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Production of Bacterial Cellulose from Wine by-Products

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***Abstract*— Wine cultivation and production play a vital role in the agricultural industry and serve as a cornerstone of the rural economy in the Mediterranean region. Surprisingly, Greece, despite its relatively small land area, manages to hold 2% of the international market. In 2020 alone, 120 million tonnes of wine were produced worldwide, with 50 million tonnes coming from European vineyards. Greece, in particular, harvests 600,000 tonnes of grapes annually for the production of both red and white wine. In addition to wine production, the winemaking process creates a significant amount of waste. This waste is often disposed of in open spaces, leading to environmental pollution. Bacterial cellulose has a wide range of current applications and has great potential for future use. Bacterial cellulose has a wide range of current applications and has great potential for future use. Its numerous and unique properties make it valuable in a variety of industries, including food production, biogenerics, medicine and other technical fields.**

Keywords— winemaking; wine by products; circular economy; Bacterial cellulose

I. INTRODUCTION

The food sector is responsible for generating a huge amount of waste and by-products, estimated at around 95-98 million tones per year. Specifically, wine lees, which make up about 2% to 6% of the total volume of wine, consist mainly of various components such as ethanol, tartaric acid, yeast cells, polysaccharide complexes, polyphenols, and inorganic matter [1]. If we take into account the total volume of wine sludge produced worldwide in 2023, it amounts to a staggering 10. 32 million hectoliters. This significant quantity is a challenge for countries where winemaking plays a critical role in their economy, as the efficient management and utilization of this winemaking waste, known as lees, is of paramount importance. This is a major environmental and social challenge for our culture [7].

1. Wine-related waste

European Regulation 337/79 defines lees as the residual material remaining after completion of the must fermentation process and after approved biological treatments and filtration or centrifugation of the fermented product. The term "sludge" is also used to describe the mixed substance formed during the transport of the white wort prior to fermentation [6]. Throughout the winemaking process, the components of the lees are gradually reduced through maceration and their composition varies depending on factors such as the type of grape, the stage of vinification and the specific operations [23]. After the completion of the vinification process, the remaining lees can be classified as a waste product with various characteristics. These include a low pH level, low electrical conductivity and a high concentration of phosphorus and potassium [15]. However, they are low in micronutrients and heavy metals. In addition, wine lees have a high demand for biochemical and chemical oxygen, which makes them a source of environmental pollution. Enzymes found in the lees also help to break down the polyphenolic substrate, resulting in the production of valuable phenols such as gallic acid. Because of these properties, the leaching of the lees is extremely difficult. The high variability of the compounds present in the wine lees

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allows it to be used as a fermentation activator after enzymatic or thermal treatment or as a source for obtaining pure mannoprotein extracts, the use of which improves the stability and sensory properties of wines [88]. Therefore, ageing wine in contact with lees can change the sensory characteristics of the finished wine.

Characteristic parameters of the lees	
Parameters	***Range of prices***
Total dissolved solids (mg/l)	171500 ± 22300
Total volatile solids (mg/l)	152700 ± 12800
Suspended solids (mg/l)	145300 ± 15200
COD (mg/l)	122000 ± 8400
BOD5 (mg/l)	67500 ± 6250
Ph	4.2 ± 1

Table 1 . Characteristic parameters of the lees

In the traditional aging procedure, only a little portion of the sediment left over from the vinification process is employed. Ageing wine in these sediments, known as lees, is typically done with sediments obtained mostly from the fermentation process. These lees are composed primarily of microorganisms related with winemaking, such as yeasts and bacteria [5]. Furthermore, tartaric acid and inorganic materials make up a lesser proportion of the lees' makeup. The ageing of sparkling wine, which occurs after the foaming process, is an example of how wine lees are used in winemaking [21].

In the case of red wine, the presence of colloidal lees prevents the precipitation of tannin and anthocyanin complexes, resulting in improved colour stability [22]. Contact with the lees also enhances the body and roundness of the wine, creating a less astringent taste. Despite these positive effects during the ageing process, there is a risk of sulphur and unpleasant odours, mainly due to the consumption of oxygen by dead yeasts and the growth of harmful microorganisms. In addition, oenolapses may also be responsible for the presence of precursors and enzymes that, under favourable conditions, can lead to the synthesis of biogenic amines [90]. These amines, in turn, pose a potential risk to consumers due to their physiological effects.

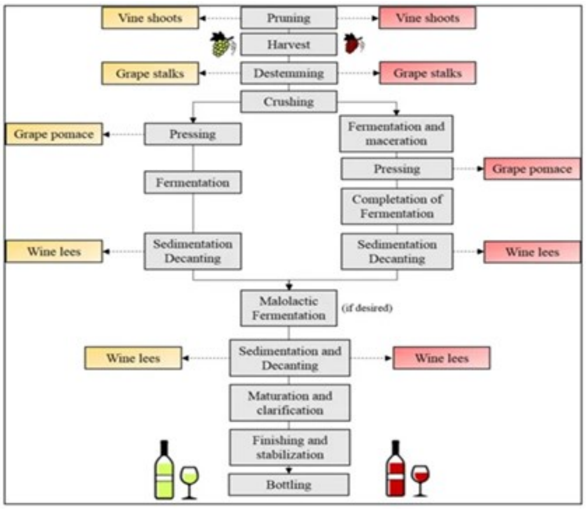

Fig. 1: A diagram showing the steps involved in making wine lees.

According to the European Council Regulation, the lees must be shipped for ethanol production. This ethanol is rich in aromatic compounds derived from wine and can be used in the production of alcoholic beverages. However, this practice is becoming less attractive due to dwindling financial support from Europe. Some wineries choose to accumulate wine lees together with other by-products of winemaking to produce animal feed or compost, mainly as a means of reducing disposal costs rather than as a waste recovery strategy. The heterogeneous composition of wine sludge has led to its analysis for various purposes [10]. The presence of certain compounds in wine lees has generated commercial interest from the pharmaceutical and cosmetics industries. In addition, the high nutrient content of the wine sludge allows the creation of a culture medium for the growth of lactic acid bacteria. The mineral content of wine sludge has also made it a valuable resource for biodistillation experiments [29].

Over the last decades, numerous studies have shown that wastewater from winemaking has the potential to be a valuable alternative raw material to produce various bioproducts. Contrary to current practices, the proper use of waste produced by the winery could lead to significant socio-economic benefits. Given the rising energy costs and increasing demand for environmentally friendly materials, it is vital for energy-intensive industries to address their energy issues using waste materials [30].

Much more research into sustainable or alternative materials is required, as a large portion of the consumption of arable land and natural resources is unsustainable. Interest in bacterial cellulose as a novel functional material has grown. The scientific community has recently directed its attention toward developing appealing waste management strategies that offer superior nutrients for the development of bacterial cellulose, due to economic, social, and environmental factors [54]. Determining the natural polymer's characteristics based on the planned usage is the main goal of research. One biopolymer that is abundant on Earth is cellulose, which accounts for an astounding 1.5 trillion tonnes of biomass produced yearly.

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2. Bacterial cellulose

One of the most important components of plants' skeletons is the carbohydrate cellulose [16]. Since cellulose makes up more than half of all the carbon in the plant world, it stands out as the most abundant carbohydrate due to the enormous amount of plants that covers the planet [2]. It's a plentiful, versatile polymer with intriguing features and attributes. This homopolymer, which is made up of repeated D-glucose units, has a solid, linear chain structure. In particular, cellulose is recognized for its hydrophilic character, its lipophilicity, its capacity to be broken down by physical processes, its vast variety of chemical modification possibilities and its ability to produce flexible and partly crystalline threads [3,12].

Microorganisms and plants synthesize cellulose in two primary steps: first, they combine glucose units to create beta-1,4-glucan chains, and then they synthesis and crystallize those chains [4]. The following figure provides an illustration of this process.

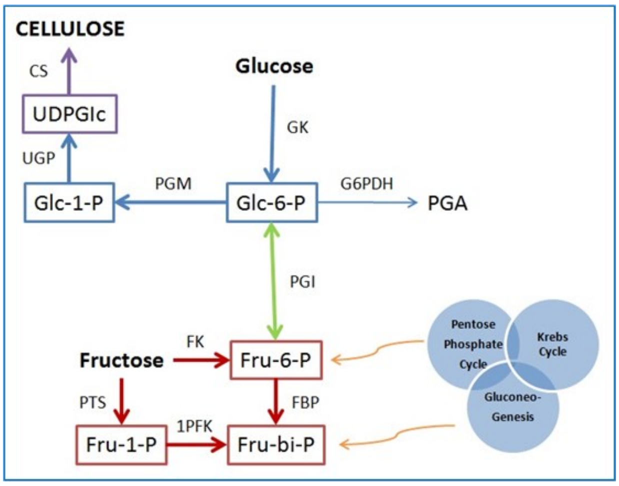

Fig. 2: Biosynthetic pathway of cellulose in microorganism cells [24].

An enzyme called cellulose synthase, which links glucose molecules in a particular pattern known as 1,4-beta-glucan chains, facilitates the manufacture of cellulose. Nevertheless, a thorough knowledge of how the polymerization process transforms glucose units into glucan chains remains lacking [19,20].

Cell division results in the formation of a fiber network. This network progressively transforms into a BC, or branching cell membrane, which is visible on the culture medium's surface [8]. The pace of bacterial cell multiplication affects the creation of this dense network. Furthermore, the rate of bacterial cell growth is influenced by environmental parameters as physico-chemical conditions, carbon source availability, culture medium composition, and oxygen levels. These factors also affect network connectivity [9,28]. As a consequence, a gelatinous suspension forms on the culture medium's surface. Water is present in each layer of bacterial cellulose, and when this water is eliminated, chemical groups are free to create new hydrogen bonds between neighboring cellulose chains [11,27]. This procedure makes the cellulose structure more crystallinity.

II. MATERIAL AND METHODS

The production of BC was studied using different renewable raw materials derived from non-recyclable materials from the winery that are usually disposed of in the environment and create environmental problems. The first step involves the evaluation of several commercial monosaccharides such as glucose, fructose and galactose, as well as a disaccharide called lactose. The purpose of this assessment was to determine which carbon sources could be catalyzed by the microbial strain [18]. The bacterial strain used in this study was K. Sucrofermentans, DSM No.1597. At the same time, appropriate chemical analyses were carried out in order to determine the composition of the brewery and winery waste. The second step of the study aimed to identify the most efficient source of coal for BC production. A standard winemaking protocol was followed for the five white grape varieties and a similar protocol for the four red varieties. These substrates were used as alternative carbon sources for the production of bacterial cellulose. In addition, the main reagents used to conduct the experiment were:

1. Yeast extract
2. Peptone
3. Citric acid
4. Na2HPO4

III. ANALYSIS

All the wines produced were subjected to the necessary classical analyses for the determination of total and active acidity (pH), volatile acidity, reducing sugars and alcohol content.

The study aimed to investigate the effect of these substrates on bacterial cellulose production. In all fermentations, Hestrin-Schramm medium supplemented with glucose (referred to as HS-glucose) was used as a base [13,25]. The initial sugar concentration was maintained at 20 g/L throughout the experiments. The nutrient substrate used is derived from sustainable raw material and falls into the category of industrial waste, by-products or process by-products. Each fermentation process lasted 13 days and the production of bacterial cellulose, sugar consumption, FAN (free amino nitrogen) and water holding capacity (WHC) were analyzed at specific time intervals.

IV. III. RESULTS AND DISCUSSION

Based on the data obtained from the experiments, it was determined that glucose and fructose substrates showed greater efficiency in bacterial cellulose production compared to galactose and lactose. In particular, the highest BC concentration, reaching 0. 92 g/L, was achieved using glucose, resulting in a yield of 0. 045 g/g. Similarly, fructose also showed satisfactory BC production, with a yield of 0. 03 g/g. In contrast, galactose and lactose showed lower fermentation efficiency in terms of concentration and yield. The bacterial strain used in the study found it difficult to efficiently

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metabolize these carbon sources. This is confirmed by extensive research that reports limited BC production when lactose is used as a carbon substrate as this disaccharide lacks the gene responsible for beta-galactosidase, an enzyme necessary for the hydrolysis of lactose [14,17].

The graph below shows a comprehensive analysis of the C/FAN ratio after stabilizing the pH at the optimum value of 5. Notably, when the C/FAN ratio was set at 17. 3, a remarkable 52% reduction in total sugar consumption was achieved, resulting in a yield increase of only 0. 06 g/g. Although a slight reduction in FAN was observed during the first five days of fermentation, this reduction was negligible. Moreover, while productivity and yield were relatively low compared to the subsequent C/FAN ratios, the satisfactory sugar consumption can be attributed to the pH setting at 5

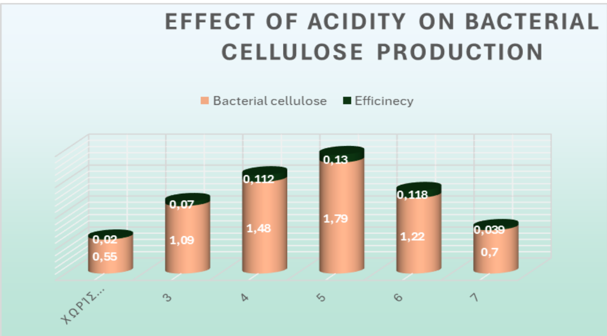

Fig. 3: Effect of different C/FAN values on bacterial cellulose production and yield

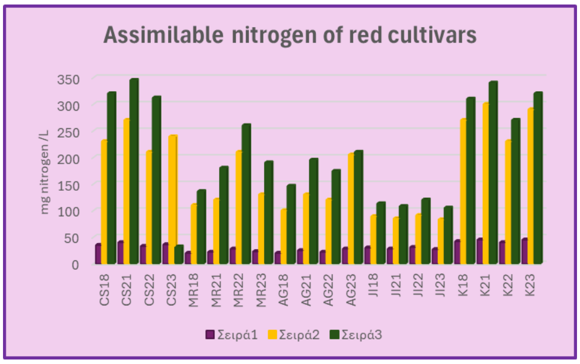

Fig. 4: Assimilable nitrogen by yeasts in mg nitrogen per liter in red cultivars.

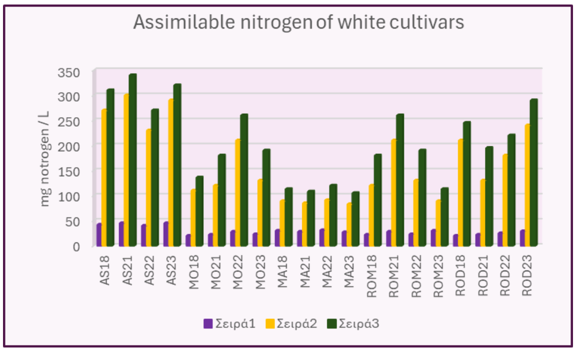

Fig. 5: Assimilable nitrogen by yeasts in mg nitrogen per liter in white cultivars.

V.CONCLUSION

After extensive research, it was confirmed that the bacterial cellulose formed in the by-products of vinification is mainly influenced by two critical factors: the acidity of the by-products and the specific type of grape used. In the analysis of the pooled data, it became apparent that sugars are consistently consumed over a pH range of 5. 1 to 5. 4. In particular, this pH range includes notable white grape varieties such as Cabernet Sauvignon, Merlot and Xinomavro. In addition, the use of sugars in this pH range shows remarkably high rates, ranging from 81% to 83%. In addition, nitrogen levels are significantly reduced during the fermentation process, eventually leading to the formation of resistant membranes. The efficiency of this whole process has been quantified at 0. 18 g/g.

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