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DEPURATION OF CITRUS PROCESSING WASTEWATER IN AERATED LAGOONS: OPTMISATION OF OPERATIVE CONDITIONS AND DESIGN OF AN AUTOMATED CONTROL SYSTEM

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Abstract/ Sommario

Aerated lagoon systems are widely adopted as a robust solution for the treatment of agro-industrial wastewaters, particularly where operational simplicity, low capital costs and high hydraulic flexibility are required. Their performance is strongly influenced by oxygen transfer efficiency, organic load and nutrient availability. In high-strength effluents characterised by inhibitory compounds and extreme variability of conditions, the optimisation of aeration strategies is essential for treatment efficiency and energy sustainability.

This Ph.D. research explores the optimisation and scale-up of aerated lagoon systems for the treatment of citrus-processing wastewater (CPW). This high-strength agro-industrial effluent is characterised by high chemical oxygen demand (COD), acidic pH, severe COD/N imbalance and the presence of essential oils and phenolic compounds.

The research was structured into two sequential phases: (i) laboratory-scale optimisation, and (ii) pilot-scale validation based on development and implementation automated aeration control system of dissolved oxygen (DO).

At laboratory scale (in 100-L reactors), a one-factor-at-a-time experimental design was adopted to independently evaluate the influence of aeration regime, essential oil concentration and COD/N ratio on depuration efficiency. The results demonstrated that continuous aeration, partial reduction of essential oil concentration (80% dilution with restoration of organic matter) and intermediate nitrogen supplementation significantly enhanced COD and phenol removal, fastened pH stabilisation and promoted microbial activity. These findings identified a robust operational configuration suitable for scale-up.

The optimal conditions were subsequently experimented to a 1000-L pilot-scale system consisting of two parallel tanks: one operated under constant aeration and the other equipped with DO-based automated control. Both reactors achieved effective organic matter degradation and phenolic reduction, confirming the scalability of laboratory findings. However, when treating real high-strength CPW, dissolved oxygen concentrations remained persistently below the control threshold due to extremely high oxygen demand. As a consequence, the automated system continuously worked, similarly to the fixed-aeration tank, without an effective aeration modulation or measurable energy savings.

These results demonstrate that while aerated lagoons are highly resilient for the treatment of high-strength citrus wastewater, DO-based aeration control is constrained by intrinsic oxygen demand under extreme loading conditions. Automated aeration strategies are therefore more suitable for medium- or low-strength effluents, where dissolved oxygen concentrations can periodically exceed control thresholds and enable effective modulation of blower operation.

Overall, this research provides an integrated experimental framework for lagoon optimisation and scale-up, clarifies the operational limits of DO-based automation, and contributes to the rational design of sustainable lagoon systems for agro-industrial wastewater treatment.

I sistemi di lagunaggio aerato rappresentano una soluzione ampiamente diffusa ed affidabile per il trattamento di reflui agro-industriali, in particolare laddove sono richieste semplicità gestionale, flessibilità idraulica e contenuti costi di investimento/gestione. Le prestazioni di tali sistemi dipendono strettamente dall'efficienza del trasferimento di ossigeno, dal carico organico dei reflui e dall'equilibrio dei nutrienti del refluo trattato. In presenza di effluenti ad elevato carico organico, caratterizzati da alta concentrazione composti inibenti e forte variabilità delle condizioni operative, l'ottimizzazione delle strategie di aerazione diventa determinante per l'efficienza depurativa e la sostenibilità energetica del processo.

La presente ricerca ha valutato l'ottimizzazione e l'"upscaling" di sistemi di lagunaggio aerato per il trattamento delle acque reflue prodotte dalla lavorazione degli agrumi (ARA). Tali effluenti agro-industriali presentano un alto carico organico, caratterizzato da elevato COD, pH acido, forte squilibrio del rapporto COD/N e presenza di oli essenziali e composti fenolici.

La ricerca si è articolata in due fasi successive: (i) ottimizzazione su scala di laboratorio e (ii) validazione su scala pilota e sviluppo/implementazione di un sistema di controllo automatico dell'aerazione basato sulla misura dell'ossigeno disciolto (OD) in tempo reale.

Nella fase di laboratorio (reattori da 100 L), è stato adottato un approccio sperimentale "one-factor-at-a-time" per valutare indipendentemente l'effetto del regime di aerazione, della concentrazione di oli essenziali e del rapporto COD/N sull'efficienza depurativa. I risultati hanno evidenziato che l'aerazione continua, la riduzione del 80% della concentrazione di oli essenziali con ripristino del carico organico e un apporto equilibrato di azoto migliorano significativamente la velocità di rimozione del COD e dei composti fenolici, favoriscono la stabilizzazione del pH e supportano l'attività microbica. Tali condizioni operative sono state individuate come configurazione ottimale per il passaggio alla scala superiore.

Tali condizioni ottimali sono state quindi applicate a un impianto pilota da 1000 L costituito da due vasche parallele: una soggetta con aerazione continua a portata costante ed una dotata di sistema automatico di modulazione dell'aerazione basato sulla misurazione dell'OD. Entrambe le vasche hanno mostrato una rapida neutralizzazione del pH e un'elevata rimozione della sostanza organica, confermando la trasferibilità dei risultati ottenuti in laboratorio. Tuttavia, nel trattamento di ARA reali ad alto carico organico, la concentrazione di ossigeno disciolto è rimasta costantemente al di sotto della soglia di controllo a causa dell'elevatissima domanda di ossigeno. Di conseguenza, il sistema automatico ha operato in modo continuo, analogamente alla vasca di controllo, senza consentire un'effettiva modulazione dell'aerazione né un risparmio energetico misurabile.

I risultati dimostrano che i sistemi di lagunaggio aerato sono altamente efficaci nel trattamento delle acque reflue agrumarie anche in condizioni di carico organico elevato, ma che il controllo automatico basato sul DO è limitato dall'elevata domanda di ossigeno intrinseca a tali reflui. La modulazione automatica dell'aerazione risulta più vantaggiosa nel trattamento di reflui a carico medio o ridotto, in cui le concentrazioni di ossigeno possono superare periodicamente le soglie di controllo, consentendo una reale flessibilità operativa.

Nel complesso, la ricerca fornisce un quadro sperimentale integrato per l'ottimizzazione e l'upscaling di sistemi di lagunaggio aerato, chiarisce i limiti operativi del controllo basato sull'OD e contribuisce alla progettazione razionale di sistemi a maggiore consapevolezza energetica per il trattamento di reflui agro-industriali.

CHAPTER 1: INTRODUCTION

1.1 The citrus sector on a global scale

Citrus fruits represent one of the most widely cultivated crop groups worldwide and play a fundamental role in global agriculture, particularly in tropical and subtropical regions (Panwar et al., 2021; Schimmenti et al., 2013). According to the most recent FAO statistics, global citrus production reached approximately 169 million tonnes in 2023, confirming a long-term growth trend in the sector (FAO, 2025). More detailed estimates from the USDA Foreign Agricultural Service indicate that, considering the main commercial categories (oranges, tangerines/mandarins, lemons/limes and grapefruit), global output for the 2023/24 season is expected to reach around 103.7 million tonnes (USDA FAS, 2024).

Market analyses confirm the progressive expansion of the citrus sector, which is forecasted to get a global market value of f approximately EUR 123 billion by 2025, with an expected annual growth of around 6% up to 2030 (Market Research Future, 2025). About three-quarters of global citrus production output is consumed fresh, while 20-25% is processed to produce juices and concentrates, jams, candied fruit, dehydrated products, essential oils, pectin, flavouring extracts, and various bio-based commodities (Mahato et al., 2019). Major processing industries are in Brazil, the United States, Mexico, Argentina, Mainland China, and Spain, where highly mechanised operations support large-scale transformation.

Mediterranean countries, particularly Spain and Italy, hold a strategic position in supplying both fresh fruit and raw citrus for processing, thanks to favourable climatic conditions and long-established supply chains (FAO, 2023). From an economic and social perspective, the citrus industry plays a key role in supporting rural development and employment across producing regions. In countries such as Italy and Spain, processing activities are deeply rooted within the socio-economic fabric, where cooperatives and family-owned firms contribute to the dynamics of local agro-industrial systems (Rivera et al., 2024; Schimmenti et al., 2013). The cultural value of citrus cultivation is equally significant: landscapes dominated by citrus orchards, especially in Mediterranean areas, such as Sicily, Valencia Region, and Andalusia, are emblematic of regional identity, gastronomy, and traditional knowledge (Zech-Matterne and Fiorentino, 2017).

Overall, the global citrus industry represents a dynamic and economically important sector that combines long-standing agricultural traditions with increasingly advanced processing technologies. Its scale and complexity underscore the need for efficient and sustainable wastewater management strategies, an issue that becomes central in the subsequent sections of this thesis.

1.2 The citrus industry

Industrial processing of citrus fruits relies predominantly on four species - orange, mandarin, lemon, and grapefruit - that together account for about 98% of the transformation chain (Satari and Karimi, 2018; Zheng et al., 2016). Oranges remain the most consumed and processed citrus fruit, accounting for more than half of global production and representing the dominant share of fruit processed worldwide. Estimates indicate 52-55% of total harvests and nearly 80% of citrus fruit in the industrial transformation chain (FAO, 2023; USDA FAS, 2024).

Citrus processing typically involves sequential operations, such as fruit washing, sorting and grading, juice extraction, essential oil recovery, concentration, drying, packaging, and storage. The design and level of automation of these technological configurations are widely variable depending on plant size, fruit type, and desired product specifications (Ferreira-Leitão et al., 2010; Marín et al., 2007; Torquato et al., 2017; Zema et al., 2019a). An example of a citrus production chain diagram is shown in Fig. 1.1

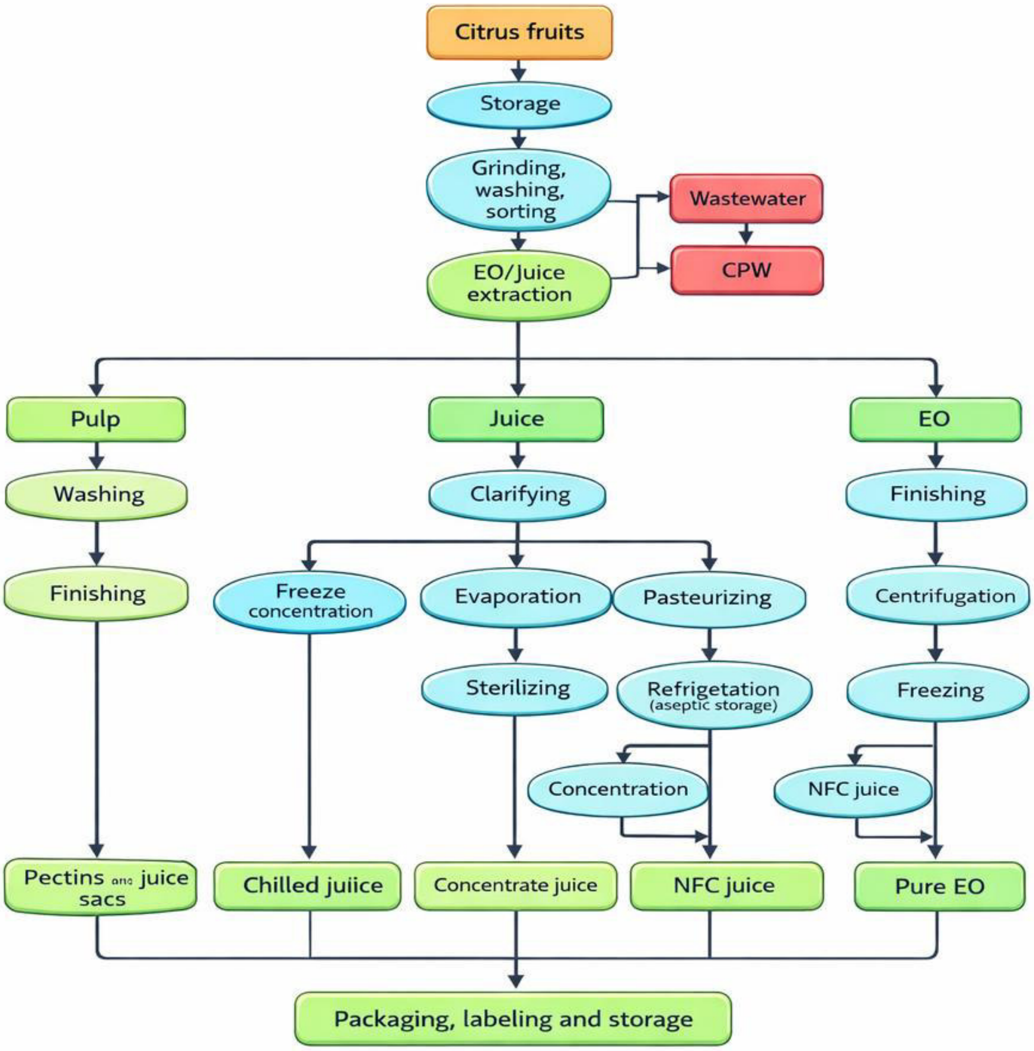


Figure 1.1 - Scheme of citrus processing cycles, products and by-products (adapted from Zema et al., 2018)

Regardless of the adopted technology, citrus transformation produces considerable amounts of residues. Between 50% and 70% of the initial fruit mass is converted into residues (Wikandari et al., 2014; Zema et al., 2019a). Many of these residues, however, are valuable feedstock to produce essential oils, natural flavourings, pectin, dietary fibres, bioactive compounds, and biofuels. This highlights the importance of citrus processing within the broader context of the bio-based and circular economy (Medina-Herrera et al., 2024; Pourbafrani et al., 2010; Welti-Chanes et al., 2025).

Solid residues of citrus processing include rotten fruit, peels, seeds, and pulp fractions (Corsino et al., 2021a; Zema et al., 2019a). Moreover, the citrus industry produces large volumes of wastewater (hereafter referred to as "citrus processing wastewater" and indicated as "CPW") from fruit washing, equipment cleaning, cooling systems, essential-oil extraction, and peel-drying operations. On a global scale, the annual production of CPW exceeds approximately 700 million cubic metres (Lucia et al., 2022), which is furthermore concentrated over short periods due to the seasonality of citrus growth cycles (Zema et al., 2019a).

1.3 Outlines on the characteristics of citrus processing wastewater

CPW represents one of the most complex agro-industrial effluents, and in this section only its main characteristics are briefly introduced, while detailed analyses will be developed in the following chapters. CPW complexity arises from its strong production variability and qualitative heterogeneity, including acidity, high organic content, and the presence of biologically inhibitory compounds. This effluent is produced throughout the citrus-processing chain, including fruit washing, peel abrasion, essential oil and juice extraction, product concentration, and cleaning-in-place operations. The chemical composition of CPW is strongly influenced by the type of citrus fruit processed, technological configuration of the industrial plant, and, to a lesser extent, by the seasonal nature of production campaigns; these factors makes CPW stream highly variable both in quality and quantity (Lucia et al., 2022; Zema et al., 2019a) , creating discontinuous hydraulic and organic loading in receiving water bodies.

From a chemical approach, CPW is characterized by abundant soluble sugars, pectin, and organic acids that undergo rapid microbial fermentation, leading to instability during storage and treatment (Suri et al., 2021). The high content of essential oils - particularly d-Limonene - is one of the major issues of CPW management. These compounds induce a strong antimicrobial activity to the effluent, inhibiting growth and survival of microbial biomass that is essential for biodegradation (i.e., in intensive biological depuration processes) (Gupta et al., 2021; Lin et al., 2024). In addition, CPW contains noticeable amounts of suspended solids, including peel fragments, fibrous residues, and colloidal particulate matter, which contribute to turbidity and increase the biochemical oxygen demand (Zema et al., 2019a). Another critical issue of CPW is its marked variability in pH. Acidic conditions are typically associated with juice residues, while alkaline conditions result from cleaning operations based on potassium hydroxide solutions. The variability in pH destabilizes microorganisms and require adequate buffering strategies through equalisation to ensure effective treatment (Tamburino et al., 2007). Furthermore, CPW is generally nutrient-deficient (especially nitrogen and phosphorus), which can limit microbial growth unless supplemented (Zekri and Obreza, 2014a).

1.4 Management and depuration problems of citrus processing wastewater

From an environmental standpoint, the uncontrolled discharge of CPW can result in oxygen depletion and ecotoxic effects linked to essential oils in receiving water bodies as well as soil degradation, and odour emissions. These effects underline the potentially severe environmental impacts of CPW and highlight the need for robust, adaptable, and cost-effective treatment technologies, able to cope with its intrinsic variability and inhibitory nature (Cinardi et al., 2025; Zema et al., 2019a).

CPW depuration poses several critical challenges. In this section only the main treatment-related issues are briefly introduced, while the following chapters will examine them in greater detail. Unlike the previous paragraph- focused exclusively on the intrinsic characteristics of CPW- here the emphasis is placed on how such characteristics translate into operational and technological obstacles for wastewater treatment systems. A first major issue concerns the extremely high organic loads typically found in CPW. Although the organic fraction is largely biodegradable, its rapid fermentation can cause sudden acidification, destabilising both aerobic and anaerobic biological processes and reducing their operational robustness (Suri et al., 2021). In addition, essential oils and monoterpenes released during peel rupture and oil extraction introduce biologically inhibitory compounds that impair microbial activity even at low concentrations. Their presence often leads to reduced process efficiency, prolonged start-up periods, and the need for dilution or pre-treatment to mitigate toxicity (Calabrò et al., 2024; Gupta et al., 2021; Lin et al., 2024).

A second depuration challenge arises from the strong temporal and compositional variability of CPW. Seasonal production, discontinuous operation, and the alternation between acidic effluents (from juice extraction) and alkaline cleaning solutions (from Cleaning-In-Place, CIP, operations) generate marked fluctuations in pH and organic load. These oscillations can damage microbial consortia, compromise sludge floc structure, and hinder the maintenance of stable biological activity (Tamburino et al., 2007). Seasonal shutdowns further complicate system management, as biomass decay during off-season periods may reduce treatment performance at the beginning of each new campaign (Lucia et al., 2022).

Additionally, CPW tends to be poor in nutrients, particularly in nitrogen and phosphorus, which are essential for microbial growth. Without supply or co-treatment with nutrient-rich effluents, biological processes cannot achieve adequate biomass development or good depuration efficiency (Zekri and Obreza, 2014a). Operational issues are also linked to the presence of suspended solids, peel residues, and fibrous particles, which can cause sedimentation problems, clogging of mechanical equipment, and uneven oxygen transfer in aerobic units (Zema et al., 2019a).

From an environmental and operational standpoint, these factors highlight the difficulty of applying conventional biological technologies to CPW without significant energy inputs, chemical reagents, or continuous supervision (Cinardi et al., 2025). The combination of high organic loads, inhibitory compounds, nutrient imbalance, and strong variability requires treatment solutions that are robust, flexible, and capable of maintaining performance under non-ideal and fluctuating conditions.

1.5 Knowledge gaps and research patterns

From these characteristics, it is evident how lagoon-based systems are a flexible and effective technology within integrated circular-economy frameworks, enhancing the viability of downstream resource-recovery processes (Lucia et al., 2022; Wainaina et al., 2019).

However, although lagoon-based systems have been applied to a wide range of effluents, their viability has often been questioned in the case of agricultural, industrial, and livestock wastewater, where operational stability and treatment efficiency are hard to achieve (Chan et al., 2009; Kimball, 1999; Trias et al., 2004). Historically, most research on lagooning has been concentrated in the United States (Gloyna, E.F, 1971; Mara, 2009), while European experiences are still limited. This sectorial and geographical imbalance has contributed to a fragmented knowledge, with large scientific evidence on municipal wastewater and marginal attention on agro-industrial effluents (namely the CPW) (Calabrò et al., 2024).

Past research has highlighted innovative approaches to improve lagoon performance: for instance, alternative aeration schemes to improve treatment efficiency (Zema et al., 2016a); incorporation of aquatic plants to enhance pollutant removal (Tuleibah et al., 2015); integration with complementary technologies such as phyto-depuration to combine the beneficial effects of the two systems (Denisi et al., 2021a); use of polishing ponds to improve final effluent quality and stabilise residual organic matter (Khorsandi et al., 2014a); hybrid systems combining anaerobic reactors with aerated lagoons (Govahi et al., 2012a). Modelling tools have also been developed to predict lagoon performance under different scenarios and to support more rational design and management strategies (Godini et al., 2021a).

Despite these contributions, existing studies remain largely descriptive, often focusing system efficiency rather than developing optimisation strategies or operational guidelines. Limited attention has been also paid to the aerated lagooning of agri-food effluents, for which the lagoon systems may play a pivotal role overcoming the drawbacks of conventional depuration plants. Only a few investigations have addressed citrus wastewater specifically, providing initial but incomplete evidence on lagoon performance, acclimation dynamics, and the interaction between essential oils and microbial communities (Andiloro et al., 2013a; Bombino et al., 2009; Tamburino et al., 2007; Zema et al., 2016a, 2012a).

The resulting research needs are therefore two-fold. First, there is a clear need to update and expand experimental understanding of lagoon performance in treating citrus industry effluents. In this regard, key knowledge gaps may concern: (i) the optimisation of operational parameters to increase the depuration efficiency of lagoons; and ii) the optimisation of oxygen transfer by aeration to the stored wastewater to reduce the energy needs and lower the associated costs. Second, there is a practical need to translate this knowledge into operational guidelines that can support technicians and practitioners in designing and managing lagoon systems for CPW.

Addressing these gaps is essential not only to advance scientific knowledge but also to support the technical applicability of lagooning in citrus processing industries. A deeper and more targeted characterisation of lagoon performance on CPW will contribute to the development of robust, flexible, and sustainable treatment models tailored specifically to this effluent (Calabrò et al., 2024).

1.6 Research objectives and innovative aspects

The need for this study has been inspired by the critical issues outlined in Sections 1.3 and 1.4 as well as the research gaps reported in Section 1.5. We felt the need to advance the scientific and technical understanding of lagoon-based depuration for CPW, given the importance of the citrus processing industry - and more in general, of citrus production sector - in Southern Italy as well as the pressing need to overcome the economic and environmental drawbacks of the treatment of its effluents. In our opinion, as discussed in Section 1.5, lagoon-based treatments may be a challenging option for this purpose, thanks to their advantages, robustness, low energy demand, and tolerance to fluctuating loads. Surprisingly, despite these beneficial aspects their application to citrus effluents has been insufficiently explored so far, leaving a lack of systematic experimental evidence and operational guidelines.

1.6.1 General and specific objectives

Against this background, the general objective of this research is to develop and test an aerated lagoon treatment model specifically tailored to CPW through a combination of laboratory experimentation with upscaling, producing an automated pilot-scale prototype.

The specific objectives are:

1) to evaluate the performance (i.e., increase in pH, and removal of organic matter and phenols) of an aerated tank simulating the aerobic layer of a full-scale lagoon under controlled laboratory conditions;
2) to identify those operational conditions (aeration rate and duration, dilution of essential oils, and COD/N ratio) that optimise process stability;
3) to characterise the microorganisms' communities that develop in the treated wastewater throughout the depuration process;
4) to translate laboratory insights – i.e., the best operation parameters identified at point 2) - into the design of an automated aerated small-scale lagoon, based on an adaptive dissolved oxygen (DO) that modulates aeration in response to theoretical and real-time oxygen demand;
5) to evaluate the depuration performance during the start-uo, without the use of any inoculum, of the upscale lagoon developed at point 4) to propose a pilot-scale prototype that could be used at the rela scale in citrus processing industries/districts.

These objectives comply with goals of Task 3.2.4 (Spoke 3 - WP 3.2.) of the "Agritech" project (CN_00000022) funded by the "National Research Centre for Agricultural Technologies " (Mission 4, Component 2, Investment 1.4), which gave the financial support of the study.

1.6.2 Novel aspects

The innovative contribution of this study emerges from its thematic focus (i.e., the CPW treatment in aerated lagoons) and its experimental approach (i.e., addressing the research gaps in Section 1.5, particularly about the need for mechanistic understanding, parameter optimisation, and strategies suited to lagoon operation). This contribution may promote the diffusion of lagoon technologies in agro-industrial contexts. In more detail, the novel aspect of the research lies in:

(i) Focus on citrus processing wastewater, an effluent largely underrepresented in the scientific literature on lagoons despite its industrial relevance in Southern Italy and the Mediterranean basin; previous studies (Andiloro et al., 2013a; Bombino et al., 2009; Tamburino et al., 2007; Zema et al., 2016a, 2012a) have offered only preliminary insights into lagoon performance with CPW, that is without systematic experimental investigations;

(ii) Experimental evaluation of aerated lagoon performance during its start-up under controlled conditions, which is less common compared to full-scale monitoring in the scientific literature; while lagoons have historically been viewed as empirical, natural systems, this research applies a structured, quantitative approach to assess the lagoon functioning and depuration performance;

(iii) Development of an automated DO-based aeration control strategy, in the proposed pilot lagoon, which will incorporate an adaptive system to adjust air flow to actual oxygen requirements; this concept has been rarely applied to lagoon systems, which traditionally rely on fixed, energy-intensive aeration patterns;

(iv) Integration of laboratory and pilot-scale experiments; the thesis follows a two-step experimental strategy:

1. Laboratory-scale investigation (controlled aerated lagoon reactor, multiple test series, inhibitory stress tests, variable aeration regimes).
2. Pilot-scale prototype design, based on previous laboratory results and targeted to practical transferability to industrial contexts.

These objectives and novel aspects establish the scientific framework for the experimental programme described in Chapters 4 and 5, which detail the laboratory trials, the development of the automated pilot system, and the evaluation of lagoon performance under CPW-specific conditions.

1.7 Thesis structure

Building upon the background, research needs, and objectives outlined in the previous sections, the work is organised into eight chapters, each addressing a specific component of the research pathway, from the identification of the problem to the experimental validation and upscaling of the proposed technology.

Chapter 1 provides the conceptual background of the dissertation. This part outlines the global importance of the citrus processing industry, the distinctive physico-chemical characteristics of CPW with their environmental impacts, and the major depuration challenges associated with its management. The chapter also reviews the main treatment technologies applied to citrus effluents, highlighting the strategic role of lagoon-based systems (Section 1.5), the research gaps (Section 1.5), and the novel contribution and objectives of the study (Section 1.6). Chapter 2 reports an in-depth analysis of the state of the art of lagoon-based treatment systems. This chapter is based on a systematic review and meta-analysis of the international literature. This review/meta-analysis has been carried out to support the methodological framework defined in the PNRR initial research plan and focusing on treatment efficiencies, design parameters, aeration strategies, microbial dynamics, and applicability of lagoons to agro-industrial effluents. The chapter provides quantitative outcomes derived from the analysed dataset, and critical considerations from the results. The review/meta-analysis acts as the scientific baseline for the experimental phases described in Chapters 4 and 5.

Chapter 3 focuses on CPW characteristics and contextualises the experimental work within a real industrial scenario. The first section characterises the depuration problems associated with citrus processing effluents, i.e., rapid acidification, presence of inhibitory compounds, nutrient imbalance, and production seasonality, by integrating literature evidence with field observations. The second section illustrates the case-study citrus processing industry, detailing the layout and working of the treatment line, the characteristics of the effluent, and the operational constraints that motivate the development of alternative lagoon-based solutions.

Chapter 4 reports the laboratory-scale investigation to test the efficiency of aerated lagoons treating the CPW of the case study under controlled conditions. Building on the objectives defined in Chapter 1, this chapter describes the experimental devices, the tested aeration schemes, monitoring tools and analytical methods, and operation conditions. The results include the treatment performance and process stability under different operating scenarios. This phase was the first step toward validating the pilot-scale system of improved lagoon prototype.

Chapter 5 addresses the start-up (without inoculum) upscaling phase, moving from laboratory-scale experimentation to the design, implementation, and testing of an automated aerated lagoon system in the case study. This chapter describes the engineering development of the pilot unit, the automated dissolved oxygen dosing strategy, and the optimisation criteria applied from lab-scale findings. Results encompass operational dynamics, energy requirements, treatment efficiency, and system response to the wastewater organic load through a comparison of traditional and novel aeration strategies.

Chapter 6 focuses on the development of the pilot-scale lagoon system equipped with an automated dissolved oxygen (DO)-based aeration control strategy. This chapter describes the iterative design process of the automation system, including the initial configuration, the design of the control architecture, and the final implementation based on a Modbus communication protocol and ON/OFF control logic. Attention is paid to sensor–controller integration and operational monitoring.

Chapter 7 reports the pilot-scale upscaling tests performed under real operating conditions. The optimised operational parameters identified at laboratory scale were implemented in two 1000-L reactors, operated under automated and fixed aeration regimes, respectively. The chapter analyses the temporal evolution of pH, COD, TN, phenolic compounds, dissolved oxygen, temperature, highlighting the robustness of aerated lagoon treatment under high organic loads and the operational limitations of DO-based aeration control in extremely high-strength wastewater conditions.

Finally, Chapter 8 summarises the main findings of the research and outlines the overall conclusions. The chapter discusses the practical implications of lagoon optimisation, defines the operational boundaries of DO-based automation, and provides perspectives for future research and full-scale implementation within sustainable agro-industrial wastewater management frameworks.

CHAPTER 2: STATE OF THE ART OF LAGOONING AS WASTEWATER TREATMENT SYSTEM

2.1 Foreword

As anticipated above, a systematic review/meta-analysis of studies about wastewater lagooning was carried out to delineate the state-of-the-art as well as the practical implications and research needs from literature evidence. The related chapter is a restatement of the scientific article published on "Journal of Environmental Management" in 2024 (Calabrò et al., 2024).
Lagoon-based treatment systems represent one of the oldest and most consolidated approaches for wastewater depuration and remain widely adopted across different contexts, including municipal, agro-industrial, and livestock sectors. Their persistence in modern wastewater engineering stems from a combination of simplicity, robustness and adaptability, features that make lagooning particularly attractive for effluents characterised by high variability, strong seasonality and high organic loads. Although lagoons are often perceived as simple basins where natural purification occurs, their functioning results from a complex interplay of physical, chemical and biological mechanisms (Calabrò et al., 2024).
A clear understanding of these mechanisms is essential for interpreting lagoon performance, evaluating their applicability to specific wastewaters such as CPW, and defining appropriate design and operational strategies.

2.2 Classification

Lagoon systems are generally classified according to oxygen availability, (i.e., DO), and dominant metabolic processes (Butler et al., 2017; Kayombo et al., 2005). This classification is useful to understand how different designs exploit ecological stratification and microbial activity to achieve wastewater depuration.
Anaerobic lagoons represent the most oxygen-deprived configuration. Being deeper units, they limit oxygen penetration and create conditions favourable to hydrolysis, acidogenesis, and methanogenesis. These lagoons are particularly suited to high-strength effluents rich in biodegradable organic matter and solids, such as livestock or fruit-processing wastewaters. Their ability to handle concentrated loads and generate biogas is an advantage, but they are often associated with odour emissions and require careful management of sludge accumulation and loading rates (Houghton and Mara, 1992; Mara, 2009).
Moving from strictly anaerobic conditions to systems that balance different metabolic pathways, facultative lagoons combine aerobic activity in the upper layers with anaerobic degradation near the bottom. The surface zone benefits from photosynthetic oxygenation and atmospheric diffusion, while deeper layers sustain fermentative and methanogenic processes. This dual nature makes facultative lagoons one of the most widespread configurations worldwide. They are valued for their operational stability, resistance to load fluctuations, and relatively low maintenance requirements. Their long hydraulic retention times allow substantial removal of organic matter and suspended solids, making them suitable for both municipal and agro-industrial effluents (Butler et al., 2017; Kayombo et al., 2005; Mara, 2009).
A further step in the evolution of lagoon systems is represented by aerated lagoons, which incorporate mechanical aeration to enhance oxygen transfer. While aeration increases treatment capacity and mitigates odour emissions, these systems generally maintain some degree of stratification, preserving both aerobic and anaerobic zones. Aerated lagoons thus represent a hybrid between natural stabilization ponds and activated-sludge systems, combining ecological tolerance with improved process control. They are particularly important for wastewaters with high organic loads or inhibitory compounds, where natural oxygenation alone would be insufficient (Calabrò et al., 2024; Zema et al., 2016a).
Finally, at the downstream end of treatment trains, maturation or polishing ponds are shallow basins designed to refine effluent quality. Their main functions include pathogen reduction through solar irradiation, nutrient uptake via algal and microbial activity, and further removal of fine suspended solids. These ponds are often used as tertiary treatment steps, ensuring compliance with discharge standards or enabling safe reuse of effluents in agriculture (Denisi et al., 2021a; Khorsandi et al., 2014a).
In practice, these lagoon types are rarely used in isolation. They are frequently arranged in series, forming

multi-stage treatment trains capable of addressing different purification requirements. Such configurations exploit the complementary functions of each lagoon type, allowing the treatment of complex wastewaters that require both high organic load removal and adequate polishing before discharge or reuse (Gloyna, E.F, 1971; Mara, 2009).
Table 2.1 presents the classification of lagoon systems, together with their typical treatments, microbial conditions, and the main operational parameters and wastewater properties.
Figure 2.1 illustrates the vertical stratification patterns that characterise each lagoon typology described above, complementing the design parameters summarised in Table 2.1.

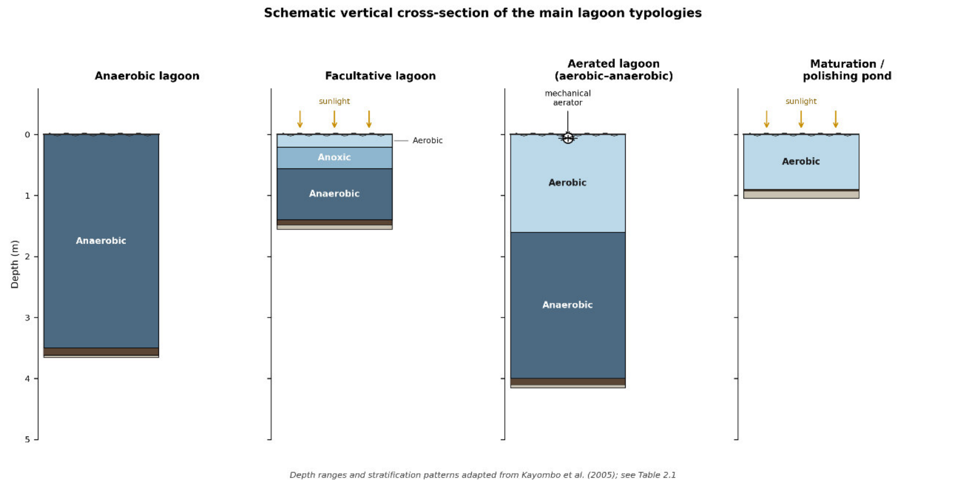


Figure 2.1 - Schematic vertical cross-section of the main lagoon typologies (anaerobic, facultative, aerated, and maturation/polishing), showing indicative depths and stratification patterns adapted from Kayombo et al. (2005) and Table 2.1.

Table 2.1 - Design criteria for lagoon systems

Lagoon Type	Organic Loading Rate [kg BOD/ha·d]	Hydraulic Retention Time [d]	Depth [m][a]	BOD_5 Conversion Efficiency [%]	Typical Influent	Microbial Conditions	Stored Wastewater Properties
Anaerobic	225–1124	20–180	2.4–4.6	50–85	Raw agri-food wastewater and sludge	Microorganisms live in the absence of oxygen, using organic matter as substrate and electron acceptor	BOD: 100–300 mg/L SS: 80–160 mg/L Algae: –
Facultative	17–85	20–180	0.91–1.83	80–95	Raw or partially treated wastewater	Microorganisms use DO from atmosphere or mechanical aeration	BOD: 20–50 mg/L SS: 160–400 mg/L Algae: 40–160 mg/L
Aerated (Aerobic–Anaerobic)	34–112	10–20	3.05–6.07	80–95	Raw or partially treated wastewater	Aerobic microorganisms in upper layer; anaerobic in lower layer	BOD: 20–50 mg/L SS: 100–350 mg/L Algae: 10–40 mg/L
Aerated (Aerobic)	899–2809	3–10	0.91–3.05	80–95	Primary effluent; tertiary	Microorganisms adapt to DO fluctuations	BOD: 20–30 mg/L SS: 250–300 mg/L Algae: –
High-rate Aerobic	67–225	3–6	0.91–1.83	80–95	Soluble organics and nutrients; tertiary	DO is overabundant relative to microbial needs	BOD: 20–30 mg/L SS: 150–350 mg/L Algae: 80–200 mg/L

[a] Depth values converted from imperial units. *Adapted from Kayombo et al., 2005.*

2.3 Functioning principles of lagoons

Lagoons are typically shallow basins, either excavated in the ground or equipped with impermeable liners, designed to retain wastewater for extended hydraulic residence times. These long retention periods allow a progressive transformation of organic matter through aerobic, anoxic and anaerobic pathways, which occur simultaneously in the same basin due to stratification phenomena. Natural processes govern much of lagoon operation: sunlight enhances surface oxygenation through photosynthesis, wind promotes mixing and reaeration, while thermal gradients drive the vertical distribution of oxygen and microbial populations (Gloyna, E.F, 1971; Mara, 2009).

One of the fundamental drivers of lagoon functioning is oxygen dynamics. Oxygen enters the system predominantly through atmospheric reaeration, wind-induced mixing, and photosynthetic oxygen release by algae in the illuminated upper layers. As a result, the spatial and temporal distribution of dissolved oxygen (DO) becomes a key operational parameter, shaping microbial pathways, stabilising organic matter removal, and controlling odour formation. Typical DO concentrations vary widely depending on depth, climatic conditions, and time of day: while surface layers may reach supersaturation during peak photosynthesis, deeper zones rapidly become oxygen-depleted, giving rise to a vertically stratified ecological structure (Henze et al., 2008; Houghton and Mara, 1992).

A defining characteristic of lagoon systems is the development of ecological stratification, a vertical differentiation of redox conditions driven by light penetration, oxygen diffusion, and organic load. The upper layers, directly exposed to sunlight and atmospheric oxygen, tend to remain aerobic and sustain algal communities, nitrifying bacteria, and heterotrophic oxidisers. Below this surface zone, oxygen concentrations progressively decrease, creating anoxic regions where facultative organisms carry out denitrification and other intermediate metabolic pathways. At greater depths, the environment becomes fully anaerobic, supporting fermentation, hydrolysis, and methanogenesis. This coexistence of aerobic, anoxic, and anaerobic processes within a single treatment unit provides lagoons with remarkable resistance to hydraulic and organic fluctuations and enables them to sustain multiple degradation pathways simultaneously, an operational flexibility rarely attainable in high-rate biological reactors, which rely on tightly controlled conditions to prevent process failure (Calabrò et al., 2024; Henze et al., 2008; Houghton and Mara, 1992; Mara, 2009).

Temperature and climatic conditions strongly influence lagoon behaviour. In warm climates, higher temperatures increase microbial activity and favour the volatilisation of odorous compounds and inhibitory molecules, whereas shallow depths promote efficient gas exchange at the air-water interface. Conversely, lower temperatures or limited solar radiation reduce oxygenation and may increase the risk of anaerobic odours. Daily and seasonal fluctuations in irradiance and temperature modulate biological processes, often leading to distinct diel cycles in dissolved oxygen and pH (Walsby et al., 2001; Yoshikawa and Furuya, 2006).

Overall, lagoons function as self-organising biological systems that rely on natural stratification, long retention times, and a diverse microbial community.

2.3.1 Physical and biochemical processes in lagoons

Lagoon systems operate through a combination of physical and biochemical mechanisms that collectively sustain wastewater treatment. The main processes include: (i) sedimentation, (ii) aerobic decomposition, (iii) anaerobic fermentation, (iv) bacterial-algal symbiosis, (v) oxygen transfer across the water surface, and (vi) evaporation (Calabrò et al., 2024). Each of these mechanisms is associated with specific microbial conditions, operational parameters, and properties of the stored wastewater.

A lagoon is a complex ecological system that, even in the absence of wastewater supply, undergoes daily and seasonal variations in water quality. Daily changes are driven by photosynthetic activity under variable light conditions, which modulates dissolved oxygen (DO) availability (Walsby et al., 2001; Yoshikawa and Furuya, 2006). Seasonal fluctuations, in turn, reflect broader climatic variability, including air temperature and rainfall, which influence microbial kinetics, stratification, and evaporation losses.

Among the physical processes, sedimentation plays a central role: suspended solids settle to the bottom, forming a sludge layer that acts as a reservoir of active biomass and contributes to long-term anaerobic degradation. In the upper layers, aerobic decomposition dominates. Bacteria oxidise biodegradable organic compounds into simpler molecules such as carbon dioxide (CO_2), ammonia (NH_3), and phosphate (PO_4^{3-}), using DO as the electron acceptor to sustain their metabolism. In deeper zones, where light and oxygen are absent, anaerobic fermentation takes place. Complex organics are degraded into methane (CH_4), carbon dioxide (CO_2), hydrogen (H_2), ammonium ($\mathbf{NH_3}$), and hydrogen sulphide (H_2S), with the latter often

responsible for unpleasant odours (Houghton and Mara, 1992).
A distinctive feature of lagoons is the development of bacterial-algal symbiosis. In water layers with sufficient sunlight and low turbidity, algae produce oxygen during photosynthesis, which supports heterotrophic bacterial metabolism. In return, bacteria release CO_2 and nutrients that sustain algal growth. This mutualistic interaction enhances treatment efficiency but also introduces daily oscillations: oxygen accumulates during daylight and is consumed at night, leading to alternating aerobic and anoxic phases (Calabrò et al., 2024).
The transfer of oxygen across the water surface further contributes to lagoon performance. The rate of oxygen diffusion depends on the difference between the actual dissolved oxygen concentration and the saturation concentration of clear water, and is influenced by turbulence, temperature, and salinity (Tchobanoglous et al., 2002).
Finally, evaporation represents a minor but context-dependent process. In cool and humid climates, water loss through evaporation is negligible in the overall mass balance. However, under hot and arid conditions, or in lagoons subject to seasonal use, evaporation can significantly affect water balance and treatment efficiency (Sinn et al., 2022).
Taken together, these mechanisms illustrate the ecological complexity of lagoon systems. By hosting multiple microbial guilds adapted to different redox conditions and by integrating physical, chemical, and biological processes, lagoons achieve pollutant removal in a resilient and self-regulating manner.

2.3.2 Factors influencing lagoon performance

The performance of lagoon systems is governed by a combination of design parameters, operational practices, and environmental conditions. Among these, hydraulic residence time (HRT) is the most influential, as it determines the extent of biological transformation and the settling of suspended solids. It is important especially because it defines how long wastewater remains in the lagoon before discharge. In practical terms, HRT corresponds to the ratio between the lagoon volume and the daily inflow of wastewater. If the retention time is too short, microorganisms cannot reproduce or adapt effectively, leading to a decline in biological activity. Conversely, longer retention times allow microbial populations to stabilise and complete their metabolic cycles, though they require larger lagoon volumes (Calabrò et al., 2024).
Equally important is the organic loading rate (OLR), which must be carefully balanced to avoid oxygen depletion and odour formation. Excessive loading, particularly under warm climatic conditions, can accelerate anaerobic activity and the release of unpleasant compounds such as hydrogen sulphide (H_2S), compromising lagoon stability (Cirelli, 2003). Given the critical role of HRT and OLR, achieving an appropriate balance between these parameters is essential to enhance wastewater treatment efficiency, sustain an active microbial community within the lagoon, and optimise the required lagoon volume (Calabrò et al., 2024).
As already mentioned, DO represents a critical operational parameter, as it directly regulates the balance between aerobic and anaerobic processes. Adequate DO concentrations sustain bacterial oxidation of organic matter and nitrification, while low DO levels favour fermentative pathways and odour generation. DO availability is influenced by lagoon depth, surface area, algal photosynthesis, and external aeration strategies, making it a central indicator of lagoon performance (Henze et al., 2008).
Depth and surface area further shape stratification patterns, light penetration, and oxygen transfer. Shallow lagoons favour atmospheric diffusion and algal photosynthesis, while deeper basins promote anaerobic processes and sludge accumulation.
Climatic factors, including temperature, wind, and solar radiation—directly affect microbial kinetics and algal productivity. Warmer temperatures accelerate biological reactions, while solar radiation sustains photosynthetic oxygenation. Wind contributes to surface mixing, enhancing oxygen transfer and reducing stratification. Seasonal variability in these drivers can cause fluctuations in lagoon performance (Henze et al., 2008).
In the case of aerated lagoons, the aeration strategy becomes decisive. Continuous, low-rate, or intermittent aeration influences oxygen distribution, mixing patterns, and energy consumption. Optimised aeration regimes have demonstrated improved performance in agro-industrial applications, offering a balance between treatment efficiency and operational sustainability (Zema et al., 2016a).

2.4 Wastewater characteristics relevant to lagoon operation

Agro-industrial effluents, and particularly CPW, pose distinctive challenges for biological treatment due to their complex composition. They are typically rich in soluble sugars, organic acids, suspended solids, and hydrophobic essential-oil compounds. These constituents undergo rapid fermentation, which can induce significant pH fluctuations and generate conditions that are inhibitory for sensitive microbial populations.

Among the essential oils, d-Limonene is especially problematic: it destabilises microbial membranes and interferes with both aerobic and anaerobic metabolic pathways, thereby reducing the performance of conventional treatment systems (Niaounakis and Halvadakis, 2006; Rajagopal et al., 2013).
Further complications arise from seasonal variability in wastewater generation and from nutrient imbalances, which limit microbial growth and adaptation. Such factors often render high-rate biological reactors unstable, as they depend on tightly controlled operating conditions and are less tolerant to inhibitory shocks. Lagoon systems, by contrast, are naturally more compatible with these effluents. Their extended hydraulic retention times provide the buffer needed for microbial communities to acclimate to inhibitory compounds, while vertical stratification creates ecological niches that support diverse microbial guilds. Additional processes such as volatilisation of hydrophobic compounds and adsorption onto sediments contribute to toxicity attenuation, mitigating the inhibitory effects of substances like d-limonene. Moreover, lagoons can function effectively under discontinuous feeding regimes, which aligns well with the seasonal nature of citrus-processing campaigns.
These combined attributes explain the renewed scientific interest in lagoon systems for CPW treatment, despite initial scepticism regarding their suitability for high-strength industrial effluents (Chan et al., 2009; Kimball, 1999; Trias et al., 2004). By leveraging natural acclimation, stratification, and ancillary attenuation mechanisms, lagoons demonstrate a sustainability that conventional high-rate systems often lack.

2.5 Advantages of lagooning

Lagoon-based treatments are among the most robust, cost-effective, and adaptable solutions for the management of CPW. As outlined in section 1.6.2, their use has drawn the attention of scientists and grown significantly in the urban and industrial contexts, particularly in Mediterranean and semi-arid regions, including agri-food wastewater. Compared with intensive biological treatments, lagoons offer structural, operational, and environmental advantages that align well with the specific constraints of CPW, including high organic loads, inhibitory compounds, and hydraulic fluctuations (Calabrò et al., 2024; Lucia et al., 2022; Zema et al., 2019a).

2.5.1 Operational robustness and tolerance to variable loads

One of the main advantages of lagoon-based systems is their intrinsic robustness under variable hydraulic and organic loading conditions. CPW composition noticeably fluctuates due to the alternate presence of acidic juice residues, alkaline Clean-In-Place (CIP) solutions, and streams rich in EOs, produced by the industrial machines. Conventional biological reactors, especially those based on activated sludge, are generally unable to maintain stable performance under such rapid variations, suffering from floc disruption, foaming, or toxic inhibition. Lagoons, by contrast, provide a large buffering capacity due to their long hydraulic residence time (HRT) and large storage volume, which smooth out the fluctuations and prevent abrupt changes in the microbiological conditions (Lucia et al., 2022; Zema et al., 2019a).
The pronounced microbial stratification that naturally develops in lagoon systems, ranging from aerobic surface layers to anaerobic deep zones, further enhances the treatment capacity of these systems. This stratification allows coexistence of different functional strains (aerobes, facultative anaerobes, fermenters, methanogens), enabling multiple degradation pathways (Calabrò et al., 2024). Such ecological heterogeneity makes lagoon environments less vulnerable to inhibitory compounds such as d-limonene (Gupta et al., 2021; Lin et al., 2024).
Long hydraulic retention times (HRTs), often exceeding several weeks or lasting even months, allow microbial populations to remain in the system long enough to gradually adapt to the specific characteristics of citrus effluents. Evidence from agro-industrial lagoons shows that this prolonged exposure promotes the selection of microbial communities capable of tolerating essential oils and phenolic compounds, thereby reducing their inhibitory effects. As a result, lagoon systems tend to maintain stable treatment performance even during short-term spikes in influent concentration (Calabrò et al., 2024; Lucia et al., 2022).

2.5.2 Low energy demand and economic sustainability

A structural advantage of lagoon systems is their very low energy consumption, which is an essential factor for small and medium citrus processing plants operating with limited technical staff and constrained budgets. Intensive biological systems require continuous and high-rate aeration to support microbial oxidation, often resulting in energy demands up to four times higher when applied to high-strength effluents, such as CPW (Calabrò et al., 2024). Lagoons, particularly facultative or partially aerated systems, operate with minimal mechanical energy input: aeration, when present, is intermittent and primarily aims at preventing odour formation and at enhancing oxygen diffusion.
Investment costs for lagoon construction are significantly lower compared with other conventional technologies, because lagoons typically require earthworks rather than specialised reactors, pressurised

piping, or membrane units. As highlighted in recent analyses, installation and operational costs for lagoon treatments frequently are lower compared to activated sludge or membrane bioreactors, especially when scaled over the short duration of citrus production season (Zema et al., 2019a).
Another economic advantage lies in the reduced need for skilled operators. Lagoon systems have minimal mechanical devices and lack the complex sensors, pumps, and blowers typical of intensive systems. This lower operational complexity makes lagoon systems attractive for decentralised industrial districts, cooperatives, and small firms that cannot rely on full-time supervision (Calabrò et al., 2024).

2.5.3 Enhanced environmental performance and ecosystem integration

Of the several environmental benefits of lagoons, many are particularly valuable in citrus-producing regions where land availability and climatic conditions favour natural treatments. Their large surface area supports oxygen diffusion, sunlight-driven disinfection, and development of macrophytes or biofilms that contribute to pollutant removal. In warm climates typical of Mediterranean citrus districts, hot temperatures accelerate microbial kinetics, enhancing COD removal and promoting the degradation of essential oils and other volatile compounds (Calabrò et al., 2024).
Beyond pollutant removal, lagoons can work as small-scale ecosystems. Photosynthetic activity by algae and aquatic plants supplies oxygen to the stored water, reducing aeration needs. Natural settling removes suspended solids and colloidal compounds, which are abundant in CPW due to peel residues and residual fibrous particles. This passive clarification reduces downstream load if lagoons are used as a preliminary step before advanced treatment.
Furthermore, lagoons exhibit a natural capacity to handle inhibitory or recalcitrant compounds. Essential oils and monoterpenes tend to be diluted in the large, stored water volume, adsorbed onto organic sediment or volatilised at the water-air interface, leading to concentration reductions before biological degradation. These mechanisms allow lagoons to gradually attenuate toxicity, one of the main constraints in intensive depuration systems (Lucia et al., 2022).
From a land-use perspective, lagoon installations can be easily integrated with agricultural landscapes, sometimes doubling as biodiversity-supporting habitats. In citrus-producing regions facing climate stress, lagoon systems also create storage volumes that buffer inputs of stormwater or enable controlled release to subsequent treatment or reuse stages.

2.5.4 Seasonal flexibility and suitability for discontinuous operation

As mentioned above, seasonality is one of the most critical issues of CPW management. Citrus production typically lasts 3-5 months per year, leaving wastewater treatment plants idle for the remaining months. Intensive biological processes suffer from such stop-and-go operation, because microbial populations undergo decay during downtime due to the lack of feeding substrates. This leads to long and unstable start-up phases at the beginning of each citrus production-wastewater depuration campaign. In contrast, lagoon systems can tolerate seasonal inactivity much better. Their large water volume prevents complete drying, and residual biomass stored in sludge layers (although limited due to the limited sludge settlement) provides *inoculum* for quick system reactivation. The long HRT and stable sediment layers allow microbial communities to persist even in low-influent periods, noticeably reducing the start-up time compared with activated sludge processes (Zema et al., 2019a).
This seasonal flexibility also enables lagoons to buffer variations in flow and organic load, providing a more stable influent to downstream units. By acting as an equalisation stage, they improve the performance of subsequent treatments—such as aerobic polishing, constructed wetlands, or biorefinery processes, and are therefore well suited as primary units in hybrid treatment configuration (Calabrò et al., 2024).
Moreover, lagoon systems can also play a central role within integrated treatment and valorisation chains. Particularly when combining depuration and resource recovery, lagoons can act as conditioning stages for agricultural reuse by reducing BOD/COD and suspended solids to levels under soil phytotoxicity threshold.

2.6 Drawbacks of lagooning

Lagoon-based treatment systems, despite their recognised robustness and operational simplicity, present several intrinsic drawbacks that may limit their applicability, particularly when compared with more intensive biological processes. One of the most significant limitations is the extensive land area required to achieve the long hydraulic residence times necessary for effective treatment. This constraint becomes particularly relevant in densely populated regions or in areas where land availability or economic value is a limiting factor (Mara, 2009).
Lagoon performance is also highly sensitive to climatic conditions. Temperature, solar radiation, and wind directly influence microbial kinetics, algal photosynthesis, and oxygen transfer, resulting in pronounced

seasonal fluctuations in treatment efficiency (Metcalf et al., 2004). During colder periods or under unfavourable weather conditions, these systems may experience reduced degradation rates and lower overall performance.
Compared with activated sludge systems, lagoons typically exhibit slower reaction rates and offer limited process control. This makes them less suitable for wastewaters requiring rapid, stable, or tightly regulated treatment (Metcalf & Eddy, 2014). Odour generation represents an additional critical issue, particularly under high organic loading or oxygen-limited conditions, where anaerobic pathways may dominate and lead to the release of reduced sulphur compounds (e.g., H_2S) and volatile fatty acids (Mara, 2009).
Another drawback is the gradual accumulation of sludge at the bottom of lagoons. Over time, this sludge layer can reduce effective volume, impair hydraulic performance, and require periodic removal. Sludge extraction is often operationally demanding, costly, and disruptive, especially in large or ageing lagoon systems.
Finally, conventional lagoon systems generally lack real-time monitoring and automation, resulting in limited adaptability to variable influent characteristics. This limitation is particularly problematic for agro-industrial wastewaters, which are characterised by strong temporal variability, episodic peak loads, and fluctuating organic strength (Metcalf et al., 2004).
Taken together, these drawbacks highlight the need for targeted optimisation strategies, such as controlled aeration, nutrient management, improved monitoring, and partial automation, to enhance treatment efficiency, process stability, and energy sustainability. These considerations provide the rationale for the experimental approach adopted in the present study.

2.7 A systematic review and a meta-analysis of lagoon systems

2.7.1 Materials and methods of the systematic review and meta-analysis

The analysis presented in this chapter is based on a systematic review and a quantitative meta-analysis of lagoon-based wastewater treatment systems, conducted to consolidate existing knowledge and identify performance trends across different lagoon typologies and wastewater categories. The methodological framework follows the structure adopted in the reference review (Calabrò et al., 2024), extended and reorganised to suit the analytical needs of this thesis.
This section synthesizes the main outcomes of both components, systematic and quantitative, offering a critical interpretation of lagoon behaviour, performance drivers, and recurrent operational constraints. Such analysis constitutes the conceptual foundation for the experimental research presented in chapters 4–7 of this thesis.

2.7.1.1 Literature search strategy

The bibliographic research was conducted across major scientific databases, including Scopus®, Web of Science®, Google Scholar®, and ScienceDirect, to ensure comprehensive coverage of lagoon systems applied to wastewater treatment. Search strings combined keywords such as lagoon, stabilisation pond, aerated lagoon, facultative pond, anaerobic pond, wastewater, agro-industrial effluents, citrus wastewater, and treatment performance. In addition, specific abbreviations frequently used in the literature (e.g., LAG for lagoon and STAB POND for stabilisation pond) were included to capture all relevant records. The search aimed to identify peer-reviewed studies on lagoon systems for wastewater treatment published between 2008 and 2023, thereby covering recent developments while excluding outdated operational contexts.
No restrictions were applied regarding the country of publication, thereby minimising geographic or disciplinary bias. The search was performed on titles, abstracts, and keywords to maximise coverage.
The initial search retrieved several hundred records. A first screening excluded technical reports, bachelor's, master's, and doctoral theses, documents that typically do not undergo peer review, as well as papers not published in English. After this step, 131 papers remained. A second screening discarded studies lacking quantitative information or reporting insufficient data (fewer than five operational parameters, as defined in Section 2.7.2). Ultimately, 36 papers were retained for detailed analysis (Fig. 2.3).

2.7.1.2 Inclusion and exclusion criteria

Studies were retained in the review when they described full-scale, pilot-scale, or bench-scale lagoon systems operating under realistic hydraulic residence times and depths. To ensure comparability, at least one performance parameter had to be reported, such as COD, BOD_5, TSS, nutrient removal, pathogen reduction, oxygen dynamics, or sludge behaviour. Furthermore, papers were required to provide sufficient detail on design variables, including depth, surface area, HRT, OLR, and aeration regime. Eligible studies covered a wide range of applications, from municipal and livestock effluents to industrial and agro-industrial wastewaters, with particular attention to citrus-processing wastewater (CPW).

By contrast, studies were excluded if they consisted solely of theoretical modelling without experimental validation, if they relied on laboratory micro-reactors too small to reproduce stratification phenomena, or if they lacked essential operational or performance data. This rigorous filtering ensured that the final dataset was both methodologically sound and directly relevant to lagoon operation.

2.7.1.3 Data extraction and classification

For each selected study, the following variables were extracted and organised into a structured dataset:

- Lagoon type (anaerobic, facultative, aerated, maturation/polishing)
- Wastewater category (municipal, livestock, agro-industrial, mixed industrial)
- Geographical location and climatic context
- Design parameters (depth, surface area, HRT, OLR, aeration intensity)
- Operational conditions (temperature, DO, inflow variability)
- Performance indicators (COD/BOD removal, nutrient removal, pathogen reduction)

Lagoon systems were classified according to the criteria described in Section 2.2, enabling comparison across functionally homogeneous categories.

2.7.1.4 Bibliometric Analysis

The selected papers were further classified according to bibliometric criteria, including year of publication, country of origin, publishing journal, and citation metrics. Citations were considered both in absolute terms and normalised per year of publication, calculated as the ratio between the total number of citations and the years elapsed since publication. This classification provided insights into the temporal evolution of lagoon research, the geographic distribution of contributions, and the journals most active in disseminating knowledge on lagoon systems. (Table 2.2 and Fig. 2.2).

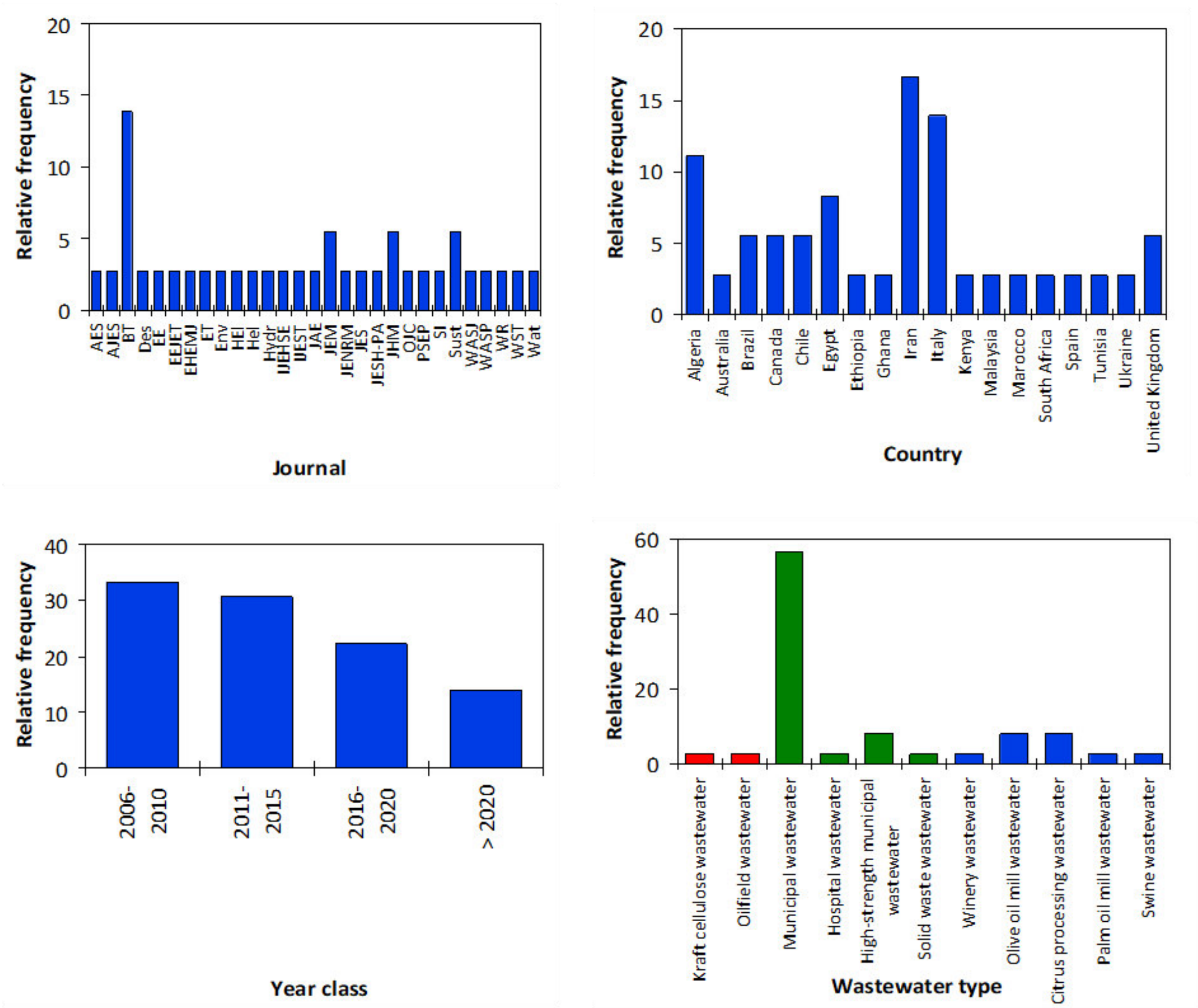


Figure 2.2 - Features of the 36 papers selected for the systematic review. (Calabrò et al., 2024)

Legend: EEJET = Eastern-European Journal of Enterprise Technologies; AES = Acta Ecologica Sinica; AJES = American Journal of Environmental Sciences; BT = Bioresource Technology; Des = Desalination; EE = Ecological Engineering; Env = Environments; EHEMJ = Environmental Health Engineering and Management Journal; ET = Environmental Technology; Hel = Heliyon; Hydr = Hydrobiologia; IJEST = International Journal of Environmental Science and Technology; IJEHSE = Iranian Journal of Environmental Health Science & Engineering; JAE = Journal of Agricultural Engineering; JENRM = Journal of Energy and Natural Resource Management; JEM = Journal of Environmental Management; JES = Journal of Environmental Science; JESH-PA = Journal of Environmental Science and Health, Part A; JHM = Journal of Hazardous Materials; OJC = Oriental Journal Of Chemistry; PSEP = Process Safety and Environmental Protection; SI = Scientia Iranica; Sust = Sustainability; Wat = Water; WR = Water Research; WST = Water Science & Technology; WASP = Water, Air and Soil Pollution; WASJ = World Applied Sciences Journal

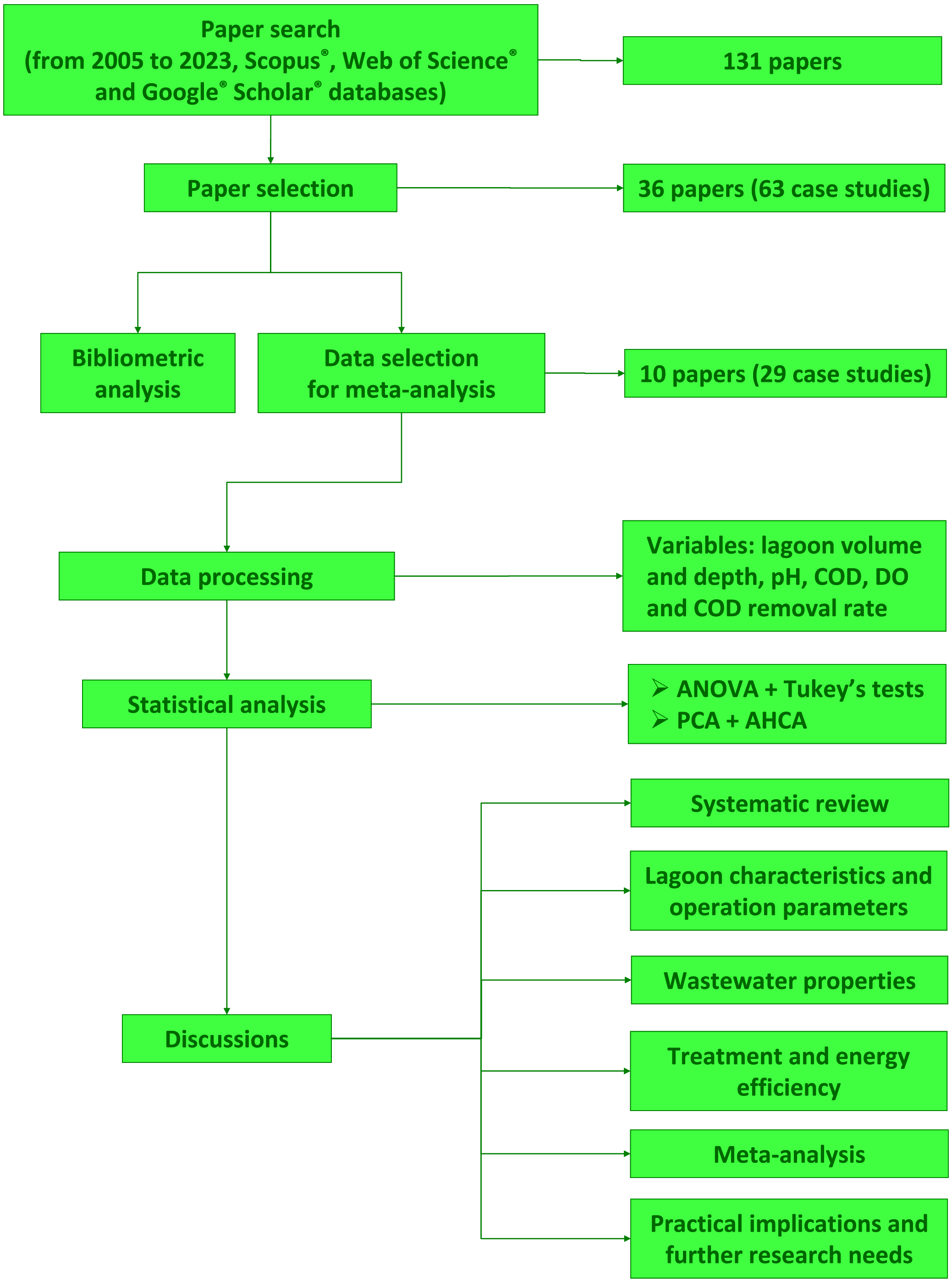
Table 2.2 - Citation analysis of the 36 papers selected for the systematic review (Calabrò et al., 2024).

Paper #	Author(s) (year)	Journal	Citations*	
			total	per year
1	Ali et al. (2020)	Hel	15	5.0
2	Ammar et al. (2017)	OJC	0	0.0
3	Andiloro et al. (2013)	JAE	9	0.9
4	Andiloro et al. (2021)	Env	3	1.5
5	Bachi et al. (2022)	Sust	1	1.0
6	Bansah and Suglo (2016)	JENRM	0	0.0
7	Denisi et al. (2021)	Sust	3	1.5
8	Edokpayi et al. (2021)	HEl	23	11.5
9	Farzadkia et al. (2014)	EHEMJ	27	3.0
10	Furtado et al. (2009)	Hydr	86	6.1
11	Ghazy et al. (2008)	AJES	0	0.0
12	Godini et al. (2021)	PSEP	35	11.7
13	Govahi et al. (2012)	IJEST	27	2.3
14	Hammadi et al. (2016)	AES	1	0.1
15	Jail et al. (2010)	JHM	23	1.6
16	Jarboui et al. (2010)	JHM	54	3.9
17	Khorsandi et al. (2014)	WST	6	0.7
18	Lian et al. (2022)	Wat	2	2.0
19	Malovanyy et al. (2018)	EEJET	0	0.0
20	Martins et al. (2013)	BT	27	2.7
21	Mburu et al. (2013)	JEM	82	8.2
22	Mehmood et al. (2009)	BT	77	5.5
23	Montalvo et al. (2010)	BT	21	1.5
24	Moradhasseli and Mohamadi (2014)	SI	1	0.1
25	Amengual-Morro et al. (2012)	JEM	63	5.3
26	Naddafi et al. (2009)	IJEHSE	30	2.0
27	Ragush et al. (2015)	EE	40	5.0
28	Beyene and Redaie (2011)	WASJ	0	0.0
29	Shpiner et al. (2009)	BT	40	2.9
30	Tuleibah et al. (2009)	JES	0	0.0
31	Türker et al. (2009)	Des	12	0.9
32	Wallace et al. (2016)	WR	62	8.9
33	Wong et al. (2009)	BT	51	3.6
34	Xavier et al. (2009)	JESH-PA	24	1.7
35	Zema et al. (2012)	ET	28	2.5
36	Zema et al. (2016)	WASP	15	2.1

Notes: EEJET = Eastern-European Journal of Enterprise Technologies; AES = Acta Ecologica Sinica; AJES = American Journal of Environmental Sciences; BT = Bioresource Technology; Des = Desalination; EE = Ecological Engineering; Env = Environments; EHEMJ = Environmental Health Engineering and Management Journal; ET = Environmental Technology; Hel =

Heliyon; Hydr = Hydrobiologia; IJEST = International Journal of Environmental Science and Technology; IJEHSE = Iranian Journal of Environmental Health Science & Engineering; JAE = Journal of Agricultural Engineering; JENRM = Journal of Energy and Natural Resource Management; JEM = Journal of Environmental Management; JES = Journal of Environmental Science; JESH-PA = Journal of Environmental Science and Health, Part A; JHM = Journal of Hazardous Materials; OJC = Oriental Journal Of Chemistry; PSEP = Process Safety and Environmental Protection; SI = Scientia Iranica; Sust = Sustainability; Wat = Water; WR = Water Research; WST = Water Science & Technology; WASP = Water, Air and Soil Pollution; WASJ = World Applied Sciences Journal.

Figure 2.3 - Flowchart of the procedure adopted for the bibliographic review and meta-analysis. (Adapted from (Calabrò et al., 2024))



2.7.1.5 Statistical processing and meta-analysis procedure

Meta-analysis was adopted to quantitatively assess whether specific drivers influence lagoon performance across different studies, and to test the statistical significance of such effects (Girona-García et al., 2019; Gurevitch et al., 2018; Zema and Lucas-Borja, 2023). Since none of the case studies reported all operational parameters, a subset was selected to ensure consistency, including lagoon volume, depth, pH, COD, DO, and COD removal rate. These variables were identified in 29 case studies reported across 10 papers out of the initial set of 36 (Fig. 2.3).

To derive weighted performance estimates, efficiencies were normalised when necessary, ensuring comparability between systems operating under different influent loads and climatic conditions. A two-way ANOVA was then applied to lagoon volume, depth, pH, COD, DO, and COD removal rate, with wastewater type (municipal vs. agri-food) and aeration regime (present vs. absent) as independent factors. Industrial wastewater was excluded from the analysis due to insufficient replication. To satisfy the assumptions of variance equality and normal distribution, datasets were subjected to normality tests or square-root transformations, and Tukey's pair-wise test at $p < 0.05$ was used to evaluate the statistical significance of differences among groups.

Correlation analyses explored the relationships between removal efficiency and operational drivers such as depth, temperature, and aeration intensity, while outlier conditions (e.g., extreme climatic events or loading shocks) were identified separately to avoid bias in the aggregated results. Principal Component Analysis (PCA) was performed on the selected parameters, with the first two principal components retained as uncorrelated synthetic variables explaining at least 75% of the variance. Pearson's correlation matrix was used to extract eigenvalues and eigenvectors, and variable associations were quantified through loadings. PCA was complemented by Agglomerative Hierarchical Cluster Analysis (AHCA), grouping case studies with similar characteristics according to key drivers, using Euclidean distance as the similarity–dissimilarity measure (Chatterjee et al., 2020).

Where datasets provided sufficient replication, regression analyses were performed to model the influence of design and climatic factors on lagoon performance, while qualitative synthesis was applied for parameters lacking sufficient replication. All statistical tests were conducted using XLSTAT software, release 2019 (Addinsoft, Paris, France).

2.7.2 Results of the meta-analysis

2.7.2.1 Overview of the selected studies and bibliometric analysis

The systematic review and meta-analysis compiled evidence from studies published over the last five decades, thereby reflecting the progressive evolution of lagoon technologies and their application to different wastewater categories. A considerable proportion of contributions originated from regions characterised by extensive agricultural and agro-industrial activity, particularly North and South America, where lagooning has historically been adopted as a low-cost and relatively simple treatment option (Gloyna, E.F, 1971; Mara, 2009). Despite this geographical bias, the selected studies encompassed a broad spectrum of effluents, including municipal wastewater, livestock manure, food-processing wastewater, and citrus-derived effluents, thus highlighting the versatility of lagoon systems across heterogeneous contexts (Calabrò et al., 2024).

A critical observation emerging from the dataset is the limited number of studies specifically addressing agro-industrial wastewater, and an even smaller subset devoted to CPW. This imbalance reinforces the research gaps identified in Chapter 1, particularly concerning the performance of lagoon systems under high-strength, inhibitory, and seasonally variable conditions typical of CPW (Andiloro et al., 2013a; Bombino et al., 2009; Tamburino et al., 2007).

The reviewed studies also varied significantly in terms of lagoon configuration (anaerobic, facultative, aerated, and polishing ponds), operational scales (laboratory, pilot, and full-scale), climatic conditions, and monitoring duration. Such heterogeneity required careful harmonisation of data before quantitative synthesis, including the standardisation of influent and effluent parameters and the calculation of removal efficiencies. This methodological effort ensured that the meta-analysis could provide robust comparisons across lagoon types and wastewater categories, while also identifying recurrent operational constraints and performance drivers (Calabrò et al., 2024).

From a bibliometric perspective, the 36 selected papers were published in 29 different international journals, with Bioresource Technology accounting for the highest share (13.9% of the total). Other journals such as Journal of Environmental Management, Journal of Hazardous Materials, and Sustainability also played a significant role in disseminating research on lagoon systems. The temporal distribution of publications revealed a concentration of studies between 2008 and 2020, reflecting renewed interest in natural treatment systems as sustainable alternatives to intensive technologies. Citation metrics further highlighted the impact of pioneering works, with several papers achieving high citation rates relative to their year of publication, thereby shaping subsequent research directions (Calabrò et al., 2024).

Geographically, most studies originated from Italy, Algeria, and Iran, confirming the relevance of lagoon systems in Mediterranean and semi-arid contexts where climatic conditions and agricultural activity favour their adoption. However, the relative scarcity of European contributions compared to North American experiences underscores the need for further investigations tailored to Mediterranean agro-industrial effluents, particularly citrus wastewater (Calabrò et al., 2024).

Overall, the bibliometric analysis and qualitative overview converge in identifying lagoons as versatile yet under-researched systems for agro-industrial applications. The imbalance between municipal and agro-industrial case studies, combined with the limited focus on CPW, provides a strong rationale for the experimental research developed in the subsequent chapters of this thesis.

2.7.2.2 Physico-chemical characteristics of wastewater

The influent quality reported across the reviewed studies exhibited a high degree of variability, reflecting the intrinsic diversity of wastewater sources. Municipal wastewaters generally presented moderate COD and BOD concentrations, whereas livestock and agro-industrial effluents were characterised by considerably higher organic loads, often exceeding the design capacities of conventional biological reactors (Niaounakis and Halvadakis, 2006; Rajagopal et al., 2013). Agro-industrial wastewaters frequently displayed COD values surpassing several thousand mg/L, accompanied by high levels of suspended solids, oils and greases, and biodegradable carbohydrates.

Fruit-processing effluents revealed marked compositional variability driven by seasonal production cycles. CPW exemplifies this variability, as it contains high concentrations of sugars, pectic residues, volatile organic compounds, and inhibitory monoterpenes such as d-limonene, which can compromise microbial activity and reduce treatment efficiency (Bombino et al., 2009; Tamburino et al., 2007).

The bibliometric dataset confirms this heterogeneity: pH values ranged between 3.40 and 9.55, with a mean of 6.85 ± 1.68, highlighting the acidic nature of many agro-industrial effluents compared to municipal ones (Martins et al., 2013; Xavier et al., 2009b). COD concentrations spanned from 0.018 g/L in municipal wastewater (Wallace et al., 2016) to 150 g/L in citrus effluents (Andiloro et al., 2013a) with an average of 9.98 g/L and a standard deviation of 27.1 g/L. Total Volatile Solids ranged between 0.9 and 600 g/L, with an average of 170 ± 213 g/L. DO concentrations were consistently below 10.8 mg/L, with the lowest values (0.1 mg/L) measured in lagoons treating CPW (Zema et al., 2016b, 2012a).

Nutrient concentrations were generally imbalanced, with frequent deficiencies in nitrogen and phosphorus, particularly in fruit- and vegetable-processing effluents. These imbalances have significant implications for lagoon performance, as nutrient limitations can slow microbial growth and reduce organic matter degradation unless external supply or co-treatment strategies are implemented (Calabrò et al., 2024).

2.7.2.3 Lagoon performance: COD, BOD, suspended solids, and nutrient removal

The performance analysis revealed substantial variability in removal efficiencies across lagoon types and wastewater categories. Lagoons achieved moderate to high organic matter removal, with typical COD reductions ranging from 50–85% depending on lagoon configuration, retention time, and influent characteristics (Calabrò et al., 2024; Mara, 2009). The bibliometric synthesis reported an average COD removal rate of 67 ± 20.6%, with extremes ranging from 10% (Edokpayi et al., 2021) to 98% (Ali et al., 2020). Anaerobic lagoons showed the highest removal in high-strength wastewaters but were more prone to odour issues. Facultative lagoons achieved balanced performance with greater operational stability, while aerated lagoons consistently reached the highest COD and BOD removal values due to enhanced oxygen transfer (Zema et al., 2016b).

Suspended solids removal was driven primarily by sedimentation processes. Facultative and maturation ponds provided the best performance, reflecting calm hydraulic conditions and long residence times. Nutrient removal showed considerable variability. Nitrogen reduction was generally modest in anaerobic and facultative systems, although denitrification in anoxic layers contributed to partial removal. Phosphorus removal remained low across most studies, consistent with the limited sedimentation and chemical-binding mechanisms typical of lagoon systems (Henze et al., 2008). Overall, lagoons are primarily suited for organic load reduction and solids removal, with nutrient removal representing a secondary benefit rather than a design objective

2.7.2.4 Comparison among lagoon types

The comparative analysis of lagoon configurations highlighted distinct performance profiles that reflect both their design principles and operational constraints. Anaerobic lagoons proved particularly effective in treating high-strength wastewaters, achieving substantial COD removal through fermentative and methanogenic pathways. However, their sensitivity to organic overloading and the frequent occurrence of odour emissions limited their broader applicability (Houghton and Mara, 1992). Facultative lagoons, by contrast, emerged as the most widely adopted configuration, owing to their robustness and the coexistence of aerobic and anaerobic processes. This dual activity enabled them to buffer fluctuations in organic load while maintaining moderate COD and BOD removal, complemented by efficient sedimentation of suspended solids.

Aerated lagoons consistently outperformed other systems in terms of COD and BOD reduction. Enhanced oxygen transfer not only improved tolerance against inhibitory compounds such as limonene but also prevented odour formation, although these benefits came at the cost of higher energy requirements (Calabrò et al., 2024; Zema et al., 2016b). Finally, maturation or polishing lagoons were primarily designed for tertiary treatment, improving disinfection, nutrient uptake, and effluent clarity, especially when positioned downstream of facultative or aerated units (Denisi et al., 2021b; Khorsandi et al., 2014b). Taken together, these findings confirm that aerated lagoons represent the most effective option for industrial effluents, a conclusion that directly supports the rationale of this research project.

2.7.2.5 Influence of operational parameters

HRT emerged as the dominant parameter influencing lagoon efficiency. Reported values ranged between 1.69 and 133 days, with a mean of 25.5 ± 29.9 days. Longer HRTs were consistently associated with higher COD, BOD, and suspended solids removal efficiencies, particularly in high-strength industrial wastewaters where extended retention allowed microbial acclimation and stratification (Calabrò et al., 2024). OLR varied between 0.01 and 3.5 gBOD5/L-d, with an average of 0.66 ± 1.04 gBOD5/L-d. Excessive OLR often resulted in oxygen depletion, increased fermentation and odour generation, and reduced COD and solids removal efficiencies.

Depth and surface area also influenced performance. Shallow lagoons favoured photosynthesis and oxygen diffusion, while deeper systems enhanced anaerobic processes but risked mixing limitations. Aeration strategy emerged as one of the strongest determinants of lagoon efficiency. Continuous aeration increased removal efficiency but raised energy costs, whereas intermittent or low-rate aeration provided a favourable compromise, improving oxygenation while maintaining microbial stratification (Zema et al., 2016b) Aerated lagoons demonstrated higher resistance to inhibitory loads, supporting their application to CPW.

2.7.2.6 Synthesis and interpretation

The synthesis of the reviewed studies highlights several recurring patterns that are crucial for understanding lagoon performance. First, operational conditions such as loading rate, aeration strategy, and climatic factors emerged as decisive drivers of efficiency, often outweighing structural parameters alone. Second, aerated lagoons consistently demonstrated superior performance compared to non-aerated systems, particularly when applied to high-strength industrial effluents. Their resistance against inhibitory compounds and seasonal variability makes them especially important for agro-industrial contexts. Finally, the persistent lack of data concerning citrus-processing wastewater confirms the research gap that motivates the present thesis.

Taken together, these findings illustrate that lagoon systems possess inherent robustness and flexibility, but their optimisation requires a deeper understanding of operational dynamics. This need for targeted research provides the rationale for the experimental work presented in Chapters 4 and 5, where citrus-derived effluents are investigated under controlled lagoon conditions.

2.7.2.7 Synthesis of the Meta-Analysis findings

The meta-analysis offered a quantitative perspective on lagoon performance, complementing the qualitative synthesis of the literature. Statistical testing through two-way ANOVA revealed that most monitored parameters did not differ significantly across lagoon and wastewater types, except for pH and DO. The pH was strongly influenced by wastewater category, while DO varied significantly with wastewater type, aeration, and their interaction. The response variable, COD removal rate (CODRR), was significantly different only between municipal and agro-industrial effluents, confirming the influence of wastewater composition on treatment outcomes (Table 2.3).

Correlation analysis provided further insights into the relationships among lagoon and wastewater variables. Lagoon depth and volume were highly correlated ($r = 0.94$), and both showed strong associations with influent COD concentrations ($r \approx 0.90$). CODRR exhibited a moderate positive correlation with DO ($r = 0.55$), while pH was positively correlated with DO ($r = 0.65$). These associations suggest that oxygen availability and acidity jointly influence organic matter removal, reinforcing the importance of aeration and influent characteristics in determining lagoon efficiency (Table 2.4)

Principal Component Analysis (PCA) clarified the structure of the dataset by identifying two principal components that together explained 81.7% of the total variance. Lagoon volume, depth, and COD loaded strongly on the first component, while pH, DO, and CODRR were most influential on the second. This separation highlights the dual role of structural parameters (volume, depth, COD) and operational/biological parameters (pH, DO, CODRR) in shaping lagoon performance (Figure 2.4)

Cluster analysis further distinguished four groups of case studies. Most aerated agro-industrial lagoons, along with one aerated municipal case, clustered together, reflecting the strong influence of aeration on high-strength effluents. A second cluster consisted of a single case of aerated agro-industrial wastewater, representing an outlier with unique operational conditions. The remaining clusters grouped municipal wastewater lagoons, both aerated and non-aerated, with one exception falling into the agro-industrial cluster (Figure 2.4).

Overall, the meta-analysis confirmed that lagoon performance is shaped by both design parameters and operational conditions. Aerated lagoons treating agro-industrial effluents formed a distinct cluster, underscoring their resistance and efficiency compared to non-aerated systems. These findings reinforce the rationale for focusing on citrus-processing wastewater in the present thesis, where aeration strategies are expected to play a decisive role in overcoming inhibitory loads and seasonal variability.

Table 2.3 - Results of two-way ANOVA applied to the main lagoon characteristics, wastewater properties and COD removal rates of 29 case studies in 10 reviewed papers.

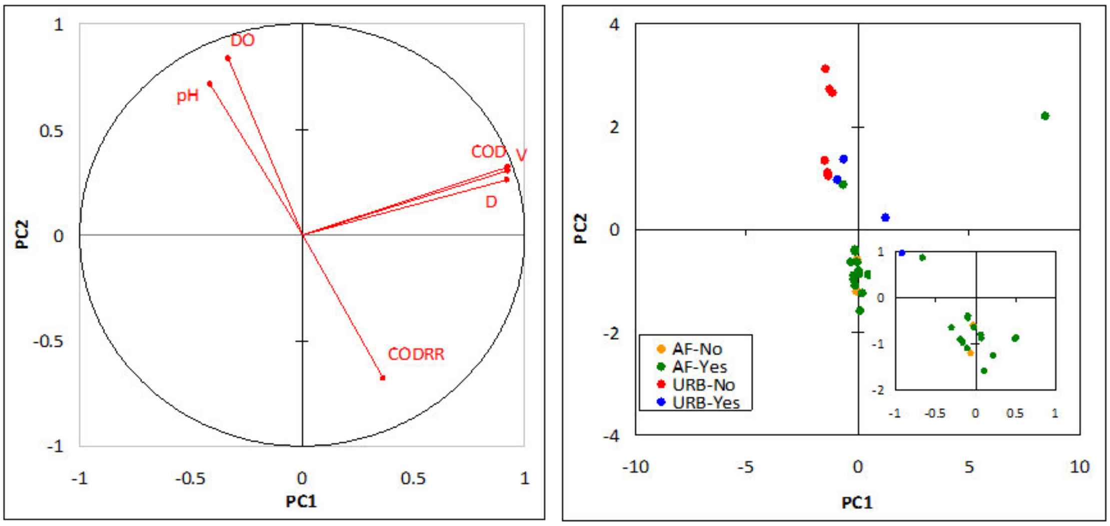
Factor	F	Pr > F
Lagoon volume (V)		
Wastewater type	0.379	0.544
Aeration	0.063	0.804
Wastewater type x aeration	0.085	0.774
Lagoon depth (D)		
Wastewater type	0.578	0.454
Aeration	0.464	0.502
Wastewater type x aeration	0.006	0.941
pH		
Wastewater type	105.952	**< 0.0001**
Aeration	0.454	0.507
Wastewater type x aeration	2.097	0.160
COD		
Wastewater type	0.019	0.891
Aeration	1.530	0.228
Wastewater type x aeration	0.417	0.524
Dissolved oxygen (DO)		
Wastewater type	99.364	**< 0.0001**
Aeration	12.228	**0.002**
Wastewater type x aeration	15.873	**0.001**
COD removal rate (CODRR)		
Wastewater type	6.245	**0.019**
Aeration	2.582	0.121
Wastewater type x aeration	2.500	0.126

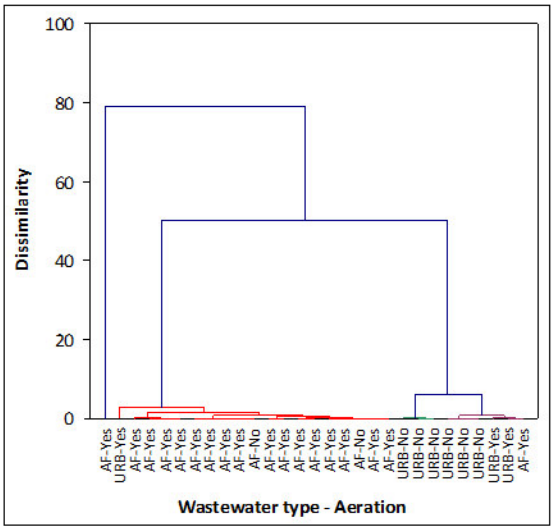
Note: values in bold highlight significant differences (p < 0.05).

Table 2.4 - Pearson's matrix reporting the coefficient of correlation between pairs of variables of lagoons of 29 case studies in 10 reviewed papers.

Variables	*V*	*D*	*pH*	*COD*	*DO*	*CODRR*
V	1	0,943	-0,171	0,889	-0,066	0,107
D		1	-0,190	0,899	-0,042	0,082
pH			1	-0,132	0,648	-0,403
COD				1	-0,094	0,208
DO					1	-0,549
CODRR						1

Figure 2.4 - Boxplot (a), scores on the first two Principal Components (PC1 and PC2) calculated by the Principal Component Analysis (b), dendrogram (c) calculated by the Agglomerative Hierarchical Cluster Analysis with cluster composition (d) of 29 case studies in 10 reviewed papers. Legend: DO = dissolved oxygen; COD = chemical oxygen demand; CODRR = COD removal efficiency; V = lagoon volume; D = lagoon depth; AF = agri-food wastewater; URB = municipal wastewater; yes = aerated lagoon; no = non-aerated lagoon; the number of case studies in each cluster is reported between brackets





Clusters			
C1	*C2*	*C3*	*C4*
AF-Yes (16)	AF-Yes (1)	URB-Yes (2)	URB-No (3)
AF-No (2)		URB-No (3)	
URB-Yes (1)		AF-Yes (1)	

2.7.3 Critical considerations

The systematic review and meta-analysis provided a comprehensive picture of lagoon systems applied to different wastewater categories, but they also revealed several weaknesses and inconsistencies that limit the generalisability of findings. These critical considerations highlight the heterogeneity of the literature, the sensitivity of lagoon performance to environmental and operational conditions, the methodological gaps in current research, and the uncertainty surrounding design criteria and operational guidelines (Calabrò et al., 2024).

2.7.3.1 Heterogeneity and fragmentation of the literature

The bibliographic search returned only 36 peer-reviewed papers, most of which originated from a limited number of countries, and about one third were published in Q1 or Q2 journals, confirming the scientific relevance of lagooning within environmental engineering (Calabrò et al., 2024). The temporal trend showed a decline in publications over the last decade compared to the previous one, possibly reflecting the maturity of more sophisticated treatment technologies and stricter regulations in high-income regions, particularly concerning nutrient removal (Cirelli, 2003).

Geographical fragmentation was evident: lagoons remain widely used in North America, where more than 50% of wastewater treatment plants, over 8000 facilities, are lagoon systems, and they represent the primary choice for small remote communities in Northern Canada (Epa, 2011; Ragush et al., 2015) In contrast, European experiences are scarce, and Mediterranean contexts remain under-represented. A concentration of studies was observed in arid and semi-arid regions, where the reuse of non-conventional water resources is increasingly important due to competition among industrial, agricultural, and domestic sectors (Arora, 2019; Aydinalp and Cresser, 2008).

From a typological perspective, most studies focused on municipal wastewater, while agro-industrial effluents were rarely investigated. This imbalance is surprising given the viability of lagoon systems for such effluents, especially considering the technical problems and low efficiency of conventional depuration technologies such as activated sludge plants (Xavier et al., 2009b; Zema et al., 2012a). CPW remains particularly under-represented, with only sporadic and often outdated studies available (Andiloro et al., 2013a; Bombino et al., 2009; Tamburino et al., 2007).

2.7.3.2 Sensitivity to environmental and operational conditions

Lagoon performance proved highly sensitive to environmental drivers such as temperature, solar radiation, and wind speed, yet many studies failed to adequately characterise these variables (Walsby et al., 2001; Yoshikawa and Furuya, 2006). COD removal efficiencies declined markedly under temperate climates unless aeration was incorporated, while nutrient removal, particularly ammonium oxidation, was strongly linked to dissolved oxygen availability and hydraulic retention time. Dissolved oxygen concentrations varied between 0.1 mg/L in lagoons treating CPW and 10.8 mg/L in kraft cellulose effluents, underscoring the variability across wastewater types (Xavier et al., 2009b; Zema et al., 2012a).

Monitoring periods averaged 350 days, but only ten studies extended beyond one year. Longer observation times are crucial to capture seasonal variability, as demonstrated by Türker et al. (2009) (Türker et al., 2009b), who monitored anaerobic lagoons in Cyprus for four years and showed that BOD and suspended solids could be maintained below discharge limits over extended periods. Sedimentation and sludge dynamics were rarely monitored, despite their importance for long-term stability.

2.7.3.3 Methodological weaknesses and limited mechanistic insight

Most studies adopted a performance-oriented approach, focusing on influent and effluent monitoring without investigating the internal dynamics of lagoon systems. Microbial characterisation was largely absent, even though stratification and acclimation are central to lagoon performance (Henze et al., 2008; Houghton and Mara, 1992). The behaviour of inhibitory compounds such as d-limonene and phenolics, highly important for agro-industrial wastewaters, was poorly documented. Long-term studies were scarce, limiting the understanding of seasonal effects, sludge accumulation, and system sustainability.

Aeration strategies were inadequately described: aerated lagoons were present in more than 50% of studies, reflecting both their theoretical higher removal rates and the need to account for energy costs (Tamburino et al., 2007) . et parameters linked to energy management—such as air flow rates, aeration duration, and specific energy power, were rarely reported. Only Andiloro et al. (2013) and Zema et al. (2012; 2016) provided energy consumption data, ranging between 0.16 and 0.57 kWh/kgCOD removed, enabling a careful quantification of aeration efficiency (Andiloro et al., 2013a; Zema et al., 2016a, 2012a). Laboratory studies often simulated only the upper layer of lagoons, neglecting the beneficial processes associated with large volumes such as dilution and complete mixing (Tamburino et al., 2007).

2.7.3.4 Wastewater properties and lagoon performance

The properties of wastewater strongly influenced lagoon behaviour. Municipal effluents generally exhibited COD values between 20 and 1800 mg/L, while agro-industrial wastewaters ranged between 0.3 and 272 g/L, and landfill leachates between 2.5 and 67 g/L (Denisi et al., 2021b; Godini et al., 2021b). pH values were typically acidic in agri-food effluents, often below 3–4, but lagoons progressively increased pH towards neutrality or alkalinity through oxidation of organic acids (Andiloro et al., 2013a; Montalvo et al., 2010; Paini et al., 2022). Dissolved oxygen concentrations varied widely, with lower ranges in landfill leachate and agri-food wastewater (0.1–2 mg/L) and higher values in municipal and industrial effluents (up to 10.8 mg/L in aerated layers;(Xavier et al., 2009b)).

Treatment efficiencies also varied considerably. COD removal rates ranged from 35–50% in lagoons treating landfill leachate (Ghazy et al., 2008; Martins et al., 2013) to 92–99% in maturation lagoons treating municipal effluents with low COD concentrations (Ali et al., 2020; Türker et al., 2009b). On average, municipal lagoons achieved 64.6% COD removal, industrial lagoons 72.8%, and agro-industrial lagoons 66.8% (Calabrò et al., 2024). Aerated lagoons consistently outperformed non-aerated ones (72.5% vs. 58.8%), confirming the beneficial effects of aeration. However, combined systems often achieved the best results, as shown by Govahi et al. (2012), who reported COD removal efficiencies up to 94% in integrated anaerobic–aerated lagoon systems (Govahi et al., 2012b).

Compliance with national discharge standards was another critical issue. Several studies reported effluent quality meeting regulatory thresholds for BOD, COD, and TSS (Bansah and Suglo, 2016; Mahdi et al., 2014; Naddafi et al., 2009; Ragush et al., 2015; Tuleibah et al., 2015; Türker et al., 2009b; Xavier et al., 2009b). In contrast, others highlighted poor compliance due to excessive loads, inadequate design, or seasonal algal blooms (Edokpayi et al., 2021; Moradhasseli and Mohamadi, 2014; Wallace et al., 2016).

2.7.3.5 Uncertainty in operational guidelines and design criteria

Given the fragmentation of the literature, existing design criteria remain largely empirical. Classical guidelines (Gloyna, E.F, 1971; Mara, 2009) continue to be widely cited, but they do not fully account for high-strength industrial effluents, inhibitory compounds, energy optimisation in aerated lagoons, or the needs of circular-economy frameworks. The meta-analysis revealed clear trends, such as improved COD removal with higher HRT and reduced OLR, but the variability of results prevented the derivation of universal thresholds (Calabrò et al., 2024). This lack of operational clarity is especially problematic for agro-industrial effluents, including CPW, which require tailored lagoon designs capable of handling toxicity, rapid acidification, and seasonality.

2.7.3.6 Implications for future research

The systematic review and meta-analysis collectively highlight the need for integrated, long-term experimental studies that evaluate lagoon performance under realistic industrial conditions. Most of the available evidence is based on short monitoring periods, laboratory simulations, or pilot-scale experiments, which, while useful, fail to capture the seasonal variability, sludge accumulation, and resistance of full-scale lagoon systems (Türker et al., 2009b). Future research should therefore prioritise extended observation campaigns that document lagoon behaviour across multiple operational cycles, including shutdowns and reactivations, to better understand biomass adaptation and system stability.

Mechanistic investigations are also required to complement performance-oriented studies. Stratification dynamics, microbial community structures, dissolved oxygen profiles, and acclimation to inhibitory compounds remain poorly characterised, despite their central role in lagoon efficiency(Henze et al., 2008; Houghton and Mara, 1992). Attention should be devoted to agro-industrial effluents, where compounds such as d-limonene and polyphenols exert bacteriostatic effects that can compromise intensive treatment plants but may be mitigated in lagoons through dilution and microbial adaptation (Amaral et al., 2008; Bombino et al., 2009; Ruiz and Flotats, 2014).

Aeration strategies represent another critical research frontier. Although aerated lagoons were present in more than half of the reviewed studies, parameters such as air flow rates, aeration duration, and energy input were rarely quantified (Tamburino et al., 2007). The few available datasets reported energy requirements between 0.16 and 0.57 kWh/kgCOD removed, values comparable or even lower than those observed in activated sludge plants (Andiloro et al., 2021a; Zema et al., 2016a, 2012a). Systematic testing of aeration regimes, continuous, intermittent, or seasonally adjusted, at laboratory, pilot, and full scale is essential to identify efficient, low-energy operational configurations.

Finally, future studies should explore the integration of lagoon systems within circular-economy frameworks. Evaluations of lagoon compatibility with biorefinery pathways—including volatile fatty acid production, anaerobic digestion, membrane concentration, and agricultural reuse—could expand the role of lagoons beyond conventional depuration, transforming them into multifunctional platforms for resource recovery (Calabrò et al., 2024).

2.7.3.7 Insights from the meta-analysis

The meta-analysis provided further evidence of the structural differences between lagoon systems treating municipal and agro-industrial effluents. A significantly lower pH was consistently observed in agri-food wastewater compared to municipal effluents, a result attributable to the higher content of sugars, oils, greases, and fats, which are degraded into organic acids during treatment (Calabrò et al., 2024)This acidity not only reflects the intrinsic composition of agro-industrial effluents but also explains the slower acclimation of microbial communities and the greater variability in treatment outcomes.

Dissolved oxygen concentrations also showed marked differences. Municipal wastewater treated in non-aerated lagoons exhibited significantly higher DO values compared to both agro-industrial effluents and aerated lagoon systems. This counterintuitive result may be explained by the low efficiency of oxygen transfer under reduced saturation deficits, which limits oxygen availability in systems with high organic loads. The variability in DO and pH proved decisive in the clustering analysis, which discriminated agro-industrial wastewater (both aerated and non-aerated) into distinct clusters (C1 and C2), while municipal effluents—regardless of aeration—formed separate clusters (C3 and C4) (Calabrò et al., 2024).

These findings confirm that lagoon performance cannot be generalised across wastewater categories. Instead, the physicochemical properties of the influent, particularly acidity and oxygen demand, play a central role in shaping treatment efficiency. The clustering patterns reinforce the need for wastewater-specific design criteria, as lagoons treating agro-industrial effluents behave fundamentally differently from those applied to municipal wastewater.

2.7.4 Practical implications

The combined evidence emerging from the systematic review and meta-analysis indicates that lagoon-based systems remain a technically viable and environmentally sustainable option for the treatment of agro-industrial wastewaters. Their performance is consistently enhanced when aeration is applied, confirming the central role of oxygen transfer in accelerating organic matter degradation. Several studies demonstrated that aerated lagoons can achieve removal rates several-fold higher than non-aerated units, even under high-strength or inhibitory wastewater conditions (Andiloro et al., 2021a, 2013a). This improved efficiency has been attributed to the favourable oxygen transfer environment of lagoons and the coexistence of partial anaerobic niches that continue to operate even under aerated conditions.

Despite the additional energy demand associated with aeration, lagoons generally require only a fraction of the power consumption typical of activated-sludge systems. Reported values suggest aeration power inputs close to 10 % of those required in conventional high-rate processes, thereby preserving the low-energy character that makes lagoons economically attractive. Some authors proposed intermittent aeration, particularly during nights or weekends when energy costs are lower, as a strategy to further reduce operating expenses without compromising treatment efficiency. This approach has been shown to decrease energy consumption by up to 40% in certain configurations (Zema et al., 2012a) although the effectiveness of intermittent aeration remains case-specific and not universally superior to continuous operation (Andiloro et al., 2021a).

Beyond aeration strategies, research has explored several optimisation measures aimed at improving lagoon performance and reducing operational constraints. Modifications such as positioning diffusers at mid-depth to intentionally maintain a two-layer structure have been shown to preserve treatment efficiency by retaining an anaerobic bottom zone while still supporting aerobic degradation in the upper layer (Zema et al., 2016a). Other enhancements include the use of baffles to minimise short-circuiting, increase hydraulic retention, and provide additional biofilm surface area; such interventions have resulted in measurable improvements in BOD removal (Lian et al., 2022).

A recurrent theme in the reviewed literature concerns the ability of lagoons to tolerate inhibitory compounds typical of agri-food wastewater, polyphenols in olive mill effluents, essential oils in citrus wastewater, tannins in winery wastewaters. Unlike high-rate biological systems, lagoons appear capable of progressively acclimating their microbial communities to high concentrations of inhibitory molecules (Jarboui et al., 2010; Montalvo et al., 2010; Zema et al., 2012b). Adaptation dynamics, however, remain poorly characterised. In some cases, microbial acclimation markedly accelerated anaerobic processes (Calabrò et al., 2018) while in citrus wastewater the influence of adapted inocula appeared less pronounced (Zema et al., 2016a). This inconsistency highlights the need for deeper investigation into microbial ecology, inhibitory thresholds, and adaptation mechanisms.

The literature also reveals a paucity of studies on microbial community composition, despite its central role in lagoon functioning. Only a limited number of investigations have analysed microbial populations or phytoplankton communities. These works highlight the importance of monitoring microbial indicators to detect under-loading, over-loading, or toxic conditions (Amengual-Morro et al., 2012; Furtado et al., 2009). The absence of systematic microbiological assessments represents a significant knowledge gap, especially when lagoons receive wastewaters containing pathogens or potentially toxic biological species.

Integration of lagoons with other technologies also emerges as a promising direction. Several studies document the successful coupling of lagoons with constructed wetlands, anaerobic reactors, or polishing ponds, particularly for nutrient removal or effluent reuse (Denisi et al., 2021b). When effluent quality cannot meet discharge standards, soil application may represent a suitable alternative, provided that the impacts of residual inhibitory compounds and pathogens are thoroughly evaluated (Jail et al., 2010a; Zema et al., 2019b).

Overall, the literature points to several priority areas for future research, including:
(i) definition of optimal organic loading rates and maximum tolerable concentrations of inhibitory compounds;
(ii) assessment of aeration strategies under different climatic, hydraulic, and compositional conditions;
(iii) systematic characterisation of microbial populations and their role in lagoon performance;
(iv) evaluation of lagoon integration into circular-economy frameworks, particularly for wastewater reuse and resource recovery.

By addressing these gaps, future studies may support the development of more efficient, predictable, and sustainable lagoon-based treatment models, especially for complex agro-industrial effluents such as CPW.

CHAPTER 3: MANAGEMENT OF CITRUS WASTEWATER: CHARACTERISTICS, ENVIRONMENTAL IMPACTS AND LEGISLATION CONSTRAINSTS

3.1 Foreword

Citrus-processing industries generate complex wastewater streams whose treatment requires a detailed understanding of both their intrinsic physico-chemical characteristics and the industrial processes from which they originate. The performance and stability of biological treatment systems are strongly influenced by the nature of these effluents, which combine high concentrations of biodegradable organic matter with inhibitory compounds, marked pH variability, and significant seasonal fluctuations. For these reasons, an accurate characterisation of citrus wastewater and a thorough analysis of the industrial context in which it is produced are essential prerequisites for designing and evaluating suitable depuration strategies.

In this chapter, a comprehensive assessment of the depuration challenges associated with citrus-processing wastewater is first provided, based on scientific literature and sector-specific evidence (Section 3.2). This review identifies the critical pollutants, operational constraints, and environmental impacts that justify the need for robust and resilient treatment technologies. The description of the selected case study, the citrus processing industry "Amedeo Giovanni S.r.l."- Reggio Calabria (Southern Italy), together with the analysis of its industrial configuration and wastewater management, is presented in the following chapter (Chapter 4).

By integrating general depuration issues with the specific industrial configuration of the case study, this chapter establishes the conceptual and operational framework that underpins the micro-aerated lagoon investigations presented in subsequent chapters.

3.2 Characteristics of citrus processing wastewater

CPW represents one of the most challenging effluent streams within the agro-industrial sector, particularly in Mediterranean regions where citrus production is seasonally concentrated and technologically diverse. These effluents originate from a combination of washing, peeling, essential-oil extraction, juice extraction and concentration, and cleaning-in-place (CIP) operations (Tamburino et al., 2007; Zema et al., 2019a). Each stage contributes differently to the overall wastewater matrix, generating a complex mixture of acidic, organic-rich, and occasionally alkaline streams.

CPW typically exhibits high chemical oxygen demand (COD) values ranging from 5,000 to 70,000 mg L^{-1} and biochemical oxygen demand (BOD_5) values between 2,000 and 40,000 mg L^{-1} (Calabrò et al., 2024). The pH is generally acidic (3–5) due to the presence of citric and other organic acids, while the total suspended solids (TSS) content can reach 8,000 mg L^{-1}, consisting mainly of peel fragments, pulp residues, and pectins (Zema et al., 2019a). The wastewater is rich in sugars (5–25 g L^{-1}) and soluble carbohydrates that are easily biodegradable but lead to rapid fermentation and acidification.

A distinctive feature of these effluents is the presence of essential oils, especially d-limonene, a hydrophobic monoterpene that exhibits strong antimicrobial activity (Calabrò et al., 2024). These oils inhibit microbial activity in both aerobic and anaerobic processes at relatively low concentrations (50–200 mg L^{-1}) (Zema et al., 2019a). Furthermore, CPW is generally poor in nitrogen and phosphorus, which limits microbial growth and affects biological treatment efficiency (Ioppolo et al., 2020).

Wastewater generation rates vary widely among citrus-processing plants, depending on the scale of operation, fruit type, and degree of water reuse. Reported values range from 1 m^3 t^{-1} to 17 m^3 t^{-1} of fruit processed (Zema et al., 2019), with water consumption largely dictated by fruit washing, oil extraction, and cleaning operations. This broad variability underscores the difficulty of establishing a single treatment strategy suitable for all citrus industries.

3.2.1 Quantitative variability

Citrus-processing activities in the Mediterranean countries are highly seasonal. Typically, they occur between October and May, corresponding to the harvesting period. This strong seasonality results in discontinuous wastewater generation and large fluctuations in both hydraulic and organic loadings (Calabrò et al., 2024). During the active season, wastewater flow may reach 30–50 $m^3 day^{-1}$ in medium-sized facilities, while off-season production is negligible (Tamburino et al., 2007).

Water consumption per unit of processed fruit varies widely depending on the type of processing, the amount of citrus handled, and the overall water management strategy of the plant (Zema et al., 2019a). Consequently, wastewater volumes increase with freshwater use during processing.

Temporal variability occurs at different scales. Inter-annual variability is linked to fluctuations in agricultural yields and market demand for fresh fruit. Intra-annual variability includes seasonal, monthly, weekly, and daily changes. In Mediterranean regions, more than 70% of citrus destined for processing is concentrated between February and April, with a peak in March (Zema et al., 2019a, 2012b). Monthly variability is influenced not only by the amount of fruit processed but also by unit water consumption, which depends on fruit quality and plant management practices (Zema et al., 2019a). A typical monthly trend is shown in Fig. 3.2, based on a Sicilian plant with a capacity of 40,000 tons per year (Tamburino et al., 2007).

Weekly and daily fluctuations are also significant, mainly due to plant inactivity during nights and weekends. The weekly peak coefficient, defined as the ratio between the average flow rate during the 12 working hours of the peak production day and the weekly average flow rate, typically ranges between 3 and 4 (Tamburino et al., 2007). Figure 3.3 illustrates a typical daily distribution of citrus wastewater flows.

From a management perspective, this discontinuous loading pattern often leads to hydraulic shocks and metabolic stress in biological treatment systems. Conventional reactors such as activated sludge or UASB units are particularly vulnerable due to limited solids retention and poor buffering capacity. In contrast, extensive treatment systems (e.g., lagoons, constructed wetlands) demonstrate higher resistance to seasonal fluctuations, as their long hydraulic retention times and natural buffering capacities help mitigate shock loads (Calabrò et al., 2024).

Figure 3.2 - Typical monthly distribution of CPW (Tamburino et al., 2007)

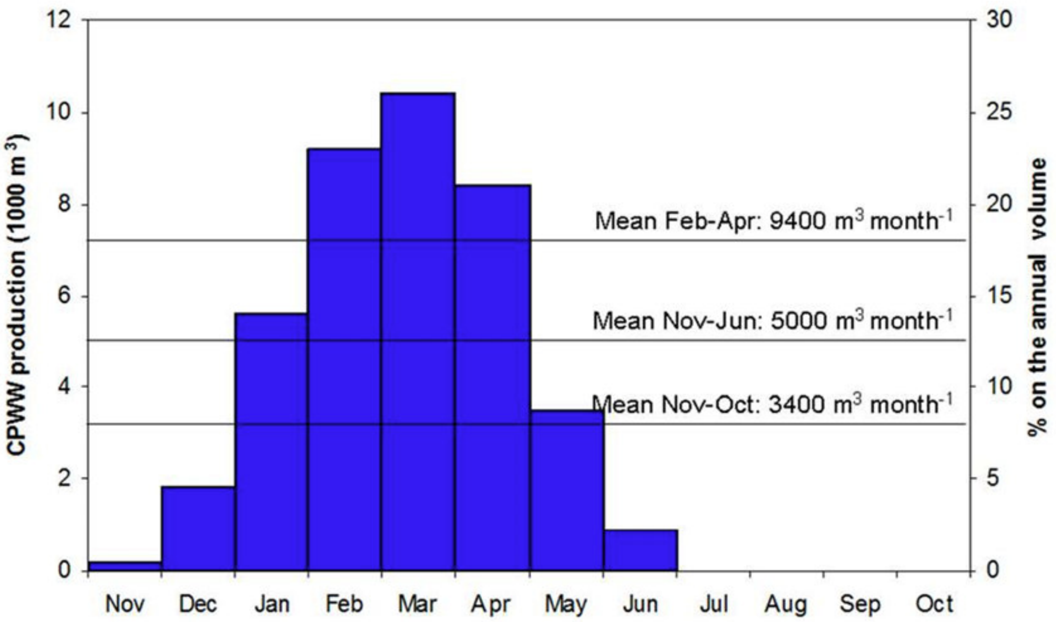
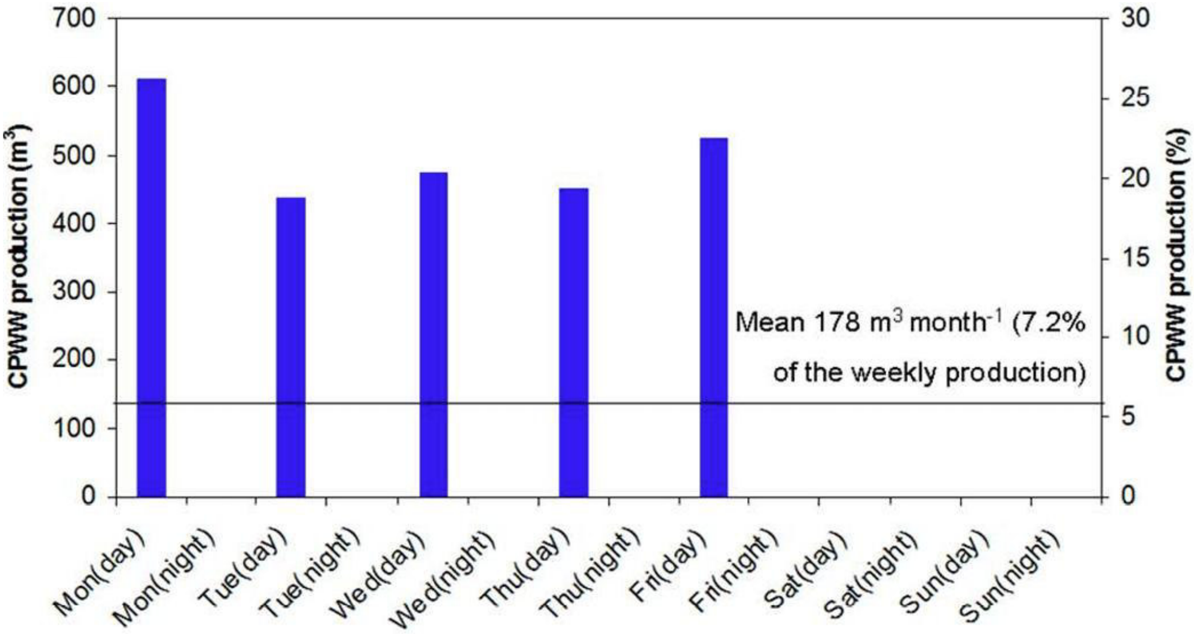


Figure 3.3. - Typical daily distribution of CPW (Tamburino et al., 2007)



3.2.2 Qualitative variability

The chemical composition of citrus-processing wastewater is highly variable and reflects both the technological configuration of the plant and the type of fruit processed. According to recent literature (Lucia et al., 2022; Tamburino et al., 2007; Zema et al., 2019a), the effluent is a heterogeneous mixture of streams with distinct physico-chemical properties, which can be broadly categorised as follows:

- Washing waters, typically low in COD (1,000–3,000 mg L^{-1}) but rich in suspended solids, peel fragments, and soil particles.
- Essential-oil extraction waters, containing emulsified oils, bioflavonoids, and hydrophobic compounds (mainly d-limonene, α-pinene, and γ-terpinene), with COD up to 30,000 mg L^{-1}.
- Juice-extraction and refining effluents, highly concentrated in soluble sugars, pectins, and organic acids; the most biodegradable fraction, often exceeding 50,000 mg L^{-1} COD.
- Condensates from concentration units, large volumes, low COD (<500 mg L^{-1}), but may contain volatile organics.
- Alkaline CIP waters, characterised by high conductivity, strong alkalinity (pH > 11), and residual detergents.

Because these streams are often discharged together, the overall effluent exhibits extreme variability in pH, organic content, and toxicity (Zema et al., 2019a). Rapid shifts between acidic and alkaline conditions can compromise the stability of microbial populations and lead to the precipitation of solids or emulsification of oils.

Table 3.1 - Main qualitative characteristics of wastewater from citrus processing industry (from literature data)

Parameter	*(Koppar and Pullammanappallil, 2013)*	*(Parish et al., 1986)*	*(Andiloro et al., 2013a)*	*(Zema et al., 2012b)*	*(Zema et al., 2016b)*
pH	4.6–4.8	4.8	3.3–4.0	4.2–4.4	5.1–5.5
TS (g L^{-1})	3.5 **	–	–	–	–
COD (g L^{-1})	8.6 *	9.0	21.2–27.2	5.0	6.6
TKN (g L^{-1})	0.06	0.3	–	–	–
EO (g L^{-1})	–	2.8	0.3–1.0	0.5	0.6

*= referred to soluble COD; ** = referred to total dissolved solids.

3.2.3 Inhibitory compounds and biological limitations

A distinctive feature of CPW is the presence of essential oils and phenolic compounds released during the mechanical abrasion and extraction of citrus peels. Among these, limonene is the dominant monoterpene, often accompanied by minor fractions of α-pinene, γ-terpinene, and linalool. These hydrophobic molecules are responsible for the characteristic aroma of citrus fruits, but they also exert strong antimicrobial and inhibitory effects on microorganisms involved in biological treatment systems (Ioppolo et al., 2020; Tamburino et al., 2007; Zema et al., 2019a).

Among all inhibitory compounds, d-Limonene, the main monoterpene of citrus essential oil, represents the most critical challenge to biological depuration processes. Due to its hydrophobic nature, limonene easily partitions into microbial cell membranes, altering their structural integrity and permeability. Once incorporated into the lipid bilayer, it disrupts the arrangement of membrane lipids, increasing fluidity and causing the leakage of intracellular components such as ions, cofactors, and nucleotides.

This structural damage compromises selective permeability and interferes with membrane-bound enzyme systems involved in oxidative phosphorylation and electron transport, reducing ATP generation and impairing energy metabolism (Han et al., 2019). The loss of energy balance leads to metabolic collapse and cell lysis, especially in sensitive microbial species (Ioppolo et al., 2020).

In aerobic systems, limonene causes foaming, sludge bulking, and the loss of microbial activity, while in anaerobic digestion, it inhibits both acetoclastic and hydrogenotrophic methanogens, leading to the accumulation of volatile fatty acids, process acidification, and reduced methane yields (Calabrò et al., 2024). Concentrations above 100 mg L^{-1} can decrease biogas production by up to 70%, and even lower concentrations may delay reactor start-up and recovery after inhibition (Tamburino et al., 2007; Zema et al., 2019a).

Beyond monoterpenes, phenolic compounds, including flavonoids, phenolic acids, and tannins, constitute another major class of inhibitory molecules present in citrus-processing wastewater. These compounds originate mainly from peel tissues, albedo, and pulp, where they occur as natural antioxidants. During fruit washing, pressing, and juice extraction, part of these phenolics' dissolves into the aqueous phase, contributing to the inhibitory burden of the effluent (Ioppolo et al., 2020; Kaur et al., 2024).

Phenolic compounds are redox-active substances capable of chelating transition metal ions and interfering with oxidative enzymes such as peroxidases, laccases, lipoxygenases, cyclooxygenases, NADPH oxidases, and xanthine oxidases, which are essential for the degradation of organic matter and the regulation of oxidative metabolism. By donating protons and electrons, phenolics modulate the cellular redox balance, but their strong antioxidant capacity can paradoxically disrupt the regulation of reactive oxygen species within microbial cells. This imbalance may trigger oxidative stress, membrane lipid peroxidation, and inhibition of key enzymatic pathways, ultimately affecting microbial growth and organic matter turnover (Andrés et al., 2023; Liu et al., 2023; Mukherjee et al., 2024).

In both aerobic and anaerobic processes, phenolic compounds have been shown to inhibit microbial respiration, reduce the rate of substrate oxidation, and destabilise activated-sludge flocs. Under anaerobic conditions, phenolics interact synergistically with limonene and other terpenes, amplifying the inhibition of methanogenic pathways and resulting in decreased methane yield and reactor instability. This effect is particularly critical in citrus-processing wastewaters, where high concentrations of d-limonene (>50 mg L^{-1}) exert a bacteriostatic action that can block microbial biomass, while phenolics contribute to the imbalance of microbial communities and the disruption of ecological stability. As highlighted by Zema et al. (2019), the combined presence of terpenes and phenolics explains the frequent instability of biological treatment plants, whereas Ioppolo et al. (2020) further demonstrated that these compounds, although providing organic carbon that can stimulate microbial growth, simultaneously exert selective pressures that inhibit respiration and alter the composition of soil and sludge microbial communities (Ioppolo et al., 2020; Zema et al., 2019a).

The combined inhibitory effects of monoterpenes and phenolics therefore represent one of the key barriers to the efficient biological treatment of citrus wastewater. Effective management of these compounds requires adequate equalisation, gradual microbial acclimation, or pre-treatment strategies such as adsorption, advanced oxidation, or dilution, aimed at reducing toxicity and improving process sustainability.

3.2.4 Rapid acidification and pH instability

Citrus-processing wastewater is characterised by a high concentration of fermentable sugars and organic acids, which makes the effluents extremely prone to spontaneous anaerobic fermentation even during short storage periods. Under oxygen-limited conditions, microbial activity rapidly converts these substrates into volatile fatty acids, mainly acetic, propionic, and butyric acids, together with minor fractions of ethanol and lactate. This uncontrolled acidogenesis leads to a sharp decrease in pH that can fall below 4 within only a few hours (Zema et al., 2019a). It is important to notice that this uncontrolled acidification occurs under anaerobic storage conditions, whereas, in properly aerated treatment systems (activated sludge, MBR), microbial activity follows aerobic pathways that stabilise pH and prevent volatile fatty acids (VFA) accumulation. Nevertheless, uncontrolled acidogenesis not only increases oxygen demand but also inhibits hydrolytic and methanogenic activity, thereby compromising the stability and efficiency of biological treatment processes. In severe cases, excessive VFA accumulation results in reactor souring, a condition in which the microbial community loses functional balance, methane production collapses, and recovery becomes extremely difficult (Masotti, 2002; Metcalf et al., 2004).

The instability of citrus wastewater composition is further exacerbated by the alternation between acidic effluents from juice extraction and alkaline streams generated during CIP operations. CIP solutions, typically based on potassium hydroxide (KOH) with pH values ranging from 11 to 13, when mixed with acidic wastewater, result in strong oscillations of pH that destabilise microbial adaptation and compromise the integrity of sludge flocs (Corsino et al., 2018; Tamburino et al., 2007). These rapid fluctuations induce osmotic stress, alter the morphology of activated sludge, and increase the risk of washout of sensitive microbial populations. Since microbial consortia generally grow within a narrow pH range (6.5-7.5), such instability needs efficient equalisation and buffering systems to keep the process stable.

Several strategies have been proposed to mitigate these inhibitory effects. Equalisation tanks and chemical buffering (e.g., addition of bicarbonates or phosphates) are commonly used to stabilise influent characteristics before biological treatment. More advanced approaches include two-stage anaerobic digestion, where acidogenic and methanogenic phases are physically separated, thereby reducing the risk of souring and enhancing resistance to pH shocks (Lukitawesa et al., 2018; Rosas-Mendoza et al., 2018).

Membrane bioreactors (MBRs) and aerobic granular sludge (AGS) systems have also been investigated as promising alternatives, offering improved biomass retention and tolerance to fluctuating conditions (Corsino et al., 2021b). Pre-treatment strategies, such as dilution with low-strength wastewater, adsorption onto activated carbon, or advanced oxidation processes (e.g., ozonation, Fenton reaction), have been proposed to stabilise citrus wastewater prior to biological treatment, reducing toxicity and improving overall process reliability (Garcia et al., 2019).

Ultimately, the strong variability in pH and organic load makes CPW one of the most challenging agro-industrial effluents to manage. Effective solutions require integrated approaches that combine equalisation, buffering, selective pre-treatment, and innovative reactor configurations to ensure long-term stability and alignment with circular economy principles.

These operational instabilities, together with the high organic and inhibitory load of CPW, not only complicate biological treatment but also amplify the environmental risks associated with improper management or insufficient depuration. To better understand the implications of inadequate treatment and the necessity for robust depuration technologies, the following section examines the environmental impacts of untreated citrus-processing wastewater.

3.2.5 Nutrient imbalance

CPW is typically characterised by a remarkable nutrient imbalance, mainly due to its extremely high organic load and limited concentrations of nitrogen and phosphorus. This results in C: N:P ratios far from the stoichiometric requirements for optimal microbial growth, which are generally close to 100:5:1 for aerobic processes and 350:7:1 for anaerobic systems (Metcalf & Eddy, 2014). Several studies have reported that agro-industrial effluents rich in soluble sugars, pectins, and essential oils - such as those from citrus processing- are carbon-rich and nutrient-poor, with nitrogen and phosphorus being often below the minimum need for a stable biomass development (Calabrò et al., 2024; Zema et al., 2019a).

This imbalance can significantly affect biological treatment. When nitrogen and/or phosphorus are insufficient, microbial metabolism becomes nutrient-limited, leading to reduced cell synthesis, slower degradation rates, and lower tolerance to inhibitory compounds, such as limonene and phenolics (Bustamante et al., 2014). In high-strength agro-industrial wastewaters, nutrient deficiency has been identified as one of the main causes of process instability, particularly in systems with high oxygen demand or rapid biomass turnover (Metcalf et al., 2004).

Lagoon systems, however, exhibit a greater tolerance to nutrient imbalance compared with intensive biological reactors. Their long hydraulic retention times (HRTs), gradual loading patterns, and stratified microbial communities allow for slower but more robust metabolic adaptation, even under suboptimal nutrient conditions (Calabrò et al., 2024; Zema et al., 2019a).

The presence of sediment layers and biofilms provides additional microhabitats where nutrient recycling - by mineralisation, ammonification, and phosphorus release - can partially compensate for the low nutrient content of CPW (Godini et al., 2021b).

Nevertheless, nutrient limitations can still constrain lagoon performance, especially during periods of high organic loading, when microbial demand for nitrogen and phosphorus increases. Aerated lagoons, in particular, may experience oxygen-driven acceleration of metabolic rates, which amplifies nutrient requirements and may lead to incomplete or slow COD removal when nitrogen and/or phosphorus becomes limiting (Khorsandi et al., 2014b; Tuleibah et al., 2015).

To address these issues, several strategies have been proposed in the literature, including controlled nutrient supply, co-treatment with nutrient-rich residues or waste, or integration with systems capable of enhancing nutrient availability, such as constructed wetlands or polishing ponds (Denisi et al., 2021b; Govahi et al., 2012b). These approaches aim to restore balanced stoichiometry and improve the overall stability and efficiency of lagoon-based treatment.

In the context of CPW, nutrient imbalance represents a critical operational constraint, influencing both microbial activity and system performance. Understanding and managing this imbalance is therefore essential for optimising lagoon performance, particularly in hybrid configurations where aeration and seasonal variability play a decisive role.

3.3 Environmental impacts of untreated citrus wastewater

When inadequately treated or directly discharged into the environment, CPW exerts significant pressure on aquatic, terrestrial, and atmospheric systems. Its high organic load, acidity, and the presence of biologically inhibitory compounds result in severe oxygen depletion, eutrophication, odour generation, and ecotoxicity in receiving ecosystems (Ioppolo et al., 2020; Zema et al., 2019a).

As previously debated, CPW typically exhibits COD values up to 70,000 mg L^{-1} and BOD_5 up to 40,000 mg L^{-1}, which are several orders of magnitude higher than the discharge limits established by national regulations. The rapid microbial degradation of such concentrated organic matter leads to intense oxygen consumption, producing anoxic or anaerobic conditions in surface waters and sediments. Under these circumstances, the oxidation of organic matter shifts towards fermentation, generating reduced compounds such as methane (CH_4), hydrogen sulphide (H_2S), and volatile fatty acids, which contribute to greenhouse gas emissions and strong odour nuisance (Calabrò et al., 2025).

The combination of high oxygen demand and hydrophobic compounds suppresses aquatic respiration and reduces biodiversity. Essential oils, especially limonene, form thin films on the water surface that limit oxygen diffusion and interfere with gas exchange (Zema et al., 2019a). These oils and associated terpenes are acutely toxic to fish and invertebrates, as they inhibit respiratory enzymes and compromise gill membrane integrity (Bakkali et al., 2008). Phenolic compounds also contribute to aquatic toxicity by generating oxidative stress and inhibiting microbial enzymatic activity (Lucia et al., 2022).

In many Mediterranean regions, small citrus-processing plants still practise land spreading of untreated effluents. The high content of soluble organics, salts, and surfactants alters soil structure, reduces permeability, and promotes anaerobic microzones (Zema et al., 2019a). The presence of phenolics and terpenes causes phytotoxicity, manifested as inhibited seed germination and reduced root elongation in sensitive crops (Lucia et al., 2022).

Prolonged application can also modify soil microbial communities, suppressing nitrifying bacteria and promoting fermentative or sulphate-reducing species (Calabrò et al., 2025).

The spontaneous fermentation of stored citrus wastewater produces volatile organic compounds (VOCs) and gases such as CH_4, CO_2, and H_2S, responsible for unpleasant odours and potential greenhouse gas emissions (Pasquarelli Federica et al., 2024). Under warm climatic conditions typical of southern Italy, anaerobic decomposition proceeds rapidly, producing high emission rates of VFAs, aldehydes, and sulphur-containing compounds (Lucia et al., 2022; Lukitawesa et al., 2018).

3.4 Wastewater treatment options for citrus processing wastewater

CPW has been the subject of extensive scientific and technological attention due to the aforementioned issues and the need for an environmentally sustainable and low-cost management. Over the last decades, several management options have been developed and tested in field and at laboratory, pilot, and full scale. These options can be broadly grouped into intensive biological processes, extensive or natural systems, agricultural reuse, biorefinery-oriented approaches, and advanced or emerging technologies (Calabrò et al., 2024; Lucia et al., 2022; Zema et al., 2019a). Each category is characterized by advantages and limitations when applied to CPW.

3.4.1 Intensive biological treatments

Intensive biological treatments are designed to operate under highly controlled conditions, with high biomass concentrations and continuous energy input. Their goal is high removal efficiency of organic matter and nutrients, but, when applied to CPW, these processes face specific challenges linked to the effluent composition. Intensive biological systems can achieve high removal efficiencies when operated under controlled conditions, but their applicability to CPW is limited by several factors, discussed below. For small and medium citrus industries, these constraints make intensive systems costly and difficult to manage without continuous supervision, auxiliary chemicals, and advanced technical expertise.

3.4.1.1 Activated Sludge and Conventional Aerobic Systems

The activated sludge process is one of the most widespread treatments for wastewater, especially in urban areas. AS relies on the action of microbial groups suspended in aeration tanks, where organic matter and nutrients are oxidised and removed. In the case of CPW, however, AS systems show severe limitations: i) the high biochemical/chemical oxygen demand (BOD/COD) requires intense aeration, which translates into high energy costs; ii) nutrient imbalance without external supply, with COD/N/P ratio much over 200/5/1 suggested for regular processes (Metcalf et al., 1991); and iii) essential oils, particularly d-limonene, disrupt the microbial mass and cause foaming, while the inhibition of nitrifying microorganisms compromises nitrogen removal (Tamburino et al., 2007; Zema et al., 2019a).

Energy demand is another major constraint. While AS plants treating municipal effluents typically require ~0.5 kWh per kg COD removed, plants for CPW depuration may need ~2.0 kWh per kg COD due to high organic loads and strong aeration requirement to maintain dissolved oxygen above 2–3 mg L^{-1} (Calabrò et al., 2024; Zema et al., 2019a).

These issues also derive from the fact that intensive biological plants for CPW often replicate design criteria from municipal wastewater treatment, neglecting the specific characteristics of citrus effluents. This leads to low efficiency and expensive management. The main drawbacks include process instability due to variability in CPW composition, frequent breakdowns during peaks of essential oils and low pH, high costs - sometimes exceeding 1-2% of the annual budget of small citrus industries, - and long start-up times needed to develop sufficient microbial biomass. Start-up problems are particularly important in seasonally operated plants, which remain inactive outside the citrus-processing period.

3.4.1.2 Membrane Bioreactors and Membrane Processes

Membrane bioreactors (MBRs) integrate AS with microfiltration (MF) or ultrafiltration (UF) membranes, allowing for excellent separation of solids and pathogens. Their main advantage is the retention of high biomass concentrations, which improves treatment efficiency and effluent quality.

Applied to CPW, MBRs show promising results, but membrane fouling remains a critical issue, exacerbated by suspended solids and lipophilic compounds. Operational costs are also high, and sensitivity to inhibitory molecules, such as d-limonene, limits their depuration efficiency (Corsino et al., 2021a). In the context of citrus-processing wastewater, recent studies highlight the potential of combining MBRs with aerobic granular sludge (AGS) pretreatment. AGS + MBR bench-scale systems achieved almost complete COD removal, though higher resistance to filtration may shorten membrane service life (Di Trapani et al., 2019). Despite these problems, pilot studies and hybrid configurations (e.g., AGS + MBR) demonstrate that innovative adaptations may improve performance, though their scalability remains uncertain (Calabrò et al., 2024; Corsino et al., 2021a; Zema et al., 2019a).

Beyond wastewater treatment, membranes are increasingly used in citrus processing for juice clarification and concentration, improving product quality and enabling recovery of valuable compounds such as pectin, oligosaccharides, and flavonoids. COD reductions of 75-95% have been reported in pilot-scale applications, suggesting that membrane biorefining could represent a promising route for future CPW valorisation (Chen et al., 2017; Garrido-Cardenas et al., 2020; Yan et al., 2018).

3.4.1.3 Anaerobic Digestion

Anaerobic digestion is attractive for CPW because of low energy requirements and potential biogas recovery. Several reactor layouts have been designed and tested, including upflow anaerobic sludge blanket (UASB), expanded granular sludge bed (EGSB), and continuous stirred tank reactors (CSTRs). These systems rely on methanogenic bacteria to convert organic matter into methane. However, monoterpenes, such as d-limonene, in CPW disrupt microbial membranes, leading to volatile fatty acid (VFA) accumulation and reactor acidification (Calabrò et al., 2024; Lukitawesa et al., 2018; Wainaina et al., 2019). Rapid fermentation of sugars and pectins further accelerates acidogenesis, destabilising the process.

To mitigate these effects, two-phase anaerobic digestion (TPAD) has been proposed, separating acidogenesis from methanogenesis. This configuration improves stability but increases system complexity and operational requirements (Wainaina et al., 2019). Co-digestion with other agro-industrial effluents has also been suggested as a way to balance nutrients and dilute inhibitory compounds (Calabrò et al., 2018; Lucia et al., 2022). For CPW, co-digesting citrus effluents with substrates richer in nitrogen and phosphorus, such as livestock manure, cheese whey, or municipal sludge, has been shown to improve process stability, enhance methane yields, and reduce the inhibitory impact of monoterpenes. The addition of co-substrates not only contrasts the intrinsic nutrient imbalance of CPW but also buffers pH fluctuations and reduces the rapid acidification due to fermentable sugars. Several studies report that appropriate mixing ratios can significantly increase the tolerance of anaerobic biomass to limonene, enabling higher organic loading rates and better reactor performance (Bolzonella et al., 2006; Wainaina et al., 2019).

3.4.2 Extensive and natural treatments

Extensive or natural treatment systems represent low-energy, low-maintenance alternatives particularly suited for agro-industrial effluents and small-to-medium citrus-processing facilities. Unlike intensive biological systems, these technologies rely on long hydraulic retention times (HRTs), external aeration, and natural microbial stratification, which make these systems more tolerant to fluctuations in flow and chemical composition of CPW (Zema et al., 2019a). Their reduced operational complexity and lower energy demand have attracted growing interest in regions where citrus wastewater production is seasonal and industrial plants lack full-time and skilled technical personnel to run conventional depuration plants. Indeed, lagoons and constructed wetlands (the most common plants for natural treatments) are considered low-cost alternatives to intensive systems, requiring less investment in construction and management. Their efficiency, however, is strongly dependent on climatic conditions, and they demand extensive land areas and long hydraulic retention times (Calabrò et al., 2024).

3.4.2.1 Stabilisation and aerated lagoons

Stabilisation lagoons are among the most widely applied natural systems for agro-industrial effluents. Their large volume allows buffering of high and rapidly variable hydraulic and organic loads, while the natural stratification between aerobic and anaerobic zones supports diversified microbial processes. In CPW treatment, lagoon systems have demonstrated tolerance to inhibitory compounds, thanks to their long solids retention time (SRT) and the capacity for self-adaptation under variable influent characteristics (Lucia et al., 2022).

With a specific focus on lagoons, their use has been proposed for the treatment of wastewater from different sources, including municipal and agro-industrial effluents (Jail et al., 2010b; Travieso et al., 2010). Research has shown that lagoons can serve as a suitable alternative to activated sludge plants, particularly in rural areas hosting small industrial and urban settlements (Juanicó and Milstein, 2004; Salgot et al., 2006). However, in developed regions exposed to severe water and land pollution or subject to strict disposal standards, lagoons have often been replaced by more recent technologies such as advanced oxidation processes (Rizzo et al., 2019).

Aerated lagoons use low-intesity mechanical aeration, increasing oxygen transfer without requiring energy as activated-sludge processes. This configuration enhances COD removal and limits uncontrolled fermentative activity—such as excessive VFA accumulation and odour-producing anaerobic reactions—by maintaining sufficient oxygen transfer (Calabrò et al., 2024). Literature reports COD removal efficiencies between 60% and 85% depending on lagoon configuration, climate, and organic load (Zema et al., 2019a). Despite these advantages, some authors have highlighted the limited viability of lagoons for agricultural and industrial effluents (Chan et al., 2009; Kimball, 1999; Trias et al., 2004). More recent studies have explored improvements such as alternative aeration schemes (Andiloro et al., 2021b; Zema et al., 2016a), the use of aquatic plants to enhance treatment effectiveness (Tuleibah et al., 2015), and integration with other processes, such as phyto-depuration or polishing ponds (Denisi et al., 2021a; Khorsandi et al., 2014a). Nevertheless, scarce attention has been devoted to aerated lagooning of agro-food effluents, and further research is needed to provide technicians with operational guidelines and to identify current gaps (Andiloro et al., 2013a; Bombino et al., 2009).

3.4.2.2 Constructed wetlands

Constructed wetlands (CWs) are considered an environmentally sustainable option for tertiary or secondary treatment of CPW. They run through a combination of plant uptake, filtration, sedimentation, and microbial degradation within the rhizosphere. Vertical (VSSF) and horizontal subsurface flow (HSSF) wetlands have both been tested, although their application to raw CPW is limited due to clogging risk of soil substrate (due to high suspended solids), and the presence of essential oils, toxic for plants (Lucia et al., 2022).

When applied after pre-treatment, CWs can achieve effective removal of residual suspended solids, nutrients, and organic matter, also improving effluent quality for reuse in irrigation. However, their large land requirements and high sensitivity to hydraulic overloads remain constraints for large-scale CPW management.

3.4.2.3 Advantages and limitations of extensive systems

Extensive treatment systems offer important benefits, particularly in citrus-processing industries, where operational simplicity and cost-efficiency are essential. Their low energy demand, high buffering capacity against high hydraulic and organic loads, and natural tolerance to inhibitory compounds make these systems well suited for seasonal and decentralised installations. At the same time, these systems present limitations, such as the need for large land areas, long hydraulic retention times, and removal rates that are generally lower compared to intensive biological systems. In configurations with low aeration rates, unpleasant odours may also occur (Calabrò et al., 2024).

Despite these limitations, extensive lagoon systems remain among the most promising solutions for CPW, especially for small-scale industries (Calabrò et al., 2024; Lucia et al., 2022; Zema et al., 2019a). These considerations provide the rationale for a deeper examination of lagoon technologies, which is developed in the following sections.

3.4.3 Agricultural reuse

The reuse of agro-industrial effluents in agriculture has gained increasing attention as a strategy to reduce freshwater consumption, close nutrient loops, and promote circular-economy principles. In the case of CPW, land application has historically been practiced in several citrus-producing regions, particularly in Mediterranean and semi-arid climates, where water scarcity encourages the use of alternative water sources (Cinardi et al., 2025; Zema et al., 2019a). However, despite its potential benefits, the agricultural reuse of CPW shows significant agronomic, environmental, and regulatory challenges.

From an agronomic perspective, CPW contains appreciable amounts of readily biodegradable organic matter, residual sugars, potassium, and micronutrients, which in principle may contribute to soil fertility. Several studies have reported short-term increases in soil organic carbon and microbial activity following controlled CPW irrigation, associated with the rapid mineralisation of soluble organics (Cinardi et al., 2025; Zekri and Obreza, 2014b). Limited nutrient content, especially nitrogen and phosphorus, reduces the risk of eutrophication when compared with livestock or municipal effluents, making CPW relatively appealing for fertigation in low-input cropping systems (Lucia et al., 2022; Zema et al., 2019a).

Despite its potential benefits, CPW reuse is constrained by the presence of acidic organic acids, suspended solids, and inhibitory compounds, particularly d-limonene. These substances may exert phytotoxic effects, inhibit seed germination, and alter soil microbial dynamics when applied under undiluted form or in large quantities with improper dilution (Gupta et al., 2021; Ioppolo et al., 2020; Lucia et al., 2022; Zema et al., 2019a). Experimental evidence from field and greenhouse trials has documented reduced germination rates in sensitive crops, transient inhibition of soil respiration and nitrification, accumulation of lipophilic compounds in the rhizosphere, and progressive soil acidification after repeated applications of untreated CPW. Moreover, the high BOD can induce localized anaerobic conditions in fine-textured or poorly aerated soils, leading to odour emission and reduced stability of soil structure (Cinardi et al., 2025; Zema et al., 2019a). Operational strategies to mitigate these risks include dilution with freshwater (from 1:3 to 1:10), neutralisation to prevent abrupt changes in soil pH, settling or screening to remove coarse suspended solids, and intermittent application schedules to avoid soil saturation and allow microbial recovery (Food and Agriculture Organization of the United Nations (FAO), 2010; Lucia et al., 2022; Zekri and Obreza, 2014b) While effective, these actions increase the operational complexity of CPW supply to soil and may reduce the economic attractiveness of reuse, particularly for small-scale producers.

From a regulatory standpoint, the European Union imposes strict requirements on the agricultural reuse of industrial wastewater. The Water Framework Directive (2000/60/EC) and Regulation (EU) 2020/741 on water reuse establish quality standards and risk-assessment procedures. Untreated CPW generally fails to meet these rules due to its high organic load and inhibitory compounds (European Commission, 2020). In Italy, the Legislative Decree 152/2006 prohibits the land application of industrial effluents that do not comply with discharge limits, effectively limiting the use of raw CPW for irrigation unless adequately treated. These constraints have limited large-scale adoption of CPW reuse, which is left confined to controlled experimental contexts.

In summary, agricultural reuse is a theoretically attractive option for CPW valorisation, promoting resource recovery and reducing wastewater volumes requiring treatment. However, chemical instability, phytotoxicity, and regulatory barriers limit its practical applicability. As a result, this option is generally feasible only after other treatments, often in combination with natural or extensive systems. Nowadays, CPW agricultural reuse is still minor compared to biological or lagoon-based technologies in modern citrus industries.

3.4.4 Valorisation of added-value compounds in biorefinery

In recent years, biorefinery-oriented strategies have increasingly been recognised as another promising path for the valorisation of CPW. This approach uses CPW as a complex biochemical substrate rich in fermentable carbohydrates, organic acids, phenolics, and volatile compounds. Therefore, the effluent becomes a potential feedstock for integrated conversion processes that align with the principles of the circular bioeconomy, combining environmental protection with the recovery of bio-based products (Calabrò et al., 2024; Lucia et al., 2022; Zema et al., 2019a).

3.4.4.1 Fermentative conversion and bioenergy production

The high concentration of readily biodegradable carbohydrates and pectin-derived oligomers makes CPW suitable for microbial fermentation. Several studies have investigated the production of volatile fatty acids (VFAs), bioethanol, biosurfactants, organic acids and biopolymers through controlled fermentative pathways (Wainaina et al., 2019).. VFAs, in particular, are versatile compounds with applications in nutrient removal, bioplastic synthesis (polyhydroxyalkanoates), and anaerobic chain elongation. However, the feasibility of fermentation processes is strongly constrained by the presence of d-limonene, which exerts membrane-disrupting and inhibitory effects on fermentative and methanogenic microorganisms. Consequently, strategies such as partial detoxification, dilution, or selective removal of essential oils are often necessary to stabilise microbial activity and prevent process failure (Zema et al., 2019a). Co-digestion with nutrient-rich or less inhibitory effluents is another effective approach to mitigate toxicity and balance the intrinsic nitrogen deficiency of CPW (Lucia et al., 2022).

3.4.4.2 Recovery of bioactive and high-value compounds

CPW contains a wide range of bioactive molecules with high market value in the food, cosmetic, and pharmaceutical industries. Pectin, flavonoids, oligosaccharides, polysaccharides, terpenes, and essential oils are among the most important compounds that can be recovered from CPW, although their extraction is often limited by dilution and process complexity (Zema et al., 2019a). Several pilot-scale studies have demonstrated the feasibility of recovering pectin, hesperidin-rich fractions, and limonene using membrane separation, adsorption, or solvent-free extraction technique. Although yields are often limited by variability of CPW composition, the selective extraction of such compounds may significantly reduce toxicity while contributing to the economic viability of integrated treatment processes (Šafranko et al., 2023).

3.4.4.3 Production of platform chemicals

CPW can be converted into platform chemicals, such as citric, succinic, and lactic acids, or into VFA-rich broths suitable for downstream applications, including bioplastic precursors and microbial oils. Mixed-culture fermentation under controlled pH conditions has been shown to enhance VFA accumulation and limit the inhibitory effects of essential oils, providing a promising route for integrated resource recovery (Wainaina et al., 2019).

3.4.4.4 Integration into multi-product biorefineries

Emerging approaches propose cascade biorefinery systems, wherein CPW undergoes sequential valorisation steps. These may include the recovery of essential oils and flavonoids, fermentation to biofuels or VFAs, anaerobic digestion of residual streams, and nutrient recovery coupled with water reuse. Such integrated systems maximise resource efficiency and minimise the volume of wastewater requiring final treatment. Nevertheless, their implementation remains largely confined to pilot scale, hindered by high capital costs, process complexity, and the seasonal availability of citrus feedstock (Ioppolo et al., 2020; Satari and Karimi, 2018).

3.4.4.5 Advantages and limitations

Biorefinery-oriented systems enable the recovery of high-value bioactive compounds while simultaneously reducing the pollutant load entering conventional treatment stages. As aforementioned, these systems support the implementation of circular-economy principles, fostering industrial recovery paths and resource efficiency, and offer opportunities for energy recovery through bioethanol, biogas, and biohydrogen production. However, several constraints hinder their large-scale adoption: inhibition by monoterpenes and phenolics make fermentation and digestion processes unstable or lower their productivity, while the need for pretreatment increases operational complexity. Economic viability remains strongly dependent on market conditions and plant scale, and most applications are still confined to laboratory or pilot studies.

Overall, biorefinery-based strategies represent a promising yet still emerging pathway for CPW management. Their integration with established depuration technologies - such as lagoon systems, anaerobic digestion, or membrane processes - may enable hybrid configurations that are able to combine environmental protection and resource valorisation. Future research should prioritise scaling up pilot systems, optimising detoxification methods, and conducting techno-economic and life cycle assessments to evaluate feasibility under real industrial conditions (Calabrò et al., 2024; Lucia et al., 2022; Zema et al., 2019a).

3.4.5 Advanced and emerging treatment technologies

Advanced and emerging treatment technologies have attracted growing interest in the management of CPW, particularly in contexts where conventional biological systems exhibit limited performance due to the aforementioned factors. These approaches are designed to enhance pollutant removal efficiency, improve effluent quality for reuse, and support integrated treatment paths that combine depuration with resource recovery.

3.4.5.1 Advanced oxidation processes

Advanced Oxidation Processes (AOPs), including ozonation, Fenton and photo-Fenton reactions, UV/H_2O_2, and photocatalysis, have been extensively studied for the degradation of recalcitrant organic compounds in agro-industrial wastewaters. These methods rely on the generation of hydroxyl radicals, which are highly reactive and capable of oxidising complex molecules, such as monoterpenes, phenolics, and pigments. AOPs have been shown to achieve substantial reductions in COD, colour, and toxicity, making these processes particularly effective as final treatments prior to discharge or reuse. Nevertheless, their large-scale application is constrained by high energy demand, reagent consumption, and operational costs. In addition, incomplete oxidation may result in the formation of intermediate by-products that require further treatment (Rizzo et al., 2019).

3.4.5.2 Electrochemical and electrocoagulation processes

Electrochemical treatments, including electro-coagulation, electro-oxidation, and electro-Fenton, have demonstrated promising results in the treatment of high-strength agro-industrial wastewater. Electrocoagulation, in particular, facilitates the removal of suspended solids, colloids, and lipophilic compounds through the in-situ generation of iron or aluminium hydroxides. Applications to CPW have reported noticeable efficiencies in reducing turbidity, suspended solids, and essential oil droplets, while simultaneously improving biodegradability. Despite these advantages, the processes are limited by electricity consumption, electrode passivation, and sludge production, which may hinder their economic feasibility (Feng et al., 2016).

3.4.5.3 Adsorption and ion-exchange processes

Adsorption techniques using activated carbon, polymeric resins, or biochar have been explored to remove inhibitory compounds in CPW. These methods are often used as pre-treatments to detoxify the effluent before biological treatment or fermentation. Ion-exchange processes can further support nutrient recovery or selective removal of undesirable compounds. Although adsorption and ion-exchange processes are effective in removing inhibitory compounds, they require periodic regeneration or replacement of adsorbents, which increases operational costs and may limit upscaling (Cassano et al., 2018).

3.4.5.4 Membrane technologies and hybrid systems

Beyond conventional membrane bioreactors, emerging membrane processes such as nanofiltration, reverse an forward osmosis, and membrane distillation have been tested for CPW valorisation and water recovery. Nanofiltration and reverse osmosis can produce permeates for reuse in cleaning operations or irrigation, while concentrating bioactive compounds in the retentate. Forward osmosis and membrane distillation shows lower fouling tendency and can be integrated into hybrid systems with anaerobic digestion or fermentation. However, membrane fouling is still a major constraint due to the high concentration of organics and suspended solids typical of CPW, requiring frequent cleaning and maintenance (Pulido and Martínez-Férez, 2017).

3.4.5.5 Emerging biological processes

Recent research has investigated novel biological approaches designed to overcome CPW toxicity. Aerobic granular sludge systems have shown improved tolerance to high organic and hydraulic loads and inhibitory compounds, while anammox-based processes offer combined nitrogen removal and organic load reduction. Microalgae-bacteria consortia have also been tested, enabling simultaneous nutrient uptake, oxygen production, and biomass generation. Although promising, these processes remain largely at pilot or laboratory scale and require controlled conditions to maintain stable microbial communities and granule structure (Sánchez-Zurano et al., 2021).

3.4.5.6 Advantages and limitations of advanced technologies

Advanced and emerging technologies provide significant opportunities for CPW management. These systems can achieve high removal efficiencies of recalcitrant pollutants, reduce toxicity, and enable partial water reuse or recovery of valuable compounds. Moreover, their integration within biorefinery frameworks offers potential synergies between depuration and resource valorisation. However, their full-scale application is hindered by high capital and operational costs, energy-intensive processes, sensitivity to seasonal fluctuations, and the need for specialised technicians. Industrial uses remain limited, and further research is thus required to optimise these systems for real-world conditions. Despite these challenges, advanced technologies represent valuable components of hybrid treatment trains, particularly as regulatory standards become more stringent and industries want to implement circular-economy strategies.

3.5 Law and regulatory constraints

European and national legislations strictly regulate the discharge of agro-industrial effluents. The EU Water Framework Directive (2000/60/EC) (European Parliament & Council, 2000) and the Industrial Emissions Directive (2010/75/EU) (European Parliament & Council., 2010) require that all industrial discharges meet specific quality standards to protect aquatic ecosystems.

In Italy, Legislative Decree No. 152/2006, Part III, Annex 5, sets the maximum allowable limits for industrial wastewater: COD ≤160 mg L^{-1}, BOD_5 ≤40 mg L^{-1}, pH 5.5–9.5, and oils and greases ≤10 mg L^{-1} (Republic of Italy, 2006).

Raw citrus wastewater typically exceeds these limits by one or two orders of magnitude (Lucia et al., 2022; Tamburino et al., 2007; Zema et al., 2019a).

The management of citrus-processing wastewater operates within a rigorous legal and institutional framework designed to protect water bodies and promote sustainable industrial practices. Due to its high organic load and toxicity, CPW is classified as an industrial effluent subject to specific discharge and treatment requirements under both European, national and regional law.

3.5.1 European legislation

At the European level, several directives and regulations form the backbone of wastewater governance:

- Water Framework Directive (2000/60/EC, Art. 4): establishes the overarching goal of achieving "good ecological status" for all water bodies. It requires Member States to adopt integrated river basin management plans and regulate industrial discharges accordingly (European Parliament & Council, 2000).
- Urban Wastewater Treatment Directive (91/271/EEC, Art. 11; Annex III): extends its scope beyond municipal effluents to include biodegradable industrial wastewaters from specific sectors, such as the manufacture of fruit and vegetable products. Article 11 stipulates that such discharges must be subject to prior authorization and, if necessary, pre-treatment before entering public sewerage systems or municipal treatment plants (Council of the European Union., 1991).
- Industrial Emissions Directive (2010/75/EU, Art. 14): requires industries to adopt Best Available Techniques (BAT) to minimize pollution and ensure integrated environmental protection (European Parliament & Council., 2010).
- Waste Framework Directive (2008/98/EC, Arts. 4–11): promotes waste prevention, reuse, and recycling, encouraging valorisation of organic residues, including agro-industrial effluents (European Parliament & Council, 2008).
- Regulation (EU) 2020/741 (Arts. 2 and 4): sets minimum quality standards for the reuse of treated wastewater in agriculture. Its provisions aim to guarantee safety for the environment, humans, and animals, aligning with circular economy principles (European Parliament & Council., 2020).

Together, these instruments create a multi-layered framework that compels citrus-processing industries to adopt advanced treatment technologies and valorisation strategies. Compliance is not optional: untreated citrus effluents, with COD and BOD_5 values far exceeding regulatory thresholds, cannot be legally discharged without adequate treatment.

3.5.2 Italian legislation

In Italy, wastewater management is governed by Legislative Decree No. 152/2006 ("*Testo Unico Ambientale"*), which consolidates environmental regulations (Republic of Italy, 2006).

- Article 101, paragraph 7, letter c: regulates the assimilation of certain industrial effluents to domestic wastewater. This applies to companies engaged in agronomic utilization and in activities related to the transformation or valorisation of agricultural products, provided that such activities are considered normal and complementary to the production cycle, and that raw materials originate predominantly from the cultivation of land available to the company (Republic of Italy, 2006).
- Annex 5, Table 3: sets strict emission limits for industrial discharges: COD ≤160 mg L^{-1}, BOD_5 ≤40 mg L^{-1}, suspended solids ≤80 mg L^{-1}, pH between 5.5 and 9.5, and oils and greases ≤10 mg L^{-1}. Citrus wastewater, with COD values up to 70,000 mg L^{-1} and BOD_5 up to 40,000 mg L^{-1}, exceeds these thresholds by one or two orders of magnitude, making direct discharge into surface waters legally prohibited (Republic of Italy, 2006).

- Article 74: defines agronomic utilization as the management of livestock manure, residues from olive processing, and wastewaters from farms and small agri-food enterprises. The purpose is to exploit the nutrient content of effluents for irrigation or fertigation (Republic of Italy, 2006).
- Article 112 and Ministerial Decree of 25 February 2016: specify that agronomic utilization is permitted only for small agri-food companies producing less than 4,000 m^3 of wastewater per year and nitrogen loads not exceeding 1,000 kg annually

To comply with these requirements, industries must obtain an Autorizzazione Unica Ambientale (AUA), which integrates all environmental permits and monitoring obligations. However, for small and medium-sized enterprises (SMEs), compliance is often difficult due to the seasonal nature of citrus production. Indeed, citrus-processing wastewater is produced mainly during short seasonal campaigns, causing strong fluctuations in flow. This variability makes conventional continuous-flow treatment plants difficult to operate efficiently.

In late 2024, by the issue of Law 191/2024, the Italian Environmental Code was amended. While emission thresholds in Annex 5 did not change, the reformation introduced (Governo della Repubblica Italiana, 2024):

- Simplification of procedures for environmental authorization ("AUA", "AIA", "VIA"), reducing administrative tasks for SMEs.
- Acknowledgement of the refined wastewater concept (in Italian, "acque affinate"), that is the treated effluents that can be legally reused for irrigation under controlled conditions.
- Stronger integration of effluent management with circular economy principles, encouraging valorisation of agro-industrial effluents.

This means that citrus-processing industries not only must comply with discharge limits but also should take into account the possibility of reusing treated wastewater in agreement with EU Regulation 2020/741 and the new Italian laws.

3.5.3 Regional legislation (Calabria)

Beyond national legislation, regional governments play a crucial role in defining specific rules for wastewater management. The Region of Calabria, one of Italy's main citrus-producing areas, has adopted regulations to govern the agronomic use of effluents and agro-industrial wastewaters.

With Deliberation of the Regional Government No. 119 of 31 March 2021, Calabria approved the *Disciplinare per l'utilizzazione agronomica degli effluenti di allevamento, dei digestati e delle acque reflue* and the *Programma di azione per le zone vulnerabili e non vulnerabili ai nitrati di origine agricola* (Regione Calabria, 2021). These measures, aligned with the EU Nitrates Directive (91/676/EEC) and Legislative Decree 152/2006, establish technical criteria for the storage and agronomic utilization of effluents, including those produced by small agro-food industries.

Key provisions include:

- Specific procedures for agronomic use of wastewater, ensuring nitrogen loads do not exceed thresholds set to protect groundwater.
- Obligation of prior communication: companies intending to use effluents for agronomic purposes must submit a preventive notification to the regional Department of Agriculture via the SUAP (Sportello Unico per le Attività Produttive), at least thirty days before starting the activity.
- Differentiated measures for vulnerable and non-vulnerable zones, depending on nitrate pollution risk.
- Integration with AUA requirements, ensuring consistency with national permitting procedures.

For citrus industries in Calabria, this means wastewater cannot be freely discharged or reused without compliance with the regional the regional guideline (*disciplinare)*. Instead, effluents must be managed according to agronomic utilization rules, with strict monitoring of nitrogen loads and seasonal application limits. This regional legislation represents an important step in harmonizing environmental protection with the valorisation of agro-industrial by-products, encouraging their use as fertilizers while preventing groundwater contamination.

Following the national law issued in 2024, regional governments, such as in Calabria Region, will need to adapt their agronomic guidelines to the new category of refined wastewater, integrating rules for reuse with existing nitrate and nitrogen load thresholds.

3.5.4 Compliance challenges and future perspectives

The BAT Reference Document for the Food, Drink and Milk Industries (JRC, 2019) recommend technical measures such as segregation of wastewater streams, primary treatment, and biological processes adapted to seasonal operation. Yet economic constraints and technical limitations frequently hinder SMEs from implementing these solutions (Ioppolo et al., 2020).

Recent policy developments, including the EU Green Deal and Italy's National Recovery and Resilience Plan (PNRR), encourage innovation in wastewater treatment and valorisation. These initiatives promote the integration of depuration processes with energy recovery and nutrient recycling, fostering a transition from linear to circular water management. In citrus-producing regions such as Sicily and Calabria, regional authorities increasingly support valorisation pathways—such as anaerobic digestion, lagooning, and biorefinery approaches—that combine environmental protection with resource recovery (Zema et al., 2019; Calabrò et al., 2024).The new law reinforces the trend towards circular water management, positioning reuse of treated wastewater (that is, treated effluents meeting the quality requirements for agricultural reuse)as a strategic option for agro-industrial sectors. For citrus industries, this opens new perspectives for integrating depuration with resource recovery. The challenges discussed throughout Section 3.1—spanning chemical complexity, environmental risks, regulatory pressures, and technological limitations—converge in real industrial practice. The following section introduces the case study of Amedeo Giovanni S.r.l., detailing the industrial processes, wastewater generation, and depuration strategies that motivated the experimental research described in Chapters 4 and 5.

CHAPTER 4: ANALYSIS OF THE CASE STUDY

4.1 The citrus processing company "Amedeo Giovanni s.r.l." - Reggio Calabria, Italy: history and mission

The present case study focuses on Amedeo Giovanni S.r.l, a citrus processing company located in Reggio Calabria, Italy (Via A. Garibaldi Var. 26, CAP 89135).

The origins of Amedeo Giovanni S.r.l. date back to 1890, when the founder's grandfather started a small artisanal business dedicated to the extraction of essential oils from citrus fruits using manual methods. At that time, the extraction was performed entirely by hand, using specialized tools known as "trade instruments", such as knives for cutting the fruit, sharp spoons ("cavatori") for removing the pulp, and natural sea sponges to absorb the oil from the peels. The oil was then squeezed and decanted to obtain the traditional "sponge essence," a valuable product appreciated worldwide in its various scents, orange, lemon, bergamot, and more.

Over the decades, the company has evolved from a small craft enterprise into a modern, technology-driven citrus processing facility, now managed by the fifth generation of the Amedeo family. The historical legacy is combined with continuous innovation, ensuring that traditional know-how coexists with state-of-the-art processing techniques and a strong environmental commitment.

The company's mission is centred on the valorisation of citrus fruits through sustainable processing. Its philosophy combines craftsmanship with industrial precision, aiming to produce high-quality, natural products while preserving the environmental integrity of the territory. Through constant research and technological upgrades, Amedeo Giovanni S.r.l. continues to expand its product range (essential oils, juices, and processed peels), maintaining compliance with HACCP standards and promoting the responsible use of resources. These features make the facility representative of medium-scale citrus processing industries in the Mediterranean region and highly suitable for studying wastewater generation and treatment.

4.2 Production processes

The company operates a fully integrated citrus processing plant equipped with three flexible processing lines. These lines handle various citrus fruits, including lemon, bergamot, sweet and bitter orange, mandarin, clementine, Sicilian lime, citron, and grapefruits.

The plant performs all production stages, including raw fruit inspection, essential oil extraction, juice extraction, and peel processing. Rigorous quality control is applied at each stage, in compliance with HACCP standards. Over time, the company has expanded its product portfolio to include essential oils, juices, and peel derivatives, with a focus on full utilization of the raw materials.

Figure 4.1 provides an overview of the industrial workflow adopted by Amedeo Giovanni S.r.l., illustrating the sequential operations involved in juice and concentrate production and highlighting the associated critical control points.

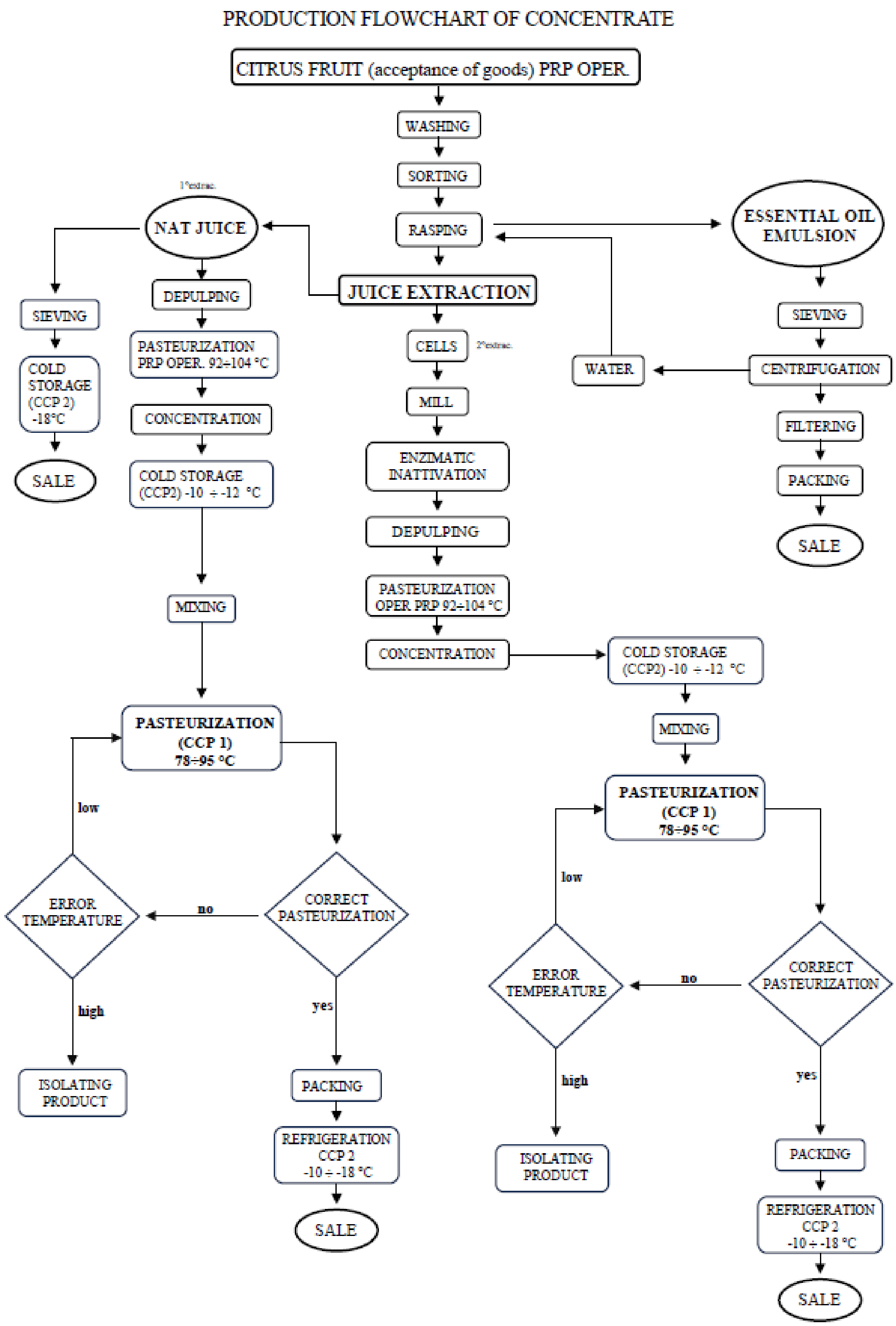
The company processes approximately 40 tons of fresh citrus per day during the peak season (October–May), corresponding to an annual production of around 6,400 tons. Daily water consumption during the production season is approximately 46 m^3/day, for a seasonal total close to 8,300 m^3/year.

4.2.1 Reception of raw materials

Raw citrus fruits (oranges, mandarins, lemons, and bergamots) are initially received and inspected for quality, compliance, and hygiene. Non-food materials (packaging, cleaning agents) are stored separately to avoid cross-contamination. Transportation and storage conditions, including temperature monitoring and documentation of cleaning cycles, are strictly controlled in accordance with HACCP guidelines and EU regulations.

Fruits are washed in potable water, removing dirt, leaves, and debris. Selection is performed visually to discard fruits that do not meet quality standards. Fruits are then transported via basket conveyors to a brush washer, where rotating brushes combined with water jets remove leaves, twigs, dirt, and damaged fruits. The brush washer is made of stainless steel and includes 27 brushes, two suction rollers, and a discharge roller. Water consumption in this stage is approximately 1.2 m^3 per 10 tons of fruit. Generated wastewater consists of washing water containing solids, sugars, peel fragments, fine particulate matter, and trace organic residues released during the mechanical cleaning of the fruits. Hygiene and sanitation are strictly maintained to minimize microbial contamination.

Figure 4.1 - Process flow diagram of the juice and concentrate production line at Amedeo Giovanni S.r.l.
The diagram summarizes the main operational stages of fruit reception, washing, sorting, rasping, juice extraction (first and second pressing), enzymatic inactivation, depulping, pasteurization, concentration, cold storage, and packaging, including critical control points (CCP1 and CCP2).



4.2.2 Essential oil extraction

Essential oil is recovered through a system that punctures the oil cells located in the citrus peel in the presence of a continuous water flow. The fruits rotate between parallel and adjacent rollers equipped with sharp points. Each roller is composed of multiple axial metal discs with pointed teeth along their perimeter, approximately 2.5 million puncturing points per machine. The rollers operate inside a water tank that partially submerges the fruits during the extraction phase, with the first four rollers and the following ones rotating at different speeds.

The fruits are transferred from one roller gap to the next by an intermittent rotary feeder. At the outlet, a stainless-steel roller conveyor (dryer) removes any residual essential oil through a water spray.

The two streams of oil-water emulsion are filtered to remove solid deposits using a finisher designed for citrus essential oil mixtures. The filtered emulsion is then pumped to a feed tank supplying the centrifuges. The centrifugation system consists of a primary and a secondary automatic-discharge centrifuge arranged in series: the first (self-cleaning) unit removes most of the sludge and water, while the second performs the final clarification of the essential oil.

The purified essential oil is collected, while the aqueous phase is sent to a three-compartment settling tank (Florentine flasks). From there, it is pumped back to the essential oil extractor for recirculation and reuse.

Water consumption in this phase is approximately 1 m^3 per 10 tons of processed fruit. The resulting effluent contains bioflavonoids, essential oils, and juice residues, with a typical pH of around 6.

4.2.3 Juice extraction

Juice extraction is carried out using a spellalbedo mechanical extractor, which processes citrus fruits previously deoiled in the essential-oil extraction step. The extractor separates the juice while simultaneously cleaning the peels, which are later destined for candied-peel production. The fruits are first cut into two halves by a fixed knife positioned between two counter-rotating cylinders coated with a rasp-type metal surface. Each half is then pressed against a height-adjustable perforated stainless-steel plate located beneath the cylinders and shaped to match their profile.

The juice passes through the perforated plate and is collected in a closed tank below, from which it is pumped to a two-stage juice refiner designed to remove remaining solids. The extractor is equipped with an adjustable device that separates membranes and residual pulp from the albedo, producing clean peels of controlled thickness suitable for further processing.

Cleaned peels exit through a dedicated outlet, while membranes and pulp residues are conveyed via a screw feeder to the loading hopper of a twin-screw press for residual juice recovery. In the first treatment zone of the press, drainage occurs because of the combined translational–rotational motion of the counter-rotating screws and a slight pressure increase generated by a wedge-shaped element. In the subsequent pressing zone, true mechanical pressing is performed, driven by screw geometry and by a backpressure regulation device located at the terminal head of the press. The resulting solid residue (pastazzo) is collected and used as an animal-feed product.

At the end of each working day, the entire extraction line undergoes sanitation through a 3 m^3 washing cycle with a 0.3% KOH solution, which produces an alkaline wastewater stream with a pH of approximately 12.

Wastewater from juice extraction therefore includes:

– dilute juice residues,
– membrane and pulp residues,
– wash water from extractors, presses and pipelines,
– alkaline CIP effluents.

These contribute significantly to the overall organic load, due to their high concentrations of sugars, pectins, organic acids, and suspended solids.

4.2.4 Juice concentration

Juice obtained from both the first and second pressing steps is transferred to temporary storage tanks and subsequently pumped into a falling-film evaporator. Within this system, the juice flows downward as a thin film along the inner walls of vertically arranged tubes preheated by steam. This configuration maximizes the heat-transfer surface area and, together with the reduced pressure inside the evaporator—which lowers the boiling point of the liquid—allows for rapid and energy-efficient evaporation.

The concentrated juice, having lost a substantial portion of its water content, is collected at the bottom of the evaporator and sent to a heat exchanger for cooling before being stored in refrigerated tanks at −10 to −12 °C. The water vapor generated during evaporation is conveyed to a condenser, where it is either discharged or recovered depending on operational requirements.

The concentration step produces a significant volume of condensate, equivalent to approximately five-sixths of the incoming natural juice, amounting to roughly 24 m³ per day under typical processing conditions. Additional wastewater streams are generated during associated cleaning operations, including 3 m³ of preliminary washing water, 3 m³ of a 0.3‰ KOH alkaline cleaning solution (with a pH of approximately 12), and 3 m³ of rinse water.

Wastewater produced during this phase is generally less concentrated than juice-extraction effluents but contributes to the overall hydraulic load and introduces variable pH conditions due to alkaline cleaning cycles.

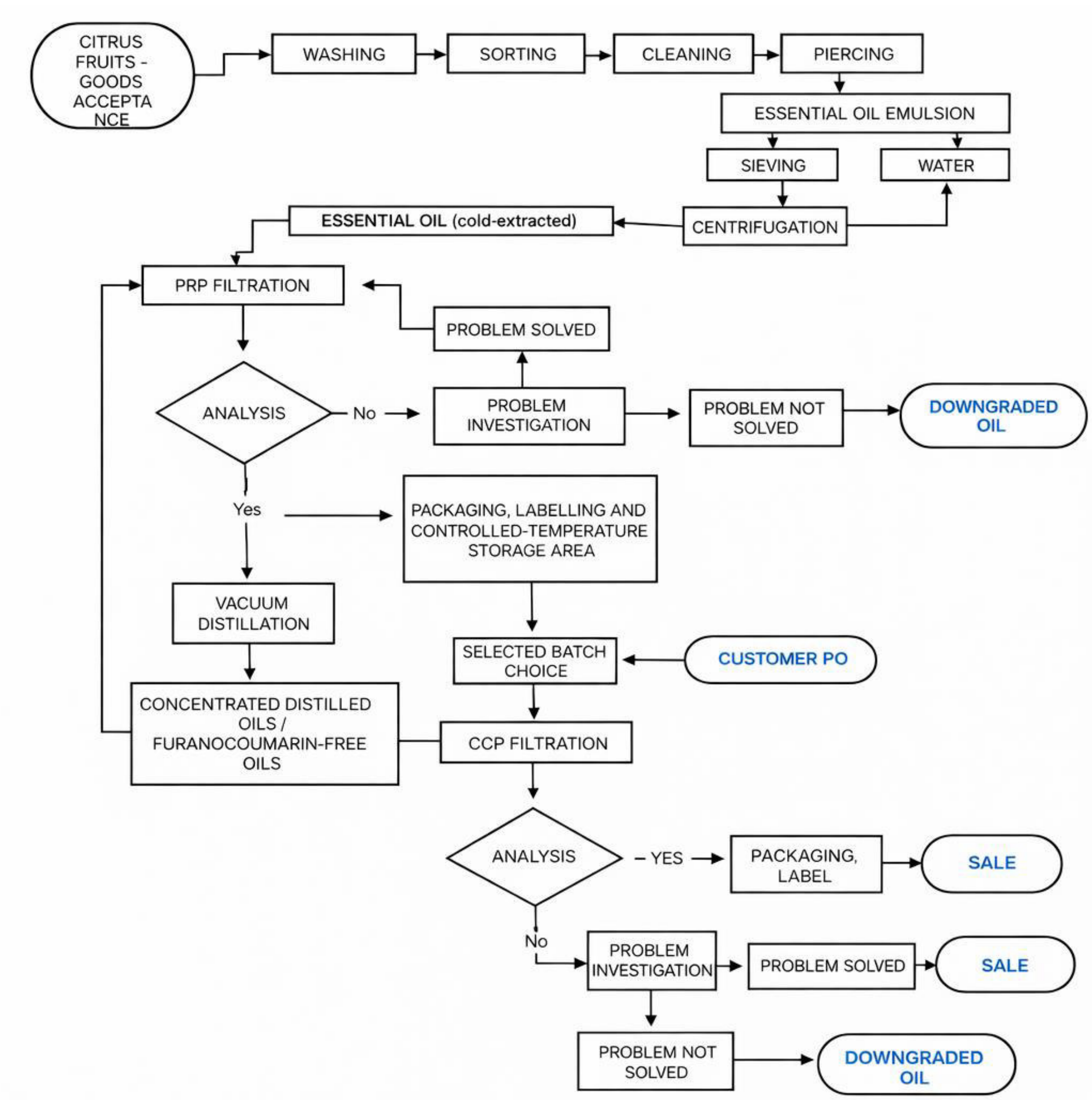


Figure 4.2 - Process flow diagram of the essential oil extraction line.
The diagram illustrates the sequential steps of cold extraction, sieving, centrifugation, filtration, quality checks, vacuum distillation, batch selection, packaging, and storage.

4.2.5 Storage, pasteurization, and packaging

After the concentration stage, the juice is transferred to insulated stainless-steel storage tanks designed to operate under controlled low-temperature conditions. The concentrated product is maintained at −10 to −12 °C, preventing microbial proliferation and preserving its chemical and sensory properties during storage. Temperature monitoring systems and alarm controls ensure continuous compliance with HACCP requirements and maintain the integrity of the cold chain.

Before distribution, the product undergoes a second pasteurization step, during which it is heated to 80–90 °C for approximately 2 minutes. This heat treatment ensures the inactivation of residual microbial populations and stabilizes the concentrate prior to packaging. Pasteurization is typically performed using inline plate heat exchangers, which allow for rapid heating and cooling, minimizing thermal degradation and ensuring high product quality.

Following pasteurization, the juice is packaged using certified food-grade materials selected according to the distribution format. Packaging options include heat-sealed plastic bags, 200-L stainless-steel drums, and 1000-L refrigerated tanks, all compliant with EU food-contact legislation. For bulk transport, the concentrate is pumped into refrigerated tanker trailers, ensuring continuous temperature control throughout the supply chain.

The packaging line incorporates several quality-control procedures, including visual inspection, sealing integrity verification, traceability labelling, and batch registration. These operations guarantee product safety and facilitate rapid identification of production lots if required by regulatory authorities.

Although this stage generates relatively low-strength wastewater compared with extraction and concentration, it produces continuous streams associated with equipment rinsing, line washing, accidental juice losses, and surface cleaning. These effluents contribute to the overall hydraulic load and typically consist of diluted organic residues with variable pH depending on the sanitation protocol adopted.

4.3 Wastewater generation and characteristics

4.3.1 Origin of wastewater streams

The citrus-processing operations carried out at Amedeo Giovanni S.r.l. generate a complex mixture of wastewater streams originating from several stages of the production chain. The first significant contribution derives from fruit washing, where high-pressure water jets and brush rollers remove soil, leaves, peel fragments, and organic material adhering to the fruit surface. The resulting washing water typically contains suspended solids, sugars, pigments, and fine particulate matter released during mechanical abrasion.

A second major source of wastewater is the essential oil extraction stage. The puncturing and abrasion of citrus peels in a continuous water bath produce emulsified mixtures of water, essential oils, bioflavonoids, and fine suspended solids. Even after filtration and centrifugation, part of this mixture becomes wastewater. These effluents are of particular environmental concern because they contain hydrophobic compounds, such as d-Limonene, which are known to inhibit microbial activity and can negatively affect biological treatment processes.

Additional wastewater streams originate from the juice-extraction line. During pressing, separation, refining, and cleaning of the extraction equipment, residual juice, pulp fragments, pectins, and organic acids are released into the process water. These effluents exhibit very high organic loads due to the abundance of readily biodegradable sugars and soluble organic compounds, and they are often characterised by substantial suspended solids. Their acidic nature (pH 4-5) contributes to the overall variability of the wastewater matrix and may promote acidification if not properly buffered.

Further contributions arise from the concentration phase, where the falling-film evaporator produces significant volumes of condensate. Although this condensate is generally less contaminated than other process streams, it adds considerable volume to the total effluent. Daily cleaning operations constitute an additional and important source: sanitation activities employ alkaline KOH solutions (0.3‰), generating wastewater with pH values around 12. When combined with acidic streams from juice extraction, these alkaline effluents introduce pronounced pH fluctuations and increase the need for adequate equalisation.

4.3.2 Current wastewater management at the industrial site

The company Amedeo Giovanni S.r.l., located in Reggio Calabria, operates in the citrus-processing sector and provides a representative example of a medium-sized enterprise in Southern Italy. Like many agro-industrial facilities in the Mediterranean basin, it faces the challenge of managing large volumes of CPW generated during seasonal campaigns. As already mentioned, CPW is characterized by high organic loads, strong variability in composition, and the presence of inhibitory compounds such as essential oils. These features make its treatment particularly complex and require robust technological solutions. In order to comply with Italian legislation (D.Lgs. 152/2006) and European directives on industrial effluent discharge, the company has invested in a modern Membrane Bioreactor (MBR)-based wastewater treatment plant This technological choice reflects the need for high depuration efficiency, compact design, and the possibility of partial reuse of treated water in industrial operations.

Influent characteristics

Analytical data provided by the company (Tab. 4.1) confirm the complexity of CPW. The influent wastewater shows COD values up to 8000 ppm (≈ 640 kg/day) and BOD_5 values up to 7000 ppm (≈ 560 kg/day). Nutrient concentrations are relatively low, with total nitrogen (TKN) below 20 ppm and phosphorus below 15 ppm, while surfactants are present at around 10 ppm. These values are consistent with the literature, which describes CPW as nutrient-deficient, highly biodegradable, but also rich in inhibitory compounds such as d-Limonene. (Lucia et al., 2022; Zema et al., 2019b). The seasonal nature of citrus processing further complicates treatment, concentrating wastewater generation over short periods and requiring systems capable of coping with hydraulic and organic shock loads.

Table 4.1 - Influent and effluent parameters of the case study plant

Parameter	Influent	Effluent
COD (ppm)	< 8000	< 160
BOD_5 (ppm)	< 7000	< 40
pH	variable	6.5–8.5
Suspended solids (ppm)	high	< 80
Phosphorus (ppm)	< 15	< 10
Nitrogen (ppm)	< 20	< 15 (NH_4^+), < 20 (NO_3^-)
Surfactants (ppm)	± 10	< 2

4.3.2.1 Treatment process description

The treatment line is organised into several sequential stages, each designed to address the specific challenges of CPW. (Fig.4.3).

1. Preliminary screening and hydraulic lifting. At the inlet, coarse solids such as peel fragments, pulp residues, and fibrous materials are removed through mechanical screening. This step prevents clogging of pumps and membranes and ensures continuous flow. Hydraulic lifting devices then convey the wastewater to the equalisation basin.
2. Equalisation basin. Wastewater is then conveyed to an aerated equalization basin, dimensioned for a daily hydraulic loading of approximately 80 m³/day and peaks of 4–4.5 m³/h. This unit homogenises hydraulic and organic loads, buffers daily and seasonal fluctuations, stabilises pH, and allows partial sedimentation of coarse solids. Continuous aeration prevents anaerobic conditions and odour formation, while pH monitoring ensures dosing, when necessary, of corrective chemicals. This stabilisation step reduces variability before biological treatment and is essential for protecting downstream units.
3. Biological oxidation (activated sludge reactor). From the equalisation tank, the flow enters the biological oxidation stage, where aerobic mesophilic bacteria degrade organic matter under continuous aeration. The process promotes nitrification and denitrification, reducing nitrogen compounds and enhancing overall depuration efficiency. Aeration is provided by blowers housed in soundproof cabins, limiting noise emissions to below 72 dB(A). Nutrient supply (nitrogen and phosphorus) is occasionally required to sustain microbial growth, given the imbalance typical of CPW.
4. Membrane Bioreactor (MBR) clarification. Clarification is achieved through a Membrane Bioreactor, which filters the mixed liquor through tangential membranes. This advanced configuration retains suspended solids, colloids, and pathogens, producing high-quality effluent suitable for discharge into surface waters or partial reuse in industrial processes. Compared with conventional sedimentation, the MBR system ensures superior effluent quality and compact design, though it requires careful management of fouling phenomena.
   Operational data confirm its effectiveness:
   - COD reduced from ≤ 8000 ppm to < 160 ppm
   - BOD_5 reduced from ≤ 7000 ppm to < 40 ppm
   - Suspended solids limited to < 80 ppm
   - Phosphates reduced to < 10 ppm
   - Surfactants reduced to < 2 ppm
   - Effluent pH stabilised between 6.5 and 8.5
5. Excess sludge generated during biological oxidation is stabilised aerobically and mechanically dewatered. The resulting biosolids can be valorised as soil amendments, contributing to circular-economy principles and reducing disposal costs. This agronomic reuse is particularly important in Mediterranean contexts, where organic amendments are valued for improving soil fertility.

6. Finally, wastewater passes through a compliance and discharge stage, where samples are taken in the final inspection chamber. Discharge is authorised only if values comply with regulatory limits for sewer discharge (Tab. 3 Allegato 5, D.Lgs 152/06). Mandatory controls include pH, COD, oils and greases, total suspended solids, and detergent residues.

4.3.2.2 Effluent quality and environmental impacts

The treated effluent (Table 4.1) meets regulatory standards, with COD reduced to below 160 ppm and BOD_5 values falling under 40 ppm. Suspended solids are limited to less than 80 ppm, and nutrient concentrations remain within regulatory thresholds: phosphates below 10 ppm, ammonium nitrogen under 15 ppm, nitrates below 20 ppm, and nitrites below 0.6 ppm. Surfactants are reduced to less than 2 ppm. These results demonstrate compliance with Italian discharge standards and highlight the potential for partial reuse of treated water, reducing freshwater consumption and supporting sustainable industrial practices (Calabrò et al., 2025).

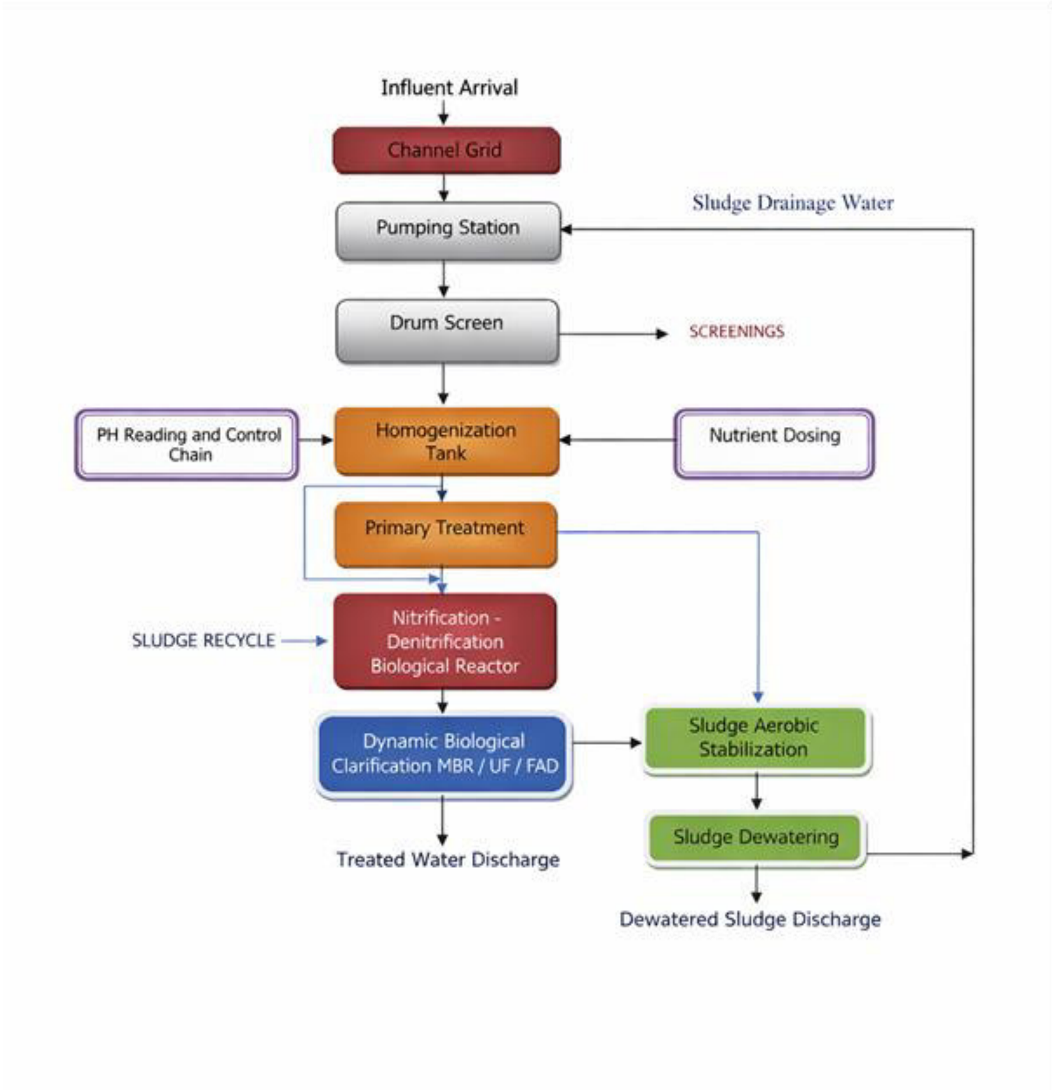
The plant design also considers broader environmental impacts. Emissions to the atmosphere are limited to nitrogen, carbon dioxide, and water vapor, while noise emissions are controlled through soundproofing. Thermal emissions are negligible, as wastewater temperatures remain close to ambient levels. Solid residues are managed through sludge stabilization and dewatering, with potential agronomic reuse. These features align with best available technologies (BAT) for agro-industrial wastewater treatment and reflect the company's commitment to environmental protection.

4.3.2.3 Critical discussion

The case study highlights several strengths of the adopted configuration. The plant achieves high depuration efficiency, with COD and BOD reductions exceeding 95%, and its compact design makes it suitable for medium-sized enterprises with limited space. The possibility of partial reuse of treated water reduces dependence on freshwater resources, while sludge valorisation supports circular-economy strategies.

Nevertheless, challenges remain. Energy demand for aeration increases operational costs, and nutrient imbalance requires external supply of nitrogen and phosphorus to sustain microbial growth. Seasonal variability complicates process stability and biomass management, suggesting that future improvements should focus on integrating biorefinery-oriented strategies. Such approaches could enable the recovery of volatile fatty acids, biopolymers, or bioenergy from CPW, enhancing sustainability and economic viability (Welti-Chanes et al., 2025).

Figure 4.3 - Flowchart of the wastewater treatment process (Company data, 2019).



CHAPTER 5: LABORATORY-SCALE TESTS

The laboratory-scale investigation was specifically planned to identify the optimal operational conditions to maximise the depuration efficiency of aerated lagoon systems treating CPW. The laboratory system was designed to reproduce the operational functioning of the aerobic upper layer of lagoon systems under controlled conditions.

Among the peculiar characteristics of CPW, discussed in Chapter 2, high organic loads, acidic pH, imbalanced COD/N ratios, and the presence of inhibitory compounds (such as EOs) may be the crucial issues for its depuration. These features often compromise the stability, efficiency, and energy sustainability of conventional activated sludge systems, thereby motivating the investigations of aerated lagoon-based solutions. Aeration is among the main cost drivers in wastewater treatment systems (Zema et al., 2016a, 2012b) (including lagoons). To explore the influence of each of the aforementioned factors on the depuration efficiency of lagoons, the experimental design followed a one-factor-at-a-time approach, allowing the independent evaluation of three key operational parameters:

- aeration regime
- EO concentration
- COD/N ratio.

In other words, predefined air flow volumes and durations (regulating the aeration rates), dilution factors and addition of nitrogen were applied, to isolate and independently assess the effects of oxygen availability, EO concentration, and nutrient balance on organic matter degradation and process stability (measured by pH).

This approach enabled the identification of the specific role of each parameter in controlling microbial activity, inhibition process, and overall treatment performance.

5.1 Material and methods

5.1.1 Experimental Setup

The laboratory system consisted of 18 cylindrical tanks (hereafter also indicated as 'reactors'), each with a working volume of 100 L and made of inert plastics. The selected reactor volume is consistent with previous laboratory investigations on lagoon-based systems treating agro-industrial wastewater (Andiloro et al., 2021b).

All reactors were operated at room temperature, without external thermal control, to reproduce the environmental conditions typically of lagoon systems in Mediterranean climates. Aeration was supplied through small spherical (5-cm diameter) fine-bubble diffusers placed at the bottom of each tank and connected to a blower with a constant airflow rate of 50 L h^{-1}, both commonly used in home aquariums.

This aeration configuration ensured adequate oxygen transfer to support aerobic degradation in the water column, while allowing the formation of anoxic or anaerobic micro-zones, a characteristic feature of lagoon systems widely documented in the literature (Calabrò et al., 2024; Zema et al., 2016a).

Water mixing was exclusively due to aeration, and no mechanical stirring was applied. This preserved lagoon-like hydrodynamic conditions where mixing is primarily driven by air supply and natural convection rather than by forced agitation. Avoiding mechanical stirring promotes the development of vertical stratification, a feature highlighted by Calabrò et al. (2024) as fundamental for lagoon functioning, influencing microbial distribution, redox zonation and the progression of organic matter degradation. (Calabrò et al., 2024).

Each experimental condition was tested in duplicate. This low number of replicates is justified by two reasons: i) funding limitations; and ii) the results of a previous experience, carried out by the same authors, which demonstrated that a extremely low (i.e., two) number of replicates does not affect data accuracy and thus reliability when system behaviour is stable and reproducible (Calabrò et al., 2022). Based on those findings, duplicate reactors were considered sufficient to ensure robust and representative results.

As a direct consequence of this experimental design, the error bars reported in the results (Figures 5.2, 5.3 and 5.5) reflect the variability captured by only two replicates. This does not contradict the reliability of the duplicate design discussed above: while n = 2 was shown to yield accurate and representative mean values under stable, reproducible system behaviour, it remains, by definition, a less precise estimator of the underlying variance than designs with three or more replicates — these are two distinct statistical properties. With n = 2, the sample standard deviation is a comparatively imprecise estimator of the true variability, which mechanically widens the error bars compared to designs with three or more replicates; this should be taken into account when interpreting the magnitude, rather than the direction, of the observed variability.

All reactors were operated in parallel under identical geometric and environmental (i.e., weather) conditions, enabling a direct comparison of treatment performance among the different operational scenarios (Figure

5.1). The tanks operated in batch mode for approximately 13 weeks (October 2024- January 2025).
The depuration efficiency of wastewater was analysed measuring the reduction in COD, TN, phenols (as a proxy for inhibitory compounds) and the variations in pH.

5.1.2 Wastewater characteristics

The influent used throughout the experimental campaign consisted of citrus processing wastewater collected from the industrial company 'Amedeo s.r.l. - Reggio Calabria'.
Raw CPW was collected directly from the processing line, after the mechanical screening, and stored for a short time before use. The main physico-chemical characteristics of CPW - pH, chemical oxygen demand (COD), Total solids (TS), Total Volatile Solids (TVS) and nutrient concentrations (TN) - are reported in Table 5.1. All reactors were fed in batch mode at the beginning of each experimental test series.

Table 5.1 - Initial characteristics of the raw CPW used in the experiments

Parameter	Initial Value	Unit	Notes
pH	6.1	–	Measured at sampling
COD	4000	mg/L	Raw, unfiltered sample
TS	2800	mg/L	Total solids
Total Nitrogen (TN)	1.5	mg/L	Used for COD/N calculation
COD/N ratio	2666.7	–	Calculated as COD / TN
Total Phenols	2.1	mg/L	Used to evaluate toxicity

Throughout this thesis, the nutrient balance of the wastewater is expressed as the COD/N ratio (chemical oxygen demand to total nitrogen), rather than the conventional C/N ratio based on organic carbon, in order to avoid ambiguity and to ensure direct comparability with the analytical parameters actually measured (Table 5.1).

5.1.3 Experimental design and operating conditions

The experimental campaign was structured into three tank groups, each characterised by specific operating conditions. In each group, one operational parameter was varied at a time, and a reference condition was assumed as a control, while the other parameters were kept constant with the aim of isolating the single operational parameter (Table 5.2). A schematic representation of the experimental design adopted for the laboratory-scale tests is reported in Figure 5.1

Figure 5.1 - Experimental design of laboratory scale test

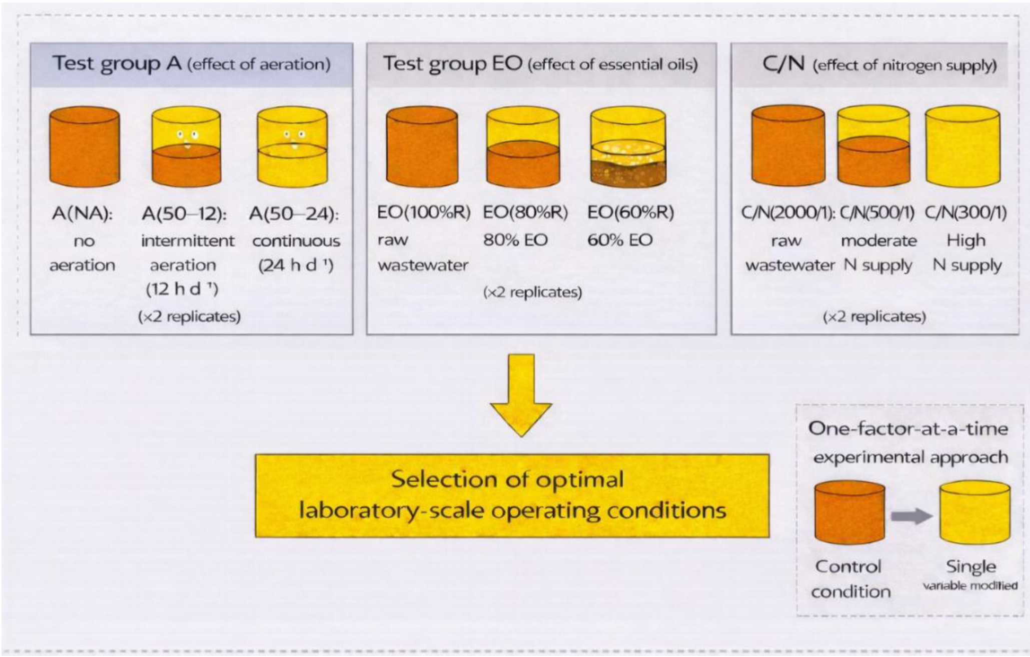


Table 5.2 - Experimental design for the laboratory-scale tests on the treatment of citrus processing wastewater by aerated lagoons.

Tank group	Tank	Operation conditions
A (effect of aeration)	A(50-24)	50 L/h for 24 h (permanent aeration)
	A(50-12)	50 L/h for 12 h (intermittent aeration)
	A(NA)	No aeration (control)
EO[1,5] (effect of essential oils)	EO(100%R)	Raw wastewater concentration (control)
	EO(80%R)	80% of EO concentration in raw wastewater[2]
	EO(60%R)	60% of EO concentration in raw wastewater [2]
COD/N (effect of nitrogen supply)	COD/N(2000/1)	Raw wastewater COD/N (control)
	COD/N(500/1)	Ideal for lagoon systems (Andiloro et al., 2013a; Zema et al., 2016a)[3,4]
	COD/N(300/1)	Tolerated by lagoon systems (Andiloro et al., 2013a; Zema et al., 2016a)[3,4]

Notes: 1 - EO = essential oils; 2 - corrected by wastewater dilution and sugar addition to keep the organic matter content equal among tanks; 3 - corrected by urea addition until the desired value; 4 - source (Andiloro et al., 2013b; Zema et al., 2016a).

5.1.3.1 Tank group A - Effect of aeration

This tank group was planned to evaluate the influence of oxygen availability on the depuration efficiency of aerated lagoons treating CPW. Three aeration rates were tested:

- A(50-24): continuous aeration (50 L h^{-1} for 24 h d^{-1})
- A(50-12): intermittent aeration (50 L h^{-1} for 12 h d^{-1})
- A(NA): no aeration (control condition).

These conditions were selected to represent, respectively, the upper limit of aerobic degradation, an energy-saving intermittent strategy, and the natural behaviour of CPW in anaerobic-facultative conditions.

5.1.3.2 Tank group EO - Effect of Essential Oils

This tank group was planned to evaluate the influence of the inhibitory effect of EOs on the depuration efficiency of aerated lagoons treating CPW. Three concentrations of EO were tested, each corresponding to a specific dilution rate of CPW:

- EO(100%R): raw wastewater (control condition).
- EO(80%R): 80% CPW.
- EO(60%R):60% CPW.

To obtain these concentrations of EO while maintaining the same amount of available organic matter, sugar (as easily degradable organic substrate) was added in the tanks. This adjustment was necessary to isolate the inhibitory EO without simultaneously reducing the substrate available for microbial degradation. Otherwise, dilution would have reduced concentrations of both EO and organic matter.
Sugar was fully dissolved in the diluted wastewater before the start of the experiment to ensure homogeneous distribution of organic matter.

5.1.3.3 Tank group COD/N - Effect of nitrogen supply

This tank group was planned to evaluate the effect of carbon to nitrogen balance on the depuration efficiency of aerated lagoons treating CPW. Three COD/N ratios were tested:

- COD/N: 2000/1 (control condition).
- COD/N: 500/1.
- COD/N: 300/1.

Nitrogen was supplied in the COD/N tank group using urea, $CO(NH_2)_2$. This additive was selected due to its ready solubility, low cost, and common use as a nitrogen source in biological wastewater treatment. Urea provides nitrogen in a chemically stable form and releases ammoniacal nitrogen upon hydrolysis, supporting microbial growth in systems where the natural COD/N ratio is excessively imbalanced, as in the CPW.
Urea was added at the beginning of the experiment, and it was fully dissolved in the wastewater to ensure homogeneous nitrogen availability. This approach allowed the system to reach the desired COD/N ratios without altering the organic matter concentration, thereby isolating the effect of nitrogen supply on microbial activity and organic matter degradation.

5.1.4 Chemical analyses

The biochemical characterisation of the wastewater treated in the 18 tanks was performed on samples collected at the start of the experiment (immediately after the wastewater preparation) and once a week until the end of experiment. Since no mixing system was installed, the CPW was manually mixed before each sampling. Therefore, observed non-monotonic behaviour may be explained by random, non-systematic errors affecting individual measurements.
The samples were immediately transported to the laboratory, frozen at −20 °C according to standard protocols and analysed as soon as possible.
The pH was measured through a digital pH meter (WTW 3320). COD, Total Nitrogen and phenolic compounds were determined by using pre-dosed cuvettes (Merck Millipore COD Cell Test 114555 and Ammonium Cell Test 114559, respectively). Phenolic compounds were assumed as an indirect measure of toxic compounds in wastewater (e.g. polyphenols). This choice is supported by two reasons:

- the analytical facilities available for this study ("Laboratorio di Idraulica Agraria") are not equipped with chemical analytical instruments or advanced chromatographic instrumentation (e.g., GC-MS or GC-FID) required for direct toxic compounds determination. Therefore, the use of phenolic compounds as a proxy does not aim to quantify these compounds in absolute terms. This methodological choice is consistent with approaches reported in the scientific literature on citrus wastewater characterisation and treatment (Lucia et al., 2022).
- both phenols and EO originate from the rupture of oil glands and the release of secondary metabolites during citrus fruit processing. Some studies on citrus wastewater report that phenolic compounds, terpenes and EO share a common biochemical origin and are simultaneously released during fruit washing, brushing and juice extraction (Lucia et al., 2022; Modica et al., 2024).Although they belong to different chemical families, phenols and EO often exhibit parallel concentration trends during biological degradation, as both are susceptible to microbial oxidation, volatilisation and adsorption phenomena (Lucia et al., 2022). For this reason, phenolic compounds are frequently used as operational markers of EO dynamics when direct chromatographic quantification is not feasible.

Specifically, COD was determined using WTW pre-dosed cuvette tests (range 500-10 000 mg/L), based on a photometric method compliant with DIN ISO 15705 and equivalent to EPA 410.4, APHA 5220 D and ASTM D1252-06 B. The method quantifies the oxygen required to oxidise organic matter in the sample and is widely used as a key indicator of wastewater pollution load.
Each kit includes 16 mm digestion cuvettes pre-filled with the necessary reagents. A sample volume of 1.0 mL

was added to each cuvette, which was then tightly sealed and mixed vigorously to ensure complete reagent–sample contact. The cuvettes were subsequently digested in a thermoreactor (WTW CR 4200) at 148 °C for 120 min, following the manufacturer's protocol. After digestion, the cuvettes were allowed to cool naturally to room temperature for at least 30 min; rapid cooling with cold water was avoided to prevent thermal shock and glass breakage.

Before photometric measurement, cuvettes were inspected to ensure they were clean and free from external residues. Any turbidity formed during digestion may lead to overestimation of COD values; therefore, careful handling was adopted.

Measurements were performed using a photometer (WTW Photoflex Std.), which automatically recognised the test via the integrated 2D barcode (Live ID), ensuring correct calibration, lot traceability and method selection.

TN was quantified using WTW pre-dosed cuvette tests (photometric method, measuring range 0.5–15.0 mg N/L). The analytical procedure is based on an oxidative digestion step analogous to DIN EN ISO 11905-1, which converts all nitrogen-containing compounds, organic nitrogen, reduced nitrogen, ammonium, nitrite and nitrate, into nitrate. The resulting nitrate is then transformed into a photometrically-detectable compound, allowing accurate quantification of total nitrogen in wastewater samples.

A preliminary digestion step was performed to mineralise all nitrogen species. For this purpose, 10 mL of sample were poured through a pipette into an empty 16 mm round cuvette, to which the reagents supplied in the kit were added in sequence (solid reagent N-1K followed by liquid reagent N-2K). The cuvette was then sealed, mixed thoroughly and placed in the thermoreactor at 120 °C for 60 min. After digestion, the cuvettes were allowed to cool gradually to room temperature, with gentle swirling after approximately 10 min to ensure homogeneity.

For the colorimetric reaction, 1.0 mL of the pretreated sample was transferred into a reaction cuvette containing the appropriate reagent (N-3K), mixed carefully, and allowed to react for 10 min. The cuvette was then placed in the photometer for measurement.

Absorbance was measured using the photometer equipped with a 2D Live ID barcode reader that automatically recognises the test, loads the appropriate calibration curve, and records lot number and expiry date for full traceability.

Phenolic compounds were quantified using Spectroquant® pre-dosed cuvette tests (photometric method, measuring range 0.10-2.50 mg/L). The method is based on the reaction between phenol and phenolic derivatives with a thiazole reagent, forming a red-violet azo dye whose absorbance is proportional to the phenolic concentration.

Before analysis, the pH of each sample was checked to ensure the acceptable range (pH 2-11). When necessary, the pH was dropwise adjusted using dilute sodium hydroxide or sulfuric acid. A volume of 10 mL of sample was then pipetted into a 16 mm reaction cuvette and mixed. The reagents supplied with the kit were added sequentially (solid reagents Ph-1K and Ph-2K), with vigorous shaking after each addition to ensure complete dissolution. The reaction was allowed to proceed for 1 min at room temperature.

After the reaction time, the cuvette was placed in the photometer, aligning the reference mark on the cuvette with that of the instrument. The photometer automatically recognised the test through the integrated 2D Live ID barcode, ensuring correct method selection, calibration curve loading, and full traceability of lot number and expiry date.

5.1.5 Microbiological analyses

Microbiological analyses were performed on citrus-processing wastewater samples collected at the beginning of the treatment (T0) and after 13 weeks (T13w). Total microbial count (TMC) was determined using Plate Count Agar (PCA, BioKar Diagnostics, Beauvais, France) under both aerobic and anaerobic incubation conditions. For each sampling event, appropriate serial dilutions were prepared and plated in duplicate. Aerobic plates were incubated at 25 °C for 72 h, while anaerobic plates were incubated under oxygen-free conditions using sealed jars with anaerobic gas-generating sachets.

After incubation, colonies were enumerated and expressed as Log CFU mL^{-1}) Representative colonies were randomly selected and observed under an optical microscope (Olympus BX53, Tokyo, Japan) to assess cell morphology. Observations included cell shape, arrangement and motility, allowing a qualitative characterisation of the microbial community present in the wastewater.

5.1.6 Statistical analyses

A one-way ANOVA with repeated measures (at each survey date throughout the 13 weeks of the

experiments), followed by pairwise comparisons using Tukey's tests ($p < 0.05$), was applied to pH, COD, TN and phenols (response variables) to evaluate the statistical significance of the differences among the operation conditions (aeration type, EO concentration, COD/N, considered as explanatory factors with the three levels of Table 5.2) in each tank group.
Moreover, a one-way ANOVA followed by Tukey's tests ($p < 0.05$) was applied to the differences between the initial (time 0) and final (13 weeks) values of pH, COD and phenols, assumed as measures of depuration efficiency for those variables, adopting the same explanatory factor as the previous ANOVA.
Finally, a two-way ANOVA followed by Tukey's tests ($p < 0.05$) was applied to the differences between the initial (time 0, T0) and final (13 weeks, T13w) counts of microbial colonies, adopting the same factor as the previous ANOVA (first factor) as well as the incubation condition (two levels: aerobic and anaerobic) as the second factor, including their interaction.
The equality of variance (by Levene test) and normal distribution (by Shapiro-Wilk test) underpinning ANOVA were evaluated. If needed to meet these assumptions, the data were square root-transformed. Since ANOVA with repeated measures met Mauchly's test, the assumption of data sphericity was not violated.

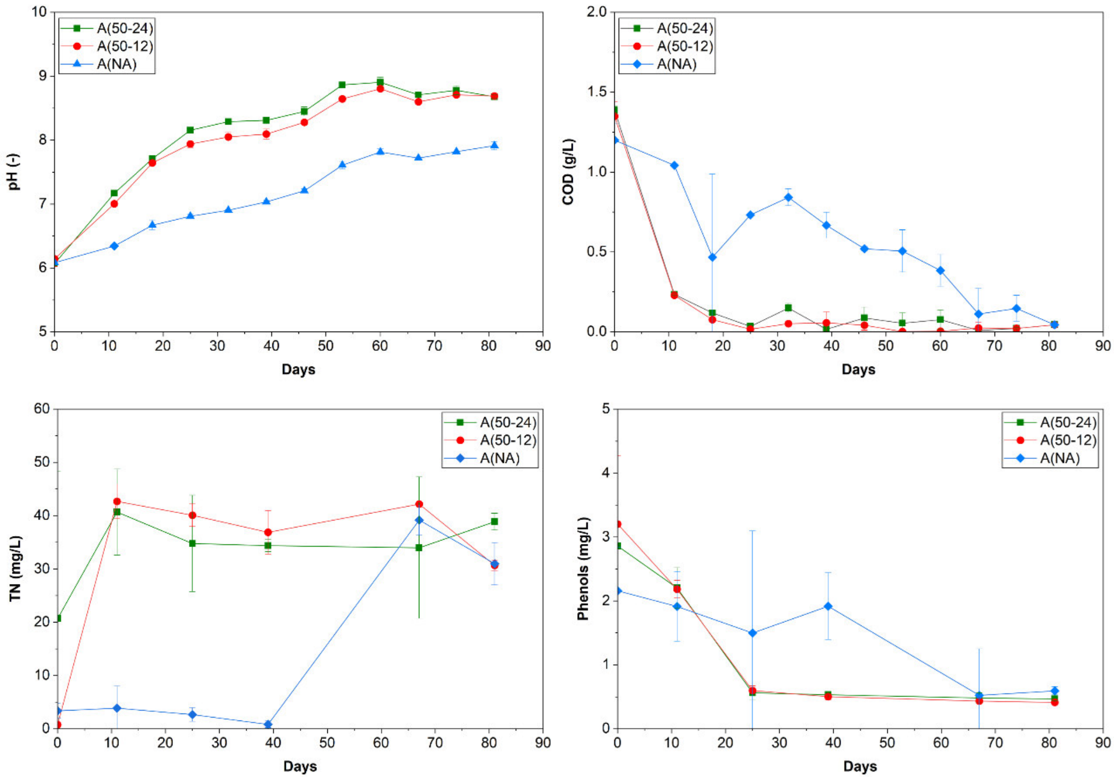
5.2 Results and discussion

As explained in Section 5.1.1, error bars throughout this section reflect the variability of duplicate measurements (n = 2) rather than data inconsistency.

5.2.1. Tank group A (effect of aeration)

Under all conditions, pH progressively increased from acidic values of the raw wastewater toward alkaline conditions. However, the rate and extent of this increase markedly differed among treatments.
In more detail, the aerated reactors, A(50-24) and A(50-12), rapidly reached stable alkaline values close to 8.7-8.9, whereas the non-aerated system, A(NA), exhibited a slower and more limited rise, stabilising below pH 8 (Figure 5.2). These observations are fully supported by the statistical analysis of final depuration efficiencies, which revealed significant differences among aeration regimes for pH ($p = 0.01$) ((Figure 5.3),(Table 5.3)).

Figure 5.2 - Temporal evolution of pH, COD, total nitrogen (TN), and phenols in the laboratory tank group A (effect of aeration).

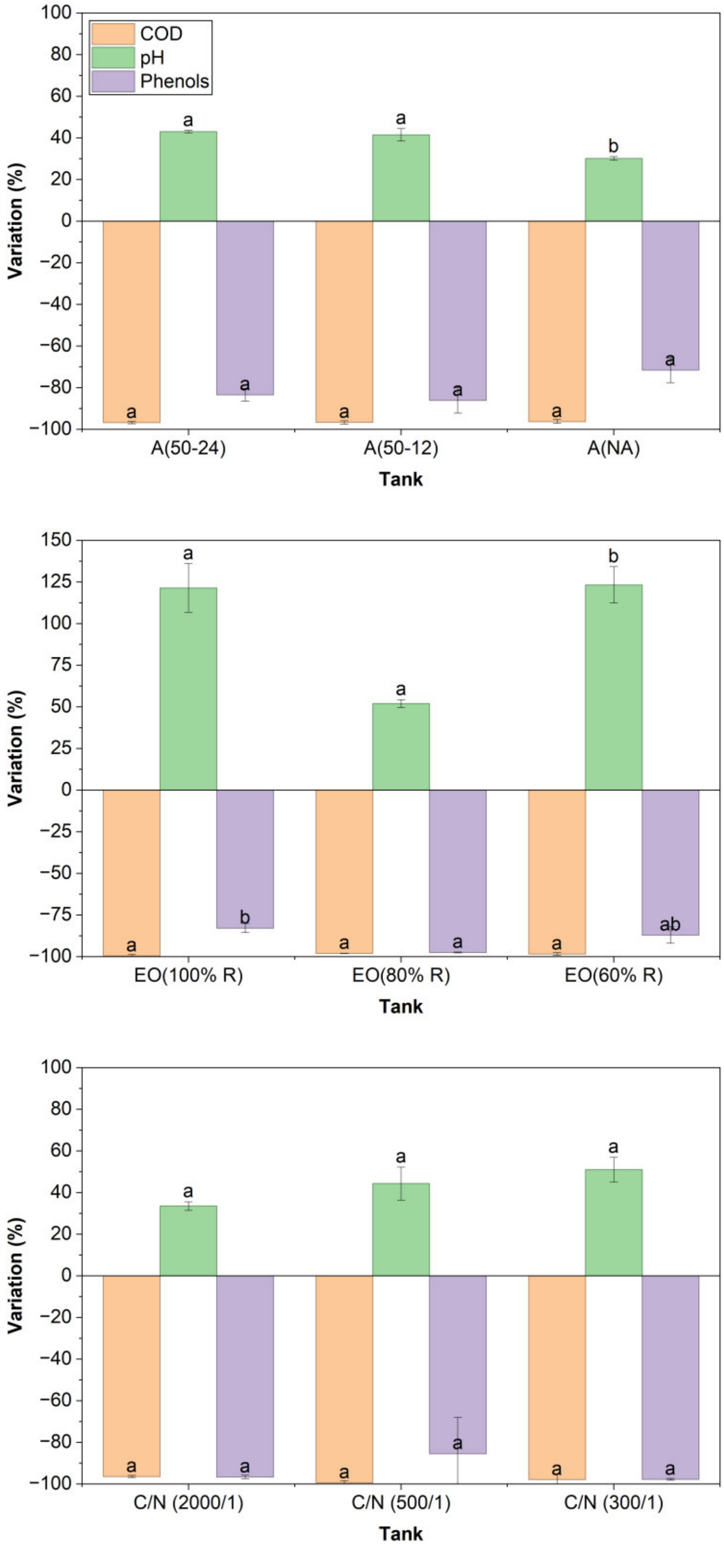


Aeration therefore exerted a measurable and statistically identifiable effect on pH correction, confirming its central role in promoting microbial activity and buffering capacity. Similar behaviour has been reported in aerated lagoon systems treating citrus-processing effluents, where oxygen availability enhances metabolic stability and accelerates pH recovery (Andiloro et al., 2013b).
COD evolution differed among the aeration conditions. Also, for this parameter, the tanks A(50-24) and A(50-12) showed a rapid decrease during the first weeks of operation, followed by stabilisation at low residual concentrations. The non-aerated condition, A(NA), exhibited a slighter and more regular decrease in COD (Figure 5.2). Despite these clear differences in process dynamics, the final COD removal efficiencies did not differ significantly among the three aeration regimes ($p = 0.77$). (Figure 5.3, Table 5.3). This indicates that aeration primarily influences the kinetics of organic acid degradation rather than the efficiency of COD removal. The faster decline observed in aerated systems compared to the tank without aeration is consistent with the enhanced oxygen transfer and stimulation of aerobic microbial pathways described by Andiloro et al. (2013b), who reported accelerated degradation rates in aerated lagoon systems treating citrus effluents. However, the convergence of final COD values across treatments demonstrates that, under the conditions tested and the long time elapsed from the start of the experiment, oxygen availability did not translate into statistically higher final removal performance (Andiloro et al., 2013b). However, 65 days of treatment were needed for the A (N/A) tank to reach the same level of treatment observed after about 20 days in the aerated tanks.
About the TN trends, aerated reactors, A(50-24) and A(50-12), showed stable concentrations after a sudden initial increase, while, in the non-aerated condition, A(NA), TN increased during the later stages of the experiment, suggesting accumulation under oxygen-limited conditions (Figure 5.2). This can be explained by a faster hydrolysis under aerobic conditions.
Phenolic compounds decreased under all aeration conditions, but this removal was clearly enhanced by oxygen availability. The decrease was initially much higher in the early stage and slower in the last weeks under aerated conditions, A(50-24) and A(50-12), whereas the reduction trend was relatively stable in the non-aerated reactors, A(NA) (Figure 5.2). Nevertheless, the final removal efficiencies of phenols were statistically comparable ($p = 0.13$), confirming that aeration accelerates the transformation of phenolic compounds but does not significantly affect the degradation level (Figure 5.3, Table 5.3). This behaviour aligns with the oxygen-dependent degradation pathways of aromatic compounds described in previous studies on citrus-processing wastewater (Andiloro et al., 2013b).
Overall, while the depuration efficiency for COD and phenols was similar among the three tanks (on average 97% and 80%, respectively, the efficiency of pH correction was significantly higher for the aerated tanks (41-43%) compared to anaerobic conditions (30%) (Figure 5.3 and Table 5.3).
The comparable final performance observed under continuous and intermittent aeration indicates that high treatment efficiency can be kept even with reduced aeration intensity. This finding is consistent with the observations of Zema et al. (2016), who demonstrated the feasibility of intermittent aeration strategies in lagoon-based systems, highlighting their potential to reduce energy consumption while maintaining effective oxygen transfer (Zema et al., 2016a).
Finally, the results demonstrate that aeration exerts a strong influence on the dynamics of CPW treatment, accelerating pH correction, COD degradation and phenol transformation, while its effect on final removal efficiencies is limited to pH after a time longer than 30-45 days. These outcomes emphasise the importance of oxygen availability for process stability and microbial activity, while also highlighting the potential for optimising aeration regimes to balance performance and energy consumption.

Table 5.3 - Results of one-way ANOVA applied to depuration efficiency of the aerated tanks for pH, COD, and phenols in the laboratory-scale tests.

Response variable	DF	Sum of Squares	Mean Square	F Value	Prob>F
	Tank group A - Effect of aeration				
COD	2	0.33	0.16	0.283	0.77
pH		199	100	29.683	0.01
Phenols		239	119	4.275	0.13
	Tank group EO - Effect of essential oil				
COD	2	2.56	1.28	3.343	0.17
pH		6586	3293	29.305	0.01
Phenols		227	114	10.588	0.04
	Tank group COD/N - Effect of nitrogen supply				
COD	2	8.87	4.44	1.824	0.30
pH		311	155	4.559	0.12
Phenols		186	93	0.923	0.49

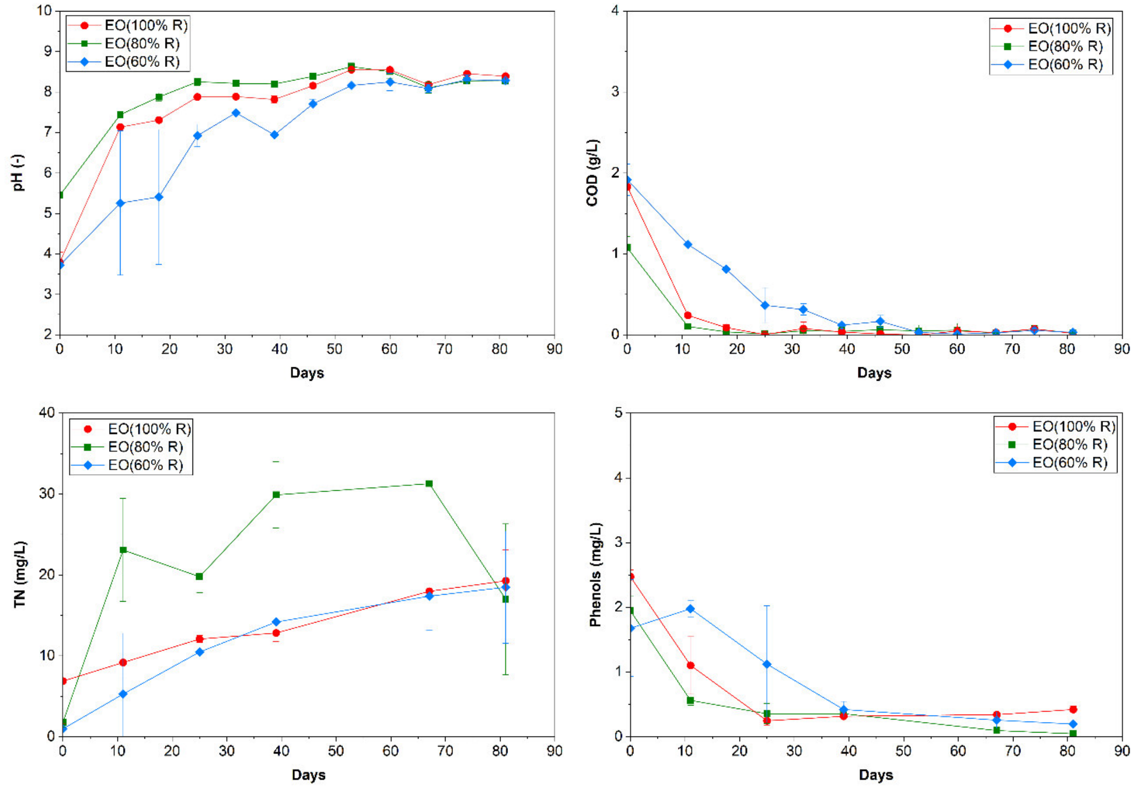
Figure 5.3 - Depuration efficiency of the aerated tanks for pH, COD, and phenols in the laboratory-scale tests. Different letters indicate significant differences after Tukey's tests ($p < 0.05$).



5.2.2 Tank group EO (effect of essential oil)

The pH increased in all treatments, but, for the reactors treating diluted wastewater, tanks EO(80% R) and EO(60% R), this increase was faster and significant (close to 120%, from 3.97-3.68 to 8.27-8.41 and from 3.81–3.62 to 8.21–8.36, Figure 5.4) compared to raw CPW, tank EO(100% R), which showed a slower trend (+52% from 5.50–5.40 to 8.27–8.30). In all conditions, the acidic pH became alkaline at the end of the treatment. This behaviour is confirmed by the statistical analysis, which detected significant differences among treatments for pH ($p = 0.01$), with both diluted conditions forming a homogeneous group distinct from EO(80% R) (Table 5.3, Figure 5.3).

Figure 5.4 - Temporal evolution of pH, COD, total nitrogen (TN), and phenols in the laboratory tank group B (effect of essential oil).



About COD, diluted wastewater, tanks EO(80% R) and EO(60% R) showed a rapid COD removal during the first month and stabilised below 0.5 g/L. In contrast, the raw wastewater, tanks EO(100% R), exhibited a more delayed COD degradation. These results suggest that essential oil primarily affects the kinetics of degradation, delaying the onset of microbial activity at higher concentrations.

In the undiluted reactors, TN values were always higher compared to the tanks with dilution. Although phenols decreased in all conditions, the removal rates were initially slower in the tanks with the lowest dilution, EO(60% R) compared to the undiluted wastewater, EO(80% R), and to wastewater with moderate dilution, EO(80% R) (Figure 5.4). While the efficiency of organic matter degradation (-99% of COD) was statistically similar for all tested conditions, phenols were removed more efficiently in EO(80%R) compared to EO(60%R) and EO(100%R) (Figure 5.4 and Table 5.3).

The results of tests in tank group EO clearly demonstrate that essential oil concentration plays a decisive role in shaping the biodegradability of CPW. The progressive improvement in process stability and reaction kinetics observed under diluted conditions confirms the inhibitory nature of citrus essential oils, whose high concentrations are known to interfere with microbial membrane integrity and enzymatic activity, delaying the onset of aerobic degradation and reducing overall biodegradability (Andiloro et al., 2021b, 2013b). This inhibitory effect is particularly evident during the early stages of treatment, where reactors receiving undiluted wastewater shows delayed pH correction, slower COD degradation and higher TN accumulation compared to diluted systems.

Dilution noticeably reduced these inhibitory pressures, allowing microbial communities to acclimate more rapidly and earlier initiate degradation processes. This is consistent with previous findings on limonene-rich wastewaters, where high essential oil content has been associated with delayed microbial activation and reduced metabolic efficiency (Andiloro et al., 2013b). In this study, one of the diluted conditions- tank (EO(80% R), intermediate content of EO- showed faster COD removal kinetics and more rapid pH increase, indicating that lowering essential oil concentration facilitates the onset of aerobic degradation pathways, similarly to what has been reported for other lagoon-based systems operated under attenuated inhibitory loads (Zema et al., 2016a).

Phenolic compounds exhibited a particularly strong sensitivity to essential oil concentration. Although all treatments achieved substantial phenol removal, the temporal patterns remarkably differed: one of the diluted systems- tank EO(60%R), lowest content of EO -showed slower initial degradation, while the undiluted wastewater ultimately achieved lower final removal efficiency compared to diluted conditions. This behaviour suggests that essential oil concentration does not remarkably influence both the accessibility of phenolic substrates and the acclimation dynamics of the microbial community. The non-significant sensitivity of phenolic compound removal to essential oil concentration is in line with previous observations of synergistic inhibitory effects in CPW lagoons, where essential oil and phenolic fractions jointly modulate microbial activity (Andiloro et al., 2021b).

Overall, the results indicate that dilution of essential oil content or partial attenuation of its effects is important but not essential for restoring effective biological activity in CPW treatment systems, particularly during the early operational stages. Reducing essential oil concentration to moderate levels mitigates inhibitory effects, accelerates microbial acclimation and enhances process stability, especially for parameters such as pH and COD, in agreement with the evidence reported for intermittently aerated lagoon systems treating agro-industrial effluents (Zema et al., 2016a). At the same time, the higher final phenol removal observed under the intermediate dilution condition, EO(80%R), suggests that partial mitigation of inhibitory effects, rather than complete removal or excessive dilution, may promote optimal co-metabolic interactions between essential oil constituents and phenolic substrates (Andiloro et al., 2021b, 2013b).

These findings highlight the complex interplay between essential oil concentration, microbial inhibition and substrate-specific degradation pathways, and underscore the importance of managing essential oil levels to optimise lagoon-based CPW treatment.

5.2.3 Tank group COD/N (effect of nitrogen availability)

The temporal evolution of the monitored parameters indicates that nitrogen availability did not substantially modify the behaviour of the reactors during CPW treatment. A progressive increase in pH, quicker in the early degradation process, was measured in all reactors, but without any significant differences linked to nitrogen availability (Table 5.3). At the end of the cycle, all tanks showed similar pH values, from 8.37, tank COD/N(300/1), to 8.79, tank COD/N(2000/1). The increase was on average 43% (Figure 5.5).

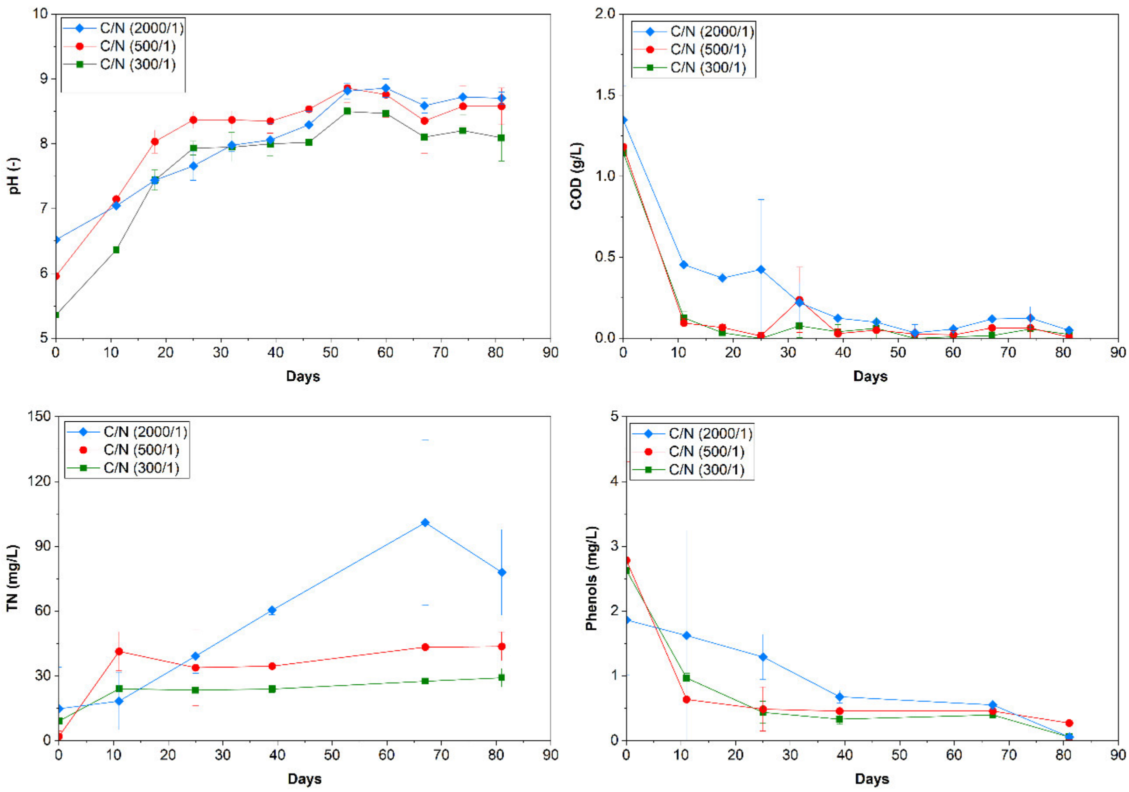
Neither COD nor phenols removal was enhanced by nitrogen addition. Both parameters decreased from 1.35 g L^{-1}, for COD, tank COD/N(2000/1), 1.19 g L^{-1}, tank COD/N(500/1) and 1.15 g L^{-1}, tank COD/N = 300/1, to 0.05 g L^{-1}, COD/N 2000/1, 0.01 g L^{-1}, COD/N 500/1, and 0.03 g L^{-1}, COD/N = 300/1.The decrease rate was higher in the early process, and slower after the third week (Figure 5.5). The total reductions throughout the cycle were 98% for COD and 93% for phenols, both non-significant (Figure 5.5). About the temporal evolution of TN, this trend was relatively stable in the reactors with the lower content, tanks COD/N(300/1) and COD/N(500/1) and increasing in the tank COD/N(2000/1) (Figure 5.5).

In this study nitrogen supply did not produce statistically significant differences under the tested conditions. This contrasts with the widely recognised role of nitrogen availability recognised as a potential controlling factor in the biological treatment of high-strength agro-industrial wastewater. Literature indicates that optimal COD/N ratios typically fall between 80:1 and 200:1 for lagoon-based systems, supporting balanced microbial growth and sustained metabolic activity (Andiloro et al., 2013b; Zema et al., 2016a). The absence of significant differences in this study suggests that the initial nitrogen content of the wastewater may have been sufficient to support microbial activity, or that the microbial community was able to adapt effectively across the tested COD/N range.

Overall, the results indicate that, within the tested conditions, nitrogen availability did not act as a limiting factor for COD or phenol removal. The high removal efficiencies achieved across all treatments demonstrate the sufficient activity of the microbial community and suggest that lagoon-based systems may tolerate broader COD/N variability than previously assumed, if baseline nitrogen levels remain above critical thresholds.

Three complementary explanations can be proposed for the limited impact of nitrogen supply observed in this study. First, the raw CPW already contained a basal nitrogen content sufficient to sustain heterotrophic assimilation at the organic loads tested, since the COD/N requirement of actively growing heterotrophs is typically much lower than the nominal ratios reported for design purposes in the literature, which are conservative values intended to also cover microbial maintenance and safety margins(Andiloro et al., 2013; Hamza et al., 2019; Zema et al., 2016). Second, the very high removal efficiencies already achieved under nitrogen-limited conditions indicate a ceiling effect: once organic matter removal approaches its practical maximum, additional nitrogen supply has little room to produce a further measurable improvement, regardless of its true biological benefit. Third, essential oils and phenolic compounds, rather than nitrogen, were identified as the dominant inhibitory factor constraining microbial activity (Section 5.2.2); under toxicant-controlled conditions, microbial performance is governed primarily by the degree of inhibition rather than by nutrient availability, meaning that additional nitrogen cannot compensate for a constraint that is not nutritional in nature.

Figure 5.5 - Temporal evolution of pH, COD, total nitrogen (TN), and phenols in the laboratory tank group C(effect of nitrogen supply).



5.2.4. Microbiological aspects

The microbiological analyses performed at the start (T0) and at the end of the cycles (after 13 weeks, T13w), revealed the presence of a heterogeneous bacterial community in all experimental conditions (Figure 5.6). The microscopic observations confirmed that all colonies belonged to the domain 'Bacteria' and included both rod-shaped and coccoid morphotypes, occurring either as single cells or arranged in chains. Motile and non-motile forms were observed, indicating the coexistence of different physiological groups within the microbial consortium.

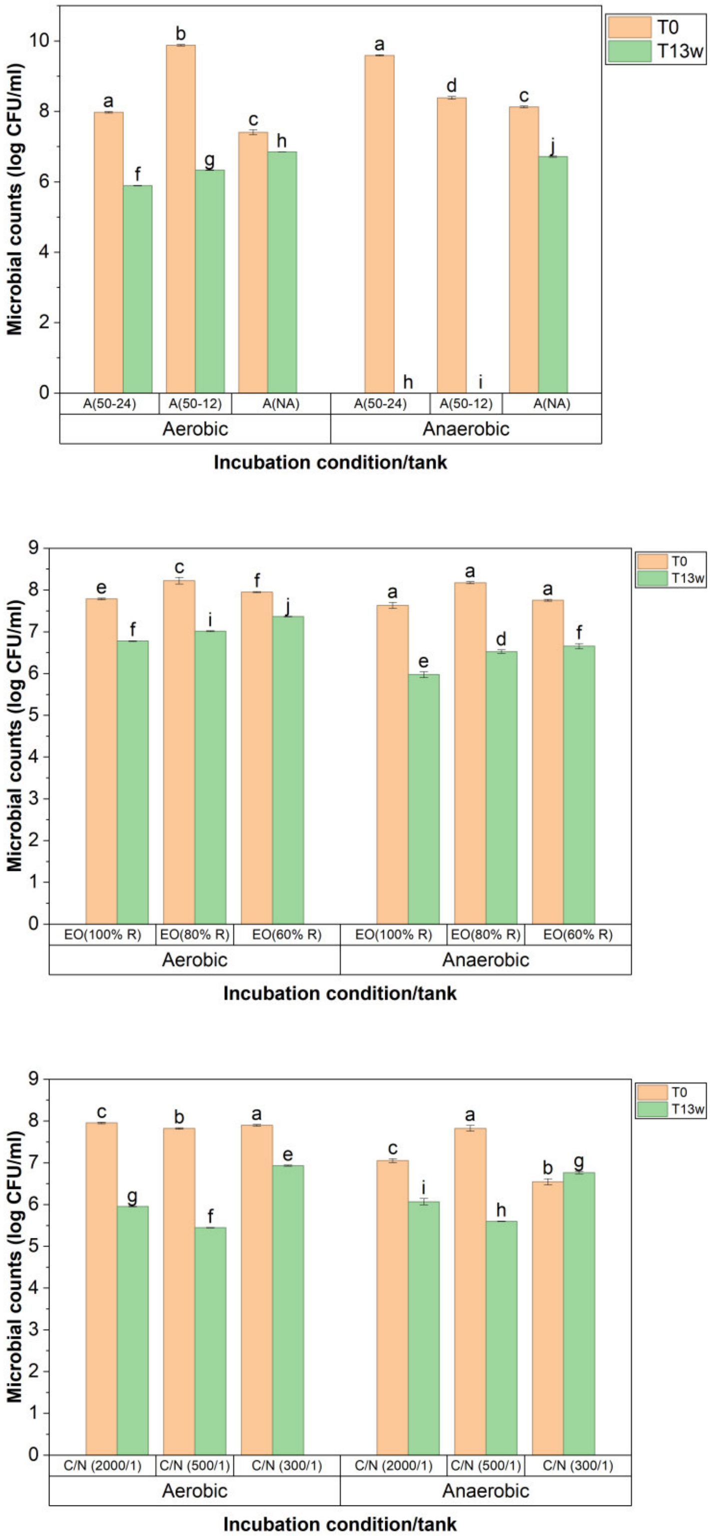
Quantitative data, expressed as log CFU/ml, showed that, in general, the total microbial count significantly decreased from T0 to T13 under both aerobic and anaerobic incubation conditions and over time. Moreover, the quantitative reduction was significantly variable among the tanks for each tank group, and the general trend was consistent across all reactors (Table 5.4). The reduction was higher for tanks with higher concentrations of essential oils or with limited oxygen availability, suggesting that both factors contributed to constraining microbial proliferation (Figure 5.6). This decline reflects the progressive adaptation of the microbial community to the chemical characteristics of CPW, which is known to contain inhibitory compounds such as essential oils and phenolic substances.

Table 5.4 - Results of two-way ANOVA applied to microbial counts at the experiment start (T0) and after 13 weeks (T13w) in the three laboratory tank groups.

Factors	DF	Sum of squares	Mean squares	F	Pr > F
	T0				
Tank	8	17.8	2.23	1173.414	< 0.0001
Condition	1	0.55	0.55	287.024	< 0.0001
Tank x Condition	8	11.6	1.45	761.483	< 0.0001
	T13w				
Tank	8	120	15.0	15328.637	< 0.0001
Condition	1	33.9	33.9	34773.195	< 0.0001
Tank x Condition	8	80.5	10.1	10320.309	< 0.0001

In more detail, in the tank group A, the aerated reactors, tanks A(50-24) and A(50-12), showed a higher microbial load compared to the non-aerated condition, particularly under continuous aeration, tank A(50-24) (Figure 5.6). This behaviour is in part consistent with the chemical results, which showed a more efficient increase in pH in the aerated systems. The higher microbial abundance observed under aerobic incubation at T13w compared to T0 indicates that the oxygen availability supported the growth of an active microbial population capable of oxidising organic acids. In contrast, this specialization of microbial mass did not result in more efficient removal of COD and phenols. Under anaerobic incubation, microbial counts at T0 also differed significantly among tanks, and all reactors exhibited a significant reduction by T13w. Both time and tank effects were significant, mirroring the aerobic pattern.
From a chemical standpoint, anaerobic reactors showed slower pH increases and similar COD and phenol removal efficiencies compared to the aerated systems. The microbiological decline under anaerobic conditions is therefore consistent with the chemical evidence that anaerobic metabolism was not sufficiently active to counteract the inhibitory nature of CPW. The similarity in microbial decline between aerobic and anaerobic conditions suggests that the inhibitory compounds present in CPW exerted a dominant effect over the metabolic advantages provided by oxygen availability.
In the tank group EO, the microbial load decreased proportionally to the initial essential oil concentration under aerobic incubation, but not under anaerobic conditions at the experiment start. After 13 weeks, tank EO(100% R), showed the highest reduction in microbial population, while tanks EO(80% R) and EO(60% R), exhibited higher microbial counts and therefore a lower reduction (Figure 5.6). Specifically, under aerobic incubation, at T0, microbial counts differed significantly among tanks EO(100% R), EO(80% R) and EO(60% R), confirming the tank effect. After 13 weeks, all reactors showed a significant decrease in microbial abundance, indicating a strong time effect. Differences among tanks persisted at T13w.
Chemically, dilution of essential oils did not significantly improve COD or phenol removal, despite the higher microbial counts observed in the diluted reactors. This indicates that the inhibitory effect of essential oils influenced microbial abundance but did not translate into enhanced chemical degradation. The microbiological data therefore support the chemical evidence that essential oil concentration affects microbial viability more than treatment performance. Under anaerobic incubation, microbial counts at T0 differed significantly among tanks, and all reactors exhibited a significant reduction by T13w. Both time and tank effects were significant. The anaerobic conditions did not show improved removal efficiencies in diluted EO treatments, consistent with the microbiological observation that higher microbial counts in diluted reactors did not correspond to enhanced degradation performance. The combined results suggest that essential oil concentration modulates microbial abundance but does not determine chemical removal efficiency under either incubation condition.
In the tank group C, nitrogen availability influenced initial microbial abundance. After 13 weeks, COD/N(300/1) maintained the highest residual microbial counts under both incubation conditions, whereas COD/N(500/1) showed the most pronounced decline (Figure 5.6).

Figure 5.6 - Microbial counts at the experiment start (T0) and after 13 weeks (T13w) in the three laboratory tank groups



In detail, under aerobic incubation, at T0, microbial counts differed significantly among tanks COD/N(300/1), COD/N(500/1) and COD/N(2000/1), confirming the tank effect. After 13 weeks, all reactors showed a significant decrease in microbial abundance, indicating a strong time effect. Differences among tanks persisted at T13w.

Nitrogen supply did not produce significant differences in COD or phenol removal, despite the higher initial microbial counts in the nitrogen-amended reactors. This suggests that nitrogen availability influenced initial microbial abundance but did not sustain microbial activity over time. The microbiological decline therefore aligns with the chemical evidence that nitrogen was not a limiting factor for treatment performance. Under anaerobic incubation, microbial counts at T0 differed significantly among tanks, and all reactors exhibited a significant reduction by T13w. Both time and tank effects were significant.

Chemically, nitrogen supply again did not lead to improved removal efficiencies, consistent with the microbiological observation that nitrogen availability did not prevent the decline in microbial abundance. The combined results indicate that the microbial community adapted similarly under both incubation conditions, regardless of nutrient supply.

Across all tank groups and incubation conditions, microbial counts showed a consistent and significant decline from T0 to T13w, while differences among tanks remained significant throughout the experiment. Despite these microbiological variations, the chemical performance of the reactors (COD, phenols, pH evolution) did not follow the same patterns, indicating that treatment efficiency was not directly driven by microbial abundance. Overall, the results suggest that the inhibitory nature of CPW exerted a dominant influence on microbial viability, while chemical removal processes proceeded largely independently of biomass levels.

5.3 Implications for lagoon optimisation and upscaling

The laboratory-scale investigation provides indications of the operational conditions that maximise the performance of aerated lagoon systems treating CPW towards the design of the pilot-scale layout described in the following chapter.

Regarding aeration, the results (tank group A) clearly demonstrate that continuous aeration is the most effective operating condition, but only for a more rapid wastewater oxidation, although no-significant difference is induced by the aeration duration (permanent rather than intermittent). These findings indicate that, for the upscaling, continuous aeration should be adopted as the reference operating mode, as it provides the most reliable control of wastewater acid conditions.

Concerning the effects of EO (tank group B), the experiments showed how the essential oil concentration is a driver of lagoon performance. Reduction of EO levels improved pH recovery and accelerated degradation kinetics, confirming the inhibitory role of citrus essential oils on microbial activity. However, final COD removal efficiencies remained comparable among treatments, and phenol removal did not show a consistent proportional response to dilution

Among the tested conditions, the intermediate dilution level, 80% of raw wastewater, proved to be the most effective, except for organic matter reduction, which was less sensitive to EO presence. This configuration achieved a favourable balance between toxicity mitigation and substrate availability, while avoiding the excessive dilution (which may reduce the substrate for microbial mass). These results indicate that, for pilot-scale operation, a reduction of EO concentration to approximately 80% of raw wastewater, combined with restoration of the organic load, represents the optimal compromise between process efficiency and operational feasibility.

About the COD/N ratio (tank group C), the nitrogen availability was not a critical parameter for lagoon optimisation. Results clearly show that all COD/N ratios do not exert significant differences in the metabolic activity. In other words, nitrogen supply did not substantially enhance organic matter and phenolic compound removal or pH correction. However, the moderate nitrogen supply, sufficient to reduce the COD/N ratio to an intermediate range, should be adopted in pilot-scale operation, avoiding both nitrogen limitation and unnecessary nitrogen excess.

Overall, the results of the three tank groups allowed the identification of the following set of optimal operational conditions for upscaling:

- continuous aeration, A(50-24)
- partial reduction of essential-oil concentration to approximately 80% of raw wastewater, EO(80% R)
- no nitrogen supply, COD/N(2000/1).

This combination does not maximise performance in absolute terms, since high removal efficiencies were achieved under multiple conditions, but represents the most balanced operational compromise, ensuring process stability, manageable energy demand and limited chemical input. The laboratory-scale results therefore provide a rational and experimentally grounded basis for the design of the pilot-scale system, where these conditions will be implemented and further evaluated under dynamic loading and automated aeration control.

CHAPTER 6: DEVELOPMENT OF THE PILOT LAGOON SYSTEM WITH AUTOMATED AERATION CONTROL

6.1 Introduction

The development of an automated aeration control system is a fundamental step in the transition from laboratory-scale experiments to full-scale implementation of optimized lagoon-based treatment for agri-food wastewater, such as the CPW. As demonstrated by the laboratory tests discussed in Chapter 5, oxygen availability can drive organic matter degradation, pH stabilisation and microbial activity, while, at the same time, the pumped air flow represents a key parameter of economic sustainability in aerated lagoon systems. The traditional functioning of lagoons typically relies on continuous aeration, supplying oxygen at a constant rate that does not consider the actual biochemical demand of the reactor. However, although simple and robust, this approach is intrinsically inefficient, as it does not account for temporal variations in biological oxygen demand and often results in oxygen limitation during peak activity or unnecessary energy consumption during low-load phases (Henze et al., 2008; Mara, 2009; Tchobanoglus et al., 2003; Zema et al., 2016a). During phases of high microbial activity, fixed aeration rates may be insufficient to prevent oxygen limitation, whereas, during low-activity phases, excessive aeration results in unnecessary energy consumption without measurable gains in treatment performance. These limitations are particularly pronounced in CPW treatment, due to the strong temporal variability of organic load, pH, and inhibitory compounds, such as EOs.

To overcome these constraints, the pilot-scale lagoon proposed in this study was conceived as a demand-driven system, in which aeration is supplied only when required and in response to real-time process conditions. The core of this approach is the implementation of an automated control strategy based on real-time measured dissolved oxygen (DO), a parameter directly linked to microbial metabolism and degradation kinetics in aerobic lagoon systems.

The development of the automated aeration system was not straightforward and required an iterative design process. Initial attempts to integrate a multiparameter controller with the selected DO sensor revealed critical compatibility issues that prevented stable signal acquisition and reliable control. These challenges ultimately led to a complete redesign of the control architecture, resulting in a robust and fully functional automated system suitable for long-term pilot-scale operation.

This chapter describes the evolution of the automation strategy, from the initial design concept to the final operational configuration adopted in the pilot plant. Particular attention is devoted to the rationale behind component selection, sensor-controller integration, control logic implementation and monitoring capability, as these elements are central to the scalability and transferability of the proposed solution.

6.2 Initial automation concept and system requirements

The automation strategy was defined based on the outcomes of the laboratory-scale tests, which demonstrated that continuous aeration, partial reduction of essential oil concentration and balanced nutrient availability maximise treatment performance. While continuous aeration proved effective at lab scale, its direct translation to pilot scale would likely lead to excessive energy consumption under fluctuating biological demand.

The automation system was therefore designed to fulfil the following requirements: (i) continuous real-time monitoring of dissolved oxygen concentration; (ii) dynamic modulation of aeration based on DO thresholds; (iii) implementation of a simple and robust ON/OFF control logic with hysteresis; (iv) compatibility with long-term operation in high-strength wastewater; (v) minimal maintenance and operational complexity.

These requirements reflect the overarching goal of the research: developing lagoon systems that are not only efficient, but also technically simple, robust and suitable for real agro-industrial contexts.

6.3 Design of the automation system

6.3.1 Conceptual architecture

The automated aeration system was built around a self-designed multiparameter controller, which integrates multiple sensors and manages actuators through configurable relay outputs. The intended architecture is linear and conceptually straightforward:

- dissolved oxygen probe → controller input
- controller logic → relay output
- relay output → blower ON/OFF control

A conceptual block diagram of the initial configuration is reported in Figure 6.1

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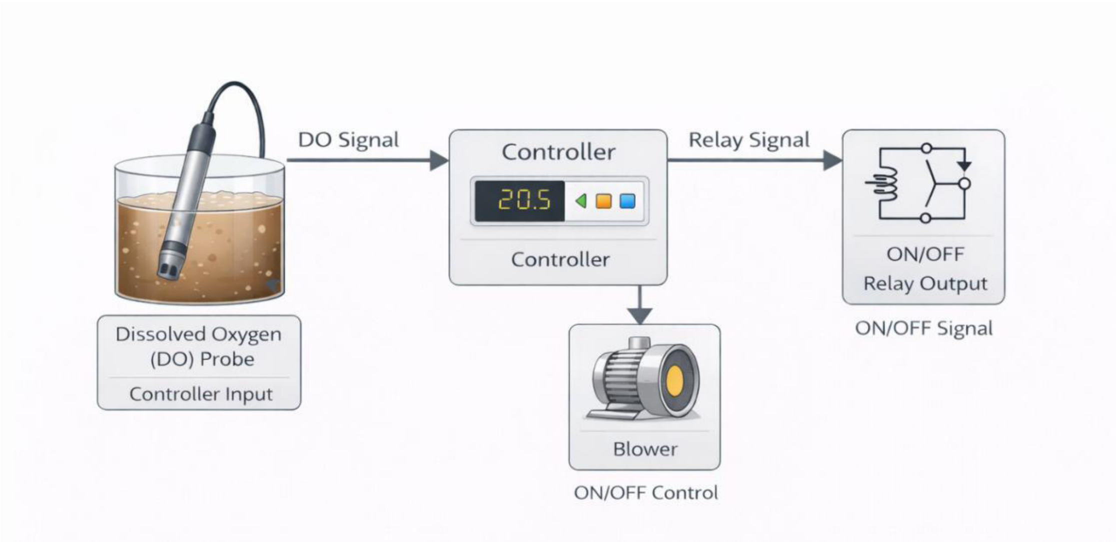
Figure 6.1 - Conceptual diagram of the initial automation configuration.



No mechanical mixing devices were installed in the experimental tank, and hydraulic circulation was provided exclusively by aeration, in accordance with hydrodynamic conditions typical of traditional lagoons.

6.3.2 Design constraints and selection of the controller

The controller was required to:

- to communicate via Modbus RTU RS485
- to read DO values directly from the optical sensor
- to implement ON/OFF control with configurable hysteresis
- to provide relay outputs suitable for blower control
- to allow monitoring and data logging during experimental operation.

Based on these requirements, the Pixsys® STR571 was selected as the control unit. This is a compact Modbus Master terminal with integrated relays and a graphical display.

6.3.3 Operative system architecture

The operative automated system consists of:

- an OPTOD optical dissolved oxygen sensor (Aqualabo/Ponsel®).
- a Pixsys® STR571 Modbus Master controller.
- a centrifugal blower (230 V AC) controlled via relay Q1.

Figure 6.2 reports a photograph of the final operative configuration of the automated aeration system.

Figure 6.2 - Final operative configuration of the automated aeration system

6.3.4 Technical characteristics of the OPTOD sensor and Pixsys STR571 controller

The dissolved oxygen probe installed in the pilot plant is an OPTOD (Optical Dissolved Oxygen) sensor manufactured by Aqualabo/Ponsel®. The instrument operates on luminescence-quenching technology, which offers several advantages over conventional amperometric probes. In particular, the OPTOD sensor does not consume oxygen during measurement, does not require either membranes or electrolyte, and exhibits very limited signal drift over time. These characteristics make the sensor especially suitable for long-term deployment in high-strength wastewater, where stability and low maintenance needs are essential.

The main technical specifications include digital communication via Modbus RTU (RS485), an operating voltage range of 5-12 V DC, and a dissolved oxygen resolution of 0.01 mg/L. The probe also incorporates automatic temperature compensation and is built with a robust housing designed for industrial and wastewater applications. These features make the OPTOD sensor particularly suitable for automated lagoon operation, where access for routine maintenance may be limited, and continuous and reliable DO monitoring is required.

The Pixsys STR571 is a compact Modbus Master terminal developed for industrial automation and process control, and it acted as the central unit for implementing the DO-based aeration strategy. The device communicates via RS485 (COM1) using the Modbus Master protocol and can simultaneously manage up to eight Modbus variables, ensuring full compatibility with the OPTOD sensor.

The controller is equipped with two relay outputs (2 A, 250 V AC), three digital inputs, and a wide supply range (24-230 V AC/DC), which facilitates integration into heterogeneous industrial environments. An OLED graphical display provides visualization of process variables, while configurable ON/OFF alarms with hysteresis allow definition of control thresholds. These characteristics enabled the implementation of a stable and efficient control logic of aeration, ensuring reliable operation of the pilot-scale lagoon system.

6.3.5 Implementation of the aeration control logic

Aeration control was implemented through relay Q1, connected to the blower power circuit. The control strategy was based on a DO-driven ON/OFF logic using predefined threshold values., as schematically illustrated in Figure 6.3. In the present study, the dissolved oxygen thresholds were set at min(DO) = 0.001 mg/L and max(DO)= 1.2 mg/L. The lower threshold was introduced for configuration purposes within the controller settings, whereas the effective operational control was governed by the upper threshold (1.2 mg/L).

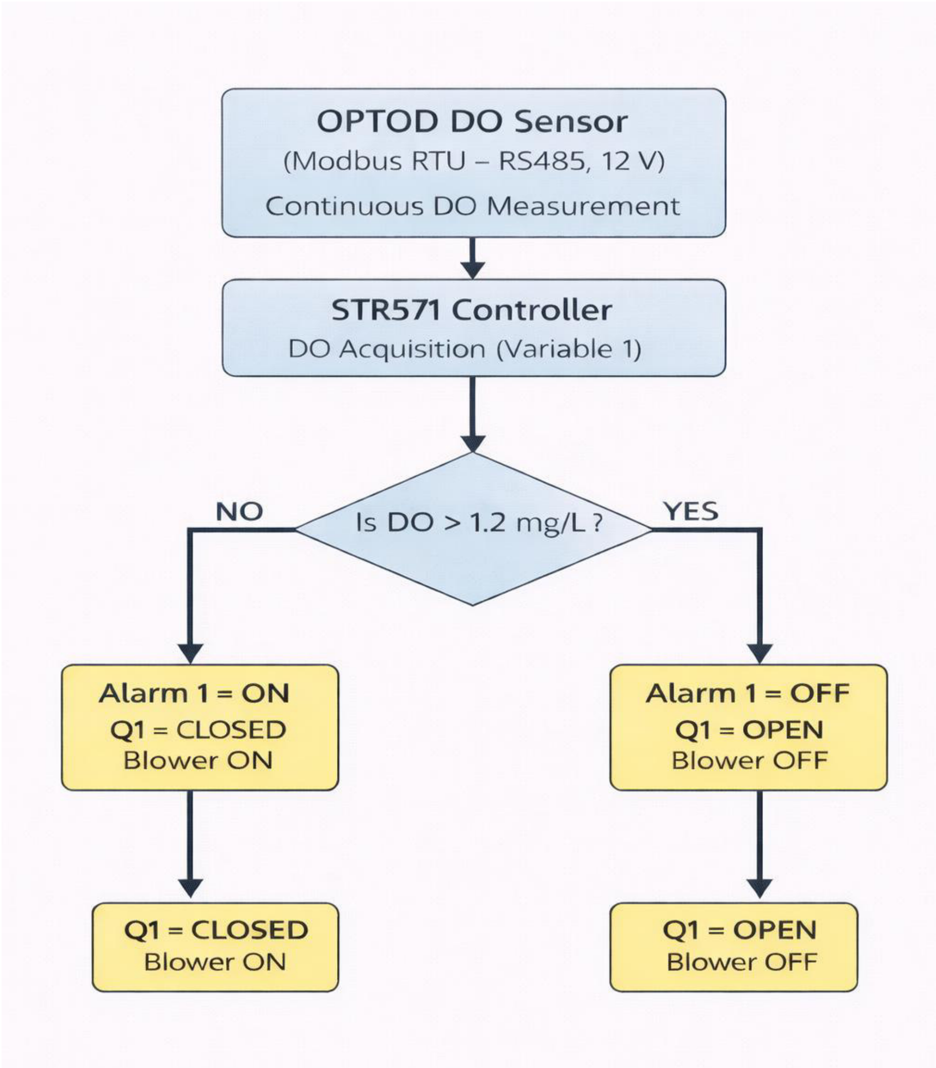
Specifically:

- when DO exceed 1.2 mg/L, the relay Q1 opened and the blower was switched OFF
- when DO remain below 1.2 mg/L, the relay Q1 remained closed and the blower was ON

Therefore, although the controller allowed the configuration of both minimum and maximum limits, the implemented strategy operated effectively as a single-threshold ON/OFF control system, without a significant hysteresis band.

This simple control logic was selected to ensure robustness and operational stability under high-strength wastewater conditions. Moreover, this strategy ensures that oxygen is supplied only when biochemically required, preventing both oxygen limitation and unnecessary energy consumption.

Figure 6.3 - Flowchart of the aeration control logic designed for the planned pilot-scale



6.6 Monitoring and data acquisition

The STR571 controller exposes relay status through Modbus register 900, enabling external systems to monitor aeration events. The STR571 shows the state of its relays through Modbus register 900:

- Bit 0 = Q1
- Bit 1 = Q2
- 0 = OFF, 1 = ON

Through the USB port, the controller was connected to a PC acting as a Modbus client, allowing real-time acquisition of:

- dissolved oxygen concentration
- relay ON/OFF status
- time records of aeration cycles.

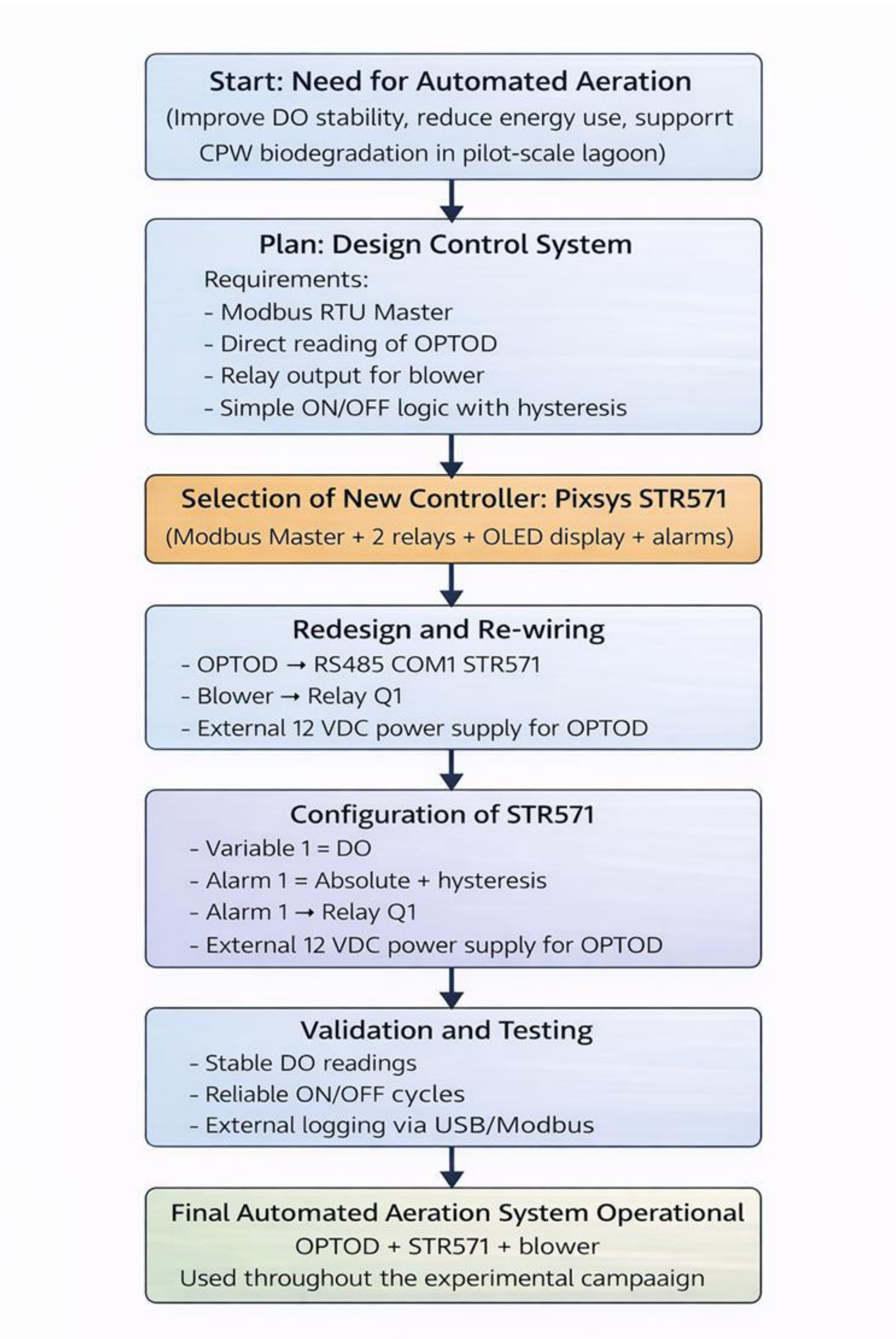
The flow-chart in Figure 6.4 summarises the sequence of decisions, diagnostic steps, and design actions undertaken during the project, illustrating how the process evolved through refinement and highlighting the central role of system compatibility analysis in achieving a robust and functional control architecture.

6.7 Role of automation in the transition to upscaling

The development of the automated aeration control system represents the conceptual and technical bridge between laboratory-scale experiments and pilot-scale operation. By linking aeration directly to dissolved oxygen demand, the system translates experimental knowledge into an adaptive operational strategy, aligning treatment efficiency with energy sustainability. The final system theoretically provides accurate DO monitoring, robust blower control and comprehensive data acquisition capabilities, forming the technological foundation for the pilot-scale experiments presented in the following chapter.

While laboratory tests identified optimal operating conditions under fixed settings, automation enables their dynamic application under realistic and variable conditions. New experimental data were used to validate aeration patterns during pilot-scale operation and to support the interpretation of treatment performance discussed in Chapter 7. This approach embodies the core objective of the research: moving beyond static design towards intelligent, responsive lagoon systems suitable for real agro-industrial wastewater management.

Figure 6.4 - Flowchart of the engineering process leading to the final automated aeration control system



CHAPTER 7: UPSCALING TESTS

The pilot-scale experimentation was the second and final experimental phase of the research framework and was designed to validate, under realistic operating conditions, the prototype of the optimized-automated lagoon system, working with the optimal parameters identified during the laboratory-scale investigation (Chapter 5) and validating the aeration control logic (Chapter 6).
As aforementioned, the laboratory results clearly identified a combination of continuous aeration, partial reduction of essential oil (EO) concentration through dilution, and moderate nitrogen supply as the most effective strategies for enhancing the depuration of CPW.
The pilot-scale system was therefore designed to translate these optimal conditions into a larger volume while introducing real-time process control based on dissolved oxygen (DO) monitoring. The primary objective of this phase was to evaluate whether automated aeration, dynamically adapted to wastewater oxygen demand, could ensure stable treatment performance while improving energy efficiency compared with traditional constant-aeration operation.

7.1 Materials and Methods

7.1.1 Pilot-scale reactor and experimental layout

Pilot-scale experiments were conducted in two cylindrical tanks, each with a working volume of 1000 L, made of inert plastics. The tanks were installed in an open space to reproduce natural and outdoor environmental conditions, typical of lagoon systems in Mediterranean regions.
The two tanks run under two aeration strategies, and their functioning was optimised according to the most effective parameters experimented in the previous experimental phase (Chapter 5):
Tank 'A' (automated-optimised lagoon): equipped with a dissolved oxygen probe and an automated control system regulating aeration flow rate and on/off cycles in real time (Chapter 6).
Tank 'C' (control-optimised lagoon): operated with constant aeration at a fixed airflow rate of 50 L m^{-3} h^{-1} for 24 h d^{-1}, representative of conventional continuously aerated lagoon operation.
For Tank A, the dissolved oxygen control threshold was set at 1.2 mg L^{-1}. The controller logic operated as a single threshold ON/OFF system: aeration was switched OFF when DO exceed 1.2 mg L^{-1} and remained ON when DO was equal to or below this value. Although a minimum value (0.001 mg L^{-1}) was configured for technical purposes, the effective operational control was governed exclusively by the upper threshold (1.2 mg L^{-1}). Both tanks worked in batch mode and received the same influent wastewater (with 80% dilution) and chemical additive (sucrose and urea). This dual configuration allowed direct comparison between optimised operation and traditional fixed aeration. Moreover, the optimisation of operation parameters in both tanks allowed testing the possible beneficial effects of automation control of aeration.
Inoculum was not added in order to simulate the start-up of the lagoons, which is often the most critical phase of the depuration process. Both tanks were filled only with CPW (as described before) and the biological community spontaneously developed from the indigenous microorganisms that are naturally present in the raw effluent.

7.1.2 Influent wastewater and operating conditions

The influent was the CPW collected from the same industrial facility described in Chapter 5. Raw CPW was collected directly from the processing line, after the mechanical screening, and stored before use. The main physicochemical characteristics of raw CPW, including pH, chemical oxygen demand (COD), nutrient concentrations (TN), are presented in Table 7.1. Due to technical constraints (low amounts of solids), TS were not measured.

Table 7.1 - Main physicochemical characteristics of the raw citrus-processing wastewater (CPW).

Parameter	Initial Value	Unit	Notes
pH	4.1	–	Measured at sampling
COD	4200	mg/L	Raw, unfiltered sample
T	27	°C	Measured at sampling
Electrical Conductivity (EC)	3,3	µS/cm	-
Total Nitrogen (TN)	1.5	mg/L	-
COD/N ratio	2800	–	Calculated as COD / TN
Total Phenols	3.5	mg/L	Used as proxy for toxicity

As outlined above, based on the outcomes of the laboratory-scale optimisation, the following operating conditions were applied to both pilot tanks:

- Essential oil concentration: reduced to approximately 80% of the corresponding concentration of raw wastewater by dilution.
- Organic matter restoration: sucrose addition to restore COD losses due to dilution with clear water.
- Nitrogen supply: urea addition to achieve a moderate COD/N ratio (200:1) consistent with optimal microbial activity.

Upscaled chemical doses were calculated proportionally to the increase in reactor volume from 100 L to 1000 L. Based on laboratory-scale calculations, the sucrose dose was 1786 g sucrose per tank. Sucrose was fully dissolved in the diluted wastewater before reactor filling to ensure homogeneous distribution. Nitrogen was supplied using urea, adding 105 g urea per tank. Urea was dissolved directly into the wastewater prior to the start of the experiment to ensure uniform nitrogen availability.

7.1.3 Monitoring and analytical methods

The pilot-scale system was monitored throughout the experimental period by periodic sampling and physico-chemical analyses. Samples (100 ml) were collected at regular intervals (more or less 9 intervals of 14 days) and pH, COD, TN, and phenolic compounds were analysed as in the previously laboratory-scale tests. Analytical methods and instrumentation were those described in Chapter 5, ensuring comparability between laboratory- and pilot-scale results.

7.1.4 Statistical analyses

To evaluate the differences in pH, COD, TN, and phenols between the two tanks, a Wilcoxon signed-rank test was applied. This non-parametric approach was selected because the data consists of paired temporal observations, where each measurement in tank C corresponds to a simultaneous measurement in tank A - and does not assume a normal distribution, which is particularly important for parameters that often exhibit skewed profiles.

The test was conducted on the series of differences between the two tanks at each time point. The null hypothesis is that the median of these differences is zero. This paired analysis effectively controlled for external temporal variables (e.g., fluctuations in weather parameters) that simultaneously influenced both tanks. All statistical analyses were performed with a significance level of $p = 0.05$. To complement the p-values, the magnitude of the differences was visualized using time-series plots to confirm the persistence of observed trends over the study period.

7.2 Results & discussion

Before analysing the temporal evolution of the observed parameters, it is worth noting that the maintenance of suitable dissolved oxygen conditions in both pilot tanks is a fundamental operational aspect of the depuration process. More specifically, throughout almost the entire experimental period, dissolved oxygen concentrations remained extremely low in both tanks. Initial DO values were 0.003 mg/L in Tank A and 0.002 mg/L in Tank C and remained below 0.005 mg/L for more than two months. Only at the end of the third month, when the organic load had already been largely depleted, higher DO values were recorded (4.55 mg/L in Tank A and 4.55 mg/L in Tank C).

Because DO never exceed the control threshold (1.2 mg/L) during the earlier treatment phase, the aerator in Tank A never switched off. The DO-based controller was therefore unable to modulate aeration, and Tank A operated under continuous aeration, exactly like Tank C. This behaviour does not indicate an out-of-work of the control system. Instead, it reflects a structural limitation: the oxygen demand generated by citrus-processing wastewater was consistently higher than the oxygen transfer capacity of the installed aeration system. Under these conditions, DO-based aeration control cannot function as planned. These results indicate that maintaining aerobic or semi-aerobic conditions during lagoon start-up would require higher aeration compared to the set-point in the present pilot configuration. This persistent oxygen limitation must be kept in mind when interpreting all subsequent process trends. It is also important to clarify that no inoculum from external sources - i.e., a microbial mass adapted to the same conditions as in the present depuration process - was added to both tanks at the start of the experimental period. Both lagoons were filled exclusively with CPW, and microbial communities spontaneously developed from the indigenous microorganisms in the raw effluent.

The absence of an acclimated inoculum contributed to the initial acidogenic conditions observed in both tanks presumably intensified the oxygen demand during the early stages of the process. This factor, combined with the extremely high organic load, further explains the persistently low DO concentrations recorded throughout most of the monitoring period. Therefore, the biological start-up relied entirely on the natural adaptation of the native microbial population, which required time to establish stable oxidative pathways.

The temporal evolution of pH, COD, TN and phenolic compounds is represented in Figure 7.1. In more detail, both tanks showed a marked increase in pH from strongly acidic initial values (4.12 in Tank A and 4.04 in Tank C) to final alkaline conditions around 9.5. This behaviour reflects the progressive consumption of organic acids and the establishment of oxidative pathways. Tank A reached near-neutral conditions earlier than Tank C, suggesting a smoother transition from acidogenic to oxidative metabolism. This difference is consistent with slightly more homogeneous oxygen distribution in Tank A, even though aeration modulation never occurred. The slower pH rise in Tank C indicates a more gradual neutralisation under fixed aeration. Overall, the pH evolution confirms that microaerobic conditions were sufficient to drive the system toward oxidative stability, despite the extremely low DO.

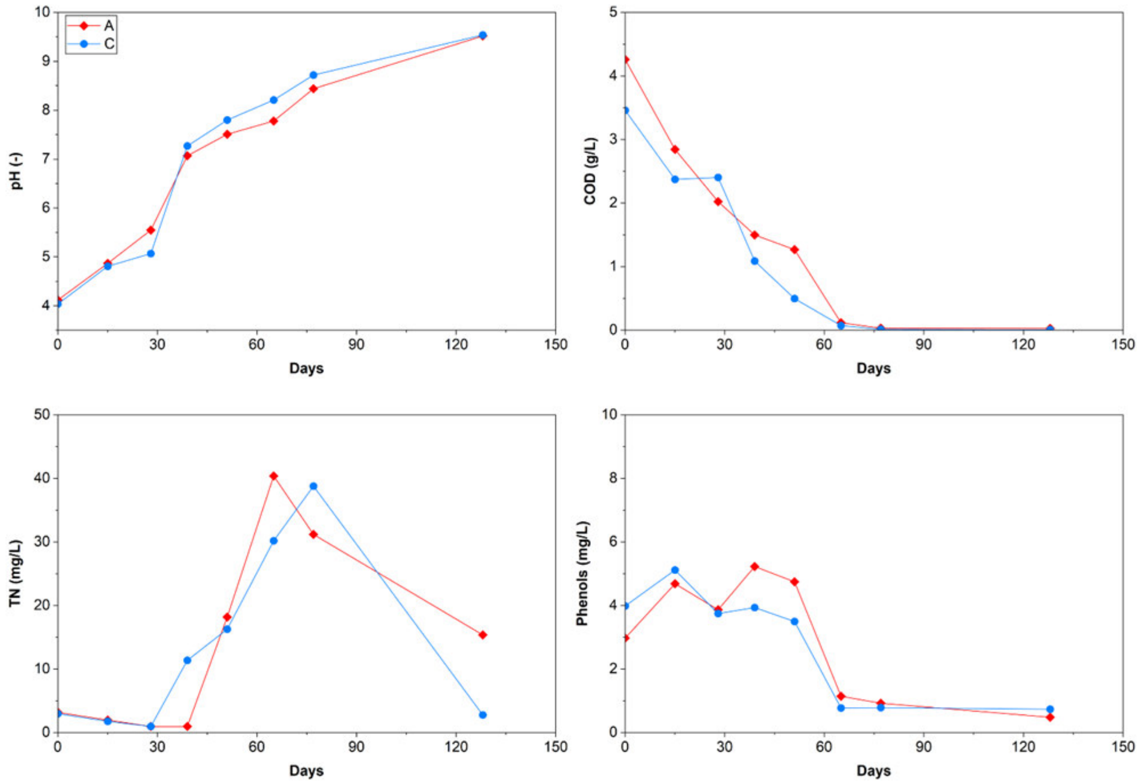
COD removal was substantial in both tanks, confirming the high biodegradability of citrus-processing wastewater. Tank A showed a faster initial COD decline (from 4.26 g/L to <0.1 g/L), while Tank C exhibited a slower but still effective reduction (from 3.46 g/L to similar final values). Although both systems reached comparable final COD concentrations, Tank A maintained slightly lower COD levels for most of the monitoring period. This behaviour reflects smoother oxidation dynamics and more stable microbial adaptation. Importantly, these differences cannot be attributed to aeration switching, which never occurred, but rather to more uniform mixing and oxygen dispersion in Tank A. The overall COD removal confirms that aerated lagoons remain robust even under oxygen-limited conditions, provided that the organic matter is highly biodegradable.

Total nitrogen concentrations exhibited different patterns in the two tanks. In Tank A, TN decreased from approximately 3.2 mg/L to around 1 mg/L and remained relatively stable. In Tank C, TN showed larger fluctuations, including transient accumulation up to significantly higher concentrations. The more stable TN profile in Tank A suggests a more consistent redox environment, whereas the variability in Tank C may reflect intermittent oxygen limitation and less stable nitrification pathways.

Although the reactors were covered, these were still exposed to sunlight, which may have favoured limited growth of photosynthetic microorganisms, this factor is unlikely to explain the pronounced TN fluctuations observed in Tank C. Algal or cyanobacterial activity typically leads to gradual nitrogen assimilation rather than sharp oscillations. The observed TN variability is more consistently attributed to redox instability and intermittent oxygen limitation, which affected nitrification pathways in the fixed-aeration system. These observations reinforce the conclusion that oxygen distribution rather than aeration modulation was the main factor influencing the differences recorded between the two systems.

Phenolic compounds decreased progressively in both tanks. Tank A reached lower concentrations (from 2.98 to 0.49 mg/L) compared to Tank C (from 3.99 to 0.74 mg/L), although the final values were close each to other. The faster phenol attenuation in Tank A indicates more favourable conditions for the degradation of aromatic compounds, despite the absence of aeration switching. This is particularly worth to be noted given the inhibitory nature of phenolics. Overall, phenolic removal confirms that aerated lagoons can effectively attenuate inhibitory compounds even under microaerobic conditions.

Figure 7.1 - Temporal evolution of pH, COD, total nitrogen (TN) and phenols in tank A and C.



7.3 Indicative treatment cost estimation

An indicative energy-based cost estimate was calculated from the actual aeration power consumption recorded during the experimental campaigns, in order to quantify the treatment cost per unit volume of treated wastewater.

The pilot-scale system (1000 L reactors) used a blower rated at 8 W, operated under continuous aeration for the duration of the monitored batch cycle (approximately 126 days, corresponding to nine 14-day sampling intervals). This yields an energy consumption of 24.2 kWh per treated batch (1 m^3), equivalent to an aeration energy cost of approximately **6.05 €/m^3**, based on a reference Italian industrial electricity tariff of 0.25 €/kWh("Arera," 2026). For comparison, the laboratory-scale reactors (100 L, 3 W blower, 13-week batch cycle) yielded a considerably higher indicative cost of 16.4 €/m^3, reflecting the disproportionately long aeration time relative to the small, treated volume that is typical of bench-scale batch testing rather than of continuous industrial operation; the pilot-scale value is therefore considered the more representative estimate for a scaled-up application.

Expressed in terms of specific energy demand, the pilot-scale system consumed approximately 5.8 kWh per kg of COD removed (COD reduced from 4.26 g/L to below 0.1 g/L in Tank A, corresponding to ≈4.16 kg COD removed per 1000-L batch). This value exceeds both the general benchmark reported for conventional activated sludge plants (~0.5 kWh/kgCOD, Section 3.4.1.1) and the CPW-specific estimate of ~2.0 kWh/kgCOD reported in the literature (Calabrò et al., 2024; Zema et al., 2019). This discrepancy is attributable to the experimental design rather than to inherent process inefficiency: aeration was maintained continuously for the entire monitoring period, whereas COD removal (shown in Figure 7.1) was largely achieved well before the end of the cycle. A shorter, load-responsive aeration period (consistent with the automated dissolved-oxygen control strategy tested in this study) would be expected to substantially reduce both the energy cost and the €/m^3 figure reported above. This cost estimates aeration energy only; it does not include capital costs, labour, or reagent costs (e.g., urea, sucrose), and should be interpreted as an order-of-magnitude indication rather than a full economic assessment.

7.4 Implications of the pilot-scale upscaling tests

The pilot-scale investigation confirmed the technical feasibility of aerated lagoon systems for treating high-strength CPW under real operating conditions. Both reactors achieved progressive pH neutralisation and effective removal of COD, TN and phenolic compounds, demonstrating that biological degradation processes were successfully established even under oxygen-limited conditions.

However, the comparison between the two aeration strategies revealed a key operational limitation: DO-driven aeration control can only be effective when wastewater characteristics allow dissolved oxygen to periodically rise above the control threshold. Under the exceptionally high organic loads of undiluted CPW, this condition was never met, preventing the activation of aeration pauses in Tank A.

In addition, the absence of an acclimated inoculum further intensified the initial oxygen demand and contributed to the persistent oxygen-limited conditions observed during the start-up. Since microbial communities had to develop entirely from the indigenous population in the raw wastewater, the system required a longer adaptation period, when DO remained constantly below the control threshold.

These findings indicate that DO-based aeration control is effective only when the organic load is high enough to generate measurable oxygen consumption, but not high enough to keep dissolved oxygen permanently close to zero. Under the conditions tested in this study, this balance was not achieved, preventing the controller from operating as planned.

CHAPTER 8: CONCLUSIONS

This research investigated the applicability, optimisation and scalability of aerated lagoon systems for the treatment of citrus processing wastewater (CPW), a highly challenging effluent characterised by high organic load, acidic pH, imbalanced nutrient ratios and the presence of inhibitory compounds such as essential oils and phenolic substances. The study combined laboratory-scale experimentation, pilot-scale validation and the development of an automated aeration control system, providing an integrated and process-oriented assessment of lagoon-based treatment strategies for agro-industrial wastewater.

Laboratory-scale experiments demonstrated that treatment performance is strongly governed by two operational parameters (namely oxygen availability and essential oil concentration) but less by nitrogen imbalance. Continuous aeration promoted rapid pH neutralisation and, to a much lesser extent, enhanced organic matter degradation, while partial dilution of CPW significantly reduced microbial inhibition caused by essential oils. In contrast, nitrogen supply did not emerge as a critical factor to overcome the extreme COD/N imbalance of raw CPW, since microbial activity was equally stable and the degradation of both organic matter and phenolic compounds were not significantly improved. The combined optimisation of these parameters identified a robust operational window characterised by continuous aeration, 50% reduction of essential oil concentration with organic matter restoration, and intermediate nitrogen supply (which was, however, performed to reduce that imbalance).

Pilot-scale tests confirmed that the optimal configuration identified at laboratory scale can be successfully transferred to larger volumes, ensuring stable biological performance under real operating conditions. Aerated lagoons proved to be reliable systems capable of supporting biological degradation even when treating high-strength citrus wastewater. The introduction of DO-based automated aeration control enhanced process stability control improved operational monitoring and provided a stable control framework but also revealed important operational constraints. Under extremely high organic loads, oxygen demand remained persistently high, preventing effective modulation of aeration and limiting potential energy savings. This finding delineates the operational boundary of DO-based automation, highlighting that its effectiveness depends strongly on wastewater strength and oxygen demand dynamics.

Overall, the results demonstrate that aerated lagoon systems represent a technically robust and operationally simple solution for citrus-processing wastewater treatment. At the same time, the study provides critical insight into when and how automation strategies can be effectively integrated, supporting rational design choices for full-scale implementation.

The main take-home messages of this research can be summarised as follows:

- Aerated lagoons are resilient systems capable of treating citrus-processing wastewater despite its high organic load and inhibitory composition, confirming their suitability for agro-industrial contexts where simplicity and robustness are required. Oxygen availability is the primary driver of treatment performance, since continuous aeration ensures rapid pH stabilisation and effective organic matter degradation, particularly during start-up and under inhibitory conditions. -
- Essential oil concentration does not appreciably limit biodegradability of organic acids, but partial dilution of CPW contributes to reducing microbial inhibition and enhances process stability, while excessive dilution does not provide proportional benefits when organic matter is restored.
- Nutrient imbalance is not a major bottleneck in CPW treatment, but moderate nitrogen supply is necessary to sustain microbial growth and enzymatic activity, improving degradation of both organic matter and phenolic compounds.
- Laboratory-scale optimisation is a reliable tool for upscaling, provided that operational parameters are carefully transferred and validated under real environmental conditions.
- Automated DO-based aeration control improves operational robustness, but its capacity to deliver energy savings is constrained under high-strength wastewater conditions where oxygen demand remains continuously high.
- The effectiveness of automation depends on wastewater characteristics: DO-based aeration modulation is more suitable for lower-strength or more variable effluents, where oxygen concentrations can fluctuate around control thresholds.
- Automation should be viewed as a context-dependent optimisation tool, not as a universal

solution, and must be integrated based on a clear understanding of process kinetics and oxygen demand.

This work contributes to bridging the gap between experimental research and practical application of lagoon-based wastewater treatment systems. By combining process optimisation, pilot-scale validation and control system development, the study provides a comprehensive framework for designing aerated lagoons that are not only effective but also adaptable to different operational contexts.

Future research should focus on extending automated aeration strategies to wastewater streams with lower and more variable organic loads, evaluating long-term energy balances, and integrating modelling tools to predict oxygen demand and optimise control thresholds. In this perspective, aerated lagoons, when properly designed and intelligently operated, can represent a sustainable and scalable solution for the treatment of agro-industrial wastewater within circular economy frameworks.The future of lagoon-based systems lies not in increasing mechanical complexity, but in combining biological robustness with intelligent, demand-driven operation.

REFERENCES

Ali, A.E., Salem, W.M., Younes, S.M., Kaid, M., 2020. Modeling climatic effect on physiochemical parameters and microorganisms of Stabilization Pond Performance. Heliyon 6, e04005. https://doi.org/10.1016/j.heliyon.2020.e04005

Amaral, C., Lucas, M.S., Coutinho, J., Crespí, A.L., do Rosário Anjos, M., Pais, C., 2008. Microbiological and physicochemical characterization of olive mill wastewaters from a continuous olive mill in Northeastern Portugal. Bioresour. Technol. 99, 7215–7223.

Amengual-Morro, C., Niell, G.M., Martínez-Taberner, A., 2012. Phytoplankton as bioindicator for waste stabilization ponds. J. Environ. Manage. 95, S71–S76. https://doi.org/10.1016/j.jenvman.2011.07.008

Ammar, Z., Ahmed Abdelhafid, B., Ali, D., 2017. The Effects of Hydraulic Retention Time on Organic Loading Rate in Efficiency of Aerated Lagoons in Treating Rural Domestic Wastewater at El-Oued (South-East Algeria). Orient. J. Chem. 33, 1890–1898. https://doi.org/10.13005/ojc/330434

Andiloro, S., Bombino, G., Denisi, P., Folino, A., Zema, D.A., Zimbone, S.M., 2021a. Depuration performance of aerated tanks simulating lagoons to treat olive oil mill wastewater under different airflow rates, and concentrations of polyphenols and nitrogen. Environments 8, 70. https://doi.org/10.3390/environments8080070

Andiloro, S., Bombino, G., Denisi, P., Folino, A., Zema, D.A., Zimbone, S.M., 2021b. Depuration performance of aerated tanks simulating lagoons to treat olive oil mill wastewater under different airflow rates, and concentrations of polyphenols and nitrogen. Environments 8, 70. https://doi.org/10.3390/environments8080070

Andiloro, S., Bombino, G., Tamburino, V., Zema, D.A., Zimbone, S.M., 2013a. Aerated lagooning of agro-industrial wastewater: depuration performance and energy requirements. J. Agric. Eng. 44, 0–5. https://doi.org/10.4081/jae.2013.s2.e166

Andiloro, S., Bombino, G., Tamburino, V., Zema, D.A., Zimbone, S.M., 2013b. Aerated lagooning of agro-industrial wastewater: depuration performance and energy requirements. J. Agric. Eng. 44, 0–5. https://doi.org/10.4081/jae.2013.s2.e166

Andiloro, S., Bombino, G., Tamburino, V., Zema, D.A., Zimbone, S.M., n.d. Aerated lagooning of agro-industrial wastewater: depuration performance and energy requirements. J. Agric. Eng. 7.

Andrés, C.M.C., Pérez de la Lastra, J.M., Juan, C.A., Plou, F.J., Pérez-Lebeña, E., 2023. Polyphenols as Antioxidant/Pro-Oxidant Compounds and Donors of Reducing Species: Relationship with Human Antioxidant Metabolism. Processes 11, 2771. https://doi.org/10.3390/pr11092771

Arora, N.K., 2019. Impact of climate change on agriculture production and its sustainable solutions. Environ. Sustain. 2, 95–96. https://doi.org/10.1007/s42398-019-00078-w

Aydinalp, C., Cresser, M.S., 2008. The effects of global climate change on agriculture. Am.-Eurasian J. Agric. Environ. Sci. 3, 672–676.

Bachi, O.E., Halilat, M.T., Bissati, S., Al-Ansari, N., Saggai, S., Kouadri, S., Najm, H.M., 2022. Wastewater Treatment Performance of Aerated Lagoons, Activated Sludge and Constructed Wetlands under an Arid Algerian Climate. Sustainability 14, 16503. https://doi.org/10.3390/su142416503

Bakkali, F., Averbeck, S., Averbeck, D., Idaomar, M., 2008. Biological effects of essential oils--a review. Food Chem. Toxicol. Int. J. Publ. Br. Ind. Biol. Res. Assoc. 46, 446–475. https://doi.org/10.1016/j.fct.2007.09.106

Bansah, K.J., Suglo, R.S., 2016. Sewage treatment by waste stabilization pond systems. Methods 10, 5.

Beyene, H., Redaie, G., 2011. Assessment of waste stabilization ponds for the treatment of hospital wastewater: the case of Hawassa University Referral Hospital. World Appl. Sci. J.

15, 142–150.
Bolzonella, D., Battistoni, P., Susini, C., Cecchi, F., 2006. Anaerobic codigestion of waste activated sludge and OFMSW: The experiences of Viareggio and Treviso plants (Italy). Water Sci. Technol. 53, 203–211. https://doi.org/10.2166/wst.2006.251
Bombino, G., Tamburino, V., Zema, D.A., Zimbone S.M., 2009. Depuration of citrus processing wastewater in aerated biological ponds. Proc. Congr. Ital. Assoc. Agric. Eng. Ischia Italy 1216.
Bustamante, M.A., Moral, R., Bonmatí, A., Palatsí, J., Solé-Mauri, F., Bernal, M.P., 2014. Integrated Waste Management Combining Anaerobic and Aerobic Treatment: A Case Study. Waste Biomass Valorization 5, 481–490. https://doi.org/10.1007/s12649-013-9260-9
Butler, E., Hung, Y.-T., Suleiman Al Ahmad, M., Yeh, R.Y.-L., Liu, R.L.-H., Fu, Y.-P., 2017. Oxidation pond for municipal wastewater treatment. Appl. Water Sci. 7, 31–51. https://doi.org/10.1007/s13201-015-0285-z
Calabrò, P.S., Folino, A., Maesano, M., Pangallo, D., Zema, D.A., 2022. Exploring the Possibility to Shorten the Duration and Reduce the Number of Replicates in Biomethane Potential Tests (BMP). Waste Biomass Valorization. https://doi.org/10.1007/S12649-022-01893-9
Calabrò, P.S., Fòlino, A., Tamburino, V., Zappia, G., Zema, D.A., 2018. Increasing the tolerance to polyphenols of the anaerobic digestion of olive wastewater through microbial adaptation. Biosyst. Eng. 172, 19–28.
Calabrò, P.S., Pangallo, D., Ferreri, M., Pedullà, A., Zema, D.A., 2025. Organic Waste and Wastewater Sludge to Volatile Fatty Acids and Biomethane: A Semi-Continuous Biorefinery Approach. Recycling 10, 125. https://doi.org/10.3390/recycling10040125
Calabrò, P.S., Pangallo, D., Zema, D.A., 2024. Wastewater treatment in lagoons: A systematic review and a meta-analysis. J. Environ. Manage. 359, 120974. https://doi.org/10.1016/j.jenvman.2024.120974
Cassano, A., Conidi, C., Ruby-Figueroa, R., Castro-Muñoz, R., 2018. Nanofiltration and Tight Ultrafiltration Membranes for the Recovery of Polyphenols from Agro-Food By-Products. Int. J. Mol. Sci. 19, 351. https://doi.org/10.3390/ijms19020351
Chan, Y.J., Chong, M.F., Law, C.L., Hassell, D.G., 2009. A review on anaerobic-aerobic treatment of industrial and municipal wastewater. Chem. Eng. J. 155, 1–18. https://doi.org/10.1016/j.cej.2009.06.041
Chatterjee, R., Debnath, A., Mishra, S., 2020. Vermicompost and Soil Health, in: Giri, B., Varma, A. (Eds.), Soil Health. Springer International Publishing, Cham, pp. 69–88. https://doi.org/10.1007/978-3-030-44364-1_4
Chen, J., Cheng, H., Wu, D., Linhardt, R.J., Zhi, Z., Yan, L., Chen, S., Ye, X., 2017. Green recovery of pectic polysaccharides from citrus canning processing water. J. Clean. Prod. 144, 459–469. https://doi.org/10.1016/j.jclepro.2016.12.140
Cinardi, G., D'Urso, P.R., Cascone, G., Arcidiacono, C., 2025. Citrus Waste Valorisation Processes from an Environmental Sustainability Perspective: A Scoping Literature Review of Life Cycle Assessment Studies. AgriEngineering 7, 335. https://doi.org/10.3390/agriengineering7100335
Cirelli, G.L., 2003. I trattamenti naturali delle acque reflue urbane. Sist. Ed. Arzano.
Corsino, S.F., Di Trapani, D., Capodici, M., Torregrossa, M., Viviani, G., 2021a. Optimization of acetate production from citrus wastewater fermentation. Water Resour. Ind. 25, 100140. https://doi.org/10.1016/j.wri.2021.100140
Corsino, S.F., Di Trapani, D., Capodici, M., Torregrossa, M., Viviani, G., 2021b. Optimization of acetate production from citrus wastewater fermentation. Water Resour. Ind. 25, 100140. https://doi.org/10.1016/j.wri.2021.100140
Corsino, S.F., Di Trapani, D., Torregrossa, M., Viviani, G., 2018. Aerobic granular sludge treating high strength citrus wastewater: Analysis of pH and organic loading rate effect on kinetics, performance and stability. J. Environ. Manage. 214, 23–35. https://doi.org/10.1016/j.jenvman.2018.02.087

Council of the European Union., 1991. Directive 91/271/EEC of 21 May 1991 concerning urban waste-water treatment, OJ L.
Denisi, P., Biondo, N., Bombino, G., Folino, A., Zema, D.A., Zimbone, S.M., 2021a. A Combined System Using Lagoons and Constructed Wetlands for Swine Wastewater Treatment. Sustainability 13, 12390. https://doi.org/10.3390/su132212390
Denisi, P., Biondo, N., Bombino, G., Folino, A., Zema, D.A., Zimbone, S.M., 2021b. A Combined System Using Lagoons and Constructed Wetlands for Swine Wastewater Treatment. Sustainability 13, 12390. https://doi.org/10.3390/su132212390
Di Trapani, D., Corsino, S.F., Torregrossa, M., Viviani, G., 2019. Treatment of high strength industrial wastewater with membrane bioreactors for water reuse: Effect of pre-treatment with aerobic granular sludge on system performance and fouling tendency. J. Water Process Eng. 31. https://doi.org/10.1016/j.jwpe.2019.100859
Edokpayi, J.N., Odiyo, J.O., Popoola, O.E., Msagati, T.A., 2021. Evaluation of contaminants removal by waste stabilization ponds: A case study of Siloam WSPs in Vhembe District, South Africa. Heliyon 7. https://doi.org/e06207.%2520https://doi.org/10.1016/j.heliyon.2021.e06207
Epa, U., 2011. Principles of design and operations of wastewater treatment pond systems for plant operators, engineers, and managers. U. S. Environ. Prot. Agency Off. Res. Dev.
European Parliament & Council., 2020. Regulation (EU) 2020/741 on minimum requirements for water reuse, OJ L.
European Parliament & Council., 2010. Directive 2010/75/EU on industrial emissions (integrated pollution prevention and control)., OJ L.
European Parliament & Council, 2008. Directive 2008/98/EC on waste and repealing certain Directives, OJ L.
European Parliament & Council, 2000. Directive 2000/60/EC establishing a framework for Community action in the field of water policy., 2000/60/EC.
FAO, 2025. Countries by Total Citrus Production in 2023 - Global Statistics and Insights. FAO.
FAO, 2023. Citrus | Markets and Trade | Food and Agriculture Organization of the United Nations [WWW Document]. MarketsAndTrade. URL https://www.fao.org/markets-and-trade/commodities-overview/food-and-agriculture-market-analysis-(FAMA)/citrus/en (accessed 11.20.25).
Farzadkia, M., Ehrampoush, M.H., Sadeghi, S., Kermani, M., Taghi, M., Ghelmani, V., Mehrizi, E.A., 2014. Performance evaluation of wastewater stabilization ponds in Yazd-Iran.
Feng, Y., Yang, L., Liu, J., Logan, B.E., 2016. Electrochemical technologies for wastewater treatment and resource reclamation. Environ. Sci. Water Res. Technol. 2, 800–831. https://doi.org/10.1039/C5EW00289C
Ferreira-Leitão, V., Gottschalk, L.M.F., Ferrara, M.A., Nepomuceno, A.L., Molinari, H.B.C., Bon, E.P.S., 2010. Biomass Residues in Brazil: Availability and Potential Uses. Waste Biomass Valorization 1, 65–76. https://doi.org/10.1007/s12649-010-9008-8
Food and Agriculture Organization of the United Nations (FAO), 2010. Wastewater treatment and use in agriculture, FAO Irrigation and Drainage Paper 47. FAO, Rome.
Furtado, A.L.F.F., Calijuri, M. do C., Lorenzi, A.S., Honda, R.Y., Genuário, D.B., Fiore, M.F., 2009. Morphological and molecular characterization of cyanobacteria from a Brazilian facultative wastewater stabilization pond and evaluation of microcystin production. Hydrobiologia 627, 195–209. https://doi.org/10.1007/s10750-009-9728-6
Garcia, C.F.H., de Souza, R.B., de Souza, C.P., Fontanetti, C.S., 2019. Effluent from Citrus Industry: Toxic Parameters of Orange Vinasse. Water. Air. Soil Pollut. 230, 201. https://doi.org/10.1007/s11270-019-4260-4
Garrido-Cardenas, J.A., Esteban-García, B., Agüera, A., Sánchez-Pérez, J.A., Manzano-Agugliaro, F., 2020. Wastewater Treatment by Advanced Oxidation Process and Their Worldwide Research Trends. Int. J. Environ. Res. Public. Health 17, 170. https://doi.org/10.3390/ijerph17010170

Ghazy, M.M., Senousy, W.M., Aatty, A.M., Kamel, M., 2008. Performance evaluation of a waste stabilization pond in a rural area in Egypt. Am. J. Environ. Sci. 4, 316. https://doi.org/10.3844/ajessp.2008.316.325
Girona-García, A., Ortiz-Perpiñá, O., Badía-Villas, D., 2019. Dynamics of topsoil carbon stocks after prescribed burning for pasture restoration in shrublands of the Central Pyrenees (NE-Spain). J. Environ. Manage. 233, 695–705. https://doi.org/10.1016/j.jenvman.2018.12.057
Gloyna, E.F, & W.H.O., 1971. Waste stabilization ponds. WHO Geneva 175.
Godini, K., Azarian, G., Kimiaei, A., Dragoi, E.N., Curteanu, S., 2021a. Modeling of a real industrial wastewater treatment plant based on aerated lagoon using a neuro-evolutive technique. Process Saf. Environ. Prot. 148, 114–124. https://doi.org/10.1016/j.psep.2020.09.057
Godini, K., Azarian, G., Kimiaei, A., Dragoi, E.N., Curteanu, S., 2021b. Modeling of a real industrial wastewater treatment plant based on aerated lagoon using a neuro-evolutive technique. Process Saf. Environ. Prot. 148, 114–124. https://doi.org/10.1016/j.psep.2020.09.057
Govahi, S., Karimi-Jashni, A., Derakhshan, M., 2012a. Treatability of landfill leachate by combined upflow anaerobic sludge blanket reactor and aerated lagoon. Int. J. Environ. Sci. Technol. 9, 145–151. https://doi.org/10.1007/s13762-011-0021-7
Govahi, S., Karimi-Jashni, A., Derakhshan, M., 2012b. Treatability of landfill leachate by combined upflow anaerobic sludge blanket reactor and aerated lagoon. Int. J. Environ. Sci. Technol. 9, 145–151. https://doi.org/10.1007/s13762-011-0021-7
Governo della Repubblica Italiana, 2024. Legge 191/2024 – Modifiche al Codice dell'Ambiente.
Gupta, A., Jeyakumar, E., Lawrence, R., 2021. Journey of Limonene as an Antimicrobial Agent. J. Pure Appl. Microbiol. 15, 1094–1110. https://doi.org/10.22207/JPAM.15.3.01
Gurevitch, J., Koricheva, J., Nakagawa, S., Stewart, G., 2018. Meta-analysis and the science of research synthesis. Nature 555, 175–182. https://doi.org/10.1038/nature25753
Hammadi, B., Bebba, A.A., Gherraf, N., 2016. Degradation of organic pollution aerated lagoons. In an arid climate: the case the treatment plant Ouargla (Algeria). Acta Ecol. Sin. 36, 275–279. https://doi.org/10.1016/j.chnaes.2016.05.002
Han, Y., Sun, Z., Chen, W., 2019. Antimicrobial Susceptibility and Antibacterial Mechanism of Limonene against Listeria monocytogenes. Molecules 25, 33. https://doi.org/10.3390/molecules25010033
Henze, M., Loosdrecht, M.C.M. van, Ekama, G.A., Brdjanovic, D., 2008. Biological Wastewater Treatment. IWA Publishing.
Houghton, S.R., Mara, D.D., 1992. The Effects of Sulphide Generation in Waste Stabilization Ponds on Photosynthetic Populations and Effluent Quality. Water Sci. Technol. 26, 1759–1768. https://doi.org/10.2166/wst.1992.0619
Ioppolo, A., Laudicina, V.A., Badalucco, L., Saiano, F., Palazzolo, E., 2020. Wastewaters from citrus processing industry as natural biostimulants for soil microbial community. J. Environ. Manage. 273, 111137. https://doi.org/10.1016/j.jenvman.2020.111137
Jail, A., Boukhoubza, F., Nejmeddine, A., Sayadi, S., Hassani, L., 2010a. Co-treatment of olive-mill and urban wastewaters by experimental stabilization ponds. J. Hazard. Mater. 176, 893–900. https://doi.org/10.1016/j.jhazmat.2009.11.120
Jail, A., Boukhoubza, F., Nejmeddine, A., Sayadi, S., Hassani, L., 2010b. Co-treatment of olive-mill and urban wastewaters by experimental stabilization ponds. J. Hazard. Mater. 176, 893–900. https://doi.org/10.1016/j.jhazmat.2009.11.120
Jarboui, R., Sellami, F., Azri, C., Gharsallah, N., Ammar, E., 2010. Olive mill wastewater evaporation management using PCA method: case study of natural degradation in stabilization ponds (Sfax, Tunisia). J. Hazard. Mater. 176, 992–1005. https://doi.org/10.1016/j.jhazmat.2009.11.140
Juanicó, M., Milstein, A., 2004. Semi-intensive treatment plants for wastewater reuse in irrigation. Water Sci. Technol. 50, 55–60. https://doi.org/10.2166/wst.2004.0088
Kaur, S., Singh, V., Chopra, H.K., Panesar, P.S., 2024. Extraction and characterization of phenolic

compounds from mandarin peels using conventional and green techniques: a comparative study. Discov. Food 4, 60. https://doi.org/10.1007/s44187-024-00139-y
Kayombo, S., Mbwette, T.S.A., Katima, J.H.Y., Ladegaard, N., Jørgensen, S.E., 2005. Waste stabilization ponds and constructed wetlands design manual. UNEP-IETC Dan. Int. Dev. Agency Danida 1–59.
Khorsandi, H., Alizadeh, R., Tosinejad, H., Porghaffar, H., 2014a. Analysis of nitrogenous and algal oxygen demand in effluent from a system of aerated lagoons followed by polishing pond. Water Sci. Technol. 70, 95–101. https://doi.org/10.2166/wst.2014.194
Khorsandi, H., Alizadeh, R., Tosinejad, H., Porghaffar, H., 2014b. Analysis of nitrogenous and algal oxygen demand in effluent from a system of aerated lagoons followed by polishing pond. Water Sci. Technol. 70, 95–101. https://doi.org/10.2166/wst.2014.194
Kimball, D.A., 1999. Citrus Processing, Citrus Processing. https://doi.org/10.1007/978-1-4615-4973-4
Koppar, A., Pullammanappallil, P., 2013. Anaerobic digestion of peel waste and wastewater for on site energy generation in a citrus processing facility. Energy 60, 62–68. https://doi.org/10.1016/j.energy.2013.08.007
Lian, Y., Coggins, L.X., Hay, J., van de Ven, A., Ghadouani, A., 2022. Effect of Attached Growth on Treatment Performance in Waste Stabilization Ponds. Water 14, 3245. https://doi.org/10.3390/w14203245
Lin, H., Li, Z., Sun, Y., Zhang, Y., Wang, S., Zhang, Q., Cai, T., Xiang, W., Zeng, C., Tang, J., 2024. D-Limonene: Promising and sustainable natural bioactive compound. Appl. Sci. 14, 4605.
Liu, C., Tan, L., Zhang, K., Wang, W., Ma, L., 2023. Immobilization of Horseradish Peroxidase for Phenol Degradation. ACS Omega 8, 26906–26915. https://doi.org/10.1021/acsomega.3c01570
Lucia, C., Laudicina, V.A., Badalucco, L., Galati, A., Palazzolo, E., Torregrossa, M., Viviani, G., Corsino, S.F., 2022. Challenges and opportunities for citrus wastewater management and valorisation: A review. J. Environ. Manage. 321, 115924. https://doi.org/10.1016/j.jenvman.2022.115924
Lukitawesa, Wikandari, R., Millati, R., Taherzadeh, M.J., Niklasson, C., 2018. Effect of Effluent Recirculation on Biogas Production Using Two-Stage Anaerobic Digestion of Citrus Waste. Molecules 23, 3380. https://doi.org/10.3390/molecules23123380
Mahato, N., Sinha, M., Sharma, K., Koteswararao, R., Cho, M.H., 2019. Modern Extraction and Purification Techniques for Obtaining High Purity Food-Grade Bioactive Compounds and Value-Added Co-Products from Citrus Wastes. Foods 8, 523. https://doi.org/10.3390/foods8110523
Mahdi, F., Mohammad Hassan, E., Shahram, S., Majid, K., Vahid, G., Ehsan Abouee, M., 2014. Performance evaluation of wastewater stabilization ponds in Yazd-Iran.
Malovanyy, M., Zhuk, V., Sliusar, V., Sereda, A., 2018. Twostage treatment of solid waste leachates in aerated lagoons and at municipal wastewater treatment plants. East.-Eur. J. Enterp. Technol. 1, 23–30. https://doi.org/10.15587/1729-4061.2018.122425
Mara, D., 2009. Waste stabilization ponds: Past, present and future. Desalination Water Treat. 4(1–3), 85–88.
Marín, F.R., Soler-Rivas, C., Benavente-García, O., Castillo, J., Pérez-Alvarez, J.A., 2007. By-products from different citrus processes as a source of customized functional fibres. Food Chem. 100, 736–741. https://doi.org/10.1016/j.foodchem.2005.04.040
Market Research Future, 2025. Citrus Fruits - Worldwide | Statista Market Forecast [WWW Document]. Statista. URL http://frontend.xmo.prod.aws.statista.com/outlook/cmo/food/fruits-nuts/fresh-fruits/citrus-fruits/worldwide (accessed 11.20.25).
Martins, C.L., Fernandes, H., Costa, R.H., 2013. Landfill leachate treatment as measured by nitrogen transformations in stabilization ponds. Bioresour. Technol. 147, 562–568. https://doi.org/10.1016/j.biortech.2013.08.085

Masotti, L., 2002. Depurazione delle acque reflue, Edagricole. ed. Bologna, Italy (in italian).
Mburu, N., Tebitendwa, S.M., Van Bruggen, J.J.A., Rousseau, D.P.L., Lens, P.N.L., 2013. Performance comparison and economics analysis of waste stabilization ponds and horizontal subsurface flow constructed wetlands treating domestic wastewater: A case study of the Juja sewage treatment works. J. Environ. Manage. 128, 220–225. https://doi.org/10.1016/j.jenvman.2013.05.031
Medina-Herrera, N., Martínez-Ávila, G.C.G., Robledo-Jiménez, C.L., Rojas, R., Orozco-Zamora, B.S., 2024. From Citrus Waste to Valuable Resources: A Biorefinery Approach. Biomass 4, 784–808. https://doi.org/10.3390/biomass4030044
Mehmood, M.K., Adetutu, E., Nedwell, D.B., Ball, A.S., 2009. In situ microbial treatment of landfill leachate using aerated lagoons. Bioresour. Technol. 100, 2741–2744. https://doi.org/10.1016/j.biortech.2008.11.031
Metcalf & Eddy, A., 2014. Wastewater Engineering Treatment and Resource Recovery. McGraw-Hill Education.
Metcalf, L., Eddy, H., Tchobanoglous, G., 1991. Wastewater engineering: treatment, disposal, and reuse.
Metcalf, L., Eddy, H.P., Tchobanoglous, G., 2004. Wastewater engineering: treatment, disposal, and reuse. McGraw-Hill New York.
Modica, G., Strano, T., Napoli, E., Seminara, S., Aguilar-Hernández, M., Legua, P., Gentile, A., Ruberto, G., Continella, A., 2024. Qualitative traits and peel essential oil profiles of 24 Italian and international lemon varieties. Food Biosci. 59, 103881.
Montalvo, S., Guerrero, L., Rivera, E., Borja, R., Chica, A., Martín, A., 2010. Kinetic evaluation and performance of pilot-scale fed-batch aerated lagoons treating winery wastewaters. Bioresour. Technol. 101, 3452–3456. https://doi.org/10.1016/j.biortech.2009.12.101
Moradhasseli, M., Mohamadi, S., 2014. Performance evaluation of a facultative aerated lagoon for the purpose of reviewing the design parameters. Sci. Iran. 21, 231–240.
Mukherjee, M., Chakraborty, T., Banerjee, S., Pandit, S., 2024. Bio-Degradation of Phenol and Phenolic Compounds, in: Pandit, S., Kumar, L., Mathuriya, A.S., Jadhav, D.A., Ray, S. (Eds.), Advancements in Bio-Systems and Technologies for Wastewater Treatment. Springer International Publishing, Cham, pp. 207–224. https://doi.org/10.1007/978-3-031-58331-5_11
Naddafi, K., Hassanvand, M., Dehghani, F.E., Faezi, R.D., Mostofi, S., Kasaee, N., Nabizadeh, N.R., M. Heydari, 2009. Performance evaluation of wastewater stabilization ponds in Arak-Iran.
Niaounakis, M., Halvadakis, C.P., 2006. Olive Processing Waste Management: Literature Review and Patent Survey. Elsevier.
Paini, J., Benedetti, V., Ail, S.S., Castaldi, M.J., Baratieri, M., Patuzzi, F., 2022. Valorization of wastes from the food production industry: A review towards an integrated agri-food processing biorefinery. Waste Biomass Valorization 1–20.
Panwar, D., Saini, A., Panesar, P.S., Chopra, H.K., 2021. Unraveling the scientific perspectives of citrus by-products utilization: Progress towards circular economy. Trends Food Sci. Technol. 111, 549–562. https://doi.org/10.1016/j.tifs.2021.03.018
Parish, M. e., Braddock, R. j., Graumlich, T. r., 1986. Chemical and Microbial Characterization of Citrus Oil-Mill Effluent. J. Food Sci. 51, 431–433. https://doi.org/10.1111/j.1365-2621.1986.tb11148.x
Pasquarelli Federica, Mariniello Aniello, Romano Fabiana, Oliva Giuseppina, Belgiorno Vincenzo, Naddeo Vincenzo, Zarra Tiziano, 2024. Hydrogen Sulphide and Odour Emissions Control in Wastewater Treatment Plants (WWTPs) by an Integrated Sustainable Biotechnological System. Chem. Eng. Trans. 112, 187–192. https://doi.org/10.3303/CET24112032
Pourbafrani, M., Forgács, G., Horváth, I.S., Niklasson, C., Taherzadeh, M.J., 2010. Production of biofuels, limonene and pectin from citrus wastes. Bioresour. Technol. 101, 4246–4250. https://doi.org/10.1016/j.biortech.2010.01.077
Pulido, J.O., Martínez-Férez, A., 2017. About the recovery of the phenolic fraction from olive mill

wastewater by micro and ultracentrifugation membranes. Chem. Eng. Trans. 60, 271–276.
Ragush, C.M., Schmidt, J.J., Krkosek, W.H., Gagnon, G.A., Truelstrup-Hansen, L., Jamieson, R.C., 2015. Performance of municipal waste stabilization ponds in the Canadian Arctic. Ecol. Eng. 83, 413–421.
Rajagopal, R., Saady, N.M.C., Torrijos, M., Thanikal, J.V., Hung, Y.-T., 2013. Sustainable agro-food industrial wastewater treatment using high rate anaerobic process. Water 5, 292–311.
Regione Calabria, 2021. Deliberaz. G.R. Calabria 31/03/2021, n. 119-Disciplinare per l'utilizzazione agronomica degli effluenti di allevamento, dei digestati e delle acque reflue [WWW Document]. Boll. Legis. Tec. URL https://www.legislazionetecnica.it/7329342/normativa-edilizia-appalti-professioni-tecniche-sicurezza-ambiente/deliberaz-gr-calabria-31-03-2021-n-119/nitrati-origine-agricola (accessed 2.19.26).
Republic of Italy, 2006. Decreto Legislativo 3 aprile 2006, n. 152 "Norme in materia ambientale", Gazzetta Ufficiale n. 88 del 14 aprile 2006 - Supplemento Ordinario n. 96. Gazzetta Uff. Ital. 1, 172.
Rivera, D., Navarro, J., Camarero, I., Valera, J., Rivera-Obón, D.-J., Obón, C., 2024. Citrus: From Symbolism to Sensuality—Exploring Luxury and Extravagance in Western Muslim Bustān and European Renaissance Gardens. Arts 13, 176. https://doi.org/10.3390/arts13060176
Rizzo, L., Malato, S., Antakyali, D., Beretsou, V.G., Đolić, M.B., Gernjak, W., Heath, E., Ivancev-Tumbas, I., Karaolia, P., Lado Ribeiro, A.R., Mascolo, G., McArdell, C.S., Schaar, H., Silva, A.M.T., Fatta-Kassinos, D., 2019. Consolidated vs new advanced treatment methods for the removal of contaminants of emerging concern from urban wastewater. Sci. Total Environ. 655, 986–1008. https://doi.org/10.1016/j.scitotenv.2018.11.265
Rosas-Mendoza, E.S., Méndez-Contreras, J.M., Martínez-Sibaja, A., Vallejo-Cantú, N.A., Alvarado-Lassman, A., 2018. Anaerobic digestion of citrus industry effluents using an Anaerobic Hybrid Reactor. Clean Technol. Environ. Policy 20, 1387–1397.
Ruiz, B., Flotats, X., 2014. Citrus essential oils and their influence on the anaerobic digestion process: An overview. Waste Manag. 34, 2063–2079. https://doi.org/10.1016/j.wasman.2014.06.026
Šafranko, S., Šubarić, D., Jerković, I., Jokić, S., 2023. Citrus By-Products as a Valuable Source of Biologically Active Compounds with Promising Pharmaceutical, Biological and Biomedical Potential. Pharmaceuticals 16, 1081. https://doi.org/10.3390/ph16081081
Salgot, M., Huertas, E., Weber, S., Dott, W., Hollender, J., 2006. Wastewater reuse and risk: Definition of key objectives. Desalination 187, 29–40. https://doi.org/10.1016/j.desal.2005.04.065
Sánchez-Zurano, A., Rodríguez-Miranda, E., Guzmán, J.L., Acién-Fernández, F.G., Fernández-Sevilla, J.M., Molina Grima, E., 2021. ABACO: a new model of microalgae-bacteria consortia for biological treatment of wastewaters. Appl. Sci. 11, 998.
Satari, B., Karimi, K., 2018. Citrus processing wastes: Environmental impacts, recent advances, and future perspectives in total valorization. Resour. Conserv. Recycl. 129, 153–167. https://doi.org/10.1016/j.resconrec.2017.10.032
Schimmenti, E., Borsellino, V., Galati, A., 2013. Growth of citrus production among the Euro-Mediterranean countries: political implications and empirical findings. Span. J. Agric. Res. 11, 561–577. https://doi.org/10.5424/sjar/2013113-3422
Shpiner, R., Liu, G., Stuckey, D.C., 2009. Treatment of oilfield produced water by waste stabilization ponds: Biodegradation of petroleum-derived materials. Bioresour. Technol. 100, 6229–6235. https://doi.org/10.1016/j.biortech.2009.07.005
Sinn, J., Agrawal, S., Orschler, L., Lackner, S., 2022. Characterization and evaluation of waste stabilization pond systems in Namibia. H2Open J. 5, 365–378. https://doi.org/10.2166/h2oj.2022.004
Suri, S., Singh, A., Nema, P.K., 2021. Recent advances in valorization of citrus fruits processing waste: a way forward towards environmental sustainability. Food Sci. Biotechnol. 30,

1601–1626. https://doi.org/10.1007/s10068-021-00984-y
Tamburino, V., Zema, D.A., Zimbone, S.M., 2007. Depuration processes of citrus wastewater. Proc. 3rd Int. Symp. CIGR Sect. VI "Food Agric. Prod. Process. Innov. Naples Italy.
Tchobanoglous, G., Burton, F.L., Metcalf, Stensel, H.D., 2002. Wastewater Engineering: Treatment and Reuse, Metcalf&Eddy, Inc., McGraw-Hill Company. ed. New York.
Tchobanoglus, G., Burton, F., Stensel, H.D., 2003. Wastewater engineering: treatment and reuse. Am. Water Works Assoc. J. 95, 201.
Torquato, L.D.M., Pachiega, R., Crespi, M.S., Nespeca, M.G., de Oliveira, J.E., Maintinguer, S.I., 2017. Potential of biohydrogen production from effluents of citrus processing industry using anaerobic bacteria from sewage sludge. Waste Manag. 59, 181–193. https://doi.org/10.1016/j.wasman.2016.10.047
Travieso, L., Sánchez, E., Borja, R., Benítez, F., León, M., Colmenarejo, M.F., 2010. Evaluation of a Laboratory and Full-Scale Microlage Pond for Tertiary Treatment of Piggery Wastes. http://dx.doi.org/10.1080/09593330.2004.9619347 25, 565–576. https://doi.org/10.1080/09593330.2004.9619347
Trias, M., Hu, Z., Mortula, M.M., Gordon, R.J., Gagnon, G.A., 2004. Impact of Seasonal Variation on Treatment of Swine Wastewater. Environ. Technol. 25, 775–781. https://doi.org/10.1080/09593330.2004.9619368
Tuleibah, H.E., Salem, M.A., Khalafallah, A.A., El Gerum, H.A., 2015. The Use of Some Aquatic Plants to Improve the Effluents of an Aerated Lagoon System. J. Environ. Sci. 18, 89–108.
Tuleibah, H.E., Salem, M.A.M., Khalafallah, A.A.M., n.d. THE USE OF SOME AQUATIC PLANTS TO IMPROVE THE EFFLUENTS OF AN AERATED LAGOON SYSTEM.
Türker, U., Okaygün, M., Almaqadma, S.J., 2009a. Impact of anaerobic lagoons on the performance of BOD and TSS removals at the Haspolat (Mia Milia) Wastewater Treatment Plant. Desalination 249, 403–410. https://doi.org/10.1016/j.desal.2009.06.044
Türker, U., Okaygün, M., Almaqadma, S.J., 2009b. Impact of anaerobic lagoons on the performance of BOD and TSS removals at the Haspolat (Mia Milia) Wastewater Treatment Plant. Desalination 249, 403–410. https://doi.org/10.1016/j.desal.2009.06.044
USDA FAS, 2024. Citrus: World Markets and Trade | USDA Foreign Agricultural Service [WWW Document]. URL https://www.fas.usda.gov/data/citrus-world-markets-and-trade-07252024 (accessed 11.20.25).
Wainaina, S., Lukitawesa, Kumar Awasthi, M., Taherzadeh, M.J., 2019. Bioengineering of anaerobic digestion for volatile fatty acids, hydrogen or methane production: A critical review. Bioengineered 10, 437–458. https://doi.org/10.1080/21655979.2019.1673937
Wallace, J., Champagne, P., Hall, G., 2016. Multivariate statistical analysis of water chemistry conditions in three wastewater stabilization ponds with algae blooms and pH fluctuations. Water Res. 96, 155–165. https://doi.org/10.1016/j.watres.2016.03.046
Walsby, A.E., Dubinsky, Z., Kromkamp, J.C., Lehmann, C., Schanz, F., 2001. The effects of diel changes in photosynthetic coefficients and depth of Planktothrix rubescens on the daily integral of photosynthesis in Lake Zürich. Aquat. Sci. 63, 326–349.
Welti-Chanes, J., Hernández-Meléndez, O., Morales-de la Peña, M., Bárzana, E., 2025. Dual process for improved recovery of essential oil and pectin from citrus waste. J. Chem. Technol. Biotechnol. https://doi.org/10.1002/jctb.7902
Wikandari, R., Millati, R., Cahyanto, M.N., Taherzadeh, M.J., 2014. Biogas Production from Citrus Waste by Membrane Bioreactor. Membranes 4, 596–607. https://doi.org/10.3390/membranes4030596
Wong, Y.S., Kadir, M.O.A.B., Teng, T.T., 2009. Biological kinetics evaluation of anaerobic stabilization pond treatment of palm oil mill effluent. Bioresour. Technol. 100, 4969–4975. https://doi.org/10.1016/j.biortech.2009.04.074
Xavier, C.R., Mosquera-Corral, A., Becerra, J., Hernández, V., Vidal, G., 2009a. Activated sludge versus aerated lagoon treatment of kraft mill effluents containing β-sitosterol and stigmasterol. J. Environ. Sci. Health Part A 44, 327–335.

https://doi.org/10.1080/10934520802659646
Xavier, C.R., Mosquera-Corral, A., Becerra, J., Hernandez, V., Vidal, G., 2009b. Activated sludge versus aerated lagoon treatment of kraft mill effluents containing β-sitosterol and stigmasterol. J. Environ. Sci. Health Part A 44, 327–335.
Yan, L., Ye, X., Linhardt, R.J., Chen, J., Yu, D., Huang, R., Liu, D., Chen, S., 2018. Full recovery of value-added compounds from citrus canning processing water. J. Clean. Prod. 176, 959–965. https://doi.org/10.1016/j.jclepro.2017.12.018
Yoshikawa, T., Furuya, K., 2006. Effects of diurnal variations in phytoplankton photosynthesis obtained from natural fluorescence. Mar. Biol. 150, 299–311.
Zech-Matterne, V., Fiorentino, G. (Eds.), 2017. AGRUMED: Archaeology and history of citrus fruit in the Mediterranean : Acclimatization, diversifications, uses, AGRUMED: Archaeology and history of citrus fruit in the Mediterranean : Acclimatization, diversifications, uses, Collection du Centre Jean Bérard. Publications du Centre Jean Bérard, Naples.
Zekri, M., Obreza, T.A., 2014a. Macronutrient deficiencies in citrus: nitrogen, phosphorus, and potassium (No. SL202). University of Florida IFAS Extension.
Zekri, M., Obreza, T.A., 2014b. Plant Nutrients for Citrus Trees (No. SL253). University of Florida IFAS Extension, Gainesville, FL.
Zema, D.A., Andiloro, S., Bombino, G., Caridi, A., Sidari, R., Tamburino, V., 2016a. Comparing Different Schemes of Agricultural Wastewater Lagooning: Depuration Performance and Microbiological Characteristics. Water. Air. Soil Pollut. 227. https://doi.org/10.1007/s11270-016-3132-4
Zema, D.A., Andiloro, S., Bombino, G., Caridi, A., Sidari, R., Tamburino, V., 2016b. Comparing Different Schemes of Agricultural Wastewater Lagooning: Depuration Performance and Microbiological Characteristics. Water. Air. Soil Pollut. 227. https://doi.org/10.1007/s11270-016-3132-4
Zema, D.A., Andiloro, S., Bombino, G., Tamburino, V., Sidari, R., Caridi, A., 2012a. Depuration in aerated ponds of citrus processing wastewater with a high concentration of essential oils. Environ. Technol. U. K. 33, 1255–1260. https://doi.org/10.1080/09593330.2011.618938
Zema, D.A., Andiloro, S., Bombino, G., Tamburino, V., Sidari, R., Caridi, A., 2012b. Depuration in aerated ponds of citrus processing wastewater with a high concentration of essential oils. Environ. Technol. U. K. 33, 1255–1260. https://doi.org/10.1080/09593330.2011.618938
Zema, D.A., Calabro, P.S., Folino, A., Tamburino, V., Zappia, G., Zimbone, S.M., 2019a. Wastewater management in citrus processing industries: An overview of advantages and limits. Water Switz. 11, 1–23. https://doi.org/10.3390/w11122481
Zema, D.A., Esteban Lucas-Borja, M., Andiloro, S., Tamburino, V., Zimbone, S.M., 2019b. Short-term effects of olive mill wastewater application on the hydrological and physico-chemical properties of a loamy soil. Agric. Water Manag. 221, 312–321. https://doi.org/10.1016/j.agwat.2019.04.011
Zema, D.A., Lucas-Borja, M.E., 2023. Effects of prescribed fire on the post-fire hydrological processes in agro-forest ecosystems: A systematic review and a meta-analysis. Hydrol. Process. 37, e14957. https://doi.org/10.1002/hyp.14957
Zheng, H., Zhang, Q., Quan, J., Zheng, Q., Xi, W., 2016. Determination of sugars, organic acids, aroma components, and carotenoids in grapefruit pulps. Food Chem. 205, 112–121. https://doi.org/10.1016/j.foodchem.2016.03.007

APPENDIX

Table A.1 - Results of one-way ANOVA with repeated measures applied to pH, COD, TN and phenols in tank groups A to C carried out at the laboratory scale.

Time from experiment start (days)	DF	Sum of squares	Mean squares	F	Pr > F
Response variable: pH - Tank group A (effect of aeration)					
0	2	0,006	0,003	0,580	0,613
11	2	0,762	0,381	368,855	0,000
18	2	1,358	0,679	325,832	0,000
25	2	2,088	1,044	485,605	0,000
32	2	2,191	1,096	626,067	0,000
39	2	1,864	0,932	417,231	0,000
46	2	1,808	0,904	410,818	0,000
53	2	1.796	0.898	441.746	0.000
60	2	1,452	0,726	223,405	0,001
67	2	1,178	0,589	8833,000	< 0,0001
74	2	1,146	0,573	330,500	0,000
81	2	0,791	0,395	234,842	0,001
Response variable: pH - Tank group EO (effect of essential oils)					
0	2	3,827	1,913	70,995	0,003
11	2	5,591	2,795	2,640	0,218
18	2	6,691	3,345	3,589	0,160
25	2	1,881	0,941	33,391	0,009
32	2	0,528	0,264	243,462	0,000
39	2	1,657	0,828	82,697	0,002
46	2	0,479	0,239	55,215	0,004
53	2	0,249	0,125	27,700	0,012
60	2	0,105	0,052	2,192	0,259
67	2	0,010	0,005	0,485	0,657
74	2	0,035	0,018	3,850	0,148
81	2	0,016	0,008	1,992	0,282
Response variable: pH - Tank group COD/N (effect of nutrient supply)					
0	2	1,346	0,673	6,655	0,079
11	2	0,726	0,363	3632,167	< 0,0001
18	2	0,472	0,236	12,072	0,037
25	2	0,513	0,256	9,777	0,049
32	2	0,220	0,110	4,067	0,140
39	2	0,140	0,070	1,588	0,339
46	2	0,260	0,130	5,239	0,105
53	2	0,154	0,077	3,353	0,172
60	2	0,164	0,082	1,677	0,324
67	2	0,235	0,118	1,330	0,386
74	2	0,288	0,144	2,552	0,225
81	2	0,415	0,208	2,851	0,202
Response variable: COD - Tank group A (effect of aeration)					

0	2	0,039	0,020	2,908	0,199
11	2	0,875	0,437	20995,800	< 0,0001
18	2	0,183	0,092	1,017	0,460
25	2	0,665	0,333	2101,132	< 0,0001
32	2	0,742	0,371	307,079	0,000
39	2	0,531	0,265	71,488	0,003
46	2	0,279	0,139	73,262	0,003
53	2	0,307	0,154	21,454	0,017
60	2	0,163	0,081	18,140	0,021
67	2	0,012	0,006	0,692	0,566
74	2	0,021	0,010	4,505	0,125
81	2	0,000	0,000	0,000	1,000
0	2	0,039	0,020	2,908	0,199
Response variable: COD - Tank group EO (effect of essential oils)					
0	2	0,855	0,428	21,766	0,016
11	2	1,213	0,606	951,183	< 0,0001
18	2	0,749	0,374	6,967	0,075
25	2	0,172	0,086	5,493	0,099
32	2	0,083	0,042	10,991	0,042
39	2	0,009	0,004	2,519	0,228
46	2	0,025	0,012	3,221	0,179
53	2	0,003	0,001	0,727	0,553
60	2	0,003	0,001	0,301	0,760
67	2	0,000	0,000	3,500	0,164
74	2	0,001	0,000	0,364	0,722
81	2	0,000	0,000	0,808	0,524
Response variable: COD - Tank group COD/N (effect of nutrient supply)					
0	2	0,046	0,023	1,079	0,444
11	2	0,159	0,079	32,535	0,009
18	2	0,139	0,069	151,264	0,001
25	2	0,233	0,116	1,871	0,297
32	2	0,031	0,015	0,763	0,540
39	2	0,011	0,005	5,047	0,110
46	2	0,003	0,001	0,866	0,505
53	2	0,001	0,001	0,514	0,643
60	2	0,002	0,001	2,853	0,202
67	2	0,011	0,005	18,304	0,021
74	2	0,005	0,003	0,606	0,601
81	2	0,002	0,001	2,461	0,233

Time from start experiment (days)	DF	Sum of squares	Mean squares	F	Pr > F
Response variable: TN - Tank group A (effect of aeration)					
0	2	468,040	234,020	0,921	0,488
11	2	1909,120	954,560	30,999	0,010
25	2	1638,173	819,087	27,911	0,012
39	2	1620,970	810,485	131,394	0,001
67	2	68,853	34,427	0,559	0,622
81	2	86,493	43,247	6,800	0,077
Response variable: TN - Tank group EO (effect of essential oils)					
0	2	40,973	20,487	2,212	0,257
11	2	350,173	175,087	5,221	0,105
25	2	98,893	49,447	11,481	0,039
39	2	359,343	179,672	6,325	0,084
67	2	246,973	123,487	9,827	0,048
81	2	5,453	2,727	0,055	0,948
Response variable: TN - Tank group COD/N (effect of nutrient supply)					
0	2	165,823	82,912	0,518	0,641
11	2	573,853	286,927	3,322	0,174
25	2	256,563	128,282	1,020	0,459
39	2	1410,280	705,140	53,718	0,004
67	2	5969,973	2984,987	6,142	0,087
81	2	2512,120	1256,060	8,297	0,060

Time from start experiment (days)	DF	Sum of squares	Mean squares	F	Pr > F
Response variable: Phenols - Tank group A (effect of aeration)					
0	2	1,134	0,567	0,986	0,469
11	2	0,105	0,052	0,379	0,713
25	2	1,124	0,562	0,656	0,580
39	2	2,614	1,307	14,192	0,030
67	2	0,008	0,004	0,023	0,978
81	2	0,034	0,017	6,993	0,074
Response variable: Phenols - Tank group OE (effect of essential oils)					
0	2	0,654	0,327	1,571	0,341
11	2	2,040	1,020	13,862	0,031
25	2	0,909	0,454	1,631	0,332
39	2	0,011	0,006	0,955	0,478
67	2	0,062	0,031	5,321	0,103
81	2	0,143	0,071	80,660	0,002
Response variable: Phenols - Tank group COD/N (effect of nutrient supply)					
0	2	0,970	0,485	0,476	0,661
11	2	1,005	0,503	0,574	0,615
25	2	0,921	0,461	5,232	0,105
39	2	0,122	0,061	11,405	0,040
67	2	0,024	0,012	9,049	0,054
81	2	0,062	0,031	1,343	0,383

Table A.2-Results of Tukey's tests ($p < 0.05$) after one-way ANOVA with repeated measures applied to pH, COD, TN and phenols in tank groups A to C carried out at the laboratory scale.

Tank	Letter		
Response variable: pH - Tank group A (effect of aeration)			
Survey date: 0 days			
A(50-12)	A		
A(NA)	A		
A(50-24)	A		
Survey date: 11 days			
A(50-24)	A		
A(50-12)		B	
A(NA)			C
Survey date: 18 days			
A(50-24)	A		
A(50-12)	A		
A(NA)		B	
Survey Date: 25 Days			
A(50-24)	A		
A(50-12)		B	
A(NA)			C
Survey date: 32 days			
A(50-24)	A		
A(50-12)		B	
A(NA)			C
Survey date: 39 days)			
A(50-24)	A		
A(50-12)		B	
A(NA)			C
Survey date: 46 days			
A(50-24)	A		
A(50-12)	A		
A(NA)		B	
Survey date 53 days			
A(50-24)	A		
A(50-12)		B	
A(NA)			C
Survey date: 60 days)			
Tank-A(50-24)	A		
Tank-A(50-12)	A		
Tank-A(NA)		B	
Survey date: 67 days			
Tank-A(50-24)	A		
Tank-A(50-12)		B	

Tank-A(NA)			C	
Survey date: 74 days				
Tank-A(50-24)	A			
Tank-A(50-12)	A			
Tank-A(NA)		B		
Survey date: 81days				
Tank-A(50-12)	A			
Tank-A(50-24)	A			
Tank-A(NA)		B		
Survey date: 0 days				
EO(80% R)		A		
EO(100% R)				B
EO(60% R)				B
Survey date: 11 days				
EO(80% R)		A		
EO(100% R)		A		
EO(60% R)		A		
Survey date: 18 days				
EO(80% R)		A		
EO(100% R)		A		
EO(60% R)		A		
Survey Date: 25 days				
EO(80% R)		A		
EO(100% R)		A		
EO(60% R)				B
Survey date: 32 days				
EO(80% R)		A		
EO(100% R)				B
EO(60% R)				
Survey date: 39 days				
EO(80% R)		A		
EO(100% R)		A		
EO(60% R)				B
Survey date: 46 days				
EO(80% R)		A		
EO(100% R)		A		
EO(60% R)				B
Survey date: 53 days				
EO(80% R)		A		
EO(100% R)		A		
EO(60% R)				B
Survey date: 60 days				
EO(100% R)		A		
EO(80% R)		A		
EO(60% R)		A		
Survey date: 67 days				

EO(100% R)		A
EO(80% R)		A
EO(60% R)		A
Survey date: 74 days		
EO(100% R)		A
EO(60% R)		A
EO(80% R)		A
Survey date: 81 days		
EO(100% R)		A
EO(80% R)		A
EO(60% R)		A
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	
COD/N (500/1)	A	
COD/N (2000/1)		B
COD/N (300/1)		
COD/N (500/1)	A	
COD/N (300/1)		B
COD/N (2000/1)		B
COD/N (500/1)	A	
COD/N (300/1)	A	B
COD/N (2000/1)		B
Survey date: 32 days		
COD/N (500/1)	A	
COD/N (2000/1)	A	
COD/N (300/1)	A	
Survey date: 39 days		
COD/N (500/1)	A	
Tank-COD/N (2000/1)	A	
Tank-COD/N (300/1)	A	
Survey date: 46 days		
COD/N (500/1)	A	
COD/N (2000/1)	A	
COD/N (300/1)	A	
Survey date: 53 days		
COD/N (500/1)	A	
COD/N (2000/1)	A	
COD/N (300/1)	A	

Survey date: 60 days	
COD/N (2000/1)	A
COD/N (500/1)	A
COD/N (300/1)	A
Survey date: 67 days	
COD/N (2000/1)	A
COD/N (500/1)	A
COD/N (300/1)	A
Survey date: 74 days	
COD/N (2000/1)	A
COD/N (500/1)	A
COD/N (300/1)	A
COD/N (2000/1)	A
COD/N (500/1)	A
COD/N (300/1)	A

Tank	Letter	
Response variable: COD - Tank group A (effect of aeration)		
Survey date: 0 days		
A(50-24)	A	
A(50-12)	A	
A(NA)	A	
Survey date:11 days		
A(NA)	A	
A(50-24)		B
A(50-12)		B
Survey date:18 days		
A(NA)	A	
A(50-24)	A	
A(50-12)	A	
Survey date: 25 days)		
A(NA)	A	
A(50-24)		B
A(50-12)		B
Survey date: 32 days		
A(NA)	A	
A(50-24)		B
A(50-12)		B
Survey date:39 days		
A(NA)	A	

A(50-12)		B	
A(50-24)		B	
Survey date: 46 days			
A(NA)	A		
A(50-24)		B	
A(50-12)		B	
Survey date: 53 days			
A(NA)	A		
A(50-24)		B	
A(50-12)		B	
Survey date: 60 days)			
A(NA)	A		
A(50-24)		B	
A(50-12)		B	
Survey date: 67 days)			
A(NA)	A		
A(50-12)	A		
A(50-24)	A		
Survey date: 74 days			
A(NA)	A		
A(50-24)	A		
A(50-12)	A		
Survey date: 81days)			
A(50-24)	A		
A(50-12)	A		
A(NA)	A		
Response variable: COD - Tank group B (effect of OE)			
Survey date: 0 days			
EO(60% R)	A		
EO(100% R)	A		
EO(80% R)			
Survey date: 11days			
EO(60% R)	A		
EO(100% R)			
EO(80% R)			C
Survey date: 18 days			
EO(60% R)	A		
EO(100% R)	A		
EO(80% R)	A		
Survey date: 25 days			
EO(60% R)	A		
EO(80% R)	A		
EO(100% R)	A		
Survey date: 32 days)			

EO(60% R)	A	
EO(100% R)	A	
EO(80% R)		
Survey date: 39 days		
EO(60% R)	A	
EO(80% R)	A	
EO(100% R)	A	
Survey date: 46 days		
EO(60% R)	A	
EO(80% R)	A	
EO(100% R)	A	
Survey date: 53 days		
EO(80% R)	A	
EO(60% R)	A	
EO(100% R)	A	
Survey date: 60 days		
EO(80% R)	A	
EO(100% R)	A	
EO(60% R)	A	
Survey date: 67 days		
EO(100% R)	A	
EO(60% R)	A	
EO(80% R)	A	
Survey date: 74 days		
EO(80% R)	A	
EO(100% R)	A	
EO(60% R)	A	
Survey date: 81 days		
EO(60% R)	A	
EO(80% R)	A	
EO(100% R)	A	
Response variable: COD - Tank group COD/N (effect of nutrient supply)		
Survey date: 0 days)		
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	
Survey date: 11days		
COD/N (2000/1)	A	
COD/N (300/1)		B

COD/N (500/1)		B
Survey date: 18 days		
COD/N (2000/1)	A	
COD/N (500/1)		B
COD/N (300/1)		B
Survey date: 25 days		
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	
Survey date: 32 days		
COD/N (500/1)	A	
COD/N (2000/1)	A	
COD/N (300/1)	A	
Survey date: 39 days		
COD/N (2000/1)	A	
COD/N (300/1)	A	
COD/N (500/1)	A	
Survey date: 46 days		
COD/N (2000/1)	A	
COD/N (300/1)	A	
COD/N (500/1)	A	
Survey date: 53 days		
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	
Survey date: 60 days		
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	
Survey date: 67 days		
COD/N (2000/1)	A	
COD/N (500/1)	A	B
COD/N (300/1)		B
Survey date: 74 days)		
COD/N (2000/1)	A	

COD/N (500/1)	A
COD/N (300/1)	A
Survey date: 81days)	
COD/N (2000/1)	A
COD/N (300/1)	A
COD/N (500/1)	A

Tank	Letter	
Response variable: TN - Tank group A (effect of aeration) –		
Survey date: 0 days)		
A(50-24)	A	
A(NA)	A	
A(50-12)	A	
Survey date: 11 days)		
A(50-12)	A	
A(50-24)	A	
A(NA)		B
Survey date: 25 days)		
A(50-12)	A	
A(50-24)	A	
A(NA)		B
Survey date: 39 days)		
A(50-12)	A	
A(50-24)	A	
A(NA)		B
Survey date: 67 days)		
A(50-12)	A	
A(NA)	A	
A(50-24)	A	
Survey date: 81 days)		
A(50-24)	A	
A(NA)	A	
A(50-12)	A	

Response variable: TN - Tank group B (effect of OE)		
Survey date:0 days)		
EO(100 % R)	A	
EO(80% R)	A	
EO(60% R)	A	
Survey date: 11 days		
EO(80% R)	A	
EO(100 % R)	A	
EO(60% R)	A	
Survey date: 25 days)		
EO(80% R)	A	
EO(100 % R)	A	B
EO(60% R)		B
Survey date: 39 days		
EO(80% R)	A	
EO(60% R)	A	
EO(100 % R)	A	
Survey date: 67 days)		
EO(80% R)	A	
EO(100 % R)	A	
EO(60% R)	A	
Survey date: 81 days)		
EO(100 % R)	A	
EO(60% R)	A	
EO(80% R)	A	
Response variable: TN - Tank group C (effect of nutrient supply)		
Survey date: 0 days)		
COD/N (2000/1)	A	
COD/N (300/1)	A	
COD/N (500/1)	A	
Survey date: 11 days)		
COD/N (500/1)	A	

COD/N (300/1)	A	
COD/N (2000/1)	A	
	Survey date: 25 days	
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	
	Survey date: 39 days)	
COD/N (2000/1)	A	
COD/N (500/1)		B
COD/N (300/1)		B
	Survey date: 67 days)	
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	
	Survey date: 81days	
COD/N (2000/1)	A	
COD/N (500/1)	A	
COD/N (300/1)	A	

Tank	Letter	
	Response variable: phenols - Tank group A (effect of aeration)	
	Survey date: 0 days)	
A(50-12)	A	
A(50-24)	A	
A(NA)	A	
	Survey date: 11 days)	
A(50-24)	A	
A(50-12)	A	
A(NA)	A	
	Survey date: 25 days)	
A(NA)	A	
A(50-12)	A	
A(50-24)	A	
	Survey date: 39 days)	
A(NA)	A	
A(50-24)		B
A(50-12)		B
	Survey date: 67 days	

A(NA)	A		
A(50-24)	A		
A(50-12)	A		
Survey date: 81 days)			
A(NA)	A		
A(50-24)	A		
A(50-12)	A		
Response variable: phenols - Tank group B (effect of OE)			
Survey date: 0 days)			
EO(100% R)	A		
EO(80% R)	A		
EO(60% R)	A		
Survey date: 11 days			
EO(60% R)	A		
EO(100% R)	A	B	
EO(80% R)		B	
Survey date: 25 days)			
EO(60% R)	A		
EO(80% R)	A		
EO(100% R)	A		
Survey date: 39 days			
EO(60% R)	A		
EO(80% R)	A		
EO(100% R)	A		
Survey date: 67 days)			
EO(100% R)	A		
EO(60% R)	A		
EO(80% R)	A		
Survey date: 81 days			
EO(100% R)	A		
EO(60% R)		B	
EO(80% R)			C
Response variable: phenols - Tank group C (effect of nutrient supply)			
Survey date: 0 days)			
COD/N (500/1)	A		
COD/N (300/1)	A		
COD/N (2000/1)	A		
Survey date: 11days			
COD/N (2000/1)	A		
COD/N (300/1)	A		
COD/N (500/1)	A		
Survey date: 25 days)			
COD/N (2000/1)	A		
COD/N (500/1)	A		
COD/N (300/1)	A		
Survey date: 39 days			
COD/N (2000/1)	A		
COD/N (500/1)	A	B	
COD/N (300/1)		B	
Survey date: 67 days			
COD/N (2000/1)	A		
COD/N (500/1)	A	B	

COD/N (300/1)		B
Survey date: 81 days		
COD/N (500/1)	A	
COD/N (300/1)	A	
COD/N (2000/1)	A	

Table A.3- Results of t-tests applied to pH, COD, TN and phenols in tank groups A to C carried out for the upscaling experiments.

t-test parameter	Value
Response variable: pH	
Difference	-0.075
Observed value (t)	-0.077
Critical value (|t|)	2.145
DF	14
Two-tailed p-value	0.940
α	0.05
Response variable: COD	
Difference	0.269
Observed value (t)	0.378
Critical value (|t|)	2.145
DF	14
Two-tailed p-value	0.711
α	0.05
Response variable: TN	
Difference	0.888
Observed value (t)	0.120
Critical value (|t|)	2.145
DF	14
Two-tailed p-value	0.906
α	0.05
Response variable: EO	
Difference	0.185
Observed value (t)	0.201
Critical value (|t|)	2.145
DF	14
Two-tailed p-value	0.844
α	0.05