الجمهورية الجزائرية الديمقراطية الشعبية وزارة التعليم العالي والبحث العلمي

**PEOPLE'S DEMOCRATIC REPUBLIC OF ALGERIA**

**MINISTRY OF HIGHER EDUCATION AND SCIENTIFIC RESEARCH**

**Academic Year 2024/2025**

**Higher School of Biological Sciences of Oran (ESSB d'Oran)**

**Department of Second Cycle**

# Educational Handout

## Subject : Workshop

Workshop of agro-industrial residues Valorisation

**Level:** 2nd year of the second cycle

**Specialty:** Enzyme engineering

**Field:** Biotechnology

**Domain:** Life and Natural Sciences


**Prepared by:**

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**Subject taught during the Academic Year :**

**2023-2024**

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# Foreword

This handout is intended for first-year students in the second cycle at the Higher School of Biological Sciences in Oran (Specialty: Biotechnology, Option: Enzyme Engineering). It serves as a manual for students with a solid background in biochemistry, microbiology, and fundamental enzymology.

The workshops on agro-industrial residue valorization aim to convert waste into valuable products, thereby reducing both environmental and economic impacts. Among these residues, coffee grounds and sawdust are particularly significant. Coffee grounds, often mistakenly regarded as waste, are a valuable resource due to their rich and diverse chemical composition. They can be utilized in various fields, such as energy production, materials science, and nutraceuticals. Additionally, they serve as substrates for microorganism cultivation or as compost to support organic farming. Sawdust is another important substrate, primarily used for cellulose extraction—a key component in bioplastic manufacturing. Bioplastics are sustainable alternatives to conventional plastics, being biodegradable and derived from renewable resources. By combining these substrates, valorization workshops can generate high-value-added products, contributing to waste reduction, promoting sustainability, and fostering the development of new economic markets.

These workshops also aim to teach students collaborative skills and problem-solving strategies. Such initiatives exemplify the circular economy concept, where every waste product is transformed into an opportunity for value creation, thereby encouraging more responsible environmental development.

## Workshop details

**Institution** : Higher School of Biological Sciences of Oran

**Department :** Second cycle

**Speciality :** Enzyme engineering

**Field :** Biotechnology

**Domain:** Natural and life sciences

**Semester : 2**

**Credits :** 4

**Rating :** 2

**Semester Hours :** 45 h

**Personal work :** 55 h

**Practical Leader :** Dr. Yasmine AIT HAMADOUCHE

**Subject:** Workshop of Agro-industrial residues Valorization

**Prerequisites:** In Biochemistry, Microbiology and Fundamental enzymology.

## Introduction

Agro-industrial waste, a by-product of agriculture-based industries, is typically rich in lignocellulosic materials and bioactive compounds. However, the management of these wastes is often inadequately regulated, leading to uncontrolled disposal practices. This has resulted in negative impacts on both ecosystems and the economy. Consequently, extensive research has focused on extracting valuable compounds from these wastes. As a result, cellulose derived from agro-industrial residues has emerged as a renewable resource with diverse applications **(Ahmad Khorairi *et al*., 2021)**.

Extensive research has focused on extracting cellulose from agro-industrial waste.
Cellulose is a widely available biopolymer, renowned as the primary raw material for paper production. It has a consistent chemical composition, is biodegradable, and renewable. However, when derived from plant materials, cellulose exhibits a complex physical and morphological structure **(Hansson *et al.,* 2009, Siró and Plackett, 2010).**

In recent years, **cellulose fibers** have gained increasing attention for their potential in producing nanocellulose. This versatile material boasts a range of physicochemical properties, offering diverse applications. However, its isolation demands specialized methodologies that need to be tailored to each specific raw material **(Ribeiro *et al.,* 2019).**

**Nanocelluloses**, with dimensions between 1–100 nm **(Joshi et al., 2024)**, are derived from cellulose fibers using mechanical, chemical, or enzymatic methods. They serve as excellent reinforcing agents in composite materials like biofilms and plastic packaging. However, their production is costly and often involves environmentally harmful conditions, such as acid hydrolysis with sulfuric acid, which requires high temperatures and produces toxic residues **(Liu *et al.*, 2016 ; Ribeiro *et al.*, 2019).**

In recent years, there has been significant interest in using agro-industrial residues like cashew apple bagasse, rice straw, and spent coffee grounds (SCG) to develop nanomaterials **(Moghadam and Moeenfard, 2024).** SCG, a by-product of coffee brewing, is particularly promising due to its large annual production of about 60 million tons, making it an inexpensive and sustainable resource **(Forcina *et al*., 2024)**. The lignocellulosic components of SCG, including hemicellulose (39.1 %), cellulose (12.4 %), and lignin (23.9 %) **(Singh *et al.,* 2023),** can be converted into valuable nanomaterials. Notably, nanocellulose (NC) stands out for its exceptional properties, such as a large surface area, high elasticity, light weight, biodegradability, and biocompatibility **(Frost and Foster, 2020).**

## Objectives of the Practical Work

**At the end of this workshop, students should be able to :**

1. Recycling and recovery of agro-industrial waste (fruit and vegetable peels, coffee grounds and eggshells) for composting

2. Valorization of agri-food residues generated by the agri-food sector in the Oran region

3. Present the potential of agro-industrial residues as a valuable resource for the development of new products and materials.

4. Raise awareness of the importance of sustainability and waste reduction in the agri-food sector.

5. Develop design thinking skills through group work, with the aim of solving problems and transforming them into innovative solutions.

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## Techniques :

1. How to compost with a wooden composter
2. Extraction techniques for cell wall polymers from various substartes
3. Purification techniques for cellulose such centrifugation and filtration.
4. Synthesis techniques for Cellulose Nanocrystals, a commonly used biopolymer.
5. Formulation technique for bioplastics, such as blending with plasticizers and casting or extruding the mixture into a desired shape.

## 1. Visit to a Food Processing Plant and Collection of residues

Organizing an educational field trip to visit a food processing and waste collection facility in Oran offers numerous benefits for students. Such a visit would provide them with practical insights into industrial processes related to the agri-food sector, while also exposing them to challenges and innovations in waste management. These immersive experiences enhance theoretical learning by offering a tangible perspective on production chains and technologies employed in the industry. Furthermore, this initiative would raise awareness among students about environmental issues, such as sustainable waste management and valorization, which are particularly relevant in the Algerian context. By combining academic learning, practical training, and awareness of local challenges, this field trip would contribute to preparing future professionals to meet the needs of the agri-food sector and promote sustainable practices.

## 2. Brainstorming session on recycling ideas for collected residues

Waste valorization is a process that transforms waste materials into valuable resources, thereby reducing their environmental and economic impacts. Students from the enzymatic engineering cohort were divided into two groups (G1 and G2). Group 1 (G1) was further split into four subgroups (A, B, C, and D), each comprising teams of four students. These teams collaborated to develop ideas for repurposing waste, structuring their concepts, and sharing them with others. At the end of this exercise, each subgroup presented its proposals, which included:

- **Team A : Recycling**

Transforming waste such as coffee grounds, banana peels, potato skins, and sawdust into raw materials for bioproduct manufacturing.

- **Team B : Composting**
  Utilizing organic waste to produce natural fertilizers.

- **Team C : Energy Recovery**
  Converting waste into thermal or electrical energy through incineration or biogas production.
- **Team D : Bioenergy and Bioinputs**
  Repurposing agricultural residues like cocoa pods and cashew shells into fuel or natural fertilizers.

Following these presentations, a debate among the teams highlighted the importance of organizing brainstorming workshops, visiting waste valorization sites, and consulting experts in the field. These activities aim to deepen students' understanding of the challenges and opportunities

***Teams of Brainstorming session***

| Group 1 | | Group 2 | |
|---|---|---|---|
| AOUNALLAH RANIA | A1 | FARA AFNANE | A1 |
| BACHIRI AYA | A2 | GABES IKRAM | A2 |
| BELAL WAFAE | A3 | GHEIDENE LIDYA BOUCHRA | A3 |
| | | GHORISSI LAMIS | A4 |
| BELGHOMARI KHADIDJA | B1 | HAMMOUDA HADJER | B1 |
| BENAMOR MOHAMMED SALAH | B2 | KAID OMAR ASMAA AOUICHA | B2 |
| BENHALIMA IBTISSEM FATIMA ZOHRA | B3 | KEZADRI WISSEM | B3 |
| BENMANSOUR AMINA | B4 | LARBI MOHAMMED YASSINE | B4 |
| BENSAID MARIAM | C1 | MEDIENE NASSIMA | C1 |
| BEY AMAL | C2 | NABTI NOUR EL HOUDA | C2 |
| BOUARFA AMINA | C3 | SAKA AMANE | C3 |
| BOUKHARI SELSEBIL | C4 | SEBBAR LYLIA | C4 |
| BRAHIMI MANAL | D1 | TADJ FATIMA-ZOHRA-AYA | D1 |
| DOUAIDIA AYA | D2 | ZENBOU FARAH | D2 |
| DRICI MOHAMMED | D3 | ZIDOUR WAFAA | D3 |

## 3. Feasibility Study of Ideas Proposed by Students

The students proposed ideas for the valorization of agro-industrial residues, which were discussed and further developed by the four teams. After assessing the feasibility and availability of materials and equipment within the enzymatic engineering laboratory at the "ESSBO" higher school, two main approaches were selected.

First, we opted for composting kitchen waste to produce a natural fertilizer. This was carried out in the school garden using a wooden composter constructed by my colleague Khodja Badra.

Second, the students decided to valorize coffee grounds and sawdust by extracting cellulose to produce bioplastics. This initiative was also implemented during the workshop.

## 4. Execution of projects

This workshop will focus on the composting process and the production of biomaterials using two agro-industrial substrates: coffee grounds and sawdust **(Figure 1)**

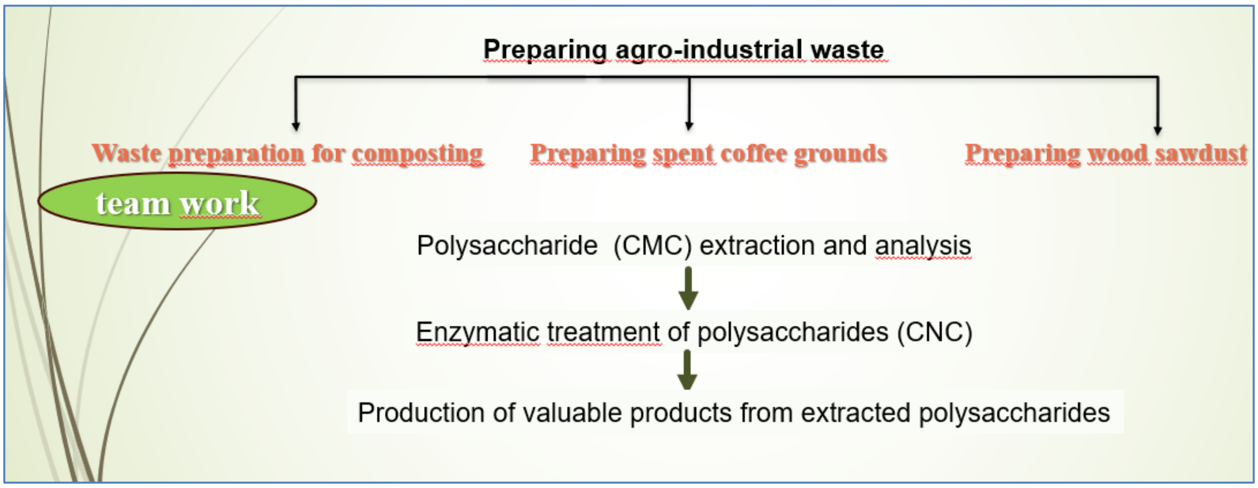


**Figure 1 :** Valorization workflow from raw waste to bioplastics

**It is essential that the student understands and adheres to the laboratory safety guidelines before commencing any experiment:**

- Familiarize yourself with the location and proper use of safety equipment, such as fire extinguishers, eye wash stations, and emergency showers.
- Do not eat, drink, or apply cosmetics in the laboratory. Always wash your hands thoroughly before leaving the laboratory.
- Always wear appropriate personal protective equipment (PPE), including a laboratory coat, gloves, safety goggles, and closed-toe shoes.
- Never work alone in the laboratory. Always inform a colleague or supervisor of your presence and activities before commencing any experiment.
- Handle all chemicals and equipment with care. Do not taste, touch, or inhale any substances unless explicitly authorized.
- Clearly label all containers and samples. Dispose of waste and hazardous materials in accordance with the instructor's guidelines.
- Keep your workstation clean and organized. Immediately report any spills, accidents, or injuries to the instructor or laboratory supervisor.
- Never conduct unauthorized experiments or modify equipment without proper training and prior permission.

## 4.1. Preparation of agro-industrial waste

### 4.1.1. Waste preparation for composting

### A) How to compost ?

**Composting** is an aerobic biological process that recycles fresh organic matter (OM) and energy contained within biodegradable waste. It is a waste management and recovery system that effectively controls and accelerates the decomposition of biomass by microorganisms (and macro-organisms) known as decomposers (**Leandro et Adriana, 2015 ; Kavita et Vinod, 2018).**

**Compost** is the end product of composting, resulting in a stabilized, sanitary material similar to dark brownish soil, enriched with humic compounds (**Zegels *et al.*, 2018**).

When the process involves earthworms acting synergistically with microorganisms, it is referred to as vermicompost **(Kavita et Vinod, 2018).**

**The objectives of waste recycling** through composting can be summarized as environmental, economic, and health interests. The following can be cited :

- Transforming end-of-life materials into a usable finished product: compost **(Fortin *et al.*, 2011 ; Faverial, 2016)**
- Reducing the mass and volume of biodegradable waste, thereby minimizing pollution (approximately 50% of the initial waste) (**Das et Keener, 1997**)
- Stabilizing solid and semi-solid organic waste (**Yulipriyanto, 2001)**
- Improving the fertility of agricultural soils by enriching them with elements that can be assimilated by autotrophic organisms (**Yulipriyanto, 2001)**
- Reducing (or even eliminating) pathogenic or phytopathogenic microorganisms that contaminate or develop on organic waste or colonize soils (**Fortin *et al*., 2011).**

### B) Decomposer microorganisms

**Bacteria** constitute the most substantial portion of the pre-existing microorganisms found in decomposing organic matter (**Strom, 1985 ; Diaz, *et al.*, 2007**). According to **Mustin (1987)**, bacteria are responsible for 80 to 90% of the microbial activity, with 40% of solid organic matter being almost entirely biodegraded by bacterial activity during the first 7 days of composting (**Strom, 1985**).

In compost, **actinomycetes** are second only to bacteria in abundance **(Mustin, 1987 ; Kaiser, 1983).** They participate in the decomposition of relatively complex organic substrates such as **cellulose, hemicellulose, and lignin** once the temperature exceeds 45 °C **(Yulipriyanto, 2001 ; Diaz *et al.*, 2007).** The decomposition activities of fungi and bacteria on easily degradable organic matter provide optimized humidity, aeration, and acidity (neutral or slightly alkaline pH) that promote the development of actinomycetes **(de Bertoldi *et al.*, 1983 ; Diaz *et al.*, 2007).**

### C) To achieve effective composting, follow these steps:

1. Place your composter in a well-ventilated area that is easily accessible **(Figure 2)**
2. Achieve Balance between Green and Brown Materials **(Figure 2)**, Green materials (kitchen waste, such as fruits and vegetables) provide nitrogen, while brown materials (dead leaves, straw) supply carbon.
3. Cut or shred waste, as smaller particles decompose more rapidly.
4. Exclude Undesirable Materials : Avoid adding meat, dairy products, fats, or animal waste, as these can attract pests and slow down the composting process.
5. Regularly mix the compost to enhance aeration, which accelerates decomposition.
6. If necessary, water the compost to ensure optimal moisture levels.
7. Add a layer of brown materials on top to reduce odors and maintain moisture.
8. Composting can take several weeks to months. Be patient and continue to maintain your compost pile.
9. Utilize the compost when it is mature, characterized by a homogeneous appearance, dark

color, earthy forest-like aroma, and a crumbly texture that breaks apart easily.

**Figure 2 :** Composting kitchen waste in a wooden composter at ESSBO

**QUESTIONS :**

**Name the main components needed for good composting**

**What are the characteristics of good compost**

**4.1.2. Preparing spent coffee grounds**

1. Gather spent coffee grounds from various sources, including residential and commercial settings such as cafeterias.
2. Transport the collected substrate to the laboratory while implementing appropriate measures to prevent contamination and moisture accumulation.
3. Grind the coffee grounds to enhance the efficiency of subsequent extraction processes.
4. Rinse the substrate repeatedly with hot water to eliminate any residual impurities until the rinse water appears clear **(Figure 3)**
5. Filter the substrate using cheesecloth to remove excess water.
6. Distribute the substrate evenly across a tray or baking sheet and dry it in an oven at 70 °C until a constant mass is achieved, indicating complete desiccation.
7. Once the substrate is fully dry, remove it from the oven and store it in an airtight container to maintain its integrity until further use.

**Figure 3 :** Preparing uesed spent coffee grounds

### 4.1.3. Preparing wood sawdust substrate

The wood sawdust was washed several times with hot water at 60 °C to remove hydrosoluble particles adhering to the surface and sap. The substrate was then dried in an oven at 70 °C until a constant mass is achieved, then crushed and sieved through a 1 to 2 mm diameter mesh **(Figure 4) (Anirudhane *et al*., 2019).**

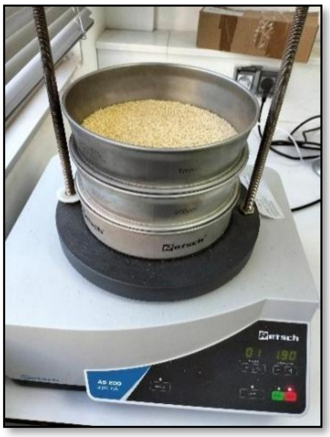

**Figure 4:** Sieving dried sawdust

### 4.2.Extraction and Analysis of Polysaccharides

#### A) Learning Objectives

In this part, the participants will be able to:

1. Distunguish between the cellulose extraction from the two substrates
2. Perform cellulose extraction from the spent coffee grounds
3. Show how polysaccharides are extracted from coffee grounds using kraft processes
4. using the soxhlet to extract extractives from sawdust
5. Show how to separate lignins from pictins and obtain pure cellulose

#### B) Principles

The treatment of **lignocellulosic** biomass with **hot water** and **nitric acid** affects the structure of the cell wall by detaching the pectins that are either highly methylated, therefore soluble in hot water, or weakly methylated and interconnected by calcium bridges, which require treatments acids.

**Ethanol precipitation** is a simple and effective method for isolating pectin from plant material and is commonly used in the food industry to obtain pure pectin for use as a gelling agent or stabilizer. Ethanol is commonly used as a precipitant for pectin due to its ability to disrupt the hydrogen bonding between pectin molecules and water. Pectin molecules are hydrophilic, meaning they have a strong affinity for water molecules due to the large number of hydroxyl groups present in their structure. When ethanol is added to a solution containing pectin, it competes with water molecules for hydrogen bonding with the pectin molecules. As a result, the pectin molecules become less soluble and start to precipitate out of solution. The degree of pectin precipitation depends on the concentration of ethanol and the pH of the solution.

**Sodium hydroxide** (**NaOH**) is commonly used in the pulp and paper industry to extract lignin from wood chips during the Kraft pulping process. Lignin is a complex polymer that provides structural support to the plant cell wall, and it needs to be removed to obtain pure cellulose fibres. **Sodium hydroxide** is a strong **alkali** that can break down the ester and ether linkages in lignin, resulting in its solubilization. When wood chips are cooked in a mixture of NaOH, the lignin is dissolved into the cooking liquor and can be separated from the cellulose fibres by filtration or centrifugation.

Sodium hydroxide (NaOH) treatment can also solubilize hemicellulose, in addition to lignin, during the Kraft pulping process. Hemicellulose is a polysaccharide that is more easily hydrolysed than cellulose, and it can be partially dissolved by NaOH under certain conditions.

During the Kraft pulping process, the NaOH solution breaks down the chemical bonds between the hemicellulose and other components of the wood, allowing it to be dissolved into the cooking liquor.

**Safety precautions !**

- Chemicals used in laboratory settings can be toxic, flammable, corrosive, and pose numerous other hazards.
- When handling acids (acetic acid, sulpheric acid, nitric acid, hydrochloric acid…) it is imperative to always add the acid to water, never the reverse, and similarly, always add sodium hydroxide to water, never the inverse.
- All use of solvents (toluene) and acids must be conducted under a chemical fume hood while wearing gloves, protective eyewear, and masks.

**Table 1 :** Pictograms and indications of danger

| **Products** | **Hazard pictograms** | **Warnings** |
|---|---|---|
| **Toluene** |  | Dangerous, harmful and irritating<br>Hazard to health<br>Easily inflammable |
| **Ethanol** |  | Easily inflammable |
| **Acetone** |  | Easily inflammable<br>harmful and irritating |
| **Sulfuric acid** |  | Hazard to health<br>Toxic<br>Corrosion |
| **Acetic acid** |  | Skin irritation and serious eye damage |
| **Nitric acid** |  | oxidizer<br>hazardous to the aquatic environment<br>Toxic<br>Corrosion |

| | | |
|---|---|---|
| **Hydrochloric acid** |  | Corosive<br>harmful and irritating |
| **Sodium hydroxid** |  | Corosive |

### C) Materials per group of Students

| **Products** | | **Equipments** |
|---|---|---|
| **Chemical** | **Biological** | Spatulas, pH meter, Stirrer plate with stirring bar, Latex gloves, Beakers, Balance, Cheesecloth, Desiccator, pumice stone, Tray or baking sheet, Oven, autoclave, Soxhlet extractor, reflux set-up |
| Sodium hydroxide , 95% ethanol, toluene, Acetic acid, nitric acid, Sulphuric acid, hydrochloric acid | food waste<br>Spent coffee grounds ;<br>sawdust ; | |

### 4.2.1. Cellulose extraction of spent coffee grounds

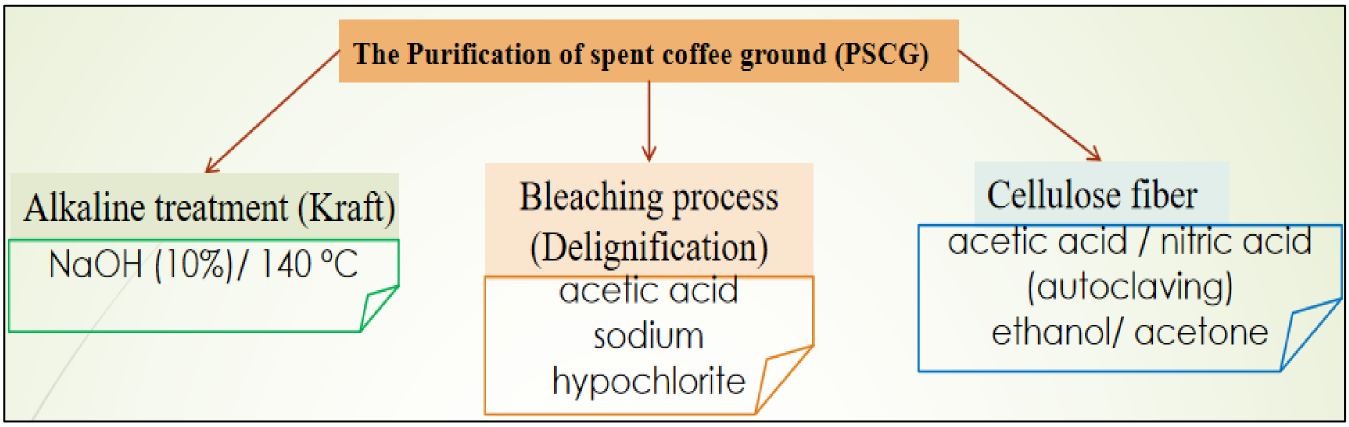


**Figure 5 :** Diagram of cellulose extraction from coffee grounds

**The Purification of spent coffee ground (PSCG) can be carried out using alkaline and acid processes :**

### A) Alkaline treatment (Kraft)

The alkali treatment process was carried out according to the procedures described in the literature (**Tapangnoi *et al*., 2022**) with few modifications.

1. In a Beaker, add 100g of SCG to 2L of a 10 % NaOH solution
2. Heat mixture at 140°C under magnetic stirring for 2h
3. Cool the mixture and filter under vacuum
4. Rinse filtrate with distilled water to completely remove the remaining hydroxide.
5. The alkali-treated SCG was later dried in an oven at 80 C for 24 h.

### B) Bleaching process (lignin extraction)

The bleaching process was carried out according to **Tapangnoi *et al*. (2022)** with few modifications. It was then performed to **remove lignin** in SCG by mixing it with a bleaching agent :

1. Add 50mL of acetic acid in 100 mL of sodium chlorite solution and 300mL distilled water at 140°C for 2h.
2. Repeat this treatment 3 times to ensure a complete removal of lignin.
3. The suspension was washed many times with distilled water till the smell of bleaching agent was removed, until a neutral pH is obtained
4. The suspension was then homogenized 500 rpm for 1 h and, dry the PSCG at 70°C until a constant weight is obtained
5. The % yield of the purification process was calculated using the following equation :

   Yield %= $\frac{Wf}{Wi} \times \mathbf{100}$

   **Wi :** weight of dried SCG

   **Wf :** the weight of dried PSCG.

**C) Purification of cellulose**

Determination of cellulose content in PSCG was carried out according to the method described by **Brendel *et al*. (2000).**

1. Mix the 10 g PSCG with 2 mL acetic acid (80% v/v) and 0.2 mL concentrated nitric acid in a bottle
2. Autoclave the mixture at 120°C for 20 min
3. After cooling, add 2.5 mL of cleaning liquid (ethanol) to the suspension.
4. Centrifuge the mixture to separate the solids (cellulose) from the liquids.
5. Repeat the cleaning process six times, using different cleaning liquids in the following order: 2.5 mL ethanol (twice), 2.5 mL deionized water (twice) and 2.5 mL acetone (twice).

**In this step, between each cleaning liquid, centrifuge and discard the supernatant**

7. After cleaning, dry the solid residue (cellulose) in an oven at 80°C for 24 hours.
8. Finally, The cellulose content was calculated as the weight percentage of solid residue to initial sample.

**QUESTION :**

What processes are used to delignify the wall of coffee grounds ? Explain the role of the alkaline agent.

### 4.2.2. Cellulose extraction from wood sawdust

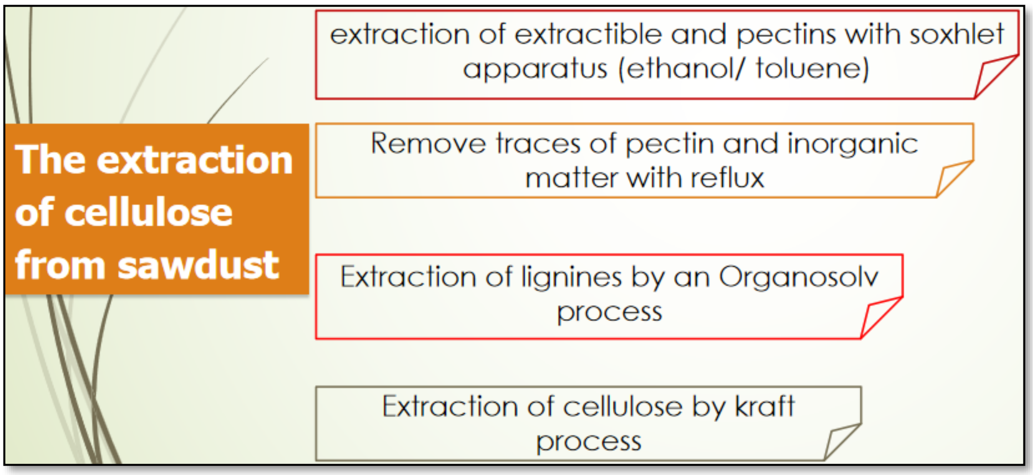


**Figure 6 :** Diagram of polysaccharides extraction from sawdust

### 4.2.2.1.Extraction of extractables and pectins

**Extractable substances** represent the only fraction that can be isolated without altering or degrading the other primary components of wood. These s

ubstances, which may hinder the pre-hydrolysis of sawdust, are removed through Soxhlet extraction. The solvents used facilitate the solubilization of these extractables present in the plant material, which are subsequently concentrated in the collection flask **(Mellouk, 2007)**

#### A) Soxhlet principle

Soxhlet extraction is a standard, conventional technique and the primary reference for evaluating the performance of other solid-liquid extraction methods **(Luque-Garcia *et al.*, 2004)**. A Soxhlet extractor is a glass device utilized in analytical and organic chemistry that facilitates the hot solvent extraction of a solid. This extraction process combines distillation with a "Soxhlet" type cartridge containing the solid product, which is impregnated with an active principle (solute) to be extracted through dissolution in a heated solvent.
The apparatus is named after its inventor, Franz von Soxhlet **(De Castro and Priego-Capote, 2010**).

#### B) Procedure of extraction

The selection of solvents is made to extract the maximum amount of extractables. A mass of wood is treated in 450 mL of a toluene/ethanol azeotropic mixture (1 :2, v/v) for 7 hours in a Soxhlet extractor to remove waxes, pigments, lipids, tannins, and fats **(Figure 7) (Rais, 2019).**
The solid was washed with distilled water and dried at 40 °C for 24 hours (**Mellouk, 2007 ; Benyoucef and Amrani, 2012).**

To extract **traces of pectin and inorganic matter**, the dried **lignocellulosic biomass** is treated (2 times) with 400 ml of **distilled water refluxed** at 85°C for 3 h **(Figure 7**) **(Rais, 2019).** The residue is filtered and washed with distilled water, then dried at 40°C for 24 h

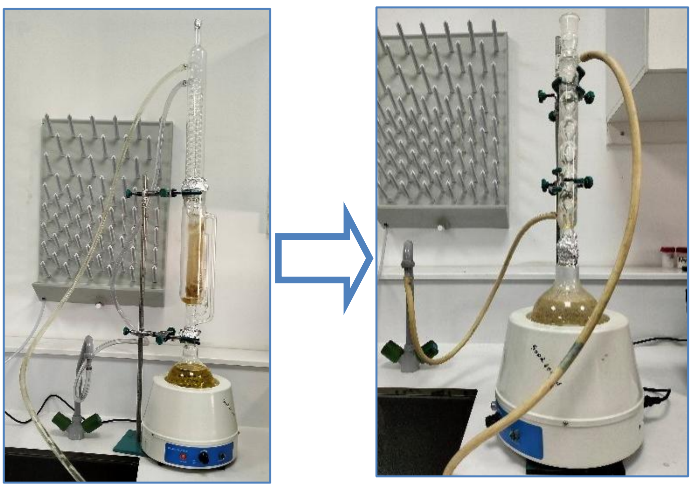

**Figure 7 :** Removal of extractables

- **The advantages of Soxhlet extraction** are as follows: the sample quickly comes into contact with a fresh portion of solvent, which aids in shifting the transfer equilibrium towards the solvent. This method does not require filtration after extraction, and it operates independently of the plant matrix **(Wolff, 1968)**
- **The most significant disadvantages** of this method, compared to other conventional techniques, include: a lengthy extraction duration and a substantial quantity of solvent consumed. This not only leads to economic losses but also raises environmental concerns **(Wolff, 1968).**

#### 4.2.2.2.Extraction of lignines by an Organosolv process

The **lignocellulosic biomass** obtained was subjected to **reflux** treatment with an **oil bath** using 400 ml of an aqueous ethanol solution (EtOH /H2O) (70/50, v/v) in the presence of sulfuric acid (1.25%) as a catalyst. The mixture was heated to 120 °C for 90 min. The residue was then filtered (**Figure 8),** washed with distilled water, and dried at 40 °C for 24 hours (**Koumba-Yoya and Stevanovic, 2016).**

The solvent used here is a mixture of water and ethanol. Water penetrates the wood to swell it and slightly degrade the hemicellulose, allowing ethanol to subsequently enter the wood to solubilize the lignin, which acts as a "glue" between cellulose and hemicellulose. Consequently, by removing this "glue" that binds hemicellulose and cellulose fibers together, a pulp is obtained, or more precisely, a liquid-solid mixture.The solid portion containing holocellulose, while the liquid portion consists of water, ethanol, lignin, and some impurities (primarily sugars).

**Holocellulose** is a mixture of cellulose and hemicelluloses. To ensure delignification, the unbleached solid obtained is treated with an aqueous solution of bleach (100 ml), and 50 ml of acetic acid is added to 300 ml of distilled water in a beaker at 60°C under agitation. This step is repeated several times (each for 45 minutes) to eliminate residual pigment traces and continues until complete **delignification** is indicated by the formation of a white pulp **(Rais, 2019).** The resulting white pulp is then vacuum-filtered and rinsed several times with distilled water until a neutral pH (pH 6-7) is achieved, followed by drying at 40°C for 24 hours **(Felissia *et al*., 2010)**

**Figure 8 :** Reflux treatment with an oil bath

#### 4.2.2.3. Extraction of cellulose by kraft process

The alkali treatment process (kraft) (**Tapangnoi *et al*., 2022**)

1. In a Beaker, holocellulose were placed in a 10% sodium hydroxide (NaOH) solution
2. Heat the mixture at 140°C under magnetic stirring for 2h
3. The cellulose obtained is filtered (hemicellulose is liquid) and rinsed with a 200 ml solution of 1% acetic acid, followed by distilled water to neutralize the pH.
4. Rinse filtrate with distilled water to completely remove the remaining hydroxide (Figure 9)
5. After filtration and drying at 40°C for 24 h, pure cellulose is obtained.
6. Derminate the yield of extracted cellulose by the following relationship:

   $Y\% = \frac{m1}{m0} \times 100$

Y% : Percentage yield.

m1 : mass in grams of extracted cellulose.

m0 : mass in grams of sawdust

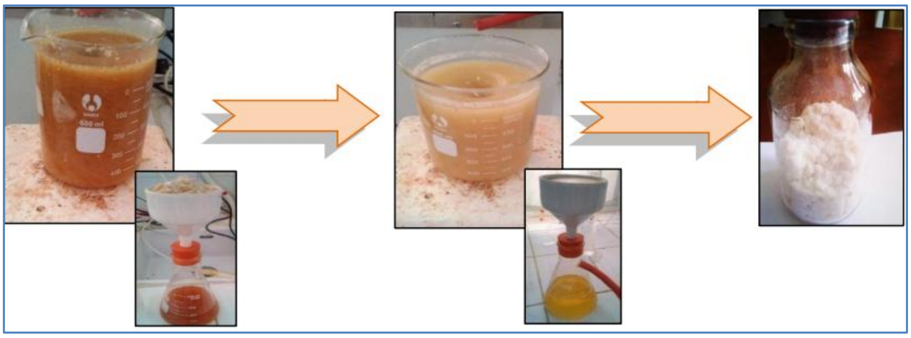

**Figure 9:** Steps for hemicellulose removal and obtation cellulose

**QUESTION :**

Correct the following statements:

- **Lignins are the major components of the sawdust wall.**
- **The extractable substances in sawdust include cellulose.**
- **Refluxing eliminates tannins and traces of pectins present in the substrate.**
- **When extracting with the Soxhlet, the inducer tube is used as the extraction chamber.**

### 4.2.3. Extraction of Cellulose nanocrystals (CNC)

#### A) Learning Objectives

In this part, the participants will be able to:

1) Explain the process of extracting nanocellulose from cellulose substrates
2) Explain the preparation of nanocrystaline cellulose bu using autoclave

#### B) Principles

Cellulose nanocrystals (CNC) are the derivative of cellulose which can be obtained through **acid hydrolysis** of cellulose, where the cellulose is exposed to **sulfuric acid** under controlled temperature and time period. For the preparation of nanocrystaline cellulose, we will use the autoclave

#### C) Materials per group of Students

| Products | | Equipments |
|---|---|---|
| **Chemical** | **Biological** | Spatulas, pH meter, Stirrer plate with stirring bar, Latex gloves, Beakers, Balance, Oven, autoclave |
| Sulphuric acid, | Microcristalline cellulose of Spent coffee grounds and sawdust | |

**Table 2 : Pictograms and indications of danger**

| **Products** | **Hazard pictograms** | **Warnings** |
|---|---|---|
| **Sulfuric acid** |  | Hazard to health<br>Toxic<br>Corrosion |

**D) Procedure**

A suspension of CNC was prepared from microcrystalline cellulose (MCC) via **acid hydrolysis** using **sulphuric acid** according to the reported method (**Lin *et al.*, 2012**)

1. (1g) of microcrystalline cellulose (MCC) was mixed with 30mL of sulphuric acid aqueous solution (63% wt) under vigorously stirring at room temperature for 1 h.
2. After that, the hydrolysis was quenching by adding iced water, 300 mL, into the mixture.
3. Te resultant mixture was frst centrifuged at 1000 rpm for 10 min to remove large particles, and then centrifuged at 11,000 rpm for 15 min to obtain cellulose nanocrystal (CNC).
4. Te obtained cellulose nanocrystal (CNC) was washed and centrifuged repeatedly for 3 times before
5. The remaining CNC suspension was autoclaved at 60°C for 1h.
6. The obtained CNC was processed by ultrasonic processor to suspension better before further application

### 4.2.4. Physico-chemical characterization of cellulosic samples

#### 4.2.4.1. Fourier Transform Infrared Spectroscopy (FTIR) Analysis

The various cellulose samples were analyzed using Fourier Transform Infrared Spectroscopy (FTIR). This analysis was conducted with a "Bruker Alpha FT-IR Spectrometer" equipped with an ALPHA single-reflection ATR (Attenuated Total Reflectance) platinum module and a diamond ATR module. The samples were examined directly without any prior preparation **(Benyoucef and Harrache, 2015).**

#### 4.2.4.2. Analysis by Estimation of Crystallinity Index via X-Ray Diffraction (XRD)

XRD is a physicochemical analytical technique used to characterize the atomic arrangement and layering of atoms within crystalline materials. It enables the identification of minerals present in each sample by analyzing how atoms are spatially organized in a crystal lattice.

Cellulose is linear and forms intramolecular and intermolecular hydrogen bonds arranged in a regular and ordered system with properties similar to crystals **(Michaud, 2003).** Cellulose is composed of individual fibrillar units consisting of long crystallite periods interrupted by completely disordered zones, designated as amorphous. It is recognized that chemical treatment affects the crystallinity rate of cellulose fibers. The crystalline zones resist attack by diluted acid, while the amorphous parts are destroyed **(Segal *et al.*, 1959).**

To determine the effect of chemical treatment on cellulose crystallinity, we opted for determining the crystallinity index using the empirical method of XRD peak height developed by **Segal et *al.* (1959)** and **Safou-Tchiama (2005).** This method involves examining changes in XRD spectra after chemical treatment. The crystallinity index (CrI) was calculated from the ratio of the peak height of 002 (I002) and the minimum height (IAM) between the 002 and 101 peaks using equation (1) **(Segal *et al.*, 1959)**. This method is useful for comparing relative differences between samples before and after chemical treatment. The sampling technique used is diffuse reflectivity. Powder samples were scanned for wavenumbers 0-4000 cm^-1 and 2θ degrees (0-50°). The X-ray diffractometer used is a D8 Advance BRUKER type, utilizing monochromatic K-alpha1 copper radiation (CuKα-radiation) (λ = 0.154 nm) at 45 kV and 40 mA in continuous scanning mode.

CrI= ((I002-IAM) / I002) x 100 (1)

The condition for constructive interference—and thus diffraction—is described by Bragg's Law: $n\lambda = 2d \sin\theta$ ; where: n is the order of reflection, λ is the wavelength of the incident X-rays (in Ångströms), d is the interplanar spacing (distance between adjacent crystal planes, in Ångströms), and θ is the angle of incidence of the X-ray beam **(Berthoumieux, 2012; Dillmann et Bellot-Gurlet, 2014).**

### 4.2.4.3.Analysis by Scanning Electron Microscopy (SEM)

The instrument used is an environmental scanning electron microscope (HITACHI TM-1000) equipped with an energy-dispersive X-ray spectrometer (EDX), allowing for the analysis of non-conductive samples such as wood. Initially, the sample to be analyzed is attached to a circular metal sample holder, which is compatible with the microscope's specimen stage. The device containing the sample is placed in the microscope's internal chamber and can be moved using micrometers to facilitate scanning **(Benyoucef and Harrache, 2015).**

The sample does not undergo prior metallization because the microscope features controlled pressure and a backscattered electron detector that produces primary electrons from a tungsten filament heated to 2700 K. The obtained micrographs enable observation of the microstructure on the surface of untreated and treated wood shavings. This observation allows for determining the effect of the chemical treatment used on the microstructure of the wood shavings **(Benyoucef and Harrache, 2015).**

#### 4.2.4.4.UV-Vis spectroscopy

Ultraviolet-visible (UV-Vis) molecular absorption spectrophotometry is a fundamental analytical technique employed for both qualitative and, more importantly, quantitative analysis of a wide range of organic and inorganic compounds. The relevant spectral regions span wavelengths from 200 to 400 nm for ultraviolet (UV) radiation and from 400 to 800 nm for visible (VIS) radiation.

The principle of this technique lies in the electronic transitions of electromagnetic energy, valence electrons are promoted from a lower energy state (En) to a higher one (En+1), or vice versa.

UV-Vis radiation interacts with matter. This interaction provides qualitative insights into molecular structure. These insights are deduced from the spectral profile. This is typically done through measurements of absorbance or transmittance across different wavelengths. Quantitative information is obtained by analyzing the intensity of absorption or emission, which correlates directly with the concentration of the absorbing species in solution **(Imelik et Védrine, 1988 ; Biémont, 2008).**

**QUESTION :** Cite other methods for chemically characterizing these materials

### 4.3. Enzymatic treatment of polysaccharides

Thermal and mechanical pre-treatments are identified as efficacious strategies for disrupting lignocellulosic bonds within the fiber fraction, thereby enhancing the surface area of the material and facilitating enzymatic interaction. This results in an improved digestibility of the ingredients. However, the intensity and duration of thermal treatments exert a significant influence on the ultimate digestibility. Mechanical pre-treatment is designed to reduce the particle size of substrates, which typically yields more digestible ingredients. Nonetheless, determining the optimal particle size of cellulose is crucial to achieve optimal nutrient and energy digestibility as an ingredient **(San Martin *et al.*, 2023).**

Concurrently, enzymatic hydrolysis possesses the potential to enhance digestibility by degrading fiber fractions. Hydrolysis involves the cleavage of bonds to produce fibers of varying sizes. This process must be consistently tailored to the characteristics of both the initial product undergoing hydrolysis and the final product to be obtained. Consequently, the optimal conditions for hydrolysis treatment are critical and necessitate precise definition.

various cellulolytic enzymes were employed to degrade the fibre fractions of cellulose. Additionally, thermal pre-treatment was assessed as a method to enhance the accessibility of cellulose and hemicellulose fractions to enzymatic action **(San Martin *et al.*, 2023).**

The initial cellulose sample was bisected. One half was reserved for subsequent analysis. The other half underwent thermal pre-treatment via autoclaving at 121 °C for 15 minutes. Both resulting subsamples were then further divided into two equal portions. One portion of each subsample was preserved in its original state for further analysis

The hydrolysis conditions were established based on the technical specifications provided in the enzyme data sheets from Novozymes. The process was conducted at a pH of 5, a temperature of 55 °C, and a stirring speed of 250 rpm for 20 hours, using a substrate-to-water ratio of 1:1 and an enzyme concentration of 1% (w/v) relative to the fiber content. Hydrolysis was carried out using a Sell Symphony 7100 Bathless Dissolution Distek system (Distek Inc., North Brunswick, NJ, USA), with precise control and monitoring of temperature, duration, and stirring speed. The pH was manually adjusted during each experimental run using 1 M NaOH, maintaining a final reaction volume of 500 mL.

To terminate the enzymatic activity, the reaction mixtures were heated to 90 °C for 15 minutes. Subsequently, the samples were centrifuged at 2650×g for 15 minutes at room temperature, yielding two distinct fractions: a solid fraction (intended for animal feed) and a liquid fraction (excluded from animal feed consideration in this study). The liquid fraction was collected from all treatments except control (CTR). This procedure was repeated in triplicate. After processing, the samples were freeze-dried and stored in sealed plastic bags until further physicochemical analyses and in vitro organic matter digestibility (IVOMD) assessments **(San Martin *et al.*, 2023).**

### 4.4. Production of Value-Added Products from Extracted Polysaccharides Learning

#### A) Objectives

In this part, the participants will be able to :

1) Explain the production process of bioplastics
2) Study biofilm from different cellulose substrates
3) Analyze the properties of the bioplastic by calculating different parameters

#### B) Principles

CNC has high aspect ratio, high surface area, and excellent mechanical properties, including high stiffness and strength. These properties make CNC a good reinforcing agent for **bioplastics**, improving their mechanical strength, thermal stability, and barrier properties.

Bioplastics, while inherently flexible and biodegradable, often require the addition of **glycerol** plasticizers to optimize their properties. These plasticizers work by weakening intermolecular hydrogen bonds within the material. However, this modification can influence both biodegradability and mechanical characteristics, creating a balance between functionality and environmental performance **(Fauziyah *et al*., 2021)**

**Starch /agar agar** plays a crucial role in the production of bioplastics, serving as a biomaterial based on biomass. It is used as a binding agent and can be transformed into biocompatible polymers, contributing to the creation of biodegradable plastics.

**Acetic acid :** plays a crucial role as a cross-linking agent in the production of bioplastics, notably by improving the structure and mechanical properties of polymers. By promoting the formation of covalent bonds between polymer chains, acetic acid helps to increase the strength, durability and thermal stability of bioplastics. It also improves their compatibility and biodegradability, which is essential for environmental applications.

**C) Materials per group of Students**

| **Products** | | **Equipments** |
|---|---|---|
| **Chemical** | **Biological** | Spatulas, pH meter, Stirrer plate with stirring bar, Latex gloves, Beakers, Balance, Tray or baking sheet, Oven, Glass Petri dishes, |
| Acetic acid, hydrochloric acid, Starch , Glycerine, agar agar | Microstalline cellulose | |

### 4.4.1. Determination of bioplastic properties

The properties of bioplastics are numerous, including those that can be realized in our laboratory: thickness, moisture content, water solubility, Water uptake and biodegradability test.

**In this workshop, we will detail various protocols for the production of bioplastic to compare their proprieties.**

- ✓ **The thickness** of the bioplastic film (2 cm × 2 cm) was determined by taking fifteen measurements at different locations using a digital Vernier caliper **(Oluwasina et *al*. 2017)** and calculated according to this equation **(Piergiovanni and Limbo, 2016) :**

$$\text{Thickness} = \frac{\text{sum of measured values}}{15}$$

- ✓ **The moisture** content was determined using Equation **(Cazón *et al*., 2017).** The initial weight ($w_1$) of the bioplastic film (dimensions : 2 cm × 2 cm) was recorded, **followed** by measuring the final weight ($w_2$) after drying the sample in an oven at 105 °C for 3 hours **(Oluwasina et *al*. 2017)**

$$MC\ (\%) = \frac{w_1 - w_2}{w_1} \times 100$$

- **Bioplastic film water solubility** was determined by measuring the mass loss of dissolved components after aqueous immersion. Samples (2 cm × 2 cm) were first dried to constant weight ($w_i$) at 105°C using a hot-air oven and cooled in a desiccator to prevent moisture absorption. The preconditioned films were then immersed in 40 mL deionized water under constant agitation (175 rpm) at 25°C for 24 hours. After immersion, residual insoluble material was collected, redried to constant weight under identical oven conditions, and weighed ($w_f$) **(Rhim et *al.*, 2007).** Water solubility percentage was calculated using **(Pajak et *al., 2019)*:**

$$\text{Water Soluble Matter}\ (\%) = \frac{w_i - w_f}{w_i} \times 100$$

- **Water uptake :** A pre-dried bioplastic sample (dried for 3h, 105 °C) (initial weight $w_0$) was immersed in water for 24 hours under continuous agitation. After immersion, the sample was removed, surface water was wiped off, and its final weight (wf) was recorded **(Oluwasina *et al.*, 2015).** The percentage of water absorbed was calculated using equation **(Jiménez-Rosado et *al*., 2019) :**

$$\text{Water uptake}\ (Wt\ \%) = \frac{W_t - W_0}{W_0}\ X\ 100$$

- **The biodegradability test** was conducted as follows: A plastic container with ten perforations on its sides and bottom was filled with 200 g of moist soil. A bioplastic sample of known initial weight ($w_0$) was buried at a depth of 3.5 cm for 24 hours. After this period, the sample was exhumed, cleaned, oven-dried at 105 °C for 3 hours, and then weighed (wt). To maintain soil moisture, 10 mL of deionized water was added to the soil in the container before reburying the sample for another 24 hours. The process of unearthing, cleaning, drying, and weighing was repeated until approximately 70% of the sample's initial weight was lost **(Oluwasina et *al*. 2017).** The biodegradability of the bioplastic was calculated based on its weight loss using Equation **(Castillo *et al.*, 2015) :**

$$WL\ (\%) = \frac{W_o - W_t}{W_o} \times 100$$

**QUESTION :** Give other bioplastic properties

We will use different protocols for bioplastic production to compare its various mechanical properties mentionned above

### 4.4.2. Utilization of additif for bioplastic Production

- First, the eggshells (additive that improves the regidity of bioplastics) ($CaCO_3$ interacts with the polymer chain) were crushed into smaller pieces and placed in sodium hypochlorite solution.
- The mixture was stirred with a glass rod until it became warm and released bubbles.
- The beaker was then covered with aluminum foil and left to stand for 24 hours.
- After this period, the sodium hypochlorite solution was removed, and the eggshells were washed with water.
- Subsequently, the eggshells were placed in an oven at 50°C and left for an additional 24 hours.
- After this drying period, the eggshells were ground using a mill until a fine powder was obtained.
- Finally, the eggshell powder was sieved through a 63 μm mesh to achieve a uniform particle size.
- For a production of bioplastic, in a volume of 50 mL of distilled water, 1g of agar and cellulose were added and continuously mixed at 70°C.
- After the initial mixture was thoroughly homogenized, 2.5 g of eggshell powder, 5 mL of glycerol, and 5 mL of acetic acid were added to the mixture. Subsequently, a milky white liquid appeared in the beaker.
- When it became sticky and nearly transparent, the mixture was spread onto aluminum foil.
- Finally, the biofilm was allowed to cool at room temperature.

### 4.4.3. Additive-free bioplastic Production (Rais, 2019)

- 1g of cellulose and 1g agar were added to 50mL of distilled water and mixed continuously at room temperature.
- After complete homogenization of the initial mixture, 2mL glycerol and 2mL acetic acid were mixed at 90°C.
- When it became sticky and nearly transparent, the mixture was spread onto aluminum foil.
- Finally, the biofilm was allowed to cool at room temperature.

### 4.4.4 Bioplastic production by neutralizing Ph

- 1g of cellulose and agar, 2mL of glycerol were added to 25mL of distilled water and mixed continuously at 150°C for 15min (no boiling).
- After complete homogenization of the initial mixture, 3mL HCl (0.1mol) and 25mL of distilled water
- At 7min, 1mL of NaOH (0.1mol) was added to neutralize
- At 15min, 2 mL of NaOH (0.1mol) was added to reduce viscosity of the mixture.
- Finally, the biofilm was left to cool in the open air for 5 minutes, then dried at 90°C for 1 hour.

**QUESTION :** additives are added to bioplastics to improve their mechanical properties. Here are a few examples

## 5. Evaluation, Discussion and Conclusion

The evaluation of this workshop for first-year students will be conducted as follows:

- **Final report (30%)** will be submitted at the end of the workshop sessions. In this report, students are expected to write a summary, introduction, describe the materials used, and outline the experimental methods. They will present the results obtained, discuss them using scientific articles, and finally conclude with a list of bibliographic references.
- **Laboratory Notebook Evaluation (20%) :** Students should record all activities conducted during the sessions, including materials, working methods, and any modifications made. They should note the results and any constraints encountered. Students are also expected to provide feedback, highlighting the strengths and weaknesses of the workshop to facilitate improvement.
- **Attendance and Participation (10%) :** Students will be assessed on their attendance, handling of equipment, seriousness, and participation
- **Quizzes (40%) :** Students will answer quizzes during the workshop to evaluate their comprehension level.

The workshop organized with students to valorize agro-industrial waste is a remarkable initiative that combines several important aspects: innovation, collaborative learning, and environmental awareness.

**a) Group Work and Creativity:**

- The fact that students worked in groups to propose waste valorization ideas undoubtedly fostered creativity and innovation. This collaborative approach allows participants to share perspectives and learn from each other.
- It also enabled students to develop essential skills such as communication, problem-solving, and collective decision-making.

**b) Socio-Economic Objective:**

- The objective of remaining within a socio-economic framework is crucial, as it implies that proposed solutions must be viable and beneficial to both society and the economy. This encourages students to consider the global impact of their ideas.
- This could include considerations of sustainability, profitability, and the potential social impact of products or services created from valorized waste.

**c) Presentation and Discussion of Ideas:**

- Learning to present and discuss ideas is a key skill in any collaborative project. This allows participants to clarify their thoughts, receive constructive feedback, and improve their proposals.
- The presence of instructors to guide and evaluate the feasibility of protocols certainly added value in terms of expertise and support.

**d) Cellulose Extraction and bioplastic Production:**

- Extracting cellulose from agro-industrial waste is a process that can be complex but highly promising. By using two different protocols, students were able to compare the results and efficiencies of each method.
- Producing bioplastics from these substrates is an excellent example of waste valorization. Bioplastics are a more sustainable alternative to traditional plastics, which can help reduce plastic waste and promote a circular economy.
- By using different procedures to produce bioplastics, students were able to analyze and compare the mechanical properties of the biofilms obtained. This is essential for understanding how to optimize the production process and improve the quality of the final product.

**In conclusion,** this workshop was an enriching experience for the students, allowing them to develop important skills while contributing to a crucial environmental cause. The valorization of agro-industrial waste to produce bioplastics is a promising field that can have a significant impact on waste reduction and the promotion of a more sustainable economy. The skills acquired by the students, combined with their creativity and commitment, are valuable assets for the future of environmental innovation

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## Appendix

During the preparation of this educational material, the **artificial intelligence tool Perplexity** was employed to translate the content into scientific English. Perplexity is an AI-powered conversational platform that integrates an advanced search engine with a conversational agent capable of analyzing and synthesizing real-time information from the web. Leveraging its natural language processing capabilities, the tool enabled the production of a precise, coherent, and contextually appropriate translation for scientific purposes, While preserving the original intent and clarity of the text. This resource facilitated a reliable translation process, complemented by an intuitive interface and access to verifiable source references.