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SCUOLA DI INGEGNERIA INDUSTRIALE E DELL'INFORMAZIONE

EXECUTIVE SUMMARY OF THE THESIS

Valorisation of food wastes by producing bacterial nanocellulose

TESI MAGISTRALE IN CHEMICAL ENGINEERING – INGEGNERIA CHIMICA

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1. Introduction

Waste generation, particularly food waste, is a major global issue, with the FAO estimating that one-third of all food produced for human consumption is wasted [1]. In the EU alone, 88 million tonnes of food waste are generated annually [2]. Traditional disposal methods include incineration, landfills, sewage, and anaerobic digestion for biogas production. However, food waste can also be repurposed into high-value products through fermentation processes or extraction of valuable components like proteins, colorants, phenolics, and sugars [3].

Bacterial Nanocellulose (BNC) has garnered significant interest due to its unique properties, such as its purity, tensile strength, biodegradability and biocompatibility, and consequent wide range of applications in various fields, including biomedical, food, and environmental sectors [4]. BNC is produced through a bottom-up synthesis method, that relies on the direct activity of specific bacterial strains that in presence of oxygen synthesize cellulose as a part of their metabolic processes [5]. 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 [6]. However, the production cost of bacterial nanocellulose using this traditional culture medium is high, with the medium itself representing 50-65% of the total process cost, limiting large scale production [7]. As sustainability becomes paramount, utilizing food waste for BNC production presents an innovative approach which allows to produce it utilising low-cost and easily available substrates while also addressing waste management [8]. This study investigates the potential of using food residues, specifically orange peels and whey, as culture media for BNC production, evaluating their effectiveness and environmental implications.

2. Materials and methods

Culture media production from food residues

Two types of food residues, orange peels and whey, were used for culture media preparation. Orange peels (OP) were sourced from the canteen of the Higher Technical School of Engineering and Industrial Design (ETSIDI) at the Polytechnic University of Madrid, while whey was obtained from a cheese production laboratory at the Higher Technical School of Forestry Engineers (ETSIM), also within UPM.

The treatment of OP involved drying at 30 °C for about 12 hours, followed by grinding to a powder. Nutrient extraction was performed using a 1:10 ratio of OP powder to distilled water, with extractions conducted at ambient temperature, 50 °C, and 100 °C to assess the optimal extraction conditions. The resulting suspension was filtrated to separate the liquid culture medium, which was then autoclaved for sterilization at 121 °C.

For whey treatment, filtration was performed to separate casein using a series of sieves. Whey was tested in both hydrolysed (H) and non-hydrolysed (NH) forms, with hydrolysation achieved using 2 mL of lactase per each litre of whey at 35-40 °C for 2 hours. After those steps, the whey medium was also sterilized at 121 °C.

Both kinds of media, from OP and whey, have been analysed with and without additives addition; when needed, additives have been added before media sterilisation. All culture media were characterised for reducing sugars concentration, employing the 3,5 dinitrosalicylic acid (DNS) essay, pH and conductivity (Ω).

BNC production and characterization

BNC production utilized the bacterial strain *Komagataeibacter sucrofermentans*. Being kept lyophilised, bacteria were activated in M10 liquid medium at 30 °C with agitation for 4 days. Nanocellulose pellets formed during the growth phase were treated to recover bacteria to create the inoculum. The subsequent cultures were performed adding 250 μL of the 1 optical density 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; the resulting BNC forms as a pellicle on the surface of the culture broth.

The production yield in g/L has been measured by weighing the membranes, after being dried in a dehydrator, and relating their dry weight to the initial liquid volume of culture medium in the Petri disk, using Equation (2.1).

$$Y\left[\frac{g}{L}\right] = \frac{W_1\,[g]}{V\,[L]} \qquad (2.1)$$

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, to quantify variability.

Membranes have been characterised by measuring their 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 holding capacity (WHC), representative of membranes water retention, has been measured as in Equation (2.2).

$$WHC\left[\frac{g}{g}\right] = \frac{W_0[g] - W_1[g]}{W_1[g]} \qquad (2.2)$$

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

BNC production optimisation

BNC production optimisation has been performed using the Design of Experiment wizard of the Statgraphics 19 software. A central composite design has been employed, belonging to the response surface design class. Moreover, the design has been chosen as face centred; although being less desirable from a statistical perspective, such design is easier to run since it involves only low, medium, and high levels of each factor. 2 centre points have been selected, randomly placed. The model used to fit the experimental data has been selected as quadratic: therefore, 10 coefficients were found.

Life cycle assessment

A Life Cycle Assessment (LCA) was conducted using the SimaPro software (9.5.0.1 version) and the Ecoinvent database (3.9.1 version) to evaluate the environmental impacts associated with BNC production from different culture media. The analysis was a laboratory scale, 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. This study is intended to help understanding and comparing the environmental impacts of BNC production from 3 different media (HS, OP and whey) and identify the best solution from the environmental impact point of view, along with the hotspots in each production chain. The functional unit for this study has been defined as 1 g of BNC produced in 7 days, to better represent the production function occurring at laboratory scale.

3. Results and discussion

BNC production from food wastes

After preliminary experiments had proved that the presence of additives into media obtained from food wastes highly boosts BNC yield, BNC production from OP for the 3 extraction temperatures (T_{amb}, 50 °C and 100 °C) has been investigated in two cases: I) with the addition of just two additives (disodium phosphate, 0.27 %w/v; citric acid, 0.115 %w/v), II) with all the additives (peptone, 0.5 %w/v; yeast extract, 0.5 %w/v; disodium phosphate, 0.27 %w/v; citric acid, 0.115 %w/v). Indeed, citric acid is a natural preservative and disodium phosphate plays as a pH buffer; therefore, they have been added to all media. The selected additives comprehend all the compounds present in the HS medium with the same concentrations, except for glucose, since it is supposed that sugars were already present in the medium, coming from orange peels. Yeast extract and peptone were added to bring nitrogen to the medium, essential for cells life. HS medium was employed as control medium. BNC production from whey has been investigated in two cases, as for the OP study: I) with the addition of just two additives (disodium phosphate, 0.27 %w/v; citric acid, 0.115 %w/v), II) with all the additives (peptone, 0.5 %w/v; yeast extract, 0.5 %w/v; disodium phosphate, 0.27 %w/v; citric acid, 0.115 %w/v).

Characterization results for the different media are reported in Table 1; they show higher reducing sugars concentration in media from orange peels than in the HS one, with the highest one found when the extraction is performed at 100 °C. pH of orange peels media, always around 5, is lower than HS's pH, that is 6.29; additionally, conductivity of orange peels media is generally lower than the one of HS. It is noteworthy that both pH and conductivity increase when all additives are incorporated.

Whey media contains higher reducing sugar levels, with significantly higher amounts for hydrolysed whey, due to the breakdown of lactose during hydrolysis, leading to greater availability of simple sugars. The pH of whey media is generally higher, resembling HS's pre-incubation pH of 6.3, with slight decreases when only citric acid and disodium phosphate are used. Conductivity is also higher in whey media compared to orange peels and HS, attributed to the additional ions from yeast and peptone, as well as the natural presence of minerals and electrolytes in whey.

Table 1: Media from orange peels characterization

	Reducing sugars [g/L]	pH [-]	Ω [mS/cm]
HS	17.67	6.29	4.76
OP (T_{amb}) I	25.45	4.97	3.36
OP (T_{amb}) II	23.11	5.44	4.27
OP (50 °C) I	22.63	5.15	3.30
OP (50 °C) II	22.46	5.30	3.84
OP (100 °C) I	25.59	4.96	3.51
OP (100 °C) II	27.86	5.19	4.10
Whey (NH) I	39.72	5.89	6.24
Whey (NH) II	40.40	6.00	6.69
Whey (H) I	49.74	5.85	6.35
Whey (H) II	52.36	5.97	6.82

Results about BNC yields are shown in Figure 1. Despite higher reducing sugars concentrations among the OP media were found when extraction was performed at 100 °C, the medium OP (100 °C) I did not show the highest yield, and no membranes formed from the OP (100 °C) II medium. BNC production from HS resulted to be lower than any case from orange peels waste. The best condition to grow BNC in terms of yield was found to be the medium T_{amb} II, yielding 3.99 ± 0.45 g/L in 7 days. The findings indicate that orange peel residue not only provides reducing sugars for BNC production but also contains other beneficial components that may enhance the overall yield. The presence of various nutrients and additives can create a synergistic effect, improving the fermentation process. However, this synergy may be compromised if some nutrients are partially degraded during extraction. At ambient temperature, this synergy appears to be optimal, resulting in higher yields of BNC.

Regarding whey, no membranes were formed by day 10 in the NH cases, likely due to the presence of lactose, which some bacteria cannot digest without specific enzymes. Although not hydrolysed whey contains other reducing sugars, they may not be readily available for bacterial utilization if lactose is predominant. In contrast, hydrolysing whey improves the availability of reducing sugars. The highest yield at incubation day 10, equal to 4.28 ± 0.36 g/L, was achieved from Whey (H) II, suggesting that yeast extract and peptone enhanced bacterial growth and metabolism, leading to increased BNC production.

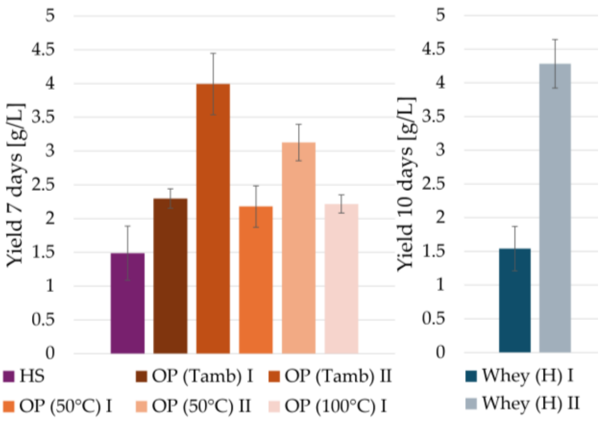

Figure 1: BNC yield from HS (7 days), orange peels (7 days) and whey (10 days)

In Figure 2, it is possible to compare the BNC membranes obtained from HS and OP (T_{amb}) II. Clearly, the membrane on the left appears to be thinner and more transparent compared to the one on the right, which looks thicker and opaquer. Moreover, the membrane from OP (T_{amb}) II looks more uniform and homogeneous, whereas the one from HS has a more irregular texture. However, WHC results indicated that the membrane from HS (135.9 g/g) displays higher water retention than the membranes obtained from OP (in particular from OP (T_{amb}) II: 66.7 g/g). The membrane in Figure 3, from Whey (H) II, demonstrates several qualities indicative of high-quality BNC, including uniformity, translucency, and a smooth surface. Its uniformity suggests a well-controlled production process, while the translucency indicates evenly distributed cellulose fibres. The WHC of membranes from whey was found to be higher than from orange peels: in particular, WHC for the Whey (H) II case resulted to be 134.3 g/g, that is comparable to what found for the HS case.

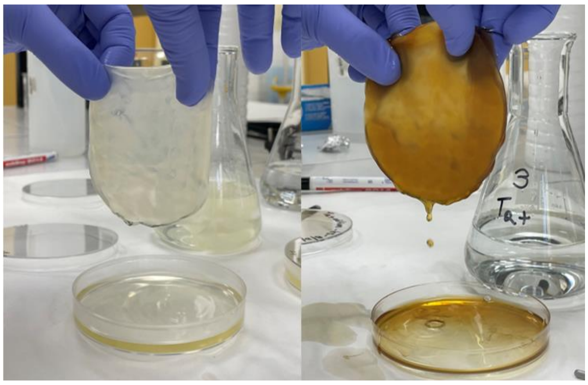

Figure 2: Membranes obtained from HS (left) and OP (T_{amb} II) (right)

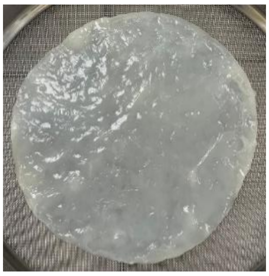

Figure 3: Membrane obtained from Whey (H) II

TEM observations of the BNC membranes from HS, OP (Tamb II), and Whey (H) revealed nanoscale fibrils, confirming successful nanocellulose production in all cases. Additionally, IR spectroscopy showed identical spectra for all three membranes, indicating that the BNC is consistent across different substrates, suggesting that its formation is independent of the substrate used.

BNC production optimisation results

Hydrolysed whey with additives (yeast extract, peptone, disodium phosphate, and citric acid) showed promising results for BNC production, both in yield and water retention of membranes. From a residue valorisation perspective, the dairy industry faces challenges in managing whey disposal due to its high organic load that creates the need for treating whey before disposing it, thereby making the valorisation of this waste more relevant and beneficial. Moreover, being liquid, whey is easier to integrate into fermentation processes than orange peels, which require extensive preprocessing. Given these advantages, this study aims to optimise BNC production from hydrolysed whey through a design of experiments approach, varying three factors, incubation days, inoculum optical density, and yeast extract concentration, at three levels, with BNC yield as the response variable. The study has been performed by incorporating only yeast extract, leveraging whey's inherent nitrogen content to reduce costs. Yeast extract enhances BNC yield due to its rich nutrient profile, proving more effective than inorganic nitrogen sources in promoting bacterial growth and cellulose production [9]. The experimental design points for the central composite design, face-centred with 2 coincident centre points is graphically visualised in Figure 4.

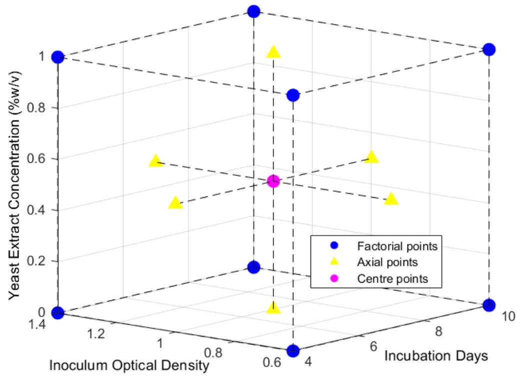

Figure 4: 3D-scatter plot for the design points.

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. The statistical analysis of the quadratic model showed a P-value of 0.0001, that indicates that the model is highly statistically significant; indeed, models with P-values below 0.05 confirm statistical significance at the 5% level. 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 an estimated yield of 2.79 g/L.

Comparison of BNC yields from two media, one with 0.5% w/v yeast extract, 0.5% w/v peptone, and an inoculum optical density of 1, and the other with 1% w/v yeast extract, no peptone, and an optical density of 1, shows that the medium with peptone produced higher yields (4.28±0.36 g/L vs. 2.88±0.44 g/L after 10 days). Despite a higher initial optical density in the peptone-free medium, cellulose production was greater in the presence of peptone, suggesting that it enhances cellulose synthesis efficiency.

Life cycle assessment results

The inventory data for BNC production were derived from laboratory experiments for three routes: orange peels, whey, and HS. Best culture conditions for BNC production from food wastes were considered to perform the assessment, specifically the medium T_{amb} II regarding orange peels and the medium Whey (H) II for whey. For orange peels, the yield was 3.99 ± 0.45 g/L in 7 days; concerning whey, the yield found for the selected medium in 7 days was 2.42 ± 0.47 g/L; lastly, for HS, the yield was 1.49 ± 0.40 g/L in 7 days. 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. The selected impact categories have been chosen as the ones recommended by the EU environmental footprint (EF) 3.1 method.

Firstly, the impacts to produce 1 L of each culture media has been assessed. The preparation of 1 L of culture medium from orange resulted in the highest impact among the three in all the 16 impact categories of the EF 3.1 method, followed by whey and, with the lowest environmental impact, HS culture medium. Indeed, orange peels pre-treatment, involves more steps than the other media, the majority of which highly energy demanding.

However, the impact assessment for BNC production, particularly for 1 g of BNC production over 7 days, reported in Figure 5, conversely showed that the HS culture medium has higher environmental impacts across all categories compared to waste-based media, due to its lower BNC yield, which increases resource consumption. Among the waste-based media, BNC production from orange peels has the lowest impact. Despite the resource-intensive preparation of the medium, its high yield offsets the energy and material costs, making it the most environmentally efficient option studied. For the climate change category, the results showed an impact of 2.36, 1.43 and 1.16 kg $CO_{2,eq}$ per gram of BNC produced in 7 days from HS, whey and orange peels, respectively.

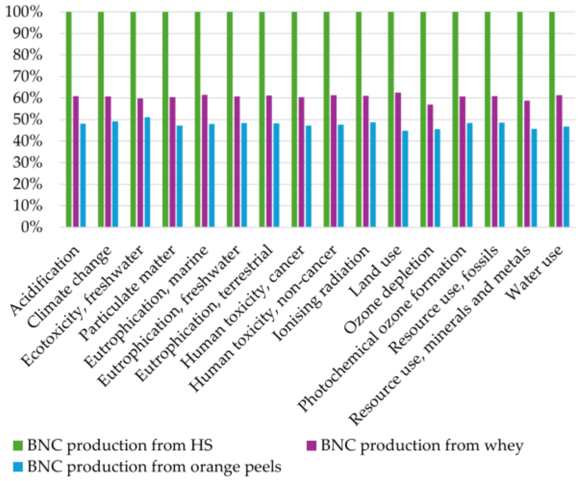

Figure 5: Environmental impacts for BNC production from HS, whey and orange peels.

4. Conclusion

This thesis investigates the potential of repurposing food waste to address the pressing issue of food waste management through a circular economy model, specifically by producing bacterial nanocellulose (BNC) from by-products such as orange peels and whey. The research demonstrated the feasibility of converting organic waste into valuable BNC. The findings revealed that BNC from orange peels was maximised when nutrients extraction happened at ambient temperature, while hydrolysed whey possesses a significant potential to produce high-quality BNC. Moreover, the best yield was found when the same additives of HS, apart from glucose, are added to the food wastes media with the same HS concentration, always finding higher productions than from HS.

The LCA results indicate that BNC production from food waste has lower environmental impacts compared to conventional media, with BNC derived from orange peels demonstrating the least environmental impact across several categories, thanks to the elevate yield. This research underscores the viability of sustainable food waste repurposing, contributing to a circular economy framework, and advocates for further investigation into refining BNC production methodologies for enhanced industrial applicability.

5. Bibliography

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