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**CHITOSAN-BASED TECHNOLOGICAL STRATEGIES FOR INDUSTRIAL WASTEWATER
TREATMENT: FROM LABORATORY RESEARCH TO PILOT-SCALE VALIDATION**

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PhD Thesis in Technology for Health

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University of Brescia

Department of Information Engineering



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Prologue

This work originates primarily from personal experiences and a growing professional awareness developed throughout my academic and working path. As a geologist, my relationship with the natural environment has always been central, both from a scientific and personal perspective. The study of geological systems inherently involves understanding natural balances, long-term processes, and the delicate interactions between water, soil, ecosystems, and human activity. This background naturally fostered a strong interest in environmental protection, sustainable resource management, and the search for solutions capable of reconciling technological development with environmental preservation. From the earliest stages of my professional experience, I found myself working in contexts directly affected by environmental contamination. Activities related to groundwater monitoring, remediation of contaminated sites, water abstraction systems, and environmental assessments provided direct exposure to the consequences of decades of intensive industrial and urban development. These experiences progressively highlighted how natural systems have often been stressed beyond sustainable limits, frequently due to short-term approaches focused primarily on productivity and economic performance.

In particular, work related to water and soil remediation, data collection from groundwater extraction wells, and the evaluation of industrial processes contributed to increasing personal awareness of the complexity of environmental impacts associated with modern production systems. Industrial processes, while often technologically advanced and optimized for efficiency, do not always fully account for long-term environmental consequences. In many cases, environmental considerations are balanced against economic targets, performance indicators, and production demands, leading to a progressive accumulation of chemical pressures on environmental systems.

Alongside these professional experiences, a growing personal interest developed toward research and innovation, particularly regarding the use of new materials and alternative technological approaches capable of improving environmental sustainability. The possibility of exploring innovative solutions, including materials derived from renewable sources or designed to reduce environmental impact, became increasingly appealing as a way to bridge the gap between environmental protection and technological advancement.

This personal and professional background naturally intersected with broader global challenges related to climate change, resource scarcity, and sustainable development. Over the past decades, international initiatives such as the Paris Agreement and subsequent environmental policies have emphasized the urgency of developing more sustainable technologies and improving resource management. The findings presented by the Intergovernmental Panel on Climate Change, particularly in the 2022 assessment, further reinforced the need for technological innovation capable of addressing emerging environmental challenges.

Within this context, water management represents one of the most critical issues of the present and future. Climate change, increasing industrial complexity, and the emergence of new contaminants are progressively altering both the availability and quality of water resources. Wastewater treatment systems, originally designed to manage a limited range of pollutants, now face increasingly complex mixtures of substances, including emerging contaminants whose environmental behavior is still being investigated.

The opportunity to work on a real industrial case aimed at improving wastewater treatment therefore represented a particularly compelling challenge. The present research originated from a concrete industrial need: the development of a sustainable and technically feasible treatment strategy for wastewater produced during the washing of flexographic printing machines. Unlike many academic investigations conducted on synthetic solutions or model contaminants, this work was conceived from the beginning as an applied research project, aimed at addressing real operational constraints and progressively developing a treatment technology suitable for industrial implementation.

Particular interest was also directed toward the use of biocompatible and sustainable materials. In this context, chitosan emerged as a promising candidate due to its biodegradability, low toxicity, and flocculating capability. Furthermore, the possibility of exploring fungal-derived chitosan, as an alternative to traditional crustacean-based sources, introduced an additional dimension of sustainability and innovation, aligning the research with emerging circular economy principles and new material supply chains.

Another distinctive aspect of this work was the decision to operate using commercially available materials and real wastewater matrices from the earliest experimental phases. This approach led to an adaptive and iterative experimental pathway, where technological development progressed through successive stages of testing, refinement, and scale-up. This methodology reflects the engineering-oriented nature of the research, where practical constraints and operational challenges played a central role in shaping the development process.

Throughout this pathway, experimentation evolved from small-scale laboratory tests to the realization of operational prototypes capable of treating progressively larger volumes of wastewater. This progressive development ultimately led to the construction of a treatment system designed for primary purification of a complex industrial effluent under realistic operational conditions.

A key contribution to this development was provided through collaboration with ISINNOVA Srl, which supported the research activities by providing technical expertise, laboratory facilities, and engineering resources. This collaboration enabled the transition from laboratory experimentation to prototype development and pilot-scale implementation, allowing the research to evolve toward applied technological innovation.

Ultimately, this work represents not only a technical investigation into wastewater treatment processes, but also a broader journey shaped by personal experiences, professional growth, and the pursuit of sustainable innovation. The search for solutions capable of balancing technological progress and environmental protection reflects both a scientific objective and a personal commitment developed throughout the research path. In this sense, the work aims to contribute, even in a limited but concrete way, to the ongoing effort toward a more sustainable interaction between human activities and the natural environment, seeking an equilibrium that should be pursued both in practical action and in the broader spirit with which we approach technological development and environmental stewardship.

Abstract (Italiano)

La crescente domanda globale di acqua pulita è minacciata dall'aumento della popolazione, dalle pressioni ambientali e dall'espansione delle attività industriali. Tra i contaminanti presenti nei corpi idrici, quelli derivati da coloranti e inchiostri industriali rappresentano circa il 10–15% delle sostanze recalcitranti. In particolare, le acque reflue generate dalla stampa flessografica costituiscono una sfida ambientale complessa, poiché contengono pigmenti persistenti, leganti polimerici, tensioattivi e altre sostanze ausiliarie che conferiscono elevata eterogeneità chimica, pH alcalino, stabilità colloidale e intensa colorazione. Queste caratteristiche riducono l'efficacia dei trattamenti convenzionali e limitano il riutilizzo dell'acqua, rendendo necessario lo sviluppo di tecnologie alternative.

Questo studio analizza l'impiego del chitosano come agente flocculante bio-based per la destabilizzazione delle dispersioni colloidali di inchiostro nelle acque reflue industriali. Il chitosano, polisaccaride cationico ottenuto dalla deacetilazione della chitina, è ampiamente utilizzato nei processi di coagulazione–flocculazione. A differenza del chitosano derivato da crostacei, il presente lavoro utilizza chitosano di origine fungina, ottenuto con minore consumo di reagenti chimici e ridotto impatto ambientale, come alternativa sostenibile e circolare.

La ricerca combina caratterizzazione fisico-chimica e ottimizzazione del processo. In una prima fase vengono studiate la composizione chimica e la stabilità colloidale degli inchiostri flessografici in ambiente acquoso. Successivamente, vengono eseguiti test batch di flocculazione per individuare le condizioni operative ottimali, valutando pH, dosaggio del polimero, miscelazione rapida e lenta e tempo di sedimentazione. L'efficacia del trattamento è determinata attraverso la rimozione del colore, la riduzione della torbidità, la variazione della conducibilità e l'abbattimento della Domanda Chimica di Ossigeno (COD) e della Domanda Biologica di Ossigeno (BOD), in relazione ai limiti normativi e alle possibilità di riutilizzo dell'acqua.

Sulla base dei risultati ottenuti, viene proposto un progetto concettuale di un impianto pilota di coagulazione–flocculazione a base di chitosano, finalizzato a valutarne la scalabilità e l'integrazione in una linea produttiva industriale. Lo studio utilizza acque reflue reali provenienti da un impianto di stampa flessografica del Nord Italia, consentendo di validare la tecnologia in condizioni operative reali e di incrementare il livello di maturità tecnologica dal TRL 1 al TRL 7.

I risultati dimostrano che il chitosano fungino favorisce efficacemente la neutralizzazione delle cariche e la flocculazione per ponte in dispersioni di inchiostro altamente stabili, garantendo una significativa rimozione dei composti cromofori e del carico ionico. Lo studio contribuisce allo sviluppo di materiali bio-based per il trattamento delle acque reflue industriali e promuove strategie circolari di gestione delle acque volte a ridurre i rischi ambientali e sanitari legati ai contaminanti persistenti derivati dalla stampa.

Abstract

The global population is facing increasing challenges in securing access to clean water due to demographic growth, environmental pressures, and the expansion of industrial activities. Among the numerous chemicals discharged into aquatic environments, those associated with industrial inks represent a particularly problematic group of contaminants. Current estimates suggest that compounds derived from dyes and ink formulations account for approximately 10–15% of recalcitrant chemicals detected in water bodies. In this context, industrial wastewater generated by flexographic printing processes constitutes a complex and increasingly relevant environmental challenge. Such effluents are characterized by persistent pigments, polymeric binders, surfactants, and auxiliary chemicals, presenting high chemical heterogeneity, alkaline pH, colloidal stability, and intense coloration, all of which limit the effectiveness of conventional treatment technologies and hinder water reuse strategies. Consequently, the development of alternative treatment approaches capable of destabilizing and removing ink-derived contaminants has attracted growing scientific interest.

This study evaluates the use of chitosan as a bio-based flocculating agent for the destabilization of ink colloidal dispersions in industrial wastewater. Chitosan is a cationic polysaccharide obtained from chitin deacetylation and widely investigated for coagulation–flocculation processes. Differently from conventional crustacean-derived chitosan, the present work investigates fungal-derived chitosan, produced with lower chemical consumption and reduced environmental impact, as a circular and potential solution sustainable alternative feedstock.

The research combines physicochemical characterization and process optimization. Initially, the chemical composition and colloidal behaviour of flexographic inks in aqueous medium are analysed to identify dominant stabilization mechanisms. Subsequently, batch flocculation tests are performed to determine optimal operational parameters, including pH adjustment, polymer dosage, mixing regime (rapid and slow mixing), and sedimentation time. Treatment performance is quantified through colour removal efficiency, turbidity abatement, conductivity variation, and reduction of Chemical Oxygen Demand (COD) and Biological Oxygen Demand (BOD), with reference to regulatory discharge limits and potential water reuse scenarios.

Based on the optimized operating conditions, a conceptual design of a pilot treatment unit employing chitosan-based coagulation–flocculation is proposed in order to evaluate process scalability and integration within an industrial production line. The investigation is conducted using a real wastewater stream from a flexographic printing facility in Northern Italy, enabling validation beyond laboratory conditions and advancing the technology readiness level from TRL 1 (basic principal) to TRL 7 (system prototype demonstration in operational environment).

The results demonstrate that fungal-derived chitosan can effectively induce charge neutralization and bridging flocculation in highly stable ink dispersions, achieving significant removal of chromophore compounds and ionic load. The study contributes to the advancement of bio-based materials for industrial wastewater treatment and supports the implementation of potential circular water management strategies aimed at reducing environmental and health risks associated with persistent printing-derived contaminants.

Chapter 1 - Thesis Introduction

The problem of industrial wastewater pollution caused by inks and dyes has gained increasing environmental and public health relevance in recent years, emerging as one of the critical challenges in the sustainable management of water resources. This issue arises within a broader historical context characterized by significant anthropogenic and climatic pressures on the hydrological cycle, which reduce water availability and amplify the effects of contaminants present in aquatic systems. In this scenario, the chemical nature of both synthetic and natural dyes—specifically designed to be stable and resistant to degradation processes—contributes to their high persistence in the environment and makes them particularly difficult to remove through conventional treatment methods. Industrial inks, moreover, cannot be considered simple solutions composed of single compounds; rather, they represent complex matrices in which pigments, surfactants, and process additives interact with one another. These interactions increase the colloidal stability of the system and further complicate wastewater treatment strategies. Although these substances do not always formally fall within the category of emerging contaminants, many of the compounds present in such effluents exhibit characteristics of persistence, mobility, and potential ecotoxicological impact. For this reason, their investigation is increasingly necessary, particularly regarding their potential effects on human health and aquatic ecosystems.

Consequently, it becomes essential to critically examine the limitations of conventional wastewater treatment technologies, biological, physical, and chemical, which, despite representing a historical cornerstone in wastewater management, often show inefficiencies when dealing with these matrices. Such limitations include high energy requirements, the production of secondary waste streams, and limited adaptability to real industrial effluents, which are frequently far more complex than the model systems typically investigated in experimental studies. Due to these challenges, there is a growing interest in treatment technologies capable of combining effectiveness, operational simplicity, and reduced environmental impact, in line with the directions outlined by the United Nations Sustainable Development Goals, particularly SDG 6 (Clean Water and Sanitation), SDG 11 (Sustainable Cities and Communities), and SDG 12 (Responsible Consumption and Production). Within this framework, flocculation emerges as a particularly promising separation strategy for colloidal contaminants and chromophoric compounds, owing to its operational flexibility and the possibility of implementation with relatively low energy consumption.

Within the broader landscape of wastewater treatment technologies and innovation perspectives in the sector, increasing attention is therefore being devoted to the exploration of biocompatible and sustainable solutions, preferably derived from by-products of other production chains or already integrated within established industrial cycles. Among these materials, chitosan represents a particularly promising candidate. Widely studied in fields such as biomedicine and biomaterials, it is also gaining attention as a potential natural flocculant for wastewater treatment applications. Understanding its potential, limitations, and conditions of applicability, both in relation to its source of origin and to the requirements of sustainability and operational simplicity, therefore represents a crucial step toward the development of more effective water treatment strategies aligned with contemporary environmental challenges.

1.1. Industrial Water Pollution from Inks

Environmental degradation represents one of the most complex and pervasive consequences of contemporary industrial development, emerging as a global phenomenon affecting air, soil, and, most critically, water resources. Water, by its very nature, constitutes the primary compartment for the accumulation of anthropogenic impacts, as it directly or indirectly receives discharges originating from industrial, agricultural, and urban activities. Over recent decades, the increasing chemical complexity of industrial effluents has highlighted the limitations of traditional management and treatment systems, emphasizing the need to rethink wastewater treatment strategies from the perspective of environmental sustainability, technical effectiveness, and adaptability to real-world matrices.

The situation is even more complex considering the global challenges currently facing modern societies. Among these, climate change represents the most significant overarching framework, as it simultaneously affects the availability and quality of water resources. Projections related to desertification processes, which are expected to affect portions of the European continent as well, indicate a progressive reduction in available water resources (IPCC, 2022), with a consequent increase in chemical and pollutant concentrations due to the reduced dilution capacity of water bodies. Moreover, more than one and a half centuries of economic and industrial development, rapid expansion of petrochemical production chains and the release of synthetic chemical substances—often produced without a full understanding of their environmental consequences—has introduced different contaminants from those considered during the early decades of the twentieth century, when the field of early wastewater treatment processes began. In fact, this was the period in which the main wastewater treatment technologies were established, technologies that nowadays are partly inefficient and often unable to meet modern environmental expectations. Nevertheless, scientific and technological progress has enabled significant improvements in analytical techniques, expanding the range of monitored parameters and allowing the detection of substances that were previously undetectable. Consequently, water quality assessments have progressively incorporated new categories of contaminants and substances, many of which prove difficult to remove using conventional treatment technologies.

Within this general context, synthetic dyes emerge as one of the most problematic classes of contaminants. A foundational contribution on water pollution caused by dyes highlights that these compounds are intentionally designed to possess high chemical and structural stability to ensure resistance to light, oxidation, and washing processes, thereby making them highly recalcitrant in environmental compartments [1]. While such stability is advantageous from an industrial perspective, it results in marked environmental persistence, rendering dyes poorly biodegradable and difficult to remove totally through conventional biological and chemical treatments. The presence of complex aromatic systems and highly stable chromophoric bonds represents one of the main reasons for the recalcitrance of these contaminants in industrial wastewater.

The environmental persistence of these compounds shifts into significant impacts on aquatic systems. Considering that dyes are widely used in numerous production sectors—including textile, construction, paper, cosmetics, and food industries—their large-scale use results in widespread environmental release. It is estimated that approximately 10–15% of the dyes used in industrial processes are discharged directly into

wastewater collection systems [2]. This proportion varies depending on the process and the chemical class of dyes involved; however, in general, a significant fraction of the dyes employed is not fixed to substrates and is therefore released directly into wastewater streams.

From a quantitative perspective, the environmental relevance of dyes is further amplified by their global production volumes. A recent review focusing on sustainable treatment technologies for dye-contaminated wastewater reports that global production of synthetic dyes reaches orders of hundreds of thousands of tonnes per year, with frequently cited estimates around 700,000 tonnes annually [3]. This aspect becomes particularly relevant in printing and ink formulation processes, where fixation efficiency may be further reduced due to the complexity of the mixtures employed.

The growing relevance of inks as aquatic pollutants must also be analysed considering contemporary production and consumption models, particularly within the textile and printing industries. According to data provided by the European Parliament, textile consumption within the European Union has significantly increased in recent years, rising from 17 kg to 19 kg per person between 2019 and 2022 [4]. At the same time, approximately 12 kg of textile products per person are discarded annually, feeding increasingly rapid production cycles typical of the fast fashion paradigm. This increase in production volumes inevitably translates into an expansion of dyeing and printing processes and, consequently, into the generation of larger quantities of wastewater containing inks and dyes.

The same report from European Parliament highlights the considerable water footprint of the textile sector, estimating that the production of a single cotton T-shirt may require up to 2,700 L of freshwater. This situation becomes particularly significant when considered alongside scientific evidence identifying the textile sector as one of the major contributors to global freshwater pollution. In particular, the European Parliament estimates that approximately 20% of global clean water pollution can be attributed to dyeing and finishing processes, underlining the central role of dyes and inks in the pressure exerted on water resources.

Scientific literature reinforces this systemic perspective by also examining other markets. A review focusing on hybrid and nanostructured technologies for dye removal in China highlights that the textile and printing industries are among the most water-intensive sectors, with typical water consumption ranging between 100 and 200 L per kilogram of processed product [5]. The authors emphasize that, given such volumes, even modest improvements in treatment efficiency could translate into significant environmental benefits on a global scale. Consequently, original compounds and the by-products generated during physical or chemical treatment processes may be detected in surface water bodies, which often represent the final receiving environments for industrial discharges. At the same time, leaching and soil infiltration processes may facilitate the migration of these substances toward groundwater systems, contributing to the wider spread of contamination [3].

The impact of dyes on aquatic ecosystems is not limited to their chemical presence but also manifests through significant alterations in the physical properties of water bodies. Even relatively low concentrations may drastically reduce water transparency, reducing solar radiation penetration and compromising the photosynthetic processes of algal and aquatic plant communities, thereby altering primary production and the balance of aquatic ecosystems [6]. This reduction in primary photosynthesis may trigger ripple effects

throughout the trophic network, affecting dissolved oxygen availability and altering the structure of biological communities by influencing both chemical and biological oxygen demand.

Scientific literature consistently highlights the toxicological potential of dyes. A systematic review on the ecotoxicological impacts of synthetic dyes reports numerous examples of acute and chronic toxicity in aquatic organisms, as well as the possibility of genotoxic and mutagenic effects associated to by-products [7]. These effects may lead to physical damage in species inhabiting aquatic ecosystems, enzymatic alterations, sterility, and larval mortality, potentially accelerating extinction processes in certain species. In particular, for azo dyes—widely used in ink formulations—the literature highlights the risk of forming aromatic amines under reducing conditions, chlorinated phenols, and other persistent metabolites, compounds known for their carcinogenic potential. This aspect contributes to defining dyes not merely as aesthetic contaminants but as true priority chemical pollutants.

The issue becomes even more complex when considering industrial inks within real wastewater matrices, depending on the production context. As previously mentioned, inks are complex mixtures that include surfactants, solvents, salts, rheological additives, and other process auxiliaries. Such chemical complexity is one of the main reasons why results obtained from simplified synthetic solutions are often not directly transferable to real industrial wastewater matrices. A review dedicated to textile and printing wastewater highlights that the presence of surfactants and other auxiliary substances contributes to increase chemical oxygen demand (COD) and biological oxygen demand (BOD), as well as to strong stabilization of colloidal dispersions, making dye removal even more difficult. This situation directly affects oxygen availability within aquatic ecosystems and as previously discussed, may create anoxic conditions that limit the proliferation of aquatic organisms. When wastewater is not properly managed, dyes may infiltrate soils and reach groundwater systems where, depending on the depth of the water table, similar environmental conditions may arise. In fact, regarding soils, change context but the outcome remains similar: chemical substances contained in dyes may influence chemical and biological oxygen demand over the long term, altering natural processes within the soil microbiome, including the carbon and nitrogen cycles [8]. This occurs due to inhibitory effects exerted by substances commonly present in inks, often including biocides, which directly affect nitrifying bacteria and decomposer fungi, with negative consequences also for soil fertility (Fig. 1.1).

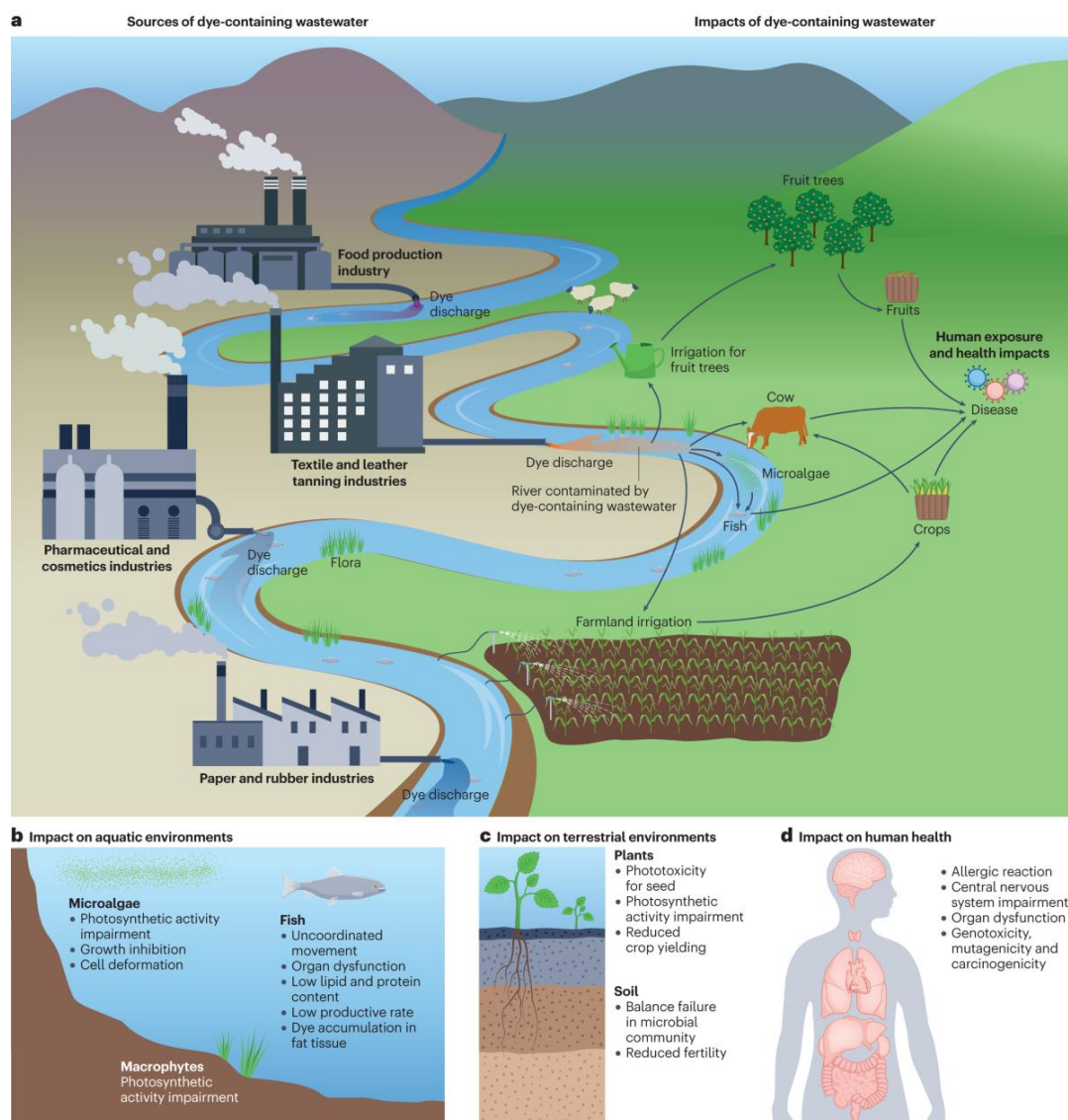


Figure 1.1 Conceptual representation of dye and ink pollution pathways from industrial activities (a) to aquatic (b), terrestrial ecosystems (c) and impact on human health (d) [9].

The environmental impact of dyes in the atmosphere needs further investigation. In 2023, the European Commission's Joint Research Centre published a study on textile production and dye synthesis processes, highlighting how these industrial chains contribute to atmospheric emissions of toxic gases, chemical vapors, and particulate matter. These include volatile organic compounds (VOCs) and formaldehyde, associated with carcinogenic mutations, as well as dust particles that mix with atmospheric particulate matter such as PM_{2.5} and PM₁₀, thereby affecting respiratory health. Due to this, the European Commission has introduced increasingly stringent regulations from 2023 onward concerning these industrial sectors and their environmental emissions. According to a study conducted by a policy think tank in 2025 on atmospheric pollutants from the textile sector [10], the vast geographical extent of these industrial activities suggests a widespread atmospheric dispersion and deposition of such substances across aquatic and terrestrial ecosystems, contributing to diffuse environmental contamination and deterioration of environmental quality.

Despite technological advances in wastewater treatment, numerous studies agree that the removal of dyes and inks remains an open challenge. A review focusing on the limitations of conventional technologies highlights that biological treatments are often ineffective against highly stable chromophoric molecules, while advanced physical and chemical approaches, although capable of achieving high removal efficiencies, are frequently associated with high operational costs, the generation of secondary sludge, and broader sustainability concerns [11]. These limitations have stimulated growing interest in alternative solutions capable of combining treatment effectiveness, operational simplicity, and reduced environmental impact.

Because of these considerations, industrial inks can be considered emerging pollutants within the context of industrial wastewater. They are not necessarily new contaminants, but substances whose environmental relevance has significantly increased in recent years due to intensified production processes, the increasing complexity of industrial matrices, and the limitations of existing treatment technologies. Understanding the environmental behaviour of inks and developing potential sustainable strategies for their removal from wastewater therefore represents a scientific and technological priority and forms the conceptual foundation of the research presented in this thesis.

1.2. Limitations of Conventional Wastewater Treatment Technologies

The treatment of industrial wastewater represents one of the most complex technological challenges within the framework of sustainable water resource management, particularly considering the increasing heterogeneity and chemical complexity of effluents generated by modern industrial processes. Current wastewater treatment technologies were historically developed in a context where wastewater matrices were characterized by relatively simple compositions dominated by easily biodegradable organic loads. However, the evolution of industrial production chains—particularly in sectors such as textiles, printing, and fine chemicals—has introduced a wide range of persistent contaminants into wastewater streams, often present in complex mixtures that challenge the effectiveness of conventional treatment technologies.

In general, wastewater treatment systems rely on a combination of biological, physical, and chemical processes, selected according to the characteristics of the effluent and the quality objectives for the final discharge (Fig. 1.2).

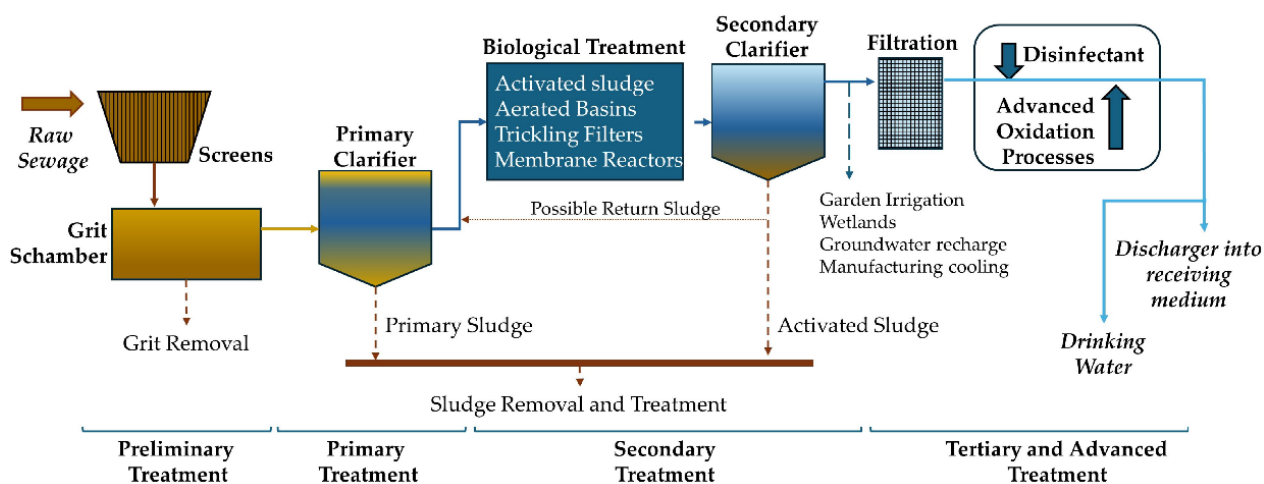


Figure 1.2. Overview of conventional wastewater treatment technologies used for dye and ink removal [12].

Despite the significant technological progress achieved in recent decades, numerous review studies agree that none of the conventional technologies is universally effective in ensuring the complete removal of emerging contaminants, particularly dyes, inks, and chemically stable organic compounds [13]. Biological treatments, which constitute the core of most industrial and municipal wastewater treatment plants, are designed to exploit the metabolic activity of microbial communities in order to degrade organic matter. However, numerous studies have demonstrated that these processes are intrinsically limited when dealing with molecules characterized by complex aromatic structures, high chemical stability, and low biodegradability. Synthetic dyes and many components of industrial inks fall within this category, exhibiting significant resistance to biological degradation and, in some cases, inhibitory effects on microbial activity [14]. Therefore, biological treatments tend to transfer or concentrate contaminants into the solid phase (sludge) rather than achieving their complete mineralization.

The situation is further complicated by the fact that the composition of modern industrial wastewater can profoundly influence the structure and functionality of microbial communities. Studies conducted on wastewater treatment plants receiving effluents from dyeing and printing processes have shown that the simultaneous presence of dyes, chemical additives, salts, and microplastics may alter biodegradation mechanisms and promote the formation of secondary contaminants or inconvenient by-products [15]. In this context, biological treatment, although remaining a fundamental technology, is often insufficient as a stand-alone purification strategy for complex effluents.

Beside biological processes, physical treatments represent another key component of conventional wastewater treatment systems. Among these, adsorption, filtration, and membrane separation processes are widely used for the removal of suspended solids, colloids, and dissolved contaminants. Adsorption is often cited as one of the most effective technologies for the removal of dyes and organic compounds, due to its operational simplicity and the possibility of achieving high removal efficiencies [13]. However, numerous studies emphasize that the effectiveness of adsorption strongly depends on the characteristics of the adsorbent material, the concentration of pollutants, and the overall composition of the wastewater matrix.

The use of conventional materials such as activated carbon is often limited by high costs, regeneration issues, and the production of secondary waste streams. Furthermore, in complex matrices, competition between different chemical species for adsorption sites may significantly reduce process efficiency [16]. This aspect is particularly relevant for wastewater containing inks, where dyes, surfactants, and additives may interact synergistically, hindering the selective removal of target contaminants.

Membrane technologies, including ultrafiltration and nanofiltration, are often considered advanced solutions for the removal of dyes and dissolved organic compounds. Although these processes can guarantee high separation efficiencies, their large-scale application is frequently limited by issues such as membrane fouling, high energy consumption, and significant maintenance costs [17]. Moreover, membrane systems do not eliminate contaminants but rather concentrate them in a reject stream that requires further treatment, effectively transferring the problem from one phase to another.

Chemical processes, including advanced oxidation processes, coagulation, and flocculation, are widely employed as primary or secondary treatments for the removal of dyes and inks. Coagulation–flocculation represents a well-established technology in the textile and printing sectors due to its operational simplicity and relatively low costs. However, several studies have highlighted that this process is mainly effective for the removal of high-molecular-weight dyes and presents significant limitations when dealing with highly soluble or low-molecular-weight species [17]. Another important limitation is the production of large quantities of chemical sludge, whose management and disposal involve considerable environmental and economic costs. Advanced oxidation processes, instead, based on the generation of highly reactive species such as hydroxyl radicals, are often proposed as solutions capable of overcoming the limitations of conventional treatments. Nevertheless, the scientific literature points out that these technologies are characterized by high operational costs, complex plant configurations, and, in some cases, the formation of potentially toxic by-products [13]. These aspects limit their applicability in continuous industrial-scale operations, particularly in contexts characterized by large volumes of wastewater and strong variability in composition.

Another critical element, often overlooked, concerns the ability of conventional treatments to manage multiple contaminants simultaneously. Studies conducted on wastewater treatment plants receiving effluents from dyeing and printing processes have shown that traditional treatment systems may be particularly ineffective in removing associated contaminants such as microplastics and chemical additives, which can act as carriers for dyes and other hazardous substances [18]. In some cases, treatment processes may even promote the fragmentation of larger particles into smaller fractions that are potentially more mobile in the environment.

Overall, the scientific literature converges on the idea that conventional wastewater treatment technologies, although representing a fundamental pillar in wastewater management, exhibit significant structural limitations when applied to complex industrial effluents rich in emerging contaminants. These limitations are not only attributable to technological inefficiencies but reflect a deeper mismatch between the characteristics of modern wastewater and the principles underlying technologies developed during the last century. This scenario highlights the need to explore alternative or complementary treatment strategies capable of combining effectiveness, sustainability, and adaptability to real wastewater matrices.

Due to these structural limitations, it is increasingly evident that overcoming the challenges associated with conventional wastewater treatment cannot rely only on incremental optimization of existing technologies but requires a broader rethinking of treatment strategies in line with international and European environmental sustainability priorities. In this context, the framework outlined by the United Nations Sustainable Development Goals—particularly those related to sustainable water management (SDG 6), sustainable cities and communities (SDG 11), responsible consumption and production (SDG 12) (Fig.1.3)—provides a clear reference for the evolution of wastewater treatment technologies, directing them toward solutions that combine effectiveness, environmental impact reduction, and process circularity. Similarly, European policies on ecological transition, circular economy, and water resource protection emphasize the need to develop treatment approaches capable of reducing the use of hazardous chemicals, limiting the generation of secondary waste, and improving overall process efficiency, while promoting the use of sustainable materials and the integration of low-impact technologies. Within this framework, research attention is progressively shifting toward innovative solutions that complement conventional treatments and can address the increasing complexity of industrial wastewater while adapting to the needs of a production system oriented toward environmental, economic, and social sustainability.



Figure 1.3. *Sustainable Development Goals in line with this work.*

1.3. Flocculation as a Pollutant Removal Strategy

Within the framework outlined in the previous chapters, it clearly emerges that industrial wastewater management is currently undergoing a critical transition phase. On the one hand, the increasing complexity of wastewater matrices—driven by the evolution of industrial production chains and the introduction of increasingly specialized chemical substances—has highlighted the structural limitations of conventional treatment technologies. On the other hand, regulatory, environmental, and social pressures require technological solutions capable of achieving high levels of efficiency without generating additional environmental impacts or simply transferring pollutants from one phase of the process to another. In this context, flocculation can be considered not only a well-established technology but also a conceptually robust strategy for addressing some of the most significant challenges associated with the treatment of complex industrial wastewater.

The central role of flocculation in water treatment lies in its ability to directly target the colloidal nature of a wide range of pollutants. As widely documented in the literature, many of the substances responsible for the high turbidity, colour, and suspension stability observed in industrial wastewater are neither fully dissolved nor easily separable through simple sedimentation. Instead, they exist in an intermediate state stabilized by surface charges and electrostatic interactions [19]. This condition, particularly common in wastewater containing inks, dyes, and chemical additives, represents one of the main obstacles to the effectiveness of traditional biological and physical treatment processes.

From a mechanistic perspective, flocculation overcomes this obstacle through a series of synergistic processes, including surface charge neutralization, compression of the electrical double layer, and the formation of bridges between particles mediated by flocculating agents. These mechanisms promote the aggregation of colloidal particles into progressively larger structures, transforming a stable suspension into a system that can be easily separated [20]. In this sense, flocculation operates at a more structural level compared with other treatment technologies, acting on the physical organization and aggregation state of pollutants rather than on their chemical transformation.

From a physicochemical point of view, flocculation is a separation process based on the destabilization and aggregation of colloidal particles suspended in an aqueous medium. These particles, which may include finely dispersed solids, pigments, dyes, and organic complexes, are generally stabilized by surface charges and electrostatic interactions that prevent spontaneous aggregation. The addition of a flocculant destabilizes the suspension through mechanisms such as charge neutralization, compression of the electrical double layer, and particle bridging, leading to the formation of larger aggregates known as *flocs* (Fig. 1.4). These flocs can then be readily separated through sedimentation, flotation, or filtration. Flocculation therefore lies at the interface between surface chemistry and transport phenomena, making it a particularly effective process for the treatment of complex matrices in which pollutants are present in colloidal or pseudo-colloidal form.

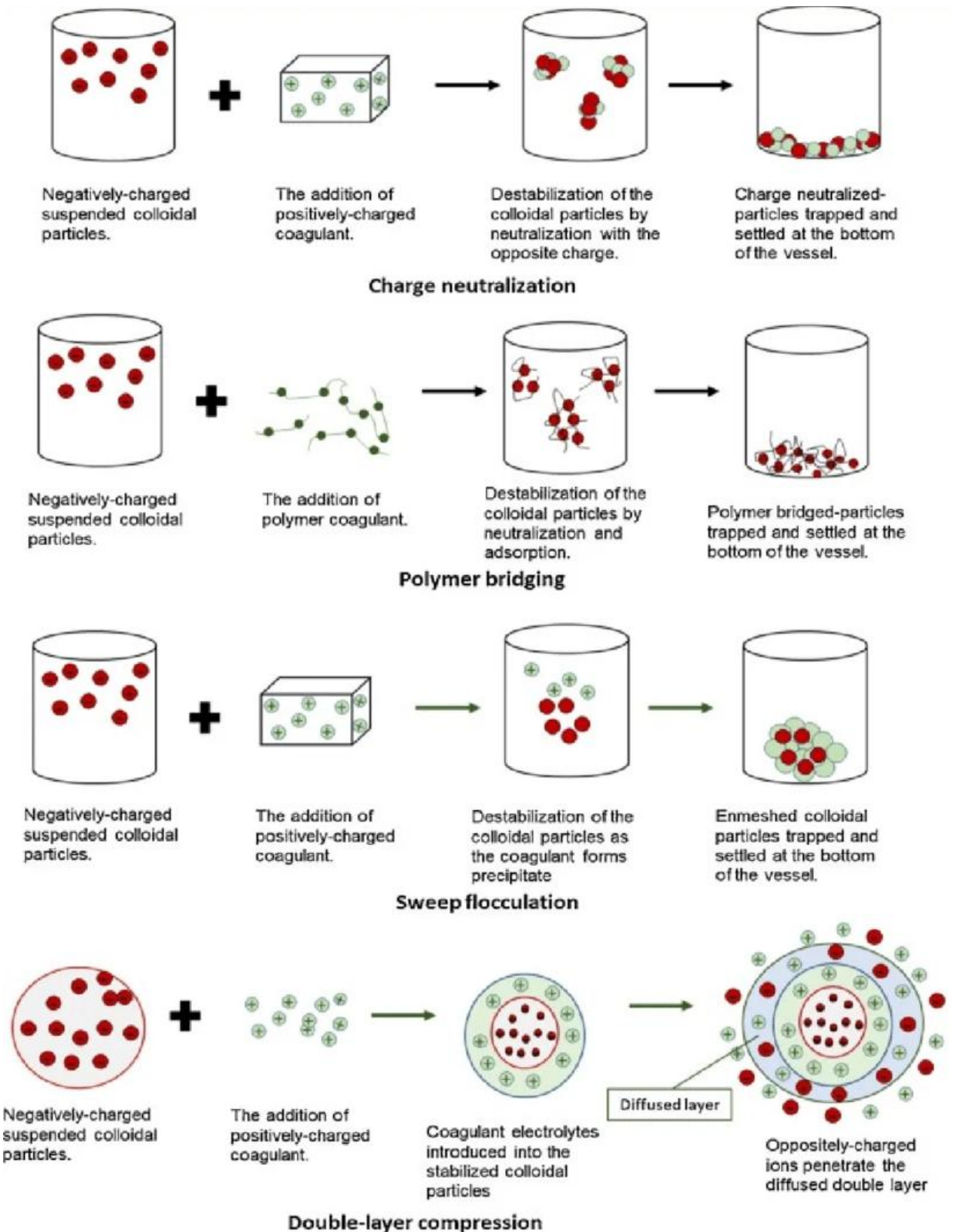


Figure 1.4. Schematic representation of the flocculation process, illustrating the destabilization of colloidal particles through charge neutralization, compression of the electrical double layer, and polymer bridging, leading to the formation of larger aggregates (flocs) that can be removed by sedimentation, flotation, or filtration [21].

An aspect of particular interest, especially in relation to complex industrial wastewater, is the operational versatility of flocculation. Unlike highly selective technologies that require detailed knowledge of the specific chemical species present, flocculation relies on general physicochemical principles that can be applied to a broad range of contaminants. This feature is particularly advantageous in the treatment of wastewater generated from the production and use of industrial inks, where the composition of the effluent may vary significantly depending on formulations, substrates, and operational conditions. Several studies have shown that flocculation can maintain good performance even under conditions of strong variation in pH, conductivity, and organic load, making it an inherently adaptable technology for real industrial applications [22].

From an application perspective, the literature reports numerous examples of flocculation being used either as a primary treatment or as a pretreatment stage aimed at reducing the pollutant load entering subsequent processes. For coloured wastewater, flocculation is often identified as one of the most effective technologies for the removal of colour and suspended solids, with removal efficiencies that can exceed 90% under optimized operational conditions [23]. This ability to achieve rapid and substantial pollutant reduction makes flocculation a fundamental tool for improving the overall efficiency of treatment systems while simultaneously reducing the energy and operational costs associated with more advanced processes. Another aspect that contributes to the relevance of flocculation is its compatibility with sustainability objectives defined at international and European levels. The process is generally characterized by low energy consumption, relatively simple plant configurations, and the possibility of being easily integrated into existing treatment systems. These characteristics are consistent with the strategies promoted by the Sustainable Development Goals, particularly those related to sustainable water resource management and the reduction of the environmental impact of industrial processes. In a context where European policies encourage scalable, low-impact technological solutions oriented toward circular economy principles, flocculation represents a mature yet highly relevant technology.

In the treatment of industrial wastewater, flocculation can be achieved using a wide range of materials that differ in origin, chemical nature, and mechanism of action, reflecting the historical and technological evolution of purification strategies. Flocculants traditionally employed in industrial applications can generally be classified into three main categories: inorganic flocculants, synthetic polymers, and materials of natural origin. Inorganic flocculants, such as aluminum and iron salts, represent the most widely used and established solution at the industrial scale due to their effectiveness, relatively low cost, and operational simplicity. These compounds mainly act through mechanisms of charge neutralization and the formation of amorphous metal hydroxides, which promote the aggregation of colloidal particles. However, as widely discussed in the literature, their use is associated with several important drawbacks, including the generation of large quantities of chemical sludge, the possible presence of residual metals in treated effluents, and a strong sensitivity to operational conditions, particularly the pH of the wastewater [24]. At the same time, inorganic flocculants, synthetic polymers and natural materials have gained increasing importance as alternative or complementary flocculating agents. Synthetic polymeric flocculants, such as polyacrylamides and their derivatives, are widely used because of their high efficiency even at low dosages and their ability to promote polymer bridging

between particles, enhancing floc formation and sedimentation. However, their limited biodegradability and the potential environmental and health concerns associated with residual monomers have stimulated growing interest in flocculants derived from natural sources (the details and an in-depth analysis about these materials will be discussed in the next chapters). Anyway, several studies and reviews highlight the potential of natural and bio-derived materials, such as clays, plant biomasses, agricultural by-products, and biopolymers. These materials offer the advantage of greater environmental compatibility while maintaining good removal performance [25]. Among these, chitosan is frequently cited as one of the most promising natural flocculants due to its polycationic nature and its ability to effectively interact with a wide range of contaminants, including dyes and pigments [26].

In recent years, increasing attention has been devoted to the development of innovative flocculants as alternatives to traditional metal salts. This trend is largely driven by the need to reduce the production of chemical sludge and to limit the introduction of additional potentially problematic substances into the treatment cycle. Within this scenario, natural polymeric flocculants have gained significant attention due to their biodegradability, low toxicity, and availability from renewable resources [27]. Chitosan is widely recognized in the literature as one of the most promising materials, owing to its polysaccharide structure and the presence of protonable amino groups that confer a strong ability to interact with negatively charged species, such as many dyes and pigments.

Recent literature, however, tends to explore the use of chitosan mainly in the form of composite or chemically modified materials, with the aim of improving flocculation performance or extending its applicability to specific classes of contaminants [28]. Although these approaches have demonstrated interesting results under laboratory conditions, they often introduce additional complexity in material formulation and in the assessment of their overall environmental impact. This aspect becomes particularly relevant in the treatment of wastewater already characterized by complex chemical compositions, such as those containing industrial inks, where the addition of further additives or modifying agents may increase system complexity rather than reduce it.

In this context, growing interest is emerging toward flocculation strategies based on the use of materials that are as simple as possible, capable of combining effectiveness and sustainability without requiring complex structural modifications or the addition of multiple coagulant aids. The direct use of natural polymers in their essential form may represent a coherent approach with the objective of reducing the overall environmental impact of the treatment process, minimizing the introduction of additional chemicals and simplifying the management of generated by-products.

Despite the widespread use of flocculation and the extensive body of literature available on the topic, several significant knowledge gaps remain. Systematic studies conducted on real and complex industrial wastewater are still limited, especially those evaluating flocculation not only in terms of pollutant removal efficiency but also in relation to the overall impact on the treatment system and on the composition of the generated sludge. Furthermore, most studies focus on model systems or on single contaminants, leaving the applicability of the process to multicomponent matrices—such as those derived from industrial inks—only partially explored. These aspects define an open research space that justifies the approach adopted in the present work.

1.4. Chitosan as a Natural Flocculant: Potential and Limitations

Within the framework of flocculation strategies oriented toward environmental sustainability, chitosan has emerged as one of the most extensively studied natural materials in the literature for wastewater treatment applications. The growing interest in this biopolymer arises from a combination of chemical, environmental, and technological factors that make it a particularly attractive candidate compared to conventional flocculants of inorganic or synthetic origin. As widely reported in numerous reviews, chitosan is a linear polysaccharide obtained through the deacetylation of chitin and is primarily composed of repeating units of D-glucosamine and N-acetyl-D-glucosamine (Fig. 1.5). The distribution and proportion of these units determine key material properties such as the degree of deacetylation, molecular weight, and solubility [29], [30].

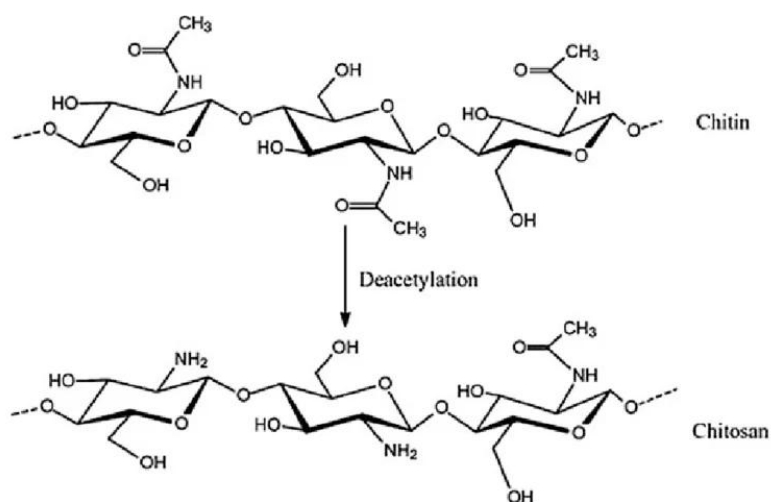


Figure 1.5. Representation of the chemical conversion of chitin into chitosan through the deacetylation process. During deacetylation, acetyl groups ($-\text{COCH}_3$) are removed from the N-acetyl-D-glucosamine units of chitin, generating D-glucosamine units with free amino groups ($-\text{NH}_2$). This structural modification confers chitosan its polycationic character in acidic aqueous environments, which is responsible for its effectiveness as a flocculating agent in wastewater treatment [31]

From a physicochemical perspective, the presence of primary amine groups along the polymer chain confers a polycationic nature to chitosan in acidic aqueous environments, making it particularly effective in interacting with negatively charged species. This property underlies its ability to act as a flocculant through combined mechanisms of charge neutralization and polymer bridging, thereby promoting the aggregation of colloidal particles, dyes, and pigments present in industrial wastewater [32]. Several experimental studies and reviews emphasize that these mechanisms make chitosan particularly suitable for the treatment of complex matrices in which pollutants are not present as isolated molecules but rather as stabilized colloidal systems, as is typically the case in wastewater containing inks and dyes [33].

Historically, the production and commercialization of chitosan have been dominated almost exclusively by marine-derived sources, particularly the shells of crustaceans such as shrimp, crabs, and lobsters. This production chain developed within the framework of valorizing by-products from the seafood industry, allowing solid waste to be transformed into a high-value-added resource. Numerous reviews highlight that crustacean-derived chitosan still represents the primary commercial source worldwide due to the relatively high availability of raw material and the maturity of industrial extraction processes ([34].

However, a more detailed analysis of production processes reveals several environmental and technological limitations associated with crustacean-derived chitosan. Traditional extraction procedures involve intensive chemical treatments, including demineralization using strong acids to remove calcium carbonate, deproteinization with concentrated alkaline solutions, and subsequent deacetylation at high temperatures.

Recent life cycle assessment (LCA) studies indicate that these processes are characterized by high energy consumption, significant use of chemical reagents, and the generation of secondary effluents that require further treatment [35]. Although several LCA studies on marine-derived chitosan production have reported, in some cases, an overall environmental balance, these findings partially contrast with the objective of developing wastewater treatment solutions that are genuinely and systemically sustainable. Impact assessments often focus on individual process stages without fully considering issues related to the long-term availability and sustainability of the raw material itself. Moreover, it is known nowadays, brought to wider public attention through documentaries and awareness campaigns promoted by activists and international organizations, that the fishing sector is under increasing pressure, with significant consequences for marine ecosystems. Intensive fishing and the expansion of aquaculture over recent decades have contributed to considerable environmental stress, raising questions about the true compatibility of such supply chains with production models oriented toward environmental sustainability and the protection of natural resources.

Beyond the environmental impact of extraction processes, the literature also highlights additional limitations related to the intrinsic variability of marine-derived raw materials. The composition of crustacean shells can vary significantly depending on species, geographic origin, and seasonality, directly influencing the properties of the resulting chitosan, including its degree of deacetylation, molecular weight, and polymer chain distribution [36]. Such variability may lead to lower reproducibility in the performance of the material as a flocculant, sometimes requiring additional purification or chemical modification steps to obtain products with controlled characteristics.

In recent years, in response to these limitations, the scientific community has increasingly explored alternative sources of chitosan, particularly those of fungal origin (fig. 1.7). Several studies and reviews highlight that chitosan naturally occurs in the cell walls of numerous fungal species, thereby eliminating the need for complete conversion of chitin into chitosan through aggressive chemical treatments [37]. Unlike crustaceans, fungi do not contain large amounts of minerals such as calcium carbonate, making the demineralization step unnecessary and allowing for a significant reduction in the use of strong acids and the generation of liquid waste streams (fig. 1.6).

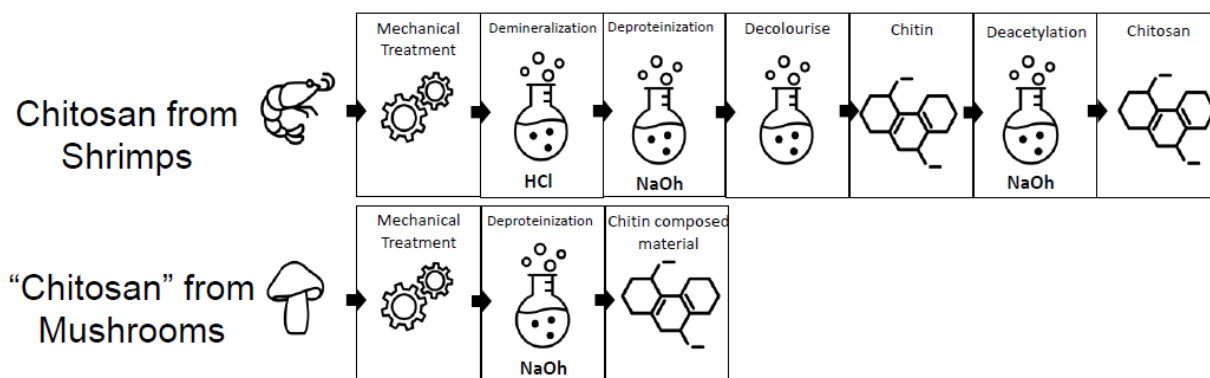


Figure 1.6. Comparison between conventional chitosan production from crustacean shells and chitosan extraction from fungal biomass. In crustaceans (e.g., shrimp shells), chitosan is obtained through multiple chemical steps including demineralization with acid (HCl), deproteinization with alkaline solutions (NaOH), and subsequent deacetylation of chitin. In contrast, fungal biomass contains chitin complexes within the cell wall, allowing the extraction of chitosan with fewer processing steps and reduced chemical treatment.

From a production point of view, fungal-derived chitosan also offers significant advantages in terms of process controllability. Fungal cultivation can be carried out under controlled fermentation conditions or in greenhouse systems, enabling modulation of parameters such as pH, temperature, and growth substrate. This level of control allows for more precise regulation of the physicochemical properties of the resulting chitosan [38]. Some studies have demonstrated that fungal chitosan can be produced with well-defined characteristics, including a more uniform degree of deacetylation and lower polydispersity compared to materials obtained from marine sources [39].

Another important aspect concerns the safety and applicability of fungal chitosan. Unlike crustacean-derived chitosan, fungal chitosan is intrinsically free of allergenic animal protein residues, which broadens its potential application range and reduces risks associated with use in sensitive contexts. This feature is frequently cited in the literature as a significant advantage, particularly from the perspective of social sustainability and regulatory acceptance (Sources, Production and Commercial Applications of Fungal Chitosan, 2022).

Despite these promising characteristics, the literature agrees that fungal chitosan remains less widespread at the industrial level compared to its crustacean-derived counterpart. The main limitations are related to currently higher production costs and the lower maturity of dedicated industrial supply chains. Nevertheless, recent studies suggest that optimization of fermentation processes and the integration of fungal chitosan production into circular economy models could significantly reduce this gap in the medium term [40].

Furthermore, the growing scientific and industrial interest in chitosan and its potential applications has encouraged the development of integrated production chains in which microbial biomass—often of fungal origin—used for the production of organic acids or bases through fermentation processes is subsequently valorized as a source of chitosan. In such systems, the residual biomass is not considered a waste by-product but rather a secondary resource, introducing an additional material recovery stage within the production chain.

Although in these cases chitosan generally still requires chemical treatments for extraction and purification, the literature highlights that the quantities of reagents required are significantly lower than those needed for extraction from crustacean biomass. This approach therefore contributes to reducing the overall environmental impact of the process, strengthening the potential of fungal-derived chitosan as a material consistent with principles of sustainability and resource valorization.

Within the specific context of industrial wastewater treatment, and particularly for complex effluents containing inks and dyes, chitosan therefore represents a natural flocculant of considerable interest, not only for its physicochemical performance but also for its potential to reduce the overall environmental impact of treatment processes. Within this framework, the systematic exploration of fungal-derived chitosan used in its simplest form—without resorting to complex chemical modifications or composite materials—represents a relevant element of innovation. This approach makes it possible to simultaneously address challenges related to the complexity of wastewater matrices and those associated with the sustainability of treatment technologies, thereby establishing the conceptual foundation for the experimental work developed in the following chapters of this thesis.

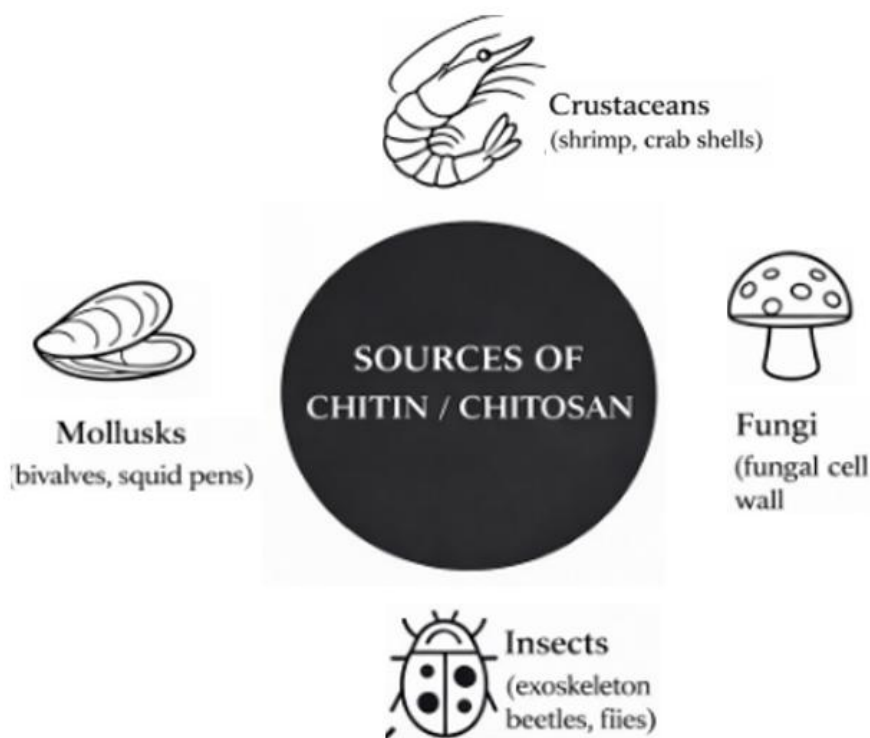


Figure 1.7. Main biological sources of chitin and chitosan, including crustaceans, fungi, insects, and mollusks. These biomasses represent renewable raw materials for the extraction of chitin and its derivative chitosan.

1.5. Thesis Objectives and Structure of the Work

Considering the framework outlined in the previous sections, it clearly emerges that the treatment of industrial wastewater containing inks represents a complex challenge, requiring solutions capable of combining removal efficiency, environmental sustainability, and operational simplicity. The critical analysis of the literature has highlighted, on the one hand, the structural limitations of conventional wastewater treatment technologies, which are often inadequate for dealing with multicomponent matrices characterized by colloidal contaminants, persistent dyes, and chemical additives; and, on the other hand, the growing interest in alternative strategies, including flocculation using naturally derived materials, as a promising approach for the treatment of such effluents. Within this context, chitosan stands out as a natural flocculant of particular interest due to its intrinsic physicochemical properties, which are widely documented in the literature. The ability of chitosan to act as a polycationic polymer in acidic aqueous environments, through the protonation of its amino groups, enables effective interaction with negatively charged species, which are typical of the dyes and pigments present in industrial inks. Numerous studies have shown that aqueous chitosan solutions, when properly prepared and dosed according to pH, concentration, and polymer molecular weight, can ensure high efficiencies in the removal of color and suspended solids, making this material a suitable candidate for wastewater treatment applications.

However, despite the extensive literature devoted to chitosan as an adsorbent or flocculant, a critical analysis of the available studies reveals several significant gaps. In particular, a large proportion of the research focuses on model systems, often consisting of synthetic solutions of single dyes, and on modified or composite materials developed to maximize removal performance. By contrast, systematic studies evaluating the use of simple chitosan solutions, employed in their essential form and without the addition of further additives, for the treatment of real and complex effluents—such as those deriving from the industrial ink production chain—remain limited. Even less frequently, such studies explicitly address the possibility of obtaining a treated effluent suitable for reuse, whether in sewer systems or, prospectively, as water intended for stormwater networks or secondary industrial uses.

So that, the main objective of this thesis is to evaluate the technical and environmental feasibility of using a chitosan solution as a flocculant for the treatment of wastewater contaminated by inks, derived from a real case study concerning a company located in the Brescia area and operating in the field of flexographic printing on cardboard. The wastewater generated by this company appears as a slightly viscous fluid, black to dark blue, and characterized by a heterogeneous chemical composition. More specifically, this work aims to demonstrate that a chitosan-based treatment system, properly characterized in terms of concentration, acidity, and operational conditions, may represent an effective and potentially sustainable solution for pollutant removal, while at the same time reducing process complexity and limiting the introduction of additional chemicals into the treatment cycle.

A further objective is to explore the potential of fungal-derived chitosan, considered a lower-impact alternative to chitosan traditionally obtained from crustaceans, in accordance with the principles of circular economy and environmental footprint reduction. In addition, the thesis aims to develop a prototype treatment system

articulated in successive stages, starting from laboratory-scale tests in which the relationship between the volume of chitosan solution and the volume of wastewater is evaluated, in order to enable subsequent process scalability. This approach seeks to transfer laboratory results toward larger operational volumes, thereby laying the foundation for possible pre-industrial implementation and for a market-oriented pathway of applied valorization.

The thesis is structured to progressively guide the reader from the theoretical and critical framework to the experimental application, culminating in the realization and installation of a pilot test system in situ at one of the facilities of the company involved in the real case study, where the industrial wastewater under investigation is generated.

Chapter thesis is organized as it follows:

- **Chapter 1** provides a general overview of the problem of industrial water pollution caused by inks, highlighting the limitations of conventional treatment technologies and introducing flocculation and chitosan as strategies and materials of interest.
- **Chapter 2** is devoted to an in-depth analysis of the physicochemical characteristics of chitosan, with particular attention to the differences among various sourcing routes and to the parameters influencing its behavior in solution. It also aims to provide a critical overview of the current state of the art and state of the technique of this approach within the field of wastewater treatment.
- **Chapter 3** describes the materials and experimental methods adopted in the study, including the characterization of the real wastewater under investigation and the operational conditions of the flocculation process.
- **Chapter 4** presents the experimental results and their discussion, including a critical analysis of system performance in terms of pollutant removal and treated effluent quality.
- **Chapter 5** summarizes the main conclusions of the work, highlighting the implications of the obtained results and outlining possible future developments of the research.
- **Chapter 6** discusses the limitations of the work and the residual limitations of the developed process, highlighting the main constraints identified during the experimental campaign and outlining possible future research and development perspectives.

Chapter 2 — State of the Art

A recent bibliometric analysis of global research on coagulation–flocculation processes for wastewater treatment has highlighted how this field currently represents one of the most dynamic areas of environmental research, with more than 8,700 publications produced between 2000 and 2023.

The study shows that the sector is strongly interdisciplinary, primarily involving environmental sciences, chemical engineering, and environmental engineering, reflecting the applied and technological nature of these processes. The analysis of keywords indicates that the most recurrent terms in the literature are coagulation, flocculation, and wastewater treatment, followed by concepts related to process mechanisms such as charge neutralization, zeta potential, adsorption, and jar test. This confirms that research is increasingly focused on understanding colloidal mechanisms and on the operational optimization of treatment processes.

At the same time, a growing interest in sustainable approaches and alternative materials has emerged. Among the keywords identified are chitosan, bioflocculant, and natural coagulants, indicating a progressive shift in research toward solutions with lower environmental impact. The study also highlights several emerging trends shaping the field, including the development of magnetic coagulants, integration with biological and membrane technologies, the application of artificial intelligence for process parameter optimization, and increasing attention to emerging contaminants such as microplastics, pharmaceutical compounds, and industrial dyes.

Overall, this evolution confirms that coagulation–flocculation is no longer considered a static conventional technology, but rather a field undergoing rapid innovation, in which research is increasingly oriented toward efficiency, sustainability, and integration with novel materials and treatment processes [41].

2.1. Industrial inks: chemical composition and environmental impact

Industrial inks represent one of the most complex categories of chemical formulations used in contemporary manufacturing processes, as they are designed to simultaneously satisfy extremely demanding aesthetic, functional, and technological requirements. Unlike many other industrial matrices, inks must ensure high color intensity, long-term stability, selective adhesion to heterogeneous substrates, and compatibility with high-speed printing systems. This multiplicity of functions is inevitably reflected in their chemical composition.

The increasing diffusion of water-based flexographic inks in the industrial printing sector, particularly for cardboard and packaging, has led to the generation of wastewater characterized by a complex and multiphase chemical composition. Formulations commonly employed in the Italian industrial context typically consist of an alkaline aqueous matrix stabilized by light amines such as ammonia and ethanolamine, containing film-forming acrylic or vinyl resins, finely dispersed organic and inorganic pigments—sometimes metal-based—glycol co-solvents, non-ionic surfactants, and biocidal preservatives.

The presence of significant amounts of insoluble pigments, stabilizing additives, and, in some cases, metals such as copper and zinc contributes to the formation of dark, slightly viscous, and chemically heterogeneous effluents characterized by high colloidal stability and potential environmental toxicity. This compositional complexity clearly indicates that the treatment of these matrices cannot be addressed through simplified approaches but rather requires a thorough understanding of the physicochemical mechanisms governing their behaviour in aqueous environments.

From a formulation perspective, the literature consistently describes inks as complex mixtures composed of pigments or dyes, polymeric binders, solvents or aqueous vehicles, and numerous functional additives [42]. Each of these classes of compounds contributes specifically to the final properties of the product, but at the same time introduces significant challenges when these substances enter the wastewater cycle.

Pigments represent the functional core of industrial inks and are generally insoluble solid materials characterized by high chemical stability and resistance to photochemical degradation. In the case of black inks, widely used in industrial and flexographic printing, characterization studies have identified the use of pigments based on amorphous carbon (lamp black, carbon black), pigments derived from carbonized organic materials (bone black, ivory black), and inorganic pigments based on metal oxides, often present in complex mixtures [43]. Spectroscopic and microscopic analyses have also shown that these pigments exhibit distinct chemical signatures but structures that are difficult to degrade once dispersed in aqueous environments.

The size of pigment particles represents another critical factor. Sampling performed on real matrices indicates the presence of particles ranging between 10 and 20 μm in diameter, stabilized within the liquid matrix by surfactants and dispersing polymers. This particle size, combined with the low apparent density of many carbonaceous pigments, favors the formation of stable suspensions and slows spontaneous sedimentation phenomena, making such systems particularly resistant to conventional physical treatments [15].

Alongside pigments, polymeric binders play a crucial role in fixing the color to the substrate and providing the printed film with the desired mechanical properties. In modern water-based inks, typical of flexographic printing on cardboard, these binders are often composed of acrylic, vinyl, or polyurethane resins dispersed in

water. The presence of these polymers gives inks a complex rheological behavior, often non-Newtonian, which results in industrial wastewater appearing as slightly viscous fluids that are difficult to treat [44]. From an environmental perspective, these polymers significantly contribute to COD values and to the persistence of organic loads.

A further level of complexity is introduced by the presence of functional additives, which are essential for the correct operation of the printing process. Among these, non-ionic surfactants play a particularly important role, acting as wetting agents, defoamers, and colloidal stabilizers. One of the most studied compounds in this category is 2,4,7,9-tetramethyl-5-decyne-4,7-diol (TMDD). Widely used in inks and coatings, TMDD exhibits high solubility in water and limited removal efficiency in conventional treatment systems [45]. From a quantitative perspective, TMDD concentrations of up to 310 µg/L have been measured in the effluents of wastewater treatment plants receiving discharges from paper and printing industries, while values up to 3300 µg/L have been detected in raw wastewater from ink and paint manufacturing facilities [45]. Although these concentrations are below acute toxicity thresholds, the persistence of this compound and the lack of data regarding chronic toxicity raise significant concerns in terms of long-term environmental exposure.

The combination of insoluble pigments, film-forming polymers, and stabilizing surfactants gives wastewater containing industrial inks a high colloidal stability, which represents one of the main challenges from a treatment perspective. Such effluents are typically characterized by intense coloration, high turbidity, variable pH, and low biodegradability, as widely documented for wastewater from the printing and dyeing industries [46], [47].

From a global perspective, the environmental impact of this sector is far from negligible. Printing and dyeing activities are responsible for up to 20% of industrial wastewater discharged worldwide, while the textile sector—different but closely related to the same problem—requires an estimated water consumption of approximately 200 L per kilogram of textile material produced [47]. In addition, annual consumption of dyes and inks exceeds 10,000 tons, a significant fraction of which ultimately enters water bodies through industrial effluents [46].

In recent years, additional concerns have emerged regarding the presence of microplastics in wastewater originating from printing and dyeing processes. Analyses conducted on complete treatment lines have shown that raw effluents may contain significant percentages of polymeric particles, including fluorosilicones, PVC, and PET, with transformations in composition occurring along the treatment process [18]. These findings suggest that wastewater containing inks may represent not only a source of chemical pollution but also a potential vector for microplastic contamination.

Dealing with flexographic printing on cardboard, these issues become particularly relevant. Wastewater generated by such processes typically appears as dark, slightly viscous, and chemically heterogeneous fluids containing a complex mixture of pigments, polymers, surfactants, and cellulosic residues. This physicochemical complexity makes these matrices particularly representative of the challenges associated with the treatment of water contaminated by industrial inks and constitutes the conceptual basis for the analysis of coagulation and flocculation processes discussed in the following sections.

Currently, one of the most established technologies for the treatment of complex dye-containing wastewater—although falling outside the broader framework of recent innovations in water treatment—is thermal treatment through evaporation followed by condensation. Although its diffusion across industrial territories is still limited and the number of companies adopting it remains relatively small, this technology is gradually expanding due to its high effectiveness in separating liquid and solid fractions. The operating principle is based on heating the wastewater to boiling temperatures, causing the evaporation of water and the subsequent condensation of vapor in a separate circuit. This process produces a highly demineralized liquid fraction free of contaminants, often comparable to high-purity water. At the same time, a solid or semi-solid residue remains in the reactor, commonly referred to as “sludge”, consisting of a concentrated mixture of pigments, additives, and other components originally present in the wastewater. Despite the high efficiency of this process in terms of separation and water quality, several important limitations exist. First, energy demand is particularly high, as the process requires the daily heating of large volumes of water to boiling temperatures, resulting in significant operational and maintenance costs. Furthermore, the condensed water, although extremely pure, often finds no real reuse within the production cycle and is typically discharged directly into the sewer system, generating a limited return compared to the costs incurred for treatment. Similarly, the solid residue produced, despite representing a complex mixture of potentially recoverable materials, is generally managed as waste without systematic material recovery.

In this sense, thermal treatment represents a highly effective technology from the perspective of physical separation, but it remains poorly optimized from an energetic, economic, and circular perspective, highlighting the need to develop alternative or complementary solutions capable of reducing energy consumption and valorizing both fractions generated by the process.

2.2 Coagulation and flocculation processes

The stability of colloidal dispersions represents the theoretical foundation of coagulation and flocculation processes and is therefore central to understanding the behavior of industrial wastewater containing inks. Such effluents can be described as complex colloidal systems in which pigment particles, polymer fragments, and stabilizing additives are finely dispersed within an alkaline aqueous matrix. Their persistence in suspension results from a delicate energetic balance between attractive and repulsive forces acting at the nanometric scale. According to classical DLVO theory (Derjaguin–Landau–Verwey–Overbeek), this balance arises from the competition between van der Waals attractive forces and electrostatic repulsion generated by the electrical double layer surrounding charged particles in an electrolyte solution. Recent studies have confirmed the predictive capability of DLVO theory in dispersed systems, showing that the total interaction energy between particles depends on the interparticle distance and typically exhibits a primary energy barrier that prevents spontaneous aggregation [48], [49].

When this barrier is sufficiently high, particles remain dispersed; when it is reduced—for instance through changes in pH or increases in ionic strength—the system may evolve toward aggregation. Zeta potential represents the electrokinetic parameter most commonly used to evaluate colloidal stability, as it reflects the

electric potential at the particle slipping plane. High absolute values, either positive or negative, indicate strong electrostatic repulsion and therefore stable dispersions, whereas values approaching the isoelectric point favor particle aggregation due to the predominance of attractive interactions [50]. In alkaline systems typical of water-based inks, deprotonation of surface functional groups and the presence of light amines increase the negative surface charge of particles, expand the electrical double layer, and consequently enhance colloidal stability. The potential distribution within the electrical double layer is described by the Poisson–Boltzmann equation, which indicates that the thickness of the diffuse layer, represented by the Debye length, is inversely proportional to the square root of the ionic strength of the medium. Increasing ionic concentration compresses the double layer, reduces electrostatic repulsion, and facilitates particle aggregation [51]. This principle constitutes the physical basis of coagulation processes based on metallic salts, which destabilize colloids by screening surface charges and lowering the energy barrier between particles. However, classical DLVO theory does not account for additional interactions that may play a significant role in real complex systems. More recent research has highlighted the importance of non-DLVO forces, including hydrophobic interactions and Lewis acid–base interactions. These contributions are incorporated into extended models (XDLVO), which combine van der Waals, electrostatic, and acid–base components to provide a more comprehensive description of aggregation phenomena [52]. In matrices containing organic pigments, polymeric resins, and surfactants, such interactions may strongly influence aggregation behavior, particularly in the presence of heterogeneous surfaces or aromatic functional groups.

Another relevant aspect concerns the structural characteristics of the aggregates formed. At low particle concentrations and moderate energy barriers, aggregation may follow diffusion-limited (DLCA) or reaction-limited (RLCA) mechanisms, producing fractal clusters with distinct mechanical and rheological properties [53]. Furthermore, systems that appear gel-like may in fact behave as “cluster fluids,” in which viscoelastic properties arise from the percolation of weakly interconnected aggregates rather than from a permanent three-dimensional network [53]. This distinction is particularly relevant in industrial wastewater, where floc formation may lead either to compact structures that sediment readily or to low-density aggregates that are difficult to separate.

Recent applications of DLVO theory to bio-based materials have also shown that quantifying the energy barrier—expressed in multiples of kT —can predict adhesion and nucleation processes on solid surfaces [54]. These findings suggest that energetic analysis of particle interactions may also help explain flocculation mechanisms induced by natural polymers, where electrostatic barrier reduction occurs simultaneously with molecular bridging phenomena.

Overall, the stability of wastewater containing inks can be interpreted as the combined result of high surface charge, an extended electrical double layer, steric stabilization induced by polymers and surfactants, and the presence of non-DLVO interactions. The controlled destabilization of such systems therefore requires processes capable of reducing the zeta potential, compressing the double layer, and overcoming the primary energy barrier, thereby enabling the formation of aggregates large enough to be removed through sedimentation

or filtration. This theoretical framework forms the basis for understanding coagulation and flocculation mechanisms, which, although closely related, rely on fundamentally different physicochemical principles.

Coagulation represents the first stage in the destabilization of colloidal dispersions and aims to reduce or eliminate the electrostatic repulsive forces that keep particles suspended. In aqueous systems containing negatively charged particles—commonly observed in alkaline industrial wastewater—the addition of inorganic coagulants based on multivalent metal salts, typically aluminum (Al^{3+}) or iron (Fe^{3+}), allows surface charge neutralization through mechanisms involving electrical double layer compression and the specific adsorption of hydrolyzed metal species [55]. Charge neutralization reduces zeta potential and lowers the DLVO energy barrier, facilitating particle approach and primary aggregation. Chemically, aluminum and iron salts undergo hydrolysis reactions in aqueous solutions, generating intermediate hydroxylated species such as $\text{Al}(\text{OH})^{2+}$, $\text{Al}(\text{OH})_2^+$, $\text{Fe}(\text{OH})^{2+}$, and polynuclear complexes. These species exhibit strong affinity for negatively charged colloidal surfaces and promote electrostatic destabilization. At appropriate pH values, hydrolysis continues until amorphous metal hydroxides such as $\text{Al}(\text{OH})_3$ and $\text{Fe}(\text{OH})_3$ are formed, precipitating as gelatinous flocs [56]. This phenomenon introduces an additional removal mechanism known as sweep coagulation, whereby colloidal particles become physically entrapped within the precipitating metal hydroxide matrix. Coagulation can therefore be interpreted as a process combining electrostatic neutralization and physical entrapment, particularly effective in systems characterized by high turbidity and suspended solids concentrations.

Nevertheless, electrostatic neutralization alone does not always generate aggregates large enough for efficient sedimentation. In this context, flocculation represents a subsequent and mechanistically distinct step. Flocculation relies primarily on high-molecular-weight polymers capable of linking destabilized particles through the mechanism known as polymer bridging [57]. In this process, segments of polymer chains adsorb onto different particle surfaces, forming larger three-dimensional structures compared with the primary microaggregates generated during coagulation.

Besides bridging, flocculation may occur through adsorption–patch mechanisms, where low- or medium-molecular-weight cationic polymers adsorb locally onto particle surfaces, generating regions of opposite charge that promote electrostatic attraction between neighboring particles [58]. Under conditions of high metallic coagulant concentration, a combined mechanism known as sweep flocculation may also occur, in which polymers enhance the growth and consolidation of previously formed metal hydroxide flocs, improving their mechanical strength and sedimentation properties.

From a structural perspective, flocculation leads to the formation of macroflocs, typically ranging from hundreds of micrometers to millimeters in size. Their morphology depends on polymer molecular weight, charge density, and hydrodynamic conditions within the system [57]. The kinetics of floc formation are influenced by parameters such as the velocity gradient (G-value), mixing time, and flocculant concentration, which collectively determine collision probability and interparticle adhesion efficiency.

A comparison between coagulation and flocculation highlights clear differences in the dominant mechanisms involved. Coagulation primarily acts at the electrostatic level by neutralizing surface charges and promoting primary aggregation, whereas flocculation operates at a structural level by increasing aggregate size through

polymer interactions and bridging mechanisms. While coagulation strongly depends on pH and metal speciation, flocculation is more strongly influenced by polymer molecular weight and conformational configuration (fig. 2.2).

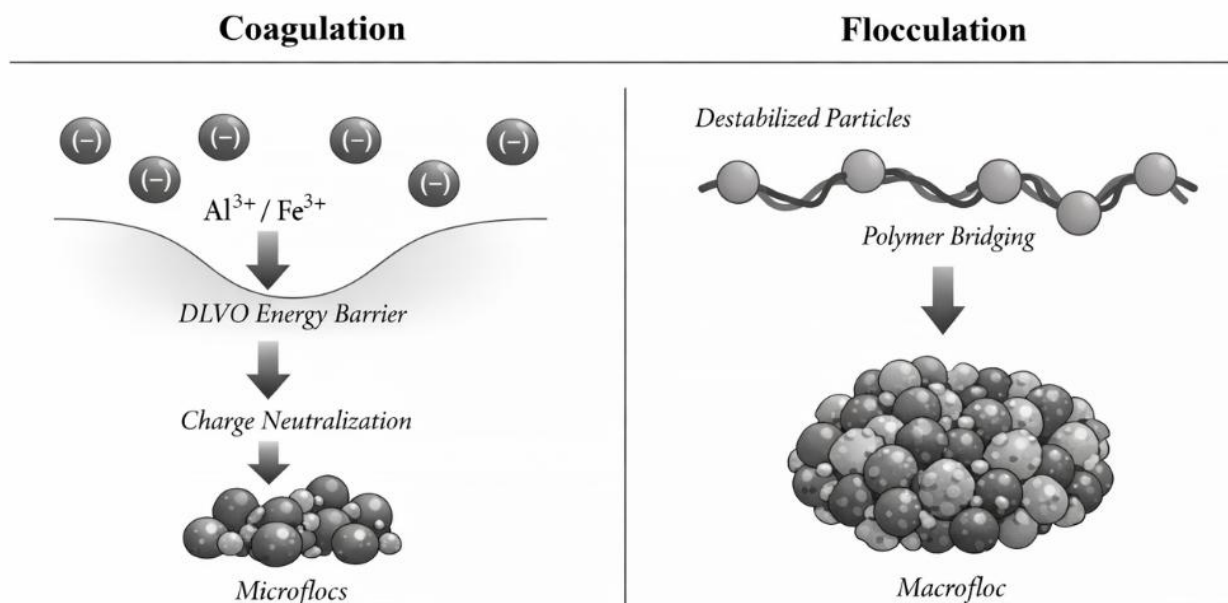


Figure 2.1. Main mechanisms involved in coagulation–flocculation processes: charge neutralization, polymer bridging, sweep flocculation and (macro/micro) flocs formation.

In many practical systems, however, these two processes are complementary rather than alternative. The combined use of inorganic coagulants and polymeric flocculants exploits the synergy between charge neutralization and molecular bridging, producing flocs that are more compact and settle more rapidly than those formed through either mechanism alone [56], [57]. In complex industrial wastewater characterized by high colloidal stability and the presence of stabilizing polymers or surfactants, such combined approaches are often required to overcome the primary energy barrier and generate structurally stable aggregates.

In summary, coagulation can be interpreted as the primary destabilization stage centered on electrostatic neutralization and metal hydroxide formation, whereas flocculation represents the structural growth phase of aggregates through mechanisms such as polymer bridging, adsorption–patch interactions and sweep flocculation. A comprehensive understanding of both processes is essential for designing effective wastewater treatment strategies, particularly in systems characterized by complex multicomponent matrices. The efficiency of coagulation–flocculation processes does not depend solely on the chemical nature of the reagents employed but is strongly influenced by operational parameters that govern floc destabilization, growth, and sedimentation dynamics. Among these, pH represents the most critical factor, as it controls both metal coagulant speciation and the charge state of colloidal particles. Several studies on industrial wastewater containing inks or dyes have shown that pH directly determines the formation of hydroxylated Al^{3+} and Fe^{3+} species and therefore their charge neutralization capacity [59]. Maximum removal efficiencies for COD, turbidity, and color are typically observed within specific pH ranges, while even moderate deviations can

increase colloidal stability or produce mechanically unstable flocs. Similarly, in the treatment of printing industrial wastewater (PIW), Simoni et al. [60] demonstrated that preliminary acidification promotes the precipitation of hydrophobic organic compounds, thereby improving the effectiveness of subsequent FeCl_3 coagulation.

Ionic strength represents another crucial parameter, as it influences the thickness of the electrical double layer and thus the energy barrier between particles. Increasing ionic concentration compresses the Debye length, reduces electrostatic repulsion, and facilitates destabilization. However, excessive ionic strength may alter floc structure and compromise mechanical stability. In wastewater containing ink nanoparticles, variations in zeta potential of only a few millivolts have been shown to significantly reduce sedimentation times [59], highlighting the strong link between ionic conditions and separation kinetics.

Flocculant dosage is another highly sensitive parameter. Insufficient doses prevent effective charge neutralization or bridging, whereas excessive doses may lead to restabilization due to charge inversion or surface saturation. In dye-containing wastewater, Wu et al. [61] demonstrated that molecularly designed flocculants can achieve removal efficiencies exceeding 99% at significantly lower dosages than conventional coagulants, reducing chemical residues and improving dewatering efficiency. Similarly, Wang et al. [62] showed that branched polymer architectures and block distribution significantly enhance bridging efficiency and produce flocs that are more compact and resistant to shear forces.

The mixing regime also plays a critical role in floc formation. Rapid mixing, characterized by high velocity gradients, ensures uniform dispersion of the coagulant and promotes rapid particle–coagulant collisions. This is followed by slow mixing, which enables the controlled growth of microaggregates into macroflocs through gentle and progressive collisions. Parameters such as the velocity gradient (G-value) and the product $G \cdot t$ (gradient multiplied by contact time) determine the probability of effective collisions and ultimately the final size of the aggregates.

Naghiyan et al. [59] reported that excessive shear stress may cause floc fragmentation, releasing ink nanoparticles with sizes ranging between 59 and 80 nm and thus deteriorating effluent quality. In contrast, Wang et al. [62] observed that branched polymer structures enhance floc resistance to mechanical stresses and improve solid–liquid separation performance.

The kinetics of floc formation are closely related to the dominant aggregation mechanism. Systems primarily governed by charge neutralization exhibit rapid aggregation but produce less structured flocs. In contrast, systems dominated by polymer bridging generate larger and more compact aggregates but require more carefully controlled mixing conditions. Simoni et al. (2025) [60] showed that in printing wastewater, consolidation periods of up to 24 hours may significantly increase the dry solids content of the filtered cake, reaching values as high as 79% when polymeric flocculants are used. This observation highlights the importance of the post-flocculation consolidation phase.

The final sedimentability of the aggregates represents the operational parameter that summarizes the overall effectiveness of the process. Flocs smaller than approximately 200 μm require more intensive filtration systems

and lead to longer separation times [60]. Conversely, larger, compact, and shear-resistant aggregates allow sedimentation times below 5 minutes and improve the quality of the clarified effluent [59].

In the specific case of ink-containing wastewater, additional challenges arise from the presence of alkaline conditions, surfactants, and stabilizing resins. High alkalinity maintains pigment particles in a strongly negatively charged state, increasing zeta potential and limiting the effectiveness of simple electrostatic destabilization. Surfactants interfere with the adsorption of coagulants onto particle surfaces by creating steric barriers that hinder bridging mechanisms. Moreover, the organic nature of many pigments and additives reduces the efficiency of conventional sweep coagulation based on metal salts, often requiring additional mechanisms involving polymeric or hydrophobic interactions [61], [62].

An efficient coagulation–flocculation process for ink-containing wastewater must therefore integrate several factors synergistically:

- precise pH control to optimize coagulant speciation and particle surface charge.
- regulation of ionic strength to compress the electrical double layer without compromising mechanical stability.
- optimal flocculant dosage to avoid restabilization phenomena.
- controlled mixing conditions to maximize polymer bridging while minimizing floc fragmentation.
- formation of mechanically stable aggregates with high sedimentation potential.

Only through an integrated understanding of these parameters can the typical limitations of alkaline and surfactant-rich wastewater be overcome, opening the way toward more biocompatible and targeted treatment solutions, particularly when natural or bio-based polymeric flocculants are considered as alternatives to conventional metallic coagulants.

2.3. Natural and synthetic coagulants–flocculants: a comparison

Coagulation–flocculation represents one of the most widely used strategies for solid–liquid separation in industrial wastewater and colloidal systems, and it can be achieved through a wide variety of materials with different chemical and structural properties. Historically, industrial treatment processes have favored the use of inorganic coagulants and synthetic polymers, such as polyacrylamide derivatives or multivalent metal salts, due to their high efficiency, relatively low cost, and adaptability to different wastewater matrices [63], [64]. However, in recent years, the literature has increasingly highlighted the environmental limitations of these materials, particularly in terms of poor biodegradability, the risk of releasing toxic monomers, and the production of secondary sludge. These concerns are directing research toward more sustainable alternatives [64], [65]. In this context, bio-based flocculants derived from natural polysaccharides, residual biomasses, or microorganisms are emerging as promising candidates because of their biodegradability, renewability, and lower environmental impact, although systematic comparisons with synthetic materials in terms of performance, stability, and industrial scalability are still needed [65], [66].

The literature reports an extremely broad and diverse range of materials classified as coagulants or flocculants, whether inorganic or synthetic organic, making it difficult to establish a classification that is truly exhaustive with respect to all possible water treatment applications. In this work have been reported the nature, mechanism, application and pro and cons of the flocculants and coagulants that are resumed in Table 2.1.

Class	Material	Chemical nature	Main mechanism	Typical applications	Advantages	Limitations
Inorganic coagulants	Aluminum sulfate ($\text{Al}_2(\text{SO}_4)_3$)	Multivalent metal salt	Charge neutralization, sweep flocculation	Drinking water clarification, turbidity removal	Low cost, high availability	Large sludge production, pH sensitivity
	Ferric chloride (FeCl_3)	Multivalent metal salt	Hydrolysis and sweep flocculation	Industrial wastewater, phosphate removal	High efficiency in organic matter removal	Corrosive, sludge generation
	Ferric sulfate	Metal salt	Charge neutralization	Water and wastewater treatment	Effective over wide pH range	Sludge disposal
Synthetic organic flocculants	Polyacrylamide (PAM)	Synthetic polymer	Polymer bridging	Sludge dewatering, wastewater clarification	Very high efficiency	Poor biodegradability
	Anionic PAM	Synthetic polymer	Bridging, electrostatic interaction	Mineral suspensions	High molecular weight	Sensitive to ionic strength
	Cationic PAM	Synthetic polymer	Charge neutralization	Biological sludge treatment	Strong electrostatic interactions	Toxic monomer concerns
	PolyDADMAC	Cationic polymer	Charge neutralization	Drinking water treatment	High charge density	Limited biodegradability
	Polyethylenimine (PEI)	Branched polymer	Charge neutralization, complexation	Heavy metal removal	Multiple amine groups	pH dependent behavior
	Epichlorohydrin–dimethylamine polymers	Cationic polymer	Electrostatic destabilization	Surface water clarification	Good floc formation	Synthetic origin

	Polyvinylamine	Functionalized polymer	Adsorption and bridging	Fine particle removal	Strong interaction with surfaces	Limited environmental compatibility
	Polystyrene sulfonate polymers	Anionic polymer	Floc strengthening	Combined coagulant systems	Improves sedimentation	Requires metal coagulants
Natural bio-based flocculants	Chitosan	Aminopolysaccharide	Charge neutralization, bridging	Dye removal, turbidity removal	Biodegradable, renewable	pH dependent solubility
	Starch derivatives	Natural polysaccharide	Polymer bridging	Wastewater treatment	Low toxicity	Lower efficiency
	Cellulose derivatives	Natural polymer	Adsorption, bridging	Industrial wastewater	Renewable material	Limited charge density
	Alginates	Natural polysaccharide	Gel formation, adsorption	Metal removal	Biocompatible	Mechanical stability
	Tannins	Polyphenolic compounds	Charge neutralization	Coagulation aid	Natural origin	Variable composition
	Moringa oleifera extracts	Plant proteins	Charge neutralization	Drinking water clarification	Low cost, natural	Organic residuals
	Opuntia ficus-indica mucilage	Plant polysaccharides	Bridging flocculation	Suspended solids removal	Sustainable	Limited industrial scale

Table 2.1. summarizes the main classes of coagulants and flocculants reported in the literature and their principal mechanisms of action.

Following the literature, classification is proposed in a progressive and multilevel way. First, it is important to note that, although coagulation and flocculation have historically been described as two distinct processes, more recent literature increasingly considers them as interdependent stages of a single colloidal transformation mechanism. Numerous studies have shown that electrostatic destabilization, adsorption, polymer bridging, and floc growth may occur simultaneously from the earliest stages of treatment, especially when multifunctional organic or polymeric coagulants are employed. Consequently, colloidal stability, aggregation kinetics, and floc development evolve continuously within the same reactive system. For this reason, the present work does not

adopt rigid distinctions based on the classical separation between the two processes but rather favors a unified interpretation of the phenomenon.

The first classification level adopted therefore concerns the chemical nature of the materials, which can be divided into inorganic compounds, synthetic organic polymers, and natural or bio-based organic materials. This distinction represents a fundamental interpretative key, as it reflects differences in particle interaction mechanisms, required operating conditions, and the environmental implications associated with their use.

From a historical perspective, inorganic coagulants–flocculants represent the oldest form of chemical water treatment. The literature shows that aluminum and iron salts were already used for water clarification in remote historical periods, owing to their ability to hydrolyze rapidly, forming positively charged hydroxylated species and precipitated metal hydroxides capable of adsorbing colloids and organic substances [41] This behavior makes them particularly effective in charge neutralization and sweep flocculation mechanisms. Even today, compounds such as ferric chloride and aluminum sulfate remain widely used industrial standards for the removal of turbidity, suspended solids, and organic matter, although they present limitations related to sludge production and sensitivity to operating conditions [55].

The development of polymer chemistry introduced a second major family of materials used in colloidal destabilization processes: organic coagulants and flocculants. Unlike inorganic coagulants, which act mainly through hydrolysis and precipitation, organic materials consist of macromolecules capable of interacting with suspended particles through more complex mechanisms, including multiple-site adsorption, polymer bridging, and the formation of surface charge domains. The introduction of polymers therefore transformed coagulation–flocculation from a process dominated by chemical precipitation into a more articulated system in which molecular structure, molecular weight, and charge density directly influence treatment performance [57].

Within organic materials, a distinction can be made between natural polymers and synthetic polymers. The former are derived from biomass or living organisms and are attracting increasing attention due to their biodegradability and sustainability; the latter, however, still represent the most consolidated and widely applied technological solution in industrial water treatment systems, owing to their high efficiency, industrial reproducibility, and the possibility of controlled molecular design. The present section focuses on the latter.

From a chemical standpoint, synthetic flocculants are generally water-soluble polymers obtained by radical polymerization in solution or emulsion, with molecular weights that may reach the order of millions of Daltons. These characteristics allow the polymer chains to extend in solution and interact simultaneously with multiple particles, promoting the formation of macroscopic aggregates that can be readily separated by sedimentation or filtration. The literature highlights that these materials were developed to improve aggregation efficiency, increase floc size, and reduce the dosages required in industrial processes [57], [67].

Among the most widely used synthetic flocculants are polyacrylamides (PAM) and their copolymers, which represent the most common family in both research and industrial applications. These polymers may be anionic, cationic, or nonionic depending on the nature of the monomers incorporated into the chain, and their high molecular weight and structural flexibility make them particularly effective in promoting bridging mechanisms between colloidal particles. Acrylamide–acrylate copolymers, for example, allow modulation of

the charge density of the material and adaptation to wastewater characteristics, making this class extremely versatile in industrial systems [57], [67]ly versatile in industrial systems [57], [67]. A second relevant category is represented by cationic acrylic copolymers containing quaternary ammonium groups, obtained through the incorporation of monomers such as DADMAC, DMAEA, or MAPTAC. These polymers carry a permanent positive charge and are particularly effective in systems dominated by negatively charged particles, such as wastewater containing organic matter, pigments, or biological sludge. The possibility of simultaneously modulating molecular weight and charge density makes these materials especially adaptable to the chemical conditions of the system and allows improved floc stability even in the [68]. Among the most industrially important cationic polymers is polyDADMAC, characterized by a cyclic structure that provides high charge density and good chemical stability. This material is widely used in drinking water clarification, surface water treatment, and sludge conditioning, often in combination with acrylamide to enhance flocculating efficiency. Its dominant mechanism is electrostatic neutralization, followed by secondary aggregation and floc growth phenomenon. Another historically important class is polyethyleneimine (PEI), a branched cationic polymer containing numerous protonable amine groups. This structure gives the material a dual function: electrostatic destabilization of particles and complexation of dissolved ionic species, making it useful in the treatment of complex industrial wastewater and in heavy metal removal. However, the dependence of its degree of protonation on the chemical conditions of the system may limit its operational predictability [57]may limit its operational predictability [57].

Synthetic flocculants also include polymers derived from epichlorohydrin and polyamines, such as epichlorohydrin–dimethylamine copolymers, which are widely used in the precipitation of organic matter and in surface water clarification. These materials carry cationic charges distributed along the polymer chain and are effective both as primary organic coagulants and as coadjuvants in combined systems. The literature also reports the use of functionalized vinyl polymers, including polyvinylamines, quaternized polyvinylpyridines, and ammonium polystyrene derivatives, which can adsorb onto mineral and colloidal surfaces and contribute to the removal of fine particles and natural organic matter. Beside cationic flocculants, synthetic anionic polymers such as polyacrylates, sulfonated copolymers, and polystyrene sulfonate-based polymers are also used. These materials are employed mainly as flocculation aids, especially in systems in which metallic coagulants or primary flocs are already present, contributing to the reinforcement of aggre[57].

The literature clearly shows that synthetic flocculants comprise a broad range of materials specifically designed to maximize solid–liquid separation efficiency and adapt to different chemical matrices. For this reason, from the second half of the twentieth century onward, such polymers have been extensively studied, developed, and applied across very different industrial contexts. Their widespread adoption has been favored by their high structural tunability, the possibility of precisely controlling molecular weight and charge density, and their demonstrated operational effectiveness, all of which have made them the technological benchmark in water treatment systems. Nevertheless, these materials also present significant drawbacks related to poor biodegradability, the potential toxicity of residual monomers, and dependence on fossil-based raw materials. A substantial portion of the literature has highlighted concerns related to the persistence and environmental

fate of the polymers used, particularly in the case of polyacrylamides (PAM). The high molecular weight typical of commercial products—generally within the range of 10^6 – 3×10^7 Da—is one of the factors supporting their effectiveness as flocculants, but it may also translate into environmental management issues when such materials are accidentally released or are not completely removed or immobilized in sludge (Xiong et al. [57]). The kinetics of floc formation are inflt al., 2018). The review by Xiong et al. [69] indeed shows that the large-scale application of PAM in water/wastewater treatment, soil conditioning, and other sectors may generate “significant environmental challenges,” precisely because degradation may be slow and highly dependent on the physicochemical conditions of the system, making the assessment of polymer fate and possible transformation products particularly relevant.

In parallel, the toxicological literature has historically emphasized the need to evaluate the biological effects associated with organic flocculants. For example, Takigami et al. [70] tested different classes of flocculants—cationic, anionic, amphoteric, and nonionic—using assays based on microalgae and bacteria, reporting that algal growth inhibition for various flocculants occurred in the order of 1–20 mg/L, with the main contribution attributed to the high-molecular-weight fraction ($>100,000$ Da). Although these findings do not provide a generalized condemnation of the entire class of synthetic polymers, they support the idea that, in addition to process performance, the environmental fate and possible biological effects of the employed materials should also be considered, especially in intensive-use scenarios or in complex real matrices.

These concerns are further reinforced by considerations related to supply-chain sustainability and the energy and environmental impacts of synthetic materials, which are often strongly dependent on industrial production and on fossil-derived feedstocks or energy-intensive chemical processes. A useful example is the LCA study by Bajdura et al. [71], which evaluated the environmental impact of a flocculant produced from polystyrene waste using the EF 3.0 method recommended by the European Commission and implemented in SimaPro. The authors reported, for the production scenario, a projected electricity demand of 60 kWh to produce 100 kg of flocculant (PSP), highlighting the importance of electricity as a contributor to impact categories and including a sensitivity analysis specifically for this parameter [71]. In the application phase, the study also considered the electricity consumption associated with treatment, reporting that approximately 51 kWh were consumed to treat an assumed daily volume of 3 m³ of wastewater [71]. Overall, these quantitative elements strengthen the argument that the adoption of synthetic flocculants—although highly effective from a performance standpoint—may be associated with a considerable burden in terms of production, energy demand, and residue management, and they provide a concrete rationale for the growing interest in bio-based and renewable flocculants, which should be evaluated not only in terms of efficacy but also with regard to impact reduction over the full life cycle.

Within this framework of environmental, energetic, and toxicological concerns associated with synthetic flocculants, natural organic polymers are emerging as a class of increasing interest, since they are derived from plant, animal, or microbial biomass and possess macromolecular structures intrinsically compatible with aquatic systems. Among the various biopolymers studied for water treatment applications, particular attention has been devoted to polysaccharides and biopolymers containing reactive functional groups capable of

interacting with colloidal particles through mechanisms analogous to those of synthetic polymers, but with potentially lower environmental impact.

So-called bio-based flocculants mainly include polysaccharides, microbial biopolymers, and protein derivatives, among which chitosan, cellulose and its derivatives, modified starch, alginates, natural tannins, lignin, plant proteins, and bioflocculants produced by microorganisms are particularly relevant [72]. Among the most widely studied plant-derived materials are also bioflocculants obtained from seeds and plant mucilage, such as *Moringa oleifera*, *Opuntia ficus-indica*, and *Aloe vera*, as well as vegetable tannins and mucilage extracted from natural fibres. From a performance point of view, the literature reports that many of these materials are capable of reaching efficiencies comparable to those of synthetic polymers. Natural polysaccharide-derived flocculants can achieve turbidity reductions above 90% and COD removals between 60 and 85%, while in dye-containing systems different bioflocculants have shown decolorization efficiencies ranging from 70 to 95% [72], [73]. Similarly, the use of natural mucilage has been reported with colour removal efficiencies of up to 82–83%, while *Opuntia ficus-indica* cactus has shown suspended solids removal capacities exceeding 80% in industrial effluents [74].

The effectiveness of these materials is attributed to the presence of reactive functional groups along the macromolecular chains—particularly amino, hydroxyl, and carboxylic groups—which enable multiple modes of interaction with dispersed particles. Biopolymers can indeed operate through mechanisms analogous to those of synthetic flocculants, including charge neutralization, polymer bridging, adsorption, and physical entrapment of particles, while maintaining greater environmental compatibility due to their biodegradable nature. In several studies, the reported operating dosages for these materials are on the order of 10–50 mg/L, values comparable to those of synthetic polymers, suggesting potential applicability at real treatment scale [72].

Another important aspect concerns supply-chain sustainability. Natural flocculants are often derived from agricultural or industrial residual biomass—such as wood-processing waste, fishery by-products, or agro-food residues—thereby reducing dependence on fossil raw materials and improving the environmental balance of the treatment process. Recent studies indicate that the use of biopolymers may contribute to reducing the production of persistent sludge and improving the biodegradability of process residues, thereby facilitating the integration of water treatment systems into circular economy models [72], [73].

Despite these promising features, the literature also points out several limitations associated with natural flocculants, including greater compositional variability, stronger sensitivity to operating conditions, and, in some cases, lower stability compared with synthetic materials. However, the possibility of chemical modification and functionalization of biopolymers is progressively helping to overcome these limitations, making such materials increasingly competitive in water treatment systems.

According to the different classes of materials employed in coagulation–flocculation processes, the need emerges for a comparative evaluation that simultaneously considers operational performance, chemical nature, sustainability, and raw material origin. Traditional inorganic coagulants, such as aluminum and iron salts, represent technologically consolidated solutions characterized by high operational reliability, relatively low

cost, and good effectiveness in removing turbidity and suspended solids. However, such materials also present limitations related to the production of voluminous sludge, sensitivity to pH conditions, and the possible introduction of residual metallic species into the treated system. Synthetic organic flocculants, particularly acrylamide-based polymers and cationic copolymers, offer a higher degree of process control thanks to the possibility of modulating molecular weight, charge density, and polymer chain architecture. These characteristics allow the formation of more compact flocs, reduction of required dosages, and improved system sedimentability. From a performance perspective, they still represent the technological benchmark in industrial water treatment systems. However, their dependence on fossil-based raw materials, poor biodegradability, and the risks associated with residual monomers raise significant questions concerning their environmental impact and the long-term sustainability of their production chain.

Natural or bio-based flocculants occupy a third position in this scenario, characterized by renewable origin, greater biodegradability, and lower potential toxicity. Polysaccharides, proteins, and microbial biopolymers may provide removal performances comparable to those of synthetic materials, particularly in systems dominated by electrostatic interactions or polymer bridging mechanisms. Furthermore, the possibility of obtaining such materials from residual biomass helps reduce the environmental footprint of the treatment process and supports the integration of water treatment into circular economy strategies. Nevertheless, some operational limitations remain, including compositional variability and sensitivity to the chemical conditions of the system, which require careful optimization of process parameters. Overall, the comparison among these three classes of materials highlights that no single technology represents a universally optimal solution. Rather, the choice of flocculant depends on a balance between operational efficiency, costs, compatibility with the treated matrix, and life-cycle sustainability. In this context, the growing attention toward bio-based materials does not arise solely from environmental considerations, but also from the possibility of developing solutions capable of combining adequate performance with impact reduction, making these polymers particularly attractive candidates for applications in which process sustainability plays a central role.

Among the different natural flocculants studied, chitosan stands out in a particularly significant way, as it represents a point of contact between technological effectiveness and coherence with circularity principles. Its nature as a cationic polyelectrolyte makes it possible to reproduce, with a renewable material, some of the most effective mechanisms typical of synthetic polymers, such as charge neutralization and polymer bridging, while maintaining a more favorable profile in terms of biodegradability and environmental compatibility. Moreover, the possibility of modulating operating conditions (pH, ionic strength, concentration) and the intrinsic characteristics of the material (degree of deacetylation and molecular weight) makes chitosan a particularly suitable candidate to be studied not only as a possible alternative to conventional flocculants, but also as a design platform for the development of targeted treatments for complex industrial effluents. For these reasons, chitosan is addressed in greater detail in the following sections, with particular attention to its structural properties and interaction mechanisms with colloidal dispersions and organic contaminants, thereby establishing the theoretical basis for the experimental work proposed in this thesis.

2.4 Chitosan: chemical structure, properties and interaction mechanisms

Chitosan is a natural biopolymer belonging to the class of aminopolysaccharides, obtained through the partial deacetylation of chitin, one of the most abundant structural polysaccharides in nature after cellulose. From a chemical standpoint, chitin consists of repeating units of N-acetyl-D-glucosamine linked through β -(1 $\rightarrow$ 4) glycosidic bonds, arranged in a linear structure analogous to cellulose but characterized by the presence of acetamide groups at the C-2 position. Deacetylation progressively converts these units into D-glucosamine, generating a copolymer composed of acetylated and deacetylated units, whose chemical behavior is largely governed by the presence of amino groups along the polymer chain. For this reason, the term “chitosan” does not refer to a single chemical species but rather to a family of materials with variable composition, whose properties depend on the origin of the biomass, extraction conditions, and the degree of transformation of the starting chitin [30], [75].

The main sources of chitin and chitosan include both animal and microbial matrices. Traditionally, industrial production relies on crustacean exoskeletons, in which chitin is associated with minerals and proteins and is extracted through sequential processes of acid demineralization and alkaline deproteinization, followed by a deacetylation step under strongly basic conditions [75]. Alongside this established supply chain, numerous studies have highlighted the presence of chitin and chitosan in fungal cell walls and other microorganisms, demonstrating that fungal matrices can represent an alternative source characterized by different levels of purity, crystallinity, and distribution of functional groups, with consequent variability [76], [39], [75]. These differences in origin can significantly affect the physicochemical performance of the polymer, making the primary source a relevant factor in defining its applications. Crustacean-derived chitosan is generally easier to obtain at high purity, as chitin in crustaceans is organized in relatively compact structures and can be isolated through well-established demineralization and deproteinization sequences. In contrast, in fungal matrices, chitin is an integral component of the cell wall and is structurally associated with β -glucans and mannoproteins. While mannoproteins can be relatively easily removed due to their higher susceptibility to chemical or enzymatic degradation, β -glucans form part of the structural scaffold of the cell wall together with chitin and are therefore more difficult to separate. Consequently, fungal-derived chitosan may contain residual β -glucans, sometimes in limited amounts, unless more aggressive chemical treatments are applied to improve purity, which may in turn affect the properties of the polymer.

Among the structural parameters that most strongly influence the behavior of chitosan, the degree of deacetylation (DD) represents the most critical variable. It expresses the fraction of glucosamine units along the polymer chain and thus the density of amino groups available for chemical interactions. An increase in DD leads to a higher number of protonable sites and an increase in the potential charge of the polymer in solution, directly affecting solubility, surface reactivity, and the ability to interact with anionic species. Reviews on environmental applications of chitosan highlight that amino groups constitute the main active sites of the material and that their concentration along the chain largely determines performance in adsorption processes and interactions with contaminants [30]. DD values in the range of 70–90% are commonly reported for

commercial materials, with values exceeding 95% achievable through more extensive deacetylation treatments, underscoring the importance of this parameter in material characterization [75].

In addition to DD, molecular weight (MW) is another key factor in defining the properties of chitosan in solution. It influences polymer chain configuration, solution viscosity, diffusion behavior, and the ability to establish multipoint interactions with particles and solutes. High molecular weight chains tend to adopt more extended conformations and increase the likelihood of simultaneous interactions with multiple surfaces, whereas lower molecular weights result in greater chain mobility and different interaction kinetics. The literature indicates that molecular weight and degree of deacetylation jointly influence material properties such as swelling, diffusion, and solution behavior, making these parameters essential for interpreting the variability of chitosan performance in different application contexts [30].

Regardless of its quality, chitosan exhibits selective solubility in acidic environments. Due to strong intermolecular interactions and its partially crystalline structure, the polymer is generally insoluble in neutral water and in most organic solvents. However, in the presence of dilute aqueous acids, the amino groups become protonated and the material becomes soluble, adopting a more expanded conformation in solution. In virtually all applications, chitosan is therefore pre-dissolved in acid prior to use, typically yielding solutions with concentrations ranging from 1% to 2.5% (v/v). Above these values, the solution exhibits very low pH levels, which require subsequent treatment of the treated water, particularly a neutralization step. The intrinsic protonation behavior is linked to the pKa of the amino groups, generally reported in the range of 6.3–6.5. At pH values below this range, the fraction of protonated amines increases and chitosan acquires a positive charge distributed along the chain, whereas at higher pH values protonation decreases and solubility is reduced [30]. In acidic solution, chitosan therefore behaves as a natural cationic polyelectrolyte. The protonation of amino groups generates a charge density that promotes interaction with negatively charged species, colloids, and polar surfaces, while simultaneously influencing chain conformation, rheological properties, and the ability to establish multipoint interactions. The polycationic nature of chitosan, combined with the possibility of tuning its properties through variations in pH, molecular weight, and degree of deacetylation, makes this polymer highly relevant in contemporary research on functional materials. The combination of a polysaccharide structure, reactive functional groups, and controllable ionic behavior contributes to explaining the growing scientific interest in chitosan, which is increasingly regarded as a versatile platform for environmental, biomedical, and industrial applications [30] [39].

Thanks to a unique combination of chemical, biological, and functional properties rarely found in other natural materials, chitosan is one of the most versatile and widely studied biopolymers in scientific research. Its biodegradability, biocompatibility, non-toxicity, and chemical reactivity have enabled its application across numerous fields.

Over the past decades, chitosan has been employed in a wide range of application sectors, reflecting its increasing relevance in functional materials research. In the biomedical field, it has been used to produce membranes, hydrogels, and scaffolds for tissue engineering, as well as in drug delivery systems and coatings for medical devices. The literature also reports established applications in advanced wound dressings and in

the delivery of bioactive molecules, highlighting growing interest in its use in regenerative medicine and biopharmaceutical applications [77]. In parallel, chitosan is used in the food industry as a film-forming agent, preservative additive, and edible coating, while in agriculture it has been applied as a biostimulant, antifungal agent, and carrier for controlled-release fertilizers and pesticides, with applications ranging from crop protection to post-harvest preservation [75].

In the field of materials science and advanced technologies, chitosan has been investigated as a component of separation membranes, a support for catalysts, a substrate for chemical sensors and biosensors, and a matrix for composite materials with optical, mechanical, or piezoelectric properties. Additional applications include the production of microcapsules, fibers, and nanoparticles for industrial and environmental purposes, demonstrating how the polymer has been progressively integrated into complex systems and multifunctional technologies [39]. In environmental applications, chitosan has been employed in various contexts, including the treatment of wastewater contaminated with heavy metals, dyes, and organic substances, the removal of nutrients and emerging contaminants, as well as in the production of adsorbent materials, membranes, and flocculation aids, with results documented at laboratory scale and, in some cases, at pilot scale [78]. These studies will be discussed in greater detail in the following chapters.

The widespread use of chitosan across such diverse fields highlights how this biopolymer has become firmly established among functional materials of industrial and scientific interest. Precisely because of the breadth of applications reported in the literature, a more focused discussion of its use in water treatment systems is necessary, where the material has been applied both as an adsorbent and as a coagulation–flocculation agent. Environmental applications represent one of the most dynamic development areas for chitosan, making it particularly relevant to analyze in detail its performance, operational modes, and limitations in the context of wastewater treatment.

2.5. Applications of chitosan in water treatment

In water treatment, chitosan is employed according to two main application approaches which, in many cases, coexist or are combined:

- liquid-phase coagulation–flocculation, where the polymer is dissolved (typically under acidic conditions) and acts as a cationic polyelectrolyte capable of destabilizing colloidal dispersions and/or interacting with dissolved anionic species;
- solid-phase adsorption, where chitosan (or its derivatives) is used in the form of flakes, powders, granules, gels, or composites and functions as an adsorbent.

In both configurations, process efficiency does not depend on a single parameter, but rather on the interaction between the intrinsic characteristics of the material—particularly degree of deacetylation, molecular weight, and the availability/accessibility of -NH_2 groups—and operational conditions such as pH, ionic strength, dosage, mixing, and contact/settling time, all of which must be optimized consistently with the real matrix under investigation [32].

In the case of coagulation–flocculation of colored wastewater, where pigments and dyes may be present either as dissolved species or as stabilized colloidal systems, the mechanism most frequently reported in the literature for anionic dyes is charge neutralization through electrostatic attraction/ion exchange between sulfonate groups of the dye and protonated amino groups of chitosan, accompanied by a contribution from polymer bridging that promotes macrofloc growth and separability by sedimentation or filtration. A recurring element is the existence of an optimal dosage window: as chitosan concentration increases, the residual dye concentration decreases to a minimum and then rises again due to restabilization caused by excess positive charge (overdosing), with direct practical implications for the need for accurate dosage control [79].

A classical case study on Reactive Black 5 (RB5) clearly illustrates the effect of operating pH and dosage sensitivity. For solutions containing 50 mg/L of RB5, increasing the initial pH from 3 to 7 leads to a marked increase in the optimal dose: at pH 3 the optimal dose is approximately 15 mg/L, at pH 5 about 22 mg/L, and at pH 7 it rises to about 50 mg/L, consistently with the decrease in the protonated fraction of chitosan as pH increases and, consequently, with the reduction of its “strength” as a cationic polyelectrolyte [80]. The same work also shows that, at fixed pH, the optimal chitosan concentration (OCC) can be described by a linear correlation with the initial dye concentration. Studies focusing on chitosan amount as a function of dye concentration suggest that the quantity of chitosan required increases proportionally with the dye load, highlighting a nearly stoichiometric behavior of the flocculation process. The difference between the various pH conditions is attributed to the higher degree of protonation of chitosan amino groups in more acidic environments, which increases the positive charge density of the polymer and improves its interaction efficiency with anionic species, thus allowing similar performance to be achieved with lower dosages. This aspect is particularly useful as a basis for dosage control strategies in relation to fluctuations in the influent load. From an intrinsic point of view, the same study also discusses that the use of higher-molecular-weight chitosans may slightly reduce efficiency in these systems, attributing the effect to lower effective “availability” of amino groups and reduced conformational flexibility in following the distribution of anionic charges on the dye molecules.

Another highly useful quantitative example, because it formalizes pH dependence explicitly, is the study on the removal of Acid Blue 92 (AB92) by chitosan coagulation–flocculation in tap water, where the optimum is described by the relationship:

$$\text{Opt[Chit]} = a \cdot [\text{Dye}]_0 + b$$

with coefficients that vary significantly with the initial pH. In particular, the following values are reported:

$a = 0.82$ and $b = 0.82$ at pH 3;

$a = 0.71$ and $b = 14.3$ at pH 5;

$a = 0.92$ and $b = 15.9$ at pH 7;

$a = 0.91$ and $b = 27.6$ at pH 9

(with concentrations expressed in mg/L) [79].

This parameterization makes the key design message immediately clear: for the same initial dye concentration, dosage optimization is strongly controlled by pH and, in practice, also by ionic strength and water composition, because chitosan changes its degree of dissociation and therefore its charge density. The same study explicitly notes that the pK_a of amino groups is approximately 6.3–6.4 for chitosan with a high degree of deacetylation ($\approx 90\%$), implying that at $\text{pH} \leq 5$ more than 90% of amino groups are protonated, whereas at higher pH values electrostatic effectiveness decreases and the optimal dosage window shifts accordingly [79]. From an operational point of view, the study also shows that the addition of an acidic chitosan solution may itself modify system pH depending on the type of water (demineralized vs tap water) and on buffer capacity/ionic strength, underlining the need, in real effluents, to manage pH robustly rather than simply setting it nominally [79]. Beyond colored wastewater, the literature describes a well-established use of chitosan for the removal of turbidity and mineral suspended solids, that is, solid–liquid separation in colloidal suspensions. In this context, it is particularly relevant that in slightly acidic suspensions (around pH 5), chitosan concentrations on the order of 0.1 mg/L may be sufficient to reduce the turbidity of bentonite suspensions with initial contents of a few g/L to below the threshold of 5 FTU (Formazine Turbidity Units) [32]. Besides highlighting the effectiveness of chitosan toward mineral particulates, this result emphasizes an important methodological point: the performance observed in a turbidity test cannot be directly transferred to an organic effluent or a colored wastewater, because both the dominant mechanism and the relationship between required charge density and accessibility of reactive sites change. In practice, the same review points out that performance may vary significantly depending on process conditions and intrinsic chitosan properties—namely degree of acetylation/deacetylation and molecular weight—and that accurate determination of the optimal dose becomes a central issue, especially when the system is governed by charge stoichiometry and may undergo restabilization [32].

Beside “classical” coagulation–flocculation, a very broad research field concerns the use of chitosan as an adsorbent for organic contaminants, particularly dyes, in batch experiments often aimed at estimating adsorption capacities and identifying optimal pH windows. Reviews show that the adsorption of numerous dyes onto unmodified chitosan is generally favoured under acidic conditions, where the polymer chain is cationic and the affinity toward anionic dyes increases [78]. Literature data summarized in tabular form indicate, for instance, highly variable adsorption capacities—depending on pH, particle size, and dye type—with reported values up to 1100 mg/g for Reactive Black (RB5) at pH 3, whereas under less favourable conditions (e.g., neutral pH) much lower values have been reported, such as 200 mg/g for RB5 at pH 7 in other studies. This clearly shows how acid–base conditions and the accessibility of reactive sites are also determinant when adsorption, rather than flocculation, is the dominant process [78]. From an application perspective, this evidence is important because it suggests that even when chitosan is employed as a flocculant in solution, the “chemical affinity” component—namely the specific interaction with anionic groups—may contribute significantly to overall efficiency, especially in wastewater where dissolved and colloidal fractions coexist.

A transversal aspect across the different approaches concerns the preparation of the chitosan solution and, consequently, the availability of $-\text{NH}_2$ groups. Dissolution in acid, typically acetic acid, is described as a step

that “opens” the polymer structure, increasing the accessibility and availability of amino groups, with effects on both equilibrium performance and interaction kinetics/process time [32]. In experimental studies on dyes, the procedure often includes controlled hydration and dissolution of chitosan prior to addition to the synthetic wastewater; for example, in the case of Acid Blue (AB92), a stock solution is prepared by hydrating chitosan and adding high-purity acetic acid before use in coagulation–flocculation tests [79]. This type of methodological detail is relevant because, from a scale-up perspective, the “chemistry” of chitosan cannot be separated from its “preparation engineering”: solution viscosity, homogeneity, maturation time, and dosing mode directly affect the reproducibility of the optimum and the operational stability of the process [32].

Overall, the application landscape of chitosan in water treatment highlights a common optimization logic:

- selection of chitosans with appropriate degree of deacetylation and molecular weight, in order to balance charge density and bridging capacity;
- control of pH within a range that maximizes protonation of amino groups when the target is anionic, typically under acidic to weakly acidic conditions;
- identification of a narrow optimal dosage window, avoiding overdosing that may lead to restabilization;
- definition of mixing/contact conditions compatible with aggregation kinetics and with the desired separability by sedimentation or filtration.

The available quantitative results on model systems containing dyes such as RB5 and AB92 show that the dependence on pH and influent load can be described through linear correlations useful for process control and design, whereas evidence on mineral turbidity and performance under slightly acidic conditions suggests the possibility of operating at very low dosages in specific matrices [32], [79]. From this perspective, the following section may naturally focus on applications in complex matrices and on how intrinsic variables (DD and MW) and operational variables (pH, ionic strength, dosage, and mixing/settling conditions) are combined to maximize not only color and turbidity removal, but also process robustness under real conditions.

2.6. Crustacean-derived chitosan vs fungal-derived chitosan

The comparison between crustacean-derived chitosan and fungal-derived chitosan is not simply a matter of “the same molecule from a different source.” The literature shows with sufficient clarity that biological origin and extraction pathway substantially affect the structural and performance-related parameters of the material, such as degree of deacetylation (DD), molecular weight (MW), polydispersity, and the presence of residual impurities (for example proteins, minerals, or associated polysaccharide fractions). These characteristics, in turn, determine solubility, reactivity, viscosity, mechanical properties, behavior in water, and even biological response in biomedical contexts [38], [81], [82]. In this sense, referring to “chitosan” without specifying origin, DD, and MW introduces an ambiguity that may be critical both in comparisons between studies and in industrial translation, because two “nominally” identical chitosans may show divergent performances simply due to differences in microstructure or purity [38], [81].

On the crustacean side, the most evident advantage is that the supply chain is historically consolidated and relies on an abundant raw material, namely waste from the seafood industry, including shells and non-edible fractions of shrimp and crabs. However, the very nature of this waste explains why extraction is often chemically intensive. Recent reviews report that crustacean residues typically exhibit a “mixed” composition, in which significant fractions of minerals (mainly calcium carbonate), proteins, and chitin coexist, together with pigments and lipids, with indicative ranges that may reach approximately 30–50% CaCO_3 , 30–40% proteins, and 20–30% chitin [81]. This compositional complexity makes the classical process steps almost unavoidable:

- (i) acid demineralization to remove CaCO_3 ,
- (ii) alkaline deproteinization to remove proteins,
- (iii) possible depigmentation steps, and finally
- (iv) deacetylation under alkaline conditions to convert chitin into chitosan [38], [81].

The critical point emphasized in the review literature is that these “conventional” routes tend to require harsh conditions. Deacetylation is commonly performed with highly concentrated NaOH solutions, on the order of 30–50% w/v, and at temperatures above 100 °C, which entails significant energy consumption and the production of chemical effluents requiring management [38], [81]. Moreover, the severity of the treatment introduces a technical trade-off: while harsher conditions may increase DD, they may also promote depolymerization and a reduction in MW, with direct consequences for viscosity, processability, and, in many cases, the mechanical properties of the final material [82]. In practice, the crustacean industry benefits from a robust and scalable route, but often pays for this robustness with a substantial chemical footprint and with the risk of “shaping” product properties more through degradation than through fine control.

Another recurring issue with crustacean sources is variability. Even when the production plant is stable, the raw material may vary depending on species, geographical area, and seasonality, which translates into batch-to-batch differences in DD and MW. In some fields, especially biomedical ones, this becomes a substantial reproducibility problem [38]. The consequence is not merely theoretical: if DD and MW change, then charge density and rheological behavior also change, and therefore the interaction of chitosan with proteins, cells, or contaminants changes as well. For regulated applications, this implies strict specifications and robust analytical controls [38], [82]. Added to this is the discussion regarding potential allergenicity, or at least the presence of animal-derived protein residues. The literature indicates that if the process is not optimized or if controls are inadequate, protein traces may persist and become relevant in sensitive applications [38]. This does not mean that crustacean chitosan is “intrinsically” unsuitable, but rather that the traditional supply chain requires a level of standardization and validation that is not always homogeneous across producers and commercial grades, and that this may sometimes be reflected in the differences observed among studies.

In the case of fungal chitosan, the narrative in the literature is often structured around two key concepts: bioprocess controllability and the potential reduction of chemical intensity. Fungi do not exhibit the same mineralization as shells; there is no equivalent to the structural CaCO_3 typical of crustaceans. As a result, the need for heavy demineralization is eliminated, and this, at least in principle, opens the way to milder processes

or to routes more readily integrated into biorefinery logic [38], [81]. Furthermore, production through cultivation or fermentation allows, within certain limits, modulation of product properties through the choice of species and growth conditions, reducing dependence on the seasonality typical of marine supply chains [38], [82]. Reviews dedicated to the topic emphasize that fungal sources may offer greater reproducibility and, in some cases, lower polydispersity, precisely because the input biomass is generated under controlled conditions rather than collected as a by-product of a highly variable ecological–industrial system [38].

From the perspective of measurable properties, some comparative studies make this comparison particularly concrete. In one study often cited in the literature, indicative values are reported showing higher DD for fungal chitosans than for some crustacean counterparts, for example a DD of approximately 85% for fungal chitosan compared with about 71% for crustacean chitosan in the reported comparison, although this is associated with lower MW and therefore lower viscosity [83]. This combination of high DD and lower MW may be advantageous when the objective is to maximize solubility and reactivity, for example in mildly acidic aqueous formulations or in processes requiring a rapid and controllable response, but it may become a limitation when the application requires high mechanical robustness and structural stability, typically associated with higher MW. An interesting application example comes from a comparison of alginate/chitosan membranes for wound dressing, where the use of fungal chitosan is associated with more porous morphologies and higher uptake and water vapor permeability, whereas the use of high-MW animal chitosan tends to favor better mechanical properties and greater water stability [84]. In other words, fungal chitosan may be particularly competitive where porosity, transport, and interaction with the surrounding environment are central, for example in exudate management and gas exchange in a dressing, whereas high-MW crustacean chitosan may retain an advantage when the priority is the mechanical resistance of the support [84].

A further argument often raised in favor of fungal chitosan is its “non-animal” profile. The absence of crustacean-associated proteins is considered a potential advantage in terms of perceived safety and compatibility for some applications, although, in a rigorous thesis framework, it is important to stress that safety does not derive automatically from the source, but rather from the combination of source, process, and quality control. At the same time, however, fungal sources also present a specific challenge: the fungal cell wall is a complex matrix in which chitin/chitosan is integrated with other polysaccharides, particularly glucans. This means that extraction may produce associated fractions, for example chitosan–glucan complexes, that are not always completely removed. A classical characterization study indeed shows that non-negligible amounts of glucans may remain associated with “chitinous” fractions, with reported ranges on the order of approximately 7–40% depending on the organism and the fraction analyzed, indicating that purification may be non-trivial and that the purity of fungal chitosan must be demonstrated and quantified case by case [61]. In addition, yield and even the actual presence of chitosan in the biomass may strongly depend on the species. In the same study, it was observed that a significant chitosan fraction can be obtained from *Mucor rouxii*, whereas in *Aspergillus niger* chitin but not chitosan was detected, showing that the generalization “fungi produce chitosan” is not always valid and that strain selection is crucial [85]. This point directly connects to an industrial gap: the fungal supply chain, although promising, still requires optimization in terms of scalability, inter-

producer standardization, and the definition of shared technical specifications, especially if it is to compete with crustacean-derived material in terms of volume and cost [38], [82].

For a balanced discussion, it is useful to focus clearly on the pros and cons, because both materials present real strengths and structural weaknesses. If one considers, for instance, the issue of availability and supply chain, crustacean chitosan currently benefits from a broad industrial base and a mature market, but this abundance is linked to a supply chain—fishing and seafood processing—with intrinsic variability and not always predictable availability. Fungal chitosan, by contrast, can theoretically be produced year-round through fermentation and process-control strategies, but it still has to demonstrate full competitiveness in terms of volume and cost in a broadly generalizable way [38], [82]. If one instead considers process impact and “harshness,” the crustacean route often remains anchored to intensive acid–base and high-temperature steps that generate effluents and require environmental management, whereas the fungal route has greater potential to adopt milder conditions or to integrate into biorefineries. However, this potential cannot be assumed automatically, because the complexity of the fungal wall and the need to separate associated fractions such as glucans may introduce new steps and new process costs [38], [82], [85]. With respect to standardization, the literature converges on the idea that fungal production has a conceptual advantage because growth conditions can be controlled and repeated, but, in parallel, there remains a need to define comparable analytical criteria—DD, MW, impurities, acetylation pattern—that would allow real harmonization between studies and among producers [38], [81]. Finally, on the performance side, application studies indicate that fungal chitosans may offer highly interesting functional properties such as porosity, permeability, and performance in wound-dressing contexts, whereas high-MW crustacean chitosans often retain a mechanical advantage. In practical terms, this implies that the choice of source should not be ideological, but rather guided by the functional specification of the target application [83], [84].

In conclusion, the literature supports an interpretation in which the main achievements of the crustacean supply chain lie in industrial maturity, commercial availability, and the possibility of obtaining high-MW chitosans with excellent mechanical properties, whereas the main gaps concern the chemical intensity of the processes, effluent management, raw-material variability, and issues related to impurities, allergenicity, and standardization [38], [81]. For the fungal route, the most solid achievements are the theoretical controllability of the supply chain through non-seasonal production, the possibility of obtaining materials with more uniform properties, and, in some cases, a convincing biomedical applicability profile in terms of functional performance. The main limitations, however, concern production scale and economic competitiveness, as well as the need to rigorously address the complexity of the fungal matrix—associated fractions, species dependence, and cell-wall physiology—through purification protocols and comparable analytical standards [38], [82], [85]. It follows that, at the current state of knowledge, neither source “wins” in an absolute sense. Rather, the choice between fungal and crustacean chitosan should be formulated as a rational compromise among performance requirements, such as mechanical strength versus transport properties, safety and purity requirements, process controllability, and sustainability objectives, while taking as a non-negotiable constraint the complete characterization of the material—DD, MW, and impurities—in order to ensure comparability and

reproducibility [38], [81]. In table 2.2. has resume the main differences between crustacean and fungal chitosan derived.

Later on the comparison between the two supply chains, within the specific context of water treatment, crustacean-derived chitosan currently remains the most immediately effective solution for large-scale applications, especially in flocculation and coagulation processes, where the higher molecular weight typical of commercial products favors polymer bridging between particles, floc growth, and sedimentation, thereby ensuring consolidated performance in systems with high turbidity or suspended load [30]. However, this operational superiority does not automatically imply a structural superiority of the material. Fungal chitosan, in fact, shows characteristics that may be advantageous in more advanced treatment scenarios, such as the selective adsorption of organic or metallic contaminants, the preparation of functionalized membranes or beads, and integration into biorefinery systems, thanks to greater controllability of physicochemical properties and a potentially more homogeneous composition derived from production processes managed under controlled conditions [38], [82]. From this perspective, the choice between the two sources should not be interpreted as an absolute alternative, but rather as a compromise among functional requirements, application scale, and sustainability goals. Crustacean chitosan currently appears preferable for bulk and already established industrial applications, whereas fungal chitosan emerges as a promising platform for future developments oriented toward material modularity, valorization of residual biomass, and the construction of integrated circular supply chains. Consequently, within the framework of the present thesis, the comparison between the two sources is not interpreted in terms of immediate substitution, but rather as a progressive technological evolution in which fungal chitosan may assume an increasingly relevant role as production, purification, and standardization processes become competitive at the industrial scale.

Parameter	Crustacean-derived chitosan	Fungal-derived chitosan
Raw material source	Exoskeleton waste from shrimp, crab and other marine crustaceans	Cell wall components of filamentous fungi (e.g., <i>Mucor</i> , <i>Rhizopus</i> , <i>Aspergillus</i>)
Feedstock availability	Abundant by-product of seafood industry but dependent on fishing activity and seasonal variability	Produced through controlled fermentation processes; potentially year-round production
Extraction complexity	Requires multiple chemical steps including demineralization (acid), deproteinization (alkaline) and deacetylation	Usually does not require strong demineralization due to absence of CaCO ₃ ; extraction focuses on cell wall separation
Typical mineral content in raw biomass	High (mainly CaCO ₃ , often 30–50%)	Very low mineral fraction

Typical impurities	Residual proteins, pigments, minerals	Associated polysaccharides such as β -glucans
Degree of deacetylation (DD)	Typically 70–90% depending on processing conditions	Often comparable or slightly higher in some studies ($\approx$ 80–90%)
Molecular weight (MW)	Often higher due to extraction from crystalline chitin	Frequently lower due to biological structure and extraction conditions
Polydispersity	Can vary significantly depending on raw material variability	Potentially more uniform due to controlled biomass production
Mechanical properties	Often higher mechanical strength due to higher MW	Typically lower mechanical strength
Solubility and reactivity	Good solubility in acidic conditions; behavior strongly dependent on DD and MW	Often higher reactivity due to lower MW and accessible functional groups
Allergenicity concerns	Possible traces of crustacean proteins if purification is incomplete	Generally considered non-animal origin; lower allergenic concerns
Process environmental impact	Often associated with intensive acid–base treatments and chemical waste streams	Potentially lower chemical intensity but still dependent on purification process
Industrial maturity	Highly established industrial production and global supply chain	Emerging production with increasing research interest
Typical applications	Water treatment, food packaging, agriculture, biomedical materials	Biomedical materials, functional membranes, adsorption systems, emerging environmental applications
Main advantages	High availability, scalable production, strong mechanical properties	Controlled production, non-animal origin, potentially more uniform properties
Main limitations	Variability of raw material, intensive chemical extraction processes	Lower industrial scalability and possible presence of associated polysaccharides

Table 2.2 summarizes the main differences between crustacean-derived and fungal-derived chitosan reported in the literature.

2.7. Gaps in the literature and rationale of the study

Despite the large number of studies devoted to chitosan-based water treatment technologies, most available research focuses on adsorption processes and chemically modified materials tested under controlled laboratory conditions. Consequently, a significant gap remains regarding the practical application of commercially available chitosan as a coagulation–flocculation agent for the treatment of real industrial wastewater and its

validation under progressive scale-up conditions. [86]. However, this effectiveness has almost always been demonstrated in model systems, with synthetic solutions and controlled operating conditions, whereas validation in real matrices—characterized by ionic competition, natural organic matter, and variable pH—remains limited and fragmented [86], [87].

Recent review studies also highlight that most research efforts are focused on coagulation-flocculation mechanisms, composite materials, or chemically modified chitosan, often developed to enhance specific removal performances under controlled laboratory conditions. While these approaches demonstrate promising removal efficiencies, they frequently rely on synthetic dye solutions or simplified wastewater matrices, limiting their transferability to complex industrial effluents [88]. Furthermore, the growing use of modified chitosan materials—such as grafted polymers, cross-linked composites, and functionalized derivatives—introduces additional complexity in material synthesis, cost, and environmental assessment, potentially reducing the practical feasibility of large-scale implementation [89].

However, in contrast to adsorption-based approaches and advanced material design, coagulation–flocculation using commercially available chitosan remains comparatively underexplored. Most published studies focus either on synthetic dye solutions or on modified chitosan derivatives specifically engineered to enhance flocculation performance. Consequently, there is a lack of studies investigating simple chitosan formulations applied directly to real industrial wastewater characterized by complex and variable compositions. Furthermore, only a limited number of investigations evaluate process performance beyond laboratory scale, making it difficult to assess operational robustness and industrial feasibility.

A first major gap concerns the transferability of results from batch scale to continuous scale. Most of the available studies focus on batch experiments or short-term laboratory tests, whereas systems that are more representative of process engineering, such as fixed-bed columns, continuous-flow membranes, or pilot reactors, remain poorly investigated. This limits the possibility of evaluating fundamental phenomena such as fouling, time-dependent loss of efficiency, and mechanical stability of the material, all of which emerge as critical aspects in real applications [86]. Even for chitosan-based membranes, the literature explicitly reports a lack of long-term studies in real wastewater, making it difficult to estimate material durability and the actual operational sustainability of the proposed systems [90]. In addition, several recent experimental studies confirm that chitosan-based coagulation–flocculation is effective in laboratory conditions, with removal efficiencies exceeding 80–90 % for turbidity, COD, and organic compounds under optimized parameters such as pH, mixing time, and dosage [90]. However, these investigations remain largely limited to controlled experimental setups and do not address long-term process stability or industrial scalability, thus reinforcing the gap between laboratory performance and real industrial applicability.

While the limitations associated with chitosan stability, regeneration, and coagulation-flocculation selectivity have been extensively discussed in the literature, these aspects are less relevant when chitosan is employed as a single-use coagulation–flocculation agent. In this context, the major challenges become process optimization, floc formation, sludge separation efficiency, and the transferability of laboratory-scale results to larger operational scales. The native polymer exhibits limited solubility outside the acidic range and tends to lose its

cationic charge under neutral or alkaline conditions, drastically reducing its efficiency in many natural and industrial waters. In addition, swelling phenomena, structural disintegration, and mechanical fatigue may occur during repeated regeneration cycles, especially in continuous-flow systems, where the material is exposed to hydrodynamic stress and chemical fluctuations in the matrix [90]. This explains why many studies focus on chemical modification or composite formation, implicitly showing that native chitosan, although promising, is not yet an engineeringly robust material for large-scale applications.

Another limitation identified in the literature is the low selectivity of chitosan toward specific contaminants. Although the presence of amino and hydroxyl groups enables interaction with a wide range of chemical species, this same versatility leads to competition among contaminants, which may reduce removal efficiency in complex matrices [91]. This implies that unmodified chitosan often behaves as a generic rather than a selective coagulant/flocculant, thus requiring the development of functionalized materials capable of discriminating between target species and interfering compounds.

Furthermore, recent studies investigating modified chitosan-based flocculants report high removal efficiencies for dyes and suspended solids, often exceeding 90 %, but these results are commonly obtained using synthetic dye solutions and chemically grafted polymers, limiting direct comparison with real industrial wastewater conditions [92]. This further highlights the need for studies focused on simple, industrially available materials applied directly to complex wastewater matrices.

The literature also highlights issues related to material regeneration and life-cycle sustainability. Although many chitosan-based systems can be regenerated by acidic or alkaline elution, such processes often result in polymer degradation, release of functional components, and production of secondary effluents containing concentrated contaminants, which require further treatment [86]. From an environmental point of view, conventional chitosan production based on intensive alkaline and acidic treatments also generates chemical waste streams and requires significant energy consumption, partly reducing the ecological advantage of the material [86]. This aspect shows that the concept of a “sustainable biopolymer” must be evaluated over the entire life cycle and not only during the use phase.

Another crucial gap concerns the lack of methodological standardization in experimental studies. Chitosan performances are often reported under different operating conditions in terms of pH, ionic strength, initial contaminant concentration, and characteristics of the aqueous matrix, making direct comparison among results difficult and hindering the definition of reliable reference parameters [93].

Unlike most previous studies, the present work does not aim to develop novel chitosan-based materials, but rather to evaluate the effectiveness, robustness, and scalability of a simple and commercially available chitosan-based coagulation–flocculation process under real industrial conditions. In particular, the study examines washing water derived from a flexographic cardboard printing plant located in northern Italy, characterized by a variable mixture of dyes, surfactants, and process residues generated by the cleaning of printing rollers. This matrix represents a particularly challenging real system, in which the simultaneous presence of soluble organic compounds, surface-active agents, and dispersed pigments reproduces operating conditions that are far removed from the model systems commonly investigated in the literature. In this context,

the objective of the thesis is not only to verify the effectiveness of chitosan as a flocculant, but also to test its process stability and technological transferability through a progressive scale transition, from the laboratory to increasing volumes and finally to plant-scale experimentation. Therefore, the present work focuses specifically on chitosan-based coagulation–flocculation applied to real industrial wastewater, aiming to evaluate process robustness, scalability, and industrial transferability, rather than developing novel materials under controlled laboratory conditions.

Chapter 3 — Materials and Methods

The objective of this work was not solely to verify the effectiveness of chitosan as a flocculant—whose use is already widely documented in the literature—but rather to progressively develop a treatment process applicable to a complex real matrix, evaluating its stability, reproducibility, and scalability toward increasing operational levels. Unlike many laboratory studies based on synthetic solutions or single model contaminants, the experimentation was conducted directly on industrial wastewater generated from the washing of flexographic printing machines, and the process was consistently carried out using commercially available chitosan already present on the market.

This methodological framework is consistent with technology development studies, where adaptive experimental design is used to progressively refine process performance under real operational constraints.

This approach required the development of an iterative methodology, in which the flocculant formulation, treatment sequence, and system configuration were progressively adapted based on the results obtained in previous trials. In this sense, the experimental pathway can be interpreted as a gradual technological development process, starting from small-scale batch experiments and leading to the realization of operational prototypes capable of treating progressively larger volumes of wastewater. Due to the high variability of the real industrial wastewater investigated in this study, the experimental design was intentionally developed according to an adaptive engineering approach rather than a strictly factorial experimental scheme. This strategy is commonly adopted in applied process development, particularly when dealing with complex real matrices, where preliminary identification of operational windows is necessary before systematic optimization. Therefore, variations in operating conditions across the experimental phases should be interpreted as part of a progressive process development strategy rather than as methodological inconsistency. Within this framework, the experimentation was not conceived as a linear sequence of experiments, but rather as a process organized into functional phases, each addressing a specific operational question, sometimes even revisiting the same question at different stages with increased awareness throughout the industrialization process. During the industrialization process, preliminary tests were conducted on small volumes with the aim of verifying the actual effectiveness of chitosan on real wastewater matrices, which differ in composition and behavior from the model systems commonly described in the literature. This phase allowed confirmation of the formation of separable flocs and enabled the identification of an initial relationship between flocculant dosage and the amount of sedimentable material. In the initial screening phase, process response was primarily evaluated through floc formation, phase separation, and supernatant clarification, as these parameters were the most immediately sensitive indicators for rapidly identifying promising treatment conditions. Once a sufficiently stable operating window had been identified, the assessment was extended to analytical parameters including UV–Vis absorbance, COD, conductivity, and other physicochemical indicators, thus enabling a progressively more quantitative evaluation of treatment performance. This approach allowed the identification of reproducible operating windows prior to further scale-up and process optimization.

A second phase was dedicated to process stabilization through the optimization of both flocculant formulation and operational parameters. Throughout the experimental activities, two different types of commercial chitosan were evaluated. In the initial phase, a crustacean-derived chitosan, commonly used also in food applications, was employed. Subsequently, a fungal-derived chitosan was introduced, selected not only for reasons related to raw material availability and improved traceability, but especially to ensure better reproducibility in the preparation of flocculant solutions, enhanced long-term stability, and greater compatibility with potential industrial-scale applications. Fungal-derived chitosan was therefore adopted as the reference material in the subsequent phases of the study, representing a turning point in process development also from the perspective of supply chain sustainability.

The experimental work increasingly focused on assessing the scalability of the treatment by progressively increasing treated volumes and introducing test configurations closer to real operational systems. At several intermediate stages, the maintenance of process efficiency at larger volumes, the management of solid–liquid separation, and the influence of operational parameters such as pH, mixing speed, and contact time were evaluated. This analysis was carried out both during the design phase of Prototype 1 and subsequently replicated in analogous stages of Prototype 2 (pilot scale), following the increased process understanding acquired through prior experimentation. On this basis, the treatment system implemented in Prototype 1 was structured as a two-stage process: a primary physico–chemical flocculation phase, responsible for the main removal of color and suspended matter, followed by a physical separation and polishing phase achieved through progressive filtration. In Prototype 1, additional and secondary treatment modules were also acquired and implemented to validate the feasibility of industrializing a natural polymer-based treatment system aimed at meeting discharge requirements for wastewater reuse in sewer systems. These additional modules included quartz filtration cartridges and activated carbon filters (necessary for significantly reducing COD), as well as a softening unit, implemented to remove calcium and magnesium responsible for the high hardness of the wastewater. In contrast, Prototype 2 (pilot scale) focused on refining the removal of chemical species through physico-chemical coagulation processes and improving separation efficiency without the use of additional downstream treatment modules following the primary chitosan treatment. At this stage, greater attention was devoted to engineering aspects and industrial transferability, with the system designed for potential application in a production context, equipped with electronic automation and intended for continuous operation.

The data collected and their analysis throughout the experimental campaign were conducted following an adaptive rather than factorial approach. Although this adaptive experimental strategy allowed progressive optimization of the treatment process under realistic operational constraints, it also introduced some methodological limitations. In particular, not all experimental conditions were tested under fully controlled factorial schemes, and replicate experiments under identical conditions were limited, especially during pilot-scale tests involving larger treatment volumes. This approach reflects the engineering-oriented nature of the work, where process development and scalability were prioritized over strictly statistical experimental design. Nevertheless, future investigations based on systematic factorial designs and replicated experimental conditions would further strengthen the statistical robustness of the results and improve reproducibility

assessment. To carry out this experimental pathway, a range of materials and reagents were employed, including commercial powdered chitosan used as a natural flocculant, acetic acid (80%) for polymer solubilization, cationic polyacrylamide, and distilled water for solution dilution.

Analytical reagents included cuvette-based test kits (Nanocolor) for the determination of COD, BOD₅, hardness, surfactants, ammonium, and other physicochemical parameters such as copper, calcium, and magnesium concentrations (all parameters of interest for the industrial partner generating the wastewater). Laboratory instrumentation included a UV–Vis spectrophotometer, multiparameter probes for pH and conductivity measurements, and rapid colorimetric systems. For the experimental procedures, standard laboratory equipment was used, including magnetic stirrers, technical glassware, and progressive filtration materials consisting of meshes and textile supports with different pore sizes.

The laboratory and pilot-scale activities described above, the development of the experimental pathway was supported by the technical contribution of ISINNOVA Srl, which provided access to laboratory facilities, pilot-scale equipment, and engineering expertise. The collaboration with ISINNOVA Srl played a key role in enabling the transition from laboratory-scale experimentation to prototype development, supporting the design, construction, and optimization of the treatment systems implemented throughout the study. The availability of industrial-grade instrumentation and technical resources allowed the experimental work to be conducted under conditions closer to real operational scenarios, facilitating the progressive scale-up of the process and the evaluation of engineering constraints related to mixing, solid–liquid separation, and system automation. Furthermore, the joint development approach, involving continuous interaction between research and engineering activities, contributed to shaping the adaptive experimental framework adopted in this work and supported the iterative development of the treatment technology.

3.1 Characterization and sampling of the industrial wastewater

The wastewater investigated in this study originates from the washing operations of rollers and equipment used in flexographic printing on corrugated cardboard. The washing waters, collected through the internal drainage system of the facilities, constitute a mixture containing residues of inks, detergents, and auxiliary process components. The samples analyzed in this thesis were collected from two different production plants, referred to as Plant A and Plant B, belonging to the same industrial supply chain, allowing both temporal and spatial variability of the matrix to be evaluated.

Sampling campaigns were conducted at multiple time points between 2023 and 2025, including samples intended for internal analyses (pH, conductivity, and initial ink concentration) as well as samples sent to certified external laboratories for the determination of key physicochemical parameters. The pH values were generally close to neutrality, typically ranging between approximately 6.7 and 6.9, with occasional alkaline values, whereas electrical conductivity exhibited a wider variability, ranging from approximately 1700 to over 5000 $\mu\text{S}/\text{cm}$, with peaks reaching up to 20,000 $\mu\text{S}/\text{cm}$. This variability is significant and reflects the concentration degree of the wastewater matrix.

Analyses performed by external laboratories revealed very high organic loads, with chemical oxygen demand (COD) values on the order of tens of thousands of mg/L and BOD₅ values that, although lower, were still

significant. In particular, the presence of non-ionic surfactants in considerable concentrations confirms the highly stable colloidal nature of the system, which hinders spontaneous sedimentation of pigment particles and justifies the need for controlled destabilization strategies. Elemental analyses also highlighted the recurrent presence of metals such as copper, aluminum, and calcium, at concentrations consistent with the typical composition of flexographic inks and mineral fillers used in industrial formulations. These findings are in agreement with technical datasheets of the inks used in the facilities, which indicate the presence of carbon-based pigments, phthalocyanine derivatives, and mineral additives dispersed in an alkaline aqueous phase stabilized by light amines. Internal measurements, although less comprehensive than certified laboratory analyses, allowed continuous monitoring of wastewater evolution during treatment tests and provided immediate operational indicators for process performance evaluation.

To illustrate the temporal and spatial distribution of sampling campaigns and analyses, a summary scheme was developed, synthesizing the sampling events at the different facilities and distinguishing between internal and external analyses (Figure 3.1). This representation provides an overview of the experimental basis of the study and highlights the continuity of monitoring over time.

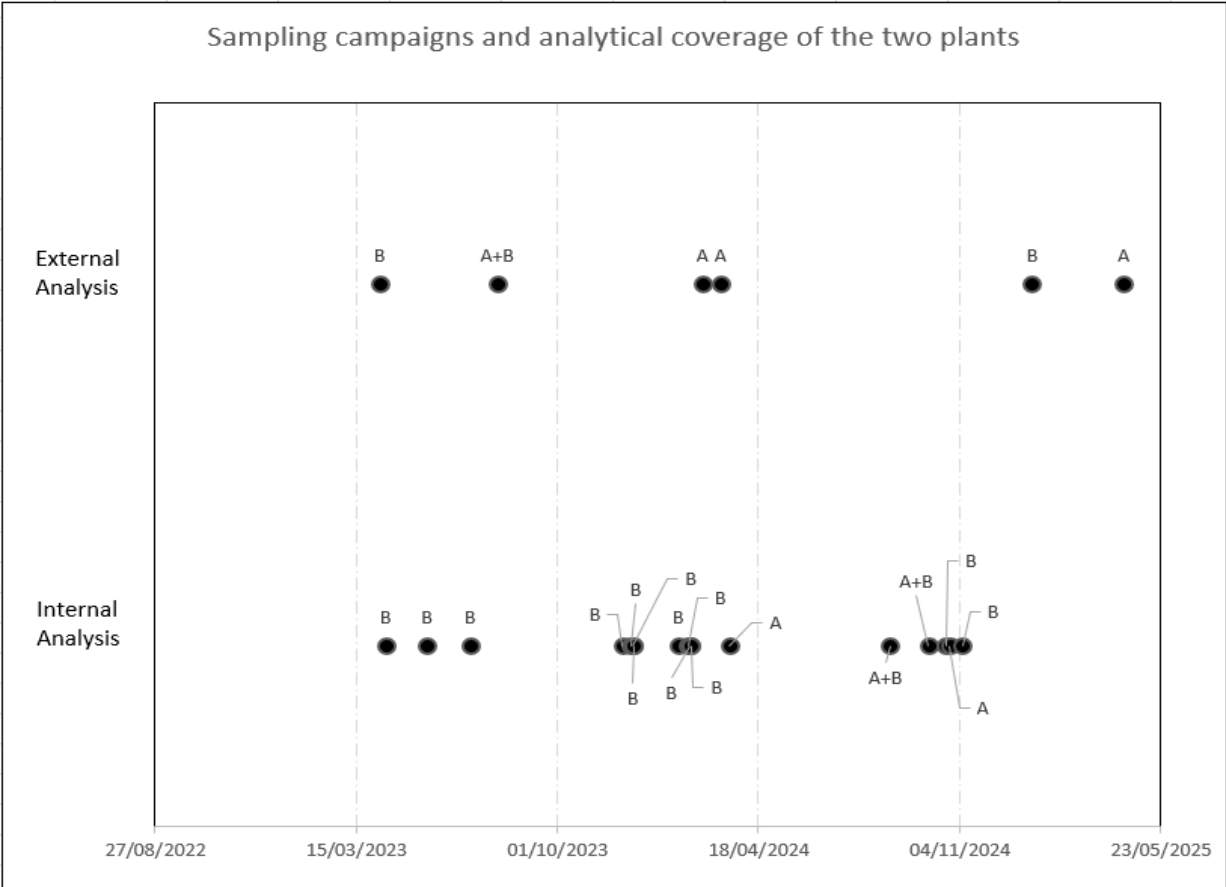


Figure 3.1. Temporal distribution of wastewater sampling campaigns and analytical activities conducted between 2023 and 2025 at the two facilities under study. The figure distinguishes between internally performed analyses and those carried out by certified external laboratories, highlighting the continuity of monitoring and the spatial variability of the wastewater matrix.

The analytical measurements were systematically performed for each experimental test, allowing a complete comparative framework between pre- and post-treatment conditions. This approach enabled evaluation of process efficiency not in absolute terms but in relation to the specific characteristics of the treated wastewater in each cycle, ensuring direct control over the evolution of the main physicochemical parameters.

The temporal distribution of analyses reflects the progression of the experimental work. In the initial phase, most determinations were focused on wastewater from Plant B, used as the reference matrix during batch tests and pre-prototyping stages due to its greater availability and reproducibility. Subsequently, with the transition to system engineering and the design of Prototype 2, the focus shifted toward wastewater from Plant A, where the pilot plant was intended to be implemented.

This shift does not represent a methodological discontinuity but rather a coherent choice aligned with the objective of validating the process under real operating conditions, assessing its robustness, adaptability, and technological transferability.

Internal analyses were further oriented by application requirements related to the development of a treatment solution for the industrial supply chain, with particular attention to parameters relevant for discharge into public sewer systems and potential water reuse, in accordance with national regulatory frameworks (Italian Legislative Decree 152/2006) (Table 3.1).

External analyses were performed by certified laboratories to obtain a comprehensive chemical characterization of the wastewater matrix, including substances regulated at the European level and determined using standardized analytical methods. Detailed methodologies and results are reported in Chapter 4.1.

Category	Parameter	Limit value	Unit
General parameters	pH	5.5 – 9.5	–
	COD	500	mg/L
	BOD ₅	250	mg/L
	Total suspended solids (TSS)	200	mg/L
	Oil and grease	40	mg/L
	Total surfactants	10	mg/L
	Electrical conductivity	Not fixed*	μS/cm
Nutrients	Ammonium (NH ₄ ⁺)	30	mg/L
	Total nitrogen (N)	40	mg/L
	Total phosphorus (P)	10	mg/L
Metals and metalloids	Iron (Fe)	2	mg/L
	Manganese (Mn)	2	mg/L
	Copper (Cu)	0.5	mg/L
	Zinc (Zn)	2	mg/L
	Nickel (Ni)	2	mg/L
	Chromium total	2	mg/L

Chromium VI	0.2	mg/L
Lead (Pb)	0.5	mg/L
Cadmium (Cd)	0.02	mg/L
Mercury (Hg)	0.005	mg/L

Table 3.1. *Electrical conductivity is not explicitly regulated by Legislative Decree 152/2006 for discharge into sewer systems and may be subject to local authority requirements depending on the receiving wastewater treatment plant.*

3.2 Materials used

3.2.1 Chitosan and reagents

In the present study, two different commercial chitosans were employed, selected in accordance with the objective of developing a treatment process potentially transferable to real industrial applications.

The first material used was a commercial crustacean-derived chitosan (Oxford Vitality), supplied in powder form with a declared purity of 90%. Available technical information indicates that the material consists primarily of poly-(1→4)-2-amino-2-deoxy-β-D-glucan, with a pH ranging between 5.5 and 7.5 in a 1% aqueous solution, and with no hazard classification according to the CLP regulation. For this material, certified data regarding molecular weight and degree of deacetylation were not available, as the product was originally intended for food and nutraceutical applications (Fig. 3.2).



Figure 3.2. *a) Oxford Chitosan; b) Acetic acid (80%); c) Chito-F.*

Crustacean chitosan solutions were prepared in acidified aqueous conditions using acetic acid as a weak solvent, in order to promote protonation of the amine groups and the consequent solubilization of the polymer. Oxford Vitality chitosan was employed during the preliminary laboratory investigations. Subsequently, the fungal-derived Chito-F (Chito-F – Fig. 3.2) formulation was selected for the scale-up phases and pilot-scale applications because of its easier preparation, commercial availability, and suitability for industrial implementation. This product consists of a mixture of fungal-derived chitosan obtained from *Aspergillus niger* and fumaric acid. According to the technical datasheet, the product contains approximately 40% chitosan, while the remaining fraction consists mainly of the acidic component.

In both cases, the chitosan–acid mixture was subjected to low-speed magnetic stirring until complete solubilization of the material was achieved, typically within approximately 60 minutes under batch conditions.



Figure 3.3. Cationic polyacrylamide used as a coagulant.

Oxford Vitality chitosan was employed exclusively during the preliminary proof-of-concept laboratory investigations, with the primary objective of understanding the behaviour of chitosan under acidic conditions and verifying its potential as a flocculating agent. These initial experiments were intentionally exploratory and focused on demonstrating the feasibility of the coagulation–flocculation process. At this stage, the chitosan dosage was optimized only with respect to floc formation and contaminant removal, without considering the final solution acidity or the potential operational implications associated with large-scale application.

Once the flocculation mechanism and the main operating principles had been established, supported by the available literature, subsequent experimental activities were carried out using the commercial fungal-derived Chito-F formulation. This material was selected for all optimization, scale-up, and pilot-scale investigations because it represented the product intended for industrial implementation. The following chapters therefore describe an iterative process development strategy focused on process optimization, scalability, and the transition from laboratory experimentation to industrial application (lab-to-market approach). In the advanced stages of the experimental work, a synthetic cationic polyacrylamide polymer in powder form was used as a flocculation aid. The product was applied in addition to chitosan, acting as a bridging agent to promote the growth and compaction of already formed flocs, thereby improving subsequent solid–liquid separation.

Prior to use, the polymer was dissolved in water and applied at concentrations ranging between 0.1% and 0.2%, with a dosage in the wastewater treatment phase of approximately 0.01–0.02% of the total volume. The solution was dosed in a subsequent step relative to the primary flocculant, in order to maintain a clear separation between the colloidal destabilization phase, attributed to chitosan, and the floc consolidation phase. The use of cationic polyacrylamide represented an auxiliary treatment introduced to improve the operational stability of the process in experimental configurations closer to application scale. The polymer solution was introduced at a later stage during the development of Prototype 2 (pilot scale), in order to keep the two mechanisms of action clearly distinct.

3.2.2 Laboratory materials and auxiliary treatments

During the experimental development of the treatment process, various laboratory materials, filtration systems, and auxiliary devices were employed, progressively selected according to the scale of the tests and the operational requirements that emerged throughout the study. In the initial stages, following wastewater flocculation with the chitosan solution, the separation between the liquid phase and the semi-solid fraction was carried out by gravity filtration using filter paper. This method was adopted as a preliminary approach to verify the formation of separable flocs and to obtain an initial qualitative assessment of filtrate clarity. To provide an indicative evaluation of the size of the aggregates formed during the flocculation process, filtration meshes with different pore sizes (100, 200, and 300 mesh) were subsequently employed. This approach allowed an empirical estimation of floc granulometry and their retention capacity on filtering supports with varying pore dimensions.

Flocculation tests were conducted in laboratory beakers, in which the wastewater was mixed with the chitosan solution under controlled agitation using a magnetic stirrer. After a sedimentation period, the supernatant liquid phase was separated from the settled semi-solid fraction (sludge) and subjected to filtration. To improve the reproducibility of solid–liquid separation, a vacuum-assisted filtration system was later introduced, consisting of a suction pump and cellulose filters. This setup enabled faster and more efficient phase separation and allowed the treatment of larger sample volumes compared to gravity filtration alone.

The quantities of reagents and wastewater used in the experiments were measured using standard graduated laboratory glassware (graduated cylinders, pipettes, and syringes), ensuring repeatability of dosing and accurate definition of volumetric ratios between wastewater and flocculant solution.

Following separation, the clarified liquid was preliminarily evaluated through visual observation of clarity and turbidity, used as qualitative indicators of treatment effectiveness. Under optimal operating conditions, this procedure revealed suspended solids removal efficiencies generally ranging between 95% and 99%. In the subsequent stages of the study, particularly during the development of larger-scale prototypes, additional separation systems and auxiliary components were introduced, including cartridge filters, filtration columns, transfer pumps, and flexible tubing. These elements were implemented as auxiliary treatments aimed at improving the removal of residual particles and stabilizing the quality of the treated effluent.

3.3 Experimental design

The experimental design of the present study was developed following a progressive approach, aimed at transferring the flocculation process from controlled laboratory conditions to operational configurations increasingly close to application scale. The main objective was not solely to verify the effectiveness of chitosan as a flocculant under ideal conditions—an aspect already well established in the literature—but rather to construct an experimental pathway capable of evaluating process stability, performance reproducibility, and the scalability of the system toward larger operational volumes.

Unlike a purely statistical experimental design, typically structured through predefined factorial matrices and systematic replicates, the present work is configured as an adaptive technological development process. Operational conditions were not fixed a priori in a definitive manner but were progressively modified and refined based on the results obtained in previous tests, following an incremental improvement strategy and a gradual approximation to real industrial conditions. The apparent heterogeneity of the experiments conducted over time therefore reflects the exploratory and iterative nature of the development process, rather than a lack of methodological planning.

During the experimental development, the treatment process was not statically defined from the initial stages, but instead emerged progressively through a pathway of validation, adaptation, and reformulation guided by the response of the real wastewater matrix. The definition of dosage parameters, flocculant solution composition, and operating conditions—following laboratory validation—was continuously correlated with the behavior of the wastewater, characterized by high physicochemical complexity and non-negligible variability.

To fully understand the nature of the experimental process, the entire work can be divided into four distinct phases of experimental maturity, resumed in figure 3.4. and as it follows:

Exploratory phase: The objective of this phase was to verify the feasibility of process industrialization starting from the real wastewater matrix and to evaluate the scalability of the treatment through batch-scale laboratory experiments. This phase included studies aimed at defining the operational window of chitosan in terms of pH, solution type, and chemical composition, as well as optimal mixing speed (rpm), contact time, and solution durability over time. All these aspects were investigated with the aim of potential commercialization of the process in a competitive market, requiring dosages aligned with economic constraints. The primary effectiveness criterion in this phase was visual observation of the process, which had to demonstrate a minimum level of separation between solid and liquid phases.

Prototyping development phase: This phase included experiments conducted at larger, “semi-industrial” scales, with treated volumes ranging from 20 L to 50 L, up to 100 L in final prototyping tests. A continuous adaptive process is evident in response to the main challenge of industrialization: wastewater variability. The dosage of the chitosan solution was adjusted depending on the nature of the wastewater and the observed effectiveness of the flocculation process, influenced by the concentration of both chitosan and acetic acid in the treatment solution. The objective of this phase was to adapt the treatment in order to meet specific threshold values for selected chemical parameters of the wastewater. This was achieved by integrating secondary treatment modules into the process, including quartz and activated carbon filtration systems, as well as a softening unit used in the final stages to remove calcium and magnesium, responsible for the high hardness of the treated wastewater. This phase demonstrated the reproducibility potential of the system and enabled process stabilization in subsequent stages. The treated wastewater was systematically analyzed for pH, conductivity, COD, and surfactants.

Formulation stabilization phase: In this phase, conducted after prototyping, greater emphasis was placed on the reproducibility of the chitosan solution, with a focus on optimizing its formulation and dosage within the

treatment process, as well as confirming the optimal pH operating window. Additionally, the minimum effective dosage of a secondary coagulant—cationic polyacrylamide (C-PAM)—was investigated. This polymer was introduced as a coagulant–flocculant aid to enhance the separation of solid and liquid phases. This phase was essential for understanding the interaction between the two flocculant/coagulant agents, effectively transforming the treatment from purely chemical (chitosan-based) to chemical–physical. Process effectiveness was evaluated through visual observation, phase separation efficiency, and measurements of pH and conductivity of the treated wastewater.

Pilot phase: The final phase focused on the design of the pilot plant and the transferability of the knowledge developed in the previous stages to a pre-industrial system. It is important to note that all tests in this phase were conducted at Plant A, using wastewater generated from that specific facility. The use of secondary treatment modules introduced during the prototyping phase was eliminated and replaced by the integration of C-PAM, which had demonstrated a significant improvement in flocculation efficiency and mechanical separation of chromophoric compounds during earlier experiments. Consequently, pilot-scale experiments were designed to validate the treatment under industrial conditions through a single-stage process, consisting of chemical–physical separation using chitosan and C-PAM, without additional downstream modules. Process effectiveness was evaluated through the analysis of key physicochemical parameters of the treated wastewater, including pH, conductivity, hardness, turbidity, surfactant concentration (cationic, anionic, and non-ionic), ammonium ion (NH_4^+), COD, and UV–Vis absorbance. The obtained values were compared with the requirements specified by the client for potential discharge or reuse within the sewer system, in accordance with the limits established by Legislative Decree 152/06. In the final stage of the experimental work, a comparative pilot-scale test was conducted using a conventional coagulant/flocculant, ferric chloride (FeCl_3), in order to evaluate the performance of the developed system against a traditionally applied treatment solution. The comparison of physicochemical parameters obtained in both cases allowed the proposed process to be positioned within a competitive application context.

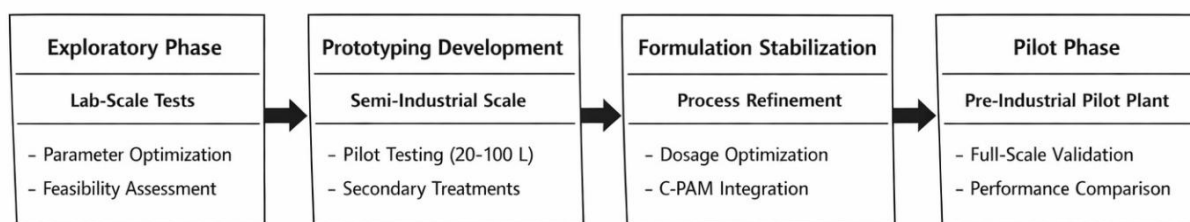


Figure 3.4. *Workflow and phases of the industrialization of wastewater treatment with chitosan*

3.3.1 Description of experimental prototypes

The process engineering development was structured into two successive technological phases, corresponding to different levels of experimental maturity of the system. In the first phase, Prototype 1 was developed,

conceived as a modular platform for process development and validation of flocculation and solid–liquid separation at an intermediate scale (20–100 L). This configuration enabled the analysis of real wastewater behavior and the progressive optimization of key operational variables, including flocculant dosage, filtration line configuration, and hydraulic parameters, leading to the identification of a stable and reproducible operating window.

Based on the knowledge gained in this phase, Prototype 2 was subsequently designed as an engineered pilot plant, developed to operate at larger volumes (≈ 200 L per cycle) and characterized by semi-automated process management. The system integrates reagent preparation and dosing, wastewater mixing, and primary mechanical separation through a drum filter, resulting in a modular structure more closely resembling an industrial application context.

The two prototypes therefore represent successive stages within the same technological development pathway: the first focused on understanding and optimizing process variables, and the second on verifying the transferability of operational conditions into a system closer to industrial application, corresponding to a technological readiness level (TRL) progression from TRL 1 to TRL 7.

3.3.1.1 Prototype 1

Prototype 1 was conceived as a modular validation platform aimed at the progressive optimization of the flocculation process and subsequent solid–liquid separation stages at intermediate scale. The system was initially designed to operate with volumes between approximately 20 and 50 L, eventually reaching, in its final configuration, treatment tests up to 100 L of wastewater.

The consolidated plant configuration, shown in Figure 3.5, consisted of a modular treatment line organized into multiple functional stages, preceded by a feed tank and a pumping system. Upstream of the line, the wastewater—after mixing with the chitosan-based flocculant solution and undergoing initial separation of settleable material—was conveyed into a collection tank and subsequently directed to downstream treatment modules. The first section of the system was dedicated to primary mechanical separation of flocculated material through polypropylene bag filters and, in more advanced configurations, polyester filters with a mesh size of approximately 250 μm . These units were designed to remove larger flocs and distribute the solid load along the treatment line. The clarified fluid was then transferred via pump to the secondary treatment section. In the final configuration, the first polishing module consisted of two filtration columns arranged in series, containing quartz and activated carbon, respectively. The quartz functioned as a granular filtration medium for the removal of residual particles, while the activated carbon was used to reduce residual organic matter, color, and part of the surfactant content present in the treated matrix. The second module consisted of a cartridge filtration line with progressively decreasing pore sizes, with a final configuration of 10 μm – 1 μm – 1 μm . This setup was adopted to distribute the residual solid load and improve the clarity of the treated effluent. In the final stage of development, a cation-exchange resin softener was also integrated, regenerated using a NaCl solution, with the aim of reducing the hardness of the treated wastewater. Sampling and control points equipped with pressure gauges were installed along the treatment line, allowing monitoring of the evolution of the main

physicochemical parameters at different stages of the process. In particular, pH, conductivity, COD, BOD₅, surfactants, hardness, ammonium, and UV–Vis absorbance were analyzed to evaluate process performance and final effluent quality. These parameters were also compared with the requirements for discharge into the public sewer system according to Legislative Decree 152/2006, with the aim of assessing the compatibility of the treated wastewater with discharge scenarios and, where possible, internal reuse in accordance with the client's operational needs. The development of Prototype 1 proceeded through a series of progressive configurations, in which flocculant dosage, module sequence, and operational conditions were continuously adjusted. In total, eleven preliminary tests were conducted, during which various treatment elements were introduced or removed, including activated carbon in batch or pseudo-column configurations and, in some cases, ozonation trials.

Overall, Prototype 1 represented a development and consolidation platform for the treatment process, through which it was possible to define a stable modular configuration, verify process scalability up to 100 L, and identify the operational conditions subsequently transferred to the pilot phase.

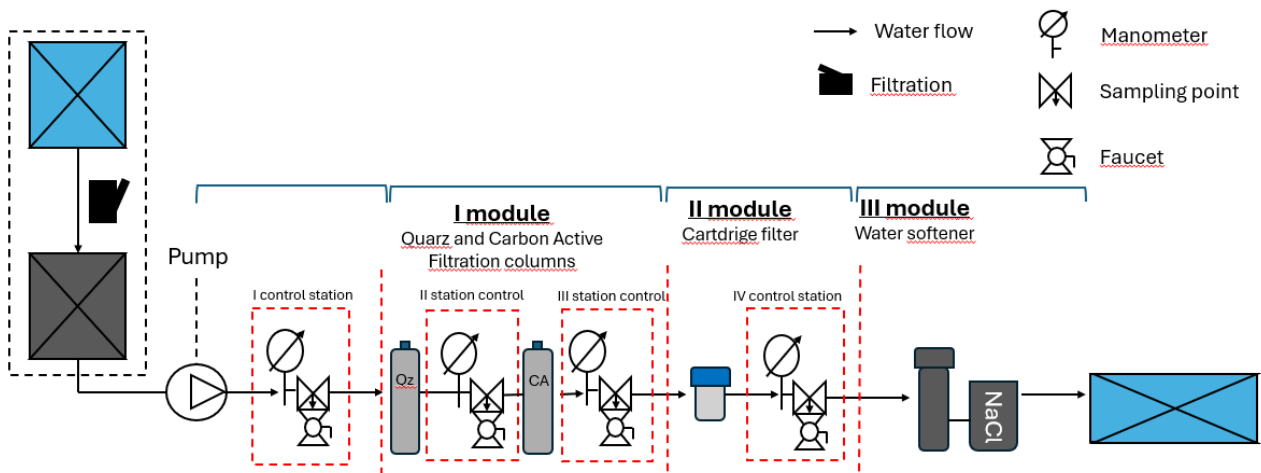


Figure 3.5.: Functional scheme of Prototype 1 for the modular treatment of post-flocculation effluent. The system consists of a pumping line followed by three modules arranged in series: (I) filtration columns containing quartz (Qz) and activated carbon (AC); (II) progressive filtration through three cartridge filters in series ($10\ \mu\text{m} - 1\ \mu\text{m} - 1\ \mu\text{m}$); (III) a cation-exchange resin softener with regeneration using NaCl. Intermediate control stations, equipped with pressure gauges and sampling points, allow monitoring of head losses and collection of samples along the treatment line (S1: post-flocculation wastewater; S2: post-quartz; S3: post-activated carbon; S4: post-cartridge filtration; S5: final effluent).

As anticipated in the previous chapters, the final configuration of Prototype 1 (Figure X) is the result of an iterative process aimed at identifying an effective and reproducible strategy for the mechanical separation of suspended solids from the wastewater following the chemical flocculation stage with chitosan. A total of eleven tests were conducted to achieve this setup, which can be grouped into three main technological phases:

Phase I – Preliminary study (0–20 L): The first phase had an exploratory nature and was aimed at transferring batch experiments to an intermediate scale. The treatment line included a feed tank, preliminary filtration using

a polypropylene filter placed downstream of chitosan mixing, a centrifugal pump for fluid transfer, and a final filtration stage carried out either through a cartridge filter or a filter press with a nominal pore size of 10 μm . The objective of this phase was to verify the possibility of maintaining solid–liquid separation efficiency as the treated volume increased, while identifying initial operational issues related to head losses, filter clogging, and flow stability.

- **Phase II – Operational stabilization (20–40 L):** The second phase focused on stabilizing the system in response to the variability of wastewater from Plant B. Pre-filtration elements were introduced, consisting of polyester filters with a mesh size of 250 μm coupled with polypropylene filters, along with experimental filtration columns containing activated carbon for the reduction of residual organic matter and surfactants. In parallel, a progressive mechanical filtration system was implemented using cartridges with decreasing pore sizes (100–60–20 μm), followed by final filtration using a filter press at 1 μm . In some configurations, oxidative treatments through ozonation were also tested, as well as the integration of activated carbon directly into the filtration cartridges.

- **Phase III – Meta-prototyping (≈ 50 L):** The third phase represented the transition toward a more structured system configuration, with a flocculant solution already optimized in terms of performance and cost-effectiveness. The treatment line included pre-filtration through polypropylene and polyester filters (250 μm), mechanical filtration using cartridges (100–50–20 μm), an integrated activated carbon module, and fine filtration through three cartridges in series (10–1–1 μm). During this phase, the filtration sequence was progressively rationalized until reaching the final modular configuration, which was subsequently adopted in Prototype 1.

Overall, these three phases enabled the definition of a stable modular treatment line based on progressive particulate removal and physicochemical polishing of the effluent, forming the technical basis for the design of Prototype 2.

3.3.1.2 Prototype 2 – Pilot system

Prototype 2, referred to as the pilot system, was conceived as a simplified and more engineered pilot plant compared to the previous configuration, with the objective of focusing exclusively on flocculation efficiency and primary mechanical separation.

Unlike Prototype 1, in which secondary polishing modules (adsorption columns, series filtration units, and a softener) were integrated, these elements were removed in the second system in order to isolate the actual contribution of the chemical phase and the subsequent drum filtration stage (Fig. 3.6).

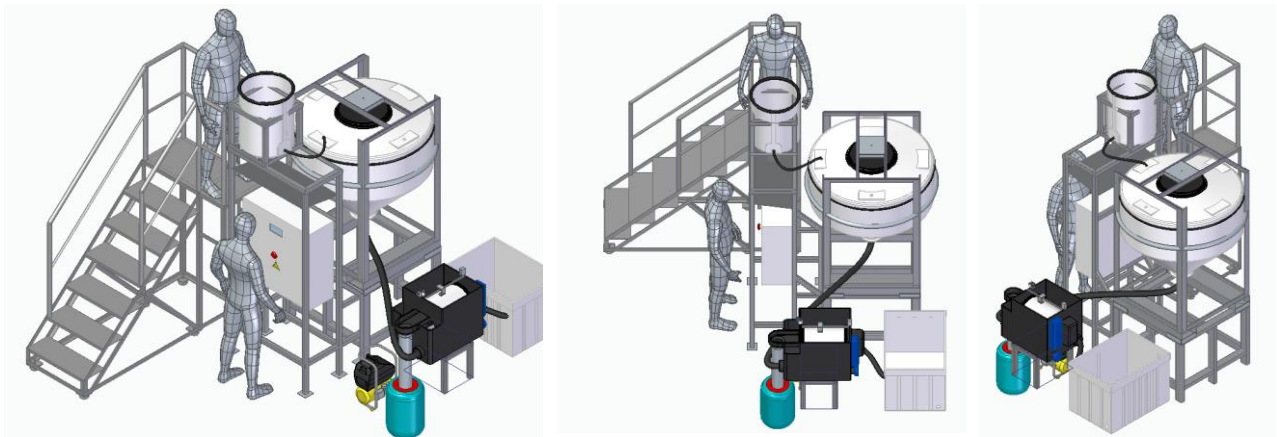


Figure 3.6. Official rendering of the pilot plant (Prototype 2), illustrating the system layout from multiple perspectives.

The system consists of a main storage and mixing tank for the wastewater, with an operational capacity of up to approximately 200 L per cycle. Within this tank, the controlled addition of the Chito-F-based flocculant solution and the polymeric coagulant aid C-PAM takes place. The tank is equipped with a mechanically driven stirrer with adjustable speed, designed to ensure proper homogenization of the wastewater and effective dispersion of the reagents. The Chito-F solution is prepared in a dedicated smaller-volume tank, also equipped with a mixing system, and subsequently dosed into the main tank. Similarly, the C-PAM solution is prepared separately and introduced into the system at a later stage relative to the primary flocculant. This configuration allows the colloidal destabilization phase and the floc growth phase to remain distinct, while still occurring within a single treatment basin.

Downstream of the mixing tank, a transfer line consisting of piping and a pumping system conveys the treated wastewater to a drum filter. This unit represents the only mechanical separation module in the system and is responsible for removing the flocculated solid fraction through surface retention of the flocs and discharge of the separated sludge. The drum filter therefore constitutes the central element of the physical treatment phase, replacing the modular sequence of progressive filtration adopted in Prototype 1. The system is completed by a control panel that manages the operation of mixers, pumps, and the drum filter, enabling semi-automated operation of treatment cycles and improving the reproducibility of operating conditions compared to the previous configuration. Overall, Prototype 2 can be described as a compact and functionally streamlined system, consisting of: a mixing tank, reagent preparation and dosing units, a pumping line, and a drum filter. The removal of secondary polishing modules allowed the experimental analysis to focus on the intrinsic efficiency of flocculation and on the capability of the primary separation system to ensure stable and reproducible removal of the solid fraction under operating conditions closer to a potential industrial application.

3.3.2 Treatment sequences

The experimental campaign is summarized in Table 3.2.

Test ID	Volume [L]	Chitosan dosage [mL/L]	Mixing time [min]	RPM	Time Contact [h]	Filtration type	Additional treatment
Exploratory Phase							
Lab 1	0.1–0.5	–	–	–	-	Gravity filtration	–
Lab 2	0.1	–	–	–	-	Gravity filtration	–
Lab 3	0.5	–	–	–	-	Gravity filtration	-
Prototype Phase							
Test I–IV	20	variable	variable	variable	4	PP filter / filter press (10 µm)	Activated carbon - ozonation
Test V–VI	30	variable	variable	variable	4	PP filter / filter press (10 µm)	Activated carbon - Biocide
Test VII–VIII	40	variable	variable	variable	2	PP filter / filter press (10 µm)	Activated carbon
Test IX–XI	50	variable	variable	variable	2	PP filter / filter press (10 µm)	Activated carbon
Prototype 1	50–100	optimized	controlled	controlled	1	Modular filtration	Activated carbon - Softener
Stabilization Formulation Phase							
Lab 4–6	–	optimized	controlled	controlled	-	Gravity filtration	–
Pilot Phase							
Prototype 2	200	optimized	controlled	controlled	1	Drum filter	C-PAM

Table 3.2. Summary of the different phases of workflow dealing with treatment sequences from the beginning of lab prelaminal test until pilot test. Variable conditions indicate parameters adjusted iteratively during the experimental campaign.

3.4 Analytical methods

Analytical determinations were carried out with the aim of monitoring treatment efficiency under different experimental configurations and assessing the compliance of the effluent with reference parameters for discharge and potential industrial reuse. Internal analyses were performed using a bench-top spectrophotometer NANOCOLOR® Advance (Macherey-Nagel), a monochromator-based instrument operating within a spectral range of 340–800 nm, employing pre-dosed cuvette tests (tube tests) from the same product line. This setup enabled rapid, reproducible photometric determinations compatible with continuous experimental monitoring. UV–Vis measurements were conducted by scanning within the available spectral range using standard cuvettes with a 10 mm optical path length. Spectrophotometric analysis was used to evaluate variations in absorbance of the wastewater before and after treatment, with particular attention to the reduction of absorption maxima associated with pigment chromophores. When necessary, samples were subjected to preliminary filtration using filter paper (5–8 µm) to remove interference caused by residual turbidity not representative of the dissolved fraction.

Chemical oxygen demand (COD) was determined using the dichromate method in pre-dosed cuvettes (NANOCOLOR® COD 4000), based on the oxidation of organic substances in an acidic environment with controlled thermal digestion followed by photometric measurement. Samples characterized by high concentrations were appropriately diluted to fall within the measurement range of the method. Results are expressed in mg/L O₂.

Biochemical oxygen demand over five days (BOD₅) was determined using a photometric method equivalent to APHA/ISO standards, with controlled incubation at 20 °C for 5 days, following sample dilution when necessary to ensure measurement reliability. This parameter was used as an indicator of the biodegradable fraction of residual organic matter.

Surfactants were determined by distinguishing between different chemical classes using dedicated cuvette tests. Anionic surfactants were measured using the methylene blue active substances method (MBAS), analogous to DIN 38409-H23 and APHA 5540C; cationic surfactants were determined using a photometric method based on disulfine blue; non-ionic surfactants were measured using the TBPE (tetrabromophenolphthalein ethyl ester) method, based on the formation of a colored complex. Concentrations were expressed in mg/L and, when required, total surfactant concentration was calculated as the sum of the individual measured fractions.

Ammonium ion (NH₄⁺) concentration was determined using the indophenol blue photometric method, based on the reaction with hypochlorite and salicylate, in accordance with APHA 4500-NH₃ F, ISO 7150-1, and DIN 38406-E5 standards. Results were expressed in mg/L NH₄⁺. Total hardness was determined using a photometric method with phthalein violet, while copper concentration was measured using a photometric method based on cuprizone. Chlorides were determined using a semi-quantitative cuvette test.

Total solids were eventually (but not always) determined using a gravimetric method. A known volume of sample (100 mL) was evaporated to dryness on a heating plate and subsequently dried at 105 °C until constant weight was achieved. The concentration of solids was then calculated by relating the residual mass to the initial

sample volume, with results expressed in g/L. This parameter proved particularly relevant during the experimental design phase, as it was used to define the ratio between flocculant solution volume and the actual mass of ink present in the wastewater.

Chapter 4 — Experimental Results

4.1 Exploratory Phase

4.1.1 Initial characterization of ink-based wastewater

The preliminary characterization of the wastewater under study was carried out on samples collected from two distinct production facilities, identified as Plant A and Plant B, both operating in the industrial ink production and processing sector (EWC 08.03.08 – aqueous liquid waste containing ink). The analytical campaigns, conducted between 2023 and 2025, made it possible to assess not only the physicochemical composition of the matrix but also its temporal variability, a key factor in the design of the chitosan-based treatment process. For Plant A, three analytical campaigns were considered (07/04/2023, 11/03/2024, and 07/04/2025). The first two campaigns highlight a relatively stable matrix, with pH values between 6.7 and 6.9, conductivity ranging from 1716 to 2540 $\mu\text{S}/\text{cm}$, TOC in the range of 6300–6700 mg/kg, ammoniacal nitrogen between 140 and 420 mg/kg, non-ionic surfactants between 116 and 284 mg/kg, and copper concentrations between 117 and 122 mg/kg. This configuration describes a wastewater characterized by a high organic load, significant presence of non-ionic surfactants, and a metallic contribution consistent with pigments and formulation additives typical of ink production.

The campaign conducted on 07/04/2025, however, represents a significantly more critical scenario, with a pH of 10.0 (alkaline matrix), COD of 28,199 mg/L, and BOD₅ of 11,244 mg/L, along with an ammonium concentration of 152 mg/kg and copper concentration of 121 mg/kg. The 2025 dataset represents a case of extreme organic load, with COD values exceeding 28 g/L, highlighting substantial production variability and a potential operational challenge linked to plant conditions. Overall, Plant A is therefore characterized by a high-strength wastewater matrix, with a pronounced surfactant contribution and significant operational fluctuations.

For Plant B, the campaigns considered were those conducted on 15/11/2024, 06/12/2024, and 22/02/2024. The analyses show pH values between 6.8 and 6.9, conductivity ranging from 3540 to 3670 $\mu\text{S}/\text{cm}$, COD between 12,250 and 14,650 mg/L, ammonium between 130 and 167 mg/kg, and copper concentrations between 88 and 95 mg/kg. Compared to Plant A, wastewater from Plant B exhibits a high organic load but generally lower than the peak values observed for Plant A in 2025, greater pH stability, and a comparable but slightly lower metal content. The surfactant fraction varies depending on production formulations but remains a structural component of the matrix.

Table 4.1 reports the data expressed as ranges derived from the analytical campaigns, along with the average values calculated for samples collected at the two plants.

Parameter	Plant A – Range	Plant A – Mean	Plant B – Range	Plant B – Mean	Unit
pH	6.73 – 10.00	7.87*	6.79 – 6.91	6.85	–
Conductivity	1716 – 2540	2128	3540 – 3670	3605	μS/cm
COD	28199*	28199**	12250 – 14650	13450	mg/L
BOD₅	11244*	11244**	5085 – 5606	5346	mg/L
TOC	6390 – 6750	6570	–	–	mg/kg
Total hardness	10.2 – 14.2	12.1	11.0 – 14.2	12.6	°F
Ammoniacal nitrogen	140 – 420	280	130 – 167	149	mg/kg
Non-ionic surfactants	116 – 284	200‡	<10 – 510	~260‡	mg/kg
Anionic surfactants	up to 52	~52‡	up to 52‡	~52	mg/kg
Cationic surfactants	<10	<10‡	<10‡	<10	mg/kg
Copper	117 – 122	119.5	88 – 95	91.5	mg/kg

Table 4.1. Ranges and mean values of the main physicochemical parameters of wastewater collected from Plant A and Plant B. * The mean pH value for Plant A is influenced by the alkaline sample collected in 2025 (pH 10); excluding this value, the average would be approximately 6.8. ** For COD and BOD₅ in Plant A, complete data are available only for the 2025 campaign; therefore, the reported “mean” corresponds to this single value. ‡ For surfactants in Plant B, the mean value is estimated based on the maximum observed concentration (510 mg/kg) and lower values (<10 mg/kg); it should therefore be considered indicative rather than statistically representative.

The comparison between the two analytical datasets highlights that both matrices are characterized by COD values exceeding 10 g/L, indicating wastewater with a high concentration of organic substances. However, Plant A shows greater temporal variability, including an alkaline scenario and organic loads exceeding 28 g/L, which can be considered a potential design “worst-case” condition. In contrast, Plant B exhibits a more homogeneous composition over the analyzed period.

From an engineering perspective, this implies that the chitosan-based flocculation process was tested both on a relatively stable matrix and on a matrix characterized by high variability and operational criticality. This dual experimental condition enhances the robustness of the results presented subsequently, allowing the evaluation of treatment efficiency not only under controlled conditions but also under scenarios of particularly high organic load and alkalinity.

Regarding the monitoring of wastewater parameters, internal analyses were systematically performed, as previously described in earlier chapters. For each experimental test—whether conducted at laboratory or prototypical scale—measurements of pH and conductivity were carried out using a multiparameter probe. Additionally, at selected stages of the experimental campaign, the concentration of ink in the wastewater

(expressed in g/L) and COD were also determined. Ink concentration was estimated gravimetrically by evaporating known volumes of wastewater and determining the dry residue. The resulting mass of solids was used as an approximation of the ink content and as a basis for defining the chitosan dosage during preliminary optimization tests. The collected data are reported in Table 4.2.

Date	Plant	pH	Conductivity (mS/cm)	Ink concentration (g/L)
13/04/2023	B	5.08	–	11.4
24/05/2023	B	6.05	–	18.5
06/07/2023	B	6.05	5.2	–
05/12/2023	B	6.75	1.9	–
13/12/2023	B	6.75	1.9	–
15/12/2023	B	6.75	1.9	–
29/01/2024	B	7.00	1.4	–
08/02/2024	B	7.00	–	–
10/02/2024	B	6.75	–	–
11/02/2024	B	6.75	–	–
20/03/2024	A	7.10	1.6	–
19/04/2024	A	–	–	–
19/04/2024	A	–	–	–
27/08/2024	A	–	–	12.8
27/08/2024	B	–	–	40.11
04/10/2024	A	7.96	1.5	12.01
04/10/2024	B	7.31	4.38	137.19
21/10/2024	B	7.01	4.8	11.02
25/10/2024	A	6.78	1.5	6.98
07/11/2024	B	7.06	4.8	15.93

Table 4.2. Overview of the main monitored parameters (pH, conductivity, initial ink concentration) during the experimental campaigns. Missing values indicate parameters not measured in the respective sampling events.

The analysis of the temporal trends of the main parameters monitored during internal controls highlights significant differences between the two matrices. Regarding pH, Plant B shows a progressive stabilization toward values close to neutrality (≈ 6.7 – 7.0), whereas Plant A exhibits more pronounced fluctuations throughout 2024, with values approaching 8. This behavior suggests greater operational variability and a possible influence of production conditions on the acid–base balance of the wastewater. Similarly, conductivity is consistently higher in Plant B during 2024 (≈ 4 – 5 mS/cm), indicating a greater dissolved ionic load, while in Plant A it remains lower and relatively stable (≈ 1.5 – 1.6 mS/cm). The trend of ink concentration (ConcINK) further highlights a marked peak in Plant B (up to 137 g/L in October 2024), compared to lower and less

variable values in Plant A. This parameter is particularly significant, as it directly reflects the variability of the pigment load and helps explain the differences observed in organic loads and matrix complexity between the two plants.

4.1.2 Study of optimal pH

In the preliminary phase of the study, an exploratory analysis was conducted to identify the pH window required to trigger the flocculation process. At this stage, no quantitative determinations of organic load removal were performed; instead, the system response was evaluated qualitatively based on visual observation of floc formation and clarification of the supernatant.

The experiments were carried out by progressively adjusting the system pH through variation of the dosage of the acidic chitosan solution. For each pH value, the evolution of the wastewater matrix and the appearance of macroscopically visible aggregates were documented photographically.

The experiment was performed in beakers containing 80 mL of wastewater from Plant B, with an ink concentration of 11.02 g/L (diluted 1:20 to enhance the visibility of flocculation). The samples were acidified with CH_3COOH to reach the target pH values, namely pH 6, pH 5, pH 4, and pH 3.5, as shown in Figure 4.1.



Figure 4.1. Experiment for the identification of the optimal pH. From left to right: raw sample, pH 6 sample, pH 5 sample, pH 4 sample, and pH 3.5 sample.

The observation of the pre-filtration samples (Figure 4.1) highlights a progressive destabilization of the wastewater matrix as pH decreases. The untreated sample (TQ) appears uniformly dark and shows no visible phase separation, confirming the high colloidal stability of the system. At pH 6 and 5, the wastewater maintains an intense coloration and a largely homogeneous dispersion, with limited evidence of macroscopic aggregation. Under these conditions, flocculation appears weak or incomplete.

A clear transition is observed at pH 4, where an initial distinction between the supernatant liquid phase and a more concentrated fraction at the bottom becomes evident. This separation becomes even more pronounced at pH 3.6, with the formation of a compact sediment and significant clarification of the supernatant.

These results indicate that effective destabilization of the colloidal system occurs within a pH window below approximately 4.5, consistent with the increased protonation of chitosan amino groups and the activation of charge neutralization and polymer bridging mechanisms.

Figure 4.2 shows the centrifuged sample, allowing a clearer visualization of floc formation and the separation between semi-solid and liquid phases.



Figure 4.2. *Visual appearance of samples after acidification, filtration, and centrifugation, highlighting floc formation and phase separation.*

Even after centrifugation, at pH values of 6 and 5 the system still appears predominantly dispersed, with intense coloration and no clear separation between the liquid and solid phases. Under these conditions, the colloidal stability of the wastewater is largely maintained, suggesting insufficient protonation of chitosan amino groups and consequently limited electrostatic interaction with pigment particles.

Below pH 4.5, a clear macroscopic change is observed: at pH 4, sedimentable aggregates begin to form and the supernatant becomes visibly clearer; at pH 3.5, flocculation is even more pronounced, with the formation of compact flocs and a well-defined separation of the solid phase. This behavior indicates that the response of the real wastewater system is not governed solely by the theoretical pKa of chitosan, but rather by the complex interaction between the protonated polymer, the surfactants present, and the dispersed pigment particles. These findings indicate the existence of a critical destabilization threshold located between pH 5 and pH 4, consistent with the need for a sufficiently acidic environment to ensure full protonation of the -NH_2 groups of chitosan and to promote charge neutralization and polymer bridging mechanisms. This observation allowed the definition of a preliminary operating window for the subsequent quantitative optimization phases of the process.

4.1.3 Influence of mixing speed and contact time

In order to identify the most favorable operating conditions for the flocculation process, a series of experimental tests was conducted by varying the mixing speed (rpm) and the contact time between the chitosan-based flocculant solution and the wastewater. The objective of this phase was to evaluate the influence of hydrodynamic conditions on colloidal particle aggregation and floc formation.

During the tests, the wastewater was treated with the flocculant solution under controlled operating conditions, systematically varying mixing speed and contact time. At the end of the mixing phase, the samples were subjected to filtration and visually evaluated to verify the occurrence of separation between the liquid and solid phases.

For these experiments, a flocculant solution volume of 0.5 mL was used in 100 mL of wastewater solution. This choice was made in order to isolate the effect of hydrodynamic conditions on the flocculation process, maintaining a constant and relatively low polymer dosage. In this way, it was possible to assess the ability of the system to generate colloidal aggregation as a function of mixing speed and contact time, avoiding the masking effect of excessive flocculant dosage on the differences between operating conditions. Therefore, the rpm–time tests should be interpreted as a preliminary operational study aimed at identifying a favorable working window for the process, while the dosage used in the prototypical phase was defined at a later stage based on the overall optimization of the treatment.

In cases where clear floc formation and a transparent or slightly yellowish filtrate were observed—indicative of effective flocculation—UV–Vis absorbance analysis was subsequently performed. Conversely, when no evident separation of the solid phase was observed and the filtrate appeared turbid or grayish, spectrophotometric measurements were considered non-representative and were therefore not carried out.

Table 4.3 reports the set of tests performed, indicating for each combination of mixing speed and contact time the observed outcome in terms of floc formation and whether absorbance analysis was subsequently conducted.

Contact time (min)	RPM	Replicate	pH	Conductivity (mS/cm)	Flocculation observed	UV–Vis analysis
30	100	1	6.98	5.49	No	No
30	150	1	7.04	5.63	No	No
30	250	1	6.97	5.74	Yes	Yes
30	100	2	6.54	5.58	No	No
30	150	2	6.40	5.59	No	No
30	250	2	7.00	5.54	Yes	Yes
30	100	3	6.38	5.52	No	No
30	150	3	5.67	5.32	Yes	Yes
30	250	3	5.75	5.30	Yes	Yes
60	100	1	6.50	5.70	No	No
60	150	1	6.43	5.80	No	No
60	250	1	6.68	5.71	No	No
60	100	2	6.27	5.49	No	No
60	150	2	5.89	5.53	Yes	Yes
60	250	2	6.30	5.51	No	No
60	100	3	5.40	5.27	No	No

60	150	3	6.57	5.58	No	No
60	250	3	5.60	5.10	No	No
120	100	1	5.57	5.16	Yes	Yes
120	150	1	5.96	5.43	Yes	Yes
120	250	1	6.23	5.53	No	No
120	100	2	6.73	5.93	No	No
120	150	2	6.65	5.81	No	No
120	250	2	6.81	5.78	No	No
120	100	3	6.23	5.61	No	No
120	150	3	5.91	5.54	No	No
120	250	3	5.86	5.58	Yes	Yes

Table 4.3. Results of flocculation tests performed by varying contact time (30, 60, and 120 min) and mixing speed (100, 150, and 250 rpm).

Overall, the formation of stable flocs was observed in 8 out of 27 tests, corresponding to approximately 30% of the tested operating conditions. This result highlights that the flocculation process is strongly dependent on hydrodynamic mixing conditions and on the contact time between the flocculant and the wastewater, confirming the existence of a specific operational window within which the process is effective.

In the samples where flocculation was effective, UV–Vis spectrophotometric analysis was subsequently performed in order to qualitatively assess the reduction of the chromophoric fraction of the wastewater.

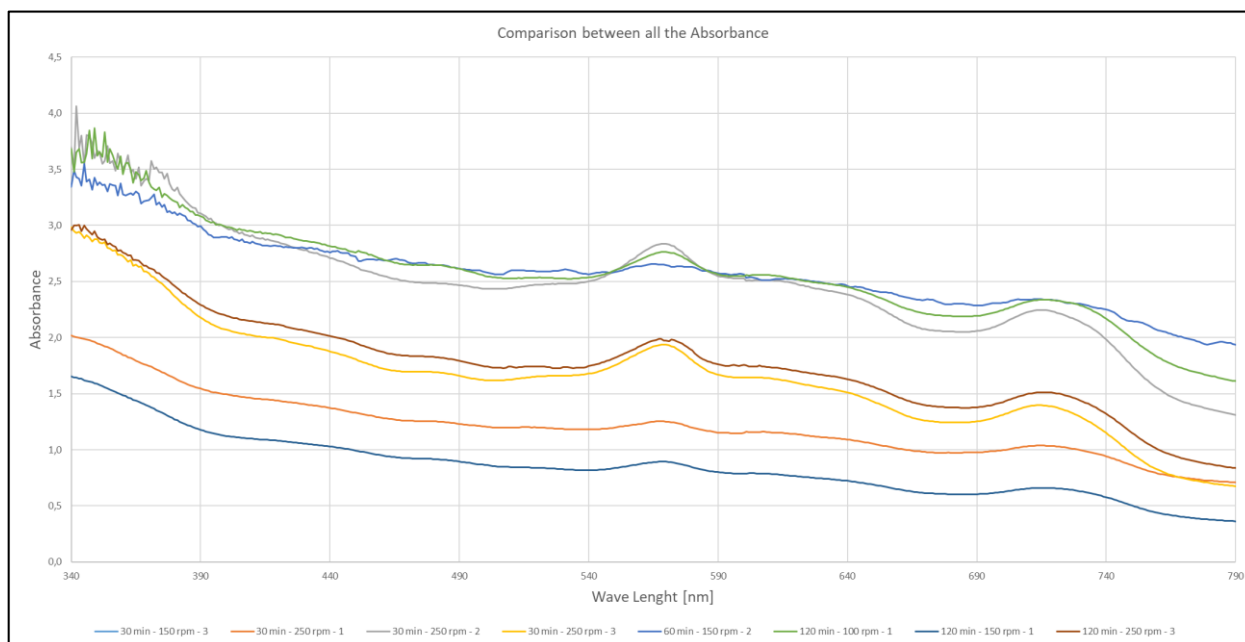


Figure 4.3. Comparison of UV–Vis absorbance spectra of samples showing effective flocculation and subsequent filtration.

The overall analysis of the experimental results shows that operating conditions significantly influence floc formation and the subsequent separation of the solid phase. In particular, specific combinations of mixing speed and contact time promote interaction between the polymer and the colloidal particles present in the wastewater, leading to the formation of more compact and easily separable aggregates. Rather than representing a purely exploratory sequence of isolated trials, the tests reported in Table 4.3 allowed the identification of a restricted operational window in which flocculation could occur. The fact that only a limited subset of the tested combinations resulted in stable floc formation supports the interpretation that process effectiveness depends on the combined effect of pH, mixing intensity, and contact time, rather than on a generic visual impression.

In general, tests conducted under intermediate mixing and contact time conditions exhibit the lowest absorbance values across the entire analyzed spectral range (340–800 nm), indicating a more effective removal of the colored fraction of the wastewater. Conversely, some tests characterized by longer contact times or less favorable mixing conditions show higher absorbance spectra, suggesting reduced separation efficiency.

A relevant aspect is that the reduction in absorbance occurs relatively uniformly across the entire spectrum, indicating that the treatment does not act selectively on a single chromophoric species, but rather results in an overall removal of the pigment fraction present in the wastewater. This behavior is consistent with the typical mechanism of chitosan-based flocculation, which relies on colloidal destabilization and adsorption of organic molecules onto the formed flocs.

Furthermore, an absorption maximum is observed in the region between approximately 560 and 580 nm, consistent with the presence of aromatic chromophoric compounds typical of ink formulations. The reduction of this signal in treated samples confirms the ability of the flocculation system to effectively remove these species from the liquid phase.

It is also observed that excessively low mixing speeds may limit contact between the flocculant and dispersed particles, while excessively high mixing conditions may lead to the breakage of newly formed flocs. Similarly, insufficient contact times do not allow the completion of aggregation processes, whereas longer times promote floc growth and stabilization.

Overall, the results allow the identification of an operational window in which the flocculation process is most effective: a contact time between 30 and 60 minutes and a mixing speed between 150 and 250 rpm. This represents an important element for defining operating conditions in the subsequent stages of process development and implementation at the prototypical scale, where a configuration of 60 minutes at 150 rpm is proposed, while 30 minutes at 250 rpm can be considered a valid alternative for reducing process time at the industrial scale.

4.1.4 Solution durability

The durability study of the flocculant solution was carried out with the aim of verifying the stability over time of the chitosan-based formulation developed during the previous experimental phases. From the perspective of potential industrial application, the flocculant solution is not prepared and used immediately but may be

stored for extended periods in storage tanks before being dosed into the wastewater. Therefore, verifying the persistence of flocculation efficiency over time represents a key step in assessing the operational transferability of the process and its compatibility with continuous or semi-automated treatment systems.

To this end, several concentrated solutions of the flocculant formulation were prepared, conventionally identified as solutions A–E (Table 4.4), and subsequently stored and tested at weekly intervals through flocculation experiments on real wastewater. The solutions were then labeled with a progressive number according to the week in which the test was performed (e.g., solution A tested in the second week = A2, in the third week = A3, and so on).

Component	Reference solution	A	B	C	D	E
Water in initial solution [% w/w]	70.78	0.78	5.78	10.78	15.78	20.78
CHITO-F [% w/w]	1.59	1.59	1.59	1.59	1.59	1.59
Acetic acid [% w/w]	27.63	27.63	27.63	27.63	27.63	27.63
Water in solution [% w/w]	70.78	0.78	5.78	10.78	15.78	20.78
Added water [% w/w]	0.00	70.00	65.00	60.00	55.00	50.00
Final Solution Composition						
Component	Reference solution	A	B	C	D	E
CHITO-F [% w/w]	1.59	5.30	4.54	3.98	3.53	3.18
Acetic acid [% w/w]	27.63	92.10	78.94	69.08	61.40	55.26
Water [% w/w]	70.78	2.60	16.51	26.95	35.07	41.56

Table 4.4. *Composition of the reference flocculant solution and of the dilution series (A–E) used in the stability study. The formulations were obtained by progressively diluting the initial concentrated solution with different amounts of water, while maintaining a constant ratio between chitosan (CHITO-F) and acetic acid. The resulting mixtures therefore differ in overall concentration but preserve the chemical interaction between polymer and acid. These solutions were subsequently tested over time to evaluate both the physicochemical stability of the formulation and the persistence of its flocculation performance under storage conditions.*

The different solutions did not represent chemically distinct flocculant formulations, but rather separate batches or preparations of the same reference solution with varying amounts of water, used to assess the persistence of flocculation performance during storage. Therefore, the experimental focus of the study was not on differences between individual formulations, but on the influence of solution concentration on stability over time, as well as on the effect of storage duration on the ability of the formulation to maintain its separation efficiency. This approach was adopted within a broader perspective of operational simplicity and practical applicability of the treatment. For each test, the wastewater was treated with the flocculant solution and subsequently subjected to filtration. In cases where flocculation was effective and the filtrate appeared clear or slightly yellowish—indicative of proper separation of the pigment fraction—the main physicochemical parameters of the filtrate were measured, namely pH and conductivity, together with UV–Vis absorbance in order to evaluate pigment removal efficiency through flocculation and filtration.

Not all tests resulted in complete separation of the pigment phase. In cases where the filtrate appeared turbid or greyish, indicating incomplete flocculation, pH and conductivity measurements were not considered representative of the separated liquid phase and were therefore not included in the summary table. This behaviour can be attributed to the delicate balance between the degree of chitosan protonation, solution viscosity, and the polymer's diffusion capability within the wastewater matrix. Under these conditions, both the most diluted and the most concentrated formulations exhibited more stable performance, whereas intermediate concentrations fell within a less favorable range for the formation of compact flocs. As a consequence, some of the tested solutions (in particular all B, C, and D solutions, as well as solution A1) are not included in the reported results, as the corresponding tests did not provide analytically reliable data for pH (Fig. 4.4), conductivity (Fig. 4.5), and UV–Vis absorbance spectra (Fig. 4.6).

The results shown in Figure 4.4 indicate that, in cases where flocculation was effective (solution A and solution E), the pH values of the filtrate consistently fall within a narrow range between approximately 5.3 and 5.6, with very limited variation across the different weeks of observation. This notable stability suggests that the chitosan-driven separation process brings the system toward a well-defined chemical window, consistent with the protonation of the polymer's amino groups and the charge neutralization mechanisms responsible for colloidal aggregation. Under these conditions, chitosan acquires a positive charge that promotes electrostatic interaction with pigment particles and colloidal fractions present in the wastewater, favoring the formation of aggregated flocs that can be easily removed by filtration.

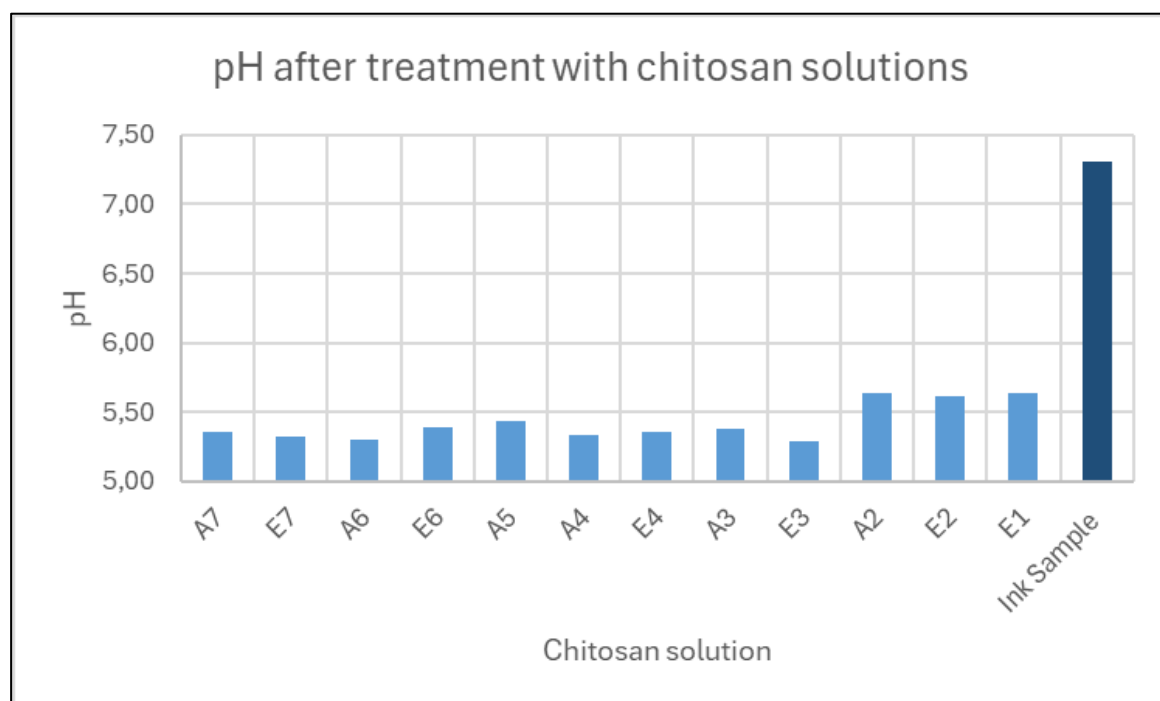


Figure 4.4. pH values of the treated effluents obtained using different chitosan-based solutions (A–E series) compared with the untreated ink wastewater. The results show a consistent acidification of the system following treatment, with final pH values generally ranging between approximately 5.3 and 5.6, indicating the influence of the acetic acid-based formulation and confirming the establishment of conditions favorable for flocculation.

The electrical conductivity of the filtrate after treatment with the different chitosan solutions shows values ranging between approximately 4.6 and 4.9 mS/cm, slightly higher than the value measured in the untreated wastewater (approximately 4.4 mS/cm) (Fig. 4.5). However, the observed variation remains limited and does not highlight significant differences between the tested solutions or across the different monitoring weeks. This behavior indicates that the treatment process primarily acts on the removal of the colloidal and pigment fractions of the wastewater, without substantially altering the ionic composition of the liquid phase. The slight increase in conductivity can be attributed to the acidic solution used to solubilize chitosan, which introduces a modest amount of dissolved ionic species into the system.

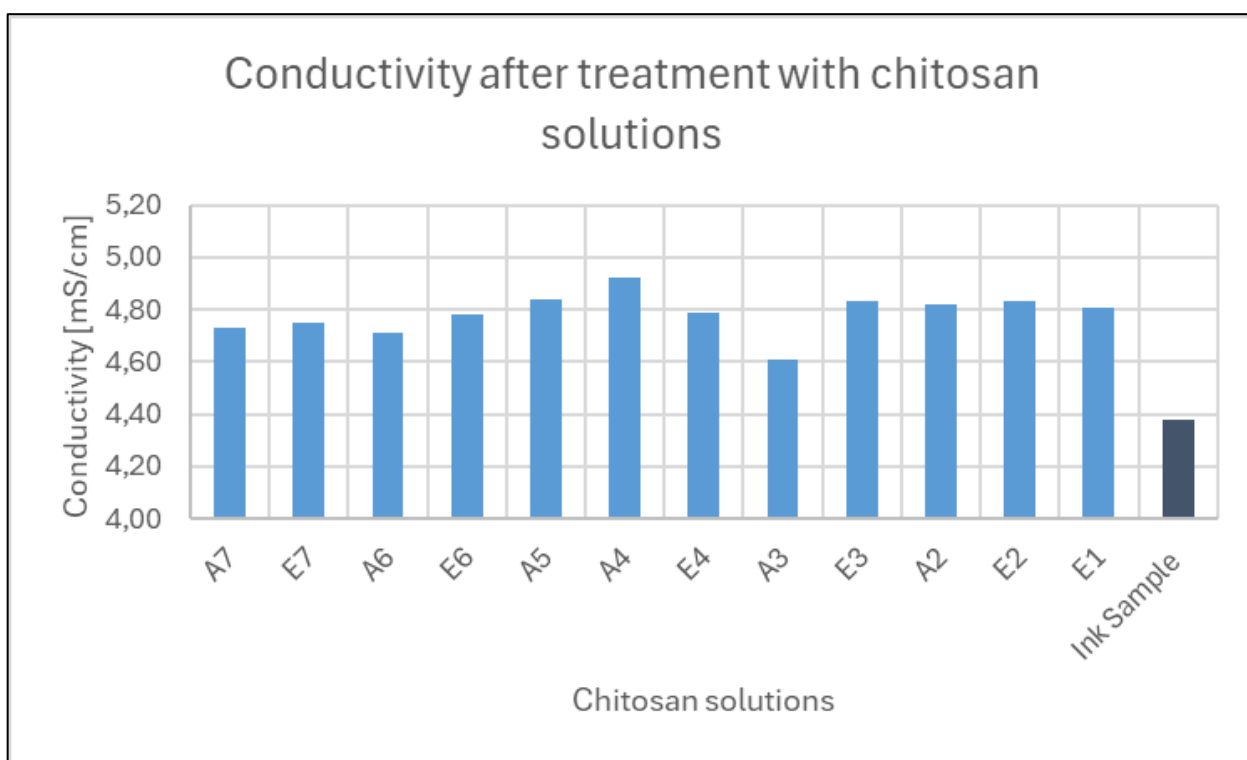


Figure 4.5. *Electrical conductivity of treated effluents obtained using different chitosan-based solutions (A–E series) compared to the untreated ink wastewater. The results show a slight increase in conductivity after treatment, attributable to the introduction of ionic species from the acidified chitosan solution, while maintaining relatively consistent values across different formulations.*

The analysis of UV–Vis spectra of the treated wastewater shows a marked reduction in absorbance across the entire analyzed spectral range (340–800 nm), indicating a significant removal of the chromophoric fraction of the matrix. As shown in Figure X, the curve corresponding to the untreated wastewater (A1) exhibits significantly higher absorbance values compared to the treated samples, highlighting the presence of pigments and aromatic compounds typical of ink-processing effluents. In contrast, samples treated with the different flocculant solutions display spectra characterized by substantially lower absorbance values, which are also highly similar to each other. The overlap of the curves corresponding to the different weeks of testing suggests

that the effectiveness of the flocculation process remains essentially constant over time, confirming the functional stability of the flocculant solution during the monitoring period. The reduction in absorbance observed across the entire spectrum is consistent with a removal mechanism based on aggregation and subsequent separation of pigment particles, rather than selective chemical degradation of colored compounds.

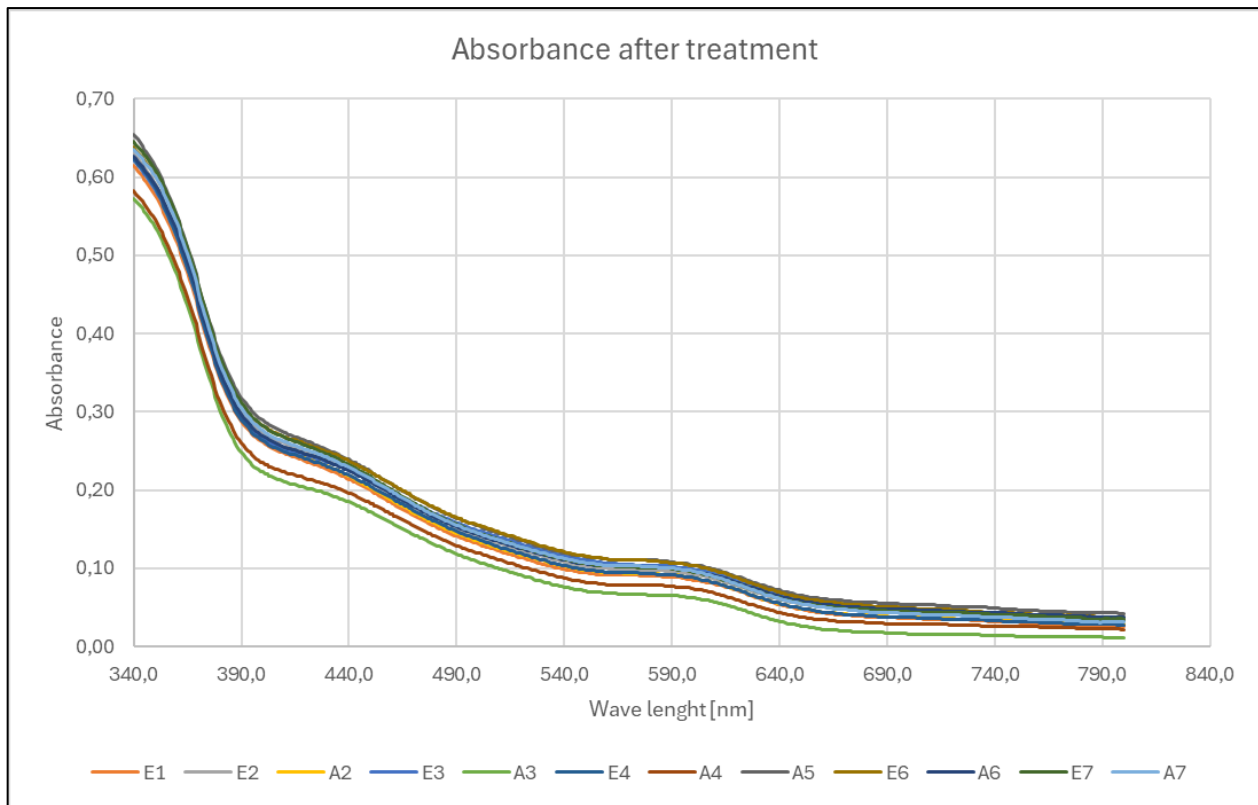


Figure 4.6. UV-Vis absorbance spectra of treated wastewater samples obtained using different chitosan-based formulations (A–E series). All treated samples show a consistent reduction in absorbance across the entire spectral range (340–800 nm), indicating effective removal of the chromophoric fraction of the wastewater. The overlap of the spectra suggests stable flocculation performance across different formulations and storage times.

Overall, the study demonstrates that the flocculant solution maintains its ability to induce separation of the pigment fraction throughout the entire observation period, indicating good operational stability of the formulation over time. This result is particularly relevant in view of potential industrial application, as it shows that the solution can be prepared, stored, and used even after several days or weeks without significant loss of flocculation efficiency. This characteristic represents a key advantage for the operational management of treatment systems based on automated dosing of flocculant solutions, such as the pilot system developed in the subsequent phases of the study.

4.2 Prototyping phase (Prototype 1)

4.2.1 Evolution of Prototype 1

The implementation of the first prototypical wastewater treatment system, as previously outlined in earlier chapters, was the result of an iterative experimental development process aimed at identifying a plant configuration capable of ensuring effective separation between the liquid phase and the flocculated solid fraction, as well as the control of key parameters to be maintained below the limits established by current Italian regulations.

The process was progressively scaled up to larger volumes through a series of pilot tests conducted on an experimental setup. This phase was carried out in the absence of complete analytical data for the untreated wastewater, relying instead on the average values obtained from analytical reports provided by external laboratories (Table X). The system performance was therefore evaluated by comparing the results obtained under different experimental configurations of the prototype, based on key parameters (pH, conductivity, COD, BOD, hardness, total surfactants, and ammonium) and UV–Vis absorbance of the treated wastewater across the various tests, as an indicator of treatment effectiveness. This approach made it possible to assess the evolution of process performance throughout the different stages of system development. The development phase of the treatment system was structured into a series of progressive experimental tests (Test I–XI), aimed at defining an effective prototypical configuration for the treatment of wastewater contaminated by ink-based matrices. The tests were conducted on increasing volumes of wastewater, with the objective of evaluating both the effectiveness of the different treatment modules and the scalability of the process. In particular, the experimental campaign was organized into three main operational phases: an initial phase at approximately 20 L, an intermediate phase at 30–40 L, and a final phase at 50 L, corresponding to the most advanced pre-prototypical configuration.

During the different tests, both operating conditions and system configuration were progressively modified, introducing or optimizing the main treatment units: chitosan-based flocculation, primary mechanical filtration, progressive filtration through cartridges with decreasing pore size, and adsorption treatments using activated carbon. The overall configuration and treatment sequence are summarized in Table 4.5. The performance analysis of the system was carried out by comparing the results obtained under the different experimental configurations, in order to evaluate the evolution of process efficiency throughout the successive stages of development.

Test	Volume [L]	Chitosan dosage [%]	Chitosan dosage [mL/L]	Operating conditions [rpm – contact time h]	Primary filtration	Secondary treatment	Additional modules
I	20	6.98	75	[250] – [1]	Polypropylene filter	10 µm filter	–
II	20	6.98	75	[250] – [1]	Polypropylene filter	Filter press (10 µm)	–
III	20	6.98	75	[250] – [1]	Polypropylene filter	Filter press (10 µm)	Activated carbon (batch)
IV	20	5.66	60	[250] – [1]	Polypropylene filter	Filter press (10 µm)	Ozone + activated carbon
V	30	7.66	83	[250] – [1]	Polypropylene filter	Filter press (10 µm)	Activated carbon + ozone
VI	30	7.66	83	[250] – [1]	Polypropylene + polyester filter (250 µm)	100 µm + 60 µm + 20 µm + filter press (10 µm)	Activated carbon around filters + biocide
VII	40	6.37	68	[250] – [1]	Polypropylene + polyester filter (250 µm)	100 µm + 60 µm + 20 µm + 1 µm	Activated carbon around filters
VIII	43	6.36	68	[250] – [1]	Polypropylene + polyester filter (250 µm)	100 µm + 60 µm + 20 µm + 1 µm	Activated carbon around filters
IX	50	0.36	3.64	[100] – [1]	Multiple polypropylene filters + polyester filter (250 µm)	100 µm + 50 µm + 20 µm	Activated carbon in mixing + serial filters (10 µm + 1 µm)
X	50	0.36	3.64	[100] – [1]	Multiple polypropylene	100 µm + 50 µm + 20 µm	Activated carbon (10 g/L)

					filters + polyester filter (250 µm)		
					Multiple polypropylene filters + polyester filter (250 µm)	Activated carbon	10 µm + 1 µm + 1 µm
XI	50	0.36	3.64	[100] – [1]			

Table 4.5. Summary of the experimental configurations adopted during the development of Prototype 1. The table reports the progressive evolution of operating conditions, chitosan dosage, and treatment modules across the different tests (I–XI), highlighting the transition from simple filtration systems to more complex multi-stage configurations integrating adsorption (activated carbon), oxidation (ozonation), and progressive mechanical filtration. This iterative approach enabled the identification of an effective and scalable treatment configuration for ink-based wastewater.

4.2.2 Performance trends across Tests I–XI

In order to evaluate the evolution of the process and the modifications introduced in the treatment sequence, the experimental results were analyzed by comparing the main physicochemical parameters of the treated wastewater, including COD, surfactant concentration, pH, and conductivity, as well as through UV–Vis spectrophotometric analysis to assess variations in absorbance associated with the presence of chromophoric compounds derived from inks. The analysis of process parameters was not conducted uniformly throughout the entire sequence of experimental tests, as the analytical plan was progressively expanded during the development phase of the prototypical system. In particular, COD determinations were performed from Test II to Test XI, while surfactant analysis was introduced starting from Test III and continued through the final tests. Regarding pH and conductivity, their monitoring was maintained consistently throughout the entire experimental phase, with the aim of evaluating the physicochemical stability of the matrix during treatment. These parameters were primarily used as process control indicators, allowing verification that the different system configurations and applied treatments did not result in significant alterations of the fundamental characteristics of the wastewater.

UV–Vis spectrophotometric analysis, aimed at evaluating absorbance trends and thus the removal of chromophoric compounds associated with the presence of ink, was systematically applied from Test III to Test XI, when the analytical protocol had become stabilized and the treatment system had reached a configuration sufficiently representative of the final operating conditions.

The different availability of analytical data between the early and later tests is attributable to the iterative nature of the experimental development phase, during which the analytical set was progressively expanded in accordance with the evolution of the prototype and the availability of materials and specific analytical kits used for laboratory determinations. This approach made it possible to focus more detailed analyses on the stages in which the system had reached a more stable and representative operational configuration, while ensuring the reliability of comparisons between different treatment setups. The overall performance trend of

the system throughout the sequence of experimental tests is illustrated in Figure 4.7, which reports the variation of COD across the different tests and highlights the progressive improvement in system performance following the introduction of secondary treatment modules.

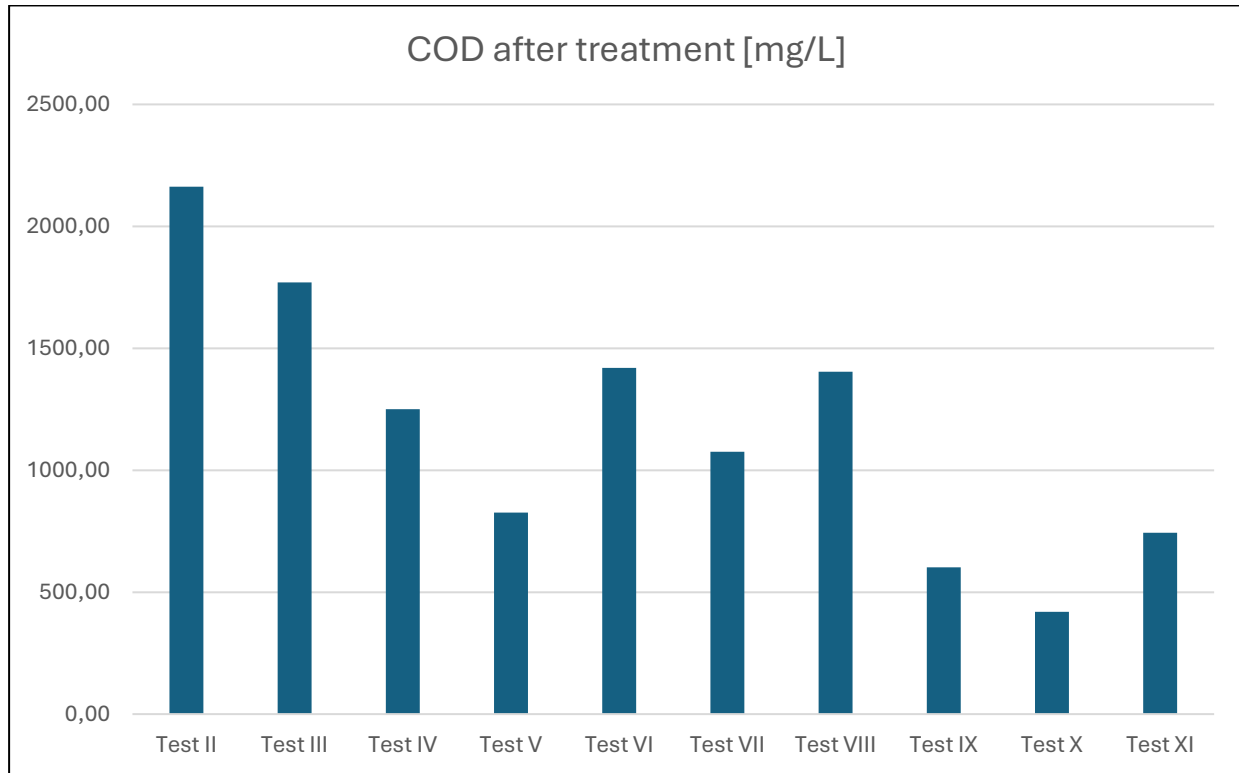


Figure 4.7. COD values of treated wastewater across Tests II–XI during the development of Prototype 1. The results show a general decreasing trend in COD along the experimental sequence, highlighting the progressive improvement of treatment efficiency with the introduction and optimization of secondary treatment modules.

Subsequently, Figure 4.8 shows the trend of total surfactant concentrations (divided into non-ionic, cationic, and anionic fractions) across the different experimental tests, a parameter of particular relevance in the case of wastewater derived from ink-containing matrices and organic additives. The comparison among the different tests allows for the evaluation of the effectiveness of the combined flocculation and adsorption processes in reducing these components of the matrix.

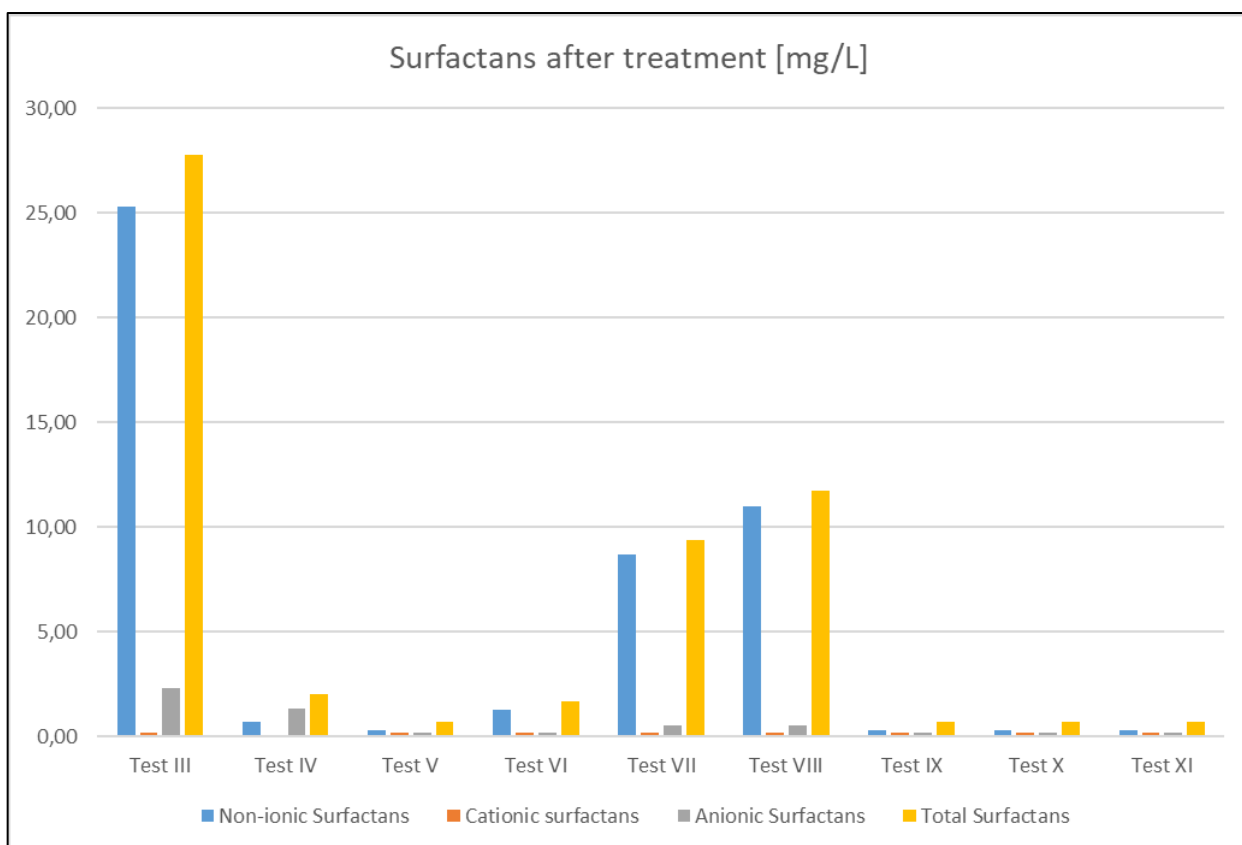


Figure 4.8. Evolution of surfactant concentrations after treatment across Tests III–XI, including non-ionic, cationic, anionic, and total surfactants. A general decrease in total surfactant concentration is observed along the experimental sequence, with an initial high load followed by a progressive reduction as the treatment configuration is optimized. The results highlight the effectiveness of the combined flocculation and adsorption processes in removing surfactant fractions, particularly non-ionic compounds, which represent the dominant component of the matrix.

The analysis of the physicochemical parameters of the treated wastewater is further complemented by the comparison of pH and conductivity values, reported in Figure 4.9, in order to assess the stability of the chemical matrix throughout the different treatment stages.

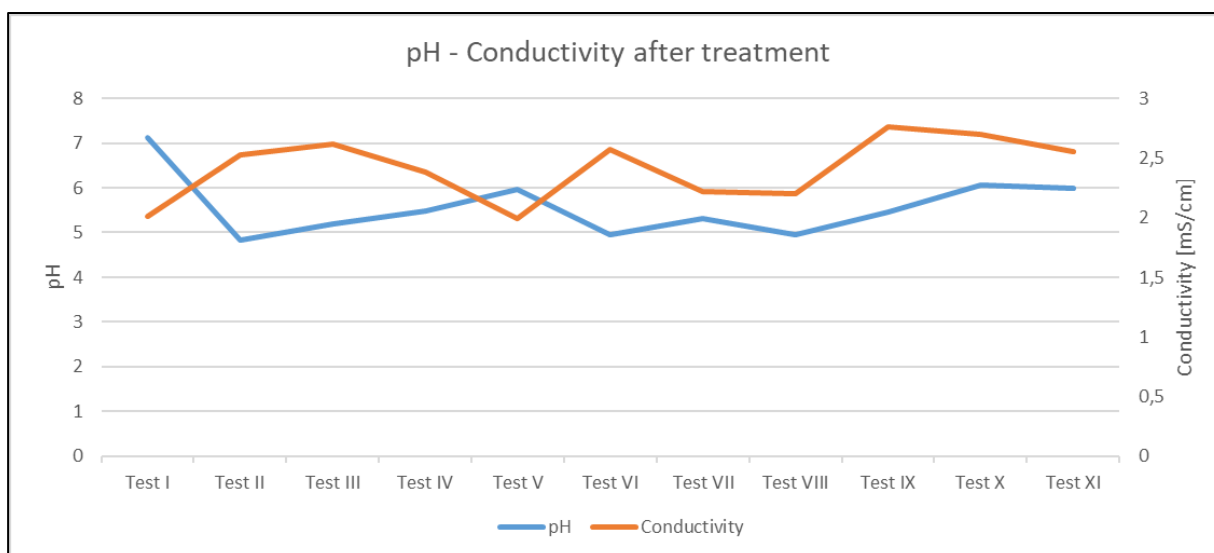


Figure 4.9. *pH and conductivity trends across Tests I–XI, highlighting the overall stability of the chemical matrix during treatment. The limited variation of both parameters confirms that the process mainly affects the particulate and organic fractions without substantially modifying the ionic composition of the effluent.*

UV–Vis spectrophotometric analysis was employed as a qualitative tool to evaluate the effectiveness of the different treatment configurations in removing chromophoric components associated with the presence of ink in the wastewater. Since the tests were conducted on wastewater samples collected at different stages of industrial production and characterized by intrinsic variability of the matrix, it was not always possible to obtain a consistent reference measurement of the absorbance of the untreated wastewater for each experiment. For this reason, the comparison of absorbance curves (Fig. 4.10) was carried out by considering the spectra of the treated effluents at the end of each process, in order to assess the relative reduction in chromophore intensity and to identify the most effective treatment configurations.

In this context, lower absorbance values across the entire UV–Vis spectral range are indicative of a higher removal of colouring species and, therefore, of improved treatment efficiency.

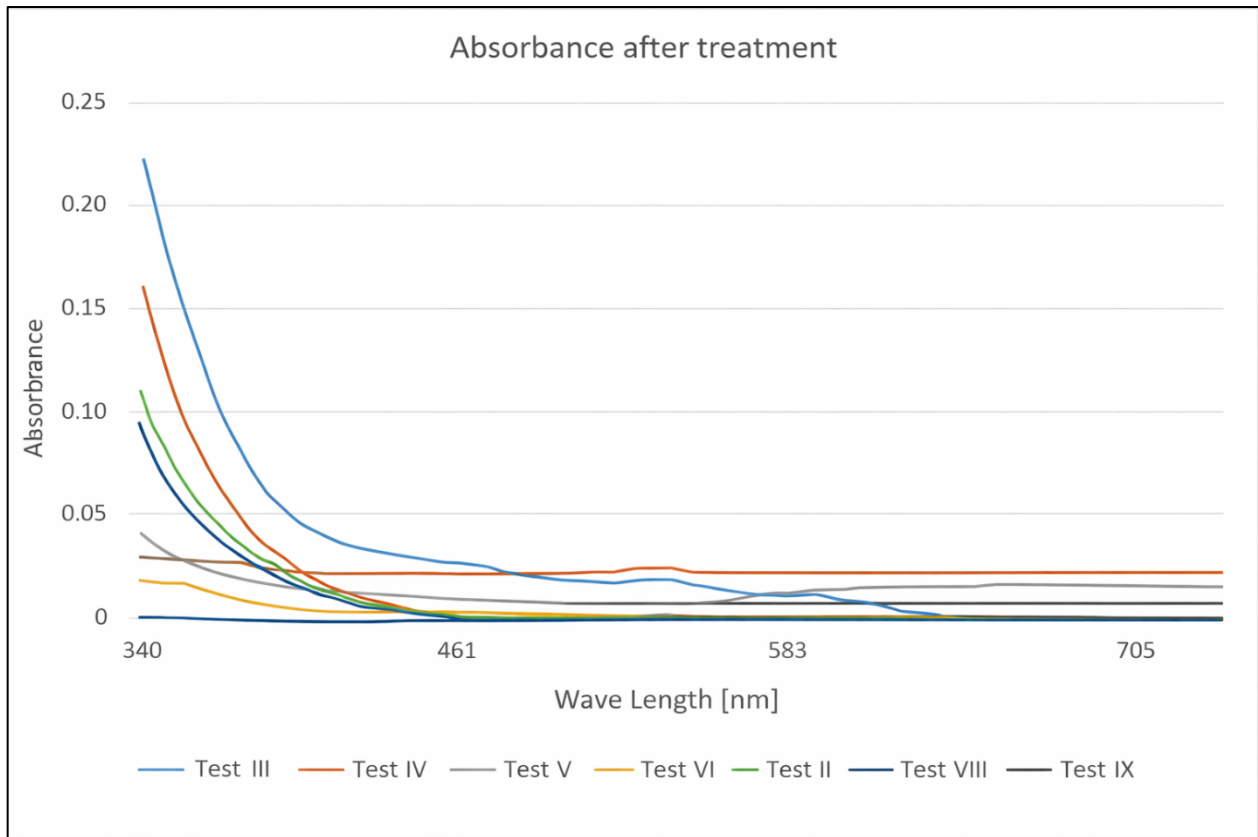


Figure 4.10. UV–Vis absorbance spectra of treated wastewater samples (Tests III–XI). All curves show a rapid decrease in absorbance at lower wavelengths followed by stabilization at near-zero values across the visible range, indicating effective removal of chromophoric compounds. The overall low and overlapping absorbance profiles suggest consistent treatment performance across different experimental configurations.

The analysis of UV–Vis absorbance spectra of the treated wastewater across the different experimental tests allows for the evaluation of the effectiveness of the adopted treatment configurations in removing the chromophoric component associated with the presence of ink in the matrix. The comparison between spectra recorded at the end of the different processes highlights significant differences in absorbance intensity across the entire analyzed spectral range (340–800 nm), indicating variations in treatment efficiency under the different experimental setups.

In general, all curves show a decreasing trend in absorbance with increasing wavelength, a typical behavior of matrices containing dye-related compounds derived from ink formulations. However, the overall spectral intensity varies significantly among the different tests, reflecting the different degrees of removal of chromophoric species achieved under the various treatment configurations.

In particular, Test III shows significantly higher absorbance values across most of the spectrum, indicating lower process efficiency in the early stages of system development. In the subsequent tests, progressive reduction in spectral intensity is observed, suggesting an improvement in treatment performance in terms of removal of compounds responsible for wastewater coloration.

The lowest absorbance values are observed especially in Tests VI, VII, IX, X, and XI, where the spectra appear almost completely flattened and close to zero for wavelengths above approximately 450–500 nm. This behavior indicates highly efficient removal of chromophoric species and a significant reduction of the colored fraction in the treated wastewater.

An important aspect emerging from the comparison of the spectra is that, despite significant variations in absorbance intensity, the overall shape of the curves remains substantially unchanged across the different tests. This suggests that the treatment process does not alter the chemical nature of the chromophoric species present in the matrix but primarily reduces their concentration through the combined processes of flocculation, filtration, and adsorption.

Overall, the spectrophotometric analysis confirms what has also been observed through other process control parameters, highlighting that the evolution of the system configuration throughout the sequence of experimental tests led to a progressive improvement in the effectiveness of chromophore removal from the wastewater. The absorbance results demonstrate a continuous enhancement in system performance across the different experimental configurations, confirming the validity of the integrated approach based on flocculation, filtration, and adsorption. These experimental findings therefore provided the technical and scientific basis for the design of a more structured and stable plant configuration, leading to the development of the final prototype described in the following section.

4.2.3 Prototype 1 Results

The effectiveness of the prototypical system was evaluated through two experimental tests conducted on different wastewater volumes, namely 50 L and 100 L, in order to assess process scalability and the stability of system performance as a function of treated volume. In both cases, the wastewater was subjected to the same sequence of treatments, including an initial mechanical filtration step, followed by secondary treatment modules consisting of filtration columns and adsorption systems based on activated carbon, and finally a polishing phase through cartridge filters.

As previously mentioned in Section 3.3.1.1, the results obtained during the preliminary tests not only led to a better understanding of the matrix separation dynamics, but also enabled further development in terms of prototypical system engineering. In particular, the analysis of the performance of the different experimental configurations highlighted the need to introduce secondary treatment modules capable of improving the removal of dissolved organic species and stabilizing the overall system performance. Downstream of the mixing phase between wastewater and the chitosan flocculant solution, and after the initial mechanical separation of flocs in the collection tank, a first filtration module consisting of a quartz column was introduced, followed by a granular activated carbon column (Hydromont filters), specifically dimensioned according to the flow rates used during the experimental tests. The passage through the quartz bed primarily served as a granular filtration step, allowing the removal of residual particles and finer flocs not retained during the initial filtration stage. This phase contributed to further reducing wastewater turbidity and stabilizing the separation of suspended solids.

Subsequently, the wastewater was conveyed into the activated carbon column, which played a key role in the removal of dissolved organic matter. In particular, the activated carbon enabled the adsorption of various chemical species present in the matrix, including aromatic compounds derived from ink formulations, residual organic solvents, formulation additives, and a significant fraction of surfactants, thereby contributing to the reduction of COD and the chromophoric component of the wastewater.

Downstream of these modules, a cartridge filtration stage was maintained, consisting of three successive steps with decreasing pore size ($10\ \mu\text{m}$ – $1\ \mu\text{m}$ – $1\ \mu\text{m}$), aimed at removing any remaining particles and further polishing the filtrate. This configuration allowed for a more efficient separation of the particulate fraction and improved the clarity of the treated wastewater (Fig. 4.11).

The final stage of the treatment process was implemented through the use of an ion-exchange resin softener, supplied by Culligan, designed to remove calcium (Ca^{2+}) and magnesium (Mg^{2+}) ions responsible for the high hardness of the matrix. Passage through the resin enabled a significant reduction in total hardness via an ion-exchange process, in which calcium and magnesium ions are replaced by sodium ions present in the resin.

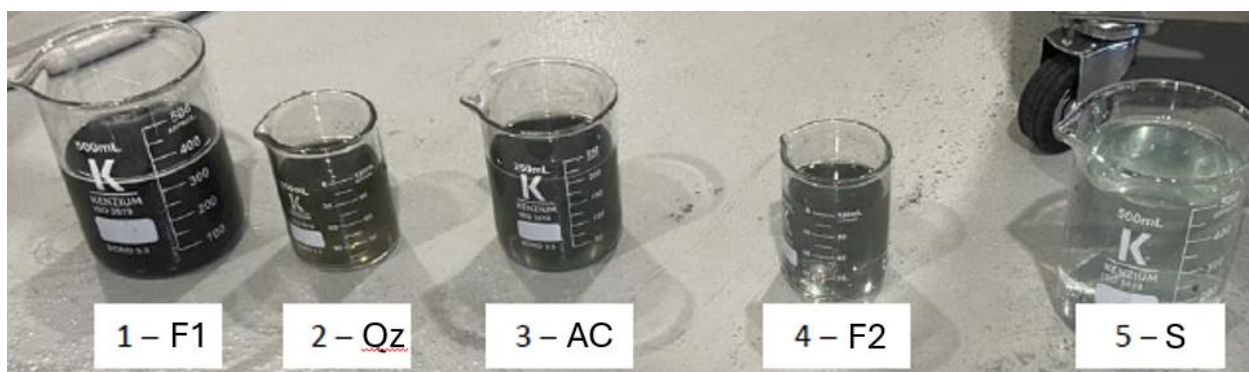


Figure 4.11. Visual appearance of wastewater samples at different stages of the treatment process: (1) F1 – first filtration, (2) Qz – quartz filtration, (3) AC – activated carbon treatment, (4) CF – cartridge filtration, and (5) S – softening. The progressive clarification of the samples highlights the effectiveness of the combined treatment sequence in removing suspended solids, colloidal fractions, and dissolved organic compounds.

The integration of these treatment modules enabled the development of a more comprehensive and stable process, capable of acting on both the particulate and dissolved fractions of the matrix. Overall, the combination of chitosan-based flocculation, granular filtration, adsorption on activated carbon, fine filtration, and softening through ion-exchange resins allowed for a significant reduction of the main contamination parameters of the wastewater, bringing the final values closer to the limits established by current discharge regulations.

The results obtained highlight a substantial reduction in organic load throughout the entire treatment process. In the test conducted on 50 L, the COD value decreased from 1381 mg/L in the raw wastewater to 1158 mg/L after the initial filtration stage, reaching 545 mg/L after passage through the adsorption column, and finally 100 mg/L in the final treatment stage (Fig. 4.12). A similar trend was observed in the 100 L test, in which the

initial COD value of 1453 mg/L was progressively reduced along the different stages of the process, reaching comparable final values close to 100 mg/L (Fig. 4.13).

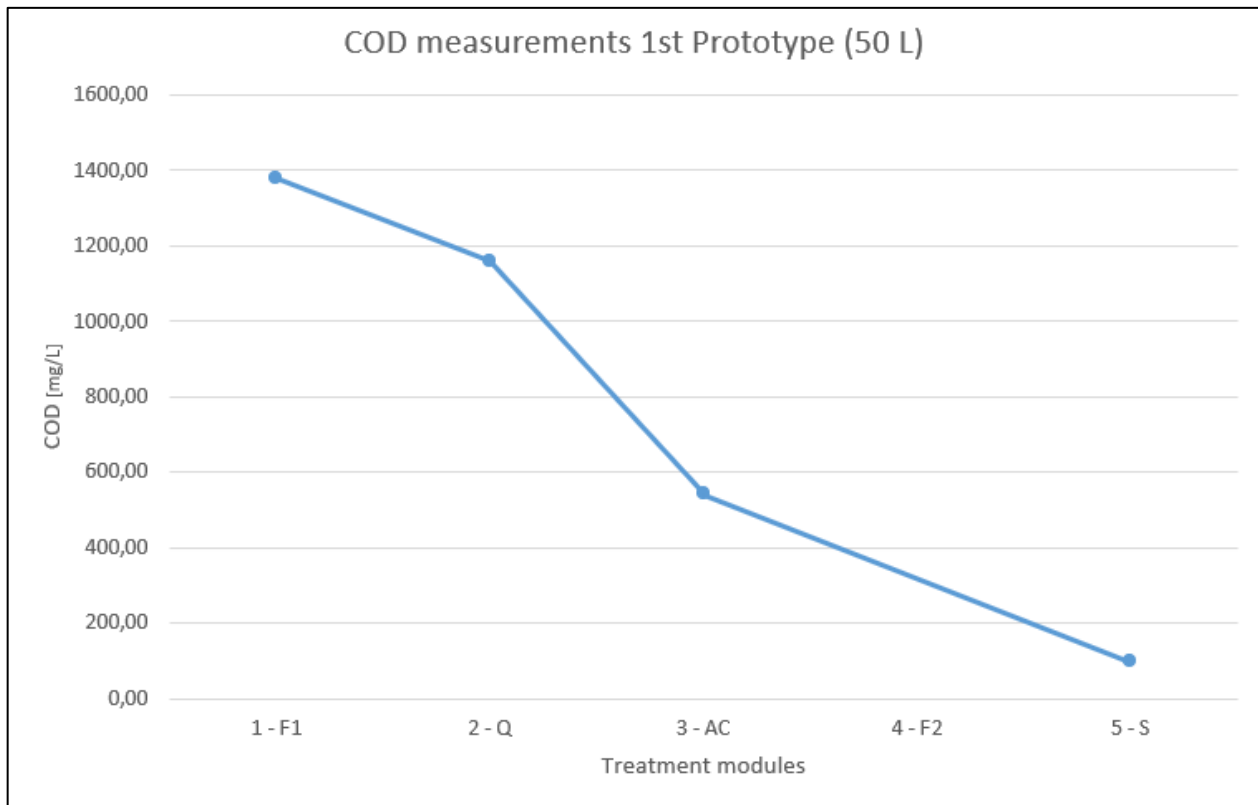


Figure 4.12. COD reduction along the treatment sequence in Prototype 1 (50 L test). The results show a progressive decrease in COD from the initial filtration stage (F1) through quartz filtration (Q), activated carbon adsorption (AC), secondary filtration (F2), and final softening (S). The most significant reduction occurs after the adsorption step, highlighting the key role of activated carbon in removing dissolved organic matter. The final COD value (~100 mg/L) indicates a substantial improvement in effluent quality.

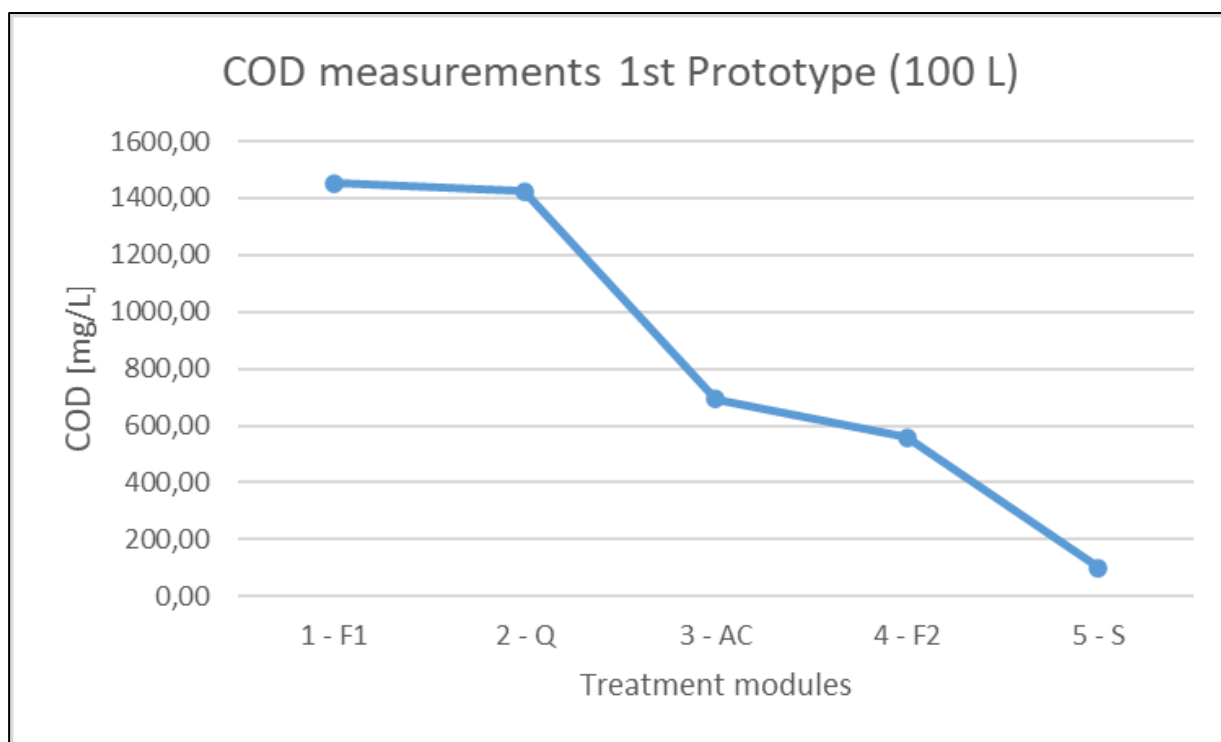


Figure 4.13. COD reduction along the treatment sequence in Prototype 1 (100 L test). The results show a progressive decrease in COD from the initial filtration stage (F1) through quartz filtration (Q), activated carbon adsorption (AC), secondary filtration (F2), and final softening (S). The most significant reduction occurs after the adsorption step, highlighting the key role of activated carbon in removing dissolved organic matter. The final COD value (~100 mg/L) indicates a substantial improvement in effluent quality (as for 50 L test).

A similar behavior was also observed for total surfactant concentration, which represents a significant component of wastewater derived from ink processing. Along the treatment process, a progressive reduction in surfactant concentrations is observed, with final values below 1 mg/L in the most advanced treatment stages in the 50 L test, as well as in the 100 L treatment (Fig. 4.14).

This result highlights the effectiveness of the combined flocculation, filtration, and adsorption processes in removing not only the particulate fraction but also a significant portion of the dissolved organic substances present in the matrix.

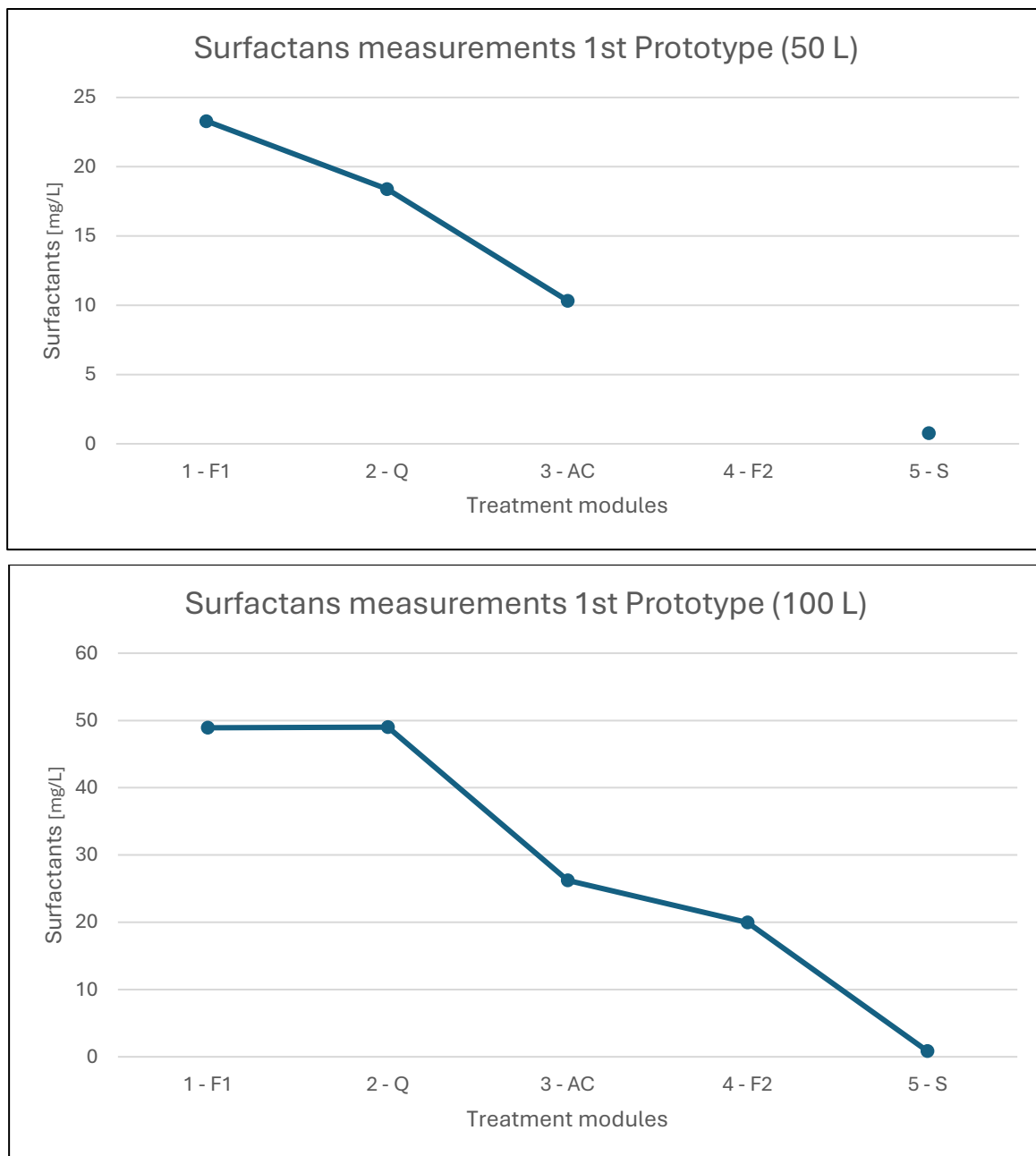


Figure 4.14. Evolution of total surfactant concentration along the treatment sequence in Prototype 1 (50 L and 100 L test). A progressive decrease is observed from the initial filtration stage (F1) through quartz filtration (Q) and activated carbon adsorption (AC), with a marked reduction achieved after the adsorption step. Final concentrations below 1 mg/L are reached after the complete treatment (F2 and softening, S), highlighting the effectiveness of the integrated process in removing surfactant compounds.

UV–Vis spectrophotometric analysis, carried out on samples collected at different stages of the process, further confirms the effectiveness of the treatment in removing the chromophoric component of the wastewater. The absorbance spectra show a progressive decrease in spectral intensity across the entire analyzed range, indicating a reduction in the concentration of coloring species associated with the presence of ink (Figs. 4.15).

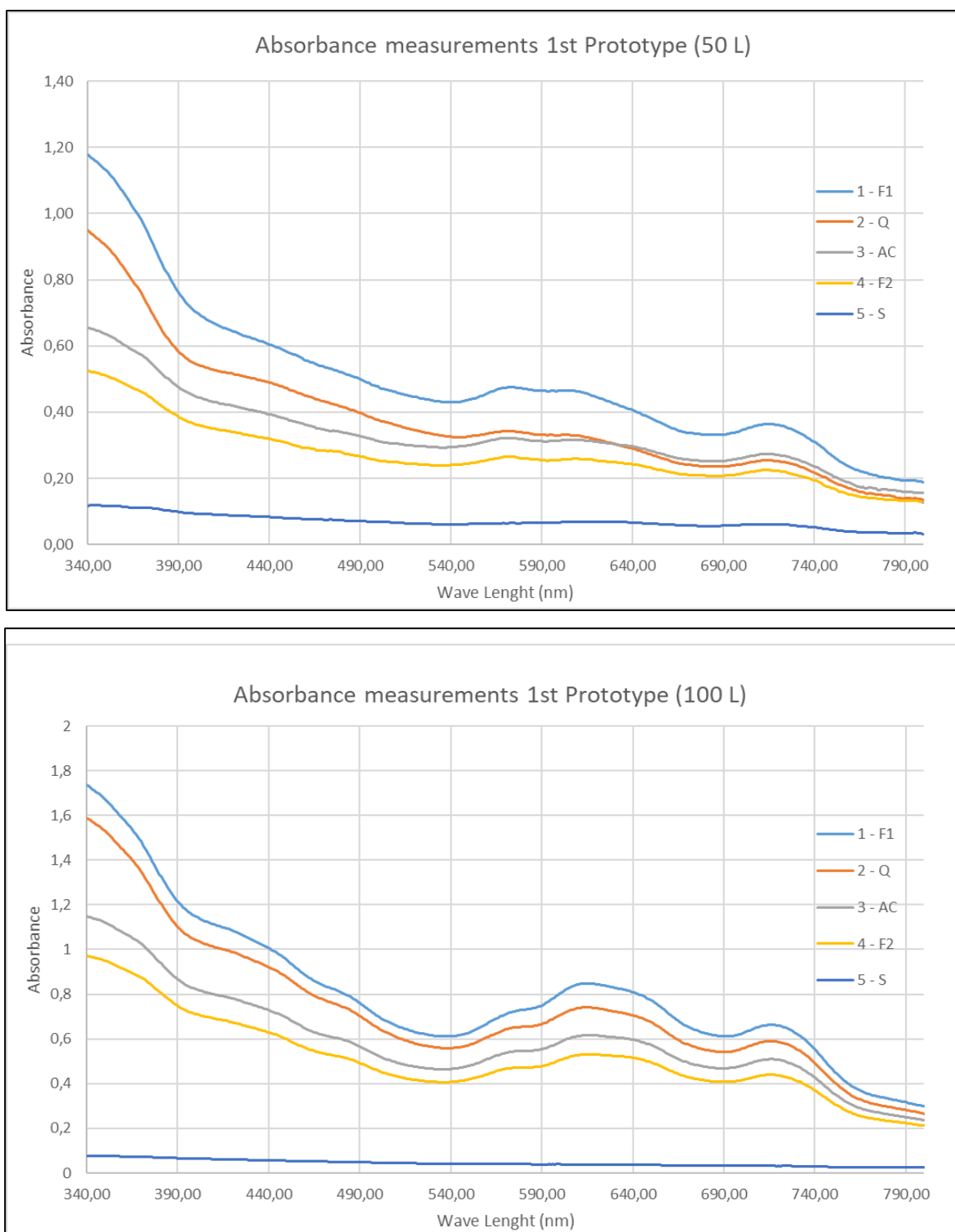


Figure 4.15. UV–Vis absorbance spectra of treated wastewater at different stages of the treatment process in Prototype 1 for both 50 L and 100 L tests. In both cases, a progressive decrease in absorbance intensity is observed along the treatment sequence, with spectra shifting downward from the initial stage (F1) to the final softening step (S). The reduction and partial convergence of the curves across the entire spectral range indicate an effective removal of chromophoric compounds associated with ink-derived wastewater. The comparable trends observed in the two tests confirm the stability and scalability of the treatment process.

In both tests, a similar spectral trend is observed across the analyzed range (340–800 nm), characterized by higher absorbance values at lower wavelengths and a progressive decrease with increasing wavelength. This behavior is consistent with the presence of chromophoric compounds in the wastewater, typically associated with ink formulations, which are generally composed of organic pigments and aromatic molecules exhibiting absorption bands in the UV–visible region.

A comparison of the spectra recorded after the different treatment steps clearly shows a progressive reduction in absorbance across the entire spectral range. The initial filtration stage (F1) leads to a first decrease in spectral intensity, mainly associated with the removal of particulate matter and flocs generated during the flocculation phase. The subsequent passage through the quartz filter (Q) further reduces absorbance by removing finer particles not retained during the initial filtration stage.

A particularly significant contribution to the reduction of the chromophoric component is observed during passage through the activated carbon column (AC). At this stage, a marked decrease in spectral intensity is recorded across the entire analyzed range, indicating the adsorption of dissolved organic compounds, including dyes, residual solvents, and formulation additives present in the matrix. This behavior confirms the central role of activated carbon in the removal of the dissolved organic fraction of the wastewater.

The subsequent fine filtration stages (F2) contribute to further polishing of the filtrate, reducing the presence of residual particles and improving the clarity of the treated wastewater. Finally, the sample collected after the entire treatment process (S) shows absorbance values close to zero over most of the spectral range, indicating a substantial removal of the chromophoric component.

The comparison between the two tests conducted on 50 L and 100 L shows a qualitatively similar behavior in terms of COD, surfactant concentrations, and spectral trends throughout the different treatment stages. Despite the increase in treated volume, the evolution of the curves and the progressive reduction of both parameter values and absorbance remain comparable between the two tests, suggesting that the process maintains good efficiency at a larger scale.

This findings indicates that the adopted prototypical configuration exhibits strong operational robustness and promising scalability, maintaining similar performance even when the treated wastewater volume is doubled. Furthermore, it suggests that the system represents a suitable technological basis for subsequent development at a pre-industrial scale.

4.3 Formulation Stabilization Phase

4.3.1 Optimal pH

During this process stabilization phase, a series of tests were conducted to evaluate different operating conditions in terms of reagent concentration and dosing volumes, with the aim of identifying the optimal pH during the operational stages, as well as the appropriate flocculant and polyelectrolyte dosages.

One of the first variables investigated during the preliminary development phase of the process was the influence of pH on the flocculation capacity of chitosan. It is well known that this natural polymer contains

amino groups that, under acidic conditions, become protonated, conferring a positive charge to the molecule. This characteristic allows chitosan to interact with colloidal particles present in the wastewater, which are generally negatively charged, promoting charge neutralization mechanisms and subsequent aggregation.

To experimentally verify this behavior, several tests were conducted on both untreated wastewater samples and samples previously acidified through the addition of acetic acid.

The experiments were performed on volumes of approximately 100 mL of wastewater, maintaining constant mixing and sedimentation times, while varying only the pH of the matrix prior to the addition of the flocculant. In the first experiment, the wastewater was treated with a chitosan solution without any modification of the initial pH ($\text{pH} \approx 7$). Under these conditions, no significant floc formation was observed, and the matrix remained visually homogeneous and strongly colored, indicating the absence of effective destabilization of the colloidal particles. Subsequently, tests were carried out on slightly acidified wastewater ($\text{pH} \approx 6$). In this case, the formation of small aggregates was observed; however, the separation between the liquid and solid phases remained incomplete, and the filtrate retained a dark coloration, suggesting that the flocculation process was not yet fully effective.

By further reducing the system pH to values close to 5, a clear improvement in flocculation was observed (Fig. 4.16). Under these conditions, the formed flocs appeared visibly larger and more compact, and during the sedimentation phase, a clear separation between the sludge phase and the supernatant was established. Increasing the chitosan dosage under these conditions further improved solid–liquid separation, confirming the key role of an acidic environment in activating the polymer, as shown in Table 4.6.

N	Wastewater condition	Initial pH	Chitosan added	Observations during mixing	Sedimentation result	Filtrate appearance	pH (post-filtration)	Conductivity [mS/cm]
1	Raw wastewater	≈ 7.0	1 mL Chito-F 1%	No floc formation observed	No evident separation	Black and turbid water	\	\
2	Acidified wastewater	≈ 6.0	1 mL Chito-F 1%	Formation of small flocs	Incomplete separation	Dark grey filtrate	5.96	2.82
3	Acidified wastewater	≈ 5.0	1 mL Chito-F 1%	Larger and more visible flocs	Clear separation between sludge and liquid	Light grey filtrate	4.78	3.04
4	Acidified wastewater	≈ 5.0	2 mL Chito-F 1%	Rapid formation of compact flocs	Effective sedimentation	Clearer filtrate	4.71	2.98

Table 4.6. Results of preliminary tests conducted to evaluate the influence of pH on wastewater flocculation using chitosan. The results show that stable floc formation mainly occurs under acidic conditions, within a pH range of approximately 4.5 to 5.5.

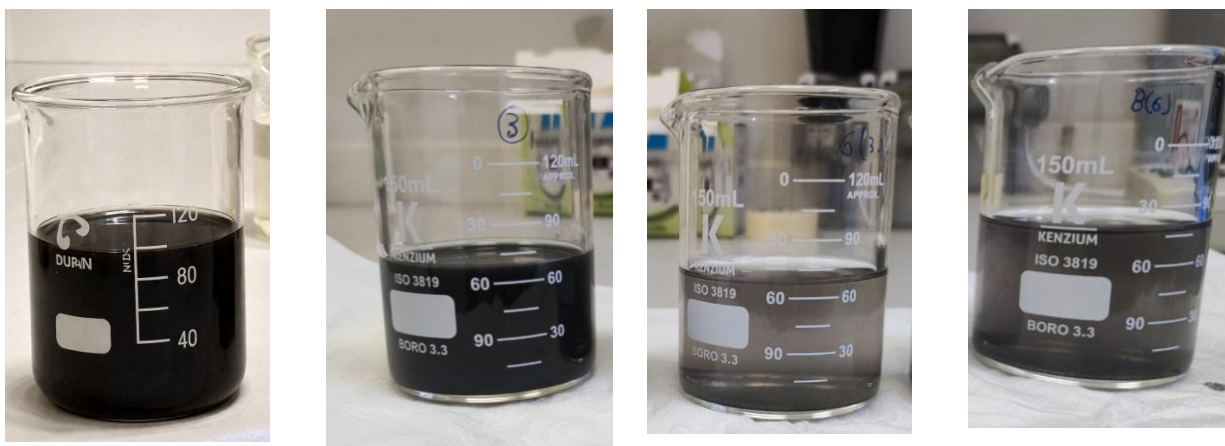


Figure 4.16. Visual comparison of wastewater samples at different pH conditions during preliminary flocculation tests with chitosan. From left to right: raw wastewater ($\text{pH} \approx 7$), slightly acidified sample ($\text{pH} \approx 6$), acidified sample ($\text{pH} \approx 5$), and acidified sample with higher chitosan dosage. The progressive clarification of the liquid phase highlights the improvement in floc formation and solid–liquid separation under acidic conditions.

The results reported in Table X clearly highlight that wastewater flocculation is strongly dependent on the pH of the system. Under near-neutral conditions, no significant destabilization of the colloidal matrix is observed, whereas floc formation becomes evident when the pH is reduced to values close to 5.

4.3.2 Chitosan solution dosage

In this preliminary phase preceding the construction of the pilot plant, the effect of chitosan dosage on floc formation and stability was also investigated. The experiments were carried out by maintaining a constant acidic pH (approximately pH 5) and varying only the volume of flocculant solution added to the wastewater (table 4.7).

The results revealed the existence of a minimum dosage threshold below which colloidal destabilization does not occur. In particular, at dosages of approximately 0.25 mL of chitosan solution per 100 mL of wastewater, no significant floc formation was observed, indicating that the amount of polymer was insufficient to neutralize the charges of the dispersed particles.

By increasing the dosage to 0.5 mL per 100 mL of wastewater, a progressive formation of visible flocs was observed, followed by sedimentation leading to the separation between the liquid and solid phases. A further increase in dosage up to 1 mL per 100 mL resulted in faster flocculation, with almost immediate formation of aggregates.

Higher dosages, in the range of 2–3 mL, produced larger flocs but did not result in a significant improvement in filtrate quality, suggesting that excessive amounts of flocculant do not provide substantial benefits to the process (fig. 4.17).

N	pH	Chitosan dose	Chitosan concentration	Observations	Final result	pH (post-filtration)	Conductivity [mS/cm]
1	≈5	0.25 mL	1.5%	No floc formation observed	No change	5.60	2.59
2	≈5	0.5 mL	1.5%	Progressive formation of flocs	Good separation	5.12	2.79
3	≈5	1 mL	1.5%	Rapid flocculation	Effective sedimentation	4.80	2.88
4	≈5	2 mL	1.25–1.5%	Large flocs formed	Filtrate still colored	4.57	2.95
5	≈5	3 mL	1.25%	Very large aggregates	Limited improvement	4.30	3.03

Table 4.7. Results of preliminary tests conducted to evaluate the effect of chitosan dosage on flocculation efficiency under acidic conditions ($\text{pH} \approx 5$). The results highlight the presence of a minimum dosage threshold required to induce effective floc formation, while higher dosages lead to larger aggregates without significantly improving filtrate quality.

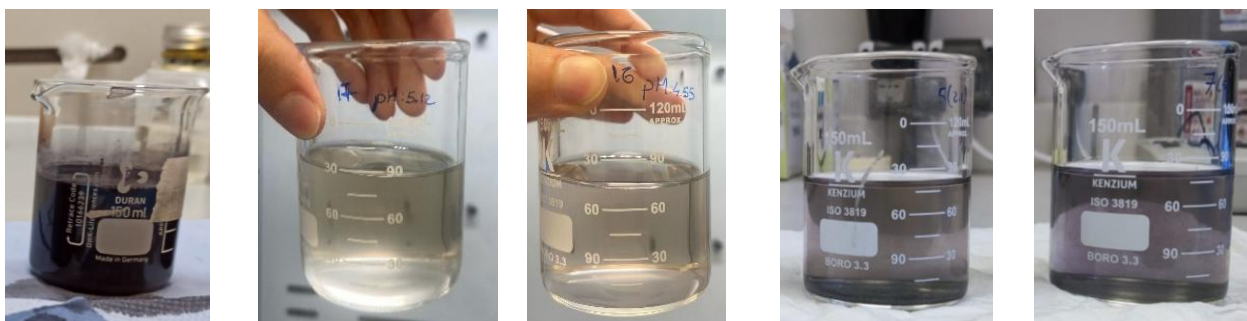


Figure 4.17. Visual comparison of wastewater samples treated with increasing chitosan dosages under acidic conditions ($\text{pH} \approx 5$). From left to right: raw wastewater and samples treated with progressively higher chitosan doses. The images highlight the evolution of floc formation, sedimentation behavior, and filtrate clarity, showing improved solid–liquid separation up to optimal dosages and limited additional benefits at higher concentrations.

4.3.3 Study of cationic polyacrylamide (C-PAM) dosage

After identifying the key role of pH and chitosan dosage in the destabilization process of the wastewater, the effect of adding a flocculating polyelectrolyte—specifically a cationic polyacrylamide (C-PAM)—was investigated, with the aim of enhancing the aggregation of microflocs generated by chitosan.

The experiments were conducted under operating conditions previously identified as favorable for flocculation, namely acidic pH (approximately 4.5–5.5) and chitosan dosages ranging between 0.5 and 1 mL of solution per 100 mL of wastewater. In table 4.8 is represented the design of experiment and parameter results. Under these conditions, chitosan was able to destabilize the colloidal matrix of the wastewater and generate visible microaggregates during the mixing phase; however, in the absence of a flocculation aid, the flocs remained relatively small and sedimentation occurred slowly and, in some cases, incompletely (Figure 4.18).

The addition of C-PAM at concentrations between 0.1% and 0.2% resulted in a clear improvement in floc growth. In the presence of the polyelectrolyte, the microflocs generated by chitosan tended to aggregate into larger and more compact structures, which were more easily sedimentable. This behavior is consistent with the typical role of polyelectrolytes in physicochemical water treatment processes, where they mainly act as bridging agents, promoting the aggregation of already destabilized particles.

The experiments also showed that C-PAM alone is not capable of inducing effective flocculation of the wastewater. In the absence of chitosan, the addition of the polyelectrolyte did not produce significant destabilization of the colloidal matrix, and the wastewater remained highly turbid. This result confirms that the process is based on a two-step mechanism: an initial coagulation phase induced by chitosan through charge neutralization of dispersed particles, followed by a flocculation phase in which the polyelectrolyte promotes aggregation and growth of the flocs.

N	System used	C-PAM dose	Chitosan dose	Observations	Result	pH (post-filtration)	Conductivity [mS/cm]
1	C-PAM	0.5 mL	—	No flocculation observed	Turbid wastewater	4.94	2.85
2	Chitosan	—	2 mL	Microflocs formation	Slow sedimentation	4.76	3.09
3	Chitosan + C-PAM	0.5 mL	2 mL	Large flocs formation	Rapid sedimentation	4.61	2.89
4	Chitosan + C-PAM	0.25 mL	1 mL	Compact flocs	Clearer filtrate	4.92	2.88

Table 4.8. Results of preliminary tests evaluating the effect of cationic polyacrylamide (C-PAM) addition on floc formation under optimized acidic conditions. The results highlight the synergistic role of chitosan and C-PAM in promoting floc growth and improving sedimentation, confirming a two-step mechanism consisting of initial coagulation (chitosan) followed by flocculation through polymer bridging (C-PAM).



Figure 4.18. *Visual comparison of wastewater samples treated under different flocculation conditions with chitosan and C-PAM. From left to right: exp. 1, exp. 2, exp. 3, exp. 4. The images highlight the effect of C-PAM addition in promoting the aggregation of microflocs into larger and more compact structures, resulting in improved sedimentation and clearer supernatant compared to treatments with chitosan alone.*

The introduction of C-PAM improved sedimentation and solid–liquid separation, making the treatment process faster and more stable. For this reason, in the subsequent stages of the experimentation, the flocculation system was structured according to an operational sequence consisting of the addition of chitosan as the primary coagulant, followed by the introduction of C-PAM as a secondary flocculating agent. Following these experiments, the chitosan solution was used in a formulation corresponding to 1.5% of the treated volume, while the anionic polyelectrolyte C-PAM was prepared at concentrations ranging between 0.1 and 0.2 g/L, with dosages between 1 and 2 mL per 100 mL of wastewater. These values were subsequently scaled during tests performed at larger volumes, corresponding to dosages between 0.1% and 0.2% of the treated volume.

4.4 Pilot Phase (Prototype 2)

4.4.1 Evolution of Prototype 2 (Pilot)

The evolution of the second prototype represents the transition from the modular experimental phase described in the previous chapters to a simplified process configuration oriented toward industrial scalability. While Prototype 1 was designed to investigate the effectiveness of different secondary treatment modules (granular filtration, activated carbon, multistage filtration, and softening), the development of Prototype 2 involved a substantial revision of the process architecture.

The experimental results obtained in the previous phase highlighted that the main contribution to the removal of the chromophoric and colloidal components of the wastewater was attributable to the physicochemical flocculation stage, whereas the secondary modules primarily played a polishing role on the treated effluent. In light of these observations, the configuration of the second prototype was redesigned by prioritizing the optimization of mixing, flocculation, and solid–liquid separation stages, reducing the number of treatment modules and simplifying the process line.

The objective of this revision was twofold: on one hand, to increase the operational robustness of the system, and on the other, to make the process more easily transferable to an industrial scale.

The pilot system was therefore configured as a batch treatment plant consisting of a main mixing tank for the wastewater, a system for preparing the flocculant solution, and a solid–liquid separation system based on mechanical filtration. Process control is managed through a control panel that allows operation of mixers, transfer pumps, and the filtration system.

The treatment process includes an initial mixing phase between the wastewater and the chitosan solution, followed by a floc growth phase through the addition of a supporting flocculating polymer (C-PAM). The typical operational sequence consists of an initial high-intensity mixing stage to promote coagulant dispersion and destabilization of colloidal particles, followed by a slower mixing phase aimed at floc growth and aggregation.

4.4.2 Results of Prototype 2 (Pilot)

The experimental campaigns were conducted on increasing wastewater volumes, reaching up to approximately 200 L per treatment cycle. Experimental observations showed progressive floc formation already during the mixing phase with chitosan, while the addition of the polyelectrolyte enhanced the aggregation of microflocs into larger and more easily sedimentable structures. After approximately 30 minutes of sedimentation, where applied, a clear separation between the clarified liquid phase and the sludge deposited at the bottom of the tank was observed.

The tests also allowed the identification of several operational criticalities related to filtration flow management and sludge removal, highlighting the tendency of the settled material to aggregate into compact masses that are difficult to remove from the system.

The functionality of the system was evaluated through three complementary types of observations:

- (i) visual observation of the formation of macroscopic flocs immediately after the addition of the polyelectrolyte, followed by efficient separation between solid and liquid phases with progressive clarification of the wastewater;
- (ii) evaluation of treatment efficiency through UV–Vis spectrophotometric analyses conducted on treated samples;
- (iii) analysis of physicochemical parameters measured after treatment, including pH, conductivity, turbidity, hardness, total surfactants (cationic + anionic + non-ionic), ammonium ion (NH_4^+), COD, chlorides, and Cu^{2+} .

The results are presented in the following sections.

During the pilot-scale experimentation, four representative tests were selected in order to describe the operational evolution of the process and compare different wastewater treatment conditions. The selection of these tests was not random, but aimed to document:

- (i) preliminary verification of system performance on diluted wastewater;
- (ii) the first treatment on raw wastewater using a standard chitosan-based formulation;
- (iii) process optimization through modification of polyelectrolyte concentration and refinement of mechanical separation;

(iv) comparison with a conventional inorganic coagulant, namely ferric chloride.

Table 4.9 summarizes the main experimental setup parameters of the four selected tests, including

Parameter	Test I	Test II	Test III	Test IV
Primary flocculant	Chitosan	Chitosan	Chitosan	Ferric chloride
Treated volume	200 L	200 L	~180 L	200 L
Wastewater type	50% wastewater + 50% water	Raw wastewater	Raw wastewater	Raw wastewater
Chito-F 1.5%	2.0 L (1 L + 1 L)	2.0 L	1.75 L	—
FeCl₃ 2.5%	—	—	—	2.0 L
C-PAM	0.75 L (0.1%)	1.0 L (0.1%)	0.875 L (0.2%)	1.5 L (0.2%)
Primary mixing	30 min	45 min	30 min	15 min
Secondary mixing	Short mixing after C-PAM addition	15 min	30 min	15 min
Sedimentation	30 min	30 min	30 min	30 min
Filtration system	Drum filter	Drum filter + 50 µm cartridge	Drum filter + 50–10–5–1 µm cartridges	Textile filters (TB65, VN927/100, Duragaf)
Main operational observations	Formation of small flocs; low filter flow rate	Well-visible flocs; improved flow with compressed air	Formation of sludge blocks at the bottom	Less compact flocs but faster filtration
Test objective	System functionality verification	Treatment of raw wastewater	Process repeatability and optimization	Comparison with conventional coagulant

Table 4.9. Summary of the main operational parameters and experimental conditions adopted in the four pilot-scale tests carried out with Prototype 2. The table reports the type and dosage of reagents, mixing conditions, sedimentation time, filtration systems, and key operational observations, highlighting the progressive optimization of the process and the comparison between chitosan-based treatment and a conventional inorganic coagulant (ferric chloride).

The four selected tests progressively illustrate the evolution of the process at pilot scale.

Test I primarily had an exploratory and operational function, allowing the assessment of system performance on diluted wastewater and the identification of initial filtration-related criticalities.

The treated wastewater solution in the batch appeared approximately as shown in Fig. 4.19a (left beaker), exhibiting a clear phase separation with the solid fraction tending to settle, as visible in Fig. 4.19b. Following filtration (right beaker – Fig. 4.19a), the water appeared clear and transparent.

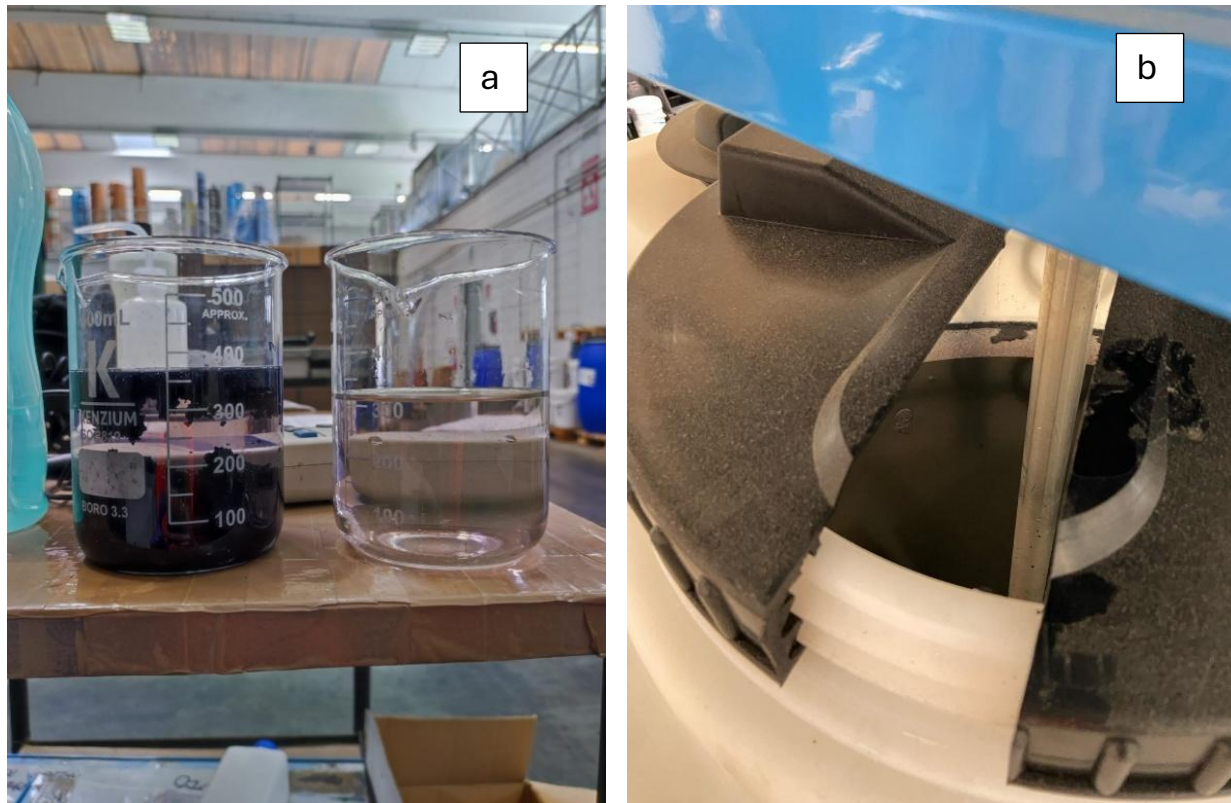


Figure 4.19 (a–b). (a) Visual comparison of wastewater before and after treatment during Test I, showing the transition from a highly colored and turbid matrix to a clarified and transparent effluent following filtration. (b) Detail of the pilot-scale filtration system during operation, highlighting the solid–liquid separation phase and the accumulation of sludge within the filtering unit.

The issues encountered during the test mainly concerned the performance of the filtration system (drum filter), which, due to structural limitations and leakage, did not retain 100% of the flocculated material, as shown in Fig. 4.20b. Furthermore, as illustrated in Fig. 4.20a, the entire system proved to be not only affected by physical losses but also relatively slow, with a flow rate of approximately 0.03 L/s (as inferred from the outlet water flow). This significantly affected the overall treatment capacity, making it poorly compatible with daily treatment requirements or, from an energy perspective, relatively inefficient compared to the achieved results. In fact, with such a flow rate, the treatment duration would be approximately 1.5 to 2 hours.

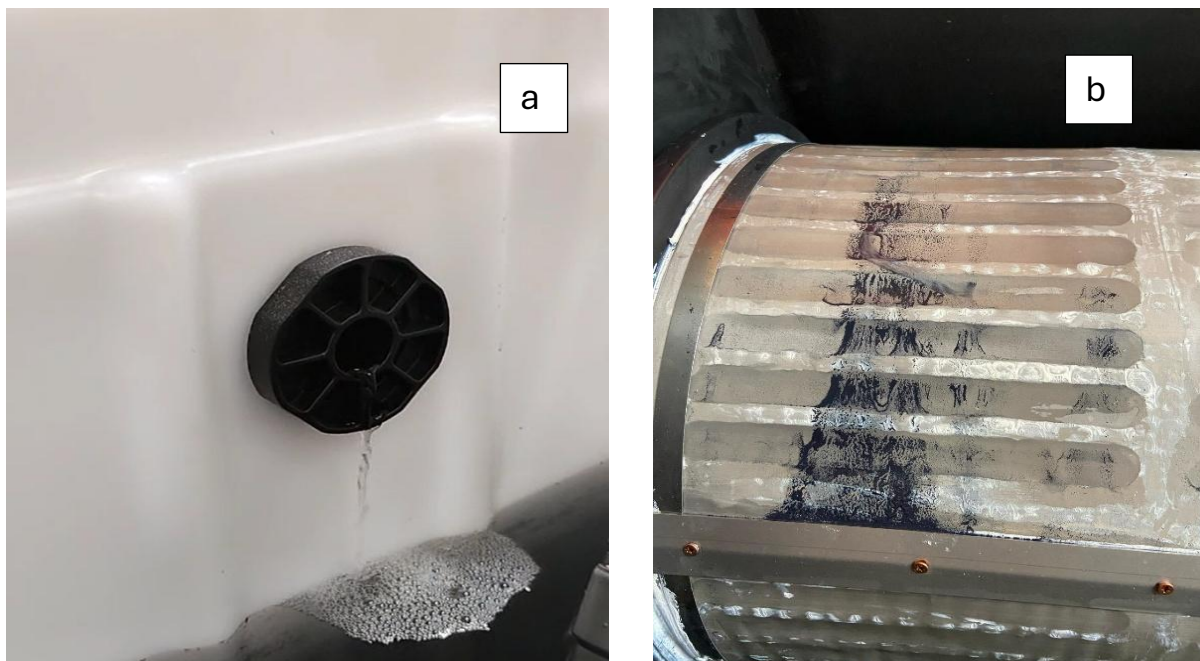


Figure 4.20 (a–b). (a) Evidence of leakage in the drum filtration system during operation, resulting in partial loss of treated effluent. (b) Accumulation of flocculated material on the surface of the drum filter, highlighting the incomplete retention of solids and the limitations of the filtration unit under the tested operating conditions.

Test II represents the first complete treatment performed on raw wastewater using chitosan, allowing the actual capability of the process to generate flocculation and solid–liquid separation under realistic operating conditions to be evaluated.

In Fig. 4.21a, the post-mixing and treatment condition with chitosan and C-PAM is shown, where phase separation is clearly visible, while Fig. 4.21b shows the water obtained after filtration. The treated water appears slightly yellowish, with small, suspended solids still present.



Figura 4.21. (a) Fase di miscelazione del refluo con chitosano, con formazione iniziale dei flocchi all'interno del reattore; (b) campione di acqua trattata post-filtrazione, caratterizzata da una colorazione giallastra e dalla presenza residua di solidi sospesi.

Regarding the system flow rate, which was 0.03 L/s in Test I, it increased to 0.08 L/s in Test II due to the use of compressed air in the drum filter (installed after the difficulties encountered in the first test). The application of an additional 50 μm mesh filter did not improve the removal of suspended solids in the treated wastewater. This is because the mesh size of the drum filter and that of the cartridge filter used were similar (30 μm vs 50 μm).

Test III represents the repeatability and optimization phase of the system, particularly with regard to the polyelectrolyte concentration and the refinement of mechanical separation through cartridge filtration. After treatment with chitosan and C-PAM, three filters were arranged in series—50, 10, and 1 μm —in order to remove suspended solids. The difference between the first and second filtration stages is shown in Fig. 4.22.

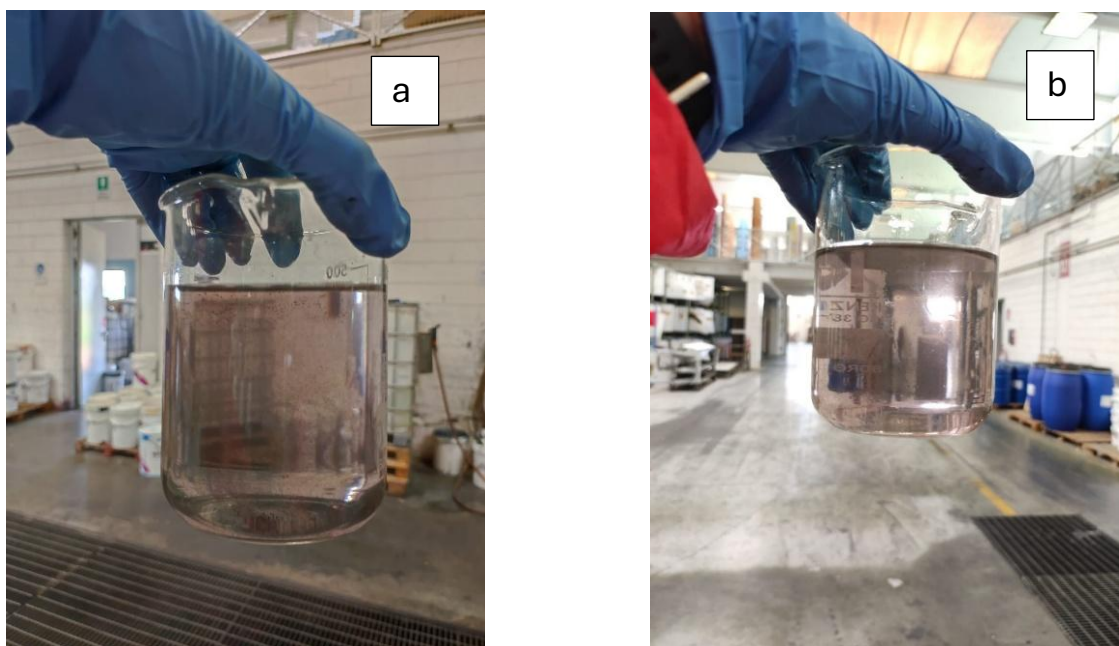


Figure 4.22. (a) wastewater sample after the first filtration stage, still characterized by high turbidity and the presence of suspended solids; (b) sample after sequential filtration (50–10–1 μm), showing a clear improvement in clarity and a reduction of suspended particles.

Test IV was carried out as a comparison with a conventional inorganic coagulant, in order to evaluate differences in performance and sludge behavior with respect to the chitosan-based treatment.

As previously mentioned, ferric chloride was used as the coagulant, applied in excess to ensure effective phase separation and to enable a comparison between an innovative treatment and a well-established one widely used in wastewater treatment.

As shown in Fig. 4.23, the treatment produced an effluent that visually appears very clear.



Figure 4.23. Wastewater sample after the treatment with FeCl_3 and filtration stage

The tests consistently resulted in a treated effluent in which most of the chromophoric compounds and the elements responsible for the dark coloration of the wastewater were removed. Based on approximate visual assessment, the colour removal can be estimated at around 95–99%. However, a significant amount of dissolved particles still remains in the effluent, affecting its overall chemical quality.

Because of not direct data about the total removal of the colour the effectiveness of the process at the pilot scale was therefore evaluated through an integrated approach, including visual observations, physical parameters (Table 4.10), and physico-chemical analyses. In particular, the system performance was not interpreted solely in terms of compliance with final regulatory limits and wastewater producer as potential reuser, but rather in terms of its ability to effectively destabilize the colloidal matrix, promote the formation of settleable flocs, and achieve a progressive separation between the liquid and solid phases.

Parameter	Test I	Test II	Test III	Test VI (FeCl ₃)	Client target	Discharge limits (152/06)
Filtrate color	Pink with suspended solids	Yellowish with small solids	Pinkish → clear after cartridge filtration	Slightly bluish	–	n.p. 1:40*
Final pH	5.55	4.66	4.68	7.91	7.2	5.5 – 9.5
Conductivity (mS/cm)	2.71	2.45	2.14	1.88	2.5	–
Turbidity (NTU)	–	3.7	2.2	1.1	0.5	–
Hardness (°f)	–	69.3	74.5	53.4	20	–
Total surfactants (mg/L)	–	26.9	25.5	22.16	≤ 4	4
Cationic surfactants (mg/L)	–	0.4	0.42	0.2	–	–
Anionic surfactants (mg/L)	–	2.2	1.95	1.26	–	–
Non-ionic surfactants (mg/L)	–	24.3	23.1	20.7	–	–
NH₄⁺ (mg/L)	–	172.5	170	121	≤ 30	30

COD (mg/L)	–	4012	4224	1302	≤ 500	500
Chlorides (mg/L)	–	0–500	0–500	0–500	≤ 1200	1200
Cu²⁺ (mg/L)	–	0.25	0.3	< 0.01	≤ 1200	0.4

Table 4.10. Summary of the main physical and chemical parameters measured for the pilot-scale treatment tests (Tests I–III using chitosan-based treatment and Test VI using ferric chloride), compared with client target values and regulatory discharge limits according to Italian Legislative Decree 152/06.

The results reported in Table 4.10 show that the chitosan-based treatment process is capable of producing an effective solid–liquid separation and a significant clarification of the wastewater, as demonstrated by the reduction in turbidity and the improvement in the visual appearance of the filtrate. However, COD and surfactant values indicate only a partial removal of the dissolved organic fraction, suggesting that the process primarily acts on the colloidal and suspended components of the matrix. The comparison with ferric chloride treatment highlights a greater reduction in COD and turbidity, but with different sludge characteristics and filtration behavior, indicating that the two systems rely on different coagulation mechanisms.

The comparison of UV–Vis absorbance spectra of the treated samples shows significant differences in the effectiveness of the various treatment configurations applied in Prototype 2 (Fig. 4.24).

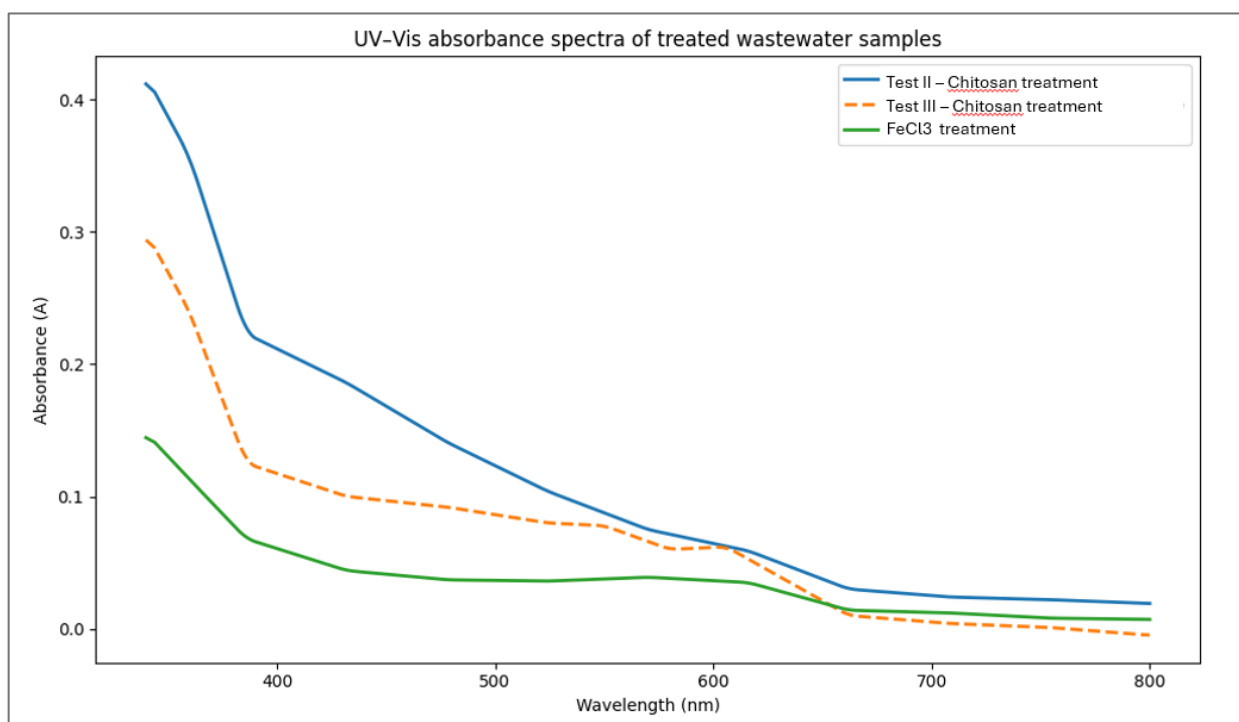


Figure 4.24. Qualitative comparison of the UV–Vis absorbance spectra of samples treated in Prototype 2: Test II (chitosan treatment), Test III (chitosan treatment under optimized conditions), and ferric chloride treatment. The graph highlights the progressive reduction of the chromophoric fraction of the wastewater and the greater effectiveness of the inorganic coagulant in reducing residual absorbance.

Test II, carried out with a chitosan-based treatment in the initial process configuration, shows the highest absorbance values across the entire spectral range considered, with a maximum in the UV region around 340–360 nm. This trend indicates the residual presence of a significant fraction of chromophoric compounds and dissolved organic substances not completely removed by the treatment.

Test III, performed under optimized operating conditions, shows an overall reduction in absorbance compared to Test II. The decrease of the curve across the entire spectrum suggests an improvement in the system's effectiveness in removing the pigment and colloidal components of the wastewater, likely attributable both to the optimization of reagent dosages and to improvements in the mechanical separation phase.

The treatment carried out with ferric chloride shows the lowest absorbance values among those considered, highlighting a greater capacity of the inorganic coagulant to reduce the residual chromophoric fraction of the wastewater. This result is also consistent with the physico-chemical data, which show a more pronounced reduction in COD and turbidity compared to chitosan-based treatments. However, the presence of a residual signal, although limited, suggests that part of the dissolved organic fraction still remains in solution.

The comparison of the spectra confirms that the treatment mainly acts on the colloidal and suspended fraction of the wastewater matrix, leading to a progressive reduction of the chromophoric component. The optimization of the chitosan-based process significantly improves treatment performance, while the comparison with ferric chloride highlights differences in effectiveness attributable to the different chemical nature of the two coagulants.

In this context, the appearance of a clear interface between sludge and water, the reduction in turbidity, the variation in absorbance, and the trend of the main physico-chemical parameters represent complementary indicators of treatment performance. The results obtained show that the chitosan-based process is capable of achieving effective solid–liquid separation and significant clarification of the wastewater, although it does not lead, under the tested conditions, to a complete removal of the dissolved organic fraction. The comparison with ferric chloride treatment further confirms that the nature of the coagulant influences not only the quality of the final filtrate but also the characteristics of the sludge and the manageability of the filtration phase.

Overall, these findings indicate that the coagulation–flocculation process based on the use of chitosan and polyelectrolyte is effective at pilot scale, enabling rapid solid–liquid separation and a significant reduction in turbidity and wastewater color. From the perspective of physico-chemical parameters, the treated water shows a significant reduction in turbidity and a decrease in the chromophoric component, highlighting the effectiveness of the flocculation process in removing colloidal particles and pigment fractions present in the wastewater. However, some parameters, such as COD, surfactants, and ammonium, remain above the discharge limits, indicating the need for further polishing treatment stages in order to achieve full regulatory compliance. It can therefore be concluded that the development of Prototype 2 has made it possible to demonstrate the feasibility of the flocculation process at pilot scale and to define the most suitable operating conditions for the destabilization of the colloidal matrix of the wastewater. The simplification of the plant layout and the optimization of chemical dosages thus represent a key step toward potential industrial-scale implementation of the process.

Chapter 5 — Discussion

5.1 Variability of the matrix and implications for the process

One of the most evident aspects that emerged during the experimental campaign was the marked variability of the wastewater matrix, both between the two facilities considered and over time within the same production site. The analytical campaigns showed significant differences in the main physico-chemical parameters, including pH, conductivity, COD, BOD₅, surfactant concentration, ammonium, and copper content, with very wide measurement ranges. This confirms that wastewater deriving from the washing of industrial inks cannot be considered a constant matrix, but rather a dynamic system whose composition depends on production operating conditions, the formulation of the inks used, and the degree of wastewater dilution. For instance, COD values ranged from approximately 12,250–14,650 mg/L in Plant B to extreme values exceeding 28,000 mg/L in Plant A, while conductivity varied from approximately 1.7 mS/cm to more than 5 mS/cm depending on sampling conditions. These variations quantitatively confirm the strong heterogeneity of the wastewater matrix and highlight the need for flexible operating conditions

The comparison between the two wastewaters from plants A and B highlighted two distinct behaviors: wastewater from plant B showed a more homogeneous overall composition, with pH close to neutrality and relatively high but fairly stable conductivity, making it a more suitable matrix for the initial stages of process optimization. In contrast, wastewater from plant A showed greater temporal variability, culminating in extreme conditions of alkalinity and very high organic load, which can be interpreted as a worst-case scenario. Moreover, it should be emphasized that the composition and quality of the wastewater were also strongly influenced by the sampling position within the storage system. In particular, samples collected from the deeper zones of the container generally showed higher concentrations of the main chemical parameters, attributable to the greater presence of pigment particles, sediments, and colloidal fractions accumulated over time. Conversely, samples collected near the surface presented, on average, lower values for the same parameters, being more diluted and characterized by a chemically less concentrated matrix. This vertical heterogeneity of the wastewater matrix highlights that the storage system does not behave as a perfectly homogeneous volume, but rather tends to develop concentration gradients between bottom and surface, with direct implications both for the representativeness of the collected samples and for the evaluation of treatment performance. This difference had direct relevance for the experimental design: on the one hand, it allowed process development under initially more controllable conditions; on the other hand, it made it possible to subsequently verify the robustness of the process in the presence of more critical matrices.

From an engineering perspective, these findings indicate that wastewater variability represents the main process variable. In such a complex system, flocculation behavior does not depend exclusively on the nature of the reagent used, but on the interaction among the polymer, pH, ionic strength of the solution, concentration of dispersed pigments, presence of surfactants, and overall organic load. It follows that apparently simple parameters, such as chitosan dosage or contact time, cannot be interpreted as absolute constants, but must be read in relation to the specific characteristics of the matrix treated in each test.

This observation helps explain why the experimental pathway did not follow a rigidly linear logic. The progressive modifications of the flocculant formulation, dosage, treatment sequence, and plant configuration should not be interpreted as elements of methodological discontinuity, but as a direct consequence of the need to adapt the process to a highly variable real matrix. In this sense, the experimentation showed that treatment transferability does not depend solely on its effectiveness under optimal conditions, but above all on its ability to maintain satisfactory performance as wastewater conditions vary.

Matrix variability also had direct implications for the choice of criteria used to evaluate process effectiveness. In the initial stages, system response was mainly assessed in terms of floc formation, supernatant clarification, and reduction of the chromophoric component, precisely because these parameters were the most immediately sensitive to differences among the wastewaters tested. Only in later stages, once a sufficiently stable operating window had been defined, was it possible to expand monitoring to chemical parameters more strictly related to discharge suitability or potential reuse.

The analyses carried out at laboratory, prototype, and pilot scale therefore indicate that chitosan-based treatment has good application potential even for complex real wastewaters, but that its implementation requires a flexible process approach capable of adapting to matrix variability. Rather than toward the definition of a single and immutable dosage, technological development therefore appears to be directed toward identifying a robust operating window within which treatment parameters can be modulated according to the characteristics of the influent wastewater.

5.2 Role of pH and operating conditions in flocculation

One of the most significant results emerging from the experimental phase concerns the decisive role of pH and mixing operating conditions in the flocculation process using chitosan. The tests carried out during the exploratory phase showed that the ability of the system to generate settleable aggregates depends critically on the chemical window within which treatment takes place, as well as on the hydrodynamic conditions governing the interaction between the flocculating polymer and the dispersed particles present in the wastewater. The results of the pH study showed that the destabilization of the colloidal matrix is strongly limited under conditions close to neutrality. In samples with pH between about 6 and 7, the wastewater matrix remained visually stable and homogeneous, without significant floc formation and with no evident separation between liquid and solid phases. This behavior is consistent with the chemical nature of chitosan, whose amino groups are poorly protonated at neutral pH and are therefore unable to develop a sufficient positive charge to effectively interact with the colloidal particles present in the wastewater.

The situation changes clearly when pH is reduced to values between about 4.5 and 5.5, a range in which progressive formation of macroscopically visible flocs and a clear separation between liquid phase and sediment are observed. Under these conditions, chitosan assumes a more protonated configuration, acquiring a positive charge that favors electrostatic interaction with pigment particles and other colloidal fractions present in the matrix. The flocculation process can therefore be interpreted as the combined result of surface charge neutralization and polymer bridging mechanisms, which lead to the formation of aggregates large

enough to be separated by sedimentation or filtration. This qualitative observation is supported by the experimental data reported in Table 4.6, where floc formation was observed only when the pH decreased from approximately 7.0 to values close to 5.0. At $\text{pH} \approx 7$ no flocculation occurred, while at $\text{pH} \approx 5$ clear phase separation was obtained. This behavior quantitatively confirms the existence of a critical operating window for effective chitosan-based coagulation.

Alongside pH, mixing conditions and contact time also proved to be determining factors for process effectiveness. Tests conducted by varying stirring speed and contact time showed that floc formation occurs only within a specific operating window. Stirring speeds that are too low tend to limit contact between the polymer and dispersed particles, reducing the probability of collision and therefore aggregate formation. Conversely, excessively intense mixing conditions can cause fragmentation of newly formed flocs, compromising flocculation stability. Similarly, the contact time between the flocculant and the wastewater significantly affects floc growth. Contact times that are too short do not allow completion of the aggregation processes, whereas longer times promote aggregate growth and compaction. The experimental results indicate that an operating window characterized by intermediate stirring intensity (about 100–150 rpm) and contact times between 30 and 60 minutes represents a favorable configuration for the formation of stable and easily separable flocs.

These results are particularly relevant in the context of prototype process development. Defining an operating window in terms of pH, stirring speed, and contact time made it possible to establish the reference conditions used in subsequent larger-scale tests, contributing to system stabilization during prototype development. At the same time, these findings confirm that flocculation with chitosan cannot be interpreted as a simple chemical dosing operation, but must be considered a complex physico-chemical process in which hydrodynamic conditions and the chemical environment of the system play a role just as important as the nature of the reagent used.

5.3 Effectiveness of chitosan on the colloidal and chromophoric fraction

The experimental results obtained during the different phases of the work show that chitosan-based treatment is particularly effective in removing the colloidal and chromophoric fraction of the wastewater, namely the component mainly consisting of dispersed pigment particles, micro-aggregates, and organic compounds responsible for the intense coloration typical of wastewaters deriving from ink processing.

Already in the preliminary laboratory tests, the formation of macroscopically visible flocs and the subsequent clarification of the supernatant represented the first qualitative indicator of the effectiveness of the colloidal matrix destabilization process. The formation of compact and easily settleable aggregates demonstrated the ability of chitosan to interact with the dispersed particles present in the wastewater, promoting their aggregation and subsequent separation from the liquid phase by sedimentation or filtration. This behavior is consistent with the typical mechanism of action of the polymer, which in an acidic environment acquires a positive charge and

favors neutralization of the surface charges of colloidal particles, accompanied by polymer bridging phenomena between adjacent particles [30].

Process effectiveness was also particularly evident from UV–Vis spectrophotometric analysis, used as a semi-quantitative indicator of chromophoric fraction removal, allowing comparison of treatment configurations on the basis of absorbance reduction across the visible range.. Comparison of the spectra of samples treated under different experimental configurations showed a significant reduction in absorbance across the entire spectral range analyzed (340–800 nm), indicating a substantial decrease in the concentration of the species responsible for wastewater coloration. Although UV–Vis analysis was mainly used as a semi-quantitative indicator, the spectra show a consistent decrease in absorbance across the entire range (340–800 nm), with treated samples approaching near-baseline values in the visible region. This corresponds to a substantial reduction of the chromophoric fraction, consistent with the visual clarification observed during treatment. In many cases, especially in the more advanced treatment configurations, the spectrum of the final sample appears almost completely flattened in the visible region, suggesting very effective removal of the dispersed pigment fraction. This behavior is consistent with the nature of the wastewater matrices considered in this study. Wastewaters deriving from ink processing contain a significant amount of organic pigments, coloring particles, and aromatic compounds which, although present in dispersed or colloidal form, contribute substantially to matrix coloration. Chitosan-induced flocculation acts mainly on this component, destabilizing the colloidal system and promoting aggregation of coloring particles into larger flocs that can be easily separated from the liquid phase.

Visual observation of the treated samples during the different experimental phases further confirms this interpretation. In several cases, treatment produced a marked visual clarification of the wastewater, consistent with the substantial reduction in UV–Vis absorbance observed after flocculation and filtration. However, these observations should be interpreted as supportive evidence of chromophoric fraction removal rather than as a fully quantified measure of colour removal efficiency. However, analysis of the physico-chemical parameters also highlights an important aspect: the significant removal of the chromophoric and colloidal component does not necessarily translate into an equally marked reduction in the dissolved organic fraction. COD, surfactants, and ammonium values, although in some cases showing a reduction compared with the initial wastewater, often remain above the limits required for direct discharge. This behavior indicates that flocculation with chitosan acts mainly on the suspended and colloidal component of the matrix, whereas the fully solubilized organic fraction largely remains in solution. This behavior is consistent with the quantitative results obtained during the pilot tests, where COD values decreased from approximately 4000–4200 mg/L to values around 1300 mg/L after treatment, while turbidity values decreased to approximately 2–3 NTU. These data confirm that the process primarily removes the colloidal fraction while leaving part of the dissolved organic load in solution.

Overall, these results suggest that chitosan is a particularly effective agent for primary separation of the pigmentary and colloidal component of the wastewater, contributing significantly to matrix clarification and chromophoric component removal. However, to obtain a more complete reduction of parameters associated

with the dissolved organic fraction, the process must be integrated with additional treatment stages, such as adsorption on activated carbon, polishing filtration, or other secondary treatment systems. From this perspective, the chitosan-based flocculation system can be interpreted not so much as a complete wastewater treatment process, but rather as a highly effective primary clarification and solid–liquid separation stage, on which any subsequent polishing phases can be built.

5.4 Significance of the auxiliary modules in Prototype 1

The development of Prototype 1 represented a fundamental stage of the experimental work, since it made it possible to evaluate not only the effectiveness of chitosan flocculation, but also the contribution of the different auxiliary treatment modules progressively introduced into the system. Unlike laboratory tests, in which the main objective was to verify floc formation and the preliminary separation of the solid phase, the prototype phase made it possible to analyze process behavior in a more complex configuration, closer to a possible plant-scale application.

During the experimental tests, the system configuration was modified several times through the introduction of different secondary treatment modules, including primary mechanical filtration systems, granular filtration, adsorption on activated carbon, and fine filtration using cartridges. The objective of this modular configuration was to progressively evaluate the contribution of each stage to the final quality of the treated wastewater and to understand which process components were actually necessary to improve the overall performance of the system.

The chitosan flocculation stage played the role of primary treatment, responsible for destabilizing the colloidal matrix and forming flocs. However, as emerged from the analyses carried out under the different experimental configurations, the separation obtained in this phase was not always sufficient to ensure complete removal of the finest particles and dissolved organic species present in the matrix. In this context, the auxiliary modules played a decisive role in improving filtrate quality.

The first significant contribution was provided by the mechanical and granular filtration systems, which made it possible to retain residual solid particles and microflocs not completely removed during the initial separation phase. Passage through filters of different particle sizes further reduced wastewater turbidity and stabilized separation between liquid and solid phases. This step demonstrated that, in the presence of complex matrices such as those deriving from ink processing, flocculation alone is not always sufficient to guarantee complete removal of the finest suspended fraction.

An even more relevant contribution was observed with the introduction of activated carbon, used as an adsorbent system for removal of the dissolved organic fraction. For instance, in Prototype 1 the COD decreased from approximately 1381 mg/L after primary filtration to about 545 mg/L after activated carbon treatment, reaching final values close to 100 mg/L. This quantitative reduction confirms the key role of adsorption in removing dissolved organic compounds. Consequently, the experimental results showed a significant reduction in COD and in the chromophoric component of the wastewater after passage through the adsorption column, indicating that an important fraction of the substances responsible for organic load and matrix coloration is not

present as suspended particles, but as dissolved molecular species. Adsorption on activated carbon therefore represented a key step in improving the chemical quality of the treated wastewater.

Finally, the fine filtration systems using cartridges of decreasing particle size performed a final polishing function on the filtrate. These stages allowed removal of any residual particles and further improved the clarity of the treated wastewater, contributing to the stabilization of system performance under the various tested configurations.

The overall analysis of the results obtained with Prototype 1 therefore shows that the treatment process cannot be interpreted as a single flocculation operation, but rather as an integrated sequence of separation and polishing operations. In this sequence, chitosan flocculation plays the role of the primary phase of destabilization and aggregation of colloidal particles, while the auxiliary modules contribute to the removal of finer fractions and the dissolved organic component.

From an engineering point of view, Prototype 1 therefore played an essential role in the technological development process, making it possible to distinguish the contribution of the different treatment stages and identify which modules were most effective in improving final wastewater quality. This modular approach also provided important indications for the subsequent pilot-system design phase, in which the process architecture was simplified by prioritizing the stages considered most decisive for matrix separation.

5.5 Stability and durability of the flocculating solution

An aspect that emerged during the experimentation, of particular relevance for a possible industrial application of the process, concerns the long-term stability of the chitosan-based flocculating solution. In real treatment systems, the flocculating solution is generally not prepared and used immediately, but is stored in accumulation tanks and progressively dosed into the wastewater through pumping or automatic dosing systems. Verification of the persistence of flocculating effectiveness over time therefore represents a fundamental step in evaluating the actual operational transferability of the process.

The durability of chitosan solutions is also one of the topics frequently discussed in the literature when the polymer is used as a coagulant or flocculant in water treatment. Several studies highlight that solution stability depends on factors such as the degree of protonation of amino groups, polymer concentration, solution viscosity, and storage conditions, all elements that may influence the ability of chitosan to interact with the colloidal particles present in the wastewater matrix [94]. In particular, solubilization of chitosan in an acidic environment generally ensures good chemical stability of the solution, but variations in concentration or viscosity may modify the flocculating behavior of the polymer over time.

The results obtained in the durability study show that the developed flocculating solution maintained good functional stability for several weeks, without evident loss of effectiveness in separating the pigmentary fraction of the wastewater. In cases where flocculation proved effective, the physico-chemical parameters of the filtrate showed remarkable stability over time. pH values systematically fell within a relatively narrow range, between about 5.3 and 5.6, suggesting that the system tends spontaneously to stabilize within a chemical window favorable to protonation of the amino groups of chitosan. Under these conditions, the polymer acquires

a positive charge that favors electrostatic interaction with negatively charged colloidal particles present in the wastewater, promoting the formation of easily separable aggregates.

Similarly, the electrical conductivity values of the filtrate showed limited variation among the different weeks of monitoring, indicating that the treatment process acts mainly through removal of the colloidal and pigmentary fraction, without causing substantial modifications to the ionic composition of the liquid phase. The slight increase observed compared with the initial wastewater may be attributed to the presence of acetic acid used for chitosan solubilization, which introduces a modest amount of dissolved ionic species into the system.

Further indications of process stability derive from the analysis of the UV–Vis spectra of the treated wastewater. The absorbance curves of the treated samples are strongly superimposable even several weeks after preparation of the flocculating solution, indicating that the effectiveness of chromophoric fraction removal remains substantially constant over time. The reduction in absorbance observed along the entire spectral range analyzed is consistent with a removal mechanism based on aggregation and subsequent separation of pigment particles rather than on selective chemical degradation phenomena.

Overall, the results obtained indicate that the developed flocculating formulation presents good operational stability over time, maintaining its ability to induce separation of the pigmentary phase even after several weeks of storage. From an operational standpoint, the possibility of preparing the flocculating solution in advance of its use represents a fundamental requirement in industrial treatment systems, in which reagents are generally stored in dedicated tanks and automatically dosed according to wastewater flow rate. This aspect is of notable application interest, since it suggests that the solution can be prepared and stored for prolonged periods without significant loss of effectiveness, thereby facilitating integration of the process into continuous or semi-automatic treatment systems based on controlled reagent dosing.

5.6 Transition to pilot scale and the role of C-PAM

The transition from Prototype 1 to the pilot system (Prototype 2) represented a key moment in process development, not only from the perspective of operational scale, but also with regard to the very nature of the treatment applied to the wastewater matrix. During the initial phases of the experimentation, the process could mainly be described as a chemical treatment based on the addition of a flocculating reagent (chitosan) with the objective of destabilizing the colloidal system present in the wastewater. In this configuration, the main action of the reagent was to neutralize the surface charges of dispersed particles, promoting aggregation of pigments and colloidal fractions present in the matrix.

With the evolution of the system and the introduction of further operational stages, the process progressively took on the typical characteristics of a physico-chemical treatment. In the field of water treatment, this term indicates a process in which the addition of chemical reagents is combined with physical operations for separating the solid phase from the liquid phase, such as sedimentation, filtration, or flotation. In the specific case of the system developed in this work, the chemical component is represented by the addition of coagulants

and flocculants (chitosan and polyelectrolyte), whereas the physical component consists of mixing operations, floc growth, sedimentation, and subsequent filtration of the treated wastewater.

The introduction of C-PAM (cationic polyacrylamide) played a central role in this transition. Whereas chitosan acts mainly as a primary coagulant, destabilizing the colloidal particles present in the wastewater through charge-neutralization mechanisms, C-PAM instead functions as a flocculating agent, favoring aggregation of the microflocs generated in the previous stage. Thanks to its high-molecular-weight polymeric structure, the polyelectrolyte is able to create bridges between already destabilized particles, promoting the formation of larger and more compact aggregates. This phenomenon, known as polymer bridging, significantly increases the size and density of the flocs, making them more easily settleable and improving subsequent solid–liquid separation. The combined use of natural biopolymers and synthetic coadjuvants is currently a strategy frequently proposed in the literature to improve floc stability and size in the clarification of complex wastewaters [95].

From an operational perspective, the process can therefore be configured as a two-stage system. In the first phase, coagulation occurs, in which chitosan destabilizes the colloidal matrix and generates microaggregates of pigment particles and dispersed organic fractions. In the second phase, C-PAM promotes floc growth and compaction, facilitating their sedimentation or removal by filtration. This sequence is typical of the physico-chemical treatments used in industrial wastewater treatment plants, where process effectiveness depends on the correct combination of chemical reagents and physical separation conditions.

The transition to pilot scale therefore made it possible to observe more clearly the importance of this integration between the two stages. During tests on volumes on the order of 200 L per treatment cycle, floc formation was already visible during the mixing phase with chitosan, but the addition of C-PAM produced a significant increase in aggregate size and a clearer separation between the clarified liquid phase and the sludge deposited at the bottom of the tank. This behavior confirmed that the system based on the combination of chitosan and polyelectrolyte is more effective and stable than the use of the coagulant alone. This improvement was also reflected in the measured parameters, with turbidity values decreasing to approximately 2–3 NTU and improved filtration flow rates compared to the initial prototype configuration. These observations quantitatively support the role of C-PAM in enhancing floc growth and separation efficiency.

At the same time, observation of the pilot-scale system highlighted some operational criticalities related to sludge management and the filtration phase, in particular the tendency of the flocculated material to aggregate into compact blocks and the limited filtration rate of the system initially adopted. These aspects showed that treatment effectiveness does not depend exclusively on process chemistry, but also on the design of the physical separation units.

Overall, the transition from the experimental prototype to the pilot system made it possible to transform the process from a simple chemical dosing operation into a true physico-chemical solid–liquid separation system, in which coagulation with chitosan and flocculation with C-PAM act synergistically with sedimentation and filtration stages. This evolution represents a fundamental step toward a possible industrial application of the

process, since it makes it possible to integrate reagent action with a separation system capable of handling wastewater volumes significantly larger than those used in laboratory tests.

5.7 Comparison with ferric chloride

In order to contextualize the effectiveness of the developed system and evaluate its competitiveness relative to technologies commonly used in industrial wastewater treatment, an experimental comparison was carried out between the chitosan-based process—possibly assisted by a synthetic polymer—and a traditional flocculant widely used in physico-chemical treatments, ferric chloride (FeCl_3). This comparison was introduced with the objective of evaluating chitosan as a non-conventional material and a potential sustainable alternative to metallic coagulants, as also suggested in recent literature [96], [97]. Ferric chloride is in fact one of the most widely used inorganic coagulants in water treatment processes, thanks to its high ability to destabilize colloidal suspensions through charge-neutralization mechanisms and the formation of insoluble metallic hydroxides, which promote aggregation and subsequent sedimentation of suspended particles and numerous organic and inorganic species present in the wastewater matrix. In this way, aggregates are formed that sediment or can be removed by filtration. However, the use of ferric salts generally also involves some operational drawbacks, including the production of relatively abundant inorganic sludge, possible alteration of the pH of the treated wastewater, and the introduction of a metallic component into the residual matrix.

During the experimental tests, ferric chloride was used as a reference treatment within the pilot plant, applying operating conditions analogous to those adopted for the chitosan-based system. The main objective of this comparison was not exclusively to evaluate the removal efficiency of the monitored chemical parameters, but also to analyze differences in system behavior in terms of floc formation, sludge management, and final effluent quality.

The results obtained showed that both systems are capable of producing a significant reduction in turbidity and in the suspended fraction present in the wastewater. However, in the case of ferric chloride, floc formation was generally faster but characterized by more compact and less structured aggregates than those observed in the chitosan treatment. By contrast, chitosan showed a greater ability to generate voluminous and relatively light flocs, favoring more gradual separation and a more homogeneous distribution of the flocculated material.

From the point of view of effluent quality, the differences between the two treatments were particularly evident in relation to the organic and chromophoric component of the matrix. The chitosan-based system showed good effectiveness in removing the colloidal fractions responsible for wastewater coloration, thanks to the polymer's ability to interact with pigments and organic molecules through combined mechanisms of charge neutralization and polymer bridging. Ferric chloride, on the other hand, showed greater effectiveness in reducing turbidity and some particulate species, but lower selectivity toward the more complex organic components. This difference is also reflected in the measured parameters, where ferric chloride treatment showed lower turbidity (1.1 NTU) and COD values (1302 mg/L) compared to chitosan-based treatments, which remained above 4000 mg/L under similar conditions. These results quantitatively confirm the higher coagulation strength of the inorganic coagulant, although with different sludge characteristics.

A further differentiating element concerns management of the sludge produced by treatment. The use of inorganic coagulants such as ferric chloride generally leads to the formation of sludges containing metallic hydroxides, which may be more difficult to manage and dispose of. By contrast, the sludges generated by chitosan treatment have a predominantly organic composition and are potentially more compatible with alternative management strategies, in line with the circular-economy approach that guided process development.

The comparison with ferric chloride therefore made it possible to highlight that the chitosan-based system can represent a valid alternative to traditional coagulants, especially in contexts in which it is desirable to reduce the use of inorganic reagents and limit the introduction of metals into the treatment cycle. At the same time, the experimental results suggest that the choice of the most suitable reagent should be evaluated case by case, taking into account the specific characteristics of the wastewater matrix and the objectives of the treatment, both in terms of removal efficiency and overall process management.

5.8 Potential valorization routes of the recovered sludge

An important aspect that deserves consideration when evaluating the overall sustainability of the proposed treatment process concerns the management and potential valorization of the sludge generated during coagulation–flocculation. Conventionally, sludge produced during industrial wastewater treatment is regarded as a waste requiring disposal, with the associated environmental and economic burdens often representing one of the major operational costs of treatment plants. However, the composition of the sludge generated in the present study suggests that alternative management strategies based on material recovery may be feasible, in line with the principles of circular economy and industrial symbiosis.

The solid fraction produced during the treatment process originates from the aggregation of suspended particles, pigments, polymeric binders, fillers and other colloidal compounds naturally present in flexographic printing wastewater. During coagulation–flocculation, protonated chitosan promotes the destabilization of colloidal particles through charge neutralization and polymer bridging mechanisms, leading to the formation of dense flocs that can be readily separated from the aqueous phase. Consequently, the recovered sludge is not simply a waste product but rather a concentrated fraction containing a significant proportion of the original raw materials employed in the ink formulation.

To better understand the composition of this material, X-ray diffraction (XRD) analyses were performed on representative sludge samples (Fig. 5.1). The mineralogical characterization (Tab. 5.1) revealed that titanium dioxide (TiO_2) represents the predominant crystalline phase, accounting for approximately 59 wt.% of the crystalline fraction (Fig. 5.2). Titanium dioxide is widely employed in the printing industry as a high-performance white pigment owing to its excellent opacity, brightness, chemical stability and resistance to ultraviolet degradation. Besides TiO_2 , the sludge contains calcite (approximately 13 wt.%) (Fig. 5.3), commonly used as a mineral filler in printing inks and paper products, together with minor crystalline phases including archerite, sodium chlorate, cristobalite, ferrihydrite and traces of goethite. Overall, the mineralogical composition indicates that the recovered material is largely constituted by inorganic pigments and fillers

originally incorporated into the printing ink formulation rather than by newly formed hazardous compounds generated during the treatment process.

Crystalline phase	Chemical formula	Phase quantity (wt.%)
Titanium dioxide (Rutile)	TiO ₂	58.89
Magnesium perchlorate	Mg(ClO ₄) ₂	16.60
Calcite	CaCO ₃	13.36
Sodium chlorate	NaClO ₃	3.80
Archerite	KH ₂ PO ₄ ·NH ₄ H ₂ PO ₄	3.28
Cristobalite (high)	SiO ₂	3.03
Ferrihydrite	Fe ₅ HO ₈ ·4H ₂ O*	0.71
Goethite	FeO(OH)	0.33
Total	—	100.00

Table 5.1. Quantitative phase composition of the recovered sludge obtained after chitosan-based coagulation–flocculation, determined by Rietveld refinement of the X-ray diffraction pattern. Titanium dioxide (TiO₂) represents the predominant crystalline phase, followed by magnesium perchlorate and calcite, indicating that the recovered material mainly consists of pigments and mineral fillers originally present in the flexographic ink formulation.

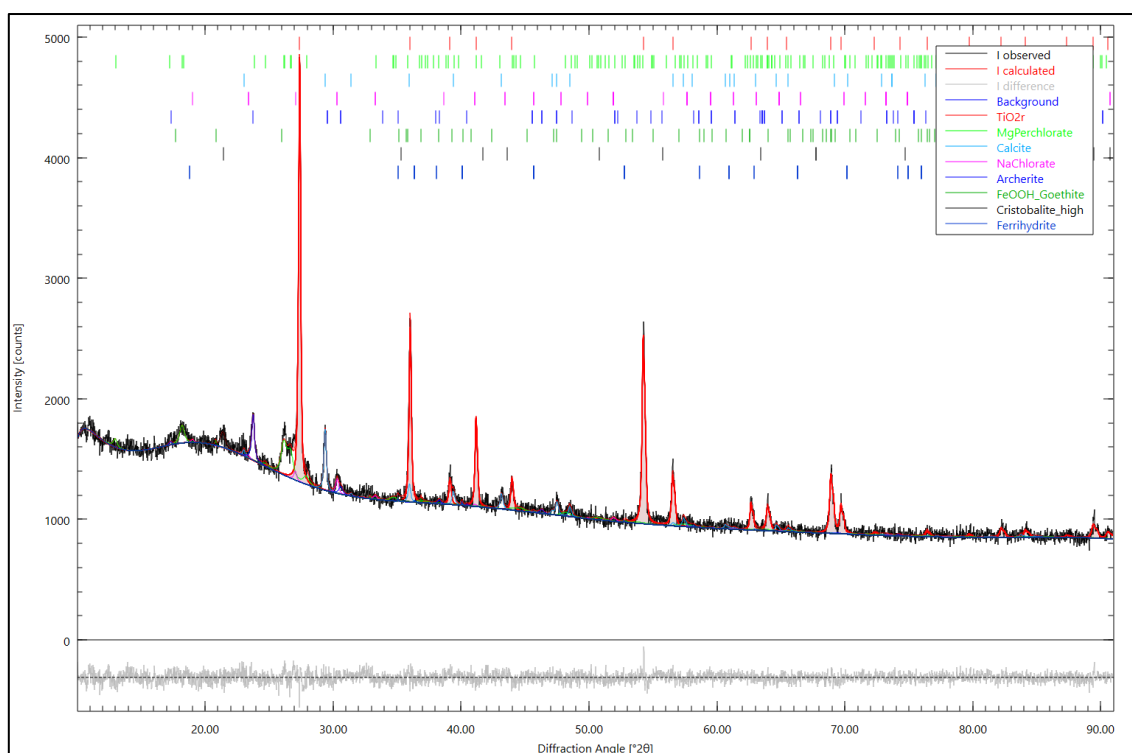


Figure 5.1. X-ray diffraction (XRD) pattern of the recovered sludge obtained after the chitosan-based coagulation–flocculation treatment of flexographic wastewater. The black line represents the experimental diffractogram, the red line the Rietveld refinement, the grey line the residual difference, while the coloured tick marks indicate the Bragg reflection positions of the identified crystalline

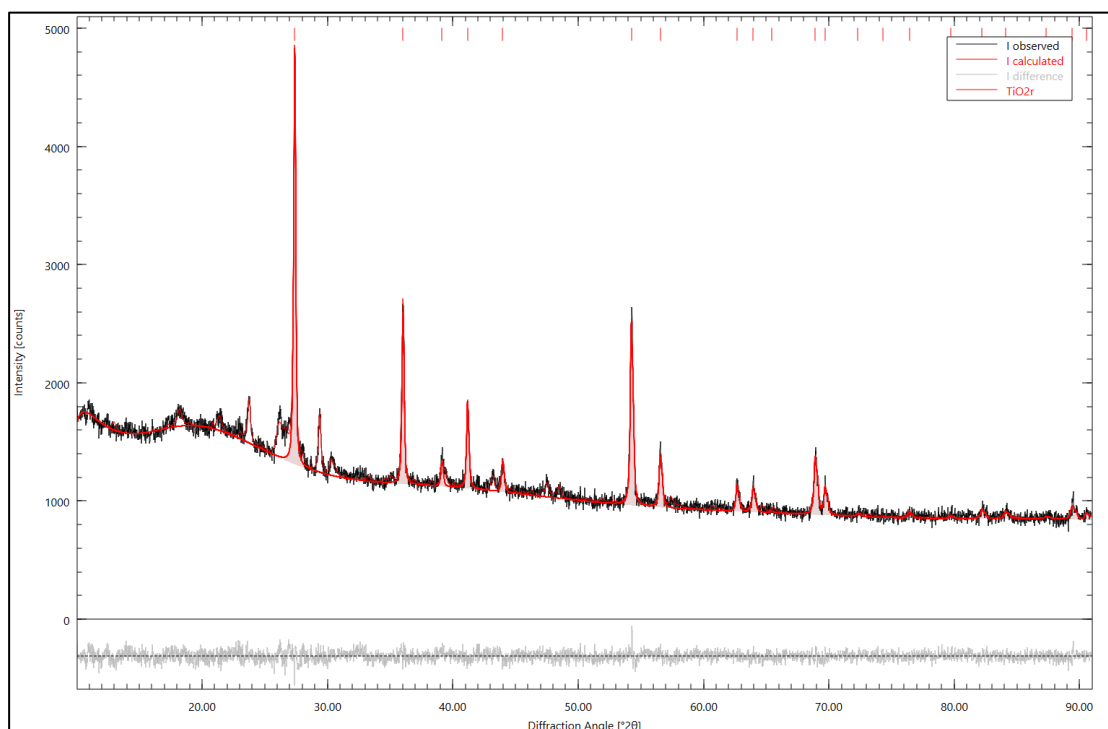
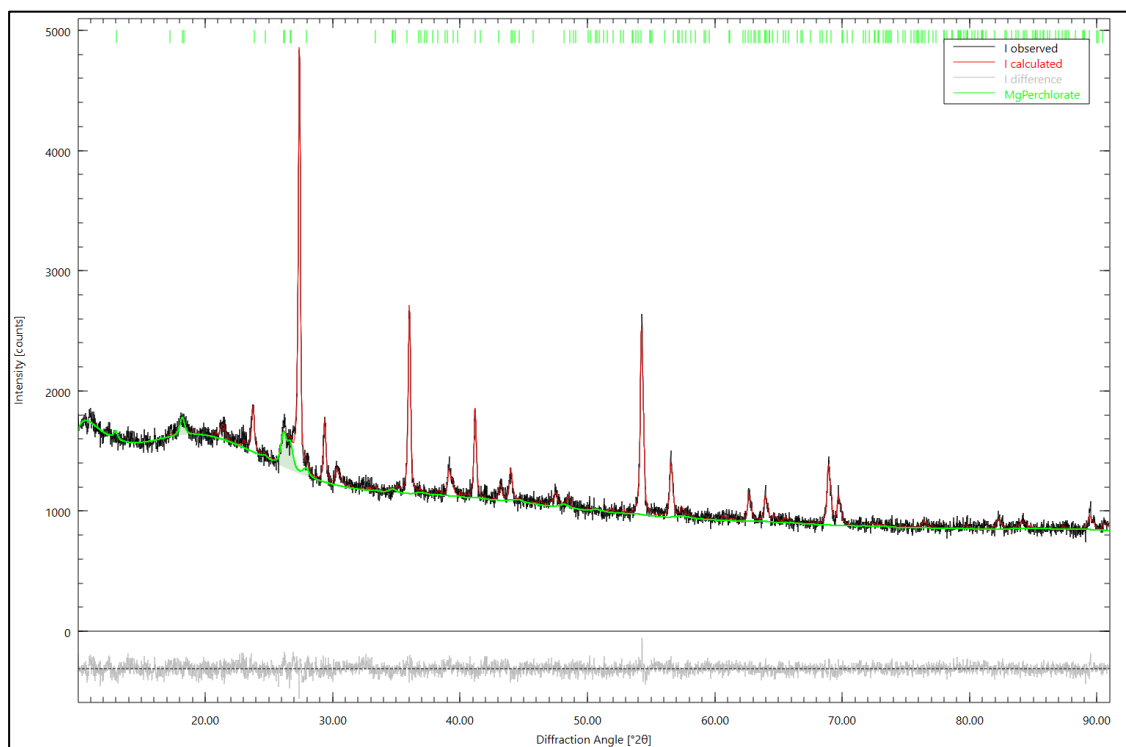


Figure 5.2 X-Ray Diffraction pattern and Rietveld refinement highlighting the TiO_2 phase identified in the recovered sludge after the coagulation–flocculation treatment.



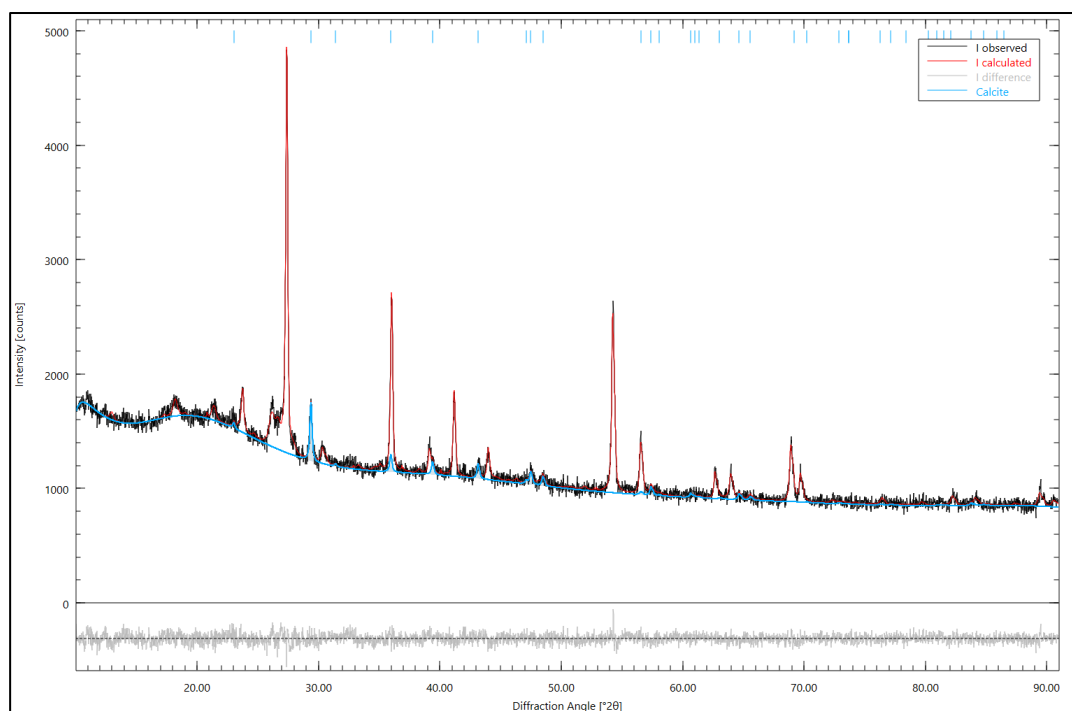


Figure 5.3 X-Ray Diffraction pattern and Rietveld refinement highlighting the Calcite and MgPerchlorate phase identified in the recovered sludge after the coagulation–flocculation treatment.

Within the framework of the present research, preliminary industrial evaluations were carried out in collaboration with a commercial manufacturer of water-based flexographic inks in order to assess the possibility of directly reusing the recovered sludge as a secondary raw material. Initial proof-of-concept experiments demonstrated that the recovered material could be incorporated into newly formulated black water-based inks. Different physical forms of the recovered product were evaluated, including both liquid and semi-liquid formulations. Although the colour strength remained significantly lower than that obtained using virgin commercial pigments, the semi-liquid recovered material exhibited more promising behaviour than the liquid formulation. In particular, preliminary tests indicated that partial substitution of virgin pigments with recovered material is technically feasible, although additional optimization is required to increase pigment concentration, colour intensity, dispersion stability and rheological performance.

From an industrial perspective, these preliminary results are particularly encouraging. Discussions with the industrial partner indicated that no comparable commercial products are currently available, suggesting that the recovered material may represent an innovative secondary raw material rather than a conventional waste. Furthermore, the expected production costs appear compatible with current manufacturing processes, potentially allowing its integration into existing industrial formulations without significant economic penalties. Such an approach would contribute simultaneously to reducing virgin raw material consumption, decreasing waste disposal requirements and improving the overall environmental sustainability of flexographic printing operations.

Nevertheless, several technical challenges remain before large-scale implementation can be considered. One of the principal issues concerns the long-term microbiological stability of the recovered material. Since recent

European regulations have progressively restricted the range of biocides available for industrial formulations, maintaining adequate preservation during storage may become increasingly challenging, particularly considering the residual organic matter contained in the recovered sludge. Additional studies should therefore investigate appropriate stabilization strategies capable of preserving product quality while maintaining compliance with current regulatory requirements. Further investigations are also required to evaluate batch-to-batch variability, long-term storage stability, rheological properties, colour reproducibility and compatibility with different commercial ink formulations. In addition, although preliminary industrial feedback has been highly encouraging, comprehensive analytical characterization—including migration studies where appropriate, chemical safety assessment and quality control procedures—will be necessary before the recovered material can be routinely classified and marketed as a secondary raw material (MPS).

Where direct material recovery is not technically or economically feasible, alternative valorization strategies may also be considered. Thermochemical conversion processes, such as pyrolysis, represent one possible option for recovering energy and carbonaceous products from the organic fraction of the sludge. Similarly, conventional energy recovery through controlled thermal treatment could reduce disposal volumes while partially recovering the calorific value associated with the residual polymeric binders and organic compounds. However, the heterogeneous composition of the sludge, including pigments, mineral fillers and residual organic substances, requires dedicated environmental and techno-economic assessments before these alternatives can be considered viable. In particular, emissions, ash composition, process efficiency and overall life-cycle impacts should be carefully evaluated to ensure that these recovery routes provide genuine environmental benefits compared with conventional disposal.

Overall, the results obtained in the present study suggest that the sludge generated during chitosan-based coagulation–flocculation should not necessarily be regarded as an unavoidable waste stream but rather as a potentially valuable resource. The combination of mineralogical characterization, preliminary industrial validation and positive commercial interest demonstrates that direct material recovery may constitute the most promising valorization pathway. Such an approach fully aligns with the principles of circular economy by transforming a treatment by-product into a reusable industrial resource, thereby simultaneously reducing waste generation, preserving raw materials and improving the overall sustainability of wastewater treatment within the flexographic printing industry.

5.9 Limitations of the work

Although the results obtained highlight the potential of the developed process and demonstrate the feasibility of using chitosan as a flocculating agent for treatment of the studied matrix, the present work has some experimental, methodological, and application-related limitations that should be explicitly discussed. The analysis of these limitations is not only an exercise in scientific completeness, but also a useful tool for correctly interpreting the results obtained and identifying possible directions for future process development. A first critical aspect concerns the intrinsic variability of the wastewater matrix analyzed. During the experimental tests, it emerged that wastewater composition can vary significantly both over time and spatially

within the storage system. In particular, relevant differences were observed between samples collected near the bottom of the tanks and samples collected in more superficial zones of the matrix. In the deeper portions of the storage container, higher concentrations of the main chemical parameters were generally found, probably due to the sedimentation of pigments, additives, and other solid fractions present in the wastewater. Conversely, samples collected in the more superficial portions often appeared more diluted and characterized by relatively better chemical quality. This heterogeneity is a typical characteristic of many complex industrial wastewaters, but at the same time it represents an experimental difficulty, since it makes it more complex to define universally optimal operating conditions.

A second limitation is related to the experimental scale of the tests carried out. Although the work followed a progressive pathway from laboratory tests on small volumes to the development of a pilot system capable of treating approximately 200 L per cycle, the process was still evaluated under controlled experimental conditions and on relatively limited volumes compared with those typically handled in an industrial plant. In a real context, industrial wastewaters are often treated in continuous mode and at significantly higher flow rates, conditions that may substantially influence system hydrodynamics, reagent distribution, and the efficiency of solid–liquid separation stages. Aspects such as continuous flow management, long-term process stability, and operational handling of separation units would therefore require further studies at demonstration or industrial scale. Furthermore, long-term operational stability under continuous industrial conditions was not investigated in this study. Extended operational campaigns would be necessary to evaluate process robustness over time, maintenance requirements, and performance variability under real industrial operating conditions. A further limitation concerns management of the solid phase generated by treatment. Flocculation with chitosan leads to the formation of a semi-solid fraction consisting of aggregates of pigments, suspended particles, organic compounds, and flocculating polymer. Although experimental observations suggest that the amount of sludge produced is generally lower than that generated by treatments based on traditional inorganic coagulants, the present work did not include an in-depth characterization of the chemical composition and physical properties of this material. Sludge management is nevertheless a central aspect in the overall evaluation of any wastewater treatment process, since it directly affects operating costs and environmental impact.

Moreover, considering the high load of suspended particles and colloidal fractions present in the treated matrix, it would be appropriate to conduct more in-depth studies on saturation and useful life of the secondary treatment modules eventually integrated into the system, such as adsorption columns, granular filters, or filter cartridges. During the experimental tests, it emerged that the high particulate concentration may lead to premature clogging or loss of efficiency in filtration and adsorption systems. A systematic evaluation of saturation times, head losses, and regeneration or replacement strategies for the filtering materials would therefore represent a fundamental step for proper plant design of the process.

From an analytical perspective, another limitation is that treatment effectiveness was evaluated mainly through global physico-chemical parameters, such as COD, turbidity, electrical conductivity, pH, surfactants, hardness, and UV–Vis absorbance. Although these indicators are widely used for monitoring water treatment processes,

they do not always make it possible to identify in detail the individual chemical species responsible for organic load or wastewater coloration. More advanced analytical techniques could help clarify more deeply the mechanisms of interaction between chitosan and the different components of the matrix. Another limitation concerns the statistical robustness of the experimental results. Due to the progressive and adaptive nature of the experimental design and the relatively large working volumes treated during pilot-scale experiments, replicate tests under identical operating conditions were limited. Although this approach reflects realistic process development conditions, further investigations including systematic replicates and statistical analysis would strengthen the quantitative assessment of process performance and improve reproducibility evaluation. A further point for reflection concerns the technological context into which the process fits. In the current industrial landscape, one of the main competing systems for the management of heavily loaded wastewaters is represented by industrial evaporators, which allow immediate separation between liquid and solid phases through evaporation and condensation processes. These systems generally make it possible to recover an aqueous fraction of high purity, often comparable to demineralized water or MilliQ water, while the residual solid component is concentrated in the form of sludge or highly dewatered residue. Compared with these technologies, the process developed in this work is proposed as an alternative based on physico-chemical separation mechanisms, potentially characterized by lower energy consumption and by a different approach to wastewater matrix management. However, direct comparison between these technologies would require further studies, particularly from energetic, economic, and operational perspectives.

The present study focused mainly on the technical feasibility of the process, while aspects such as detailed economic analysis, system energy balance, and life-cycle assessment of the treatment were not systematically investigated. These elements represent crucial factors in evaluating the sustainability and competitiveness of the process relative to existing technologies.

The limitations highlighted do not reduce the relevance of the results obtained, but rather bring to light the exploratory and technological-development nature of the work carried out. The experimental pathway undertaken made it possible to demonstrate the feasibility of using chitosan as a flocculating agent in a modular treatment system, identifying promising operating conditions and laying the foundations for further process developments toward possible industrial application. Overall, the results show that system behavior is not governed by a single variable, but by the interaction among wastewater composition, chemical treatment conditions, and plant configuration. Chitosan proved particularly effective as a destabilizing agent for the colloidal and pigmentary fraction, whereas achieving more advanced performance requires integration with secondary polishing units. The value of the work therefore lies not only in demonstrating the effectiveness of the reagent, but in defining an operating window transferable from laboratory tests to prototype and pilot scale. These limitations are typical of technology-oriented research aimed at bridging laboratory-scale studies and industrial implementation, where progressive process validation is prioritized over strictly controlled experimental designs.

Chapter 6 — Conclusions and Future Perspectives

6.1 Main conclusions

The present work aimed at the development and evaluation of a treatment process for industrial wastewater characterized by a high content of pigments, organic compounds, and colloidal particles, based on the use of chitosan as a flocculating agent followed by subsequent solid–liquid separation stages. The experimental pathway was structured in progressive phases, starting from laboratory-scale tests on small volumes and advancing to the development of an intermediate-scale pilot system, with the objective of understanding the process mechanisms and verifying its potential applicability in industrial contexts. This engineering pathway enabled the technology to evolve from a very early stage (TRL 1) to TRL 7. In terms of technological maturity, the work supported the transition from an exploratory phase, typical of low TRL levels, to validation in a pilot plant operating in a relevant environment. From this perspective, the study can be interpreted as achieving a maturity level compatible with TRL 7, having demonstrated prototype functionality under conditions significantly closer to real applications than laboratory-scale testing alone.

The originality of the present work lies in the development and validation, on a real (non-synthetic) wastewater matrix, of a progressively engineered treatment pathway based on chitosan. This led not only to the demonstration of the operating principle, but also to the definition of the main operating conditions, the long-term stability of the reagent, the role of auxiliary polishing modules, and the transferability of the process up to pilot scale.

Preliminary laboratory tests allowed identification of the key operating conditions for matrix flocculation, highlighting pH as one of the most influential variables. Under moderately acidic conditions, chitosan showed an enhanced ability to destabilize colloidal suspensions present in the wastewater, promoting the formation of visible aggregates that could be easily separated from the liquid phase. Under these conditions, significant removal of the suspended fraction and reduction of turbidity and chromophoric components were achieved.

The results also showed that chitosan is particularly effective in destabilizing colloidal fractions and dispersed pigments due to the presence of protonated amino groups, which enable electrostatic interactions with negatively charged particles in the wastewater. This behavior promotes the formation of relatively large flocs, facilitating subsequent solid-phase separation by sedimentation and filtration.

Scaling up the process led to the development of Prototype 1, conceived as a modular platform for process study and for evaluating the contribution of different treatment modules. In this configuration, the system enabled progressive analysis of the effects of different unit operations, including mechanical filtration, adsorption columns, and cartridge filters. The modular architecture demonstrated that chitosan flocculation represents the key stage of the process, while auxiliary modules primarily contribute to improving final effluent quality through removal of residual particles and dissolved organic fractions. An additional important result obtained during this phase concerns the stability of the flocculating solution over time. Durability tests showed that the chitosan solution maintained its effectiveness for up to eight weeks, suggesting the possibility of preparing the reagent in advance and managing it operationally even in industrial contexts.

Based on the knowledge acquired, a pilot plant (Prototype 2) was subsequently developed, featuring a more compact and engineered configuration capable of treating volumes of up to approximately 200 L per cycle. At this stage, the process more clearly assumed the characteristics of a physico-chemical treatment, in which colloidal destabilization induced by chitosan was combined with the use of a cationic polyelectrolyte (C-PAM), introduced to promote floc growth and compaction. The combined action of the two reagents improved process stability and facilitated solid–liquid separation through filtration systems.

Experimental comparison with a widely used traditional flocculant, ferric chloride, showed that the chitosan-based system can represent a valid alternative to inorganic coagulants. While showing comparable performance in turbidity and suspended solids removal, chitosan offered the advantage of producing predominantly organic sludge and avoiding the introduction of metals into the treated matrix, a potentially relevant aspect from a sustainability perspective.

However, it is important to note that, under the tested operating conditions, the developed treatment alone does not fully achieve the discharge limits required by Italian Legislative Decree 152/2006 for release into the sewer system. The results indicate that the chitosan-based system should be interpreted as an effective primary treatment, capable of significantly reducing suspended solids, turbidity, and part of the chromophoric fraction. In this perspective, the developed process represents a preliminary treatment stage that can be integrated with secondary polishing modules—such as adsorption systems, advanced filtration, or additional physico-chemical treatments—to meet regulatory requirements.

Overall, the work demonstrates the technical feasibility of using chitosan as a flocculating agent for primary treatment of complex industrial wastewater, while highlighting the key operational variables influencing process performance. The experimental approach, based on a progressive transition from laboratory tests to prototype and pilot systems, enabled identification of a promising treatment configuration and laid the groundwork for further developments toward industrial-scale application.

6.2 Application implications

The results obtained in this work highlight several relevant application implications for the treatment of industrial wastewater characterized by high concentrations of pigments, colloidal particles, and complex organic compounds. In particular, the use of chitosan as a flocculating agent demonstrated strong potential as a primary treatment technology, capable of significantly reducing suspended solids and part of the chromophoric component.

From an application perspective, the developed process could be integrated as a pre-treatment stage in industrial plants managing wastewater with high solid and pigment content. In such contexts, chitosan flocculation would enable initial separation of the solid fraction and reduction of particulate and colloidal loads. This preliminary step could help stabilize the matrix and improve the efficiency of downstream treatment processes by reducing clogging, premature saturation of filtering materials, and loss of efficiency in polishing units.

A particularly interesting aspect is the potential use of the process as a pre-treatment for more intensive systems, such as industrial evaporators, membrane systems, or advanced oxidation processes. The reduction of suspended solids and pigments achieved through flocculation could decrease the overall load on these technologies, improving operational stability and reducing operating costs. In particular, for industrial evaporators, the introduction of a physico-chemical pre-treatment could limit scaling and accumulation of solid material on heat exchange surfaces.

Another important application aspect concerns the nature of the reagent used. Chitosan is a natural polymer derived from chitin, widely available as a by-product of various biological industries. Its use as a flocculant therefore represents a potentially more sustainable alternative to traditional inorganic coagulants such as iron or aluminum salts, which introduce metals into the treatment system and generate chemically different sludge. In this sense, the developed process aligns with broader research trends aimed at reducing inorganic reagent use and valorizing biomass-derived materials.

From a plant design perspective, the results suggest that the process could be implemented in relatively simple configurations consisting of controlled mixing of wastewater with the flocculant followed by solid–liquid separation via sedimentation or filtration. The modularity demonstrated during the prototype phase also shows that the treatment can be easily integrated with secondary polishing units, such as adsorption columns or progressive filtration systems, depending on treatment objectives.

A particularly relevant aspect also concerns the management of the solid phase produced by the process. Sludge generated by chitosan flocculation consists mainly of aggregates of pigments and organic material embedded within the polymeric floc structure. The predominantly organic nature of this fraction could, at least theoretically, offer greater opportunities for management or valorization compared to sludge produced by inorganic coagulants. However, this aspect requires further investigation, particularly regarding chemical characterization and potential recovery or disposal strategies.

Overall, the findings suggest that the developed process represents a promising solution for pre-treatment of complex industrial wastewater, especially in contexts where rapid reduction of suspended solids and pigment content is required prior to the application of more advanced treatment technologies. Although further studies are needed to optimize the process and fully assess its economic and operational sustainability, the results indicate that the use of natural flocculants such as chitosan may offer a valuable opportunity for developing more sustainable and adaptable treatment strategies.

6.3 Residual limitations of the process

Despite the effectiveness of the developed process as a primary treatment for reducing suspended solids and the colloidal fraction, some intrinsic limitations must be considered for potential industrial-scale application. One of the main limitations concerns the ability of the process to completely remove dissolved organic fractions and stable molecular species present in the wastewater. Chitosan flocculation primarily operates through charge neutralization and aggregation of colloidal particles, making it particularly effective in removing suspended and pigment fractions. However, part of the organic load—consisting of soluble

compounds or very small molecules—remains in solution even after treatment. This behavior is typical of coagulation–flocculation processes and necessitates the use of secondary polishing modules such as activated carbon adsorption, advanced filtration, or oxidative treatments to achieve stricter discharge limits [98].

Another limitation concerns the sensitivity of the process to operating conditions, particularly pH and matrix concentration. As observed during the experiments, chitosan effectiveness strongly depends on the protonation of its amino groups, which occurs optimally under acidic conditions. Variations in pH or wastewater composition may therefore influence floc formation and process stability, requiring control systems to maintain consistent operating conditions in industrial applications.

An additional critical aspect is related to the management of separation units and auxiliary modules. High concentrations of suspended solids and pigments may lead to clogging or saturation of filtration and adsorption systems. This phenomenon was also observed experimentally, where filtration systems showed reduced permeability and increased pressure losses over time. Further studies on saturation dynamics, regeneration strategies, and material replacement would therefore be necessary for long-term process stability.

From an engineering standpoint, effective solid–liquid separation is essential to fully exploit chitosan flocculation. While the formation of large flocs is advantageous, it requires properly designed sedimentation or filtration units. Without adequate separation systems, part of the flocculated material may remain suspended or be carried downstream, reducing overall treatment efficiency.

Another limitation concerns comparison with existing industrial technologies, particularly evaporators. These systems allow complete separation of liquid and solid phases, producing highly purified water and concentrated sludge. Compared to such technologies, the developed process does not achieve the same level of purification in a single step and should instead be considered a pre-treatment technology aimed at reducing solid and organic load before further treatment.

Finally, the lack of a comprehensive economic and operational sustainability assessment represents a limitation. Although chitosan is a natural and potentially sustainable reagent, its competitiveness depends on multiple factors, including reagent cost, sludge management, maintenance requirements, and integration within existing treatment systems.

Overall, these limitations indicate that the developed process should be interpreted as a primary clarification or pre-treatment technology, highly effective for removing suspended and colloidal fractions, but requiring integration with additional polishing units to meet stricter effluent quality standards.

6.4 Future development perspectives

The results obtained in this work represent a first step in developing a chitosan-based treatment system for complex industrial wastewater. However, the process opens several potential research and technological development pathways aimed at improving efficiency and transforming the system into a more advanced platform for wastewater management and valorization.

A first development direction concerns integration between flocculation processes and adsorption systems. While chitosan effectively removes colloidal and pigment fractions, part of the dissolved organic fraction

remains in solution. The systematic integration of adsorbent materials, such as granular activated carbon or other high-surface-area materials, could enhance removal of residual organic compounds. Hybrid flocculation–adsorption systems therefore represent a promising strategy to improve overall performance. Recent studies also show that chemically modified chitosan derivatives can achieve high decolorization efficiencies under extreme conditions, confirming the polymer’s potential as a platform for high-performance flocculants [99]. A second research perspective concerns recovery and valorization of pigments present in the wastewater. Experimental observations showed that a significant portion of pigments becomes incorporated into flocs. This suggests that the process could be interpreted not only as a treatment method, but also as a separation and concentration system for pigment materials. In a circular economy framework, further studies could explore the potential reuse or recovery of these materials.

Another development line involves integration with advanced separation technologies, such as membrane systems or industrial evaporators. As discussed, the developed process can function as a pre-treatment stage, reducing suspended solids and pigments before downstream processes. This could improve system stability and reduce fouling or scaling in advanced treatment units.

Finally, an important area of interest concerns the valorization of the residual solid fraction (sludge). Unlike conventional sludge, the material produced in this process consists mainly of pigments, organic matter, and natural polymer. This composition could open possibilities for recovery, reuse, or transformation into secondary materials, although further research is required.

Overall, these perspectives suggest that the developed system could evolve from a simple treatment technology into an integrated platform for wastewater management, where solid–liquid separation becomes not only a purification step but also an opportunity for resource recovery. Such approaches are consistent with circular economy principles and strategies for reducing environmental impact.

In conclusion, this work demonstrates that chitosan represents a technically credible basis for developing more sustainable treatment strategies for industrial wastewater with high colloidal and chromophoric complexity. Although not a standalone solution for achieving discharge limits under the tested conditions, the process has shown clear effectiveness as a primary separation and clarification stage and strong potential for scaling up to prototype and pilot systems. The value of the work lies in the development of a modular, adaptable, and scalable treatment platform that opens pathways for both wastewater purification and resource recovery within a circular economy framework.

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