torre Marimon, both treatment units were validated at CTM laboratories. A study at bench-scale is necessary to test how suitable a given raw water is for direct UF without pretreatment and to test operation modes and membrane fluxes.

The UF module was validated first with deionized water and afterwards with water from the secondary effluent of WWTP Caldes de Montbui, in order to update membrane fluxes with real water. Figure 4.18 shows the obtained transmembrane fluxes (LMH) under different transmembrane pressures (TMP).

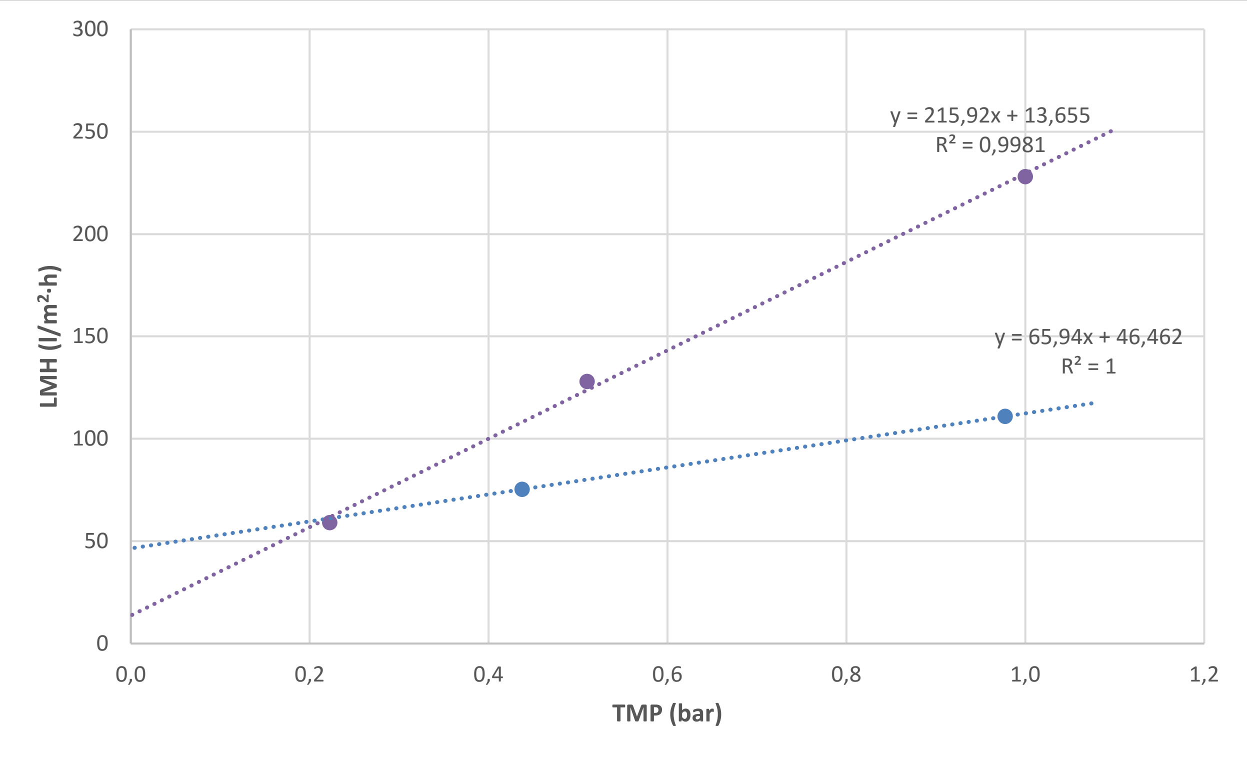


Figure 4.18 Validation of the UF membrane with distilled water (purple) and raw water (blue)

Permeability for raw water ranged between 14 and 40 L/h. This permeability was enough to achieve a daily water volume of around 200-300L. Furthermore, raw water quality is suitable for direct UF and no pre-treatment will be required (Table 4-7).

The UV module was validated by measuring the microbiological load in the influent and effluent at different D. Figure 4.19 shows aerobic plate counts and *E. coli* in the influent and at the effluent under UV doses of 53, 84 and 174 mJ/cm^2. All UV D achieved 4 log of *E. coli* inactivation, and 53, 84 and 174 mJ/cm^2 achieved reductions of 3, 3 and 4 log, respectively, of the total aerobic counts. For all doses, obtained water quality is suitable for both Class A and Class B according to the microbiological objectives set in the Australian water reuse guidelines (Table 4-4). During the present demonstration experiment, a UV D of 80 mJ/cm^2 was set. This D allows the inactivation of all pathogenic bacteria, bacterial spores, and protozoa and most of the viruses identified in section 4.6 according to scientific literature (Hijnen, W.A.M., Beerendonk, E.F. and Medema, G.J. 2006). This UV D might not be enough to inactivate adenoviruses, one of the most UV resistant organisms, thus the monitoring program of the present study will include the determination of adenoviruses of feed WW and after the UV step.

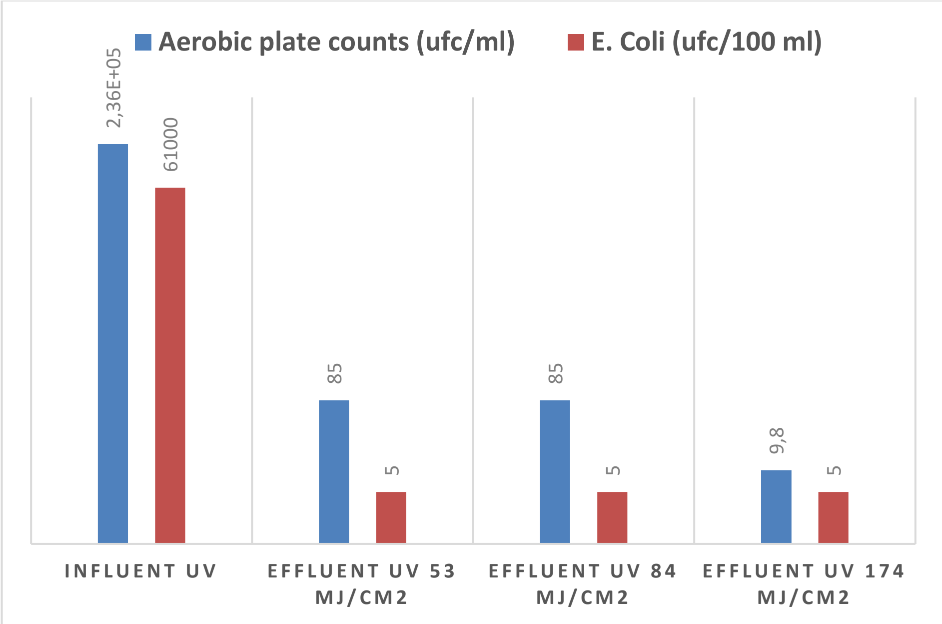


Figure 4.19 Microbiological load reduction under different UV D

The overall tertiary treatment performance was evaluated previously to its installation in Torre Marimon by the determination of some chemical and microbial parameters after each step of the treatment scheme. Table 4-11 shows water characterisation parameters of feed water, the UF effluent, the effluent after UF + the disinfection step (both chlorination and UV were tested), and effluent after UV without UF. As expected, dissolved ions, pH and conductivity are not affected by any of the treatments. UF leads to a reduction of turbidity, even though afterwards there is a slight increase on turbidity after both disinfection steps. Microbiological organisms are partially eliminated during the UF process and totally eliminated in both disinfection treatments.

Table 4-11 Initial evaluation of water quality under the different steps of the tertiary treatment

Parameter	Units	Secondary treated WW	UF	UF+ClO^-	UF+UV	UV
pH	upH	7.5	7.3	7.5	7.5	7.5
Conductivity	µS/cm	980	943	951	971	985
Turbidity	NTU	2.5	0.6	1.3	1.1	2.3
COD	mgO_2/l	< 50	< 50	< 50	< 50	< 50
NH_4^+	mg/L	< 0.1	< 0.1	< 0.1	< 0.1	< 0.1
Chloride	mg/L	173	165	167	174	173
Sulphate	mg/L	45	42	42	43	43
Nitrate	mg/L	19	18	17	17	17
Phosphate	mg/L	1.5	1.6	1.2	1.2	1.2
Aerobic counts	CFU/mL	2.05E+05	<150	0	1	4
E. Coli	CFU/mL	7600	<5	<5	<5	<5

4.7.2 Monitoring results of the reclaimed wastewater quality throughout the demonstration study

The water treatment demonstration plant in Torre Marimon was installed around mid-March 2016, was operated approximately one month for preliminary tests and then started its uninterruptedly performance on 9th May 2016 for a period of 3 months, in order to perform all demonstration studies (sections 4.8 and 4.9). Throughout this period, up to 5 sampling campaigns were performed to assess water quality, of the effluent but also of the secondary treated wastewater (SW) and the effluents after each treatment step. Microbiological and physicochemical parameters, as well as organic and inorganic toxic constituents, were determined in SW and water outputs.

Figure 4.20 shows the physicochemical quality (i.e., turbidity in NTU, TOC and TSS in mg/L) of SW, UF water output (UF), water stored after the UF treatment (Tank) and the water effluent after the overall treatment (UF+UV or reclaimed wastewater, RW). TSS were very low and close to the detection limit for all samples and effluents except for a sample after the UF treatment that we suspect it was contaminated by algae present in the permeate tubing. Regarding TOC and turbidity, a similar pattern was observed for both parameters. After the UF treatment, turbidity and TOC were reduced, in agreement to the expected UF performance (e.g., 70% reduction in turbidity). Turbidity significantly decreased after the UF ($P<0.05$). However, during water storage previously to the UV treatment, TOC was again increased likely due to a bacterial re-growth. Filtered water was kept in this storage tank during approximately 2 or 3 days. The UV disinfection did not affect the content of TOC but turbidity slightly increased, even though not significantly, probably because the UV treatment might favour the solubilisation of suspended solids. In conclusion, the overall treatment reduced significantly only the parameter of turbidity. TSS was already very low in the initial effluent and TOC, even though is reduced after the UF (from 5.7 to 4.6 mg/L), increases again during the storage (4.7 mg/L) and after the UV disinfection (4.9 mg/L).

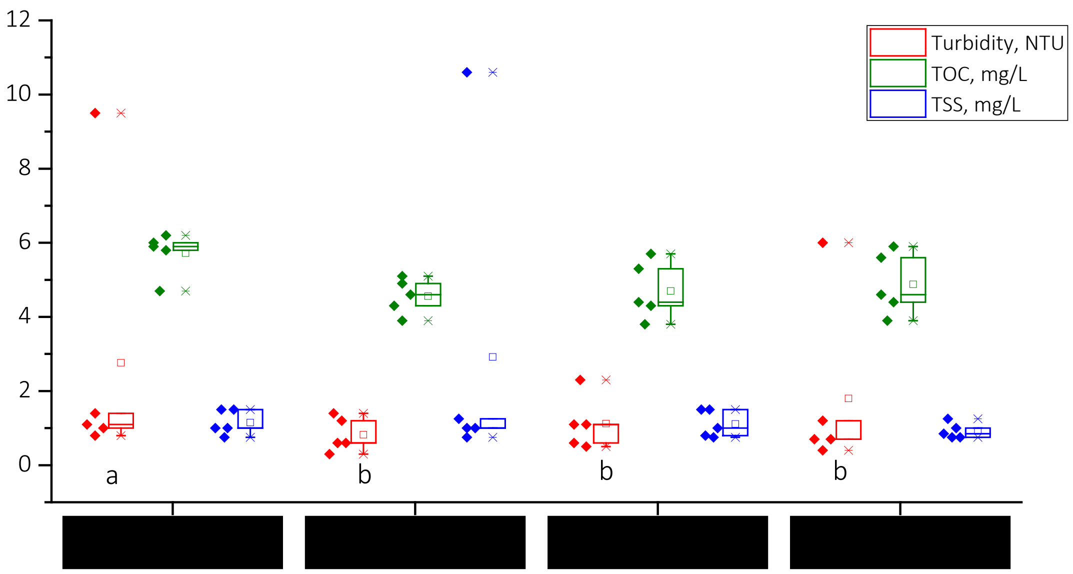


Figure 4.20 Physicochemical quality of reclaimed wastewater at each step of the water treatment scheme.
Values or boxes with different letters differ among them.

Figure 4.21 shows the microbiological quality of the same water influent and effluents shown in Figure 4.20. Aerobic counts, total coliforms and *E. coli* were analysed in the 5 samplings campaigns at each step of the treatment scheme. Not much variation in the microbiological load was observed during the UF process. However, the microbiological load increased considerably during the 2/3 days when water was stored before the UV disinfection, for all studied bacteria. However, the UV treatment achieved 5.5 and 3 log reductions of total aerobic counts and total coliforms, respectively. Analysis of variance was performed for aerobic counts with log-transformed data.

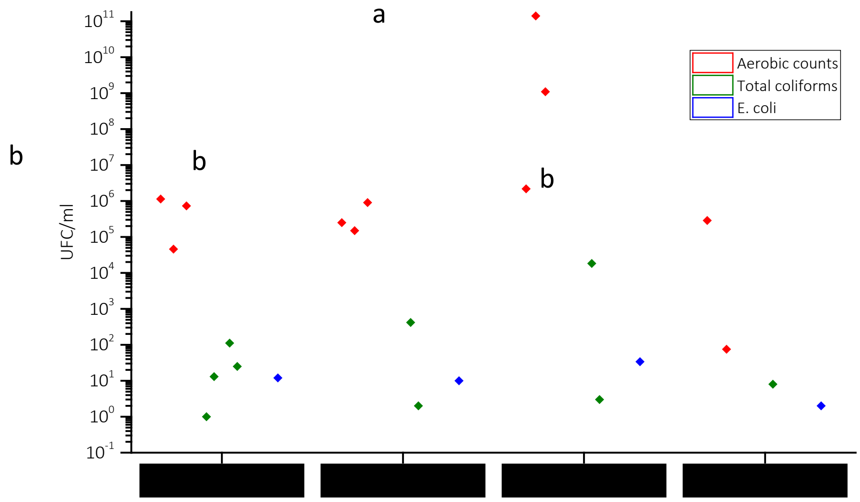


Figure 4.21 Microbiological quality of reclaimed wastewater at each step of the water treatment scheme.
Values with different letters differ among them.

Water from troughs was renewed daily. During the demonstration study with the animals, water samples from their troughs, those containing RW and tap DW, were taken for analysis. As it can be observed in Figure 4.22, after one day, water in troughs is dirty, in terms of turbidity, for all water sources. Also the content of total coliforms and *E. coli* increases in the troughs after one day (Figure 4.23). The same animals dirtied the water with ambient bacteria by approaching their mouths, or depending on the trough design, the same animal's faeces could contaminate the water. Figure 4.22 shows turbidity and significant differences between the different reclaimed effluents compared to tap DW. Tap DW had significantly lower turbidity than the UF+UV effluent (RW)($P<0.05$).

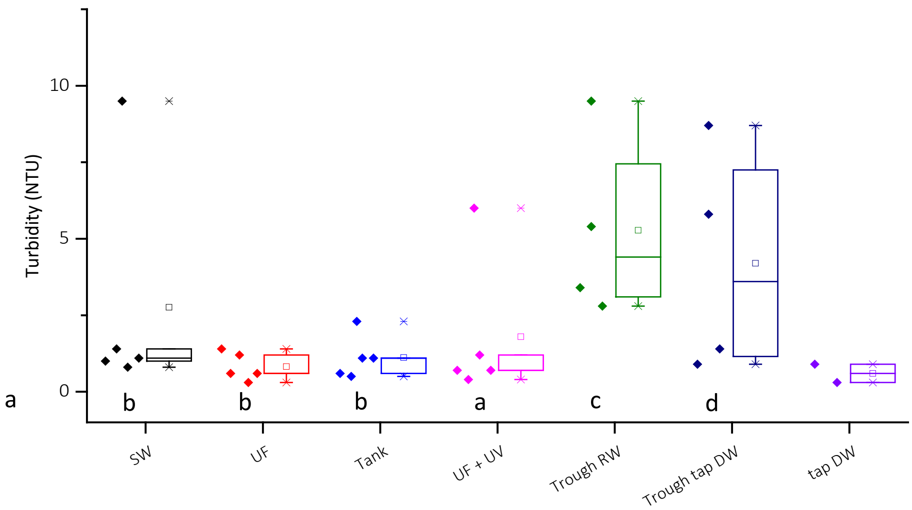


Figure 4.22 Turbidity of reclaimed wastewater at each step of the water treatment scheme with comparison to tap drinking water.
Values or boxes with different letters differ among them.

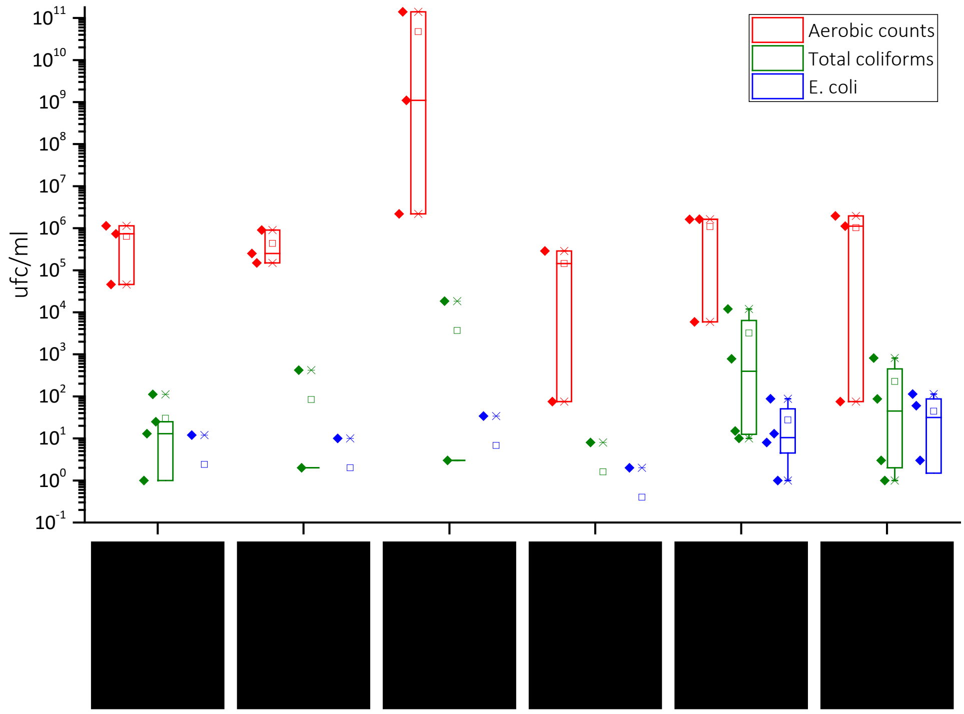


Figure 4.23 Microbiological quality of reclaimed wastewater at each step of the water treatment scheme

In all demonstration experiments, reclaimed wastewater (RW=UF+UV) was compared to tap DW from Torre Marimon in Caldes de Montbui. Thus, physicochemical parameters were also analysed in tap DW for comparison. The physicochemical characterization of tap DW and the obtained RW are shown in Table 4-12 jointly with water quality objectives set by different guidelines for livestock drinking as detailed in 4.3.1. RW quality achieved by the present demonstrative treatment fulfils most of the water quality objectives. Only certain dissolved salts exceed these objectives, such as K^+, Na^+, PO_4, and Cl^-. In any case, the overall TDS content does not exceed the recommended values shown in Table 4-2. Effluents from a WWTP usually have high levels of salinity and conductivity and this feature was expected for the RW in the present study. It is not clear from the literature how these constituents associated with salinity may affect livestock health and performance. Severe health problems associated to high levels of salt intake for prolonged periods of time are reported in the literature (Olkowski 2009). However, the present study does not present so high levels of salt, at the most, Na^+ and Cl^- concentrations may reduce water palatability and lower water intake. Regarding to the microbiological quality, obtained RW does not present any potential microbial risk. As it can be observed in Table 4-13, all reference microbiological indicators were found below threshold limits (e.g., *E. coli* below 100 org/100 mL and absence of helminth eggs). Microbial log reductions achieved by the overall water treatment scheme are not large in contrast to other reported studies. Loss of water quality occurred in some steps of the treatment scheme because of the fact that the treatment did not work continuously otherwise we had storage tanks where microbial re-growth happened. Moreover, we suspect about a possible die-off of the faecal indicator *E. coli* in the tank previously to the tertiary treatment, because *E. coli* levels in this tank are well below the secondary effluents from the WWTP reported in Table 4-7. Levels of toxic inorganic and organic constituents in RW were low and fulfil water guidelines for both human and livestock DW (Table 4-14).

Table 4-12 Physicochemical water quality of RW *versus* tap DW during the demonstration study

Parameter	Units	RW (average±stdev)	Tap DW (average±stdev)	Water quality objective	Reference
TSS	mg/L	<1.5	<1.5	<5 Class A <30 Class B	(EPA Victoria 2003)
Turbidity	NTU	1.8 ± 2.4	0.6 ± 0.4	<2 Class A No limit Class B	(EPA Victoria 2003)
TOC	mgC/L	4.9 ± 0.8	2.0 ± 0.0	<20 mgO_2/ml BOD Class B	(EPA Victoria 2003)
pH	upH	8.4 ± 0.1	7.8 ± 0.1	6-9	(EPA Victoria 2003)
Conductivity	µS/cm	1437 ± 58	570 ± 95	-	-
TIC	mgC/L	46.9	40.0 ± 6.6	-	-
TDS	mg/L	756	384 ± 189	1000	(NRC, Nutrients and Toxic Substances in Water for Livestock and Poultry 1974)
Chloride	mg/L	269 ± 9	36 ± 4.1	100	(Schlink, Nguyen and Viljoen 2010)
Sulphate	mg/L	56.7 ± 0.5	45.3 ± 8.2	150	(Schlink, Nguyen and Viljoen 2010)
Bromide	mg/L	0.5 ± 0.5	0.6 ± 0.6	-	-
Nitrate	mg/L	16.4 ± 2.0	9.9 ± 0.7	88	(Schlink, Nguyen and Viljoen 2010)
Nitrite	mg/L	<0.2	<0.2	33	(Government of New Brunswick 2014)
Phosphate	mg/L	4.5 ± 1.1	<0.2	2.15	(Schlink, Nguyen and Viljoen 2010)
Ca^{2+}	mg/L	74.8 ± 6.0	57.8 ± 7.4	100	(Schlink, Nguyen and Viljoen 2010)
Mg^{+}	mg/L	19.5 ± 0.8	18.5 ± 5.3	50	(Schlink, Nguyen and Viljoen 2010)
Na^{+}	mg/L	188 ± 3	20.3 ± 0.1	50	(Schlink, Nguyen and Viljoen 2010)
K^{+}	mg/L	20.2 ± 0.1	2.8 ± 0.2	20	(Schlink, Nguyen and Viljoen 2010)
NH_4^{+}	mg/L	<0.2	<0.2	-	-
Total Mn	µg/L	11 ± 11	2.3 ± 0.9	50	(Schlink, Nguyen and Viljoen 2010)
Total Fe	µg/L	15	5.7 ± 0.4	200	(Schlink, Nguyen and Viljoen 2010)

Table 4-13 Microbiological quality of SW and RW during the demonstration study

Parameter	Units	SW (average±stdev)	RW (average±stdev)	Log reduction
Aerobic counts	CFU/mL	6.4E+05 ± 5.5E+05	1.4E+05 ± 2.0E+05	0.65
Total coliforms	CFU/mL	30 ± 47	1.6 ± 3.6	1.3
E. coli	CFU/mL	2 ± 5	0.4 ± 0.9	0.7
Clostridium perfringens	CFU/mL	n.d.	n.d.	-
Giardia spp.				
Cryptosporidium spp.	oocysts/L	<8	<8	-
Bovine polyomaviruses[8]	GC/L	<105	<105	-

[8] analysed in the influent WW of the WWTP Caldes de Montbui, before the primary treatment

Parameter	Units	SW (average±stdev)	RW (average±stdev)	Log reduction
Human adenoviruses[9]	GC/L	7.0E+05 ± 7.4E+05	<105	5
Somatic coliphages	PFU/mL	8 ± 10	5.0 ± 5.8	0.2
Helminth eggs	egg/10L	<1	<1	-
Taenia spp. eggs	egg/10L	<1	<1	-

Table 4-14 Toxic inorganic and organic constituents in reclaimed wastewater during the demonstration study

Parameter	RW (µg/L)	Water quality objective (µg/L)	Reference
Be	<1	100	(Government of New Brunswick 2014)
B	259	5,000	(Schlink, Nguyen and Viljoen 2010)
Al	12	5,000	(Schlink, Nguyen and Viljoen 2010)
V	0.9	100	(Schlink, Nguyen and Viljoen 2010)
Cr	0.2	100	(Schlink, Nguyen and Viljoen 2010)
Mn	11	50	(Schlink, Nguyen and Viljoen 2010)
Fe	15	200	(Schlink, Nguyen and Viljoen 2010)
Co	0.3	1000	(Government of New Brunswick 2014)
Ni	4.5	250	(Schlink, Nguyen and Viljoen 2010)
Cu	6.7	200	(Schlink, Nguyen and Viljoen 2010)
Zn	34.7	5,000	(Government of New Brunswick 2014)
As	1.5	200	(Schlink, Nguyen and Viljoen 2010)
Se	<0.5	50	(Schlink, Nguyen and Viljoen 2010)
Mo	5.1	30	(Schlink, Nguyen and Viljoen 2010)
Cd	<0.1	5	(Schlink, Nguyen and Viljoen 2010)
Sb	0.6	-	-
Hg	<1	10	(Schlink, Nguyen and Viljoen 2010)
Pb	0.4	50	(Schlink, Nguyen and Viljoen 2010)
Nonylphenols	<1	-	-
PAH	<0.01	-	-
PCB	<0.01	-	-
Chlorinated pesticides	<0.01	-	-
P-containing pesticides	<0.01	-	-
Triazines, except terbutryn	<0.01	-	-
Terbutryn	0.06	-	-
Halogenated solvents	<1	-	-

4.8 Evaluation of the reuse treatment performance for livestock drinking purposes

To evaluate the potential effects of using RW for livestock drinking purposes, two different studies were performed: 1) the first consisted on evaluating cytotoxicity and inflammation markers of bovine intestinal cells in contact with RW obtained by different treatment processes (*in vitro* study); 2) a second one evaluating short-term effects and preferences on animal performance, health, and metabolism of offering RW, treated by the proposed scheme detailed in section 4.5.3, to young calves (*in vivo* study).

[9] idem above.

4.8.1 Experiments *in vitro*

The methodology of the in vitro study proposed let to explore the effects of different water types on intestinal cells cultured in plates, instead of using animals. This will allow discarding the most harmful treatments of being performed with animals. The hypothesis of the *in vitro* study is that RW from a municipal WWTP treated with different water tertiary treatment technologies may cause different cell damage depending on its quality. In this study, the cell damage was evaluated analysing several cytotoxicity and inflammation markers expressed by the *in vitro* cultured intestinal cells when are in contact with RW obtained by different tertiary treatment processes.

4.8.1.1 Materials and Methods

Jejunum cells from 11-mo Holstein bulls were cultured during 2h in 8 different types of water:

a) media culture
b) tap drinking water (tap DW)
c) water from a dairy cow trough filled with tap water (trough DW)
d) secondary WWTP effluent (SW)
e) secondary WWTP effluent + ultrafiltration (UF)
f) secondary WWTP effluent + UV disinfection (UV)
g) secondary WWTP effluent + UF + UV disinfection (UF + UV)
h) secondary WWTP effluent + UF + chlorination disinfection (UF + Cl)

The secondary WWTP effluent was obtained from the WWTP in Caldes de Montbui, and the different tertiary treatments, including UF, UV and chlorination, were performed using the UV and UF equipment described in section 4.5.3. Water quality of the different waters generated was assessed by analysing some chemical and microbiological parameters, and listed previously in this report (Table 4-11). Tap DW and media culture were used as controls. Water from a cow trough, taken after 24h of filling with tap DW, was also tested.

Jejunum cells and the different waters were cultured in 24-well plates (6 replicates per each water treatment) at 37°C under a 5% CO_2 atmosphere during 2 h. After the incubation, cells were washed and lysed with TriZol (Invitrogen) to extract RNA and quantify, by qPCR, the expression of several genes related to apoptosis (BNIP3 and CASP3), cell damage markers (heat stress proteins: HSPA1A and HSPB1), and inflammation markers (TNFα, IL-1β, and IL-10).

Data were transformed to log to assess normality to perform an analysis of variance considering the type of water used in each cultured group of cells as the main effect. Significance level was considered at P-values lower than 0.05, and lower to 0.10 a tendency.

4.8.1.2 Results and Discussion

Apoptosis markers

The expression of apoptotic gene (CASP3) was greater ($P < 0.001$) in the cells that were incubated with tap DW and UF+Cl compared to the others (Figure 4.24), being UF+UV the one with the lowest CASP3 expression. Similarly, the expression of the apoptotic gene (BNIP3) was greater ($P < 0.001$) in the cells that were incubated with tap DW, UF+Cl, trough DW, SW and UV treated WW compared to control media, UF+UV and UF treated WW (Figure 4.25).

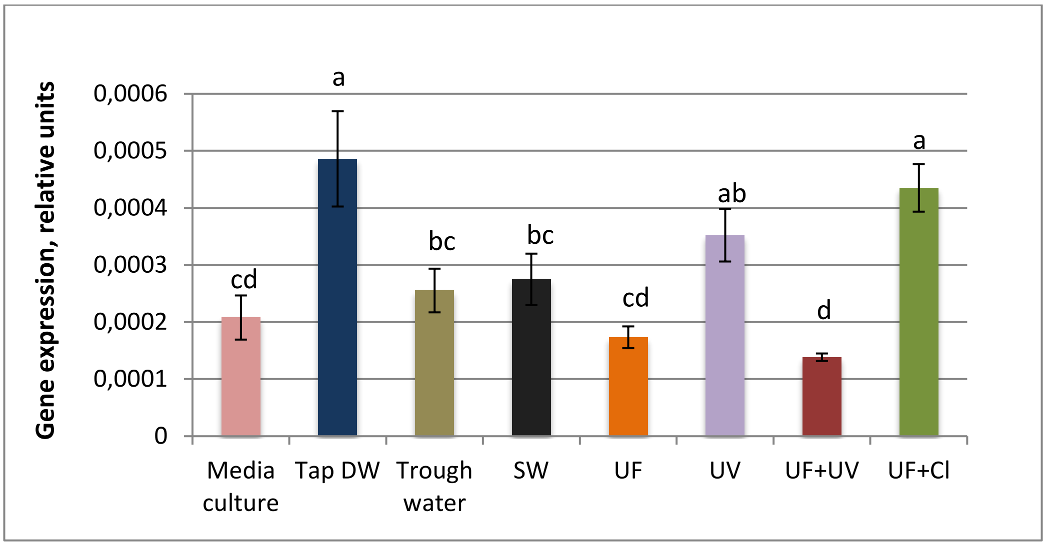


Figure 4.24 Gene expression of CASP3 of cells after incubation with different types of water quality.
Columns with different letters denote differences among type of waters.

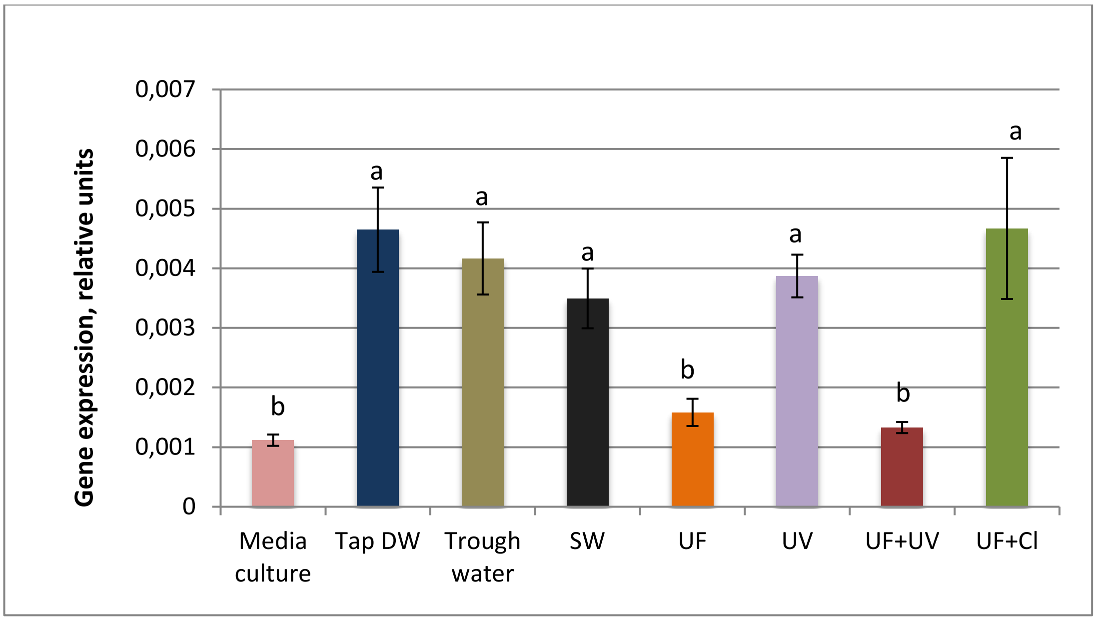


Figure 4.25 Gene expression of BNIP3 of cells after incubation with different types of water quality.
Columns with different letters denote differences among type of waters.

Cell damage markers

The expression of the gene HSPA1A was greater ($P < 0.001$) in cells that were incubated with tap DW, trough DW, and UF+Cl treated WW compared with the others, being the control media, UF, and UF+UV treated WW the ones with the lowest gene expression (Figure 4.26). In contrast, there were no differences in the expression of HSPB1 gene.

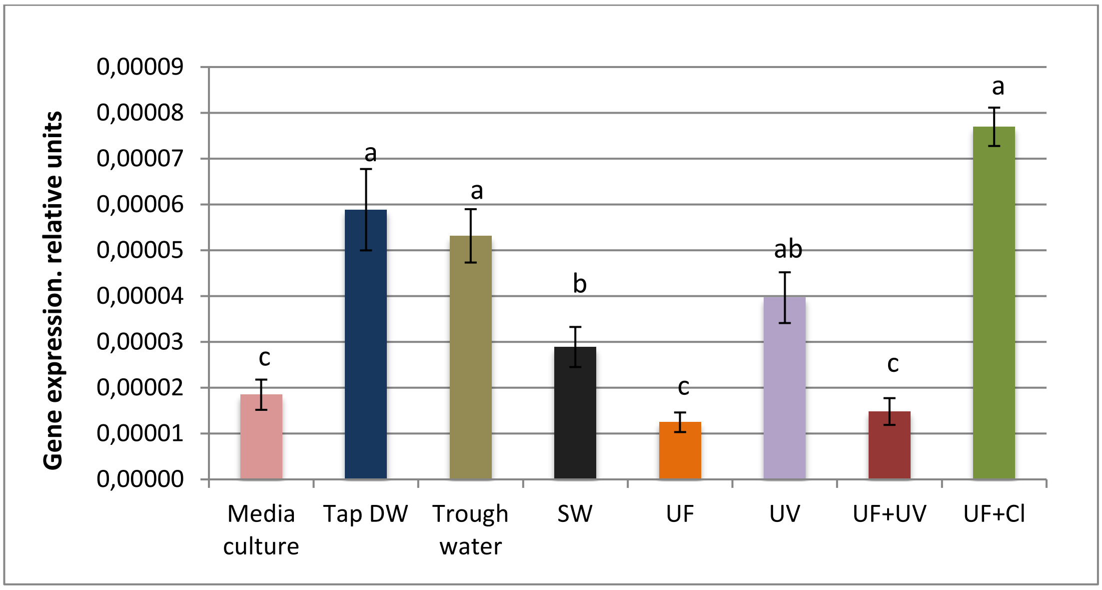


Figure 4.26 Gene expression of HSPA1A of cells after incubation with different types of water quality.
Columns with different letters denote differences among type of waters.

Inflammatory markers

The expression of the pro-inflammatory cytokine TNFα was lower in cells incubated with the control media followed by UF and UF+UV treated WW compared with all the others (Figure 4.27). In contrast, the expression of IL-1ß was the lowest in the cells incubated with control media, and the greatest in the SW (Figure 4.28). Similar to TNFα, the expression of the anti-inflammatory cytokine IL-10 was lower in cells incubated with the control media, UF and UF+UV treated WW compared with all the others (Figure 4.27).

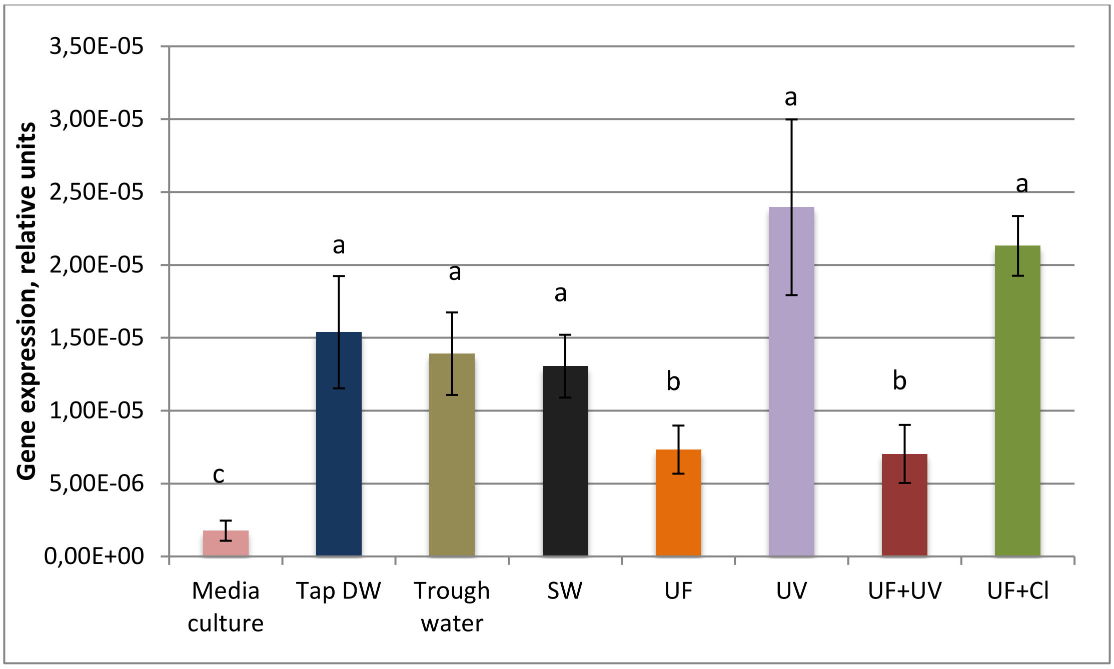


Figure 4.27 Gene expression of TNFα of cells after incubation with different types of water quality.
Columns with different letters denote differences among type of waters.

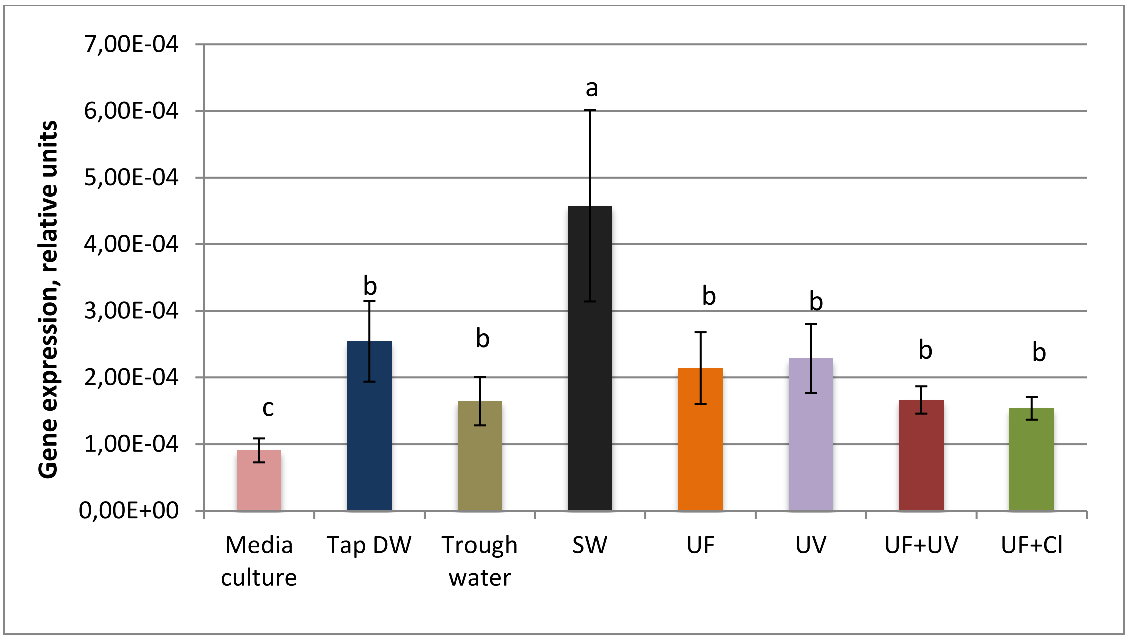


Figure 4.28 Gene expression of IL-1ß of cells after incubation with different types of water quality.
Columns with different letters denote differences among type of waters.

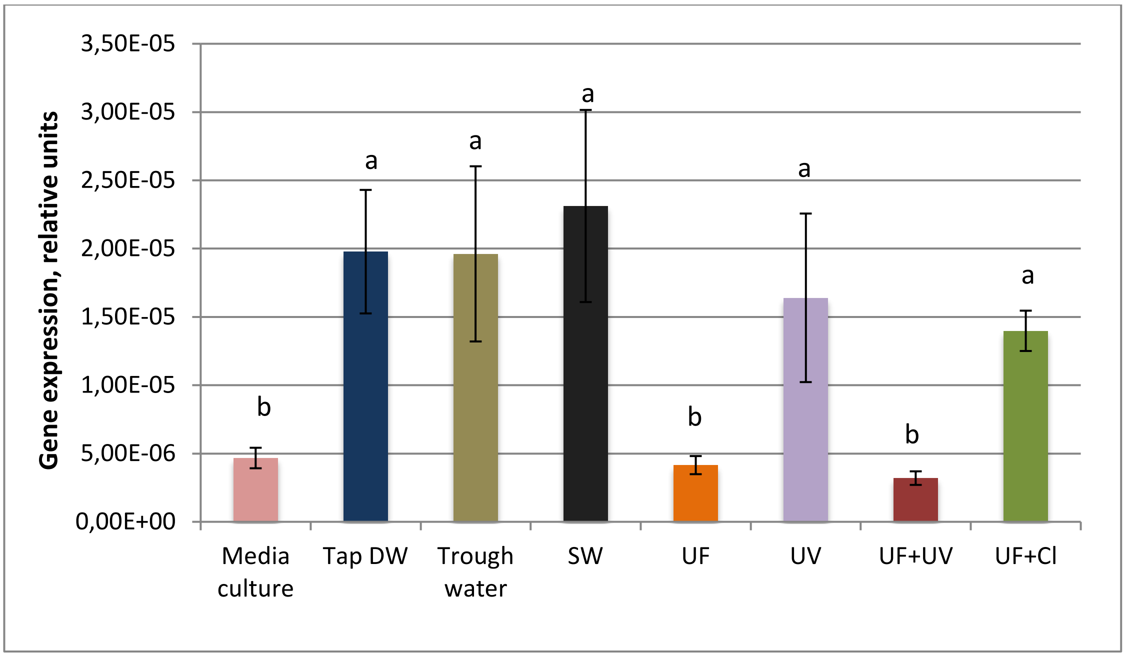


Figure 4.29 Gene expression of IL-10 of cells after incubation with different types of water quality.
Columns with different letters denote differences among type of waters.

Figure 4.30 shows a summary of the results, the darkest the colour intensity, the greatest the relative expression of the targeted gene. Generally, it could be said that apoptotic and cell damage markers indicate that the cells are suffering a stress and they are probably dying. The cells with the greatest gene expression of these markers suggest the greatest cell damage or stress. From all these data, it can be highlighted a common pattern in most of the markers. Cells incubated with tap DW, trough DW and UF+Cl treated WW had the greater expression of apoptotic, cell damage and inflammation genes TNFα and IL-10, in contrast to UF and UF+UV treated WW that generally present similar values to the control media. Curiously, the presence of chlorine in tap DW, trough DW, and in UF+Cl treated WW is a common compound found in both types of water, and it may be, in part, the cause of the negative impact of these water on intestinal cells. In the literature, it is described that chlorine disinfection by-products can cause cellular oxidative stress, and they may have carcinogenic and mutagenic properties (Yuan, et al. 2006, Richardson, et al. 2007). On the other hand, treatments that had similar results than the culture media have in common the UF treatment, which may be able to reduce the presence of toxic contaminants usually associated to particles (i.e., polycyclic aromatic hydrocarbons, heavy metals).

SW was the water with the greatest levels of IL-1ß, and it is also the water with the highest levels of *E.coli* and aerobic counts. Inflammasomes are protein complexes that merge during infections, tissue damage or metabolic imbalances, and they activate the pro-inflammatory cytokines IL-1ß and IL-18 (Latz, Xiao and Stutz 2013). Probably the mechanism explained above may be responsible of the greatest expression of IL-1ß in the SW treated cells due to the presence of more bacteria.

Figure 4.30 General view of the study results.
The more the intensity of the colour, the greater the expression of the targeted gene.

Markers	Gene	Media culture	Tap DW	Trough DW	SW	UF	UV	UF+UV	UF+ClO$^-$
Apoptosis	CASP3								
	BNIP3								
Cell damage	HSPA1A								
Inflammation	TNFα								
	IL-1ß								
	IL-10								

4.8.1.3 Conclusions

The water treatments with the lowest impact on the intestinal cells are the tertiary treatments that consisted on an UF followed by an UV disinfection and the UF treated WW without disinfection. The causes of the different results are difficult to know with the experimental design of this study, but it is clear that the UF technology contributes positively to the overall cell performance. In principle, a UF treatment is intended to eliminate suspended particles and colloids and partly reduce the microbiological load, so we cannot associate the expression enhancement produced by the UF to an elimination of the microbial load, otherwise is probably associated to the elimination of toxic chemical constituents attached to the suspended particles, which are rejected by a UF process. Although the specific compounds that caused the increase of cell damage, inflammation and apoptosis of intestinal cell primary cultures incubated with several types of water were not determined in the study, one of the common factors in some of them (tap DW, trough DW and UF+Cl treated WW) was the previous chlorination disinfection treatment. The by-products formed during the chlorination step of these waters may be pointed as a probable cause of these negative results.

4.8.2 Experiments *in vivo*

An *in vivo* experiment with animals was planned once the *in vitro* study results were finished and the UF+UV treatment resulted in a treated WW without negative health effects on the tested intestinal cells. Furthermore, the water standards defined by the Australian legislation for the use of RW in livestock were also achieved using the UF and UV treatments. Then, it was proposed to use the UF+UV treated WW as drinking water for dairy calves. A preliminary study to evaluate short-term effects of UF+UV treated WW on performance, health, and metabolism of dairy calves from 5 to 47 d of age was performed.

4.8.2.1 Materials and Methods

Animals and Treatments

A total of 18 Holstein dairy calves of 5±3.2 d of age and 40±6.3 kg of BW were gathered from several farms, and raised in the facilities of IRTA at Torre Marimon. Calves were housed individually and bedded with sawdust. Since milk replacer (MR) is prepared with milk powder and water, and calves consumed it easily, in contrast to drinking water that calves consume voluntarily, two different types of MR feeding programs were proposed in order to offer different amounts of RW to animals. The experimental design consisted on a 2x2 factorial design, being the two factors: the MR feeding program and the water source that calves consumed through both milk and drinking water. The four different animal groups are the following:

a) 4 L/d of MR diluted at 12.5% DM (4L) prepared with tap DW

b) 4 L/d of MR diluted at 12.5% DM (4L) prepared with UF+UV treated wastewater (UF+UV, RW)

c) 8 L/d of MR diluted at 12.5% DM (8L) prepared with tap DW

d) 8 L/d of MR diluted at 12.5% DM (8L) prepared with UF+UV treated wastewater (UF+UV, RW)

Concentrate and barley straw were offered *ad libitum* from the beginning of the study to 47 d of age.

Measurements and sampling during the study with animals

The features of the measurement and sampling are the following:

1. Calves were weighed at the beginning of the study and every week, and the Average Daily Gain (ADG) was calculated as:

$$ADG = (BW\ current\ week - BW\ previous\ week)/7$$

2. Milk replacer, concentrate, forage and water intake were measured daily
3. Veterinary treatments were recorded
4. Faecal consistency was evaluated daily in 1-3 scale (being 1 normal and 3 watery)
5. Blood samples were obtained at the beginning of the study, and at 35 d of study to check for the main biochemical and haematological parameters: glucose, insulin, urea, creatinine, hepatic enzymes (AST and GGT), NEFA, triglycerides, T3, and hemogram.
6. Faecal samples were obtained at the beginning and at 35 d of study to check for the presence of helminthic eggs, and *Cryptosporidium* cysts and coccidia oocysts.
7. The last day of study all animals were offered to drink during 24-h both types of water to evaluate its capacity to distinguish between tap DW and UF+UV treated WW, and to determine if calves had a preference for one of them. At 9:00 all calves were offered 5,500 mL of each type of water, then at 13:30 all water buckets were weighed and water was added if they had less than 4,000 mL in the bucket. Then, at 16:30 all buckets were weighed again and the water was completely changed with new fresh water until 9:00 of the following day, when all buckets were weighed again. Figure 4.31 shows the calf pen with three buckets: one for the concentrate, and the other two for the two types of water (blue stick = tap DW, and green stick = UF+UV).

8. Reclaimed water samples, including SW and the effluents after each treatment step (storage tank after UF, storage tank before UV, water after UV, and water from both tap DW and UF+UV troughs) were collected biweekly to analyse basic chemical and microbiological parameters such as TOC, pH, conductivity, turbidity, TSS, aerobic counts, total coliforms and *E. coli*, as was described above in section 4.7.2.
9. Reclaimed water samples were analysed at least once during the study for all microbiological risks identified above, including all reference indicators listed in Table 4-10 (e.g., human adenoviruses, bovine polyomaviruses, protozoa Giardia and Cryptosporidium, *Clostridium perfringens*, and helminth eggs).
10. Chemical water quality of UF+UV treated WW and tap DW was assessed through several campaigns performed during the study, by means of parameters such as pH, conductivity, TDS, anions, cations and other organic and inorganic trace constituents, as was described above in section 4.7.2.

Figure 4.31 Calf pen during the water preference test.
Blue stick denotes tap DW in the bucket, and green stick UF+UV (RW).

Performance and DM intake data were analyzed with a mixed-effects model with repeated measures, including milk-feeding program, type of water, weeks of study and their interactions as fixed effects. Initial BW, initial age, and farm origin were used as covariates and calf entered in the model as a random effect.

Fecal score was analyzed with a logistic regression considering milk-feeding program, type of water, and their interactions as fixed effects, and the probability of having a fecal score of 2 along all the study was estimated.

Hematological and biochemical profiles were analyzed with an analysis of variance considering initial age and initial parameter value (day 0 of the study) as covariates and milk-feeding program, type of water, and their interactions as fixed effects. Those parameters that did not follow the normality were log-transformed.

Water preferences were analyzed calculating the preference ratio of tap DW as follows:

$$\text{Preference ratio} = (\text{tap DW intake})/(\text{tap DW intake} + \text{RW intake}).$$

Where,

preference ratio > 0.5 indicates preference for tap DW
preference ratio = 0.5 indicates no water preference
preference ratio < 0.5 indicates preference for UF+UV treated WW (RW)

Preference ratios for all calves (independently of their previous water experience) were analyzed for a difference from 0.5 (lack of preference) using t-test. Then, to check the effect of the previous experience water source offered, preference ratio throughout the 24-h were also analyzed using a mixed-effect model considering time of the day as a repeated measure, type of milk feeding program and type of water, and their interactions as fixed effects.

4.8.2.2 Results and Discussion

There was not any interaction of MR feeding program and type of water on performance and feed intake parameters (Table 4-15). Calves fed with 8L of MR increased their performance, decreased concentrate intake, and improved feed efficiency in comparison to 4L-fed calves (Table 4-15). This effect was expected because the greater amount of MR offered to calves the lower the concentrate intake (Terré, Tejero and Bach 2009), and as MR is more digestible than concentrate, the feed efficiency of calves improves when more MR is offered. Calves that were offered UF+UV ($P < 0.05$) consumed less concentrate than tap DW-fed calves (Table 4-15), and this negatively affects ADG that was numerically lower in RW-fed calves compared with tap DW-fed calves. There exists a close relationship between water and concentrate intake (Kertz, Reutzel and Mahoney 1984), and low availability of water is usually related to a decrease in calf starter concentrate. Although water intake was similar in both treatments, it was numerically lower in RW than in tap DW calves, and it might explain the decrease of concentrate intake of RW calves (Table 4-15). The greater conductivity (and therefore salinity) in UF+UV treated WW compared with tap DW may have an effect on water palatability and cause lower water consumptions and animal performance. Generally, increasing the salt content in water for dairy cows decreases water and feed intake, and milk yield (Challis, Zeinstra and Anderson 1987, Solomon, et al. 1995).

Table 4-15 Performance and DM intake of calves.
Calves were fed by two different milk feeding programs (4 L/d *vs* 8 L/d of milk replacer), and two different water types (tap water *vs* reclaimed wastewater).

Water source:	Tap DW		RW		SEM[10]	Significance (p-values[11])		
Feeding program, L/d	4	8	4	8		Water (W)	Milk (M)	W x M
Number of calves	4	4	5	5	-	-	-	-
Initial age, d	5.3	3.5	4.8	5.4	1.63	0.66	0.73	0.48
Initial BW, kg	40.6	37.8	40.0	41.0	3.21	0.69	0.79	0.57
Final BW, kg	66.0	71.9	63.3	69.3	1.57	0.35	0.001*	0.60
ADG, g/d	616	778	556	692	49.1	0.14	0.003*	0.79
Water intake, L/d	3.4	3.2	2.9	2.8	0.41	0.19	0.64	0.86
Total DM intake, g/d	1,158	1,161	1,036	1,054	67.4	0.09	0.87	0.91
Milk replacer, g/d	487	861	487	872	0.02	0.72	<0.001*	0.73
Concentrate, g/d	650	282	532	170	45.6	0.01*	<0.001*	0.94
Straw, g/d	17	15	14	15	7.6	0.81	0.94	0.85
Gain to feed[12]	0.53	0.68	0.53	0.69	0.04	0.89	<0.001*	0.94

[10] Standard error of the mean
[11] water: effect of the type of water used to prepare the MR and for livestock drinking; milk: effect of the volume of MR offered to calves; WxM: effect of the interaction of W and M
[12] Ratio ADG/Total DM intake

Faecal score was used as an indicative of faeces consistency, and therefore the risk of having diarrhea. There were no differences in the probability of faecal score of 2 in any of the treatments (Figure 4.32). Similarly, the number of veterinary treatments for diarrhea and respiratory problems was similar in all treatments. These data suggested that the water treatment based on UF+UV was efficiently done, and using the secondary effluent from a WWTP ultrafiltrated and UV-disinfected do not increase the percentage of ill calves.

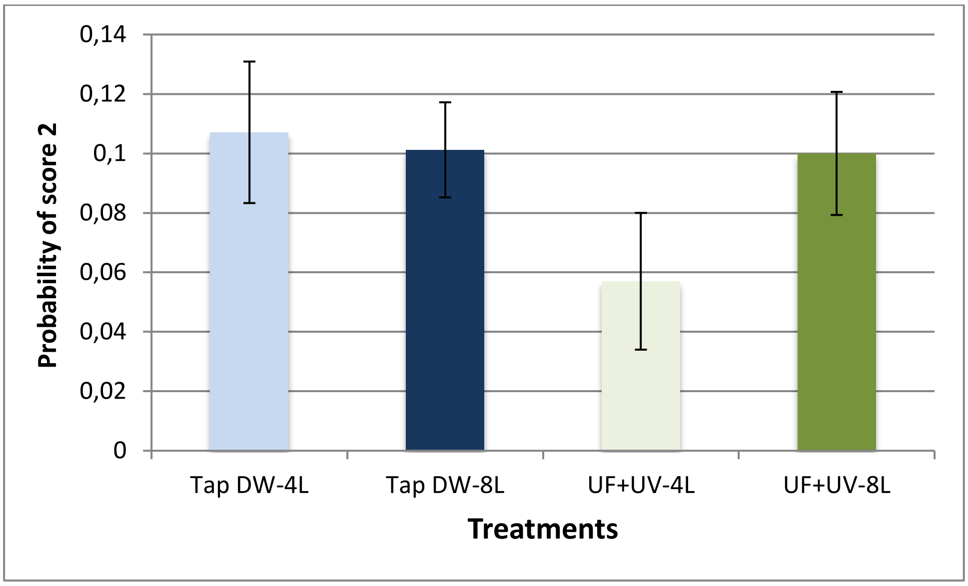


Figure 4.32 Probability of faecal consistency of score 2 in calves.
Calves were fed following two different milk feeding programs (4 L/d vs 8 L/d of milk replacer), and were fed with two different types of water (tap DW *vs* UF+UV treated wastewater, RW).

Haematological blood profiles were similar between animals fed with tap DW and UF+UV and at 4 or 8 L/d, with the exception of eosinophil and platelet counts. Eosinophil counts tended (P=0.08) to be greater in calves raised with RW than those with tap DW, and platelets counts tended (P=0.05) to be lower in RW than in tap DW calves. Mostly of the blood parameters were in the normal range of calves reported by (Brun-Hansen, Kampen and Lund 2006) and (Roland, Drillich and Iwersen 2014), with the exception of eosinophil that had greater counts and platelets that were lower in UF+UV calves of the present study compared with those healthy calves studied by (Roland, Drillich and Iwersen 2014). Conditions commonly associated with eosinophilia included hypersensitivity reactions and parasitic infections. Additional causes are neoplasia, infections, and drug reactions (Roland, Drillich and Iwersen 2014). On the other hand, platelets counts can decrease due to blood loss, toxins, drugs or infections that destroy platelets, or disorders that affect the bone marrow. The faeces of calves were checked for coccidia oocysts, nematodes eggs, and the presence of *Cryptosporidium* cysts. Only *Cryptosporidium* cysts were found at the beginning of the study (day 0, before offering RW, 44.4% of the positive calves were distributed in the tap DW treatments and 55.6% in RW treatments), and no helminth eggs or cysts were detected at 35 d of study in any of the treatments. Therefore, either other parasitic infection than coccidia, nematodes or cryptosporidia occurred in RW calves or, the presence of toxins or drugs in the WW which were not eliminated by the membrane might also be a hypothetical explanation of the changes observed in eosinophil and platelets counts. In any of the cases, the problem should be considered as mild, since a greater infection or problem would have changed more parameters of the haematological profile.

Table 4-16 Haematological profile at 35 days of study of calves.
Calves were fed by two different milk feeding programs (4 L/d *vs* 8 L/d of milk replacer), and two different types of water (tap water *vs* reclaimed wastewater).

Water source:	Tap DW		RW		SEM	Significance (p-values[13])		
Feeding program, L/d	4	8	4	8		Water (W)	Milk (M)	W x M
Erythrocytes, 10^6/µl	8.4	8.9	8.4	8.6	0.45	0.76	0.45	0.80
Hemoglobin, g/dL	8.9	9.3	9.6	8.9	0.49	0.76	0.79	0.30
Hematocrit, %	23.4	24.7	25.2	23.3	1.18	0.84	0.77	0.21
Mean corpuscular volume, fl	28.5	27.8	28.9	27.6	1.57	0.51	0.09	0.60
Leukocytes, 10^3/µl	9.4	11.1	8.1	10.2	1.66	0.50	0.25	0.93
Neutrophils[14], 10^3/µl	2.1	3.7	1.4	2.9	1.95	0.60	0.11	0.53
Lymphocytes, 10^3/µl	6.4	6.2	5.2	6.6	0.75	0.59	0.38	0.41
Monocytes, 10^3/µl	0.48	0.56	0.52	0.42	0.057	0.35	0.83	0.17
Eosinophil[3], 10^3/µl	0.16	0.32	0.49	0.74	0.481	0.08*	0.40	0.83
Basophils, 10^3/µl	0.11	0.09	0.10	0.15	0.020	0.35	0.41	0.12
Platelets, 10^3/µl	713	819	541	669	69.5	0.05*	0.11	0.88
Mean platelet volume, fl	6.7	6.4	7.2	6.7	0.70	0.56	0.57	0.85

The different biochemical parameters were measured to detect possible variations on general health status and are shown in Table 4-17. Serum glucose concentration (related to carbohydrate metabolism) tended to be greater in calves offered 8L of RW compared with those fed with 8L of tap DW, but 4L-fed calves had similar values. However, there were no differences in serum insulin concentration. In contrast, serum urea and creatinine concentrations, related to protein catabolism, and kidney damage, respectively, were similar in all treatments. On the other hand, serum AST concentration (as indicator of soft tissue damage) did not differed among treatments, but serum GGT concentration (associated to bile duct damage) was lower in 8L than in 4L-fed calves. Normal values of GGT in calves are around 20 U/L (Klinkon and Jezek 2014), and values under this level are not considered a health problem. Serum NEFA concentrations were greater in tap DW compared with RW-fed calves. Increases in serum NEFA concentrations are related to body fat mobilization. The values of serum NEFA concentration in tap DW calves are too low to be indicative of a negative energy balance. Furthermore, total DM intake was slightly greater in tap DW than in RW-fed calves, and differences in DM intake cannot explain the differences between tap DW and RW-fed calves. Serum triglycerides concentrations were greater in 8L-tap DW and 4L-RW treatments compared with 4L-tap DW and 8L-RW. These differences are difficult to interpret with the information of the study. Finally, serum thyroid hormone (T3) concentrationas greater in 8L than in 4L calves. Thyroid hormone is related to growth and to increase the basal metabolism, as 8L calves grew more than 4L calves, this difference was expected. In general, biochemical parameters are within the normal range and the differences found cannot be attributed to serious health problems.

Table 4-17 Biochemical parameters at 35 days of study of calves.
Calves were fed following two different milk feeding programs (4 L/d *vs* 8 L/d of milk replacer), and two different types of water (tap water *vs* reclaimed wastewater).

Water source:	Tap DW		RW		SEM	Significance (p-values[15])		
Feeding program, L/d	4	8	4	8		Water (W)	Milk (M)	W x M
Glucose, mg/dL	120.8	85.0	111.9	133.2	13.48	0.19	0.59	0.07

[13] water: effect of the type of water used to prepare the MR and for livestock drinking; milk: effect of the volume of MR offered to calves; WxM: effect of the interaction of W and M

[14] This parameter was log-transformed, and the values are the non-transformed means, and the p-values the ones from the log-transformed analysis

[15] water: effect of the type of water used to prepare the MR and for livestock drinking; milk: effect of the volume of MR offered to calves; WxM: effect of the interaction of W and M

Water source:	Tap DW		RW		SEM	Significance (p-values[15])		
Insulin, µg/L	0.95	1.75	1.70	3.10	1.828	0.54	0.18	0.73
Urea, mg/dL	14.0	11.9	15.0	11.6	2.37	0.90	0.29	0.78
Creatinine, mg/dL	0.92	0.85	0.93	0.91	0.047	0.54	0.38	0.60
AST, U/L	54.5	48.8	55.9	50.3	4.76	0.76	0.26	0.99
GGT, U/L	21.0	14.0	21.8	16.4	2.65	0.57	0.03*	0.75
NEFA, mmol/L	0.14	0.14	0.12	0.09	0.013	0.02*	0.42	0.32
Triglycerides, mg/dL	22.6[a]	48.9[b]	34.2[b]	19.6[a]	6.91	0.21	0.40	0.02*
Thyroid hormone, ng/dL	154.1	243.4	124.6	214.0	22.77	0.23	0.003*	0.99

Finally, the preference water test indicated that when analysing all calves together with the t-test without considering their previous experience, they had a preference for tap DW (0.76±0.297). However, the confidential limits were between 0.91±0.30 and 0.22±0.45 (limit range for no preference). Surprisingly, when analysing the effects of their previous experiences on water, those that were consuming tap DW throughout the demonstration study, afterwards did not show any preference for one of the waters during the preference test (preference ratio of 0.53±0.08), in contrast to previously RW-fed calves that had a clear preference for tap DW during the preference test (0.91±0.07, Figure 4.33). This information demonstrates firstly, the ability of calves to distinguish between the two types of water, and secondly that the RW obtained in the present demonstration study, although it does not pose any important risk for the animal health, has less acceptability and palatability than the one from the tap in Torre Marimon.

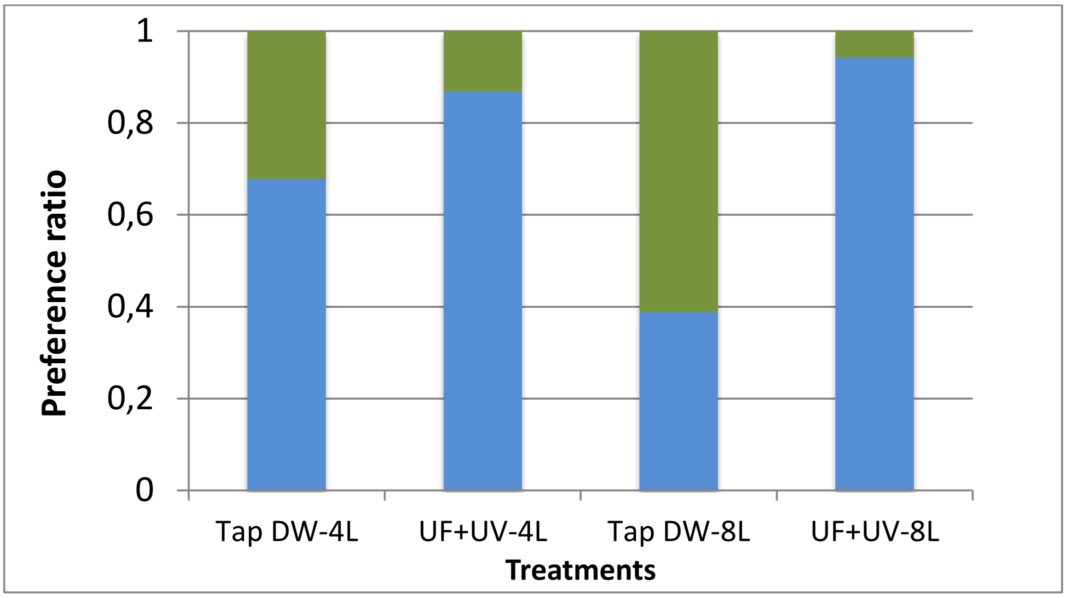


Figure 4.33 Preference ratio at 42 days of study of calves

Calves were fed following two different milk feeding programs (4 L/d *vs* 8 L/d of milk replacer), and with two different types of water (tap water *vs* reclaimed wastewater).

4.8.2.3 Conclusions

The RW obtained by the treatment scheme installed in Torre Marimon, based on a UF and a UV disinfection achieved a water quality that fulfil most of the water quality objectives set for livestock drinking except for some salts, such as Na^{+} and Cl^{-}, that they were found at concentrations around the threshold limits. It's worth mentioning that an installation at full-scale should not include intermediate storage tanks unless some kind of continuous disinfection is applied to avoid bacterial re-growth. In the *in vivo* experiment, the effects of feeding calves with RW on animal performance and health were evaluated in comparison with tap DW during a 42-d period. With regard to animal performance, animals fed with RW had lower water intakes and consequently lower concentrate intakes than those fed with tap DW, although not statistically different, but still contributing to a lower ADG in animals fed with RW than with tap DW. A lower water intake is associated to a lower palatability and acceptability of RW, probably due to the presence of certain salts. The preference test performed in the present study also confirms that the animals preferred tap DW instead of RW. Concerning effects on the animal health, non-severe alterations were observed in calves fed with RW. However, some parameters of the haematological profile such as eosinophils and platelets were found statistically different between RW and tap DW animals. Alterations found in animals fed by RW are indicative of toxins, drugs or infections, although they did not cause any illness and any other alteration in the haematological profile or in the other biochemical parameters analysed in blood.

4.9 Evaluation of the reuse treatment performance for cleaning livestock facilities

In the present study it was evaluated the potential of using RW to clean dairy calves buckets usually used to feed MR. The hypothesis of this study was that the secondary treated WW with UF and UV may be used for cleaning livestock facilities without increasing the risk of bacterial contamination. The specific objective of this study was to evaluate the microbial contamination when cleaning MR feeding buckets with the same RW than in section 4.8.2.

4.9.1 Materials and Methods

Twenty-four previously cleaned buckets were dirtied with one litre of MR at 12.5% DM concentration. After that, all buckets were emptied, but some traces of milk replacer remained in the bucket. Buckets were kept with MR residues during 48h. Then, twelve of them were cleaned with tap DW and the other twelve with RW. The cleaning protocol was the following:

1. Before cleaning a sterile swab was obtained from each bucket to evaluate the contamination before cleaning.
2. Half of the buckets were cleaned with hot tap DW, and the other half with hot RW
3. All the buckets were brushed with a clean plastic brush
4. Finally, they were rinsed with cold water, and they were open-air dried
5. Once they were dried, another swab was obtained from each bucket to check the degree of cleanness
6. All the cotton swabs were cut and mixed with 1.5 mL of 0.9% NaCl in a 1.5 mL tube. They were vortexed during 10 minutes, and 100 µL were plated in both PCA and compact dry EC cultured medias, to determine total aerobic counts, and coliform and *E.coli,* respectively. All plates were incubated at 37°C during 24 h.

Total bacteria counts data were log transformed and analysed with a mixed-effects model considering time as a repeated measure (before and after cleaning), and the water type used for cleaning and their interactions as the fixed effects of the model. The bucket enters in the model as a random effect. Total coliform and *E.coli* counts were analysed with a Chi-square test considering the presence or absence of this bacteria before and after cleaning.

4.9.2 Results and discussion

The number of total bacteria counts before cleaning was greater than after cleaning, but no differences in total bacteria, *E. coli* or coliform counts were observed after the buckets were cleaned (Figure 4.34, Figure 4.35, Figure 4.36). These data indicate that RW is useful for cleaning purposes without contaminating the cleaned material with more bacteria than when cleaning with tap DW.

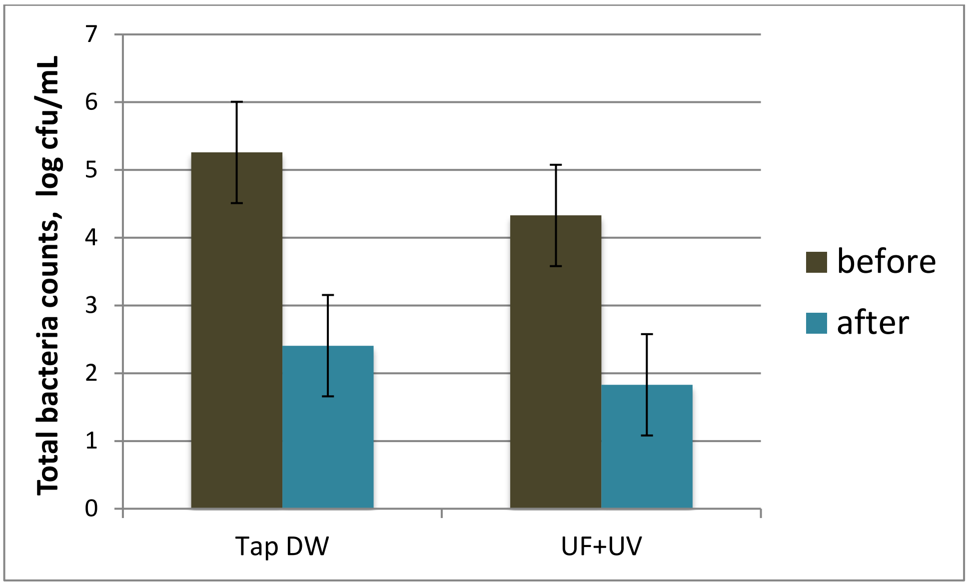


Figure 4.34 Total aerobic bacteria counts (log CFU/mL) of 5-L buckets used to feed milk replacer to dairy calves before and after cleaning them with tap DW or RW.

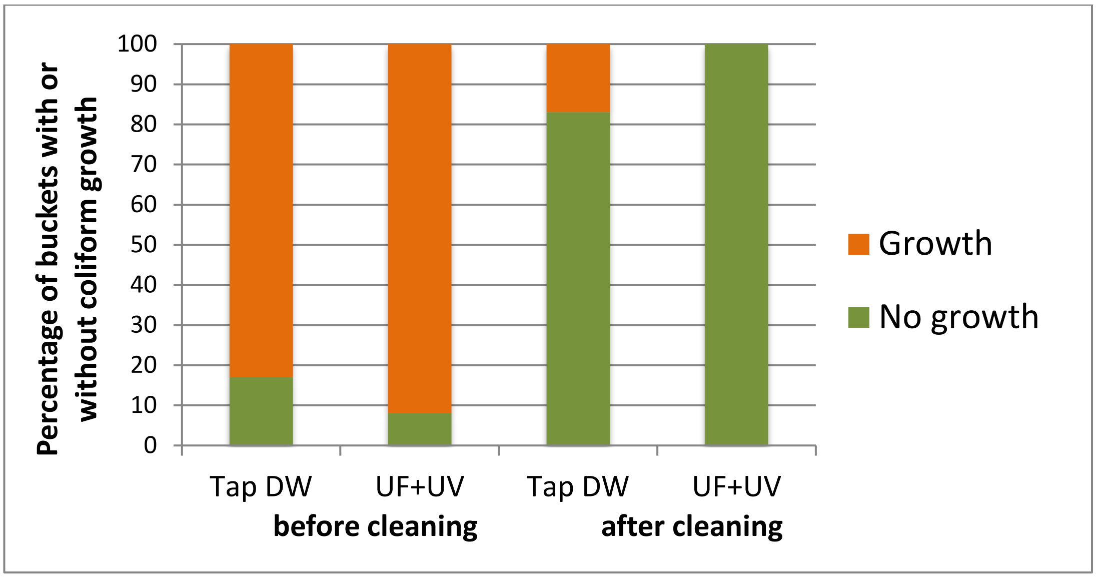


Figure 4.35 Percentage of buckets in which at least one coliform bacteria colony grew in 5-L buckets used to feed milk replacer to dairy calves before and after cleaning them with tap DW or RW.

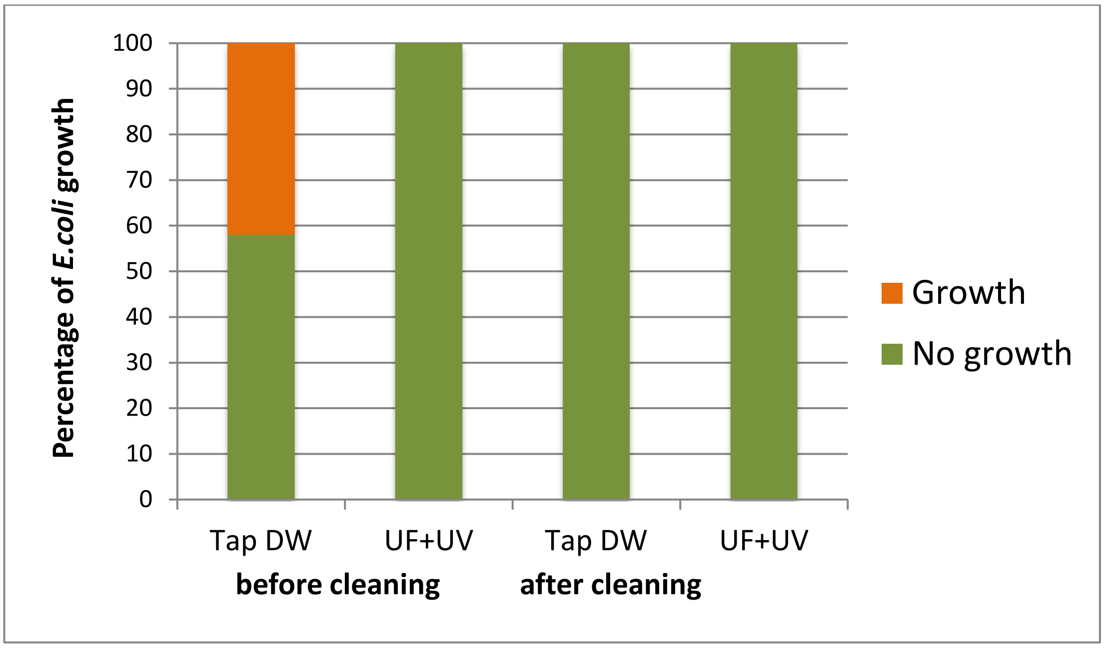


Figure 4.36 Percentage of buckets in which at least one *E. coli* colony grew in 5-L buckets used to feed milk replacer to dairy calves before and after cleaning them with tap DW or RW.

4.9.3 Conclusions

Using RW obtained by a tertiary treatment based on a UF and UV, is a good option for cleaning farm material in a calf barn because it does not increase the risk of contaminating the material with bacteria.

4.10 Economic evaluation: capital and operational costs

Costs will vary depending on the size of the farming facility and the number of animals hosted. Examples of intensive farms presented in section 4.2 had water consumptions ranging from few daily m^3 to up to more than 300 m^3/d. In the present section, both capital and operational costs were calculated for two different scenarios: a) a water treatment plant working at around 375 m^3/day, which could be the case of a rather large farm facility treating on-site their daily water demand, and; b) a larger plant treating around 3750 m^3/d, which could supply DW to several farms or other industrial facilities. Costs are based on cost estimates reported by the American Water Works Association (AWWA Subcommittee on periodical publications 2008, Cotton, et al. 2001). Prices have been updated for year 2016 by applying inflation rates provided by the U.S. Department of Labor Bureau of Labor Statistic (United States Department of Labor 2016).Table 4-18 shows both capital and operational costs for the membrane UF and UV disinfection systems for the two different plant capacities.

Table 4-18 Capital and operational costs of a tertiary treatment water plant based on membrane filtration and UV disinfection

Plant capacity		375 m^3/d		3750 m^3/d	
Capital costs	**Lifespan (years):**	**€**	**€/m^3**	**€**	**€/m^3**
Membrane UF equipment	7	204,233	0.21	700,226	0.07
Membrane UF facility	20	262,585	0.10	1,342,100	0.05
UV disinfection system	7	42,745	0.04	244,256	0.03
Operation and maintenance costs					

Membrane UF system			0.03		0.02
UV disinfection system			0.04		0.01
Total costs					
Membrane UF system			0.34		0.14
UV disinfection system			0.08		0.04
Total cost tertiary system			**0.42**		**0.18**

The capital costs which were considered in the reference study for the filtration system include the membrane equipment, a substantial number of valves, feed pumps, air compression equipment (as necessary for pneumatic valves, integrity testing, backwashing, and similar processes), and chemical cleaning equipment (i.e., tanks, metering pumps and similar equipment), including facilities for spent cleaning solution neutralization (AWWA Subcommittee on periodical publications 2008). Costs for the membrane UF facility include construction and installation, but not land acquisition/development nor intake structures nor storage. Regarding the operation and maintenance costs, these include energy, chemicals for cleaning, and membrane replacement but not equipment maintenance and repair, waste disposal or labor. Although in the original report costs are reported in 2003 dollars, the equivalent 2016 costs in euros have been upgraded using reported inflation and exchange currency rates.

For the UV system, capital costs considered in the reference study include equipment (mainly lamps, ballasts, and sleeves), and others such as building, piping, valves, electrical instrumentation, etc. (Cotton, et al. 2001). The operating costs for the UV disinfection system include power consumption, cleaning chemicals and supplies, and replacement of lamps, ballasts, and sleeves.

Considering lifespans of 7 years for the membrane and UV equipments, and 20 for the rest of the UF system, capital costs per unit of treated water resulted in 0.42 and 0.18 €/m^3, for scenarios a) and b), respectively, being the membrane filtration the treatment accounting around 80% of the overall tertiary treatment cost.

4.11 Conclusions

The evaluation of water management strategies applied in several intensive farms in Spain confirmed that the most demanding water use in farming is for drinking water (between 70 to 90%), followed by cleaning of barns and yards (5-25%), and other minority uses such as animal refrigeration during summer, and in some cases for milk cooling and cleaning of dairy machinery. The water source is usually from groundwater, public net or other sources such as dams, rivers, and treated (usually disinfected) before use to meet quality criteria.

Livestock need water of similar quality than humans to ensure animal performance and health. However, water quality requirements for animals are poorly researched and not regulated. The present demonstration study confirms that water quality determines palatability and acceptability of water by animals, and consequently animal performance in terms of feed consumption and body weight gain. The *in vivo* experiments showed that RW, which has higher content of dissolved salts than tap drinking water, was less preferred by animals and probably this is the reason that these same animals had slightly lower feed consumptions and weight gains. In contrast, intestinal cells incubated with chlorinated waters in the *in vitro* experiments showed greater expressions of genes indicating cell damage and stress than the other cells incubated with not-chlorinated RW, probably associated to the presence of toxic disinfection by-products from the chlorination process. Not severe health effects or illnesses were detected in animals fed with RW, even though some parameters indicative of toxins or infections of their hematological profile were altered. Experiments to evaluate the use of RW for cleaning purposes showed a good cleaning performance, equivalent to a cleaning with tap drinking water.

Reclaimed wastewater achieved by this demonstration study from a secondary WWTP effluent met most of the water quality objectives, set by the different guidelines checked, in terms of physicochemical and microbial quality. Only certain dissolved salts exceed these recommended values. Alterations found in the haematological profile of RW-fed animals, although they did not cause any illness, might be caused by the presence of toxic contaminants, such as micropollutants (CECs) not analysed in the present study. However, the water reuse scheme used in the present case study ensures the elimination of all microbial hazards responsible of severe illnesses in animals (viruses, helminth eggs and protozoa). This reclaimed wastewater is as efficient as potable drinking water for cleaning farming facilities.

The use of reclaimed wastewater for livestock drinking and farming cleaning does not pose an acute risk for animal health. Water reuse for livestock production represents a feasible option in case of water shortages or lack of water availability, and depending on the achieved water quality blending options could be considered. However, some considerations should be taken into account in case of water reuse for livestock drinking, because animal performance in terms of weight gain could be affected depending on the chemical quality of reclaimed wastewater.

5 Recommendations when using reclaimed wastewater in agriculture

5.1 Irrigation of food and non-food crops

Crops irrigation requires a considerable amount of water. In semi-arid regions, the cultivation of high water-demanding crops can be greatly limited by water shortage and it often occurs under water-deficiency conditions. A widespread use of reclaimed wastewater for crop irrigation may constitute a relevant benefit for the agriculture sector. However, in order to avoid risks for human health and the environment, this practice needs careful implementation and monitoring. Many studies have been carried out to investigate the effects of the presence of microbiological and chemical pollutants in the reclaimed water used for irrigation, indicating pathogens, metals and salts as the main potential sources of pollution. Based on these studies, international organisms have proposed guidelines or established standards at the local level, which, however, differ significantly from country to country and even from region to region. The EU, through the Common Implementation Strategy (CIS) for the Application of the Water Framework Directive (WFD) has recently approved the “Guidelines on Integrating Water Reuse into Water Planning and Management in the context of the WFD”. This document is a first attempt to explore the policy and planning context of reuse of treated wastewater at the European level (https://circabc.europa.eu/sd/a/4bba82bd-65b0-42d1-aa8f-19c230e0d517/CIS%20Guidelines%20on%20Water%20Reuse-final.pdf).

Despite the lack of uniformity in terms of regulations, in the case of reuse of treated municipal wastewater, a common approach can be defined for all cases, consisting in the upgrade of existing wastewater treatment plants with the introduction of a tertiary treatment. These have to be adapted to the specific WWTP, to the local limits for reuse and to the specific crops and irrigation network. Numerous pilot studies and full-scale installations demonstrated the existence of technologies suitable for producing reclaimed municipal effluents that comply with limits for reuse (Bixio, De Koning, et al. 2006, Lopez, Pollice, et al. 2006, Norton-Brandão, Scherrenberg and van Lier 2013). Within this framework, the acceptance of the final users is also important for the success of the reuse projects. The information and the involvement of farmers and stakeholders can be considered a necessary step.

The context changes completely in the case of reuse of treated industrial WW, since the characteristics of industrial effluents vary considerably on a case by case basis, so a common approach cannot be defined. Moreover, non-biodegradable compounds potentially toxic for the crops or for the biomass involved in WW reclamation processes may be present in the target WW. These factors cause the reuse in irrigation of WW with relevant industrial fractions to be adopted very seldom. Very few research activities were carried out in this field, therefore the lessons learned through the two years of monitoring of the full-scale plant in Capitanata treating WW from an agro-food industry can be useful to identify the key points in case of reuse of this type of effluents. The main barriers encountered during the operation of the WWTP and the possible solutions to overcome them are summarized below.

The main critical point observed in Capitanata was the variability of the WW characteristics. The monitoring campaign showed that changes in the industrial process that may not be considered relevant from the industrial point of view considerably affected the quality of the reclaimed WW. The solution in this case consisted in sending the extra volumes of highly polluted WW (collected separately) to external alternative treatments, and can be considered a good short-term strategy. As long-term strategy, the upgrade of the entire WWTP is being evaluated at Capitanata site, taking into account the possibility of segregating the most polluted streams. As general recommendation, the segregation of the WW streams based on the type of pollutants produced by the industrial processes would favour the efficiency and reduce the costs of the reclamation processes. Moreover, this strategy can be considered the only one sustainable with respect to salinity, considering the high costs of desalinization processes.

Even though SAR and electrical conductivity in the treated WW were often close or slightly above the limits for reuse, after 2 years of irrigation with reclaimed WW no relevant salt accumulation was observed in the soil. These results, in agreement with other studies (Morugán-Coronado, et al. 2011), indicate that salt accumulation in the soil can be neglected after short-term irrigation with salty treated WW. However, it is not possible to exclude that relevant durable long-term effects could occur. Therefore, to avoid or minimize them, a precautionary strategy that could be applied consists in alternating the use of water sources between the reclaimed agro-industrial effluent and a conventional source having a low salinity.

The treatment scheme introduced in Capitanata as polishing step for reuse purposes consisted of membrane ultrafiltration (preceded by sand filtration) and UV radiation. This scheme has been successfully applied in other studies (Pollice, Lopez, et al. 2004). However, two critical points have been found in its application for treating the secondary settled effluent of the WWTP of the company Fiordelisi: (i) a low productivity of the membranes; (ii) a residual presence of *E. coli* and suspended solids in the membrane effluent.

The first problem can be associated to the characteristics of the influent to the filtration unit, in terms of suspended solids, grease and oil. In order to have a good productivity of the ultrafiltration, when treating agro-industrial WW the oil removal step should be addressed very effectively. In the specific plant, oil and grease removal from the raw WW is performed by gravity separation. More efficient technologies such as Dissolved Air Flotation (DAF) applied to the secondary settled effluent could potentially improve the removal of both suspended solids and oil, thus improving the performance of the membrane ultrafiltration, as well.

The integrity of ultrafiltration membranes initially operated in Capitanata was severely deteriorated after a relatively short time of operation, probably due to a wrong operational setting. Membrane modules were then substituted. New modules still showed a (much smaller) residual presence of *E. coli* and suspended solids in the membrane effluent. A careful inspection of the P&I of the filtration unit revealed the possibility of a slight contamination of the membranes during backwashing/cleaning operation, which could be avoided by modifying the piping of the filtration unit. The main lesson learned from this is that residual bacterial contamination can be expected even downstream ultrafiltration membranes, due to a loss of integrity or to an imperfect design, so a disinfection process should always be applied. In Capitanata, the final UV disinfection, performed in line with irrigation, effectively removed the residual faecal contamination, also due to the high efficiency of ultrafiltration in terms of turbidity removal. Therefore, from this point of view, a tertiary treatment scheme composed of membrane ultrafiltration and UV radiation can be recommended for reuse in irrigation.

Before being used for irrigation, the three sources of water tested in Capitanata were stored in a tank for a period of up to 7 days, depending on the irrigation needs. This caused a bacterial re-growth, and an increase of the suspended solids in the water. On the other side, for the faecal indicator *E. coli,* an average decay of about one order of magnitude was observed in the tank. For large-scale applications, the bacterial re-growth can be controlled by minimizing the storage duration. When high irrigation volumes are required during short periods, a different strategy should be applied. For instance, it could be possible to store big volumes of the secondary settled effluent and then perform the tertiary treatment (both filtration and disinfection) depending on irrigation needs. This strategy would take advantage of the decay of faecal bacteria during the storage and, at the same time, minimize bacterial re-growth after ultrafiltration.

In terms of fertilization potential, the treated WW in Capitanata had a high content of potassium, but a low content of nitrogen and a very low content of phosphorus. However, the high irrigation requirements for the cultivation of tomato allowed for a relevant recovery of nitrogen and potassium from treated water sources, indicating that it is possible to reduce the amount of these nutrients that needs to be supplied through chemical fertilization. On the other side, it is important to point out that the concentration of nitrogen can be much lower in the treated agro-industrial WW than in well water, as observed in Capitanata. This is caused by two factors that are typical of the agro-industrial sector. The first is the over-fertilization of soil, which is common in intensive farming areas and causes the presence of nitrogen in groundwater. The second is the very high COD/N ratio in agro-industrial effluents. Indeed, even if vegetable processing and toilets generate effluents with relevant concentrations of nitrogen (and phosphorus, as well), most of these elements are removed during the activated sludge process to fulfil the metabolic needs of the microorganisms responsible for the biodegradation of organic substances. In order to enhance the recovery of nitrogen from effluents characterized by a high COD/N ratio, anaerobic processes could be applied instead of the conventional activated sludge process. This option would also have the advantage of limited sludge production and possible energy recovery.

Findings obtained in Capitanata with regards the reuse of secondary settled WW indicate that irrigating with reclaimed WW having a residual presence of faecal contamination does not imply a relevant contamination of the crops. Moreover, the presence of *E. coli* on plants irrigated with well water (having no *E. coli*) suggests that the effect of possible external sources of contamination can be comparable (or even higher) to the effect of contamination related to the irrigation water. Previous studies also highlighted the importance of the external environment, typically wildlife, as source of faecal contamination (Langholz and Jay-Russell 2013, Forslund, Ensink, et al. 2012, Vergine, Saliba, et al. 2015). These results may suggest a revision of some of the current national legislations for WW reuse in irrigation. Indeed, too strict limits for the faecal indicators cause the increase of the costs for reusing treated WW for irrigation, so actually limiting the development of this practice.

5.2 Livestock production

5.2.1 Water resources opportunities and matching supply and demand

Water reuse for livestock production represents a good option in case that a possible water shortage could affect the operations of a farm. Usually sites of reuse should be as close as possible to existing WWTPs or other WW sources, either municipal or industrial, or even reusing WW from the same farm facility. Under this condition, water reuse may be technically and economically feasible and the opportunities of its implementation will be higher.

It is important to develop a water demand profile as a basis of matching the supply with demand. Water demand for livestock production will be variable depending on animal species, environmental factors (different water intakes depending on season, refrigeration needs), and animal's physiological conditions (e.g., age, pregnancy/lactation). The purpose of a demand profile is to determine water demand variations, i.e. diurnal and/or seasonal, set the capacity of the treatment scheme in terms of production and operating schedules, and to determine the need of storage facilities with size and location. However, water demand for livestock production (including drinking and cleaning of yards) is rather constant throughout the year, in contrast to the demand of other applications such as crops irrigation.

5.2.2 Water quality requirements

Considerations on water quality for reclaimed water for livestock drinking are extremely important to ensure principally animal health and safety of animal products intended for human consumption, as well as to ensure adequate animal performance.

The acceptability of reclaimed water is dependent on the physical, chemical, and microbiological quality of the water. Since there are no specific legal requirements concerning water quality for livestock drinking, some reported guidelines should be considered when establishing the water quality goals:

- Livestock water quality: a field guide for cattle, horse, poultry and swine in Canada, 2009 (Olkowski 2009)
- National water quality management strategy in Australia, 2000 (Chapter 4.3 for livestock drinking water) (Australian Government Dept. of the Environment 2000)
- Water quality guideline. Volume 5, agricultural use: livestock watering, South Africa, 1996 (Department of Water Affairs and Forestry 1996)
- Sustainable water for livestock, a project done in United Kingdom, 2010 (Department of Environment, Food and Rural Affairs 2010)
- Water requirements for livestock production: a global perspective, 2010 (Schlink, Nguyen and Viljoen 2010)

The Australian guidelines for the use of reclaimed water, being Australia the only country that regulated the use of reclaimed water for livestock, should be taken into account.

However, the demonstration study performed in Torre Marimon allowed identifying the main physical, chemical and microbiological parameters of concern, which could be used as indicators of microbial hazards and to envisage palatability and acceptability of water.

Physical and chemical parameters:

- pH
- Conductivity
- TDS
- TSS
- Turbidity
- BOD
- Chloride
- Sodium
- Potassium
- Sulphur and sulphate
- Iron
- Manganese
- Nitrate and nitrite

Microbial reference indicators of pathogenic illnesses:

- Bovine polyomaviruses (in case of beef cattle)
- *E. coli*
- *Clostridium perfringens*
- *Giardia spp.*
- *Cryptosporidium spp.*
- Helminth eggs (*Taenia saginata* and *Taenia solium*)

Following the same criteria as in the Australian guidelines for water reuse in livestock production, pigs should be excluded of any reclaimed wastewater application because of the capability of certain viruses to cause porcine zoonosis.

The water quality goals are important when health issues are of concern as is in the case of livestock drinking. Desired water quality limit process selection to those systems that are capable of meeting the removal of targeted constituents.

Another concern with potential to affect livestock health are micropollutants considered as emerging contaminants. More research is needed to assess toxicological effects of these pollutants and if they have the potential to bioaccumulate in the animal tissues. This topic was beyond of the scope of the case study in Torre Marimon.

5.2.3 Technology selection and infrastructure requirements

The selection of the treatment scheme is subjected to the water quality goals, the wastewater source but also influenced by the compatibility with existing processes, hydraulic considerations, and site constraints. The physical and chemical characteristics of the secondary effluent affect the type of process to be used and the effectiveness of the tertiary treatment and disinfection. Moreover, constituents identified as potential hazards have to be sufficiently reduced or eliminated by the selected treatment scheme and, for the present water application, the provision of a multiple barrier treatment would is an important consideration.

A tertiary treatment scheme intended for the reclamation of wastewater for livestock drinking should include a disinfection but also a membrane filtration step capable to eliminate helminth eggs and spores:

- Membrane filtration: there exist membranes with different pore sizes capable to reject different water constituents sizewise. Microfiltration, with pore sizes ranging from 0.08 to 2.0 µm, retains suspended particles and some colloids and ultrafiltration, with pore sizes 0.005-0.2 µm, retains organic macromolecules, colloids, some bacteria and viruses. Both filtration systems are able to eliminate helminth eggs and protozoa cysts (diameters upper 20 µm) but ultrafiltration will additionally reduce water turbidity. Selection of a filtration technique will depend on the secondary WWTP effluent quality. Other treatment options to remove helminth parasites are sand filtration, cheaper than membrane filtration even though does not eliminate protozoa cysts, or a membrane bioreactor that combines biological treatment with an integrated membrane system.
- Disinfection step: this is necessary to achieve a reduction of active microorganisms in water, including inactivation of all identified microbial hazards. Chlorination remains as the most widely used disinfection technology despite the generation of toxic disinfection by-products, and its potential negative effects on intestinal cells. Ultraviolet disinfection technology has been shown to be equally efficient as bactericidal and virucidal agent. However, if the water reuse scheme requires of reclaimed water storage, chlorination is the desired disinfection to keep water quality during the storage and avoid bacterial regrowth.

Additionally, to improve the palatability and acceptability, a treatment of osmosis to remove dissolved salts could be applied. However, investment and operation costs of this type of treatment are rather expensive and possibly may lead to a not economically feasible solution.

5.2.4 Economic considerations

Project costs, including initial investment and operating and maintenance costs, are of major significance in the selection of the water reuse application. A feasibility analysis should be developed before the implementation of project by means of total annual costs or life cycle costs. By the present demonstration study, costs of obtaining reclaimed wastewater from a secondary WWTP effluent intended for livestock drinking have been calculated and resulted in 0.42 and 0.18 €/m^3 for different plant capacities, 375 and 3750 m^3/d, respectively, which could be the case of an onsite and decentralised system, respectively. For the decentralised system, costs of piping and water distribution network are not included on the given cost. Around 80% of the cost corresponds to the ultrafiltration step, which could be replaced for sand filtration, which may reduce this cost.

5.2.5 Sustainability considerations

Even if economically is more expensive using reclaimed wastewater than groundwater or water from the public net, agricultural systems should also be evaluated under a sustainability point of view. Nowadays, it is difficult to evaluate agriculture efficiency other than the economic, but environmental and social aspects should start to be of consideration, and they also should be economically compensated.

On the other hand, in regions where drought periods can occur, having alternative methods to produce a source of clean and no harmful water for livestock drinking purposes are necessary to avoid dramatic and non-welfare scenarios as not having water for animals or reducing river flows for the maintenance of the natural ecosystem.

6 References

Alcalde-Sanz, L., and B. M. Gawlik. *Water Reuse in Europe - Relevant guidelines, needs for and barriers to innovation.* European Commission doi:10.2788/29234., 2014.

Amador, P. P., R. M. Fernandes, M. C. Prudêncio, M. P. Barreto, and I. M. Duarte. "Antibiotic resistance in wastewater: occurrence and fate of Enterobacteriaceae producers of class A and class C β-lactamases." *J Environ Sci Health A Tox Hazard Subst Environ Eng*, 2015: 50(1), 26-39.

Amador, PP, Fernandes, RM, Prudêncio, MC, Barreto, MP, and Duarte, IM. "Antibiotic resistance in wastewater: occurrence and fate of Enterobacteriaceae producers of class A and class C β-lactamases." *J Environ Sci Health A Tox Hazard Subst Environ Eng.*, 2015: 50(1), 26-39. doi: 10.1080/10934529.2015.964602.

Angelakis, A.N., and Gikas, P. "Water reuse: Overview of current practices and trends in the world with emphasis on EU states." *Water Utility Journal*, 2014: 8: 67-78.

Asano, T., Tchobanoglous, G., Leverenz, H.L., and Tsuchihashi, H. *Water Reuse: Issues, Technologies, and Applications.* McGraw-Hill Professional Publishing, 2007.

ASTM. Standard practice for integrity testing of water filtration membrane system. West Conshohocken, Pa. Patente ASTM D6908-03. 2003.

Australian Government Dept. of the Environment. "Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Volume 1, The Guidelines." *Volume 1, The Guidelines.* 2000. https://www.environment.gov.au/system/files/resources/53cda9ea-7ec2-49d4-af29-d1dde09e96ef/files/nwqms-guidelines-4-vol1.pdf.

AWWA Subcommittee on periodical publications. «Microfiltration and ultrafiltration membranes for drinking water.» *Journal AWWA*, 2008: 100:12, 84-97.

Ayers, RS, and Westcot, DW. "Water quality for agriculture." *Irrig. Drain. FAO*, 1985: 174 pp.

Bahman, AM, JA Rooket, and JH Topps. "The performance of dairy cows offered drinking water of low or high salinity in a hot arid climate." *Anim. Prod.*, 1993: 57:23-28.

Bdour, AN., Hamdi, MR., and Tarawneh, Z. "Perspectives on sustainable wastewater treatment technolgies and reuse options in the urban areas of the Mediterranean region." *Desalination*, 2009: 237 (1–3), 162-174. doi:10.1016/j.desal.2007.12.030.

Ben Said, L., et al. "Diversity of enterococcal species and characterization of high-level aminoglycoside resistant enterococci of samples of wastewater and surface water in Tunisia." *Sci Total Environ*, 2015: 530-531: 11-7.

Bixio, D., et al. "Wastewater reuse in Europe." *Desalination*, 2006: 187, 89–101.

Bixio, D., et al. "Wastewater reuse in Europe." *Desalination*, 2006: 187, 89–101.

Boletín Oficial del Estado. "Real Decreto 1620/2007, de 7 de diciembre, por el que se establece el régimen jurídico de la reutilización de las aguas depuradas." BOE, núm. 294, de 8 de diciembre de 2007, páginas 50639 a 50661, 2007.

Bouraouia, F., and B. Grizzetti. "Modelling mitigation options to reduce diffuse nitrogen water pollution from agriculture." *Science of The Total Environment*, 2014: 468–469:1267–1277.

Brun-Hansen, H. C., A. H. Kampen, and A. Lund. "Hematologic values in calves during the first 6 months of life." *Vet. Clin. Pathol.*, 2006: 35: 182-187.

Canadian Council of Ministers of the Environment. *Canadian Water Quality Guidelines for the Protection of Agricultural Water Uses.* Canadian Environmental Quality Guidelines, 1999.

Challis, DJ, JS Zeinstra, and MJ. Anderson. "Some effects of water quality on the performance of high yielding cows in an arid climate." *Vet. Rec.*, 1987: 120:12-15.

Cirelli, G. L., S. Consoli, F. Licciardello, R. Aiello, F. Giuffrida, and C. Leonardi. "Treated municipal wastewater reuse in vegetable production." *Agricultural Water Management*, 2012: 104, 163-170.

Cotton, C.A., D.M. Owen, G.C. Cline, and T.P. Brodeur. "UV disinfection costs for inactivating Cryptosporidium." *Journal AWWA*, 2001: 82-94.

Dalton, H.R., Bendall, R., Ijaz, S., and Banks, M. "Hepatitis E: an emerging infection in developed countries." *The Lancet Infectious Diseases*, 2008: Volume 8 , Issue 11 , 698 - 709.

Department of Environment, Food and Rural Affairs. «Sustainable water for livestock.» 2010. http://sciencesearch.defra.gov.uk/Default.aspx?Menu=Menu&Module=More&Location=None&ProjectID=17363&FromSearch=Y&Publisher=1&SearchText=WU0132&SortString=ProjectCode&SortOrder=Asc&Paging=10#Description .

Department of Water Affairs and Forestry. "South African Water Quality Guidelines. Volume 5, agricultural use: livestock watering." 1996. http://www.iwa-network.org/filemanager-uploads/WQ_Compendium/Database/Future_analysis/081.pdf.

EEA. «Towards Efficient Use of Water Resources in Europe.» Report No 1/2012.

Environment Protection and Heritage Council, the Natural Resource Management Ministerial Council and the Australian Health Ministers' Conference. *National Guidelines for Water Recycling: Managing Health and Environmental Risks.* Canberra: Biotext Pty Ltd, 2006.

EPA. *Current drinking water standards (human).* Washington D.C.: Environmental Protection Agency, Office of Groundwater and Drinking Water (EPA-815-K-97-002), 1997.

EPA Victoria. «Guidelines for Environmental Management - Use of reclaimed water.» 2003.

FAO. *Irrigation and Drainage Paper, 29 Rev. 1.* Rome, Italy: Food and Agriculture Organization of the United Nations, 1985.

FAO, Food and Agriculture Organization of the United Nations. *AQUASTAT - Water uses* . August 4, 2016. http://www.fao.org/nr/water/aquastat/water_use/index.stm.

Food Drink Europe. «Data and Trends of the European Food and Drink Industry 2014-2015.» 2015. http://www.fooddrinkeurope.eu/uploads/publications_documents/Data_and_Trends_2014-20152.pdf.

Forsberg, K. J., A. Reyes, B. Wang, E. M. Selleck, M. O. Sommer, and G. Dantas. "The shared antibiotic resistome of soil bacteria and human pathogens." *Science*, 2012: 337 (6098), 1107-11.

Forslund, A., et al. "Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater." *Water Research*, 2012: 46 (18), 5917-5934.

Forslund, A., et al. "Escherichia coli contamination and health aspects of soil and tomatoes (Solanum lycopersicum L.) subsurface drip irrigated with on-site treated domestic wastewater." *Water Research*, 2012: 46 (18), 5917-5934.

Garcia, J., Rousseau, DPL., Morató, J., Lesage, E., Matamoros, V., and Bayona, JM. "Contaminant Removal Processes in Subsurface-Flow Constructed Wetlands: A Review." *Critical Reviews in Environmental Science and Technology*, 2010: 40, 561-661, doi: 10.1080/10643380802471076.

Genther, O.N., and Beede, B.K. "Preference and drinking behaviour of lactating dairy cows offered water with different concentrations, valences, and sources of iron." *J. Dairy Sci.*, 2013: 96:1164-1176.

Government of New Brunswick. *Farm Water Quality Considerations.* Agriculture, Aquaculture and Fishering, 2014.

Gullberg, E., L. M. Albrecht, C. Karlsson, L. Sandegren, and D. I. Andersson. "Selection of a multidrug resistance plasmid by sublethal levels of antibiotics and heavy metals." *mBio*, 2014: 5(5): e01918-14.

Hartley, TW. «Public perception and participation in water reuse.» *Desalination*, 2006: 187 (1-3), 115-126.

Hassen A., Mahrouk M., Ouzari H., Cherif M., Boudabous A., and Damelincourt JJ. "UV disinfection of treated wastewater in a large-scale pilot plant and inactivation of selected bacteria in a laboratory UV device." *Bioresource Technology*, 2000: 74 (2), 141-150.

Hijnen, W.A.M., Beerendonk, E.F., and Medema, G.J. "Inactivation credit of UV radiation for viruses, bacteria and protozoan (oo)cysts in water: A review." *Water Research*, 2006: 40, 3-22.

Hochstrat, R., Wintgens, T., Melin, T., and Jeffrey, P. "Assessing the European wastewater reclamation and reuse potential-a scenario analysis." *Desalination*, 2006: 188, 1-8.

Huang, J.J., et al. "UV light tolerance and reactivation potential of tetracycline-resistant bacteria from secondary effluents of a wastewater treatment plant." *Journal of Environmental Sciences*, 2016: 41, 146-53.

Jaster, EH, JD Schuh, and TN Wegner. "Physiological effects of saline drinking water on high producing dairy cows." *J. Dairy Sci.*, 1978: 61:66-71.

Jimenez, B., and Asano, T. *Water Reuse: An International Survey of Current Practice, Issues and Needs.* London, UK.: IWA Publishing., 2008.

Kellis, M., Kalavrouziotis, I.K., and Gikas, P. "Review of wastewater reuse in the Mediterranean countries, focusing on regulations and policies for municipal and industrial applications." *Glob. NEST J.*, 2013: 15 (3), 333-350.

Kertz, A. F., L. F. Reutzel, and J. H. Mahoney. "Ad libitum water intake by neonatal calves and its relationship to calf starter intake, weight gain, feces score, and season." *J. Dairy Sci.*, 1984: 67:2964-2969.

Klinkon, M., and J. Jezek. *Values of blood variables in calves. A bird's-eye view of veterinary medicine.* Dr. Carlos C. Perez-Marin (Ed.), ISBN: 978-953-51-0031, InTech, available from: http://www.intechopen.com/, 2014.

Knapp, C.W., J. Dolfing, P. A. Ehlert, and D. W. Graham. "Evidence of increasing antibiotic resistance gene abundances in archived soils since 1940." *Environ Sci Technol*, 2010: 44(2): 580-7.

Kul, O., S. Karahan, M. Basalan, and N. Kabakci. "Poliencephalomalacia in cattle: a consequence of prolonged feeding barley malt sprouts." *J. Vet. Med. A.*, 2006: 53:123-128.

Langenhoff, A., et al. "Microbial removal of the pharmaceutical compounds Ibuprofen and Diclofenac from wastewater." *Biomed Res Int*, 2013: 2013: 325806.

Langholz, J. A., and M. T. Jay-Russell. "Potential role of wildlife in pathogenic contamination of fresh produce." *Human–Wildlife Interactions*, 2013: 7 (1), 140–157.

Langholz, J. A., and M. T. Jay-Russell. "Potential role of wildlife in pathogenic contamination of fresh produce." *Human–Wildlife Interactions*, 2013: 7 (1), 140–157.

Lateef SJ, Soh BZ, and Kimura K. "Direct membrane filtration of municipal wastewater with chemically enhanced backwash for recovery of organic matter." *Bioresource Technology*, 2013: 150, 149-155.

Latz, E., T. Xiao, and A. Stutz. "Activation and regulation of the inflammasomes." *Nature Reviews*, 2013: 13, 397-411.

Lopez A, Pollice, A., Laera, G., Lonigro, A., and Rubino, P. "Membrane filtration of municipal wastewater effluents for implementing agricultural reuse in Southern Italy." *Water Sci. Technol.*, 2010: 62 (5), 1121-1128.

Lopez, A., et al. "Agricultural wastewater reuse in Southern Italy." *Desalination*, 2006: 187: 323-334.

Lopez, A., et al. "Agricultural wastewater reuse in Southern Italy." *Desalination*, 2006: 187: 323-334.

Maenz, D. D., Patience, J.F., and Wolynetz, M.S. "The influence of the mineral level in drinking water and the thermal environment on the performance and the intestinal fluid flux of newly-weaned pigs." *J. Anim. Sci.*, 1994: 72:300-308.

McKinney, C. W., y A. Pruden. «Ultraviolet disinfection of antibiotic resistant bacteria and their antibiotic resistance genes in water and wastewater.» *Environ Sci Technol*, 2012: 46 (24), 13393-400.

Morugán-Coronado, A., F. Garcia-Orenes, J. Mataix-Solera, V. Arcenegui, and J. Mataix-Beneyto. "Short-term effects of treated wastewater irrigation on Mediterranean calcareous soil." *Soil & Tillage Research*, 2011: 112, 18–26.

Mounzer, O., Pedrero-Salcedo, F., Nortes, PA., Bayona, JM, Nicolás, E., and Alarcón, JJ. "Transient soil salinity under the combined effect of reclaimed water and regulated deficit drip irrigation of Mandarin trees." *Agricultural Water Management*, 2013: 120: 23-29.

Nelson, KY, McMartin, DW., Yost, CW., Runtz, KJ., and Ono, T. "Point-of-use water disinfection using UV light-emitting diodes to reduce bacterial contamination." *Environmental Science and Pollution Research*, 2013: 20 (8), 5441-5448.

Nicolás, E., et al. "Long-term physiological and agronomic responses of mandarin trees to irrigation with saline reclaimed water." *Agricultural Water Management*, 2016: 166:1-8.

Norton-Brandão, D., S. M. Scherrenberg, and J. B. van Lier. "Reclamation of used urban waters for irrigation purposes - A review of treatment technologies." *Journal of Environmental Management*, 2013: 122, 85-98.

NRC. *Mineral Tolerance of Domestic Animals.* Washington D.C.: National Academy of Sciences, 1980.

NRC. *Nutrients and Toxic Substances in Water for Livestock and Poultry.* Washington D.C.: National Academy of Sciences, 1974.

Olkowski, A.A. *Livestock water quality : a field guide for cattle, horses, poultry and swine.* Ottawa: Agriculture and Agri-Food Canada, 2009.

Ontario Ministry of the Environment. *Water Management.* Goals, Policies, Objectives and Implementation Procedures of the Ministry of the Environment, 1984.

Palese, A. M., V. Pasquale, G. Celano, G. Figliuolo, S. Masi, and C. Xiloyannis. "Irrigation of olive groves in Southern Italy with treated municipal wastewater: Effects on microbiological quality of soil and fruits." *Agriculture, Ecosystems & Environment*, 2009: 129, 43-51.

Parsons, LR, Sheikh, B, Holden, R., and York, DW. "Reclaimed water as an alternative water source for crop irrigation." *HortScience*, 2010: 45(11):1626-1629.

Pavio, N., Meng, X-J., and Doceul, V. "Zoonotic origin of hepatitis E." *Current Opinion in Virology*, 2015: Volume 10, Pages 34–41.

Pedrero, F, Maestre-Valero, JF, Mounzer, O, Alarcón, JJ, and Nicolás, E. "Physiological and agronomic mandarin trees performance under saline reclaimed water combined with regulated deficit irrigation." *Agricultural Water Management*, 2014: 146:228-237.

Pedrero, F., et al. "Response of young 'Star Ruby' grapefruit trees to regulated deficit irrigation with saline reclaimed water." *Agricultural Water Management*, 2015: 158:51-60.

Polemio, M. «Monitoring and management of karstic coastal groundwater in a changing environment (Southern Italy): a review of a regional experience.» *Water*, 2016: 8, 148.

Pollice, A., A. Lopez, G. Laera, P. Rubino, and A. Lonigro. "Tertiary filtered municipal wastewater as alternative water source in agriculture: a field investigation in Southern Italy." *Science of the Total Environment*, 2004: 324, 201–210.

Pollice, A., A. Lopez, G. Laera, P. Rubino, and A. Lonigro. "Tertiary filtered municipal wastewater as alternative water source in agriculture: a field investigation in Southern Italy." *Science of the Total Environment*, 2004: 324, 201–210.

Pourreza, J., N. Nili, and M.A. Edriss. "Effect of saline water on eggshell quality of Leghorn and native hens." *J. Agric. Tech.*, 2000: Iran, 2:3-8.

Raisbeck, M.F., et al. *Water Quality for Wyoming Livestock & Wildlife, A Review of the Literature Pertaining to Health Effects of Inorganic Contaminants.* University of Wyoming Department of Veterinary Sciences, UW Department of Renewable Resources, Wyoming Game and Fish Department, and Wyoming Department of Environmental Quality, 2011.

Regione Puglia. «Rules and measures for the reuse of treated wastewater (in italian).» Vol. Bollettino Ufficiale della Regione Puglia. 20 de 04 de 2012. 12410–12446.

Rezvani, F., M. R. Mehrnia, and A. A. Poostchi. "Optimal operating strategies of SFDM formation for MBR application." *Separation and Purification Technology*, 2014: 124, 124–133.

Richardson, S. D., M. J. Plewa, E. D. Wagner, R. Schoeny, and D. M. DeMarini. 2007. "Occurrence, genotoxicity, and carcinogenicity of regualted and emerging didsinfection by-products in drinking water: A review and roadmap for research." *Mutat. Res.*, 2007: 636:178.

Rizzo, L., et al. "Urban wastewater treatment plants as hotspots for antibiotic resistant bacteria and genes spread into the environment: a review." *Sci Total Environ*, 2013: 447, 345-60.

Roland, L., M. Drillich, and M. Iwersen. "Hematology as a diagnostic tool in bovine medicine. ." *J. Vet. Diagnos. Invest.* , 2014: 26, 592-598.

Rosen, R. «Mass spectrometry for monitoring micropollutants in water.» *Curr Opin Biotechnol*, 2007: 18(3), 246-51.

Ruiz-Sánchez, MC, Domingo, R., and Castel, JR. "Deficit irrigation in fruit trees and vines in Spain." *Spanish J. Agri. Res.*, 2010: 8(S2), S2-S20.

Schlink, A.C., M.L. Nguyen, and G.J. Viljoen. "Water requirements for livestock production: a global perspective." *Revue scientifique et technique (International Office of Epizootics)*, 2010: 29 (3), 603-619.

Scognamiglio, R. «Transboundary management of water resources: the Italian experience in Basilicata and Apulia.» En *Food security under water scarcity in the Middle East: Problems and solutions*, de A. Hamdy, & R. Monti, 269-278. Bari: CIHEAM, 2005.

Solomon, R, J Miron, D Ben-Ghedalia, and Z. Zomberg. "Performance of high producing dairy cows offered drinking water of high and low salinity in the Arava desert." *J. Dairy Sci.*, 1995: 78:620-624.

Terré, M., C. Tejero, and A. Bach. "Long-term effects on heifer performance of an enhanced-growth feeding programme applied during the preweaning period." *J. Dairy Res.*, 2009: 76, 331-339.

Tulipano, L., and M. D. Fidelibus. "Mechanisms of groundwater salinization in a coastal karstic aquifer subject to overexploitation." *17th Salt Water Intrusion Meeting, 6-10 may.* Delft, 2002.

United States Department of Labor. *Bureau of Labor Statistics - Consumer Price Index.* August 4, 2016. http://www.bls.gov/cpi/.

Vergine, P., A. Lonigro, C. Salerno, P. Rubino, G. Berardi, and A. Pollice. "Nutrient recovery and crop yield enhancement in irrigation with reclaimed wastewater: a case study." *Urban Water Journal*, 2016.

Vergine, P., R. Saliba, C. Salerno, G. Laera, G. Berardi, and A. Pollice. "Fate of the fecal indicator Escherichia coli in irrigation with partially treated wastewater." *Water Research*, 2015: 85, 66-73.

Vergine, P., R. Saliba, C. Salerno, G. Laera, G. Berardi, and A. Pollice. "Fate of the fecal indicator Escherichia coli in irrigation with partially treated wastewater." *Water Research*, 2015: 85, 66-73.

Wu, C, Spongberg, AL, Witter, JD, and Sridhar, BB. "Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from bio-solids treated soil." *Ecotoxicol. Environ. Saf.*, 2012: 85, 104-9.

Wu, C., A. L. Spongberg, J. D. Witter, y B. B. Sridhar. «Transfer of wastewater associated pharmaceuticals and personal care products to crop plants from biosolids treated soil.» *Ecotoxicol Environ Saf.*, 2012: 85, 104-9.

Xiloyannis, C., G. Montanaro, and A. Sofo. "Water emergency. Proposal in order to contain drought damages in fruit crops [Apulia - Basilicata] (in Italian)." *Frutticoltura*, 2002: 7-8, 19-27.

Yuan, J., H. Liu, L. H. Zhou, and Lu W.Q. "Oxidative stress and DNA damage induced by drinking-water chlorination byproduct 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX) in mice." *Mutat Res.*, 2006: 609:129-136.

Zhang, D., R.E. Moreng, and D. Balnave. "Reproductive performance of artifically inseminated hens receiving saline drinking water." *Poultry Sci. 70:776-779*, 1991.

Zhuang, Y., et al. "Inactivation of antibiotic resistance genes in municipal wastewater by chlorination, ultraviolet, and ozonation disinfection." *Environ Sci Pollut Res Int*, 2012: 22 (9): 7037-44.

Zinn, R.A., Álvarez, E., Méndez, M., Montano, M., Ramírez, E., and Shen, Y. "Influence of dietary sulfur level on growth performance and digestive function in feedlot cattle." *J. Anim. Sci.*, 1997: 75:1723-178.