POLITECNICO
MILANO 1863

SCUOLA DI INGEGNERIA INDUSTRIALE
E DELL'INFORMAZIONE

Valorisation of food wastes by producing bacterial nanocellulose

TESI DI LAUREA MAGISTRALE IN
CHEMICAL ENGINEERING
INGEGNERIA CHIMICA

Author: **Marilù Sagretti**

Student ID: 10736376
Advisor: Carlo Punta
Co-advisor: Noemi Merayo Cuevas
Academic Year: 2023-24

Abstract

This thesis investigates the valorisation of food waste through the production of bacterial nanocellulose (BNC), using orange peels and whey as substrates, within the framework of a circular economy. Addressing the pressing issue of food waste management, the study aims to transform waste materials into valuable resources, thereby reducing environmental impact and promoting sustainable development.

The research involves creating suitable culture media extracting nutrients from food waste, optimizing the BNC production process by *Gluconacetobacter sucrofermentans* in static conditions, and evaluating the resulting nanocellulose in terms of yield and quality. In terms of membrane quality, water retention was found to vary depending on the medium and additives used. The production process was carefully monitored, with a particular focus on the influence of additives and culture medium creation conditions on the final BNC yield and quality. The study reveals that both orange peels and whey are effective substrates for BNC production, with optimized conditions yielding higher amounts of BNC compared to conventional methods. For orange peels, the highest yield of 3.99 ± 0.45 g/L was achieved after 7 days of incubation with the addition of yeast extract, peptone, citric acid, and disodium phosphate. For whey, hydrolysed whey with the same additives yielded 4.28 ± 0.36 g/L after 10 days. These findings indicate that food waste can be repurposed into a valuable product, contributing to waste reduction and promoting a circular economy.

Moreover, the research includes a Life Cycle Assessment (LCA) to evaluate the environmental impacts of BNC production from these waste materials. BNC production from orange peels had the lowest environmental impact, particularly in categories such as climate change and resource use, compared to the Hestrin and Schramm (HS) medium. These findings indicate that using food waste substrates for BNC production significantly reduces environmental impacts compared to traditional methods, emphasizing the potential of waste valorisation as a sustainable solution.

This thesis contributes to the ongoing discourse on circular economy practices by demonstrating the feasibility of converting food waste into high-value products like BNC. The results highlight the environmental and economic benefits of such approaches, providing valuable insights for future industrial applications and sustainability initiatives.

Key-words: Circular economy, bacterial nanocellulose (BNC), food waste, life cycle assessment (LCA).

Abstract in italiano

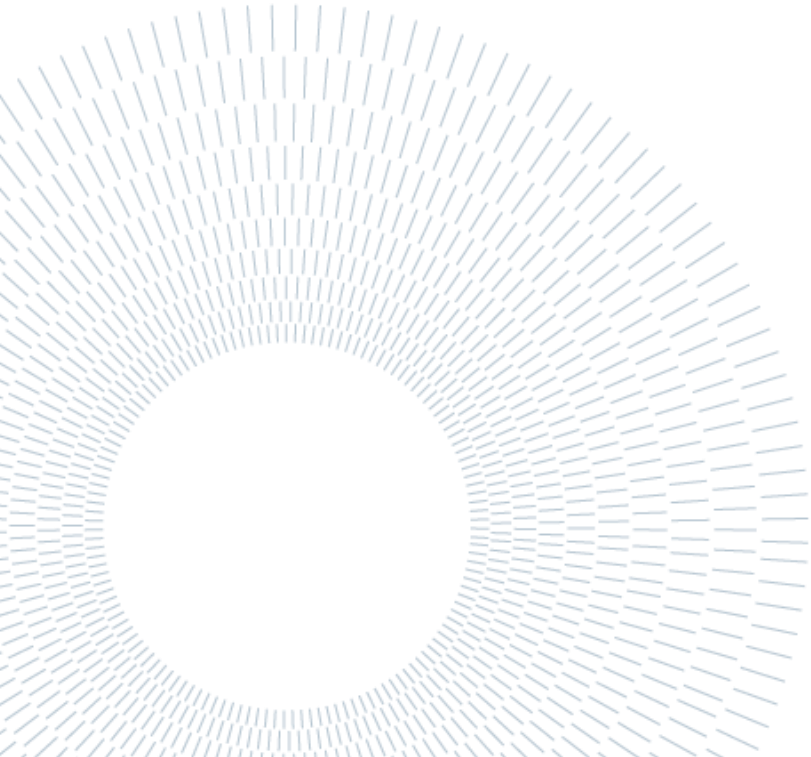
Questa tesi indaga la valorizzazione degli scarti alimentari attraverso la produzione di nanocellulosa batterica (BNC), utilizzando bucce d'arancia e siero di latte come substrati, all'interno del quadro dell'economia circolare. Affrontando la questione pressante della gestione degli scarti alimentari, lo studio mira a trasformare i materiali di scarto in risorse preziose, riducendo così l'impatto ambientale e promuovendo uno sviluppo sostenibile.

Lo studio prevede la creazione di un idoneo mezzo di coltura estraendo nutrienti dagli scarti alimentari, ottimizzando il processo di produzione di BNC tramite *Gluconacetobacter sucrofermentans* in condizioni statiche, e valutando la nanocellulosa risultante in termini di resa e qualità. Per quanto riguarda la qualità delle membrane, è stato riscontrato che la capacità di ritenzione idrica varia a seconda del mezzo e degli additivi utilizzati. Il processo di produzione è stato attentamente monitorato, concentrandosi in particolare sull'influenza degli additivi e delle condizioni di creazione del mezzo di coltura sulla resa finale e sulla qualità della BNC. Lo studio rivela che sia le bucce d'arancia che il siero di latte sono substrati efficaci per la produzione di BNC, con condizioni ottimizzate che forniscono rese superiori rispetto ai metodi convenzionali. Per le bucce d'arancia, la resa più alta di 3,99 ± 0,45 g/L è stata raggiunta dopo 7 giorni di incubazione con l'aggiunta di estratto di lievito, peptone, acido citrico e fosfato disodico. Per il siero di latte, il siero idrolizzato con gli stessi additivi ha prodotto 4,28 ± 0,36 g/L dopo 10 giorni. Questi risultati indicano che gli scarti alimentari possono essere riutilizzati per produrre un prodotto di valore, contribuendo alla riduzione dei rifiuti e promuovendo l'economia circolare.

Inoltre, la ricerca include una Valutazione del Ciclo di Vita (LCA) per valutare gli impatti ambientali della produzione di BNC da questi materiali di scarto. La produzione di BNC dalle bucce d'arancia ha avuto il minor impatto ambientale, in particolare nelle categorie come il cambiamento climatico e l'uso delle risorse, rispetto al mezzo Hestrin e Schramm (HS). Questi risultati indicano che l'utilizzo di substrati da scarti alimentari per la produzione di BNC riduce significativamente gli impatti ambientali rispetto ai metodi tradizionali, sottolineando il potenziale della valorizzazione dei rifiuti come soluzione sostenibile.

Questa tesi contribuisce al dibattito in corso sulle pratiche di economia circolare, dimostrando la fattibilità di convertire gli scarti alimentari in prodotti di alto valore come la BNC. I risultati evidenziano i benefici ambientali ed economici di tali approcci, fornendo preziosi spunti per future applicazioni industriali e iniziative di sostenibilità.

Parole chiave: Economia circolare, nanocellulosa batterica (BNC), scarti alimentari, valutazione del ciclo di vita (LCA).



Contents

Abstract ..... **i**
Abstract in italiano ..... **iii**
Contents ..... **vii**
Introduction **1**
1 Theoretical framework **3**
1.1. Valorisation of food waste 3
1.2. Bacterial nanocellulose 4
1.3. Production of bacterial nanocellulose from food waste 8
1.4. Life cycle assessment 10
2 Objective and scope **11**
3 Materials and methods **13**
3.1. Food residues treatment to produce culture media 13
3.1.1. Orange peels treatment 13
3.1.2. Whey treatment 14
3.2. BNC production 15
3.3. BNC purification 16
3.4. Culture media and nanocellulose characterization 16
3.4.1. Culture media: reducing sugars, nitrogen, phosphorous, pH and conductivity 16
3.4.2. Yield of nanocellulose production 17
3.4.3. Water retention 18
3.4.4. Morphological and structural characterisation 18
3.4.5. Chemical characterisation 19
3.5. BNC production optimisation 19
3.6. LCA of nanocellulose production: goal and scope definition 20
4 Results and discussion **23**
4.1. BNC production from orange peels 23
4.1.1. Preliminary experiment 23
4.1.2. Follow-up experiment 30

4.1.3. Comprehensive discussion and results about BNC production from orange peels 37
4.2. Bacterial nanocellulose production from whey 41
4.2.1. Preliminary experiment 41
4.2.2. BNC production optimisation 48
4.2.3. Comprehensive discussion and results about BNC production from whey 59
4.3. BNC characterization 62
4.4. Life cycle assessment of nanocellulose production: inventory, results and interpretation 67
4.4.1. Life cycle inventory 67
4.4.2. Life cycle impact assessment 69
4.4.3. Sensitivity analyses and results interpretation 74
5 Conclusion 78
Bibliography 81
A Appendix A 91
A.1. Used datasets in SimaPro 91
A.2. New datasets created in SimaPro 92
A.3. BNC production inventory data 96
A.3.1. 1 g BNC production from orange peels in 7 days 96
A.3.2. 1 g BNC production from whey in 7 days 98
A.3.3. 1 g BNC production from HS in 7 days 100
A.4. Charts 102
List of Figures 107
List of Tables 109
List of symbols 111

Introduction

Waste generation and management are among the currently most concerning topics worldwide, leading to continuous debates and concerns among the scientific community and government decision-makers to find and apply practical solutions to those issues. Each year, European countries generate about 1.3 billion tonnes of waste, that corresponds to about 3.5 tonnes of solid waste per head [1]. Since the industrial revolution, many countries have prioritized economic development regardless the consequences, leading to the problems of high resources consumption and environmental pollution (Gozgor et al., 2018). Consequently, these two issues have made environmental protection and sustainable utilization of natural resources important challenges in several countries [3]. In this context of industrial development, circular economy emerges as a valid approach to combat environmental challenges and promote a sustainable development [4]. These debates are primarily led by policy makers like the European Commission, that is making real efforts to stimulate the transition from a linear or "take-make-use-dispose" to a circular economy model in Europe (COM (European Commission), 2015). but also by organizations that operates at international level, like the Ellen MacArthur Foundation, which is a reference at international level for sustainable development and circular economy efforts (Ellen MacArthur Foundation, 2019). The European Union regulations concerning waste management have been drawn up to protect the environment against irreversible effects of harmful and toxic compound emission into the environment as well as protection of natural resources by recycling materials. Focusing particularly on the second point, circular economy represents the approach where the value of products, materials and resources is maintained in the economy for as long as possible, and the generation of waste is minimised [5]. It is operationalised by using three fundamental principles, commonly referred to as the 3R principles: Reduce, Reuse, and Recycle [7].

Among the generated waste, food waste, estimated by FAO to be one third of the total food production for human consumption [8], is a significant obstacle to sustainable development [9]. Among the yearly 1.3 billion tonnes of waste previously cited, European Union has been estimated to produce approximately 88 million tonnes of food waste per year along the supply chain [10]. The reported data are related to European Union, but the challenge of reducing food waste is recognized and embraced at a global level. Indeed, the United Nations have included in their Sustainable Development Goals the food waste topic; precisely the UN Sustainable Development Goal 12.3 targets a 50% reduction in food waste at the retail and consumer levels, in addition to reducing food losses along production and supply chains by 2030 [11].

Currently, disposal of food waste includes incineration, disposal to sewage or landfill, and, fermentation, especially anaerobic digestion for biogas and energy production [12]. However, food waste can be used to also create high-value products, by fermentation of unavoidable or inedible food waste, but also through the extraction of high-value components from the food waste, such as proteins, colorants, phenolics and sugars [13].

Food waste can be employed as a source of carbon and nutrients to produce nanocellulose [14]. Cellulose is a natural polymer, common and abundant on Earth, and nowadays is seen as a valuable alternative to plastics and other synthetic polymers, whose use is causing plastic waste accumulation and pollution levels increase in marine and terrestrial environments [15]. Cellulose can be obtained exploiting natural sources, such as wood and a large variety of plants, or can be produced by seaweed, fungi, and some species of bacteria. Due to its nanometric dimensions, bacterial cellulose is classified as nanocellulose, and, as for other types of nanocellulose, has gained particular attention due to its qualities, such as excellent surface properties, low density, good transparency and, moreover, biocompatibility, biodegradability, and non-toxic nature (Sarangi et al., 2024). Bacterial nanocellulose (BNC) has, moreover, the major advantage of its purity, being free of by-products such as lignin, pectin, hemicellulose, and other constituents of lignocellulosic materials (Portela et al., 2019).

When food waste, typically without economic value and improperly disposed, is employed in the production of a high-value product, such as bacterial nanocellulose, a more sustainable and ecologically correct process is promoted, with the double effect of both reducing food waste, and consequently promoting a circular economy, and obtaining a product with a low environmental impact.

1 Theoretical framework

1.1. Valorisation of food waste

As the challenge of food waste continues to grow, innovative solutions like Food Waste Valorisation (FWV) have become increasingly important [18]. FWV plays a critical role in the efficient use of food waste resources, offering a significant stride towards sustainable waste management. Rooted in the principles of the circular economy, this approach not only aims to reduce environmental impact but also fosters a more sustainable food system [19]. To facilitate effective decision-making in food waste management, various hierarchies have been proposed. Garcia-Garcia et al. (2017) introduced a detailed hierarchy that categorizes different management strategies (Figure 1.1). This framework helps stakeholders to identify the most sustainable methods for managing food waste, emphasizing reduction, reuse, and recycling/recovery before considering disposal [20].

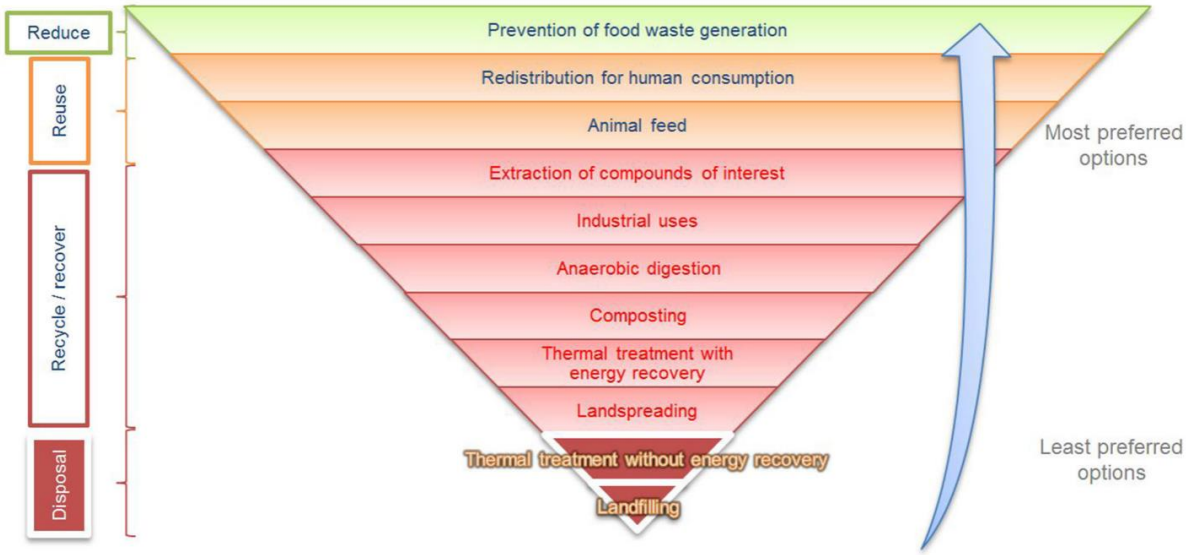


Figure 1.1: Waste hierarchy for food waste*(Garcia-Garcia et al., 2017).*

Clearly, prevention and reuse are not always a possibility; depending on the features of the food wastes, such as edibility, state, origin, complexity, animal product presence, treatment and many others, different routes can be undertaken [20]. Among the various options, it is interesting to underline the option "extraction of compounds of interest", that falls under the category "recycle/recover". Food waste possesses significant potential for generating valuable compounds, since there are dozens of

chemical and physical routes to obtain bio-compounds from food products (Granato et al., 2017). Food waste that has minimal to no economic value can be transformed into valuable resources if efficient waste resources utilization procedures are exploited. Indeed, the Food Waste Valorisation process can be performed by separation of various compounds including phenols, polysaccharides, aromatic oils, colourants, proteins, taste components, enzymes, and fibres from foods using a variety of extraction processes [22], [23], [24].

1.2. Bacterial nanocellulose

Cellulose, constituting the 35-50% of plant biomass along with pectin, lignin and hemicellulose [25], is currently recognized as the most abundant organic polymer on the planet, comprising approximately 1.5 trillion tons of the total annual biomass production. It is regarded as an almost limitless resource, vital for meeting the growing demand for products that are both environmentally friendly and biocompatible [26].

It is also non-toxic, making it a prime candidate for use as a feedstock in creating natural polymeric materials [27]. These materials could potentially replace those derived from petroleum, offering benefits such as renewability and biocompatibility. Additionally, through catalytic processes, cellulose can be transformed into chemicals and monomers that can be utilized to produce biopolymers that serve various applications across different industries [27].

Cellulose is a linear polymer, made of glucose monomers, that forms strong associations through hydrogen bonds. These intermolecular interactions are critical for the formation of polymer fibres and provide structural rigidity to the cellulose [28]. Plant and bacterial cellulose have same molecular formula, while having different physical and chemical properties. Indeed, cellulose fibres derived from plants typically have diameters ranging from 13 to 22 μm, with a crystallinity between 44% and 65% [29], while cellulose produced by bacteria, known as bacterial cellulose, consists of fibrils measuring 10 to 100 nm in diameter and showing a crystallinity of 90% [30]. Consequently, bacterial cellulose, being composed by fibrils in the nanoscale, is naturally considered nanocellulose, that is defined as cellulose particles with at least one dimension under 100 nanometres. Differently, plant nanocellulose, that can be found as cellulose nanofibers (CNF) or cellulose nanocrystals (CNC), needs to be isolated from plant fibres by means of a mechanically or chemically deconstructing strategy [31]. Bacterial nanocellulose (BNC) fibrils are moreover highly hydrophilic due to the abundance of hydroxy groups on their surface [30] and it is noted for its superior tensile strength and extensive surface area [30]. In addition, bacterial cellulose has greater purity, being free of other products such as lignin, pectin, hemicellulose, and other constituents of lignocellulosic materials [17] and greater flexibility [28] when compared to nanocellulose from plant sources.

Regarding the production methods, nanocellulose derived from plants is produced through a top-down approach, involving mechanical and chemical processes that break down plant fibres. On the other hand, BNC is created through a bottom-up synthesis method, that relies on the direct activity of specific bacterial strains that synthesize cellulose as a part of their metabolic processes. If considering their environmental impacts, BNC production has clearly an advantage, since separation processes to isolate plant-based cellulose from other compounds such as lignin and hemicellulose are quite polluting, energy expensive and could bring to the formation of dangerous compounds [28]. However, bacterial nanocellulose production at large scale is still limited, due to the lack of efficient fermentation processes [32].

BNC can be produced in presence of oxygen by various species of bacteria, such as those of the genera *Gluconacetobacter* (formerly *Acetobacter*), *Agrobacterium*, *Aerobacter*, *Achromobacter*, *Azotobacter*, *Rhizobium*, *Sarcina*, and *Salmonella* [33]. *Acetobacter* strains, specifically *A. xylinus,* are the best strains to produce bacterial nanocellulose. *Gloconacetobacter xylinus* is the commonly studied source of cellulose and can produce it exploiting a wide range of carbon and nitrogen sources [34], [35]. It is a rod-shaped, aerobic, Gram-negative organism that generates cellulose through its metabolic processes. These cellulose fibres form intricate, interwoven extracellular ribbons. This production of cellulose is not a secondary metabolic byproduct but rather a primary metabolite, indicating its central role in the bacterium's physiology [33]. This bacterium does not exhibit cellulase activity, that is the enzyme which breaks down cellulose; this can be a significant trait for organisms that produce cellulose, as it prevents them from degrading the cellulose they have just synthesized [33].

Several factors influence BNC production, with the composition of the growth medium, environmental conditions, and byproduct formation being particularly critical. Optimising the growth medium is crucial for microbial development, which in turn enhances product formation. Essential nutrients for microbial growth include carbon, nitrogen, phosphorus, sulphur, potassium, and magnesium salts. Common carbon sources for cellulose synthesis are glucose and sucrose, though other carbohydrates like fructose, maltose, xylose, starch, and glycerol have also been explored [33]. When glucose is used as the only carbon source in the fermentation medium, it serves dual roles. Firstly, it provides the necessary energy that microorganisms require for their metabolic activities. Secondly, it acts as the precursor for the synthesis of bacterial cellulose. This dual functionality of glucose makes it a critical component [36]. A notable issue with using glucose as a carbon source is the resultant production of gluconic acid. This byproduct lowers the culture's pH, adversely affecting cellulose production [36]. Nitrogen, vital for cellular metabolism, constitutes 8–14% of the dry cell mass in bacteria [33]. Different sources of nitrogen can be employed, such as yeast extract, peptone, soy tone or some residues, like corn steep liquor, that is a by-product of wet milling of corn, containing amino acids, vitamins, and minerals [37]

The most common culture medium employed to produce BNC is the Hestrin and Schramm medium (HS), that contains (%, w/v): glucose, 2; peptone, 0.5; yeast extract, 0.5; disodium phosphate, 0.27; citric acid, 0.115 [38]. The HS culture medium contains glucose as source of carbon and yeast extract and peptone as sources of nitrogen.

Among the environmental conditions affecting BNC production, culture configuration, pH, temperature, and oxygen play a pivotal role.

The most used fermentation method is the static culture, since high yields can be reached. In this configuration, BNC is formed on the surface of the culture broth in the shape of a pellicle. There are different hypotheses to this fact: it has been proposed that cellulose serves to hold the bacterium in an aerobic environment or acts as an extracellular matrix to enhance colonization by the bacterium of its substrate in nature and to prevent potential competitors from gaining access to the decaying substrates [39], [40]. Other hypotheses are about providing protection for the cells against UV radiation and helping in moisture retention to prevent drying of the substrate (Scott Williams & Cannon, 2448). However, bacterial nanocellulose can also be produced in agitated culture. When a broth culture is agitated by shaking or stirring, the bacteria grow quickly, but the production of cellulose is reduced, and the cellulose fibrils fail to form a well-organized pellicle [41]. Indeed, if the culture is incubated in an orbital shaker, spherical cellulose pellets dispersed on the surface of the medium are developed within the broth [42].

Talking about the other environmental factors, various studies agree on the fact that the optimal pH vary depending on the microorganism used and is in the range from 4 to 7 [14]. Regarding temperature influence, literature studies have underlined the dependence of productivity, morphology and crystallinity structure on temperature; generally, the ideal temperature range agreed by various authors is between 28 and 30°C [32]. Concerning oxygen influence, oxygen plays an important role in controlling aeration within the media. It has been observed that there is a clear influence of dissolved oxygen amount on both cellular metabolism and resulting polymer quality. Within the media, a low level of dissolved oxygen obstructs bacterial growth, leading to the retardation of bacterial cellulose production. A restricted oxygen supply not only collapses the production but also reduces its quality [43]. However, high levels of dissolved oxygen could bring to an excessive production of gluconic acid, that, as already underlined, could deactivate the bacterial cell growth [14].

Nanocellulose broad applicability and potential are increasingly recognized in numerous fields due to its unique characteristics. Particularly, its high purity provides it high mechanical properties, such as its remarkable tensile strength, exceeding 2 GPa [30], and its biocompatibility and biodegradability make it suitable for biomedical applications and the production of other value-added products [44].

In medical applications, BNC excels in tissue engineering and wound dressings, where its compatibility with human tissues promotes healing and supports cell growth,

particularly valuable for creating vascular grafts and skin tissue regeneration [45]. BNC characterized by high water retention is particularly suitable for wound dressings, since the ability to maintain a moist environment conducive to healing is crucial [46]. In addition to these healthcare uses, BNC's potential in controlled drug release and implant materials is further explored, emphasizing its adaptability and clinical relevance [47]. In the food packaging industry, BNC enhances food safety and extends product shelf life due to its effective barrier properties against oxygen and pathogens [48]. BNC's utility extends into the production of edible films that incorporate fruit purees, enhancing the films' sensory properties and mechanical flexibility, which significantly alters their water vapor permeability and tensile strength [49]. These films, especially when combined with fruit purees such as mango or guava, offer improved functionality for specific packaging applications [50]. The cosmetic industry could also benefit from BNC's high-water retention and skin compatibility, making it suitable for hydrating face masks and other skincare products [32]. For environmental applications, BNC provides a biodegradable alternative to synthetic polymers, reducing the environmental impact in packaging and disposable items [51], [52]. BNC's mechanical properties and transparency could also be leveraged in technological applications such as flexible electronics and high-quality acoustic diaphragms [53]. Its role in water purification systems, where its fine fibrous structure effectively filters out impurities, enhances environmental technologies [50]. Additionally, BNC is utilized in novel composites for construction and automotive applications, where its strength and biodegradability provide significant advantages over traditional materials [54].

As already mentioned, high water retention is remarkably beneficial for BNC. Water retention measures the ability of membranes to absorb and retain water, which is essential for applications requiring moisture, such as construction materials, textiles, cosmetics and medical applications.

Membranes with high water retention tend to have better mechanical properties in humid environments, including flexibility and tear resistance. These properties are important for applications where the membrane must maintain structural integrity in the presence of water [55]. In biomedical applications, the water retention capacity can influence biocompatibility and the ability to maintain a moist environment conducive to healing and cell growth [46], [56]. Water retention can also influence the filtration efficiency of nanocellulose membranes, enhancing their ability to remove contaminants or particles from aqueous solutions [57] and, being related to membrane permeability, can influence the transport of molecules through the membrane, important for applications such as water purification and molecular separation [58]. Finally, water retention can provide insights into the thermal and chemical stability of membranes, as materials that retain water stably may be less susceptible to degradation under thermal or chemical stress [59].

1.3. Production of bacterial nanocellulose from food waste

Bacterial nanocellulose is typically produced using the HS culture medium, that contains glucose as carbon source [38]. However, the production cost of bacterial nanocellulose starting from this traditional culture medium is high. Indeed, the cost of the culture medium, accounting for 50-65% of the total production cost, is an important aspect of the industrial production process that in turn defines the final price of the product [60]. Therefore, several attempts have been made in the past few years to reduce the cost of bacterial nanocellulose production by utilizing low cost, renewable, and easily available substrates in the fermentation process [36]. Utilizing waste as a carbon source to produce bacterial nanocellulose can be a valid low-cost and sustainable alternative [35], while repurposing industrial wastes like agricultural residues, dairy wastewater, brewery byproducts, textile waste, and micro-algae biomass to create a value-added product [61]. Indeed, when food waste is employed, this practice can be seen as a Food Waste Valorization technique, particularly under the recycle/recovery category.

Among food waste, vegetable waste coming from fruit processing industries has a significant impact, since those industries have huge markets and generate a large amount of peel waste and seed waste [62]. Globally, more than half a billion tons of waste are generated by the fruit processing industries every year [63]. To give an example, 20 million tons of unwanted orange peel are produced by the juice industry every year, creating disposal and fermentation issues in the environment [62].

Studies have examined various fruit waste and peels as potential sources of carbon for BNC production [61]. Utilizing pineapple, banana, orange, melon, and date peels, significant yields of BNC were obtained, demonstrating the potential of these agricultural wastes as economically viable substrates. For instance, focusing on fruit peels, banana peel yielded up to 19.46 g/L of BNC in 15 days [14], pineapple peel juice produced 2.8 g/L in 13 days [64], orange peel fluid and orange peel hydrolysate yielded up to 3.4 g/L in 8 days [65], citrus peel hydrolysate resulted in 2.33 g/100 g peel in 21 days [66], while coffee cherry husk resulted in a production of 8.2 g/L in 14 days [67]. A study by Khan et al. (2021) investigated BNC production from various fruit peel wastes, utilizing just aqueous extracts of various fruits peel (100%) as culture media or along with components of HS medium (2% peptone, 0.5% yeast extract, 0.12% citric acid, 0.27% disodium phosphate) [68]. BNC yield was observed after 16 incubation days in both types of media, although significant productions were obtained from the media containing HS components along with fruit peel wastes [68]. This study resulted in 7.69 ±0.51 g/L of BNC from pineapple peel waste, 5.76 ±1.12 g/L from banana peel waste, 4.05 ±0.05 g/L from orange peel waste and 2.11±0.15 g/L for sweet lime peel waste; which are all comparable or higher than the yield obtained in the same study from HS culture medium, that was 2.47 ±0.11 g/L in 16 days [68]. These findings

constitute only a preliminary result but indicate that fruit peels can be efficiently utilized to produce bacterial cellulose with high yields, thereby contributing to reducing agricultural resource waste.

The dairy industry, likewise, produces a by-product, cheese whey, that corresponds to the liquid stream coming from the cheese production starting from milk, specifically from the agglomeration process of casein micelles [69]. Cheese whey is considered the most important pollutant of dairy industry, particularly hazardous when designated sustainable treatments are not foreseen. Its composition is mainly water (93-95% w/w), while the dry matter fraction is composed by 66–77% (w/w) of lactose, 8–15% (w/w) of proteins, 7–15% (w/w) of minerals; and a lower portion of non-protein nitrogen (e.g., amino acids), vitamins (e.g., vitamins A, D and B5), and trace elements (e.g., zinc) [70]. Its major issue is represented by its high organic load; the rapid consumption of oxygen in the soil caused from the breakdown of proteins and sugars present in whey poses a significant disposal problem, in line with the vast amounts of volumes generated, approximately 9 L of whey per each kg of cheese produced [71]. In the last decades, a lot of efforts have been made to find viable and environmentally friendly valorisation alternatives, rather than just field disposal, especially trying to exploit cheese whey with high nutritional values [69], [72]. A sustainable solution for whey valorisation can be provided by its conversion into a cheap fermentation medium to produce varied biopolymers and other bioactive compounds, including BNC [73].

Among the studies present in literature reporting BNC production from whey, one of the most cited studies in various literature reviews is the investigation conducted by Revin et al. (2018), that explored whey as an alternative substrate and found that BNC production on whey reached 5.45 g/L (dry weight) after 3 incubation days, surpassing the 2.14 g/L obtained on the standard Hestrin-Schramm (HS) medium in the same incubation period [74]. Other studies, instead, found lower yields from whey than from conventional HS, probably since the main carbon source in whey is lactose which is a disaccharide and is not useful to bacteria producing cellulose [75]. Indeed, when lactose is the main carbon source, yields of BNC were found to be negligible, while with a hydrolytic pretreatment of whey splitting lactose in its corresponding monomers glucose and galactose, the hydrolase resulted in higher yields, up to 7 g/L [76], [77], [78], [79].

Other explorations found out that acid whey resulted in the highest yield among different waste media, such as banana peels, celery pomace, fish waste and hemp pomace [80]; moreover, it has been found that the presence of whey proteins in the medium positively influenced the BNC biosynthesis [80].

Lately, there is a lot of interest in this waste medium and some more recent studies have performed BNC production optimisation from whey by investing different parameters. For instance, Baykatar & Gursoy (2024) have studied the influence of pH, organic source concentration, amount of active culture and incubation time, finding

the best yield at pH 5.5, 25% organic source amount, 30% active culture concentration and 8 days of incubation [81]. Differently, Feng et al. (2024) studied BNC production from soy whey, adding an extra carbon source and investigated the best carbon source to add, the concentration of added carbon source, the inoculation amount, the temperature and the incubation time, finding the best yield when using sucrose as added carbon source at 8.5%, 10% inoculation amount, 32°C as fermentation temperature and 11 days of incubation [82].

1.4. Life cycle assessment

Life Cycle Assessment (LCA) is a systematic method used to evaluate the environmental impacts associated with all the stages of a product's life, from raw material extraction through materials processing, manufacture, distribution, use, repair and maintenance, to disposal or recycling. The process begins with the goal and scope definition, which determines what will be studied and to what extent, setting boundaries for the study. This is critical as it influences how comprehensive the LCA will be and is discussed by ISO 14040:2006 [83], which provides the framework and principles for conducting an LCA.

The next step involves the life cycle inventory (LCI), which is the data collection phase, compiling and quantifying inputs and outputs for a product throughout its entire lifecycle. This includes raw materials, energy use, water use, and emissions to air, water, and land. Researchers emphasize the importance of a robust LCI to ensure the reliability of the LCA results [84].

Following the inventory analysis is the life cycle impact assessment (LCIA). This phase assesses the potential environmental impacts using the LCI data. It involves selecting impact categories, category indicators, and characterization models that are appropriate for the study. This step is crucial in understanding the extent of the environmental impacts associated with different phases of the product lifecycle [85].

The final stage of LCA is the interpretation phase, where results are analysed to provide conclusions and recommendations. This phase helps decision-makers to understand the significance of the study results and make informed decisions about potential improvements [86]. The interpretation should be transparent and include a critical review, as recommended by the ISO 14044:2006 standard [87], to ensure the validity and reliability of the LCA.

Throughout the process, LCA practitioners must consider several critical factors such as data quality, geographical and time-related coverage, and the technology reviewed. The inclusion of sensitivity and uncertainty analyses is also crucial to assess the robustness of the conclusions drawn from the LCA [88].

2 Objective and scope

The primary objective of this thesis is to address the significant issue of food waste by promoting a circular economy model. Particularly, the core focus is to harness the potential of food waste residuals to develop a value-added product, thus transforming waste into a resource. This approach not only seeks to mitigate the environmental impact caused by traditional disposal methods but also promotes more sustainable methods of resource utilization.

By exploring innovative techniques to repurpose food waste, this study intends to demonstrate the feasibility of converting organic waste into valuable goods. This will involve a thorough analysis of the types of food waste that can be utilized, the transformation processes required, and exploring the applications of the created product. The purpose of this investigation is to contribute to the reduction of food waste, encourage the recovery of unused resources such as unavoidable or inedible food waste, and support the transition towards a more sustainable food system, demonstrating practical and environmental benefits of waste repurposing. The aim of this research is to provide practical solutions that can be adopted by industries, aligning with the global efforts to achieve environmental sustainability.

In particular, the issue of food waste repurposing is addressed in this study by producing bacterial nanocellulose starting from food residues, through the extraction of components from the food waste, with the purpose of exploit them as nutrients to produce bacterial nanocellulose.

Specifically, this research work will focus on the subsequent specific objectives:

1. **Creation of suitable culture media for BNC production from food waste.** The two different food wastes employed in this study are residual orange peels and whey. This first specific objective is to create a culture medium containing a complete range of nutrients, such as reducing sugars, nitrogen, and phosphorus, from different food wastes. This task will involve the development and optimisation of the culture media production processes and the subsequent evaluation of the efficiency and yield of these processes, in terms of concentration of nutrients in the final culture medium. Particularly, nutrients are extracted from orange peels waste using water, and different extraction conditions will be investigated in order to find the optimal one. Regarding whey, culture media are directly obtained from liquid whey during cheese

production; however, some pretreatments will be assessed to determine the optimal treatment.

2. **Bacterial nanocellulose production.** The second objective is to assess the production of bacterial nanocellulose using the different types of processed food wastes as feedstocks. This will include experimenting with various culture media and various conditions to determine the most effective methods for generating high-quality nanocellulose.
3. **Incubation results evaluation and characterization.** The third objective focuses on evaluating and characterizing the outcomes of the incubation processes involved in the production of bacterial nanocellulose. This includes calculating the resulting yield from different media and conditions and analysing the physical properties of the nanocellulose produced to ensure its suitability for further applications. Particularly, water retention will be evaluated, since being a pivotal parameter for nanocellulose, essential for different kinds of applications for which nanocellulose could be suitable.
4. **Life Cycle Assessment (LCA) of BNC production.** The final objective is to conduct a Life Cycle Assessment of BNC production from different wastes. This assessment will be performed for the BNC production at laboratory scale and will evaluate the environmental impact of these production processes, comparing the routes from different residues among themselves and further comparing them with BNC production from a commonly used culture medium not coming from waste. Specifically, BNC production at laboratory scale from orange peels and whey will be examined, comparing it with BNC production from the HS culture medium. The aim of this evaluation is to determine the sustainability and environmental efficiency of using waste substrates for BNC production, highlighting potential benefits and areas for improvement. Moreover, the assessment at laboratory scale could be useful to guide the scale-up of those processes at industrial scale.

3 Materials and methods

3.1. Food residues treatment to produce culture media

Two different types of food residues have been used for culture media preparation, that are orange peels and whey.

3.1.1. Orange peels treatment

Orange peels were obtained from oranges eaten daily in the canteen of the Higher Technical School of Engineering and Industrial Design (ETSIDI), belonging to the Polytechnic University of Madrid (UPM), so not from the fruit processing industry, while whey was taken from a cheese production laboratory located in the Higher Technical School of Forestry Engineers (ETSIM) of the UPM.

Orange peels pre-treatment consisted of drying the orange peels at 30°C for about 12 hours and subsequently grinding the dried peels until a powder was created. Then, extraction of nutrients from the created powder has been performed, using orange peels powder and distilled water in a ratio 1:10 (10 g of powder for each 100 mL of distilled water), while mixing for 15 minutes. The extraction has been performed at different temperatures (ambient temperature, 50°C and 100°C) to evaluate the influence of temperature on the extraction process and consequently the optimal option.

After these steps, the obtained suspension was centrifugated for 10 minutes at 4000 rpm in a centrifuge Unicen 21 supplied by Ortoalresa – Alvarez Redondo SA, to separate the liquid from the solid. The liquid part constitutes the culture medium. This step was alternatively done by filtration with sieves (Figure 3.1), that is less energy expensive but more time consuming.

Figure 3.1: Filtration to obtain the culture medium from orange peels.

Additives were added to the liquid media while stirring, when needed, and finally the media were autoclaved at 121°C to be sterilised.

3.1.2. Whey treatment

Whey pre-treatment started with filtration, to separate casein; filtration is performed using different sieves of decreasing dimension in series (Figure 3.2).

Figure 3.2: Whey filtration to separate casein

Whey has been tested as culture medium both hydrolysed and not hydrolysed. When hydrolysed, the hydrolyzation process has been done with lactase at 35-40°C for 2 hours, utilizing 2 mL of enzyme for each litre of whey to hydrolyse.

After these steps, addition of additives, when needed, and sterilization at 121°C in an autoclave have been performed. Casein dissolved in solution precipitates at temperature higher than 90°C; therefore, after sterilisation a precipitate appeared. To avoid introducing the precipitate in the culture, the medium was no longer shaken.

3.2. BNC production

The bacterial strain used to produce BNC is *Gluconacetobacter sucrofermentans*, currently renamed *Komagataeibacter sucrofermentans* CECT 7291, supplied by the Spanish Type Culture Collection (CECT).

Bacteria come lyophilised and need to be resuspended to be utilised. After been resuspended, cell growth was carried out following the conditions recommended by CECT. Particularly, bacteria were grown in a liquid medium in agitation, using approximatively 800 µL of the suspension containing bacteria for each 100 mL of medium put in 250 mL Erlenmeyer bakers. The liquid medium recommended for *Komagataeibacter sucrofermentans* growth is the M10 and contains: 25 g/L mannitol, 5 g/L yeast extract and 3 g/L peptone. The growth was performed at 30°C for 4 days, keeping the culture in agitation at 120 rpm (less than what recommended by CECT, that is 180 rpm) in an orbital incubator CTSI-070-001 supplied by Labbox Labware SI (Figure 3.3).

Figure 3.3: Cell growth in M10 medium at 30°C and 120 rpm, for 4 days.

After this period, some nanocellulose pellets were formed and needed to be cut to allow bacteria exit from the formed nanocellulose. Then, liquid and pellets in tubes were agitated at 2000 rpm for 30 minutes in a shaker Multi Reax supplied by Heidolph, to allow bacteria suspend in the liquid. At this point, filtration with a sterile gauze was performed to separate the liquid from the cellulose. The liquid was then centrifugated at 4000 rpm for 10 minutes in a centrifuge Unicen 21 supplied by Ortoalresa – Alvarez Redondo SA, to allow separation of bacteria. After removing the supernatant, bacteria were resuspended in a saline solution called Ringer's Solution (2.5 g/L NaCl, 0.105 g/L KCl, 0.12 g/L $CaCl_2 \cdot 2H_2O$ and 0.05 g/L $NaHCO_3$), and centrifugated again in the same conditions. Bacteria were resuspended in a small volume of Ringer's Solution and optical density adjusted to approximately 1 at 600 nm wavelength in a spectrophotometer Onda V-10 Plus. An optical density corresponds to 1 means 10^9 bacterial cells.

After making cells grow, the subsequent cultures were performed adding 250 µL of inoculum each 100 mL of the previously described media obtained by the wastes. In static culture mode, 9 cm Petri disks were employed and placed at 30°C in an incubator INCR-150-001 supplied by Labbox Labware SL. In each Petri disk, 35 mL of culture medium was put. Culture time varied in the experimental set and will be progressively explained throughout the text for each performed experiment.

3.3. BNC purification

The produced nanocellulose, in membranes when static culture was applied, was sterilised in an autoclave at 121°C to stop the cell growth and purified by immersion in a 1% NaOH solution at ambient temperature for approximately 30 minutes; then, it was washed with distilled water until a neutral pH of the wash solution was reached.

3.4. Culture media and nanocellulose characterization

3.4.1. Culture media: reducing sugars, nitrogen, phosphorous, pH and conductivity

Reducing sugars have been quantitatively measured with a 3,5 dinitrosalicylic acid (DNS) assay, that is a colorimetric assay that exploits the oxidation of the aldehyde or ketone group with the reduction of DNS. To perform the essay, a sample was made react with DNS in a DRB200 Reactor at 100°C for 5 minutes; then, the solution has been cooled down for 5 minutes to stop the reaction and mix with distilled water. The absorbance at 560 nm has been measured in a colorimeter DR/890 supplied by Hach Company. This method has been adapted from the method used for quantitative measurement of sugars in urine developed in 1959 by Miller G. L. [89]

Total nitrogen was measured with the Test 'N Tube (TNT) persulfate digestion method, where an alkaline persulfate digestion converts all forms of nitrogen to nitrate in a DRB200 Reactor at 105°C for 30 minutes. After the digestion, sodium metabisulfite is added to eliminate halide interferences; then, nitrate reacts with chromotropic acid under strongly acidic conditions to form a yellow complex with an absorbance at 410 nm. The concentration of total nitrogen is measured in a colorimeter DR/890 supplied by Hach Company.

Phosphorous is measured employing a Test 'N Tube (TNT) Molybdovanadate method with acid persulfate digestion. Phosphates present in organic and condensed inorganic forms need to be converted to reactive orthophosphate before analysis. A pretreatment of the sample with acid and heat provides the conditions for hydrolysis of the condensed inorganic forms; organic phosphates are converted to orthophosphates by heating with acid and persulfate in a DRB200 Reactor at 150°C for 30 minutes. Then, orthophosphates react with molybdate in an acid medium to produce a mixed phosphate/molybdate complex. In presence of vanadium, yellow molybdovanadophosphoric acid forms, with the yellow intensity being proportional to the phosphate concentration. The concentration of phosphate is measured in a colorimeter DR/890 supplied by Hach Company at 420 nm.

pH and conductivity have been measured respectively with a pH 50+ DHS pH meter and a COND 61 conductivity meter, supplied by XS Instruments.

3.4.2. Yield of nanocellulose production

When static culture mode was used and nanocellulose membranes were formed, the production yield in g/L has been measured by weighing the membranes, after being dried is a dehydrator, and relating their dry weight to the initial liquid volume of culture medium employed for each Petri disk, using Equation (3.1).

$$Y\left[\frac{g}{L}\right] = \frac{W_1\,[g]}{V\,[L]} \tag{3.1}$$

Where W_1 is the dry membrane weight and V the initial liquid volume in the Petri disk.

All investigations were performed in three replicates and the yield results were statistically analysed calculating the standard deviation of each dataset values, to quantify how much the individual data differ from the mean, as in Equation (3.2).

$$s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}\left(x_i - x_{average}\right)^2} \tag{3.2}$$

Where n is the dataset size and $x_{average}$ is the mean value of the dataset.

Error bars in plots reporting BNC yields have been put equal to standard deviations.

3.4.3. Water retention

Membranes have been weighted both humid and dry. To weight humid membranes, they were first blotted with absorbent paper to eliminate excess water.

The water content (WC), representative of membranes water retention, has been measured as in Equation (3.3).

$$WC\ [\%] = \frac{W_0[g] - W_1[g]}{W_0[g]} * 100 \tag{3.3}$$

Where W_0 is the humid membrane weight and W_1 is the dry membrane weight.

Alternative, water retention can be expressed using Equation (3.4), therefore measuring the water holding capacity (WHC).

$$WHC\ \left[\frac{g}{g}\right] = \frac{W_0[g] - W_1[g]}{W_1[g]} \tag{3.4}$$

WHC is representing the mass of water removed during drying per gram of dry BNC.

WC and WHC are two ways of expressing water retention and have been both used in this study to allow better comparison with other literature studies.

3.4.4. Morphological and structural characterisation

Besides water retention analyses, electron microscopy techniques that provide high-resolution images of microscopic structures have been employed, enabling detailed analysis of morphology and structure of BNC.

Scanning Electron Microscopy (SEM) has been used to observe the morphology and three-dimensional structure of BNC. It can reveal the fibrous structure, fibre dimensions, and surface organization of BNC, providing insights into porosity and fibril arrangement. SEM employs an electron beam to scan the surface of a sample. The electrons interact with the surface, generating signals that are converted into high-resolution images. To perform the SEM analysis, fragments of BNC have been directly obtained from the membranes and observed with a SEM Cambridge Stereoscan 360.

Transmission Electron Microscopy (TEM) operates differently from SEM; it transmits an electron beam through a sample, which must be at the nanometric scale. The transmitted electrons generate an image that reveals atomic or sub-nanometric details. In BNC, TEM has been employed to observe the internal structure of fibrils, cellulose chain alignment, and nanometric organization. Thus, while SEM is ideal for visualizing surface morphology and three-dimensional structure, TEM excels in exploring internal structures at a much finer level of detail. To create the samples for

TEM, a small piece of BNC membrane has been put in a 20 mL vial containing distilled water. The vial was then treated in a sonicator with a 3 mm diameter probe to separate nanofibers from the BNC piece, which are suitable for the TEM analysis. The sonication time has been adjusted depending on the response of the sample; in particular, the sample should be swollen in water and some nanofibers should detach from the sample, going in suspension. Therefore, the sonication should stop before completely disintegrating the sample, presumably meaning an unwanted mechanic fibrillation of the sample. The employed equipment was a Philips CM200 FEG TEM at 200 kV.

3.4.5. Chemical characterisation

Fourier Transform Infrared (FTIR) spectroscopy was employed to characterize the chemical structure of BNC membranes. FTIR spectroscopy works by passing infrared radiation through a sample, causing molecular bonds to vibrate at specific frequencies. These vibrations correspond to characteristic absorption bands, which are detected and plotted as an FTIR spectrum. The spectra were recorded using a Varian 640-IR spectrometer, with samples directly obtained from the membranes. The measurements were performed in the range of 4000-0 cm^{-1} to identify characteristic cellulose functional groups, such as the O-H stretching band, C-H bending, and C-O stretching, to assess the chemical composition and confirm the formation of nanocellulose.

3.5. BNC production optimisation

BNC production optimisation have been performed using the Design of Experiment wizard of the Statgraphics 19 software. It allows for multiple experimental factors to be manipulated, determining their effect on a desired output, in this case the production yield of BNC. It is useful to determine whether a factor influences the response and if factors interact in their effect on the response, to model the behaviour of the response as a function of the factors and to optimise the response. By manipulating multiple inputs at the same time, DOE can identify important interactions that may be missed when experimenting with one factor at a time.

A central composite design has been employed, belonging to the response surface design class, that contains a fractional factorial design with centre points that is augmented with a group of star points that allow estimation of curvature. The central composite design needs 2^n+s runs, where n is the number of experimental factors to be varied, and s is the number of star points. Moreover, the design has been chosen as face centred; although being less desirable from a statistical perspective, such a design is easier to run since it involves only low, medium, and high levels of each factor. For such designs, 1-2 centre-points are usually sufficient: in this case, 2 centre points have been selected, randomly placed. The model used to fit the experimental data has been selected as quadratic: therefore, 10 coefficients have been employed to fit experimental data. The variable to maximize was the production yield, expressed in g/L.

3.6. LCA of nanocellulose production: goal and scope definition

LCA is here employed to assess the impact of bacterial nanocellulose production, starting from different culture media. The software implied for the analysis is SimaPro (9.5.0.1 version) and the database used is Ecoinvent (3.9.1 version).

The applied methodology is suggested in several sources, such as the ISO standards 14040 and 14044. Firstly, goal and scope have been defined, aiming to explain intentions, functional unit, system boundaries and some preliminary assumptions to make a valuable, clear and transparent assessment.

This study is intended to collect the life cycle inventory data for nanocellulose produced at laboratory scale in static culture mode, from some food wastes, particularly orange peels and whey, and from the commercial culture medium HS, and therefore conduct a preliminary life-cycle assessment. This study can help to understand and compare the environmental impacts of the three production routes and identify the best solution from the environmental impact point of view, along with the hotspots in each production chain. Using the LCA results from this lab scale process can also guide the design of industrial scale BNC production to minimize energy requirements and environmental impacts.

It is a cradle-to-gate LCA, comprehending in its scope all the phases of the BNC production process, from raw materials extraction to the finished product at the factory gate, the laboratory in this case.

The system boundaries for BNC production are illustrated in the following flux diagram (Figure 3.4).

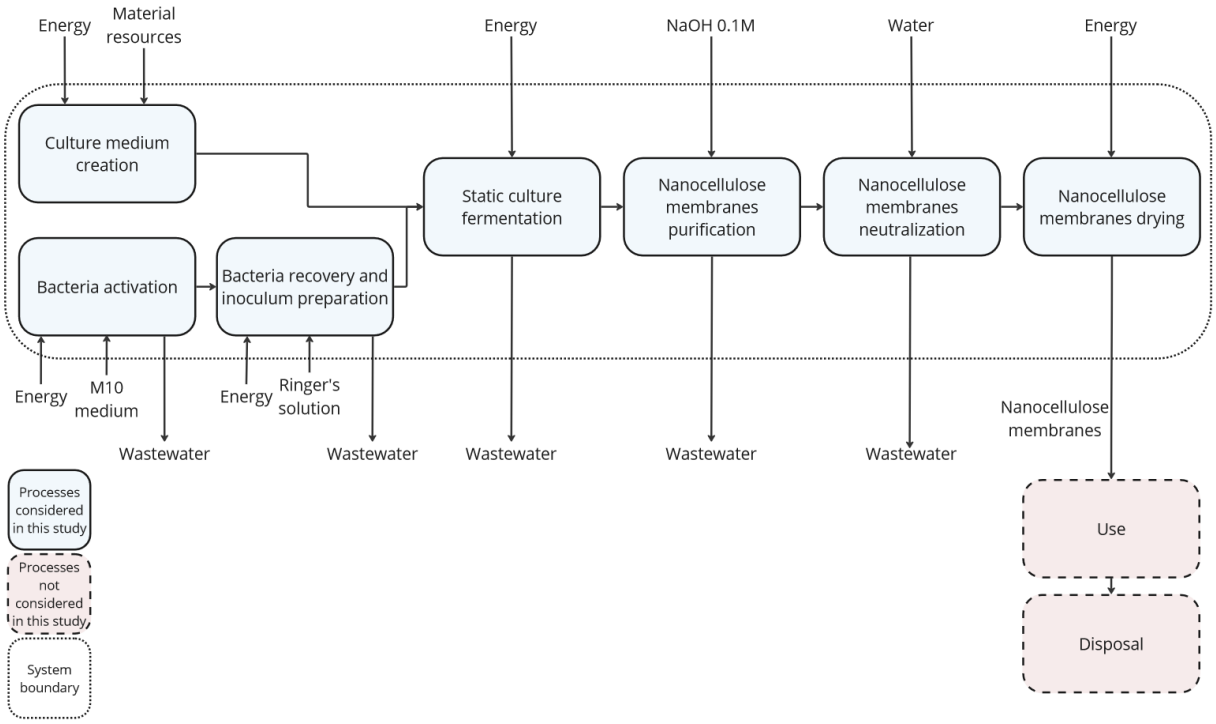


Figure 3.4: System boundary for BNC production, own illustration.

The production path includes bacteria activation, bacteria recovery and inoculum preparation, culture medium creation, static culture fermentation and then purification, neutralization and drying of the formed nanocellulose membranes. The production process analysed through the LCA methodology exactly reflects what described in sections 4.1, 4.2 and 4.3. Use and disposal phases are not considered, being this study a cradle-to-gate LCA.

As already mentioned, three different production routes have been analysed: BNC production from orange peels culture medium, from whey culture medium and from HS culture medium. They differ just for the culture medium preparation, that follows different steps depending on the employed culture medium. However, as different culture media creation conditions were investigated regarding orange peels and whey, the LCA study was performed considering the optimal ones, that is to say the conditions where the media bringing to the highest yield were created.

The functional unit quantifies the function provided by the analysed system and serves as a basis for comparing alternative systems. To enable a meaningful comparison between the three different culture media (orange peel, whey, and HS), it is essential to define a common functional unit that reflects the function or service delivered by these systems. This allows for an objective assessment of their environmental impacts, taking into account variations in observed yields and energy consumption and material requirements specific to each culture media production route.

The functional unit for this study has been defined as 1 g of BNC produced in 7 days. This functional unit was defined to better represent the production function occurring at laboratory scale.

The inventory data for the LCA were sourced directly from own laboratory experience, representing primary data that were meticulously collected and verified through the work in the laboratory. Energy consumption at each step was estimated based on the equipment used at laboratory scale.

As other research projects, this study provides lab-scale data only, which often leads to an exaggerated impact when compared to commercially available materials. Laboratory processes often lack optimisation, particularly in terms of resource efficiency, and do not benefit from economies of scale. The optimised processes and economies of scale associated with commercial production result indeed in better material and energy efficiencies. Early-stage laboratory processes often differ markedly from those in future production plants, as the vessels and equipment used in the lab are vastly different from the reactors and machinery of an industrial facility. Moreover, at this early laboratory stage, prior to the construction of a pilot plant, subsequent process steps are not interconnected, and there is no implementation of material, heat, or energy recovery [90].

Consequently, because the technology examined is only at the laboratory scale, any comparison with potentially competing materials already produced at an industrial scale would not yet be able to offer any meaningful results, not accurately reflecting the true potential of the developed nanocellulose. Scaling up to a commercial level, thereby enabling a comparison with existing technologies, could greatly enhance the accuracy of environmental assessments [91].

Indeed, LCA results from this lab-scale process can guide the design of industrial-scale BNC production, since specific steps that require optimisation at the industrial level could be identified, ensuring a more efficient and sustainable production system.

4 Results and discussion

4.1. BNC production from orange peels

This work was conducted as a study to understand if the result found in literature about the possibility to produce bacterial nanocellulose from orange peel waste was replicable and to investigate the best conditions at which a suitable culture medium could be created starting from orange peels and consequently the best conditions to produce nanocellulose.

4.1.1. Preliminary experiment

In this first experiment, nutrients extraction from orange peels, as described in section 4.1, has been performed at different temperatures to evaluate the optimal conditions at which to perform the process. The investigated temperatures were ambient temperature (25°C approximately), 50°C and 100°C. Moreover, each medium created through the extraction at a specific temperature, has been investigated with and without the addition of additives. Particularly, the additives comprehend all the compounds present in the HS medium, except for the glucose, that are (%, w/v): peptone, 0.5; yeast extract, 0.5; disodium phosphate, 0.27; citric acid, 0.115. Glucose was not added as additive since it is supposed that sugars were already present in the medium, coming from orange peels; however, yeast extract and peptone were added to bring nitrogen to the medium.

The obtained culture media was characterized by measuring reducing sugars, total nitrogen, phosphates, pH and conductivity were measured, using HS medium as control one (Table 4.1).

Regarding reducing sugars, the first observation that can be done is that all the media contain approximately the same amount of reducing sugars than the HS medium. Looking at the three extraction temperatures, in terms of reducing sugars, the optimal temperature seems to be ambient temperature, since the yield of extraction is higher. Moreover, apart from the medium created through the extraction at 50°C, the addition of additives does not influence significantly the sugars concentration, as an additional source of sugars is not added. The result for the medium $T_{50°C}$+additives is rare and difficult to explain without other analyses.

Regarding nitrogen, it can be observed that its concentration in media without additives is less than in HS. With the addition of additives, nitrogen concentration increases. Moreover, both without and with additives, there is a slight increase of

nitrogen concentration with the extraction temperature. Speaking about phosphates, their concentration in HS culture medium is the lowest one among all the media. Their concentration increases when additives are added to the media; however, in this case there is not a visible trend linking phosphates concentration and extraction temperature. Moreover, in the medium obtained at 100°C, instead, phosphates concentration is less when additives are present; this measure is not trustable, since the concentration should be at least equal or higher than the one in the same medium without additives.

pH values are notably lower in orange peels media with respect to HS medium, but quite similar among them. The addition of additives increases pH from approximately 4 to approximately 5 in all media, except for the media created through the extraction at 100°C. Conductivity values, as for pH, are lower than the conductivity value of HS medium; also in this case, additives increase conductivity to values even higher than the HS medium conductivity.

Table 4.1: Culture media from orange peels characterization in terms of reducing sugars, total nitrogen, phosphates, pH and conductivity

	Reducing sugars [g/L]	**Total nitrogen [g/L]**	**Phosphates [g/L]**	**pH [-]**	**Conductivity [mS/cm]**
HS	20.19	1.79	2.21	6.38	3.69
T_{amb}	21.34	0.70	6.02	4.10	1.83
T_{amb} +additives	21.27	1.92	6.24	5.14	4.47
50°C	19.98	0.92	5.05	4.23	1.77
50°C +additives	28.61	2.10	5.97	5.14	4.01
100°C	20.80	0.93	5.75	4.22	1.81
100°C +additives	19.78	2.22	3.41	4.2	6.04

After having characterized the initial conditions and features of the different media, the bacterial nanocellulose production was performed in all the media, previously described, with the procedure explained in detail in section 4.2., in static culture.

Reducing sugars concentration, pH, conductivity and liquid volume in the Petri disk have been monitored during the production period and registered at days 0, 3 and 7 of production (Figure 4.1: pH, conductivity, liquid volume and reducing sugars trends in time during BNC production from orange peels.). What can immediately be noticed is that parameters change more in the first 3 days that after day 3. pH decreases in time, probably due to the formation of gluconic acid, a by-product which decreases the pH and results in decreased BNC production. Additives tend to stabilize the pH of the medium. While the pH drops rapidly in all conditions, media with additives show a slightly less abrupt and more gradual decrease, indicating a buffering effect.

Conductivity increases in all conditions, suggesting ion release into the medium from the decomposition of nutrient components and microbial activity. Moreover, the produced nanocellulose is a polysaccharide; the presence of more polysaccharides in the medium could increase electrical conductivity and cellulose formation can alter the structure of the medium, making it more porous or increasing the availability of ions in the liquid. The extent and rate of increase do not seem to be influenced by the extraction temperature and the presence of additives. However, media with additives tend to have higher initial conductivity with respect to media without additives, and consequently higher final conductivity.

Liquid volume decreases under all conditions due to evaporation and liquid consumption by bacteria, but the presence of additives seem to cause greater liquid loss. This could be due to higher metabolic activity of bacteria when additives are added that causes higher liquid volume consumption.

Finally, reducing sugars have a particular variation; the general trend is that they initially decrease since are consumed by bacteria and successively increase again. Probably, something else starts to be consumed after some days of production and is transformed in reducing sugars, presumably disaccharides or polysaccharides extracted along with reducing sugars from orange peels. The only exceptions to this trend are the media $T_{50°C}$ and $T_{100°C}$+additives, in which reducing sugars monotonically increase in time, making suppose that bacteria are metabolising more disaccharides or polysaccharides than reducing sugars.

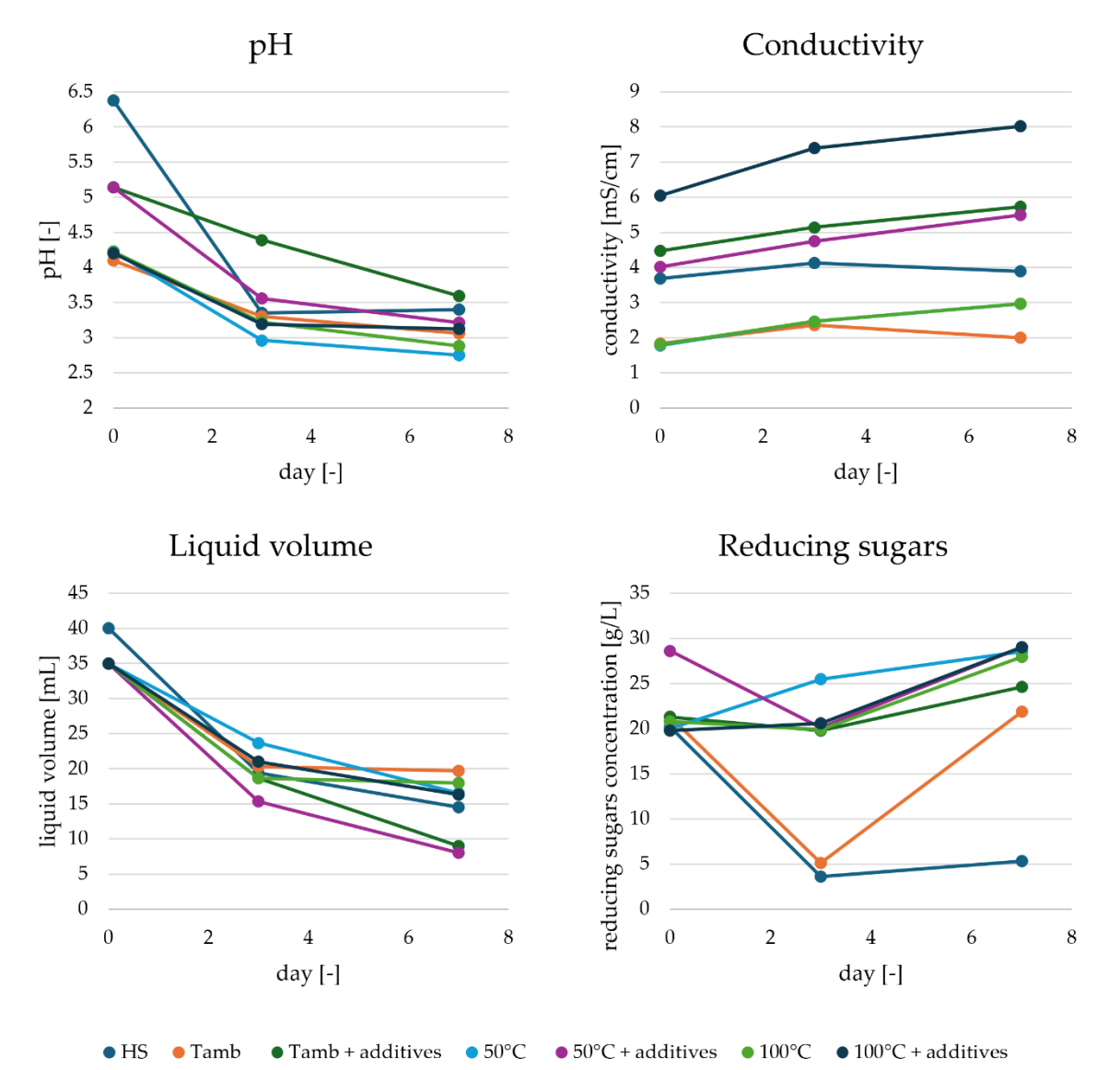


Figure 4.1: pH, conductivity, liquid volume and reducing sugars trends in time during BNC production from orange peels.

Nanocellulose yield have been measured at days 3 and 7 (Figure 4.2). It can be noticed that for the culture medium T_{amb} the yield after 7 days is less than the yield at 3 days, that is impossible since nanocellulose cannot decrease in time. This could be attributed to the fact that to measure the grams of nanocellulose at day 3 and 7 different Petri disks are employed and could be subject to slightly different environmental conditions. For instance, there can be gradients of temperature in the incubator, although the temperature is set to 30°C and this could bring to different Petri disks behave in different ways.

The same happens for the medium $T_{100°C}$; however, in this case it is possible to note the very high standard deviation of the BNC yield at day 3, so that there is a large

variability among the sample value, making the sample mean less reliable. The actual yield value at day 3 should have been presumably lower.

However, another observation possible to be done is the fact that the production seems to be faster in the first days, reaching a plateau after day 3 for almost all the media, HS included. This is not valid when additives are added, since the yield seems to be still growing after day 3, with this effect being particularly palpable in the T_{amb}+additives medium, less evident in the $T_{50°C}$+additives medium and completely disappeared in the $T_{100°C}$+additives medium.

This results in terms of yield confirmed that the best extraction temperature appears to be ambient temperature; indeed, the quantity of reducing sugars in the media without additives at the beginning of the experiment was higher in the one created from the extraction at ambient temperature. Moreover, additives significantly help the bacteria activity and consequently nanocellulose production since nitrogen and phosphorous are necessary for the cell's life, citric acid is a natural preservative and disodium phosphate is a pH buffer, that helps the media resist at pH variations.

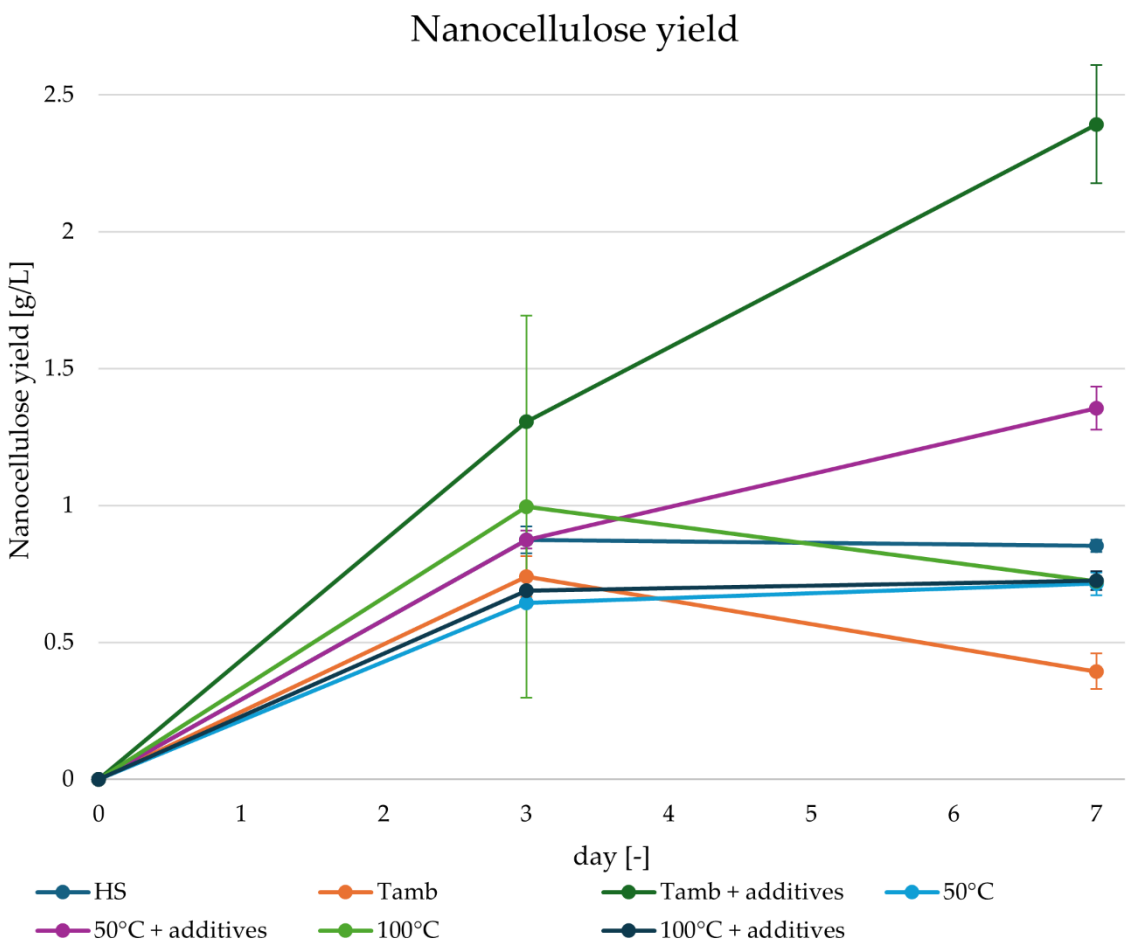


Figure 4.2: BNC from orange peels yield in time.

From this experiment, the optimal conditions for nanocellulose production from orange peel waste results to be the extraction of nutrients from orange peels at ambient temperature and the addition of additives to the resulting medium.

The final values of yield after 7 days are reported in Table 4.2: for the medium T_{amb} the reported yield is the one obtained at day 3, remarking that the value measured at day 7 is not realistic and that the real yield after 7 days is at least equal or higher than the yield at day 3.

For the three temperatures studied, without additives, the final yield is similar and just a bit lower than that for the HS medium. Additives significantly increase production yield, particularly at ambient temperature, with this effect disappearing at 100°C.

Table 4.2: BNC from orange peels yield values after 7 days of production

	Nanocellulose yield day 7 [g/L]
HS	0.85 ± 0.02
T_{amb}	0.74 ± 0.06
T_{amb} +additives	2.39 ± 0.22
50°C	0.71 ± 0.04
50°C +additives	1.36 ± 0.08
100°C	0.72 ± 0.00
100°C +additives	0.73 ± 0.03

The membranes obtained after 7 days of incubation from the commercial medium HS, used here as control medium, and from the best culture medium, T_{amb} +additives, that showed the highest yield of 2.39 g/L, are shown in Figure 4.3. Clearly from the pictures, the membrane obtained from HS appears to be thinner and more transparent compared to the one from orange peels, which looks thicker and opaquer. Moreover,

in terms of consistency, the membrane on the right looks more uniform and homogeneous, whereas the one on the left has a more irregular texture. The membrane from HS was more flexible, as evidenced by its ability to bend easily while being held. The membrane from orange peels was instead stiffer, suggesting less flexibility.

The water retention parameters of the membranes are reported in Table 4.3. It was found that the water content of BNC produced from orange peels was always higher than 98%. It indicates that the membrane structure of BNC has strong water retention ability, which is suitable for applications requiring moisture, where the material should maintain integrity in contact with water.

According to Khan et al. (2021), the average WHC of BNC membranes by *K. xylinus* from orange peel waste was 98.25 g/g [68]. This is reasonably concordant with what found during this experimentation; however, it should be noted that from the medium T_{amb} the WHC was found to be 213.9 g/g, the highest among all analysed media.

Figure 4.3: BNC membranes obtained after 7 days of incubation from HS medium on the left and from T_{amb}+additives medium on the right.

Table 4.3: Water retention parameters of BNC from orange peels after 7 days of production

	WC membranes day 7 [%]	WHC membranes day 7 [g/g]
HS	99.1	105.0
T_{amb}	99.5	213.9
T_{amb} +additives	98.9	90.2
50°C	99.2	126.9
50°C +additives	98.4	59.6
100°C	99.2	130.1
100°C +additives	99.2	130.5

4.1.2. Follow-up experiment

Due to this noticeable positive effect of additives, the experiment has been reproposed in a different manner, to further investigate on the optimal medium creation. As already explained, citric acid is a natural preservative and disodium phosphate plays as a pH buffer. Therefore, disodium phosphate and citric acid have been added to all media to investigate deeply the effects of the additives on the production yield. At each extraction temperature, two cases have been proposed: one with the addition of those two compounds (disodium phosphate, 0.27 %w/v; citric acid, 0.115 %w/v) and the other with all the additives, as in the previous experiment (peptone, 0.5 %w/v; yeast extract, 0.5 %w/v; disodium phosphate, 0.27 %w/v; citric acid, 0.115 %w/v). In this way, it is possible to investigate if the beneficial effect on nanocellulose yield comes more from peptone and yeast extract, that are the nitrogen source for cellular metabolism, or from citric acid and disodium phosphate, which help maintaining favourable the environmental conditions where bacteria live. HS is employed as control culture medium.

Measurements have only been done at the initial day and at day 7, since the main interest is to analyse the final yield of nanocellulose and to have a discussion also comprehending the results obtained from the precedent study.

The measurements at the starting day of the experimentation have been done to characterize the media; in this second research, only reducing sugars, pH and conductivity have been determined to portray the media, since being the ones analysed in time, along with the liquid volume. Table 4.4 reports the media characterization at day 0.

Looking at the concentrations of reducing sugars, these new measurements are showing higher concentrations of reducing sugars in media created from the orange peels residue than in HS, and particularly higher when the extraction of nutrients is performed at 100°C. The dissimilarity between this trend and the one found in the previous experiment could be attributed to many factors, comprehending the uncertainty of measurements but also the uncertainties related to the experimental procedures and the employed batch of orange peels.

These results are more in line with what found in literature about the yield of reducing sugars from orange peels: particularly, Kuo et al. (2019) found a reducing sugars yield extracted from orange peels at 50°C, in absence of enzymes to hydrolyse polysaccharides, of 26.62 ± 1.41 g/L [65]. Therefore, those higher reducing sugars concentrations are more trustable, with respect to what found in the previous experiment, although not so distinct. As already underlined, the additives put in the media should not vary reducing sugars; therefore, the differences in concentration between media created at ambient temperature and 100°C must depend on experimental uncertainties.

As already noticed in the previous experiment, pH of culture media from orange peels is generally lower than HS. Indeed, while HS pH value is a bit higher than 6, all media from orange peel waste have a pH around 5. Differently from the previous experiment, here citric acid and disodium phosphate are added to all media; that is why pH values of orange peels media are quite comparable.

The same discussion can be done for conductivity: the difference between media coming from extraction at the same temperature has been noticeably decreased, with lower values when only citric acid and disodium phosphate were added.

Table 4.4: Culture media from orange peels characterization in terms of reducing sugars, pH and conductivity.

	Reducing sugars [g/L]	pH [-]	Conductivity [mS/cm]
HS	17.67	6.29	4.76
T_{amb}+citric acid+disodium phosphate	25.45	4.97	3.36
T_{amb} +additives	23.11	5.44	4.27
50°C+citric acid+disodium phosphate	22.63	5.15	3.30
50°C +additives	22.46	5.30	3.84
100°C+citric acid+disodium phosphate	25.59	4.96	3.51
100°C +additives	27.86	5.19	4.10

Those 3 parameters, along with the liquid volume in the Petri disk, have been also monitored at day 7 to analyse their variation at the end of the experiment. Their behaviour in time is reported in figure 9.

The presence of citric acid and disodium phosphate stabilizes the pH, with all media showing a steady decrease, indicating a buffering effect. However, media where also yeast extract and peptone are present show a more stable pH. The more stable pH observed in media containing peptone and yeast extract is due to the complex buffering capacity provided by these additives. Their ability to neutralize pH changes resulting from microbial metabolism helps maintain a more stable and suitable environment to produce bacterial nanocellulose. Yeast extract is rich in amino acids, peptides, nucleotides, vitamins (especially B vitamins), and minerals. The amino acids

and peptides in yeast extract can buffer pH changes. Additionally, yeast extract contains other compounds, such as organic acids (e.g., glutamic acid) and phosphates, that have strong buffering properties. Peptone is a mixture of proteins and peptides obtained by digesting proteins with enzymes. These proteins and peptides can act as buffers, absorbing excess hydrogen ions (H+) and hydroxide ions (OH-) produced during microbial metabolism. Although peptone does not contain significant amounts of organic acids or nucleotides, limiting its buffering capacity compared to yeast extract, it still plays a fundamental role in providing a source of nitrogen and supporting microbial growth. Therefore, combining both peptone and yeast extract in the production medium enhance the medium ability to stabilize the pH. This stability is less pronounced in media containing only citric acid and disodium phosphate, as their buffering capacity is not as comprehensive.

Conductivity trends confirm what observed in the previous experiment: conductivity increases in time due to ion release and cellulose formation regardless the type of medium, with the only difference being in the initial value, that is higher for media containing also yeast extract and peptone. However, a decrease in conductivity is observed instead in the HS medium, contrarily to what expectable; factors such as absorption of ions or nutrients from the medium by bacterial cells, formation of biofilms that may insulate the conductivity sensor, or other environmental changes not directly related to cellulose production might be at play.

Regarding liquid volume in the Petri disk, additives do not significantly alter the trend of liquid volume reduction, indicating similar behaviour. The only noticeable exception is for the medium “100°C +additives”, in which liquid volume has not decreased much.

Concerning reducing sugars, the steepest decreases are shown by media containing also yeast and peptone, while less pronounced changes are observed by media with only citric acid and disodium phosphate. This suggests that they are more consumed when the metabolic activity of bacteria is enhanced by adding nitrogen sources. The medium “100°C+additives” shows a slight increase in reducing sugars concentration, that could be attributed to less bacteria activity or to hydrolysis of polysaccharides to reducing sugars.

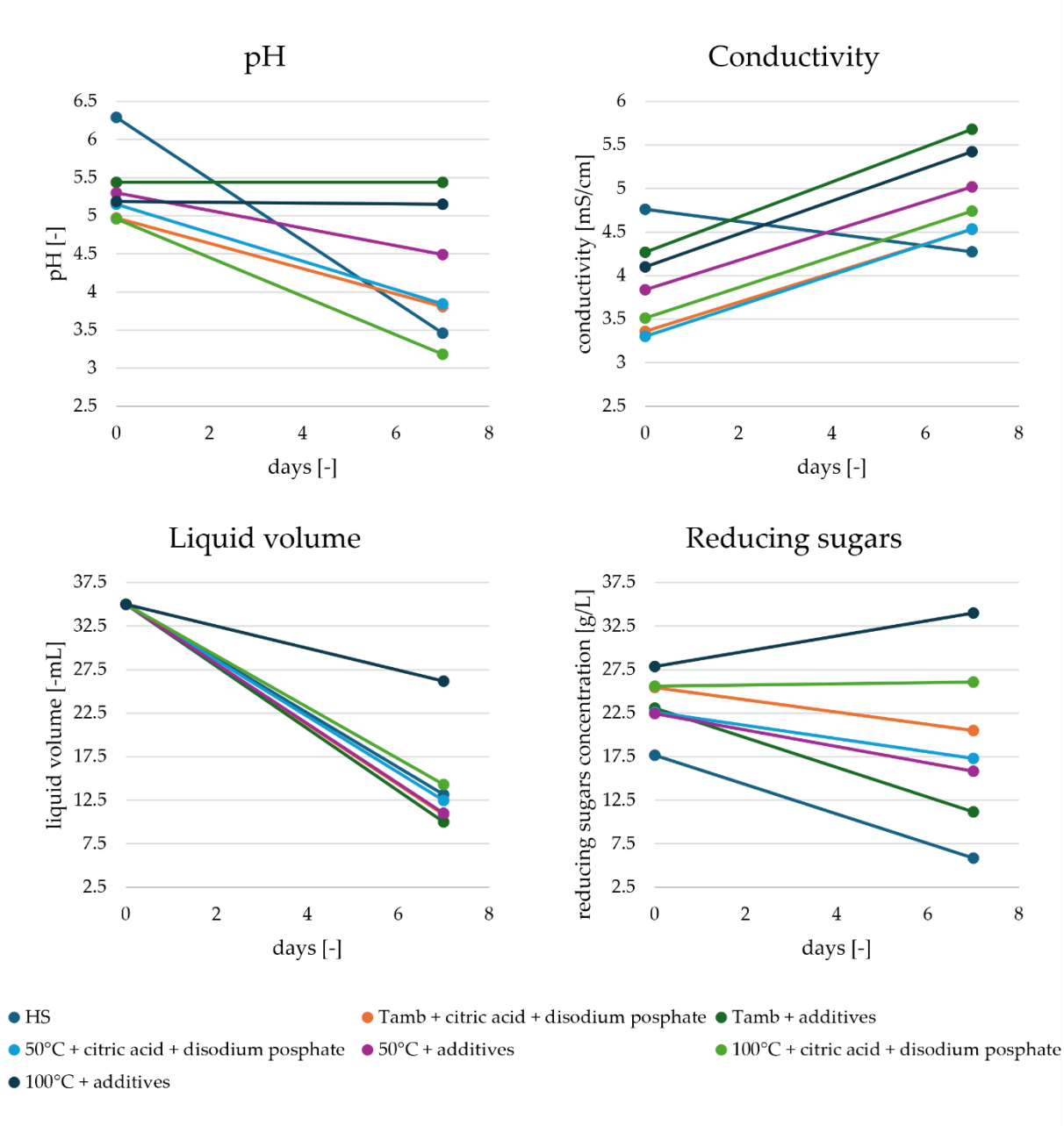


Figure 4.4. pH, conductivity, liquid volume and reducing sugars trends in time, during BNC production from orange peels

The yield for bacterial nanocellulose production, found at day 7, is reported in Table 4.5 for the different media.

In the medium "100°C+additives" no membrane was formed. However, the possibility for nanocellulose to be formed starting from this medium has already been proved in the precedent experiment. Therefore, the luck of membrane for this medium should be attributed to other factors. This is consistent with the results found for reducing sugars and liquid volume at day 7 for this medium, showing a different behaviour compared to other media.

All media showed a higher yield than HS medium, confirming that not only making bacterial nanocellulose from residues is possible, in particular from orange peels in this study, but also that higher yields are reached than from the commercial medium HS.

Table 4.5: BNC from orange peels yield values after 7 days of production.

	Nanocellulose yield day 7 [g/L]
HS	1.49 ± 0.40
T_{amb}+citric acid+disodium phosphate	2.30 ± 0.14
T_{amb} +additives	3.99 ± 0.45
50°C+citric acid+disodium phosphate	2.18 ± 0.31
50°C+additives	3.13 ± 0.27
100°C+citric acid+disodium phosphate	2.22 ± 0.14
100°C +additives	No membrane formed

Furthermore, higher yields are achieved when all additives are present; moreover, the temperature of extraction gains influence if additives are added, bringing to a higher yield at T_{amb} than at 50°C , while where yeast extract and peptone are not present the extraction temperature seems not to play a role. This conclusion suggests that, although citric acid and disodium phosphate brought to higher yields with respect to

the same conditions without any additive, yeast extract and peptone have the highest relevance.

The best condition, reaching the highest yield of 3.99 g/L after 7 days, resulted to be the extraction at ambient temperature and the presence of additives, comprehending also yeast extract and peptone. This result confirms what found in the first experiment, showing that the medium "T_{amb} + additives" is the best one among the investigated ones to grow nanocellulose.

WC and WHC were calculated for all the formed membranes and are reported in Table 4.6.

Table 4.6: Water retention parameters of BNC from orange peels after 7 days of production.

	WC membranes day 7 [%]	**WHC membranes day 7 [g/g]**
HS	99.3	135.9
T_{amb}+citric acid+disodium phosphate	98.7	75.9
T_{amb} +additives	98.5	66.7
50°C+citric acid+disodium phosphate	98.3	56.5
50°C +additives	98.3	58.9
100°C+citric acid+disodium phosphate	98.6	72.7
100°C +additives	-	-

The WC and WHC for membranes from HS and from media with all additives are comparable to what found in the previous experiment. Values regarding orange peels media with the same extraction temperature are comparable when citric acid and disodium phosphate only or all additives are added; moreover, those values are lower than what found for the same media without additives, in the previous experiment. This suggests that the presence of additives in the medium, probably of citric acid and disodium phosphate, lowers down the WHC of BNC membranes. From all culture media, the produced BNC membranes from orange peels have lower water retention if compared to the HS culture medium.

4.1.3. Comprehensive discussion and results about BNC production from orange peels

The results of BNC production of the second experiment from media with all the additives, compared with the result obtained after 7 days of production in the previous experiment, are significantly higher. It needs to be noticed that for media with all additives, the ones with peptone and yeast extract, the experimental conditions are the same than that of the first experiment; the improved results observed in the second experiment can be attributed to different orange peels batch, or more likely to the experience gained during the initial trial, suggesting that a period of practice was necessary to refine the experimental methodology and enhance accuracy. Indeed, the results for media with additives obtained in the second experiment appear more in line with literature, where BNC production from orange peel fluid medium prepared with water yielded up to 3.16 ± 0.71 g/L when yeast extract and peptone were added [65]; Khan et al. (2021) found a BNC production of 4.05±0.05 g/L from using extract of orange peel waste containing peptone, yeast extract, citric acid and disodium phosphate [68], that is exactly in line with the medium type and the results obtained in this own investigation. The same study by Kuo et al. (2019) found also a production from the same medium of 0.97 ± 0.03 g/L, when no additives are added, that is consistent with what found in the first experiment of this research.

Figure 4.5 shows a comprehensive picture of the results found after 7 days of production for media created from orange peels without any additive, with citric acid and disodium phosphate and with all additives, including yeast extract and peptone. The column bars with full colour corresponds to the first experiment, while the column bars with pattern belongs to the second experiment.

Although the yield values obtained for the cases with additives are different among the two performed experiments, the trend followed by additives addition seems to be the same.

Additives added after medium preparation at room temperature show the highest increase in BNC yield. This suggests that nutrients extracted at room temperature are

more suitable for bacterial growth and BNC production when combined with additives. Both sets of additives significantly increase BNC yield, with the combination of citric acid, disodium phosphate, yeast extract and peptone providing the highest yield.

At 50°C, additives still improve yield, but less if compared to room temperature. Formulating the medium at 50°C might partially degrade or modify nutrients, making them less effective when additives are added. The presence of citric acid and disodium phosphate alone still enhances yield significantly.

Without additives, the yield at 100°C is similar to other temperatures. However, with all additives, the yield does not improve as expected. This suggests that partial nutrient degradation at 100°C prevents achieving higher yields, even with the addition of all additives. High temperature likely degrades many essential nutrients or creates inhibitory compounds that additives cannot compensate for.

It is important to note that the lower yield observed using HS medium, which includes only reducing sugars (glucose) and the same additives used in the experiments, may suggest that other beneficial components extracted from orange peels other than reducing sugars may play a crucial role in bacterial cellulose production. Indeed, although reducing sugars are one of the significant compounds extracted from orange peels, the presence of reducing sugars in the DNS assay also at 100°C, in higher quantities than all the other media, suggests that they are not degraded at 100°C, so probably reducing sugars are not the factor determining the suboptimal outcome at this temperature. Indeed, other bio active compounds can be extracted from orange peels. Orange peels contain for instance phenolic compounds and antioxidants that have beneficial properties for bacterial health [92]. However, these molecules can be sensitive to high temperatures and degrade during extraction. Furthermore, vitamins and minerals present in orange peels are essential for bacterial metabolism and cellular growth and their stability and availability can be influenced by the extraction temperature.

This is showing that orange peel residue is not only a source of reducing sugars for BNC production, but there likely are many other components that are extracted along with sugars, bringing positive effects on the final yield; moreover, the synergy between nutrients and additives might be compromised if some nutrients are partially degraded. At room temperature, this synergy is optimal, leading to higher yields.

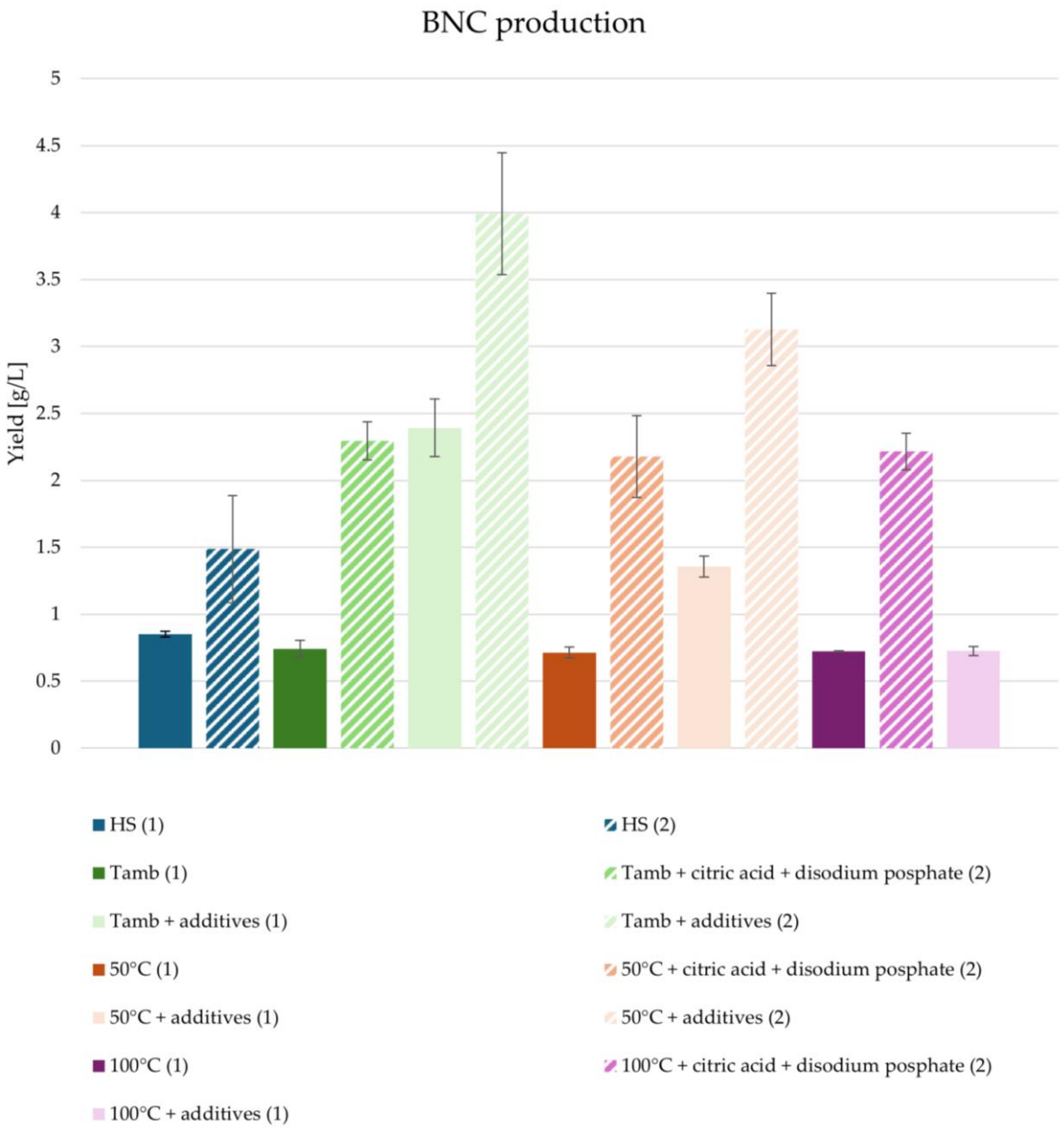


Figure 4.5: BNC from orange peels yield values after 7 days of production, comprehensive results.

Additionally, while the membranes produced from orange peels with the addition of yeast extract and peptone demonstrate high yield, they exhibit lower water retention compared to those produced using the HS medium. Specifically, the water holding capacity (WHC) of the membranes produced from orange peels, in which extraction happens at ambient temperature, was 75.9 g/g for the medium with citric acid and disodium phosphate and 66.7 g/g for the medium with all additives, which are lower than the WHC of 135.9 g/g observed for membranes produced from the HS medium. This indicates that, although the BNC yield from orange peels is high, the resulting membranes might not retain as much water as those from the commercial medium,

which could influence their suitability for certain applications requiring high moisture retention.

4.2. Bacterial nanocellulose production from whey

The objective of this study was to evaluate the efficacy of whey as a substrate to produce bacterial cellulose, analysing the resulting yield of nanocellulose.

4.2.1. Preliminary experiment

In this first experiment, different culture media produced starting from whey have been investigated for nanocellulose production. The culture media have been prepared as described in the section 4.1. Whey has been studied both hydrolysed and not hydrolysed; when hydrolysis is performed, lactose is split into glucose and galactose, so that more reducing sugars should be available for bacteria metabolism. Moreover, both hydrolysed whey and not hydrolysed whey have been analysed in two different cases: seen from the previous experiments on bacterial nanocellulose production from orange peels the positive influence of additives, the experiment for whey has been reproposed in the same manner. The hydrolysed and not hydrolysed whey have been investigated, differentiating for each one a case with only disodium phosphate (0.27 %w/v) and citric acid (0.115 %w/v) and a case where the complete series of additives is involved, comprehending yeast extract (0.5 %w/v), peptone (0.5 %w/v), disodium phosphate (0.27 %w/v) and citric acid (0.115 %w/v).

Those prepared media were then used to grow bacteria and therefore produce nanocellulose, once again in static culture. On day 0 of incubation, measurements of reducing sugars with the DNS essay, pH and conductivity were performed to characterize the culture media. The characterization results are reported in Table 4.7.

In the analysis of whey samples, both in the lactose and hydrolysed forms, significant differences are observed in terms of reducing sugar content, pH, and conductivity, depending on the additives used.

One of the most notable aspects is the reducing sugar content. Hydrolysed whey has a significantly higher reducing sugar content compared to lactose whey under both additive conditions. This increase is attributed to the greater availability of simple sugars in the hydrolysed whey, which results from the breakdown of lactose during the hydrolysis process. The high concentration of reducing sugars in whey with lactose, compared to those obtained from orange peels, can derive from the natural presence of reducing sugars, the partial degradation of lactose during production or the influence of processing conditions.

Regarding pH, the levels are slightly lower when only citric acid and disodium phosphate are used compared to all additives. However, the difference, in this case, is not so significant if compared with the characterization results of culture media created from orange peels. It can be noticed that pH values of culture media created from whey are generally higher and more similar to the pH values of HS before incubation, that was found to be 6.3.

Consistently with what found for orange peels media, conductivity is always higher when the combination of citric acid, disodium phosphate, yeast, and peptone is used compared to just citric acid and disodium phosphate. This could be due to the additional ions contributed by yeast and peptone, which increase the overall ionic strength of the solution, thereby raising its conductivity. The conductivity values are all higher with respect to orange peels media and HS: whey, indeed, naturally contains a range of minerals and electrolytes such as sodium, potassium, calcium, and magnesium. These ions contribute to the electrical conductivity of the solution.

Table 4.7: Culture media from whey characterization in terms of reducing sugars, pH and conductivity.

	Reducing sugars [g/L]	pH [-]	Conductivity [mS/cm]
Whey (lactose)+citric acid+disodium phosphate	39.72	5.89	6.24
Whey (lactose)+ additives	40.40	6.00	6.69
Whey (hydrolysed)+citric acid+disodium phosphate	49.74	5.85	6.35
Whey (hydrolysed)+ additives	52.36	5.97	6.82

These 3 parameters, along with liquid volume, have been monitored also at the end of incubation, that is 10 days after inoculation (Figure 4.6).

All culture media showed a decrease in pH, with the hydrolysed whey media exhibiting the most significant drop, indicating an enhanced microbial activity. This suggests that hydrolysis of whey increases the acidifying effect of the bacterial fermentation, likely due to the increased availability of fermentable sugars.

As noted in the previous experiments, there was a general increase in conductivity across all media, indicating an accumulation of ionic species as fermentation progressed. The observed decrease in conductivity in the medium containing hydrolysed whey with additives, contrary to expectations, could be explained by a few factors. As already discussed, one possibility is the formation of biofilms on the conductivity sensor, which can insulate the sensor and interfere with its ability to measure ionic changes accurately. Additionally, environmental changes not directly linked to cellulose production might also contribute to reduced conductivity.

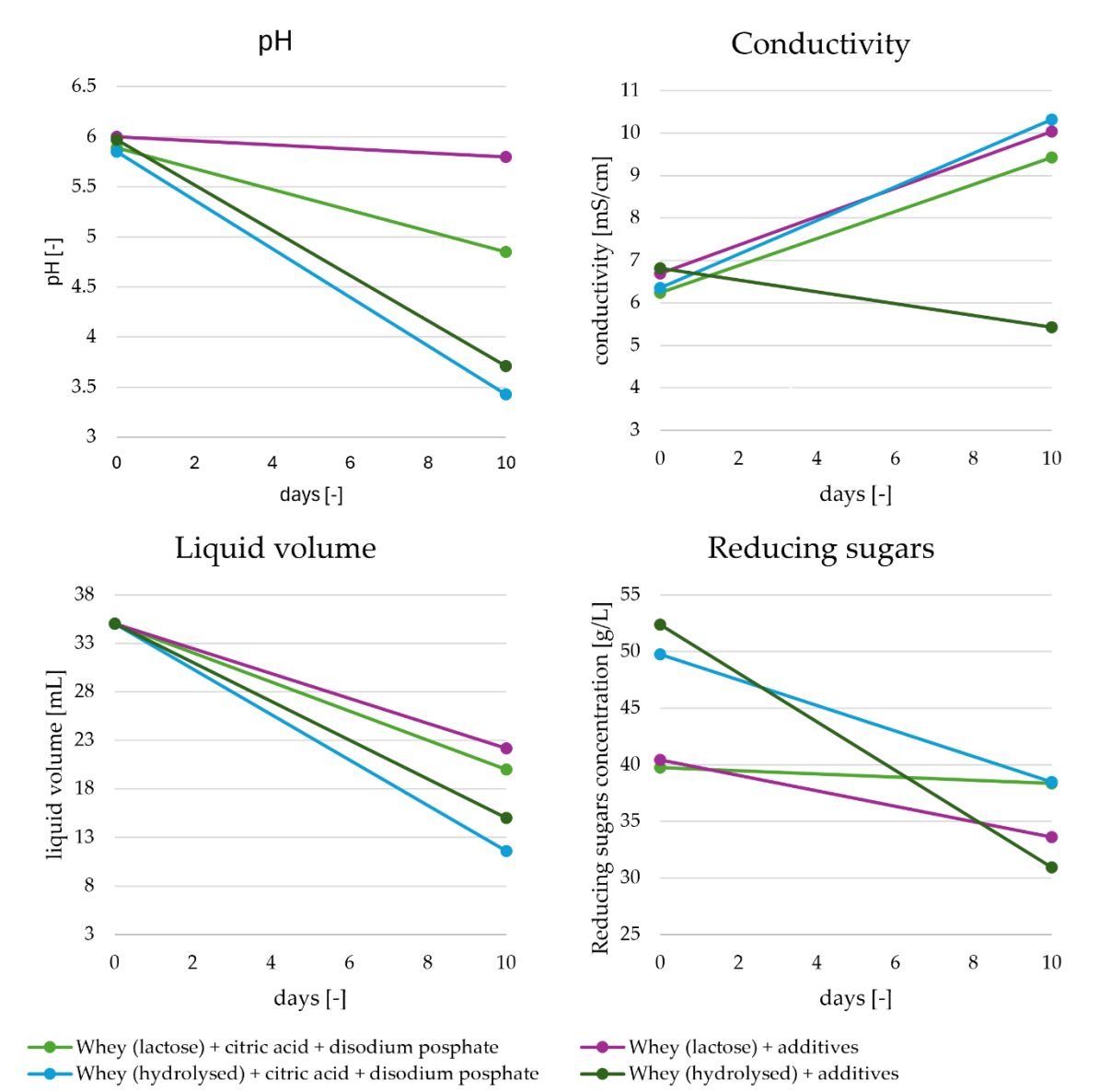


Figure 4.6: pH, conductivity, liquid volume and reducing sugars trends in time, during BNC production from whey.

A reduction in liquid volume was observed in all cases, likely due to water uptake by the BNC and evaporation. The smallest decrease was noted in the non-hydrolysed whey with additives, possibly indicating less efficient BNC production.

Concentrations of reducing sugars decreased in all treatments, confirming their utilization as a carbon source for BNC synthesis. The rate of decrease was fastest in the hydrolysed whey, aligning with its lower pH and higher conductivity readings.

BNC yields were calculated for all the four culture media cases after 10 days of incubation. The results are reported in Table 4.8.

The nanocellulose yield data from day 10 provides critical insights into the effectiveness of different whey treatments and additive combinations in supporting bacterial nanocellulose (BNC) production. For both the "Whey (lactose)+citric acid+disodium phosphate" and "Whey (lactose)+additives" cases, no BNC membrane was formed by day 10. This could be attributed to the presence of lactose in these media; not all bacteria can digest lactose due to the requirement for specific enzymes, namely β-galactosidase, which breaks lactose down into glucose and galactose. Although non hydrolysed whey was containing other reducing sugars, they might not be in a readily available form for effective bacterial utilization, especially if lactose is the predominant sugar. The stable pH in these groups likely reflects the absence of effective bacterial activity or BNC synthesis. Since BNC production typically involves acid production by the bacteria, the steady pH could be an indicator that the bacteria did not engage in significant metabolic activity. The moderate yield of BNC found for the medium "Whey (hydrolysed)+citric acid+disodium phosphate" indicates that hydrolysing whey improves the availability of fermentable sugars, as seen in the reducing sugars graph, where more significant sugar consumption was evident. This enhances bacterial metabolism to BNC production.

The setup where whey was hydrolysed and all additives were added showed the highest yield, significantly higher than the other cases. The additives (likely yeast extract and peptone) may have provided additional nutrients that enhanced bacterial growth and metabolism, leading to increased BNC production. This is supported by the observed trends in reducing sugars and pH, where there was a substantial drop in sugars and a more significant decrease in pH, indicating a highly active metabolic environment.

Table 4.8: BNC from whey yield values after 10 days of incubation.

	Nanocellulose yield day 10 [g/L]
Whey (lactose)+citric acid+disodium phosphate	No membrane formed
Whey (lactose)+ additives	No membrane formed
Whey (hydrolysed)+citric acid+disodium phosphate	1.54 ± 0.33
Whey (hydrolysed)+ additives	4.28 ± 0.36

In Figure 4.7, it is possible to observe membranes obtained from hydrolysed whey with the addition of all additives, the medium resulting in the highest yield, while neutralization with tap water. Notably, they exhibit a uniform amber colour and a thick, consistent structure, with quite regular, smooth, and rounded edges.

Figure 4.7: BNC membranes obtained after 10 days of incubation from "Whey (hydrolysed)+additives" medium during cleaning.

The membrane displayed in Figure 4.8 is one of the previously shown membranes, from hydrolysed whey with additives, after being purified and neutralised, before drying. The membrane exhibits several notable qualities indicative of high-quality BNC. Firstly, the uniformity of the membrane suggests a well-controlled and consistent production process. The translucency indicates that the cellulose fibres are uniformly distributed throughout the membrane, which is a key characteristic of high-quality BNC. Additionally, the absence of visible inclusions or irregularities further supports the conclusion that the production process was stable and precise. The surface of the membrane appears smooth and even, which is crucial for applications requiring high surface quality. Although mechanical strength cannot be directly assessed from the image, the uniform thickness and lack of visible defects suggest that the membrane likely possesses good mechanical properties.

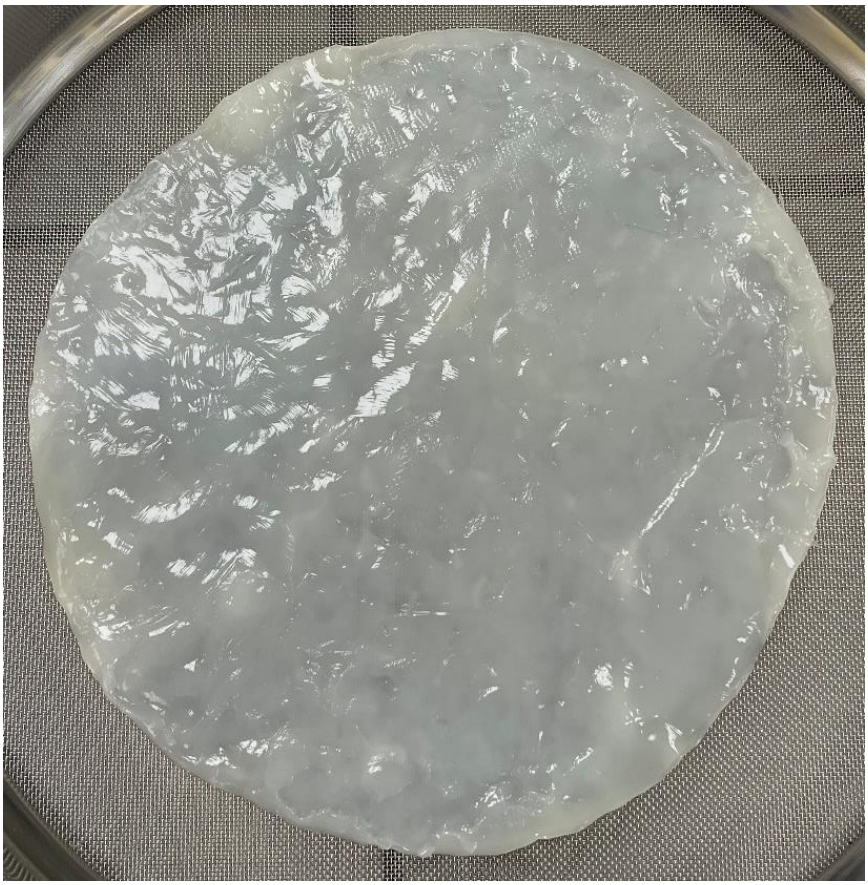


Figure 4.8: BNC membranes obtained after 10 days of incubation from "Whey (hydrolysed)+additives" medium.

To better allow comparison among BNC produced starting from residues, the experiment for the medium yielding the most ("Whey (hydrolysed)+additives" medium) has been repeated incubating for just 7 days, the same time amount employed for BNC production from orange peels waste. The result of production was found to be 2.42 ± 0.47 g/L.

Water retention of BNC was analysed through WC and WHC, that were calculated and reported in Table 4.9. WC and WHC are quite similar for the two whey media; as for BNC membranes from orange peel waste, BNC membranes from whey show same water holding capacity when only citric acid and disodium phosphate and all additives are added to the culture medium.

Table 4.9: Water retention parameters of BNC from whey after 10 days of production.

	WC membranes day 10 [%]	WHC membranes day 10 [g/g]
Whey (lactose)+citric acid+disodium phosphate	-	-
Whey (lactose)+ additives	-	-
Whey (hydrolysed)+citric acid+disodium phosphate	99.3	137.6
Whey (hydrolysed)+ additives	99.3	134.3

WC found in this study is in line with what found in literature regarding BNC produced from whey. For instance, Liu et al. (2023) found a WC of BNC by a symbiotic culture of bacteria and yeasts (SCOBY) from acid whey equal to 95.4% [93], that is just slightly lower than what found in this study. Feng et al. (2024) found the water content of BNC by *Kombucha* from soy whey to be between 83 and 92% [82].

4.2.2. BNC production optimisation

Whey, even in preliminary experiments, yielded promising results, precisely when hydrolysed with additives addition (yeast extract, 0.5 %w/v; peptone, 0.5 %w/v; disodium phosphate, 0.27 %w/v; citric acid, 0.115 %w/v), both in terms of BNC yield and water retention capacity of the membranes. Moreover, from a residue valorisation perspective, the dairy industry faces challenges in managing whey disposal due to its high biological oxygen demand (BOD) and converting whey into BNC helps reduce environmental pollution while adding economic value to a waste product. Since whey is already in a liquid form, it integrates more easily into fermentation processes

without extensive preprocessing. Conversely, orange peels need significant preprocessing, including drying, grinding, and extraction, to make their sugars available for bacterial fermentation. Observed that, it has been decided to perform a BNC production optimisation from hydrolysed whey, through a design of experiments. The goal is to develop a robust, cost-effective, and environmentally sustainable process for converting whey into high-quality bacterial nanocellulose, thus addressing both industrial waste management issues and the growing demand for nanocellulose in various applications.

Particularly, the BNC yield was the response variable to optimise and the experimental factors to be varied where incubation days, optical density of the inoculum and yeast extract concentration.

Whey already contains nitrogen due to its high protein content. Therefore, this study tries to further optimise BNC production by adding only yeast extract, without using peptone, minimising production costs while maintaining or enhancing BNC yield. The yeast extract provides additional nutrients and accessible nitrogen sources essential for microbial growth, which complements the nitrogen already present in whey. Research indicates that yeast extract significantly enhances BNC production compared to peptone. Studies on *Gluconacetobacter* strains, show that yeast extract was the most favourable nitrogen source for BNC production while using inorganic nitrogen resulted in the dramatic decreasing of the production yield. This is due to the comprehensive range of nutrients in yeast extract, including amino acids, peptides, nucleotides, and minerals, which effectively support robust bacterial growth and cellulose production [94].

Inoculum optical density has been inspected to understand number of colonies impact on BNC production, whereas the inoculum amount per Petri disk has not been varied.

All the details about the design of experiments and optimisation process were described in the methodology section 3.5. The design points for the central composite design, face-centred with 2 centre points is graphically visualised in Figure 4.9. Since the number of experimental factors equals 3, a three-dimensional scatter plot can be displayed. The 16 design points are classified into factorial points, axial points and centre points: in this case, the experiment consists of 8 runs at the corners of a cube (factorial points), 6 star points (axial points), and a replicated centre point.

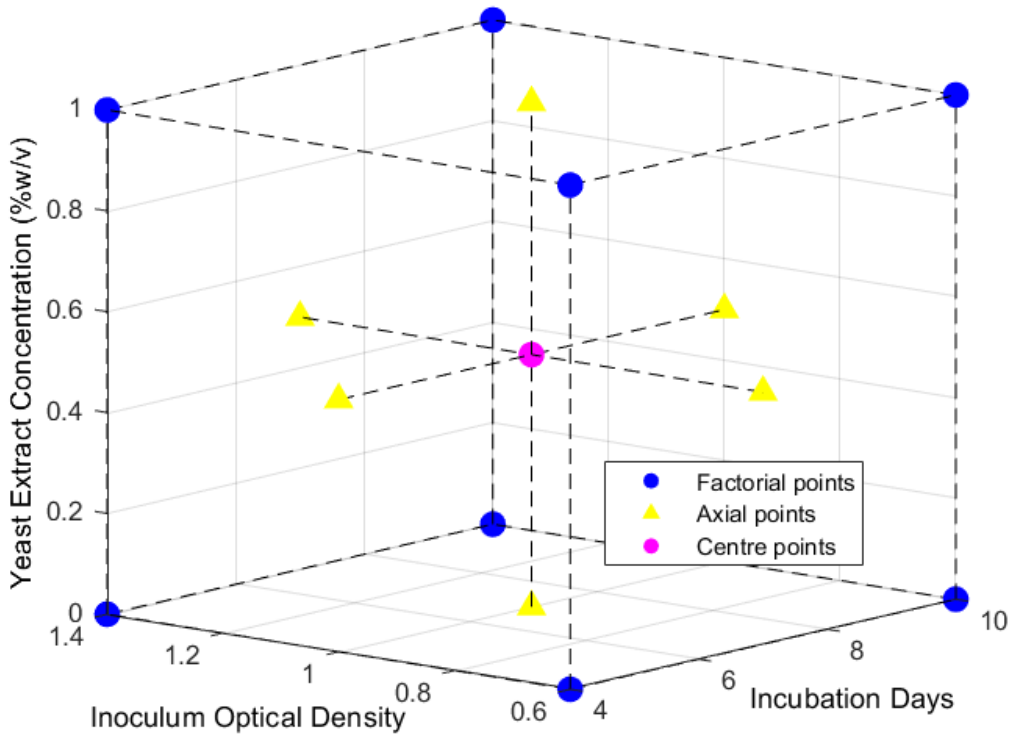


Figure 4.9: Three-dimensional scatter plot for the design points.

The experimental design worksheet is reported in Table 4.10. Incubation time was investigated between 4 and 10 days, inoculum optical density in the range 0.6-1.4 and yeast extract concentration was studied from 0 to 1 %w/v. The 16 runs were performed in triplicates and the table reports the standard deviation found for the series.

At first glance, the predicted data, obtained from the model that will be successively showed in Equation (4.1), do not closely match the mean of the experimental data, largely due to high variability observed in the experimental results. This variability in the experimental data can obscure the expected trends and highlights the importance of replicating experiments and considering variability in biotechnological processes. It needs to be underscored that for run 6, two of the three membranes were found contaminated; clearly, the contamination brought to a competition for food and oxygen with the bacteria producing nanocellulose, blocking or slowing down the production.

Table 4.10: Face-centred central composite design for evaluation of independent factors along with experimental BNC.

Run	Incubation days [-]	Inoculum optical density [-]	Yeast extract concentration [%w/v]	Observed BNC yield [g/L]	Predicted BNC yield [g/L]
1	4	1.0	1.0	2.57±0.73	2.62
2	7	1.0	0.5	1.21±0.07	1.70
3	4	1.4	0.0	0.70±0.15	0.38
4	10	0.6	1.0	1.04±0.06	1.47
5	7	0.6	0.5	1.49±0.60	1.05
6	10	1.4	0.0	0.90±0.19	1.15
7	4	0.6	1.0	2.08±0.01	1.77
8	7	1.4	0.5	1.28±0.32	1.37
9	10	1.4	1.0	2.88±0.44	2.73
10	10	1.0	0.5	2.47±0.36	1.97
11	7	1.0	0.5	1.48±0.44	1.70
12	4	0.6	0.0	0.54±0	0.99
13	4	1.0	0.5	1.38±0.31	1.74
14	10	0.6	0.0	1.18±0.31	1.24
15	7	1.0	0.0	1.52±0.28	1.27
16	4	1.4	1.0	2.53±0.01	2.51

The highest yield was found for run 9, equal to 2.88±0.44 g/L. For this run, the conditions for incubation days, inoculum optical density and yeast extract concentration were respectively 10, 1.4 and 1% w/v. The lowest yield was instead found for run 12, where incubation days, inoculum optical density and yeast extract concentration were 4, 0.6 and 0% w/v, respectively.

The membrane formed during run 9 is displayed in Figure 4.10. The membrane exhibits a translucent, gelatinous texture, indicative of its high-water content and smooth, flexible structure. The surface of the membrane is glossy, reflecting light evenly, and appears uniformly hydrated.

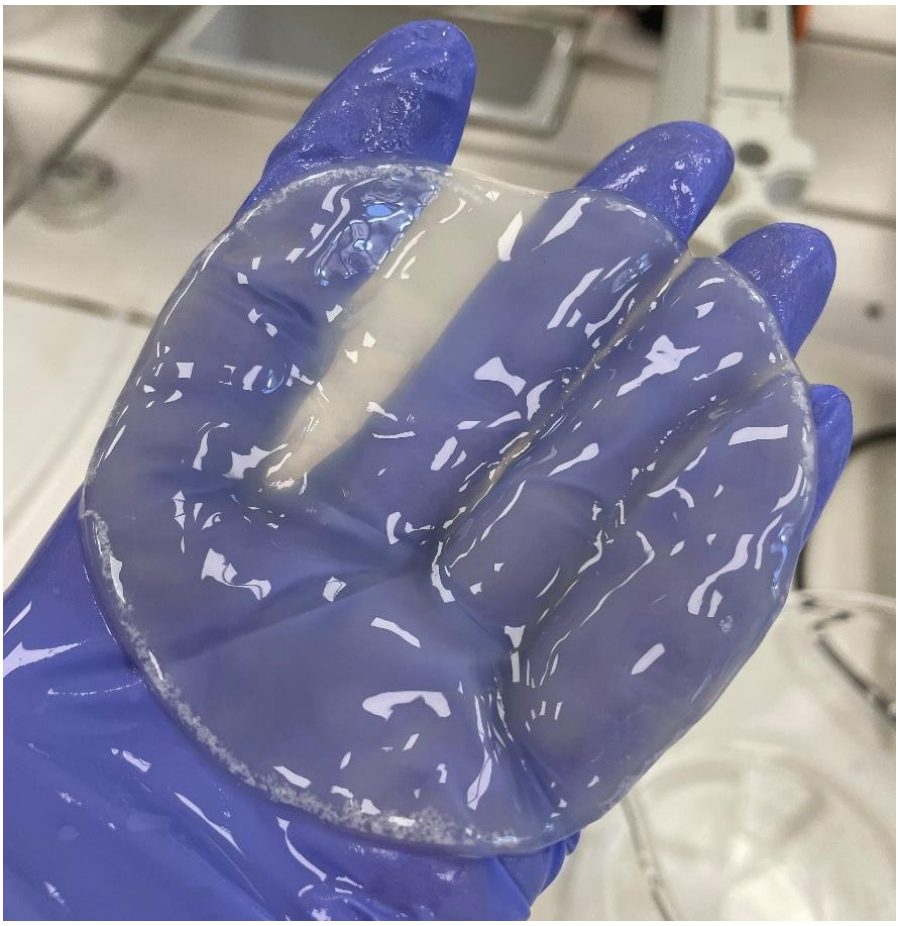


Figure 4.10: BNC membranes obtained after 10 days of incubation from run 9 (10 incubation days, 1.4 inoculum optical density and 1% w/v yeast extract concentration).

The effect of the interaction of the three variables on BNC production was visualised by drawing the contours of estimated response surface, segmented by yeast extract concentration, as reported in Figure 4.11. The yield values are reported in g/L.

From the chart, it is immediately noticeable that the optimal yeast extract concentration is 1% w/v and that the optimal incubation period corresponds to 10 days.

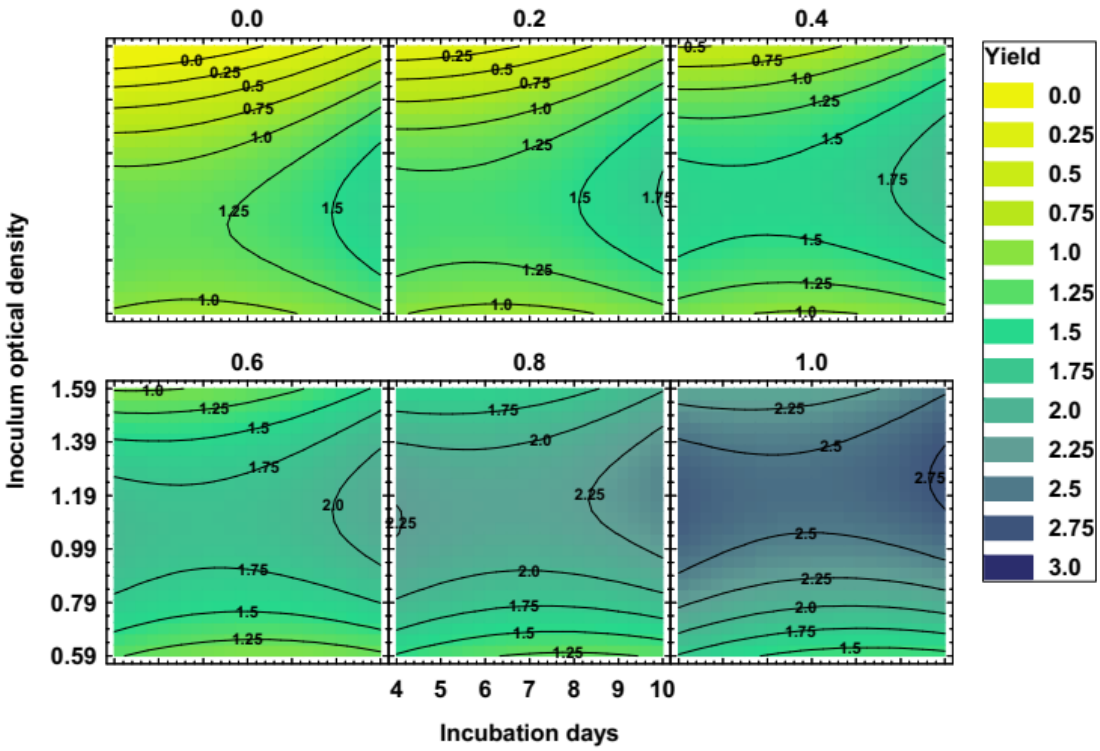


Figure 4.11: Contours of estimated response surface, segmented by yeast extract concentration.

Polynomial regression analysis was performed using BNC yield as the response value. A quadratic regression model with 10 coefficients was fitted to obtain a multiple regression model, that is reported in Equation (4.1).

$$\begin{aligned} Y = & -0.307044 - 0.26967A + 4.87381B \\ & - 0.504715C + 0.0175774A^2 \\ & + 0.109368AB - 0.0919323AC \\ & - 3.03809B^2 + 1.68806BC + 0.638249C^2 \end{aligned} \tag{4.1}$$

A, B and C represented incubation days, inoculum optical density and yeast extract concentration, respectively.

The statistical analysis of the quadratic model of the response surface is shown in Table 4.11Table 4.11: Statistical analysis of the quadratic model of the response surface.. The model indicates that no transformation was applied to the data, as specified by "none" under the Transformation row. The model degrees of freedom (Model d.f.) is 9. The P-value of 0.0001 suggests that the model is statistically significant, indicating strong evidence against the null hypothesis and affirming the relevance of the model in explaining the variability in yield. Models with P-values below 0.05, of which there are 1, indicate that the model as fit is statistically significant at the 5.0% significance level. The error degrees of freedom (Error d.f.) is 26, representing the degrees of freedom

associated with the residual error. The standard error (Stnd. error) of 0.484564 indicates the average distance that the observed values fall from the regression line, providing a measure of the model's precision. An R-squared value of 69.87% suggests that the model explains approximately 69.87% of the variability in yield, which is a substantial proportion. The adjusted R-squared value of 57.13%, which accounts for the number of predictors in the model, indicates that around 57.13% of the variability in yield can be explained by the model when adjusted for degrees of freedom.

Table 4.11: Statistical analysis of the quadratic model of the response surface.

Model	Yield
Transformation	none
Model d.f.	9
P-value	0.0001
Error d.f.	26
Stnd. error	0.484564
R-squared	69.87
Adj. R-squared	57.13

In addition, the optimal setting of the experimental factors has been determined from the maximum point of the quadratic model and showed that optimal incubation days, inoculum optical density and yeast extract concentration are 10, 1.26 and 1% w/v, respectively. At these settings, the quadratic model generates a yield of 2.79 g/L. The estimated optimal yield value of 2.79 g/L is higher than the estimated value for run 9, which was 2.73 g/L. Notably, run 9 had the highest observed yield at 2.88 ± 0.44 g/L. Therefore, it is reasonable to expect that the observed yield for the optimal settings of the experimental factors will exceed any observed yield during this experiment. This expectation arises because the quadratic model tends to underestimate the actual observed values.

To deeper investigate BNC production from hydrolysed whey when peptone is not added as additive, three conditions have been further examined in time.

In particular, the three conditions are: Min (optical density 0.6, yeast extract 0 %w/v), Control (optical density 1, yeast extract 0.5 %w/v) and Max (optical density 1.4, yeast extract 1 %w/v). The first one contains the minimum levels of both the variables inoculum optical density and yeast extract concentration, the second one contains the intermediate levels and the third one the maximum levels of both. The aim of this

investigation was to understand which condition among the three brings to higher yield, considering that yeast extract put in the medium decrease from Max to Min, but also the number of colonies inserted that could grow and develop.

At day 0, measurements of reducing sugars, total nitrogen, phosphates, pH and conductivity have been performed to characterize the media (Table 4.12). Naturally, inoculum optical density does not influence media properties at day 0; the visible differences among the media before incubation just consider differences in yeast extract concentration, going from 0 to 1 %w/v.

The three media should show similar features in terms of pH, conductivity, phosphates and reducing sugars concentration since the only one variation was yeast extract concentration. However, the Max slightly differs from the other two regarding conductivity and reducing sugars; that could be attributed to measurement uncertainties.

Concerning nitrogen, the trend confirmed what expected: increasing yeast extract concentration from 0 to 1 %w/v, total nitrogen concentration increases, specifically from 1.50 g/L to 2.68 g/L.

Table 4.12: Culture media from whey characterization in terms of reducing sugars, total nitrogen, phosphates, pH and conductivity.

	Reducing sugars [g/L]	Total nitrogen [g/L]	Phosphates [g/L]	pH [-]	Conductivity [mS/cm]
Min	67.96	1.50	5.51	6.08	8.71
Control	62.12	2.18	5.70	6.00	9.01
Max	52.19	2.68	5.07	6.00	7.82

All the aforementioned features, along with liquid volume, have been measured over time, to investigate the impact of both yeast extract and inoculum optical density on incubation (Figure 4.12).

The pH decreases rapidly during the first 4 days and then its value stabilises: the three conditions show same pH behaviour.

Conductivity increases over time, peaking around day 6-7, then stabilizes or slightly decreases.

Liquid volume decreases significantly in the first few days and then stabilizes. The condition Max shows the greatest reduction, suggesting the highest BNC production.

The concentration of reducing sugars decreases over time in all conditions. The condition with optical density 1.4 and 1% yeast extract concentration shows the highest sugar consumption, suggesting higher bacterial activity.

Nitrogen concentration gradually decreases over time. The consumption of nitrogen reflects the utilization of nitrogenous nutrients by the bacteria for growth. The similar rate of nitrogen decrease in the initial days for all three conditions can be explained by the combined effects of bacterial inoculum and yeast extract concentration. With a higher optical density, there are more bacteria present initially, which can lead to a rapid and similar rate of nitrogen consumption across all conditions due to higher metabolic activity. As yeast extract provides nitrogen, a higher initial bacterial population would consume it quickly, explaining the similar initial rates. As the fermentation progresses, conditions with more yeast extract continue to show a nitrogen decrease, reflecting the ongoing availability of nitrogen provided by the yeast extract, supporting sustained bacterial activity.

Phosphate levels remain relatively stable across all conditions. This suggests that phosphates are not significantly consumed during the process, or that their release and consumption are balanced. All conditions show similar behaviour, indicating that phosphates are not a limiting factor in BNC production.

The nanocellulose production in time is reported in Figure 4.13. The analysis presented in the graph shows the nanocellulose yield over a period of 10 days under three different experimental conditions. The purpose of the study was to determine the optimal quantity of bacterial colonies and nitrogen source for maximizing nanocellulose production. The results indicate that increasing both the optical density and the yeast extract concentration leads to a significant enhancement in nanocellulose yield. The conditions were designed so that both factors increased or decreased together: in the Min condition, both the optical density and yeast extract concentration were low; in the Control condition, both were at a moderate level; and in the Max condition, both were high.

The Max condition consistently produced the highest yield, reaching approximately 3.0 g/L by day 10. The Control condition showed moderate productivity, with a final yield of about 2.5 g/L. The Min condition, with the lowest optical density and no yeast extract, produced the least amount of nanocellulose, exceeding 1.0 g/L.

These findings suggest that optimising both the initial bacterial colony density and the nitrogen source concentration is crucial for maximizing nanocellulose yield. As both factors increase simultaneously across the conditions, their combined effect on production efficiency is evident.

The study effectively demonstrates that a higher bacterial load, combined with an adequate nitrogen source, leads to significantly higher production of nanocellulose, providing valuable insights for optimising conditions in future biotechnological applications.

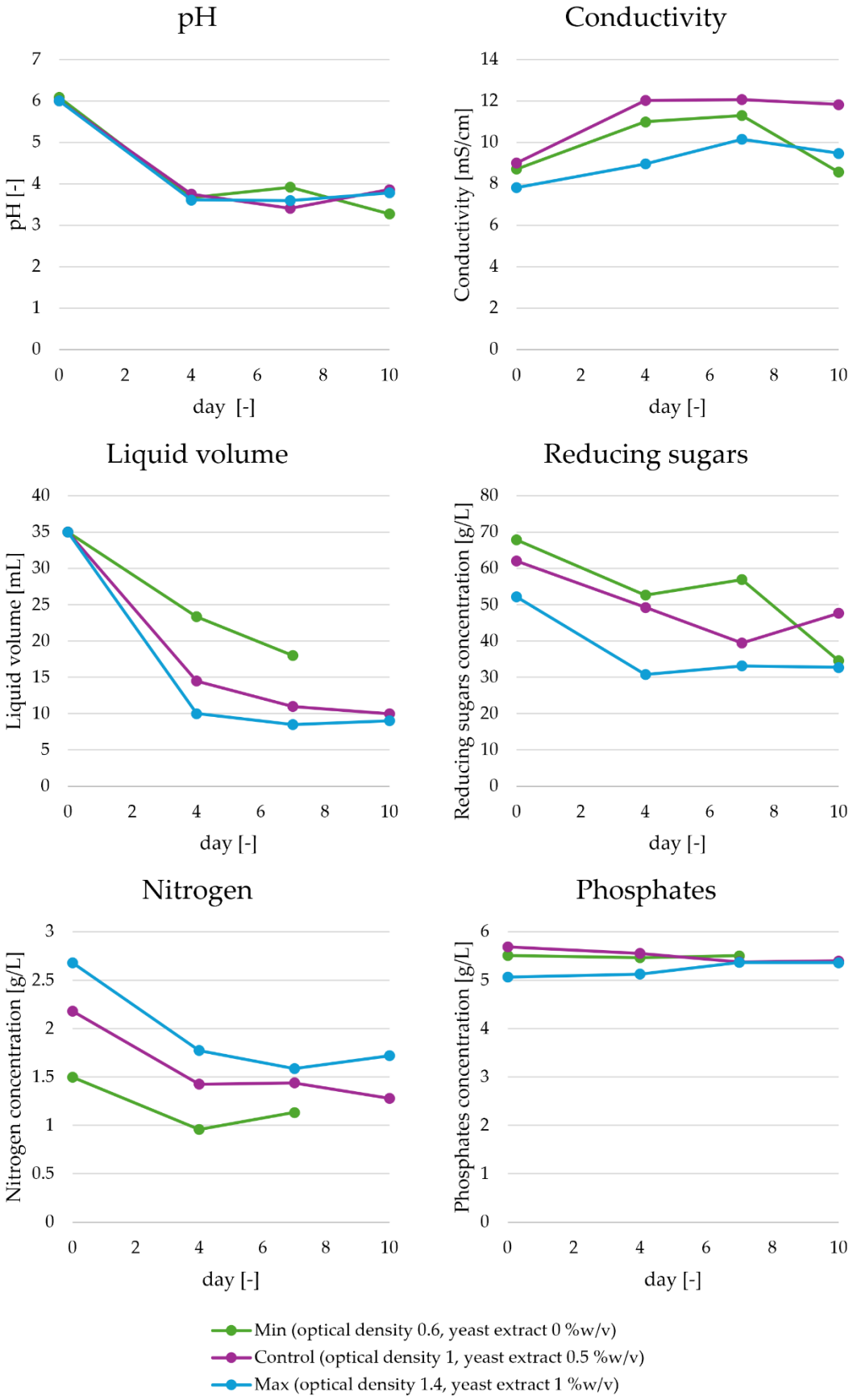


Figure 4.12: pH, conductivity, liquid volume, reducing sugars, total nitrogen and phosphates trends in time during BNC production from whey.

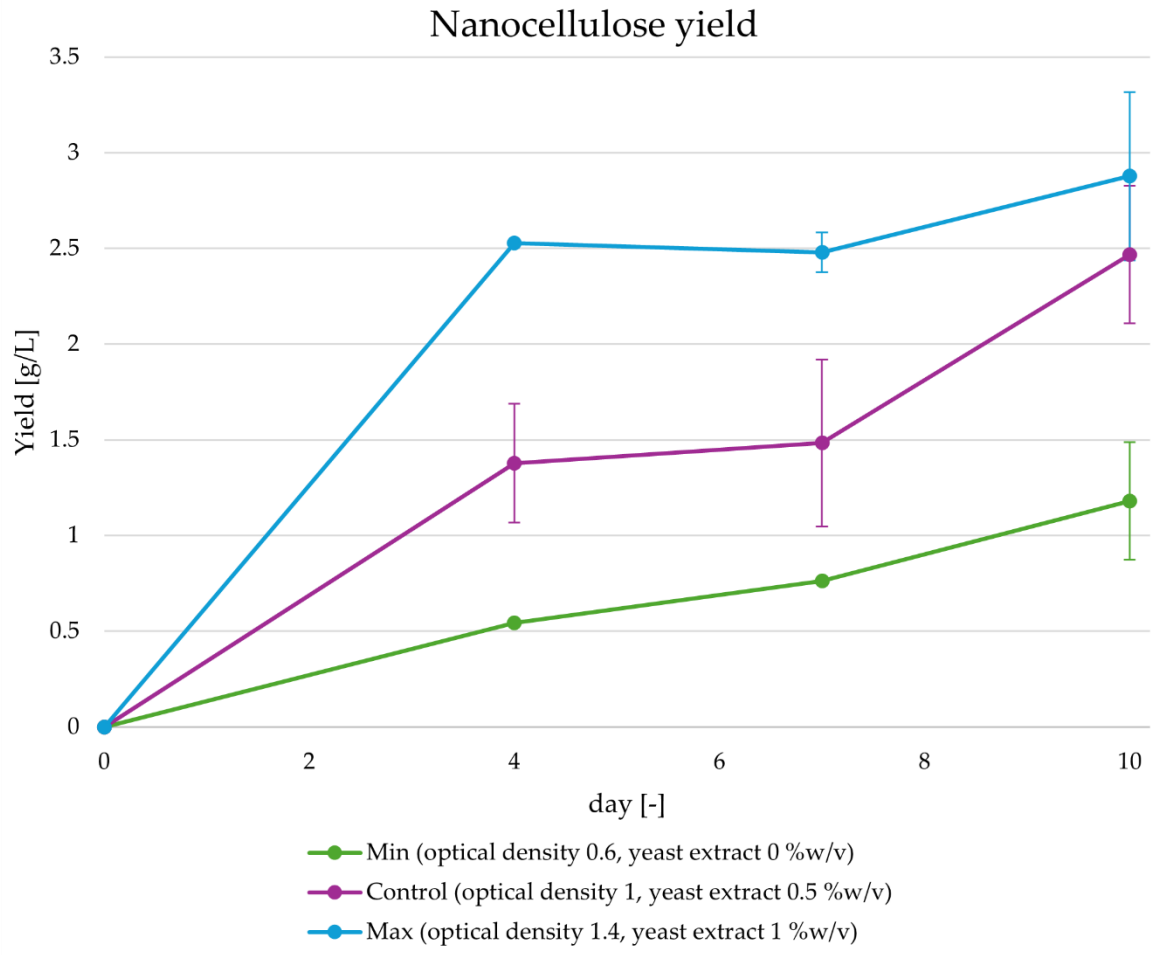


Figure 4.13: BNC from whey yield in time.

Combining these results with the optimisation results, it is possible to confirm that BNC production from hydrolysed whey, with yeast extract, citric acid and disodium phosphate addition, is enhanced by higher inoculum optical density and yeast extract addition; particularly, the optimal combination of the two variables is 1.26 and 1% w/v respectively. As anticipated, the optimal incubation period is day 10, which is the last day of this investigation. This conclusion is based on the observation that, under consistent conditions, extending the incubation time should either result in an increased BNC yield or at least maintain it at a stable level. Therefore, it is logical that the maximum yield would be achieved at the longest incubation time tested in this study. By analysing the yield trends in time, one can assess if the additional yield gained towards the end of the incubation period is significant enough to warrant extending the incubation time. This evaluation helps in making informed decisions about the optimal incubation duration for maximizing BNC production efficiently. From the chart, it appears that extending the incubation period up to 10 days does indeed result in a significant increase in yield, suggesting that a longer incubation is beneficial for maximizing BNC production.

Water retention of BNC was analysed through WC and WHC, that were calculated and reported in Table 4.13. WC and WHC are both increasing from Min to Max, indicating better water retention when higher inoculum optical density and yeast extract concentration are employed, along with better BNC yields. However, water retention resulted to be lower than what found for BNC produced from hydrolysed whey where both yeast extract and peptone are added.

Table 4.13: Water retention parameters of BNC from whey after 10 days of production.

	WC membranes day 10 [%]	WHC membranes day 10 [g/g]
Min	97.3	35.4
Control	98.2	54.3
Max	99.0	102.0

4.2.3. Comprehensive discussion and results about BNC production from whey

Based on previous experiments, it is clear that whey must be hydrolysed and that nitrogen source additives should be incorporated. This conclusion is supported by the observed yield values, which are all significantly higher with these modifications, if compared to the yields of non-hydrolysed whey or the yields of hydrolysed whey with only citric acid and disodium phosphate added. Despite optimising the medium without peptone, the maximum yield achieved is still inferior to the 10-day yield from the medium with 0.5% w/v yeast extract and 0.5% w/v peptone.

By comparing the yields from BNC produced using hydrolysed whey with the addition of 0.5% w/v yeast extract and 0.5% w/v peptone, to those from hydrolysed whey with the addition of 1% w/v yeast extract and no peptone, we can draw conclusions regarding the best medium. The yield from whey with 0.5% w/v yeast extract and 0.5% w/v peptone was 2.42±0.47 g/L at 7 days and 4.28±0.36 g/L at 10 days. In contrast, the yield from whey with 1% w/v yeast extract and no peptone was 2.48±0.10 g/L at 7 days and 2.88±0.44 g/L at 10 days. Nevertheless, considering BNC from whey with the addition of 1% w/v yeast extract and no peptone, which also has an initial inoculum optical density of 1.4 compared to 1.0 in the case of 0.5% w/v yeast extract and 0.5% w/v peptone, further insights can be derived. The higher optical density indicates more bacterial colonies, yet this does not translate to higher cellulose production.

Therefore, while the medium with peptone demonstrates superior cellulose yield, the medium without peptone, despite its higher bacterial growth, as indicated by optical density, does not achieve the same level of cellulose production. This suggests that the presence of peptone not only supports higher yield but also likely contributes to the efficiency of cellulose synthesis processes in BNC production.

The two pictures for BNC membranes at day 10 of incubation for the two scenarios, previously presented during the discussion, are here reported together to allow a better understanding and comparison (Figure 4.14).

The membrane on the right appears very transparent and uniform, with a smooth and glossy surface, indicating good quality in terms of cell structure and cellulose fibre distribution. Despite its higher optical density (1.4), the cellulose yield is lower compared to the medium with peptone.

The membrane on the left, instead, appears slightly opaquer and has some surface irregularities, possibly due to the presence of peptone influencing the structure and density of the produced cellulose. However, the yield from this medium is significantly higher, reaching 4.28±0.36 g/L at 10 days, demonstrating superior cellulose production efficiency.

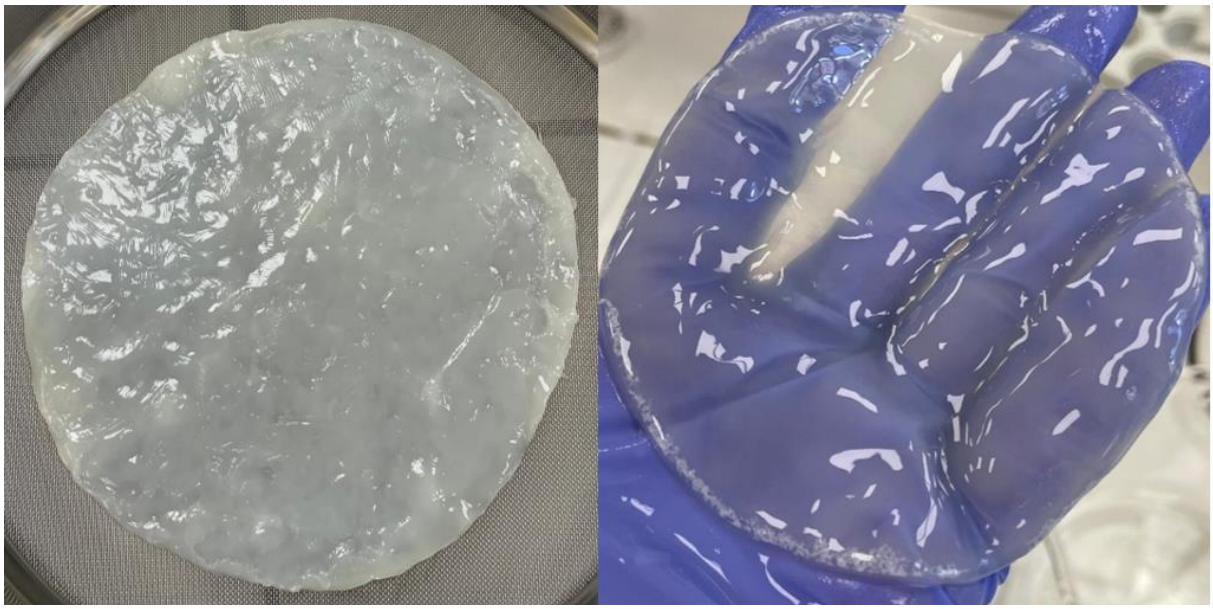


Figure 4.14: BNC membranes at day 10 of incubation obtained from hydrolysed whey with the addition of 0.5% w/v yeast extract and 0.5% w/v peptone, 1 inoculum optical density on the left and hydrolysed whey with the addition of 1% w/v yeast extract and no peptone, 1.4 optical density on the right.

The medium with 1% w/v yeast extract and no peptone, despite its lower yield, produces membranes with higher transparency and uniformity. These characteristics are indicative of a more homogeneous cellulose fibre network, which can be advantageous for applications requiring clear and consistent materials. The high initial bacterial concentration (indicated by the higher optical density) suggests that yeast extract alone can effectively support bacterial proliferation, yet this does not directly translate to higher cellulose production.

On the other hand, the medium with 0.5% w/v yeast extract and 0.5% w/v peptone yields a higher quantity of cellulose. The presence of peptone seems to enhance the metabolic efficiency of cellulose synthesis, leading to a denser and possibly stronger membrane. This suggests that peptone provides essential nutrients and growth factors that facilitate more efficient cellulose biosynthesis. The resulting membrane, though slightly less transparent, may possess superior mechanical properties, making it suitable for applications where strength and durability are paramount. Indeed, the slight opaqueness and surface irregularities may indicate a denser network of cellulose fibres, which can enhance the mechanical strength and durability of the membrane. Moreover, the membrane on the left produced with 0.5% w/v yeast extract and 0.5% w/v peptone exhibited higher water retention. This characteristic can be attributed to the denser and more intertwined network of cellulose fibres, which enhances the membrane's ability to hold water. The higher water retention can be beneficial for applications such as wound dressings, where maintaining a moist environment is crucial for healing, or in filtration systems where high moisture content can improve performance.

For applications requiring high transparency and uniformity, the medium with 1% w/v yeast extract and no peptone may be more suitable. However, for higher yield and potentially stronger membranes with higher water retention, the medium with 0.5% w/v yeast extract and 0.5% w/v peptone is preferable.

4.3. BNC characterization

Morphological and structural characterizations have been performed using SEM and TEM analyses. The characterization has been done on different types of membranes, to come up with a comparison among BNC produced from different culture media. Particularly, three types of membranes have been analysed, one produced from the HS culture medium, another from orange peels and the last one from whey.

Regarding BNC produced from wastes, the membrane analysed for the orange peels case has been chosen as the one produced from the culture medium T_{amb}+additives, where nutrients extraction from orange peels was conducted at ambient temperature and that all additives were added to the medium (yeast extract, peptone, citric acid and disodium phosphate). About BNC production from whey, the chosen culture medium was whey (hydrolysed)+additives.

The SEM and TEM analyses of BNC membranes derived from HS, orange peels, and whey substrates reveal distinct morphological characteristics at different scales.

The SEM images (Figure 4.15) provide insights into the surface morphology of the BNC membranes. The SEM analysis of the BNC samples does not reveal detailed nanometric structures due to the limited magnification capacity of the instrument. Nonetheless, SEM images confirm that all samples present as compact, non-porous films. Specifically, BNC synthesized using the HS medium displays a dense, uniform fibrillar network, indicative of a smooth and consistent surface morphology. The membrane derived from orange peels shows a subtly more irregular distribution, likely influenced by organic components in the peels. The sample produced with whey, in contrast, displays a modestly looser fibrillar arrangement, suggesting that whey's specific nutrient profile may have a minor impact on cellulose formation and packing density.

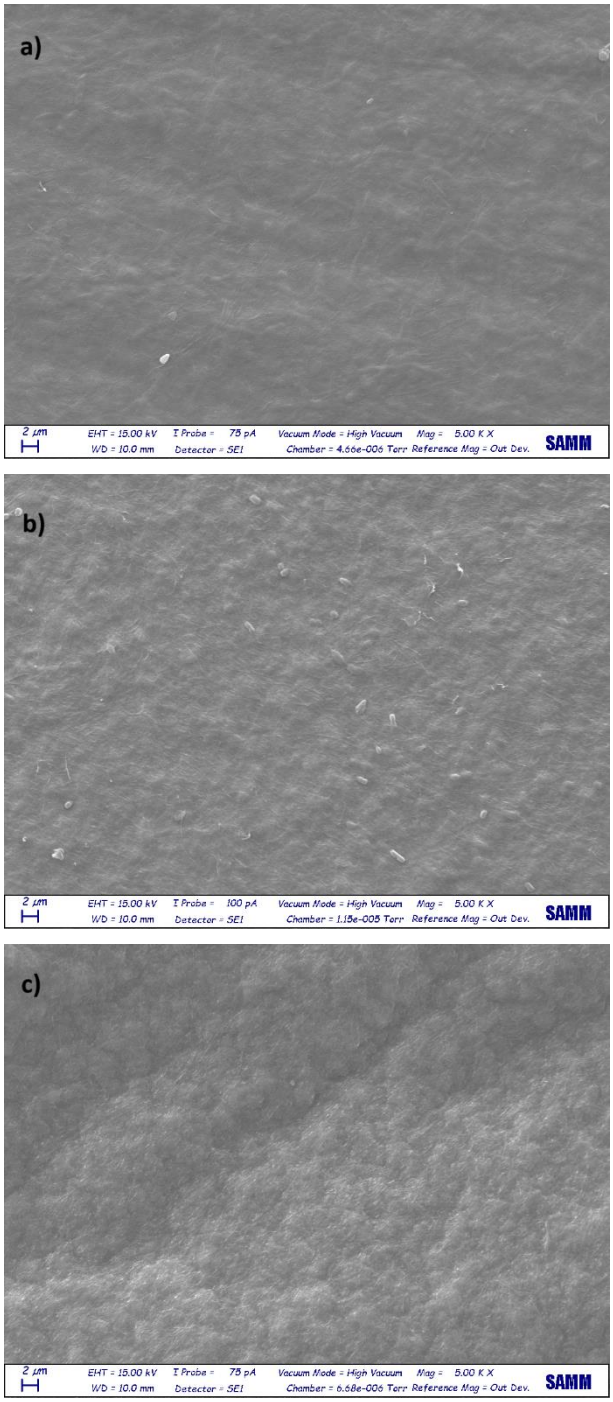


Figure 4.15: SEM analysis results for BNC membranes produced from a) HS, b) orange peels and c) whey.

The TEM images should reveal individual nanofibers, as a result of the defibrillation process applied to the samples. These TEM observations (Figure 4.16) confirm the successful production of nanocellulose, as evidenced by the presence of nanoscale fibrils. Overall, the images reflect the typical networked and elongated structure associated with nanocellulose fibres, reinforcing the material's nanoscale nature. Across all substrates, these isolated fibres demonstrate a relatively consistent

morphology, though slight variations in fibre diameter, which are in the range of 10-100 nm.

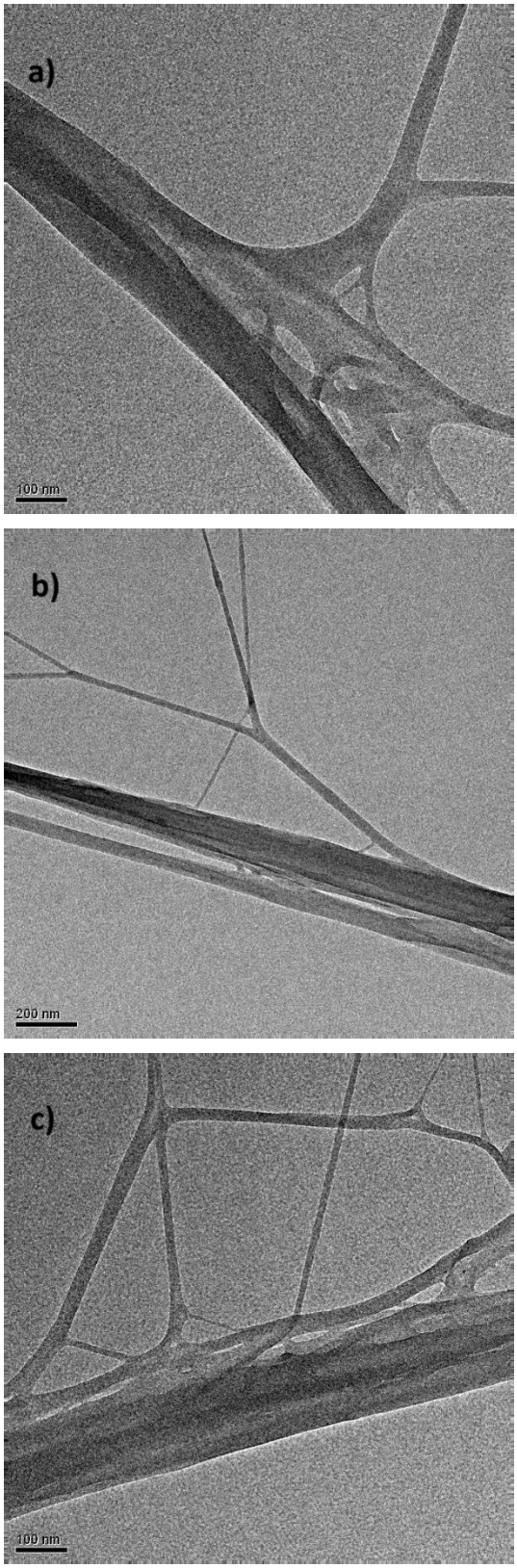


Figure 4.16: TEM analysis results for BNC membranes produced from a) HS, b) orange peels and c) whey.

The subsequent section outlines the chemical composition of BNC membranes, as determined through IR spectroscopy. The IR spectra were analysed to identify the key functional groups and structural characteristics of the BNC produced from different substrates (HS, orange peels and whey). The results are presented in Figure 4.17.

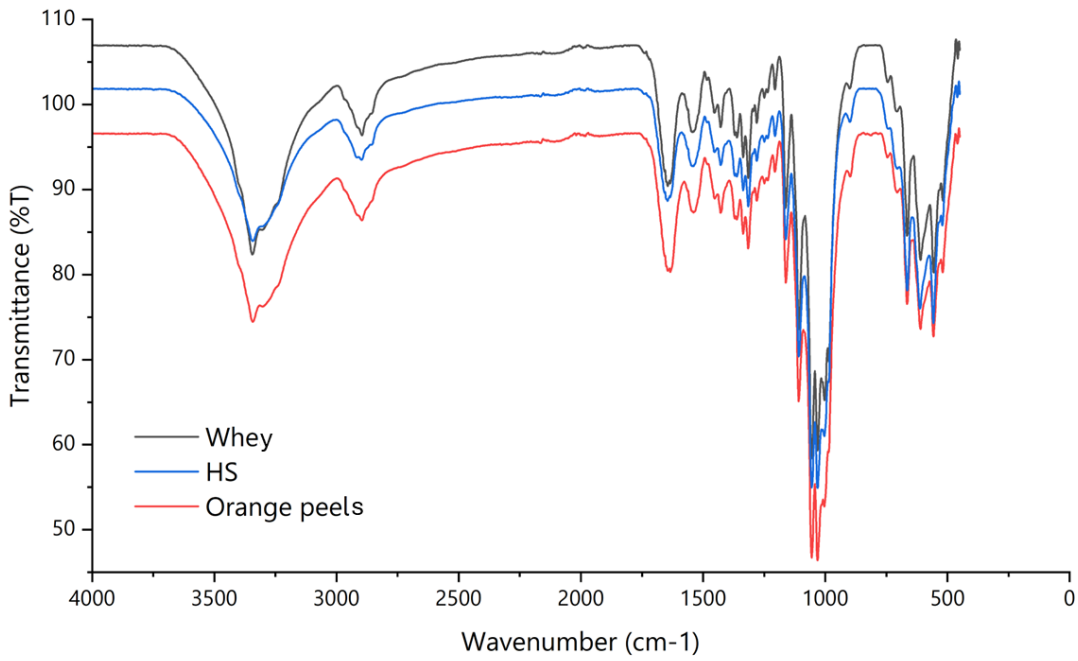


Figure 4.17: IR spectra of BNC membranes produced from HS, orange peels and whey.

The main peaks in each spectrum reveal characteristic molecular vibrations for each sample. The three spectra are similar and show different peaks, typical of cellulose, further confirming the successful production of BNC from all culture media. In particular, the region between 3200 and 3500 cm^{-1} corresponds to the O–H stretching vibrations, which are characteristic of cellulose molecules. The peak around 2900 cm^{-1} is attributed to the C–H stretching vibrations, commonly found in cellulose and other organic compounds. Its presence across all spectra suggests a consistent organic backbone in all films. The peak around 1640 cm^{-1} typically indicates the presence of C=O stretching vibrations, which might result from residual organic compounds from the substrates. The orange peel and the whey samples show a slightly more intense peak here, likely due to additional organic compounds inherent to the substrates. In some cases, this peak could also be related to O–H bending, associated with the hydroxyl groups in cellulose [95]. The minor peak around 1540 cm^{-1} is attributed to the O–H bending, as well. Finally, the region around 1000–1200 cm^{-1} is the fingerprint region of cellulose, with peaks associated with C–O stretching and C–H bending vibrations. This region shows similar patterns across all spectra, consistent with the cellulose structure in each film.

In summary, all three spectra exhibit common features, indicating that the bacterial nanocellulose produced is consistent across different substrates (HS, orange peel and whey). This suggests that the formation of nanocellulose remains the same, regardless of the substrate used.

4.4. Life cycle assessment of nanocellulose production: inventory, results and interpretation

4.4.1. Life cycle inventory

As already discussed, inventory data are primary data coming from own laboratory experience. The quantification of inputs, effluents, and yield for orange peels, whey and HS routes was performed through the experiments already discussed.

To develop the inventory for BNC production, the optimal culture conditions previously found in this research study that maximised BNC yield were considered for each of the three production routes.

Regarding BNC production from orange peels, the optimal production yield was found as 3.99 ± 0.45 g/L in 7 days, from the culture medium T_{amb}+additives, meaning that nutrients extraction from orange peels was conducted at ambient temperature and that all additives were added to the medium (yeast extract, peptone, citric acid and disodium phosphate).

About BNC production from whey, the best production was found from the medium whey (hydrolysed)+additives and was 2.42 ± 0.47 g/L in 7 days. To reach this yield, whey was indeed hydrolysed with lactase and, even in this case, all additives were added (yeast extract, peptone, citric acid and disodium phosphate).

Concerning HS, the observed nanocellulose production was 1.49 ± 0.40 g/L in 7 incubation days.

Once defined the conditions to analyse in the LCA study, insights regarding the different creation processes for the three-culture media are outlined in Figure 4.18.

Apart from the creation of the culture media, all other steps in the production routes, shown in the flux diagram in section 3.6, are identical for the three cases. Naturally, because of variations in observed yields, energy consumption and material requirements in the other steps will differ between the orange peel, whey, and HS scenarios.

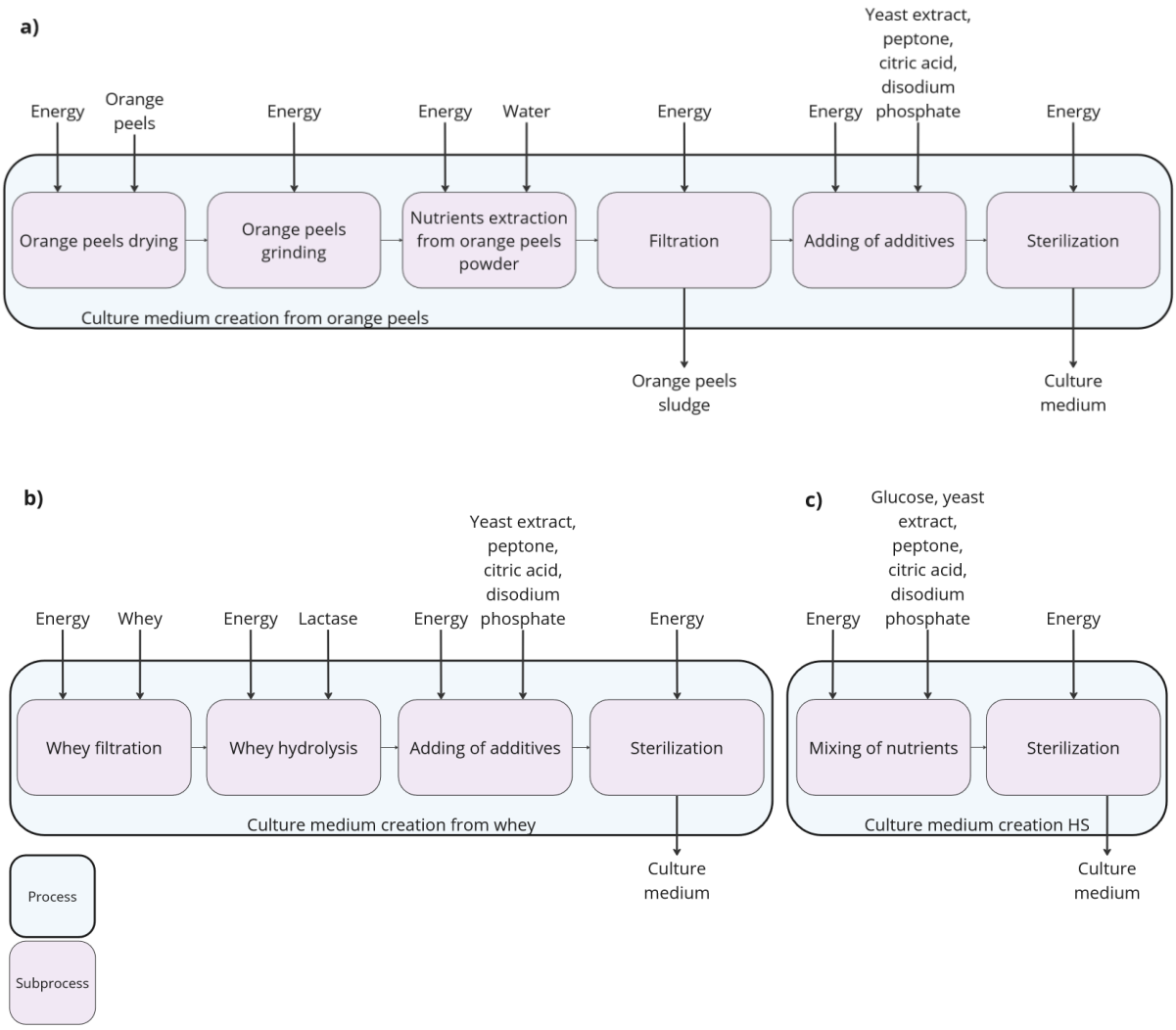


Figure 4.18: Culture media creation processes from orange peels, whey and HS.

Since two of the three routes foresee the use of waste residues as material source (orange peels and whey), it should be discussed how to allocate their impacts. Allocation in the context of LCA refers to the process of distributing the environmental impacts among different products or processes that share the same inputs or phases of production. When a process yields co-products, allocation assigns a proportion of the total environmental impact to each product. In these cases, orange peels and whey are respectively co-products of oranges or orange juice production process and cheese production process. According to ISO 14040 [83] and 14044[87] standards, allocation should be avoided whenever possible. In this case, since using waste materials as raw materials for producing BNC, there are two possibilities: consider the waste materials as zero-impact inputs because not allocating the primary production impacts to them or include only the impacts associated with the transportation and preliminary treatment of the waste materials. In this study, the first approach has been employed,

since orange peels and whey transportation and preliminary treatments are negligible in the context of the laboratory process. Moreover, this approach aligns with ISO guidelines, which recommend minimizing allocation and, when necessary, basing it on clear and justifiable criteria.

Energy requirements in each step were estimated basing on the equipment employed at laboratory scale. Specifically, energy requirements were calculated by considering the power required by the equipment, the duration of its use, and adjustments for the equipment's capacity relative to the functional unit under examination.

To give an example, the energy consumption of the incubator INCR-150-001 was calculated as follows, in Equation (4.2).

$$E = P * t * \left(\frac{C_{used}}{C_{max}}\right) \tag{4.2}$$

where P is the power required by the equipment, that is 1 kW, t is the amount of time that the equipment was used and $\frac{C_{used}}{C_{max}}$ the relation between the capacity used and the maximum load capacity of the equipment. The maximum load of the incubator typically refers to its internal volume, that is 72.625 dm^3; however, the actual usable capacity was here assumed as a percentage of this internal volume (70%), due to the need for proper airflow, heat distribution, and spacing between items. The actual used capacity was instead calculated as the culture medium volume needed to produce the selected functional unit, considering the observed yield.

Filtration steps and cutting of nanocellulose pellets for bacteria recovery are not done with equipment at laboratory scale, so they do not need any energy requirements to consider.

The inventories of BNC production at the laboratory scale starting from orange peels, whey and HS media can be found in the Appendix A, along with the datasets used in SimaPro and the new created datasets to perform the LCA.

4.4.2. Life cycle impact assessment

The selected impact categories have been chosen as the ones recommended by the EU environmental footprint (EF) 3.1, a life cycle assessment-based method which aims at assessing the environmental impacts of systems through 16 impact categories, divided in 3 main groups: environment, human health and resource consumption.

The environmental impact categories are climate change, ozone layer depletion, photochemical ozone formation, acidification, freshwater eutrophication, marine eutrophication, terrestrial eutrophication and fresh water ecotoxicity. The human health categories are ionizing radiation, respiratory inorganics, non-cancer human health effects and cancer human health effects. Lastly, the categories regarding the

resource consumption are land use, water use, fossil energy carriers resource use and minerals and metals resource use.

First, it could be interesting to just analyse impacts of 1 L culture media preparation in the three cases. Figure 4.19 reports results of impact assessment to produce 1 L of each culture media, normalised with respect to the highest one among the three. The preparation of 1 L of culture medium from orange has the highest impact among the three, followed by the preparation of 1 L of culture medium from whey and in the end, with the lowest environmental impact, the preparation of 1 L of HS culture medium. Each investigated impact category follows this trend.

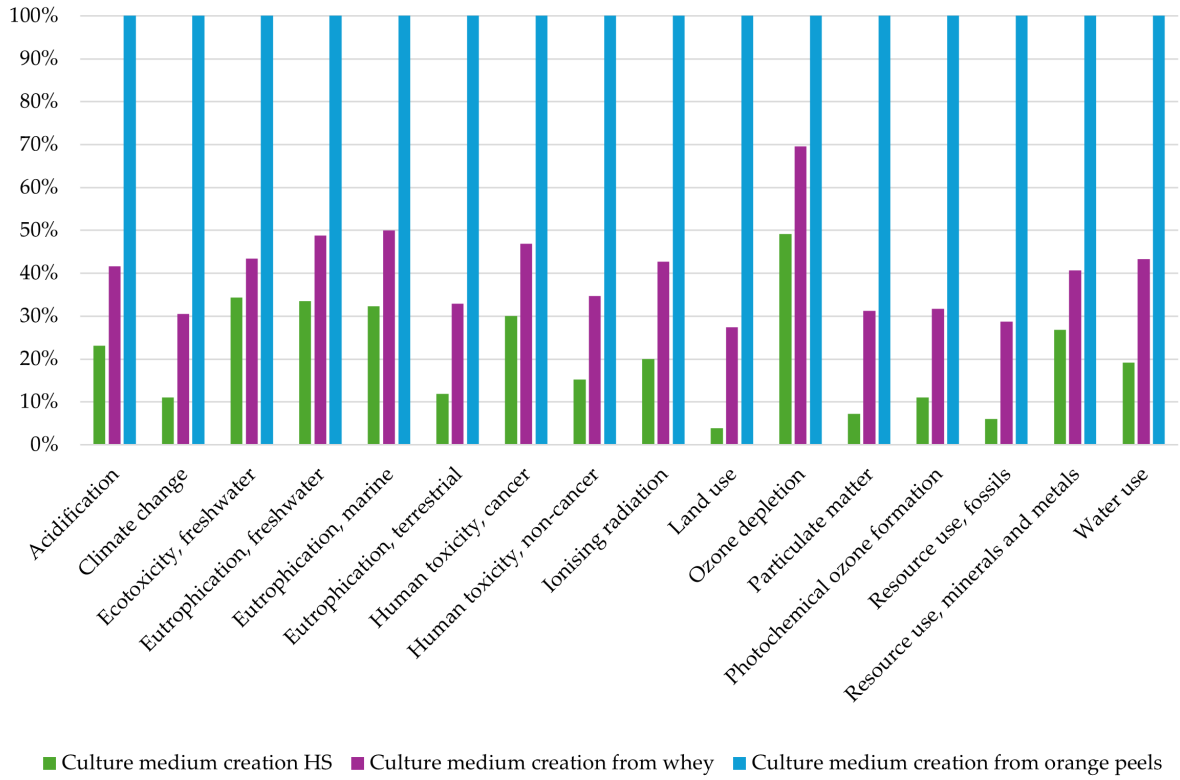


Figure 4.19: Environmental impacts of culture medium creation from orange peels, whey and HS.

Of course, HS culture medium creation in laboratory does not require significant energy consumption, apart from mixing and sterilizing and regarding material consumptions the inventory shows just HS components, that are in order of a few grams per litre, and distilled water.

Whey requires some additional steps, like hydrolyzation for 2 hours at 40°C, which needs energy and the enzyme; the following steps are the same as HS, apart from

glucose that is not added. Filtration, as already discussed, at laboratory scale does not need energy since it is not done with equipment.

Orange peels pre-treatment, instead, involves more steps, that are drying of orange peels, grinding, extraction with water for 15 minutes at ambient temperature and the same adding of additives than for the whey scenario. Likewise, filtration to remove the orange peels sludge after extraction of nutrients is not considered in the energy requirements.

However, it should be noticed that glucose for HS medium creation is supplied in the laboratory as a powder, so glucose production is assumed in this study at industrial scale, where it is probably dried, while orange peels medium creation and whey medium creation are entirely assumed at laboratory scale. As already mentioned, industrial processes typically have better efficiencies than laboratory scale processes, bringing to lower impacts, both environmentally and economically.

To better understand which step is the most impacting among the aforementioned ones, the culture medium creation from orange peels impact assessment is reported in Figure 4.20. It is noticeable that the most impacting step regarding culture medium preparation from orange peels is the drying of orange peels. Indeed, orange peels are put in the dehydrator for 12 hours, resulting in 4.2 kWh needed to dry them to create 1 L of medium. This step is the one making impacts of the culture medium significantly higher than other culture media. The orange peels sludge waste, as observable, covers a not negligible portion of total impacts. According to the selected boundary system regarding BNC production, it is considered a waste; therefore, its impacts are treated as waste. However, the possibility to treat it as a by-product should be considered; indeed, if proved with further investigation that there is a utility or economic value associated with the orange peels sludge that justifies its treatment as a by-product, allocation of environmental burdens between the main product (BNC) and the by-product (orange peels sludge) should be performed.

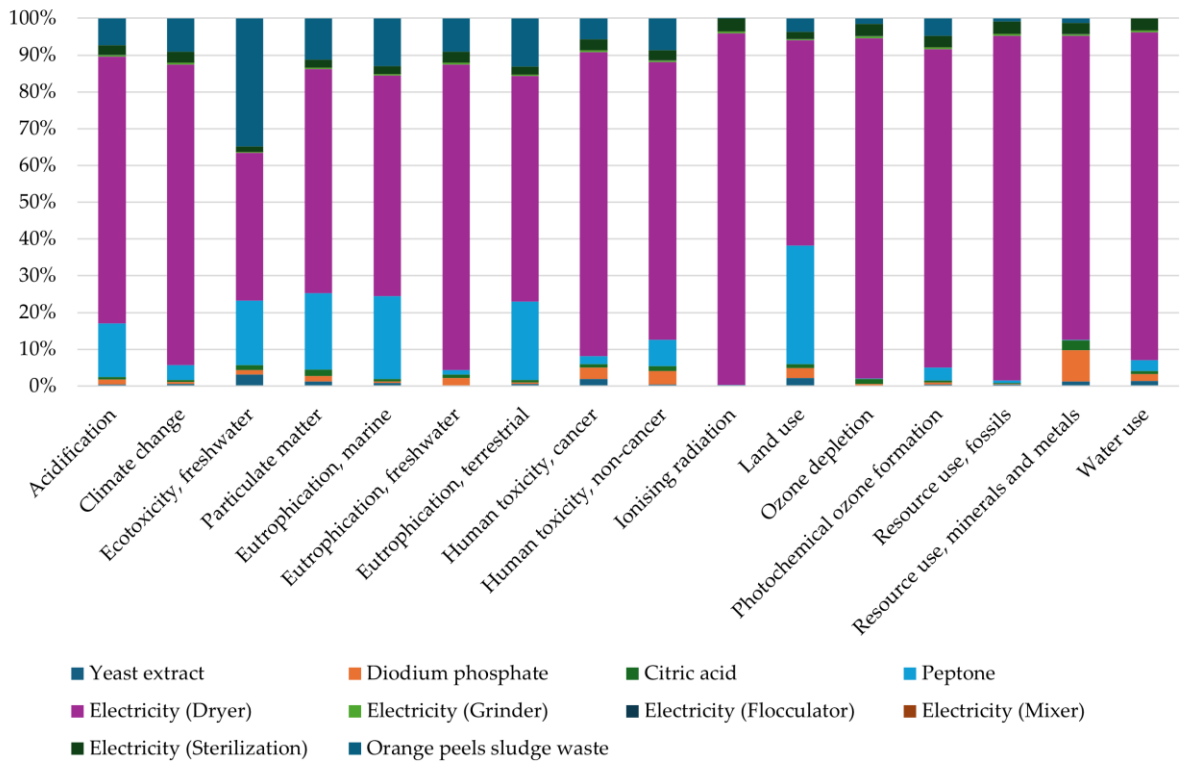


Figure 4.20: Contribution analysis for culture medium creation from orange peels.

The impact assessment comparison results for 1 g BNC production in 7 days of incubation from the three different culture media are reported in Figure 4.21. As noticeable, for each category, the impacts related to 1 g BNC production in 7 days from HS culture medium are higher than the impacts related to 1 g BNC production in 7 days from wastes. This is due to the lower yield found from HS medium, that makes energy and material resources needed to produce the same amount of BNC higher than from media created with wastes. Among wastes, BNC production from orange peels is the least impacting one. Despite the initial resource-intensive process of creating the fermentation medium from orange peels, the high yield of BNC production compensates for this, resulting in a more efficient use of energy and materials. Consequently, the environmental impacts associated with BNC production from orange peels are minimized, making it the least impactful option among the ones studied.

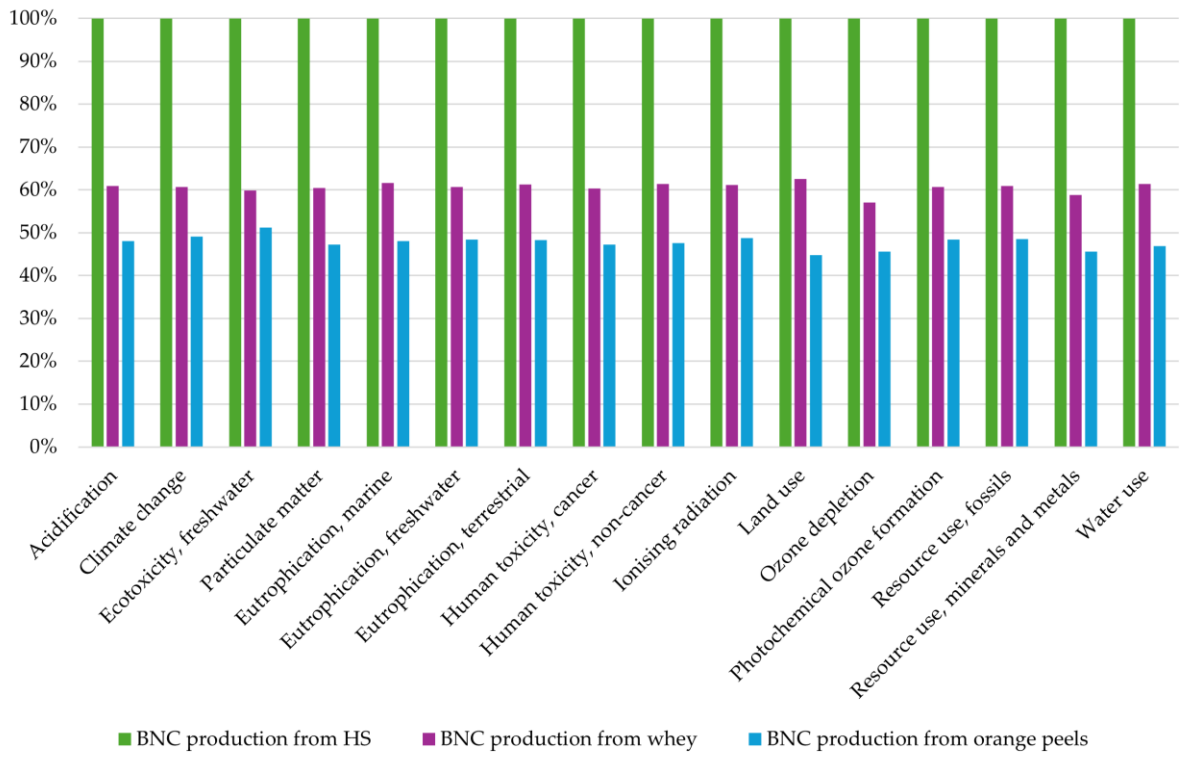


Figure 4.21: Environmental impacts for BNC production from HS, whey and orange peels.

Table 4.14 resumes environmental impacts of 1 g BNC production in 7 incubation days at laboratory scale from HS, whey and orange peels. As anticipated, BNC from orange peels consistently has the lowest environmental impact across all categories. For example, in terms of acidification, orange peels show a value of 0.0062 mol H+ eq, while HS has the highest at 0.0129 mol H+ eq.

In the category of climate change, orange peels contribute 1.16 kg of CO_2 equivalents, much lower than HS production, which emits 2.36 kg of CO_2 equivalents. This trend is reflected in other indicators like freshwater ecotoxicity (4.54 CTUe for orange peels versus 8.88 CTUe for HS) and resource use, where orange peels consume 30.2 MJ of fossil energy compared to HS's 62.1 MJ.

Orange peels also outperform other sources in water use, requiring 0.797 m^3 compared to HS's 1.70 m^3. In categories like eutrophication and human toxicity, orange peels consistently show lower impacts than whey and HS, making them the more environmentally friendly choice for BNC production across various sustainability measures.

Table 4.14: Environmental impacts for 1 g BNC production from HS, whey and orange peels in 7 days.

Damage category	BNC production from whey	BNC production from orange peels	BNC production from HS	Unit
Acidification	0.0078	0.0062	0.0129	mol H+ eq
Climate change	1.43	1.16	2.36	kg CO_2 eq
Ecotoxicity, freshwater	5.31	4.54	8.88	CTUe
Eutrophication, marine	0.0016	0.0013	0.0026	kg N eq
Eutrophication, freshwater	0.00029	0.00023	0.00048	kg P eq
Eutrophication, terrestrial	0.016	0.013	0.027	mol N eq
Human toxicity, cancer	5.94E-10	4.65E-10	9.85E-10	CTUh
Human toxicity, non-cancer	1.22E-08	9.43E-09	1.98E-08	CTUh
Land use	5.62	4.03	9.00	Pt
Resource use, fossils	37.9	30.2	62.1	MJ
Water use	1.04	0.797	1.70	m^3

The contribution analyses of BNC production from each of the three media are shown in the Appendix A, in Figure A.1, Figure A.2 and Figure A.3.

4.4.3. Sensitivity analyses and results interpretation

Energy requirements were calculated using the power indicated on the equipment. The power listed on an equipment's label generally refers to the maximum power the equipment can consume when operating at full capacity. However, this doesn't mean

the equipment always uses that amount of power. In practice, many devices consume varying amounts of energy depending on their usage and operating conditions. Many devices can operate at different intensities or settings, which directly affects their power consumption. Regarding mixers and the water distillator, they surely operate at maximum power when in use, since they operate in only an on or off state without variable power settings, so that their power consumption will be either at the maximum rated power or close to zero when off. Likewise, centrifuges and agitators are always used at maximum power, since the rotational speed is set to the maximum possible when in use. Concerning all the other equipment, they are not always employed exploiting the maximum power; therefore, a sensitivity analysis is performed on energy requirements. Specifically, different alternative scenarios are investigated, in which the energy requirement of all equipment, apart from the distillator, mixers, centrifuges and agitators are decreased from the maximum. The scenarios investigated are: -10%, -20% and -40% of energy requirements for the equipment. The results in terms of comparison among the three different BNC production methods are reported as follows (Figure 4.22).

It is possible to note that the general trend does not differ from the baseline scenario by decreasing the energy requirements. In each scenario, BNC production from HS shows higher impacts across all the categories compared to the other two methods. Certainly, the reduction in energy requirements affects all production methods, overall reducing the environmental impacts. However, BNC production from HS remains the least efficient in terms of environmental impact across all scenarios of energy reduction. Particularly, as the assumed energy requirements decrease, the environmental impacts associated with the production routes using whey and orange peels diminish more significantly than those associated with the HS route. This trend is particularly evident in the scenario with the greatest reduction in energy requirements, where the impacts of the whey and orange peel routes constitute a smaller fraction of the impacts associated with the HS route compared to the other scenarios. This observation can be attributed to the energy-requiring pretreatments needed to create culture media from waste, such as orange peels and whey, which are not necessary for HS. Consequently, overestimating the energy requirements for equipment leads to an overestimation of the environmental impacts of the orange peels and whey routes more than of the HS route. Therefore, even in the baseline scenario, which represents the worst-case overestimation of energy requirements, the results demonstrating the relative environmental benefits of using orange peels and whey are conservative. In the Appendix A, Figure A.4, Figure A.5 and Figure A.6 show how reductions in energy requirements affect 1 g BNC production in 7 days for each production route, confirming that energy requirements affect BNC production from the two residues more than from HS.

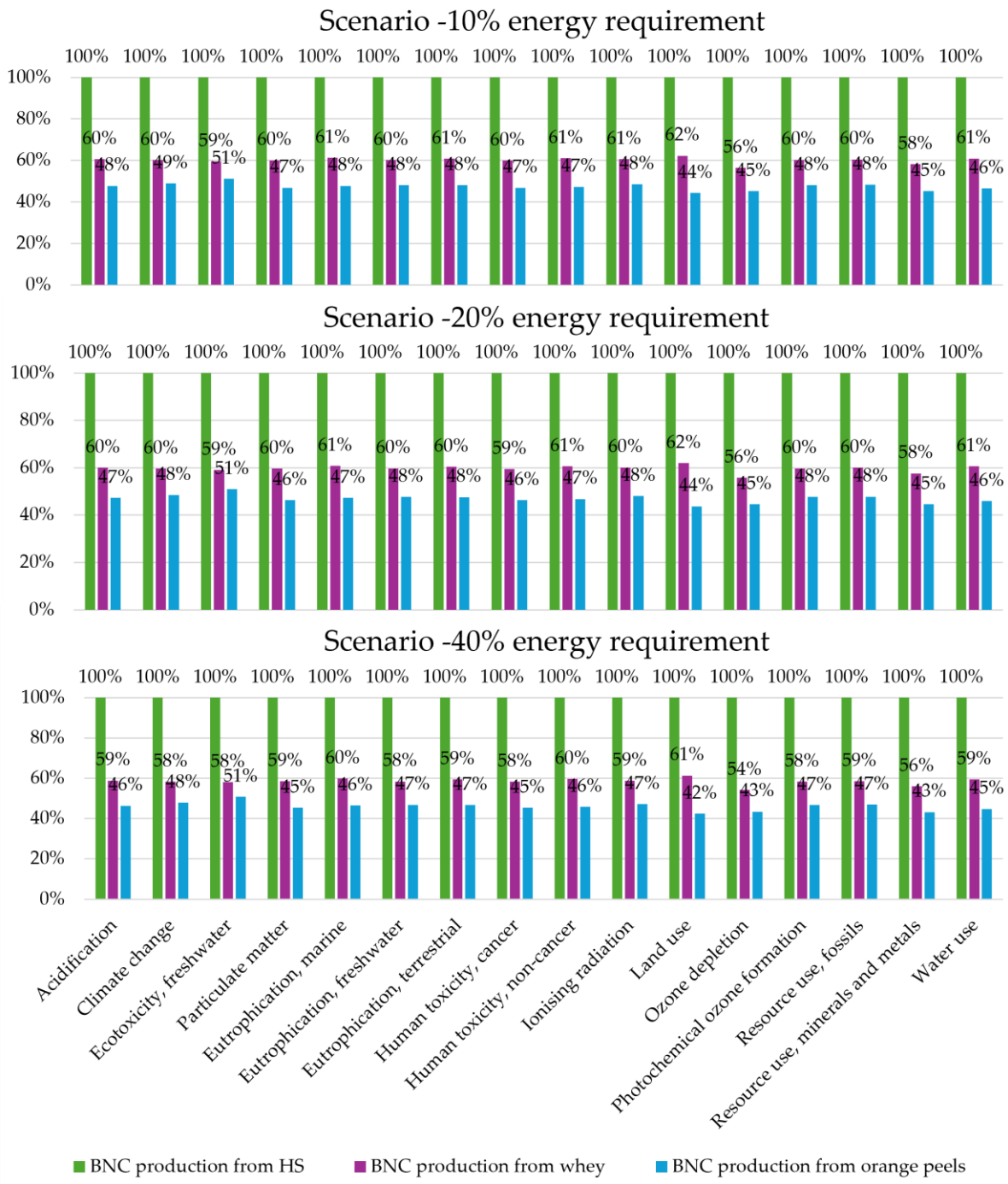


Figure 4.22: Sensitivity analyses for BNC production from HS, whey and orange peels, regarding energy requirements.

The LCA of BNC production at the lab scale provides valuable insights into the environmental impacts associated with different production methods. However, there are several limitations inherent in conducting an LCA at this scale that must be considered. Firstly, lab-scale processes often do not directly translate to industrial-scale production. The efficiencies, yields, and environmental impacts observed in the

lab may differ significantly when the process is scaled up due to factors such as process optimisation, equipment differences, and economies of scale. Resource and energy use at the lab scale are often higher per unit of product due to the use of smaller, less efficient equipment. This can result in an overestimation of environmental impacts compared to a more optimised industrial process. Additionally, the amount and type of waste generated in a lab setting may differ from industrial production. Laboratory processes may produce unique waste streams that are not representative of those in a larger scale operation, impacting the accuracy of the LCA results. For instance, in this case, membranes neutralization is done with water that is treated as a waste stream; however, at industrial scale probably a water purification and recirculation would be introduced to allow water to be recycled inside the process without generating waste. Lab-scale studies, moreover, often rely on experimental data, which can have a higher degree of variability and uncertainty. Lastly, industrial processes often undergo significant optimisation to reduce costs and environmental impacts, a step that is typically not fully realized in lab-scale studies. Extrapolating lab-scale results to predict industrial-scale impacts involves assumptions that may not hold true in a larger context. While lab-scale LCA studies are crucial for understanding the preliminary environmental impacts of new production methods, it is essential to acknowledge their limitations. The results should be interpreted with caution, and further studies at pilot or industrial scales are necessary to obtain more accurate and representative data. This will ensure a more reliable assessment of the environmental performance of BNC production methods as they move from the lab to commercial implementation.

5 Conclusion

The primary objective of this thesis was to address the significant issue of food waste by promoting a circular economy model, specifically through the production of bacterial nanocellulose (BNC) from food wastes such as orange peels and whey. This approach aimed to transform waste into a resource, mitigating environmental impacts and promoting sustainable resource utilization. The research focused on extracting valuable nutrients from food waste, optimising BNC production processes, evaluating the quality of the produced nanocellulose, and assessing the environmental impacts through a Life Cycle Assessment (LCA).

The study successfully demonstrated the feasibility of converting organic waste into valuable bacterial nanocellulose (BNC). Characterization of the culture media revealed that whey and the medium created from orange peel extraction are richer in reducing sugars compared to the HS medium, with whey showing the highest values, particularly when hydrolysed. In terms of nitrogen content, media derived from orange peels without added nitrogen sources had lower nitrogen levels than the HS medium, whereas whey naturally contained nitrogen. However, adding nitrogen sources like yeast extract or peptone increased nitrogen concentration, which is beneficial for cell growth, in both orange peel and whey media. Phosphate concentrations in media from food waste were consistently higher than in the HS medium, although they did not significantly influence BNC production.

The optimal media and conditions for BNC production were identified for both orange peels and whey. For orange peels, the highest BNC production of 3.99 ± 0.45 g/L at 7 days was achieved with ambient temperature extraction and the addition of yeast extract, peptone, citric acid, and disodium phosphate. For whey, hydrolysed whey with the same additives yielded 2.42 ± 0.47 g/L at 7 days and 4.28 ± 0.36 g/L at 10 days. These results highlight the effectiveness of using waste materials as substrates for BNC production and demonstrate higher efficiency compared to the HS medium in terms of yield.

In terms of membrane quality, the study analysed water retention. For orange peels, membranes produced from media without any additives showed water retention comparable to or higher than that of membranes from the HS medium. However, when additives were added, water retention decreased, even if the yield was notably higher. This reduction in water retention with additives might be due to the interaction of these compounds with the cellulose fibres, potentially leading to a less dense and less intertwined network. For whey, BNC produced from hydrolysed whey with only citric acid and disodium phosphate, as well as with the addition of yeast extract and peptone, showed high water retention, comparable to membranes formed from the HS

medium. Conversely, no membranes formed in media without any additives, indicating the essential role of these compounds in BNC production from whey. Therefore, no information can be drawn about whether the presence of additives decreases, increases or does not have effects on water retention of membranes from whey. However, membranes produced from hydrolysed whey with a double concentration of yeast extract, but no peptone exhibited slightly lower water retention. It is important to note that all tests with yeast extract and peptone were conducted using the proportions from the HS medium, which is 0.5% w/v for each one. This proportion likely represents an optimal combination for both yield and water retention. Altering the composition of a medium is challenging, as it requires careful balancing of nutrient concentrations to achieve the desired outcomes. The slightly lower water retention observed with the double concentration of yeast extract, but no peptone suggests that the balanced supply of nutrients provided by the standard 0.5% w/v of both yeast extract and peptone is crucial for maintaining a dense and efficient cellulose network, enhancing both yield and water retention. This demonstrates the importance of optimising the nutrient composition and conditions for BNC production to achieve not only the maximum yield but also the desired membrane quality.

The LCA results indicated that BNC production from waste residues had lower environmental impacts compared to the common medium HS. Among the waste substrates, BNC production from orange peels had the least environmental impact despite the initial resource-intensive process. This finding underscores the potential of using waste materials not only for their economic benefits but also for their environmental advantages. The environmental impact analysis for producing 1 gram of BNC in 7 days showed that BNC production from orange peels had significantly lower impacts in categories such as climate change (1.16 kg CO_2 eq) and resource use (30.2 MJ) compared to HS medium (2.36 kg CO_2 eq and 62.1 MJ, respectively).

The sensitivity analysis confirmed that the trend of lower environmental impacts for waste-derived media remained consistent even with variations in energy requirements. This robustness suggests that the use of waste substrates for BNC production is not only feasible but also environmentally beneficial. Even with reductions in energy requirements, the overall impact trends did not shift, indicating the reliability of the results.

In conclusion, this study demonstrated the practicality and environmental benefits of repurposing food waste for BNC production. By providing a detailed analysis of the processes and outcomes, this research supports the transition towards a more sustainable food system and circular economy. The findings highlight the potential for industries to adopt these methods, contributing to global efforts to achieve environmental sustainability. Future research should focus on scaling up these processes and further refining the methodologies to enhance the efficiency and applicability of BNC production from food waste residues. This will help in achieving

broader adoption and demonstrating the practical benefits of waste valorisation in industrial applications.

Bibliography

[1] A. Pawełczyk, "EU POLICY AND LEGISLATION ON RECYCLING OF ORGANIC WASTES TO AGRICULTURE," 2005.

[2] G. Gozgor, C. K. M. Lau, and Z. Lu, "Energy consumption and economic growth: New evidence from the OECD countries," *Energy*, vol. 153, pp. 27–34, Jun. 2018, doi: 10.1016/j.energy.2018.03.158.

[3] M. Song, R. Fisher, and Y. Kwoh, "Technological challenges of green innovation and sustainable resource management with large scale data," *Technol Forecast Soc Change*, vol. 144, pp. 361–368, Jul. 2019, doi: 10.1016/j.techfore.2018.07.055.

[4] J. Korhonen, C. Nuur, A. Feldmann, and S. E. Birkie, "Circular economy as an essentially contested concept," *J Clean Prod*, vol. 175, pp. 544–552, Feb. 2018, doi: 10.1016/j.jclepro.2017.12.111.

[5] COM (European Commission), "Closing the loop - An EU action plan for the Circular Economy," 2015.

[6] Ellen MacArthur Foundation, "Completing the picture: How the circular economy tackles climate change," 2019.

[7] D. Chilabade, G. Mwengula, and J. Mulopo, "Review of Opportunities and Challenges for Food Waste Circular Economy Advancement via Hydrothermal Carbonization," *Process Safety and Environmental Protection*, Mar. 2024, doi: 10.1016/j.psep.2024.03.029.

[8] FAO (Food and Agriculture Organization of the United Nations), *Global food losses and food waste : extent, causes and prevention*. 2011.

[9] C. Phonthanukitithaworn, N. Maitree, and P. Naruetharadhol, "Eco-innovation policies for food waste management: A European Union-ASEAN comparison," *Journal of Open Innovation: Technology, Market, and Complexity*, vol. 10, no. 2, Jun. 2024, doi: 10.1016/j.joitmc.2024.100295.

[10] Å. Stenmarck, C. M. Jensen, and T. Quested, "Estimates of European food waste levels RISE, UCSF Center for Vulnerable Populations," 2016, doi: 10.13140/RG.2.1.4658.4721.

[11] S. Scherhaufer, G. Moates, H. Hartikainen, K. Waldron, and G. Obersteiner, "Environmental impacts of food waste in Europe," *Waste Management*, vol. 77, pp. 98–113, Jul. 2018, doi: 10.1016/j.wasman.2018.04.038.

[12] P. Wongsirichot *et al.*, "Food wastes for bioproduct production and potential strategies for high feedstock variability," *Waste Management*, vol. 184, pp. 1–9, Jul. 2024, doi: 10.1016/j.wasman.2024.05.027.

[13] D. A. Teigiserova, L. Hamelin, and M. Thomsen, "Review of high-value food waste and food residues biorefineries with focus on unavoidable wastes from processing," *Resour Conserv Recycl*, vol. 149, pp. 413–426, Oct. 2019, doi: 10.1016/j.resconrec.2019.05.003.

[14] D. M. Rodrigues *et al.*, "A green approach to biomass residue valorization: Bacterial nanocellulose production from agro-industrial waste," Feb. 01, 2024, *Elsevier Ltd*. doi: 10.1016/j.bcab.2024.103036.

[15] B. Jayanthi, S. Vinoth, M. Hariharan, R. K. Raja, C. Kamaraj, and M. Narayanan, "Valorization of agro-industry wastes for nanocellulose fabrication and its multifunctional applications," *Biocatal Agric Biotechnol*, vol. 57, Apr. 2024, doi: 10.1016/j.bcab.2024.103124.

[16] P. K. Sarangi *et al.*, "Biotechnological innovations in nanocellulose production from waste biomass with a focus on pineapple waste," *Chemosphere*, vol. 349, Feb. 2024, doi: 10.1016/j.chemosphere.2023.140833.

[17] R. Portela, C. R. Leal, P. L. Almeida, and R. G. Sobral, "Bacterial cellulose: a versatile biopolymer for wound dressing applications," Jul. 01, 2019, *John Wiley and Sons Ltd*. doi: 10.1111/1751-7915.13392.

[18] Y. Wei *et al.*, "The complexities of decision-making in food waste valorization: A critical review," May 01, 2024, *Academic Press*. doi: 10.1016/j.jenvman.2024.120989.

[19] D. A. Teigiserova, L. Hamelin, and M. Thomsen, "Towards transparent valorization of food surplus, waste and loss: Clarifying definitions, food waste hierarchy, and role in the circular economy," *Science of the Total Environment*, vol. 706, Mar. 2020, doi: 10.1016/j.scitotenv.2019.136033.

[20] G. Garcia-Garcia, E. Woolley, S. Rahimifard, J. Colwill, R. White, and L. Needham, "A Methodology for Sustainable Management of Food Waste," *Waste Biomass Valorization*, vol. 8, no. 6, pp. 2209–2227, Sep. 2017, doi: 10.1007/s12649-016-9720-0.

[21] D. Granato, D. S. Nunes, and F. J. Barba, "An integrated strategy between food chemistry, biology, nutrition, pharmacology, and statistics in the development of functional foods: A proposal," Apr. 01, 2017, *Elsevier Ltd*. doi: 10.1016/j.tifs.2016.12.010.

[22] F. J. Barba, Z. Zhu, M. Koubaa, A. S. Sant'Ana, and V. Orlien, "Green alternative methods for the extraction of antioxidant bioactive compounds from winery wastes and by-products: A review," Mar. 01, 2016, *Elsevier Ltd*. doi: 10.1016/j.tifs.2016.01.006.

[23] P. R. More, A. R. Jambrak, and S. S. Arya, "Green, environment-friendly and sustainable techniques for extraction of food bioactive compounds and waste valorization," Oct. 01, 2022, *Elsevier Ltd*. doi: 10.1016/j.tifs.2022.08.016.

[24] S. Khalid *et al.*, "Recovery of valuable substances from food waste by ohmic heating assisted extraction -A step towards sustainable production," *Future Foods*, vol. 9, p. 100365, Jun. 2024, doi: 10.1016/j.fufo.2024.100365.

[25] R. Rinaldi and F. Schüth, "Design of solid catalysts for the conversion of biomass," 2009. doi: 10.1039/b902668a.

[26] D. Klemm, B. Heublein, H. P. Fink, and A. Bohn, "Cellulose: Fascinating biopolymer and sustainable raw material," May 30, 2005. doi: 10.1002/anie.200460587.

[27] M. Rose and R. Palkovits, "Cellulose-based sustainable polymers: State of the art and future trends," *Macromol Rapid Commun*, vol. 32, no. 17, pp. 1299–1311, Sep. 2011, doi: 10.1002/marc.201100230.

[28] J. D. P. de Amorim *et al.*, "Plant and bacterial nanocellulose: production, properties and applications in medicine, food, cosmetics, electronics and engineering. A review," May 01, 2020, *Springer*. doi: 10.1007/s10311-020-00989-9.

[29] C. Ververis, K. Georghiou, N. Christodoulakis, P. Santas, and R. Santas, "Fiber dimensions, lignin and cellulose content of various plant materials and their suitability for paper production," *Ind Crops Prod*, vol. 19, no. 3, pp. 245–254, May 2004, doi: 10.1016/j.indcrop.2003.10.006.

[30] K. Gelin, A. Bodin, P. Gatenholm, A. Mihranyan, K. Edwards, and M. Strømme, "Characterization of water in bacterial cellulose using dielectric spectroscopy and electron microscopy," *Polymer (Guildf)*, vol. 48, no. 26, pp. 7623–7631, Dec. 2007, doi: 10.1016/j.polymer.2007.10.039.

[31] M. Mariano, N. El Kissi, and A. Dufresne, "Cellulose nanocrystals and related nanocomposites: Review of some properties and challenges," Jun. 15, 2014, *John Wiley and Sons Inc.* doi: 10.1002/polb.23490.

[32] H. M. C. Azeredo, H. Barud, C. S. Farinas, V. M. Vasconcellos, and A. M. Claro, "Bacterial Cellulose as a Raw Material for Food and Food Packaging Applications," Feb. 18, 2019, *Frontiers Media S.A.* doi: 10.3389/fsufs.2019.00007.

[33] P. R. Chawla, I. B. Bajaj, S. A. Survase, and R. S. Singhal, "Microbial Cellulose: Fermentative Production and Applications," 2009.

[34] M. Moosavi-Nasab and A. Yousefi, "Biotechnological production of cellulose by Gluconacetobacter xylinus from agricultural waste," 2011.

[35] W. Jaroennonthasit, N. T. Lam, and P. Sukyai, "Evaluation of carbon sources from sugar industry to bacterial nanocellulose produced by Komagataeibacter xylinus," *Int J Biol Macromol*, vol. 191, pp. 299–304, Nov. 2021, doi: 10.1016/j.ijbiomac.2021.09.028.

[36] M. K. Poddar and P. K. Dikshit, "Recent development in bacterial cellulose production and synthesis of cellulose based conductive polymer nanocomposites," *Nano Select*, vol. 2, no. 9, pp. 1605–1628, Sep. 2021, doi: 10.1002/nano.202100044.

[37] M. Matsuoka, T. Tsuchida, K. Matsushita, O. Adachi, and F. Yoshinaga, "A Synthetic Medium for Bacterial Cellulose Production by Acetobacter xylinum subsp. sucrofermentans," 1996. [Online]. Available: https://academic.oup.com/bbb/article/60/4/575/5949229

[38] S. Hestrin and M. Schramm, "Synthesis of Cellulose by Acetobacter xylinum," The Johns Hopkins Press. Sacktor, B, 1954.

[39] M. Schramm and S. Hestrin, "Factors affecting Production of Cellulose at the Air/ Liquid Interface of a Culture of Acetobacter xylinum," 1954.

[40] S. W. Williams and R. Cannon, "Alternative Environmental Roles for Cellulose Produced by Acetobacter xylinum," 1989. [Online]. Available: https://journals.asm.org/journal/aem

[41] D. Klemm *et al.*, "Nanocelluloses: A new family of nature-based materials," Jun. 06, 2011. doi: 10.1002/anie.201001273.

[42] I. Cielecka, M. Ryngajłło, W. Maniukiewicz, and S. Bielecki, "Highly stretchable bacterial cellulose produced by komagataeibacter hansenii si1," *Polymers (Basel)*, vol. 13, no. 24, Dec. 2021, doi: 10.3390/polym13244455.

[43] D. Lahiri *et al.*, "Bacterial cellulose: Production, characterization and application as antimicrobial agent," Dec. 01, 2021, *MDPI*. doi: 10.3390/ijms222312984.

[44] J. K. Muiruri, J. C. C. Yeo, Q. Zhu, E. Ye, X. J. Loh, and Z. Li, "Bacterial cellulose: Recent advances in biosynthesis, functionalization strategies and emerging applications," *Eur Polym J*, vol. 199, Nov. 2023, doi: 10.1016/j.eurpolymj.2023.112446.

[45] M. W. Ullah *et al.*, "Advanced biotechnological applications of bacterial nanocellulose-based biopolymer nanohybrids: A review," Jan. 01, 2024, *KeAi Communications Co.* doi: 10.1016/j.aiepr.2023.07.004.

[46] N. Lin and A. Dufresne, "Nanocellulose in biomedicine: Current status and future prospect," Oct. 01, 2014, *Elsevier Ltd*. doi: 10.1016/j.eurpolymj.2014.07.025.

[47] J. Fernández-Santos, C. Valls, O. Cusola, and M. B. Roncero, "Periodate oxidation of nanofibrillated cellulose films for active packaging applications," *Int J Biol Macromol*, vol. 267, May 2024, doi: 10.1016/j.ijbiomac.2024.131553.

[48] R. Reshmy *et al.*, "Bacterial nanocellulose: engineering, production, and applications," 2021, *Taylor and Francis Ltd.* doi: 10.1080/21655979.2021.2009753.

[49] R. M. Viana, N. M. S. M. Sá, M. O. Barros, M. de F. Borges, and H. M. C. Azeredo, "Nanofibrillated bacterial cellulose and pectin edible films added with fruit purees," *Carbohydr Polym*, vol. 196, pp. 27–32, Sep. 2018, doi: 10.1016/j.carbpol.2018.05.017.

[50] A. F. Jozala *et al.*, "Bacterial nanocellulose production and application: a 10-year overview," Mar. 01, 2016, *Springer Verlag*. doi: 10.1007/s00253-015-7243-4.

[51] X. Zhai, D. Lin, D. Liu, and X. Yang, "Emulsions stabilized by nanofibers from bacterial cellulose: New potential food-grade Pickering emulsions," *Food Research International*, vol. 103, pp. 12–20, Jan. 2018, doi: 10.1016/j.foodres.2017.10.030.

[52] S. M. F. Silva, H. L. Ribeiro, A. L. A. Mattos, M. de F. Borges, M. de F. Rosa, and H. M. C. de Azeredo, "Films from cashew byproducts: cashew gum and bacterial cellulose from cashew apple juice," *J Food Sci Technol*, vol. 58, no. 5, pp. 1979–1986, May 2021, doi: 10.1007/s13197-020-04709-7.

[53] Q. Ji, C. Zhou, Z. Li, I. D. Boateng, and X. Liu, "Is nanocellulose a good substitute for non-renewable raw materials? A comprehensive review of the state of the art, preparations,and industrial applications," Oct. 15, 2023, *Elsevier B.V.* doi: 10.1016/j.indcrop.2023.117093.

[54] R. M. B. Albuquerque *et al.*, "Production of a bacterial cellulose/poly(3-hydroxybutyrate) blend activated with clove essential oil for food packaging," *Polymers and Polymer Composites*, vol. 29, no. 4, pp. 259–270, May 2021, doi: 10.1177/0967391120912098.

[55] M. Henriksson, L. A. Berglund, P. Isaksson, T. Lindström, and T. Nishino, "Cellulose nanopaper structures of high toughness," *Biomacromolecules*, vol. 9, no. 6, pp. 1579–1585, Jun. 2008, doi: 10.1021/bm800038n.

[56] B. Thomas *et al.*, "Nanocellulose, a Versatile Green Platform: From Biosources to Materials and Their Applications," Dec. 26, 2018, *American Chemical Society*. doi: 10.1021/acs.chemrev.7b00627.

[57] S. S. Nair, J. Zhu, Y. Deng, and A. J. Ragauskas, "High performance green barriers based on nanocellulose," *Sustainable Chemical Processes*, vol. 2, no. 1, Dec. 2014, doi: 10.1186/s40508-014-0023-0.

[58] N. Lavoine, I. Desloges, A. Dufresne, and J. Bras, "Microfibrillated cellulose - Its barrier properties and applications in cellulosic materials: A review," Oct. 01, 2012. doi: 10.1016/j.carbpol.2012.05.026.

[59] H. P. S. Abdul Khalil, A. H. Bhat, and A. F. Ireana Yusra, "Green composites from sustainable cellulose nanofibrils: A review," Jan. 15, 2012. doi: 10.1016/j.carbpol.2011.08.078.

[60] M. Velásquez-Riaño and V. Bojacá, "Production of bacterial cellulose from alternative low-cost substrates," Jul. 01, 2017, *Springer Netherlands*. doi: 10.1007/s10570-017-1309-7.

[61] A. Kadier *et al.*, "polymers Use of Industrial Wastes as Sustainable Nutrient Sources for Bacterial Cellulose (BC) Production: Mechanism, Advances, and Future Perspectives," 2021, doi: 10.3390/polym.

[62] A. Jayanthi Antonisamy *et al.*, "Sustainable approaches on industrial food wastes to value-added products – A review on extraction methods, characterizations, and its biomedical applications," *Environ Res*, vol. 217, Jan. 2023, doi: 10.1016/j.envres.2022.114758.

[63] J. Banerjee, R. Singh, R. Vijayaraghavan, D. MacFarlane, A. F. Patti, and A. Arora, "Bioactives from fruit processing wastes: Green approaches to valuable chemicals," Jun. 15, 2017, *Elsevier Ltd*. doi: 10.1016/j.foodchem.2016.12.093.

[64] C. Castro, R. Zuluaga, J. L. Putaux, G. Caro, I. Mondragon, and P. Gañán, "Structural characterization of bacterial cellulose produced by Gluconacetobacter swingsii sp. from Colombian agroindustrial wastes," *Carbohydr Polym*, vol. 84, no. 1, pp. 96–102, Feb. 2011, doi: 10.1016/j.carbpol.2010.10.072.

[65] C. H. Kuo, C. Y. Huang, C. J. Shieh, H. M. D. Wang, and C. Y. Tseng, "Hydrolysis of Orange Peel with Cellulase and Pectinase to Produce Bacterial Cellulose using Gluconacetobacter xylinus," *Waste Biomass Valorization*, vol. 10, no. 1, pp. 85–93, Jan. 2019, doi: 10.1007/s12649-017-0034-7.

[66] M. Güzel and Ö. Akpınar, "Production and Characterization of Bacterial Cellulose from Citrus Peels," *Waste Biomass Valorization*, pp. 2165–2175, Aug. 2019, doi: 10.1007/s12649-018-0241-x.

[67] M. U. Rani and K. A. A. Appaiah, "Production of bacterial cellulose by Gluconacetobacter hansenii UAC09 using coffee cherry husk," *J Food Sci Technol*, vol. 50, no. 4, pp. 755–762, Aug. 2013, doi: 10.1007/s13197-011-0401-5.

[68] H. Khan, V. Saroha, S. Raghuvanshi, A. K. Bharti, and D. Dutt, "Valorization of fruit processing waste to produce high value-added bacterial nanocellulose by a novel strain Komagataeibacter xylinus IITR DKH20," *Carbohydr Polym*, vol. 260, May 2021, doi: 10.1016/j.carbpol.2021.117807.

[69] I. K. Lappa *et al.*, "Cheese whey processing: Integrated biorefinery concepts and emerging food applications," 2019, *MDPI*. doi: 10.3390/foods8080347.

[70] D. Fernández-Gutiérrez, M. Veillette, A. Giroir-Fendler, A. A. Ramirez, N. Faucheux, and M. Heitz, "Biovalorization of saccharides derived from industrial wastes such as whey: a review," Mar. 01, 2017, *Springer Netherlands*. doi: 10.1007/s11157-016-9417-7.

[71] F. Carvalho, A. R. Prazeres, and J. Rivas, "Cheese whey wastewater: Characterization and treatment," Feb. 05, 2013. doi: 10.1016/j.scitotenv.2012.12.038.

[72] A. R. Prazeres, F. Carvalho, and J. Rivas, "Cheese whey management: A review," Nov. 15, 2012, *Academic Press*. doi: 10.1016/j.jenvman.2012.05.018.

[73] S. Kolesovs and P. Semjonovs, "Production of bacterial cellulose from whey-current state and prospects," 2020, doi: 10.1007/s00253-020-10803-9/Published.

[74] V. Revin, E. Liyaskina, M. Nazarkina, A. Bogatyreva, and M. Shchankin, "Cost-effective production of bacterial cellulose using acidic food industry by-products," *Brazilian Journal of Microbiology*, vol. 49, pp. 151–159, Nov. 2018, doi: 10.1016/j.bjm.2017.12.012.

[75] A. F. Jozala *et al.*, "Bacterial cellulose production by Gluconacetobacter xylinus by employing alternative culture media," *Appl Microbiol Biotechnol*, vol. 99, no. 3, pp. 1181–1190, Feb. 2015, doi: 10.1007/s00253-014-6232-3.

[76] M. Salari, M. Sowti Khiabani, R. Rezaei Mokarram, B. Ghanbarzadeh, and H. Samadi Kafil, "Preparation and characterization of cellulose nanocrystals from bacterial cellulose produced in sugar beet molasses and cheese whey media," *Int J Biol Macromol*, vol. 122, pp. 280–288, Feb. 2019, doi: 10.1016/j.ijbiomac.2018.10.136.

[77] M. Rollini *et al.*, "From cheese whey permeate to Sakacin-A/bacterial cellulose nanocrystal conjugates for antimicrobial food packaging applications: a circular economy case study," *Sci Rep*, vol. 10, no. 1, Dec. 2020, doi: 10.1038/s41598-020-78430-y.

[78] V. Kumar, D. K. Sharma, P. P. Sandhu, J. Jadaun, R. S. Sangwan, and S. K. Yadav, "Sustainable process for the production of cellulose by an Acetobacter pasteurianus RSV-4 (MTCC 25117) on whey medium," *Cellulose*, vol. 28, no. 1, pp. 103–116, Jan. 2021, doi: 10.1007/s10570-020-03519-6.

[79] I. K. Lappa *et al.*, "A comprehensive bioprocessing approach to foster cheese whey valorization: On-site β-galactosidase secretion for lactose hydrolysis and sequential bacterial cellulose production," *Fermentation*, vol. 7, no. 3, Sep. 2021, doi: 10.3390/fermentation7030184.

[80] J. Płoska, M. Garbowska, S. Klempová, and L. Stasiak-Różańska, "Obtaining Bacterial Cellulose through Selected Strains of Acetic Acid Bacteria in Classical and Waste Media," *Applied Sciences (Switzerland)*, vol. 13, no. 11, Jun. 2023, doi: 10.3390/app13116429.

[81] A. Bayraktar and C. Gürsoy, "Production of New Nano-Bacterial Cellulose with Lactobacillus rhamnosus by Using Whey Waste as Substrate with Optimization Taguchi Method, which has the potential to be used in many biomedical products," 2024, doi: 10.21203/rs.3.rs-3828016/v1.

[82] X. Feng, Z. Ge, Y. Wang, X. Xia, B. Zhao, and M. Dong, "Production and characterization of bacterial cellulose from kombucha-fermented soy whey," *Food Production, Processing and Nutrition*, vol. 6, no. 1, Dec. 2024, doi: 10.1186/s43014-023-00188-3.

[83] ISO, "ISO 14040:2006 Environmental management - Life cycle assessment - Principles and framework," 2006.

[84] J. B. Guinée, *Handbook on life cycle assessment : operational guide to the ISO standards*. Kluwer Academic Publishers, 2002.

[85] M. Z. Haushild and M. A. J. Hujibregts, "LCA Compendium—The Complete World of Life Cycle Assessment (book series) Series editors: Walter Klöpffer and Mary Ann Curran," *Int J Life Cycle Assess*, vol. 20, no. 9, pp. 1338–1341, Sep. 2015, doi: 10.1007/s11367-015-0922-7.

[86] G. Rebitzer *et al.*, "Life cycle assessment Part 1: Framework, goal and scope definition, inventory analysis, and applications," 2004, *Elsevier Ltd*. doi: 10.1016/j.envint.2003.11.005.

[87] ISO, "ISO 14044:2006 Environmental Management - Life cycle assessment - Requirements and Guidelines," 2006. [Online]. Available: www.iso.orgiTehSTANDARDPREVIEWISO14044:2006https://standards.iteh.ai/catalog/standards/sist/b3d74118-4c9f-4f3e-b556-11d704441abb/iso-14044-2006

[88] R. Heijungs and M. A. J. Huijbregts, "A Review of Approaches to Treat Uncertainty in LCA," 2004. [Online]. Available: https://scholarsarchive.byu.edu/iemssconference/2004/all/197

[89] Miller G. L., "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal Chem*, vol. 31, pp. 426–428, 1959.

[90] F. Piccinno, R. Hischier, S. Seeger, and C. Som, "Predicting the environmental impact of a future nanocellulose production at industrial scale: Application of the life cycle assessment scale-up framework," *J Clean Prod*, vol. 174, pp. 283–295, Jan. 2018, doi: 10.1016/j.jclepro.2017.10.226.

[91] F. Piccinno, R. Hischier, S. Seeger, and C. Som, "From laboratory to industrial scale: a scale-up framework for chemical processes in life cycle assessment

studies," *J Clean Prod*, vol. 135, pp. 1085–1097, Nov. 2016, doi: 10.1016/j.jclepro.2016.06.164.

[92] S. Gorinstein *et al.*, "Comparison of some biochemical characteristics of different citrus fruits." [Online]. Available: www.elsevier.com/locate/foodchem

[93] D. Liu *et al.*, "Bacterial nanocellulose production using cost-effective, environmentally friendly, acid whey based approach," *Bioresour Technol Rep*, vol. 24, Dec. 2023, doi: 10.1016/j.biteb.2023.101629.

[94] W. Soemphol, P. Hongsachart, and V. Tanamool, "Production and characterization of bacterial cellulose produced from agricultural by-product by Gluconacetobacter strains," 2018. [Online]. Available: www.sciencedirect.comwww.materialstoday.com/proceedings2214-7853

[95] R. Auta, G. Adamus, M. Kwiecien, I. Radecka, and P. Hooley, "Production and characterization of bacterial cellulose before and after enzymatic hydrolysis," *Afr J Biotechnol*, vol. 16, no. 10, pp. 470–482, 2017, doi: 10.5897/AJB2016.15486.

A Appendix A

A.1. Used datasets in SimaPro

Table A.1: Used datasets in SimaPro for each activity.

Activity	Dataset
Electricity	Electricity, medium voltage {ES}| market for electricity, medium voltage | Cut-off, U
Tap water	Tap water {RER}| market group for | Cut-off, U
Glucose	Glucose {GLO}| market for glucose | Cut-off, U
Mannitol	Sugar, from sugarcane {GLO}| market for sugar, from sugarcane | Cut-off, U
Disodium phosphate	Sodium phosphate {RER}| market for sodium phosphate | Cut-off, U
Citric acid	Citric acid {GLO}| market for | Cut-off, U
Sodium chloride	Sodium chloride, brine solution {GLO}| market for | Cut-off, U
Potassium chloride	Potassium chloride {RER}| market for potassium chloride | Cut-off, U
Calcium chloride dihydrate	Calcium chloride {RER}| market for calcium chloride | Cut-off, U
Sodium hydroxide	Neutralising agent, sodium hydroxide-equivalent {GLO}| market for | Cut-off, U
Lactase	Enzymes {GLO}| market for enzymes | Cut-off, U
Wastewater	Wastewater, unpolluted {GLO}| market for | Cut-off, U
Orange peel sludge	Biowaste {RoW}| market for biowaste | Cut-off, U

A.2. New datasets created in SimaPro

Table A.2: Distilled water process created in SimaPro.

Distilled water 1 L		
Tap water {RER}| market group for tap water | Cut-off, U	1	L
Electricity, medium voltage {ES}| market for electricity, medium voltage | Cut-off, U*	0.75	kWh

*Electricity needed to distil water (3000 W, 4L/h)

Table A.3: Sodium hydroxide 0.1M process created in SimaPro.

Sodium hydroxide 0.1M 1 L		
Distilled water	1	L
Neutralising agent, sodium hydroxide-equivalent {GLO}| market for | Cut-off, U	10	g
Electricity, medium voltage {ES}| market for electricity, medium voltage | Cut-off, U*	0.00005	kWh

*Electricity needed to mix components (0.6 W, 5 min)

Table A.4: Yeast extract process created in SimaPro.

Yeast extract 1 kg		
Yeast {GLO} | technology mix | production mix, at plant | as is | LCI result	1	kg
Transport, freight, lorry 16-32 metric ton, EURO5 {RER}| market for transport, freight, lorry 16-32 metric ton, EURO5 | Cut-off, U*	300	kgkm

*Yeast was not present in EcoInvent as a market activity; therefore, transport was added considering an average distance of 300 km

Table A.5: Peptone process created in SimaPro.

Peptone 1 kg

Whey protein concentrate {EU+EFTA+UK} | from liquid whey drying | production mix | LCI result	1	kg
Transport, freight, lorry 16-32 metric ton, EURO5 {RER}| market for transport, freight, lorry 16-32 metric ton, EURO5 | Cut-off, U*	300	kgkm

*Peptone was not present in EcoInvent as a market activity; therefore, transport was added considering an average distance of 300 km

Table A.6: M10 process created in SimaPro.

M10 1 L		
Distilled water	1	L
Sugar, from sugarcane {GLO}| market for sugar, from sugarcane | Cut-off, U	20	g
Yeast extract	5	g
Peptone	3	g
Electricity, medium voltage {ES}| market for electricity, medium voltage | Cut-off, U*	0.15005	kWh

*Electricity needed to mix components (0.6 W, 5 min) + electricity needed to sterilize the solution (1200 W, 15 min, 1/2 used capacity load)

Table A.7: Ringer's solution process created in SimaPro.

Ringer's solution 1 L		
Distilled water	1	L
Sodium chloride, brine solution {GLO}| market for | Cut-off, U	2.5	g
Potassium chloride {RER}| market for potassium chloride | Cut-off, U	0.105	g
Calcium chloride {RER}| market for calcium chloride | Cut-off, U	0.12	g
Sodium bicarbonate {GLO}| market for sodium bicarbonate | Cut-off, U	0.05	g

Electricity, medium voltage {ES}| market for | Cut-off, U*	0.15005	kWh

*Electricity needed to mix components (0.6 W, 5 min) + electricity needed to sterilize the solution (1200 W, 15 min, 1/2 used capacity load)

Table A.8: Culture medium from orange peels process created in SimaPro.

Culture medium from orange peels 1 L		
Tap water {RER}| market group for | Cut-off, U	1.5	L
Yeast extract	5	g
Peptone	5	g
Sodium phosphate {RER}| market for sodium phosphate | Cut-off, U	2.7	g
Citric acid {GLO}| market for | Cut-off, U	1.15	g
Electricity, medium voltage {ES}| market for | Cut-off, U*	4.38	kWh
Biowaste {RoW}| market for biowaste | Cut-off, U	0.65	kg

* Electricity needed to dry peels (350 W, 12 h) + electricity needed to shred peels (1500 W, 40 s, 3/2 used capacity load) + electricity needed to extract nutrients with a flocculator (19 W, 15 min, 1/2 used capacity load) + electricity needed to mix additives (0.6 W, 5 min) + electricity needed to sterilize the medium (1200 W, 15 min, 1/2 used capacity load)

Table A.9: Culture medium from whey process created in SimaPro.

Culture medium from whey 1 L		
Enzymes {GLO}| market for enzymes | Cut-off, U	2	mL
Yeast extract	5	g
Peptone	5	g

Sodium phosphate {RER}| market for sodium phosphate | Cut-off, U	2.7	g
Citric acid {GLO}| market for | Cut-off, U	1.15	g
Electricity, medium voltage {ES}| market for | Cut-off, U*	1.17505	kWh

*Electricity needed to hydrolyse lactose (2050 W, 2 hours, 1/4 used capacity load) + electricity needed to mix additives (0.6 W, 5 min) + electricity needed to sterilize the medium (1200 W, 15 min, 1/2 used capacity load)

Table A.10: Culture medium HS process created in SimaPro.

Culture medium HS 1 L		
Tap water {RER}| market group for | Cut-off, U	1	L
Glucose {GLO}| market for glucose | Cut-off, U	20	g
Yeast extract	5	g
Peptone	5	g
Sodium phosphate {RER}| market for sodium phosphate | Cut-off, U	2.7	g
Citric acid {GLO}| market for | Cut-off, U	1.15	g
Electricity, medium voltage {ES}| market for | Cut-off, U*	0.15005	kWh

*Electricity needed to mix components (0.6 W, 5 min) + electricity needed to sterilize the medium (1200 W, 15 min, 1/2 used capacity load)

A.3. BNC production inventory data

A.3.1. 1 g BNC production from orange peels in 7 days

Table A.11: 1 g BNC production from orange peels inventory in 7 days.

Bacteria activation			
Electricity	0.50	kWh	Electricity needed to incubate bacteria 4 days
M10	5.2	mL	Calculated basing on the amount of inoculum needed for culture medium from whey
Wastewater	5.2	mL	All medium is considered wastewater
Bacteria recovery and inoculum preparation			
Electricity	0.003	kWh	Electricity needed to shake tubes + electricity needed to centrifuge tubes
Ringer's solution	5.8	mL	Calculated basing on the amount of inoculum needed for culture medium from whey
Wastewater	5.2	mL	The Ringer's solution used to suspend bacteria after separation from the liquid medium M10 is considered wastewater; the Ringer's solution used to resuspend bacteria after centrifugation to create the inoculum is not considered wastewater
Culture medium creation			
Culture medium from orange peels	250.6	mL	Calculated starting from production yield in g/L of culture medium
Static culture fermentation			
Electricity	1.01	kWh	Electricity needed to incubate bacteria 7 days + electricity needed to sterilize the membranes after incubation calculated

			basing on the weight of the membranes and assuming that the sterilizer (1200 W, 15 min) can host 12 membranes
Wastewater	11.9	mL	Calculated basing on the residual liquid volume in the Petri disk
Nanocellulose purification			
NaOH 0.1M	595.2	mL	Calculated basing on the weight of the membranes and assuming 250 mL of NaOH 0.1M every 3 membranes
Wastewater	595.2	mL	All NaOH 0.1M is considered wastewater
Nanocellulose neutralisation			
Tap water	11.9	L	Calculated basing on the weight of the membranes and assuming 5 L of tap water every 3 membranes
Wastewater	11.9	L	All tap water is considered wastewater
Nanocellulose drying			
Electricity	0.83	kWh	Electricity needed to dry 1 g of membranes, calculated basing on the weight of membranes and assuming that the dehydrator (12 W, 4 hours) can host 12 membranes

A.3.2. 1 g BNC production from whey in 7 days

Table A.12: 1 g BNC production from whey inventory in 7 days.

Bacteria activation			
Electricity	0.83	kWh	Electricity needed to incubate bacteria 4 days
M10	8.6	mL	Calculated basing on the amount of inoculum needed for culture medium from orange peels
Wastewater	8.6	mL	All medium is considered wastewater
Bacteria recovery and inoculum preparation			
Electricity	0.004	kWh	Electricity needed to shake tubes + electricity needed to centrifuge tubes
Ringer's solution	9.6	mL	Calculated basing on the amount of inoculum needed for culture medium from orange peels
Wastewater	8.6	mL	The Ringer's solution used to suspend bacteria after separation from the liquid medium M10 is considered wastewater; the Ringer's solution used to resuspend bacteria after centrifugation to create the inoculum is not considered wastewater
Culture medium creation			
Culture medium from whey	413.2	mL	Calculated starting from production yield in g/L of culture medium
Static culture fermentation			
Electricity	1.66	kWh	Electricity needed to incubate bacteria 7 days + electricity needed to sterilize the membranes after incubation, calculated basing on the weight of the membranes

			and assuming that the sterilizer (1200 W, 15 min) can host 12 membranes
Wastewater	165.3	mL	Calculated basing on the residual liquid volume in the Petri disk
Nanocellulose purification			
NaOH 0.1M	980.4	mL	Calculated basing on the weight of the membranes and assuming 250 mL of NaOH 0.1M every 3 membranes
Wastewater	980.4	mL	All NaOH 0.1M is considered wastewater
Nanocellulose neutralisation			
Tap water	19.6	L	Calculated basing on the weight of the membranes and assuming 5 L of tap water every 3 membranes
Wastewater	19.6	L	All tap water is considered wastewater
Nanocellulose drying			
Electricity	1.37	kWh	Electricity needed to dry 1 g of membranes, calculated basing on the weight of membranes and assuming that the dehydrator (12 W, 4 hours) can host 12 membranes

A.3.3. 1 g BNC production from HS in 7 days

Table A.13: 1 g BNC production from HS inventory in 7 days.

Bacteria activation			
Electricity	1.38	kWh	Electricity needed to incubate bacteria 4 days
M10	14.4	mL	Calculated basing on the amount of inoculum needed for culture medium HS
Wastewater	14.4	mL	All medium is considered wastewater
Bacteria recovery and inoculum preparation			
Electricity	0.007	kWh	Electricity needed to shake tubes + electricity needed to centrifuge tubes
Ringer's solution	16.1	mL	Calculated basing on the amount of inoculum needed for culture medium HS
Wastewater	14.4	mL	The Ringer's solution used to suspend bacteria after separation from the liquid medium M10 is considered wastewater; the Ringer's solution used to resuspend bacteria after centrifugation to create the inoculum is not considered wastewater
Culture medium creation			
Culture medium HS	689.7	mL	Calculated starting from production yield in g/L of culture medium
Static culture fermentation			
Electricity	2.78	kWh	Electricity needed to incubate bacteria 7 days + electricity needed to sterilize the membranes after incubation calculated basing on the weight of the membranes and assuming that the sterilizer (1200 W, 15 min) can host 12 membranes

Wastewater	259.4	mL	Calculated basing on the residual liquid volume in the Petri disk
Nanocellulose purification			
NaOH 0.1M	1666.7	mL	Calculated basing on the weight of the membranes and assuming 250 mL of NaOH 0.1M every 3 membranes
Wastewater	1666.7	mL	All NaOH 0.1M is considered wastewater
Nanocellulose neutralisation			
Tap water	33.3	L	Calculated basing on the weight of the membranes and assuming 5 L of tap water every 3 membranes
Wastewater	33.3	L	All tap water is considered wastewater
Nanocellulose drying			
Electricity	2.33	kWh	Electricity needed to dry 1 g of membranes, calculated basing on the weight of membranes and assuming that the dehydrator (12 W, 4 hours) can host 12 membranes

A.4. Charts

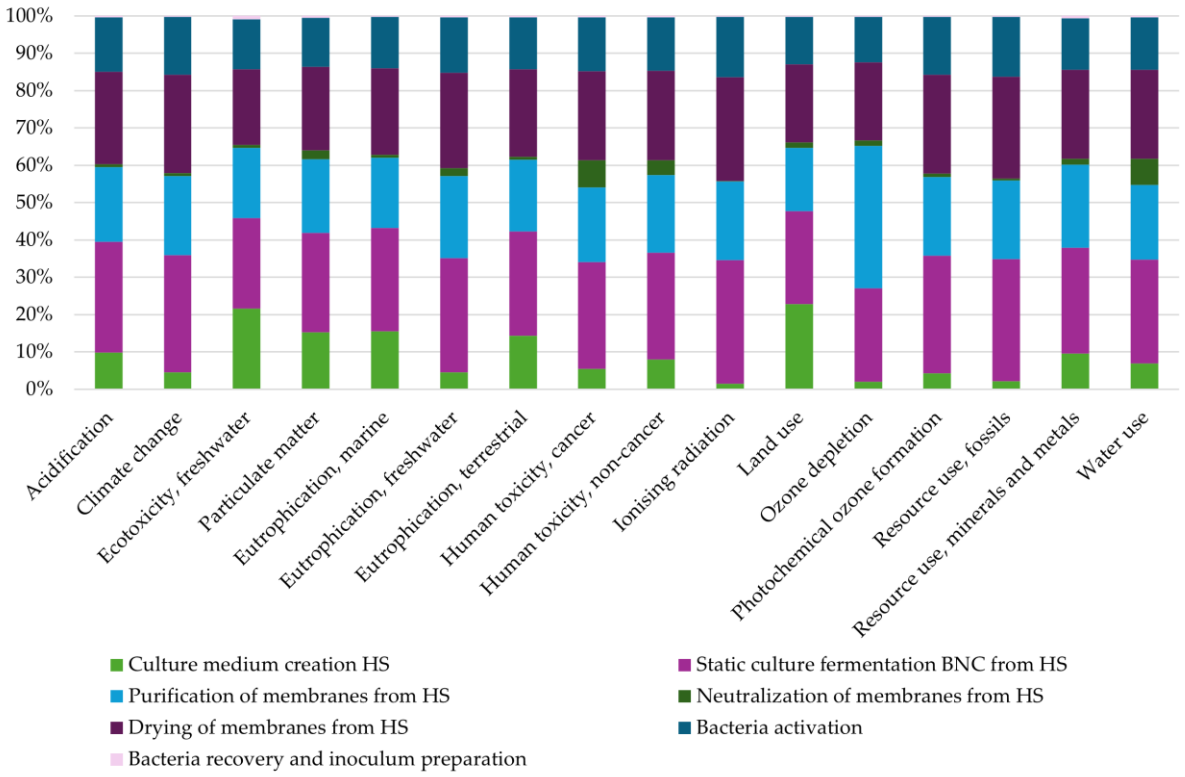


Figure A.1: Contribution analysis results for 1 g BNC production from HS in 7 days.

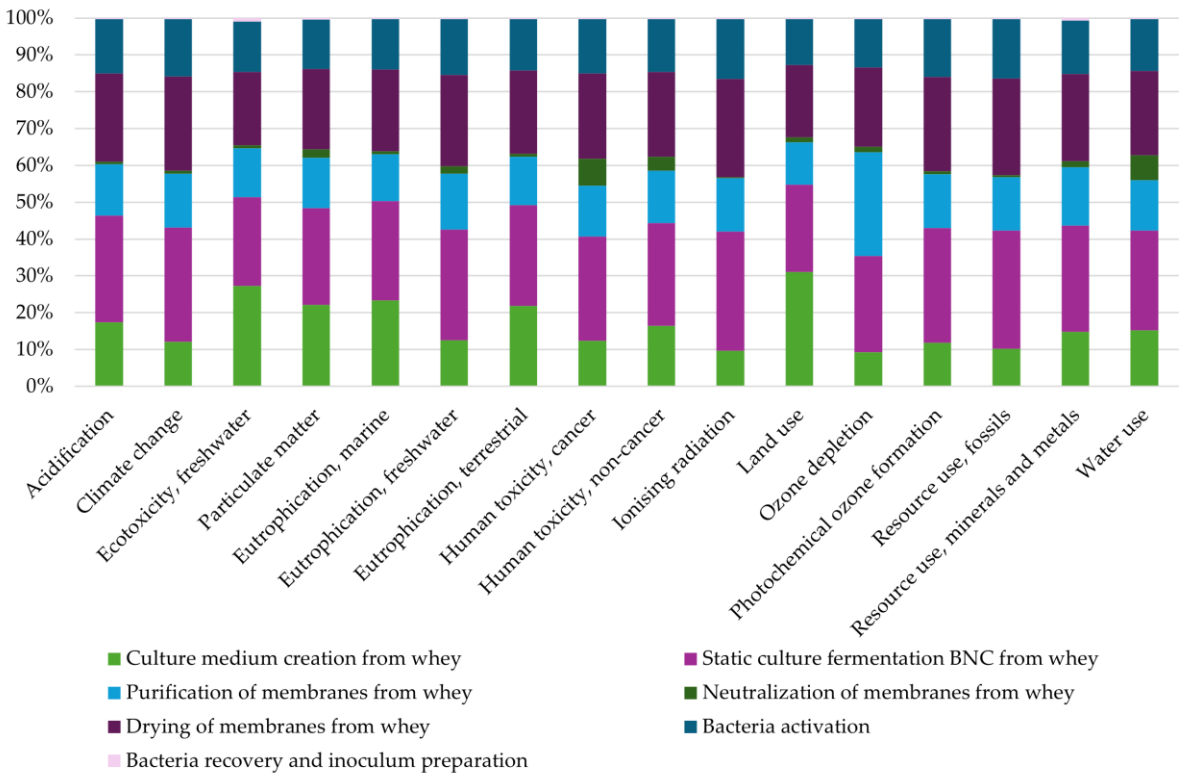


Figure A.2: Contribution analysis results for 1 g BNC production from whey in 7 days.

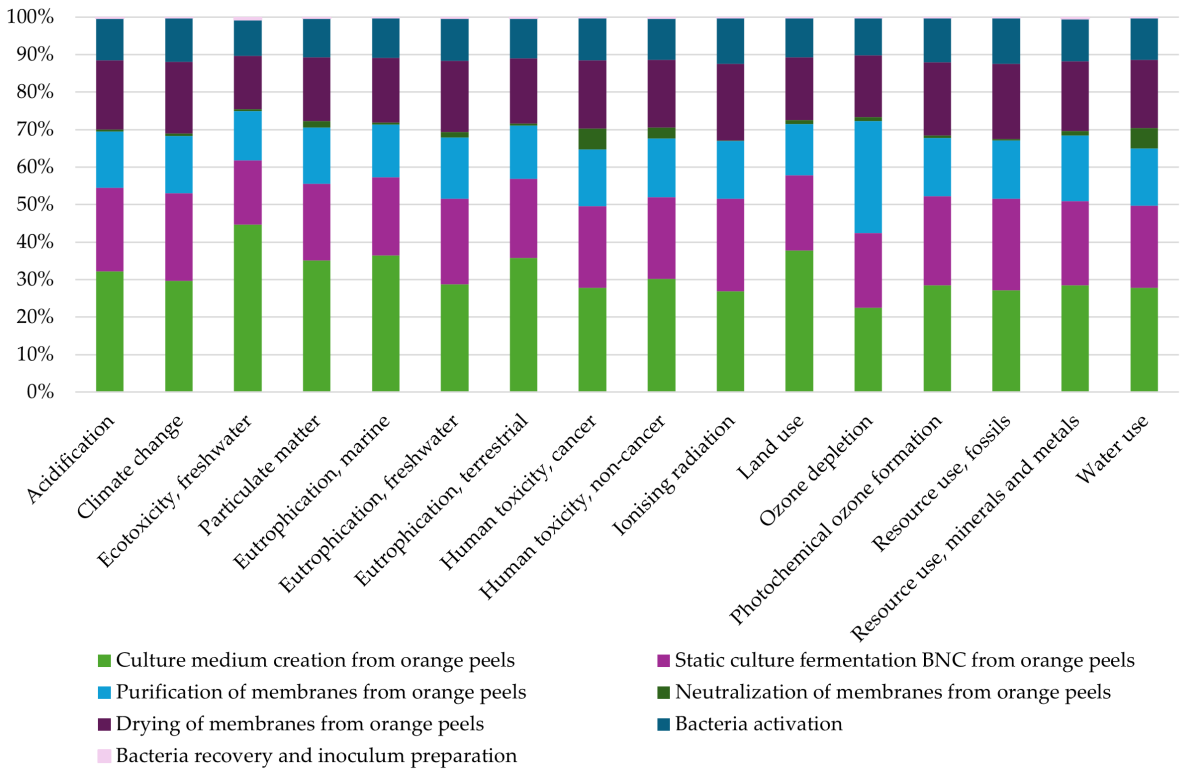


Figure A.3: Contribution analysis results for 1 g BNC production from orange peels in 7 days.

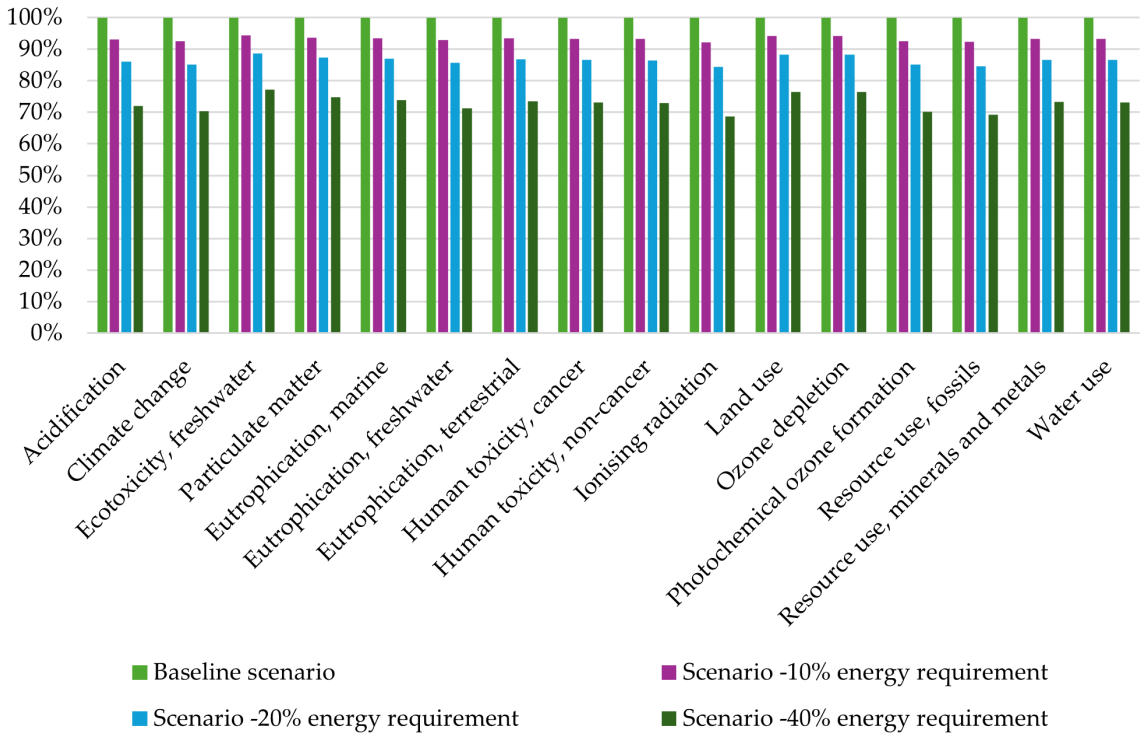


Figure A.4: Sensitivity analysis 1 g BNC production from HS in 7 days.

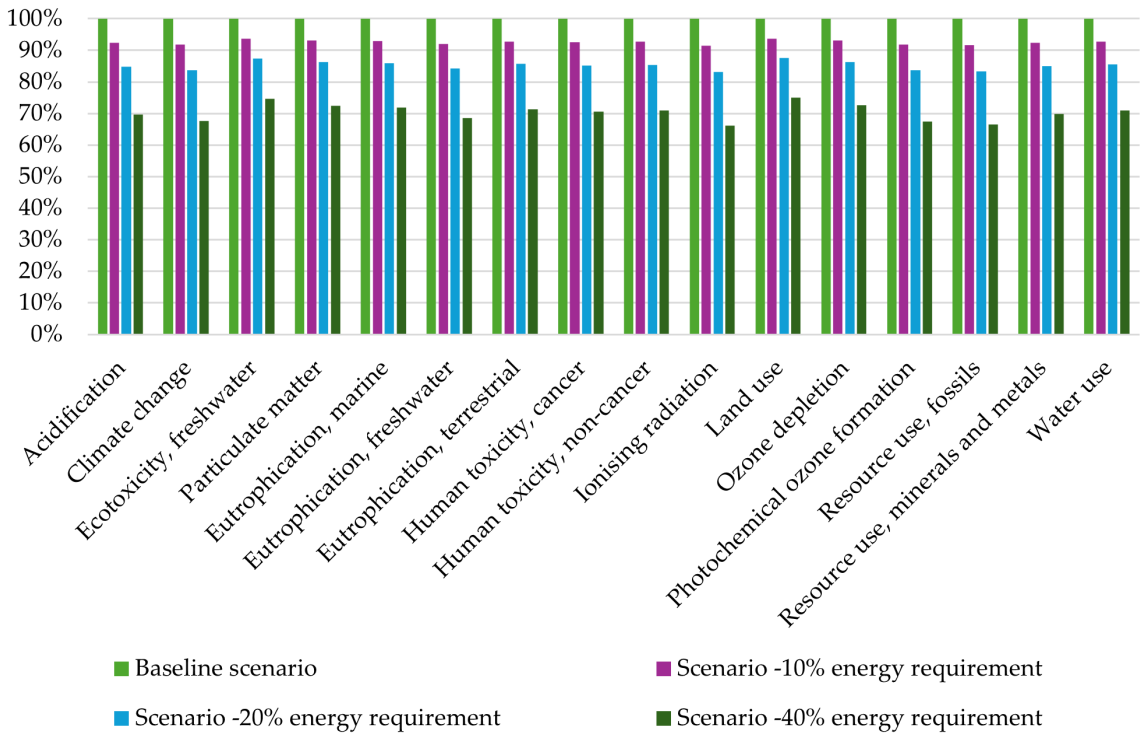


Figure A.5: Sensitivity analysis 1 g BNC production from whey in 7 days.

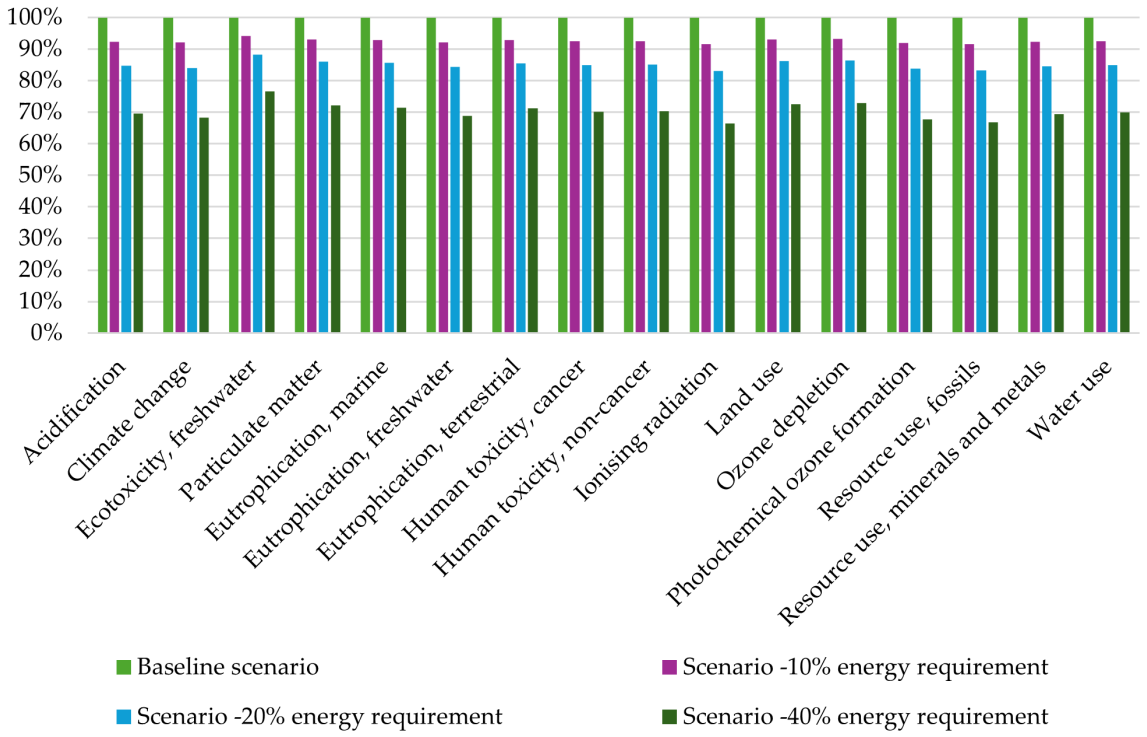


Figure A.6: Sensitivity analysis 1 g BNC production from orange peels in 7 days.

List of Figures

Figure 1.1: Waste hierarchy for food waste*(Garcia-Garcia et al., 2017).* 3

Figure 3.1: Filtration to obtain the culture medium from orange peels. 14

Figure 3.2: Whey filtration to separate casein 14

Figure 3.3: Cell growth in M10 medium at 30°C and 120 rpm, for 4 days..... 15

Figure 3.4: System boundary for BNC production, own illustration. 21

Figure 4.1: pH, conductivity, liquid volume and reducing sugars trends in time during BNC production from orange peels..... 26

Figure 4.2: BNC from orange peels yield in time. 27

Figure 4.3: BNC membranes obtained after 7 days of incubation from HS medium on the left and from T_{amb} +additives medium on the right..... 29

Figure 4.4. pH, conductivity, liquid volume and reducing sugars trends in time, during BNC production from orange peels..... 34

Figure 4.5: BNC from orange peels yield values after 7 days of production, comprehensive results. 39

Figure 4.6: pH, conductivity, liquid volume and reducing sugars trends in time, during BNC production from whey. 43

Figure 4.7: BNC membranes obtained after 10 days of incubation from "Whey (hydrolysed)+additives" medium during cleaning..... 46

Figure 4.8: BNC membranes obtained after 10 days of incubation from "Whey (hydrolysed)+additives" medium..... 47

Figure 4.9: Three-dimensional scatter plot for the design points..... 50

Figure 4.10: BNC membranes obtained after 10 days of incubation from run 9 (10 incubation days, 1.4 inoculum optical density and 1% w/v yeast extract concentration). 52

Figure 4.11: Contours of estimated response surface, segmented by yeast extract concentration..... 53

Figure 4.12: pH, conductivity, liquid volume, reducing sugars, total nitrogen and phosphates trends in time during BNC production from whey. 57

Figure 4.13: BNC from whey yield in time. 58

Figure 4.14: BNC membranes at day 10 of incubation obtained from hydrolysed whey with the addition of 0.5% w/v yeast extract and 0.5% w/v peptone, 1 inoculum optical density on the left and hydrolysed whey with the addition of 1% w/v yeast extract and no peptone, 1.4 optical density on the right. 60

Figure 4.15: SEM analysis results for BNC membranes produced from a) HS, b) orange peels and c) whey. 63

Figure 4.16: TEM analysis results for BNC membranes produced from a) HS, b) orange peels and c) whey. 64

Figure 4.17: IR spectra of BNC membranes produced from HS, orange peels and whey. 65

Figure 4.18: Culture media creation processes from orange peels, whey and HS..... 68

Figure 4.19: Environmental impacts of culture medium creation from orange peels, whey and HS. 70

Figure 4.20: Contribution analysis for culture medium creation from orange peels. . 72

Figure 4.21: Environmental impacts for BNC production from HS, whey and orange peels. 73

Figure 4.22: Sensitivity analyses for BNC production from HS, whey and orange peels, regarding energy requirements. 76

Figure A.1: Contribution analysis results for 1 g BNC production from HS in 7 days. 102

Figure A.2: Contribution analysis results for 1 g BNC production from whey in 7 days. 103

Figure A.3: Contribution analysis results for 1 g BNC production from orange peels in 7 days. 103

Figure A.4: Sensitivity analysis 1 g BNC production from HS in 7 days. 104

Figure A.5: Sensitivity analysis 1 g BNC production from whey in 7 days. 104

Figure A.6: Sensitivity analysis 1 g BNC production from orange peels in 7 days. .. 105

List of Tables

Table 4.1: Culture media from orange peels characterization in terms of reducing sugars, total nitrogen, phosphates, pH and conductivity 24

Table 4.2: BNC from orange peels yield values after 7 days of production 28

Table 4.3: Water retention parameters of BNC from orange peels after 7 days of production 30

Table 4.4: Culture media from orange peels characterization in terms of reducing sugars, pH and conductivity. 32

Table 4.5: BNC from orange peels yield values after 7 days of production. 35

Table 4.6: Water retention parameters of BNC from orange peels after 7 days of production. 36

Table 4.7: Culture media from whey characterization in terms of reducing sugars, pH and conductivity. 42

Table 4.8: BNC from whey yield values after 10 days of incubation. 45

Table 4.9: Water retention parameters of BNC from whey after 10 days of production. 48

Table 4.10: Face-centred central composite design for evaluation of independent factors along with experimental BNC. 51

Table 4.11: Statistical analysis of the quadratic model of the response surface. 54

Table 4.12: Culture media from whey characterization in terms of reducing sugars, total nitrogen, phosphates, pH and conductivity. 55

Table 4.13: Water retention parameters of BNC from whey after 10 days of production. 59

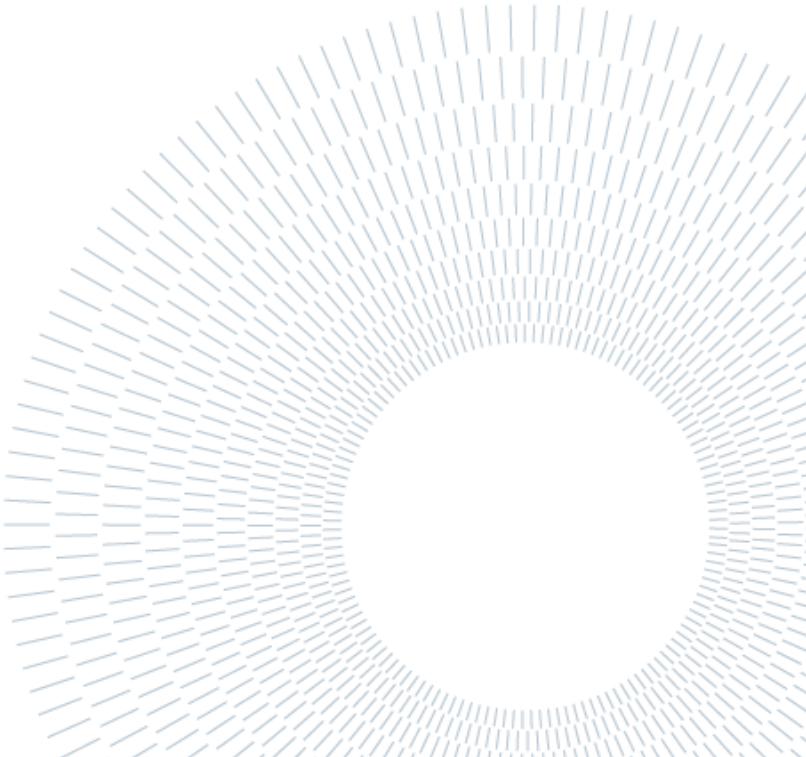
Table 4.14: Environmental impacts for 1 g BNC production from HS, whey and orange peels in 7 days. 74

Table A.1: Used datasets in SimaPro for each activity. 91

Table A.2: Distilled water process created in SimaPro. 92

Table A.3: Sodium hydroxide 0.1M process created in SimaPro. 92

Table A.4: Yeast extract process created in SimaPro. 92
Table A.5: Peptone process created in SimaPro. 92
Table A.6: M10 process created in SimaPro. 93
Table A.7: Ringer's solution process created in SimaPro. 93
Table A.8: Culture medium from orange peels process created in SimaPro. 94
Table A.9: Culture medium from whey process created in SimaPro. 94
Table A.10: Culture medium HS process created in SimaPro. 95
Table A.11: 1 g BNC production from orange peels inventory in 7 days. 96
Table A.12: 1 g BNC production from whey inventory in 7 days. 98
Table A.13: 1 g BNC production from HS inventory in 7 days. 100

List of symbols

Variable	Description	SI unit
C_{used}/C_{max}	load capacity factor	-
E	energy	J
P	power	W
t	time	s
V	volume	L
W_0	humid weight	g
W_1	dry weight	g
WC	water content	%
WHC	water holding capacity	g/g
Y	yield	g/L