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Use of Agro-Industrial Waste for Biosurfactant Production: A Comparative Study of Hemicellulosic Liquors from Corncobs and Sunflower Stalks

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Abstract: Biosurfactants have attracted considerable attention because of their lower toxicity, biocompatibility, and effectiveness over chemical surfactants. The use of renewable sources and the concept of sustainable production for such biomolecules supports the increased demand for eco-friendly products. Herein, the present study investigated corncobs (CC) and sunflower stalks (SS) as substitutes for conventional substrates in submerged fermentation with *B. subtilis*. The agro-industrial residues were submitted to an alkaline pretreatment to obtain hydrolysates rich in hemicelluloses, whose concentrations were determined at 48.8% and 65.7% for corncob and sunflower stalk liquors, respectively. The influence of different concentrations of glucose (0, 2.5, and 5%) and liquor (0, 20%, and 40%) were evaluated according to cell concentration, surface tension reduction rate (STRR), and emulsification index (EI_{24}). Biosurfactants obtained with the hemicellulose liquor of sunflower stalk showed the highest cell concentration (4.57 g/L) and STRR (58.07%), whereas the maximum values of EI_{24} (56.90% in hexane, 65.63% in toluene, and 64.86% in kerosene) were achieved by using corncob liquor. All top results were observed at 2.5% glucose, 20% liquor (CC or SS), and 1% mineral salts. Notably, excess glucose or liquor (CC or SS) negatively affected cell growth and biosurfactant performance. The results indicated the potential of corncobs and sunflower stalks as low-cost substrates to produce a high added-value biosurfactant with promising tensoative and emulsifying properties.

Keywords: agro-industrial waste; corncob; sunflower stalk; biosurfactant

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1. Introduction

Chemical surfactants are used worldwide in the most diverse application areas, which include the pharmaceutical, food, petroleum, environmental, and cosmetics industries [1,2]. This wide range of possibilities is associated with its diverse molecular structure and functional properties, since surfactants are amphiphilic molecules capable of reducing the surface and interfacial tension of different mixtures, increasing the solubility and bioavailability of hydrophobic compounds [3]. The globally most used surfactants are linear alkylbenzene sulfonate, alkyl sulfate, alkyl ethoxy sulfate, alkyl ethoxylates, alkylphenol ethoxylate, and quaternary ammonium-based compounds [4]. However, as non-biodegradable compounds,

the extensive use of surfactants results in severe accumulation in soil and aquatic ecosystems. They can depolarize microbial cell membranes and decrease nutrient absorption and oxygen acceptance, retarding microbial growth and increasing cell mutation and mortality [5]. Surfactants also affect aquatic plant growth, motility, and photosynthetic ability, besides reducing the efficiency of photochemical energy conversion, a crucial process for plant life [6,7]. In human bodies, surfactants can disrupt important enzymes, such as esterase and phosphatase, alter membrane permeability, and inhibit cellular respiration [8]. Their use can also be associated with eye and skin irritations [9].

Because of the damage linked to the non-renewable production of surfactants, these bioagents have additional advantages over conventional surfactants as they are less toxic, have high specificity and tolerance to extreme temperature, pH, and ionic strength, and have higher biocompatibility [10]. Microbial surfactants are secreted extracellularly or as part of the cell membrane. They reveal promising applications in different industrial sectors, such as cleaning, cosmetics, food, petroleum, environment, agriculture, and pharmaceuticals [11–14]. Evonik Industries, for example, launched a sophorolipid-based biosurfactant produced from *Candida bombicola*, with application in cosmetics, cleaning products, and dishwashing liquids [15]. The French startup Lipofabrik (Éléphant Vert Group subsidiary) uses *Bacillus* sp. to produce mycosubtilin and surfactin (lipopeptide biosurfactants), with pharmaceutical applications [16]. Jeneil Biosurfactant Co. also synthesized a rhamnolipid-based biosurfactant, which found a market in enhanced oil recovery and cleaning and oil recovery from storage tanks [17].

Several renewable sources can be used in fermentation processes, such as crop residues, by-products of food processing, and agro-industrial waste [18,19]. This can be observed in the work of Nalini and Parthasarathi [20], who performed a solid-state fermentation using mahua oil cake to produce biosurfactants from *Serratia rubidaea* SNAU02. Using *Pseudomonas aeruginosa* M408, Ji et al. [21], produced biosurfactants in media containing olive oil as the sole carbon source. Jadhav et al. [22] used sunflower oil refinery waste as a substrate for biosurfactant production via *Starmerella bombicola* MTCC1910. Vecino et al. [23], evaluated the utilization of cellulosic sugars extracted from vineyard pruning waste to synthesize biosurfactants with *Lactobacillus paracasei* A20. Recently, Vieira et al. [24] investigated using pineapple peel juice to produce biosurfactants from a *Bacillus subtilis* strain. This scenario opens the possibility of overcoming one of the main obstacles in the large-scale production of biosurfactants: high production costs associated with using expensive substrates. According to Makkar and Cameotra [25], agro-industrial wastes with high contents of carbohydrates and lipids are considered very useful in biotechnology processes. These wastes are derived from lignocellulosic biomasses, the most abundant and renewable feedstock available, whose utilization is a promising alternative to solve problems of food shortages, environmental pollution, and energy crises. As the main components of natural lignocellulose, cellulose, hemicellulose, and lignin form a compact structure that requires the application of a pretreatment step to break the crystalline structure—an essential step in bioconversion processes [26,27].

The primary goal of pretreatments is to break the hydrogen bonds and cross-linked hydrophobic interactions between cellulose, hemicellulose, and lignin, disrupting biomass' crystalline structure [28]. This will increase the accessibility of the polysaccharides to hydrolytic enzymes. The major pretreatment methods are divided into four different approaches, namely: physical (milling, grinding, microwaving, ultrasonication, and pyrolysis), physicochemical (steam explosion, carbon dioxide explosion, liquid hot water treatment, and wet oxidation), chemical (alkali, acid, ionic liquids, ozonolysis, and organosolv exposure), and biological (fungi, bacteria, and archaea treatment) [29,30]. According to Wyman [31], there are several criteria for selecting a suitable pretreatment, including the (i) preservation of hemicellulosic fractions; (ii) minimal formation of degradation products, such as furfural and hydroxymethylfurfural; (iii) low energy demand; and (iv) low-cost pretreatment catalysts and/or inexpensive catalyst recycling.

In the present investigation, corncobs and sunflower stalks were submitted to an alkaline pretreatment to extract hemicellulose. These two biomasses were chosen based on the large amount of agro-industrial waste discarded after the grain harvest. According to the FAO Statistical Yearbook 2021, the world corn and sunflower production is 1.15×10^6 and 4.8×10^4 thousand tons, respectively [32]. This is associated with a massive waste generation and environmental polluting agents. Considering a ratio between corn grain and corncob of 100:18 [33], approximately 20.034×10^7 tons of corncob are generated annually. On the other hand, Binici et al. [34] estimate an annual generation of 25.0×10^5 tons of sunflower stalks. Like other agro-industrial wastes, the organic potential of corncobs and sunflower stalks places them in a prominent position for the microbial production of biosurfactants for economic and environmental reasons. As abundant, renewable, sustainable, and low-cost biomasses, they are part of the concept of a circular bioeconomy [35]. In addition to reducing pollution related to improper disposal and burning practices, there is a decrease in expenses regarding industrial waste management [36].

Therefore, this work compared the use of corncobs, sunflower stalks, and a conventional carbon sources, such as glucose, as substrates for biosurfactant production by submerged fermentation. The impacts associated with the differences in culture media composition were evaluated through cell concentration, surface tension reduction, and the emulsification index. Contrary to what is commonly performed in the literature, the alkaline pretreatment used for hemicellulose extraction was applied under milder conditions of temperature, alkali concentration, and reaction time to avoid polysaccharide degradation, without losing the fractionation efficiency of the lignocellulosic complex. Until now, no report has been found comparing the role of corncob and sunflower stalk liquors as total or partial substitutes for glucose in biosurfactant production.

2. Materials and Methods

2.1. Bacterial Strain and Biosurfactant Production

The biosurfactant-producing strain *Bacillus subtilis* ICF-PC (CCMO/SE code: LMA-ICF-PC 001) was maintained on an inclined tube of nutrient agar at 4 °C.

For inoculum purposes, an aliquot of 10 mL pre-inoculum was added in 250 mL Erlenmeyer flasks containing 100 mL of 1% (*v*/*v*) glucose and 1% (*v*/*v*) of mineral salt solution (MSS), with a composition (in g/L) of NH_4NO_3 100.0; KH_2PO_4 102.0; Na_2HPO_4 142.0; $FeSO_4 \cdot 7H_2O$ 0.375; $MgSO_4 \cdot 7H_2O$ 4.93; $MnSO_4 \cdot 7H_2O$ 0.050; and $CaCl_2 \cdot 2H_2O$ 0.250 [37–39]. All samples were shaken for 24 h at 30 °C and 120 rpm.

The liquors obtained from corncobs (CC) and sunflower stalks (SS) were both used as partial carbon and nutrient sources for biosurfactant production. At this stage, experiments were carried out in 250 mL Erlenmeyer flasks containing 5 mL inoculum and 95 mL culture medium. After neutralizing the pH, the samples were kept for 72 h in a shaker for submerged fermentation at the same temperature and agitation conditions previously reported. The cell-free supernatant was obtained by separating the culture broth by centrifugation at 5000 rpm for 25 min.

It was used as a 1% MSS in all fermentative media, varying the type of liquor added, as well as the concentrations of glucose and liquor as follows: (i) 20% liquor and 2.5% glucose; (ii) no liquor and 2.5% glucose; (iii) 40% liquor and 2.5% glucose; (iv) 20% liquor and 5% glucose; and (v) 20% liquor and no glucose.

2.2. Waste Preparation

The corncobs were collected in the county of Poço Verde, State of Sergipe (10°42′11″ S 38°11′06″ W), Brazil, while the sunflower stalk was obtained at the Jacaré-Curituba Settlement, located in Poço Redondo, also in Sergipe (9°42′00.0″ S 37°44′00.0″ W), Brazil. Before submitting both wastes to the pretreatment process, they were separately crushed and dried at 45 °C for a 24 h period. The dried samples were ground in a knife mill under the following granulometric specifications: 35 mesh for characterization and 9 mesh for alkaline pretreatment. The modified Klason method [40] was used to characterize corncob

and CC liquor, while the characterization of the sunflower stalk and SS liquor followed the guidelines of the National Renewable Energy Laboratory [41,42].

Alkaline Extraction of Hemicelluloses

A mass of 10 g of each dried waste was mixed with 100 mL of NaOH solution (0.75 mol/L). Samples were shaken continuously at 50 °C for 2 h, followed by a cooling process. After neutralizing the pH of the resulting material, the hydrolysates were filtered and submitted to the characterization of carbohydrates and organic acids, furfural, and hydroxymethylfurfural, according to the same methodologies established in Section 2.2.

2.3. Cell Concentration

An aliquot of 1 mL of the fermented medium was centrifuged at 10,000 rpm for 20 min. After the supernatant removal, the precipitated mass was diluted, centrifuged again, and diluted again in 4 mL of distilled water. The bacterial growth was determined by spectrophotometry, with absorbance at 610 nm using a UV-M51 spectrophotometer (BEL Photonics, UV-VIS).

2.4. Surface Tension

The surface tension measurements were performed on Sigma 700/701 tensiometer (Attention) using the Wilhelmy plate method. The results were expressed in terms of surface tension reduction rate (STRR) and calculated from Equation (1).

$$STRR = [(ST_{H2O} - ST_{Bio})/ST_{H2O}] \times 100 \quad (1)$$

where ST_{H2O} is distilled water's surface tension, and ST_{Bio} is the biosurfactant's surface tension.

2.5. Emulsification Index

The emulsification activity of the cell-free supernatant was determined following the methodology proposed by Cooper and Goldenberg [43]. The hydrophobic compounds used were hexane, toluene, and kerosene.

2.6. Data Analysis

All the determinations were performed with three replicates. Data was used to determining means, standard deviations, and 95% confidence intervals. Therefore, the lack of intersection of confidence intervals determines a significant difference ($p < 0.05$). Data were analyzed using Microsoft® Excel® for Microsoft 365 MSO (version 2204 Build 16. 0. 15128. 20240) 64-bit.

3. Results

3.1. Chemical Composition Analysis of Corncob and Sunflower Stalk

Table 1 presents the characterization of CC and SS before the alkaline extraction. The results revealed that corncob had a higher content of hemicellulose (25.3%) and total lignin (34.7%), but a lower content of cellulose (26.2%); these results are in agreement with the results obtained in previous works [44]. Alternatively, the SS presented 35.6% cellulose, 17.1% hemicellulose, and 16.7% lignin. This distinction in compositions was expected, and it is strongly associated with the fact that different biomasses from different species have differences in their lignocellulosic compositions, cell types, and morphological characteristics [45,46]. Besides, the lignocellulosic composition also depends on the geographic region cultivated, with differences even among biomasses from the same country or region [47,48]. Table 1 also shows the presence of furan derivatives, such as hydroxymethylfurfural and furfural, in the two wastes, but at a low percentage. According to Almeida et al. [49], these compounds are considered fermentation inhibitors, causing harm to cell growth and

microorganism productivity, which is why it is so important to choose a pretreatment that does not increase the formation of these degradation products.

Table 1. Chemical composition of the corncob and sunflower stalk agro-industrial wastes before the pretreatment process.

Chemical Composition	Corncob (%)	Sunflower Stalk (%)
Cellulose	26.2 ± 0.6	35.6 ± 1.1
Hydroxymethylfurfural	0.2 ± 0.05	0.1 ± 0.03
Hemicellulose	25.3 ± 1.1	17.1 ± 0.8
Furfural	1.1 ± 0.2	0.5 ± 0.05
Total Lignin	34.7 ± 1.5	16.7 ± 1.3
Insoluble Lignin	22.5 ± 1.8	15.9 ± 1.7
Soluble Lignin	12.2 ± 1.2	0.8 ± 0.2

The hemicellulosic liquors obtained from the alkaline pretreatment of CC and SS agro-industrial wastes were also characterized, as seen in Table 2. Although the hemicellulose content was higher in the corn than in the sunflower residue, this relationship was reversed for the liquors: SS liquor had 65.7% hemicellulose, while CC liquor had 48.8%. Such an outcome can mean that the alkaline pretreatment was more effective in extracting the hemicelluloses when applied to the SS than when applied to CC, which is most likely to be the result of differences in some pretreatment parameters, such as (i) biomass crystallinity, since the lignocellulosic matrix is associated in different degrees, depending on the species and even the cultivation source; (ii) accessible internal surface area, which is dependent on the capillary structure of the cellulosic fibers; and (iii) hemicellulose and lignin content [50,51]. Moreover, in both hemicellulosic liquors, hydroxymethylfurfural and furfural showed values below the detection limits, another favorable factor for using these hydrolysates for biosurfactant production.

Table 2. Chemical composition of the corncob (CC) and sunflower stalk (SS) hemicellulosic liquors obtained after alkaline pretreatment.

Chemical Composition	CC Liquor (%)	SS Liquor (%)
Cellulose	9.8 ± 0.6	7.3 ± 0.8
Hydroxymethylfurfural	Not detected	Not detected
Hemicellulose	48.8 ± 1.2	65.7 ± 1.7
Furfural	Not detected	Not detected
Soluble lignin	13.5 ± 0.6	2.5 ± 0.8

Tables 1 and 2 show an increase in hemicellulose in the pretreated CC and SS due to the degradation of the lignin macromolecular structure. This behavior demonstrates the capacity of the extraction process to fractionate the polysaccharides efficiently, emphasizing hemicellulose. That being said, it is correct to affirm that the objective of obtaining a liquor mainly constituted of hemicellulose was accomplished, which was highly positive for biosurfactant production due to B. subtilis' ability to metabolize xylose—the second most abundant sugar in lignocellulose hydrolysates [39,52].

When comparing the amount of hemicellulose in the SS liquor with other scientific data already published, it was found that the result presented was higher than that obtained from alkaline routes by He et al. [53], whose hydrolysate had 24.1% hemicellulose. Sharma et al. [54] applied a steam explosion pretreatment in sunflower stalk samples, solubilizing 66.31% of hemicellulose, a similar result to that reported in the current study. In parallel, the content of hemicellulose in the CC liquor of the current study exceeded the maximum amount of 31.8% achieved by Du et al. [55], who applied the methodology of soaking the material in aqueous ammonia (SAA). Su et al. [56] and Yu et al. [57] used alkaline hydrogen peroxide to obtain 38.7% hemicellulose and simultaneous saccharification and co-fermentation to get 37.08% hemicellulose using corncob samples, respectively.

3.2. Effects of Glucose Concentration on Biosurfactant Production

It is well known that carbon sources play an important role in the metabolism of biosurfactant-producing microorganisms, influencing the bioproduct obtained in terms of structure, performance, and yield [58,59]. To investigate the influence of media composition on biosurfactant production, B. subtilis was cultivated in different substrate conditions. For this first step, CC and SS liquors were fixed at 20%, while the glucose content was tested at 0, 2.5, and 5.0%. Table 3 describes the results obtained for cell concentration, STRR, and emulsification index (EI_{24}) for hexane, toluene, and kerosene.

Table 3. Effect of different glucose concentrations fixing liquor content at 20% (either corncob or sunflower stalk) and supplemented with mineral salts at 1%.

Glucose (%)	Cell Concentration (g/L)			STRR (%)			EI_{24} (%)								
							Hexane			Toluene			Kerosene		
	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI
Tests with corncob liquor															
0	0.62	0.02	0.6; 0.64	42.47	0.23	42.44; 42.5	34.29	1.21	32.92; 35.66	28.66	2.32	26.04; 31.29	36.37	0.59	35.7; 37.04
2.5	2.92	0.17	2.1; 2.48	47.32	0.01	47.31; 47.33	56.90	1.60	55.09; 58.71	65.63	0.79	64.74; 66.52	64.86	0.69	64.08; 65.64
5.0	2.37	0.22	2.12; 2.62	44.58	0.03	44.55; 44.61	58.62	0.57	57.97; 59.27	25.40	0.93	24.35; 26.45	60.32	1.34	58.8; 61.84
Tests with sunflower stalk liquor															
0	1.25	0.01	1.24; 1.26	43.81	0.35	43.41; 44.21	3.70	0.05	3.64: 3.76	14.06	2.05	11.74; 16.38	7.56	0.16	7.38; 7.74
2.5	4.56	0.03	4.53; 4.6	58.07	0.06	58.0; 58.14	53.69	1.21	52.32; 55.06	3.23	0.81	2.31; 4.15	4.85	0.08	4.76; 4.94
5.0	3.57	0.07	3.5; 3.65	55.76	0.24	55.49; 56.03	5.15	0.84	4.2; 6.1	7.69	1.25	6.28; 9.11	8.95	0.67	8.19; 9.71

95% CI—95% confidence intervals; STRR—surface tension reduction rate; EI_{24}—emulsification index. There are significant differences ($p < 0.05$) between corncob and sunflower stalk liquors, for all the means.

In the absence of glucose, minimal values were obtained for cell concentration when using CC or SS liquors at 0.62 and 1.25 g/L, respectively. On the other hand, maximum cell concentration (4.56 g/L) was observed with a 2.5% glucose addition in media containing SS as a carbon source. For the corncob case, doubling the glucose content to 5% negatively affects cell concentration, decreasing it from 2.92 g/L to 2.37 g/L. Although there are different metabolic pathways for the biosynthesis of biosurfactants, hydrophilic substrates such as glucose are primarily used by the microorganisms for cell metabolism and to synthesize the biosurfactant's polar segment, whereas hydrocarbon substrates are used exclusively to produce the nonpolar segment [60]. Thus, the absence of glucose possibly harmed cell metabolism, affecting cell concentration values and the development of the polar portion of the bioproduct. Under this condition, biosurfactants presented a low performance in the emulsification tests.

Biosurfactants' abilities as surface tension reducers have been studied over the years because of the relevance of this property in industrial processes. The best result obtained was associated with SS liquor: water surface tension was reduced by 58.07% due to the biosurfactant action. This value was approximately 10% higher than that of CC liquor (47.32%) at the same concentration. Similar STRR results were found in work developed by Phulpoto et al. [61], who reported that a biosurfactant metabolized by Bacillus nealsonii SM2T reduced surface tension to 34.15 mN/m (equivalent to 47.43%, when expressed in STRR value). Using pineapple peel as a partial substitute for glucose and mineral salts in fermentation with B. subtilis, the biosurfactant extracted by Vieira et al. [62] presented a 57.71% STRR. Sharma et al. [63] produced a rhamnolipid from Franconibacter sp. IITDAS19, a bacterium strain isolated from crude oil-contaminated soil. The biosurfactant reduced the surface tension of water from 71 mN/m to 31 mN/m (STRR equivalent to 56.34%).

The analysis of the emulsification index values in Table 3 shows a significant difference ($p < 0.05$) among the hydrophobic compound used; however, in general, EI_{24} results associated with SS liquor were not relevant, except for the 53.69% EI_{24} in hexane (observed at 2.5% glucose). Now, considering the results with CC liquor, the emulsifying activity presented low values (34.29% for hexane, 28.66% for toluene, and 36.37% for kerosene) when the strain was cultivated in the absence of glucose. At 2.5% glucose, the best EI_{24} results were reported, especially for toluene (65.63%) and kerosene (64.86%). This behavior

suggests that the biosurfactant achieved higher stability and interaction with long-chain hydrocarbons (toluene) and mixtures containing both long-chain and aromatic hydrocarbons (kerosene). Nonetheless, upon increasing the glucose content again, the emulsifying activity decreased significantly, except for in the tests with hexane. The emulsification results discussed are comparable with others in the literature. Using a strain of Bacillus nealsonii, Phulpoto et al. [61] produced a biosurfactant with a 55% EI_{24} in kerosene, a value below that found in the current research (see Table 3); also using kerosene for emulsifying tests, a lipopeptide produced by Bacillus licheniformis in media containing frying oil and co-substrate glucose exhibited a 65% EI_{24} [64]. Selecting Bacillus aryabhattai and Bacillus velezensis as the biosurfactant-producing microorganisms, Singh [65] and Meena et al. [66] reported, respectively, an EI_{24} of 55% and 65.2% in toluene.

3.3. Effects of Liquor Concentration on Biosurfactant Production

This time, fixing the glucose concentration at 2.5% and evaluating other conditions for liquor concentration (0 and 40% *v*/*v*), the cell concentration, surface tension, and emulsification indexes were investigated again (see Table 4). Tests with CC and SS liquors at 20% were performed again to monitor eventual errors during the experiments.

Table 4. Effect of different liquor concentrations (either corncob or sunflower stalk), fixing glucose at 2.5% and supplementing them with mineral salts at 1%.

Liquor (%)	Cell Concentration (g/L)			STRR(%)			EI_{24} (%)								
							Hexane			Toluene			Kerosene		
	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI	$\bar{x}$	σ	95% CI
Tests with corncob liquor															
0	1.34	0.05	1.28; 1.4	41.64	0.02	41.62; 41.7	52.81	3.1	49.3; 56.32	32.98	2.18	30.51; 35.4	30.39	0.76	29.53; 31.25
20	2.84	0.04	2.8; 2.89	46.55	0.02	46.53; 46.6	56.90	1.87	54.78; 59.02	64.21	0.98	63.10; 65.32	63.08	2.49	60.26; 65.9
40	2.52	0.21	2.28; 2.76	41.03	0.16	41.12; 41.5	11.37	0.98	10.26; 12.48	19.49	1.78	17.48; 21.5	7.0	0.86	6.027; 7.97
Tests with sunflower stalk liquor															
0	2.31	0.03	2.28; 2.34	50.40	0.09	50.3; 50.5	18.49	0.22	18.24; 18.74	4.23	0.04	4.19; 4.28	12.50	2.43	9.75; 15.25
20	4.57	0.03	4.54; 4.6	56.88	1.04	55.7; 58.1	52.67	1.80	50.63; 54.71	3.05	0.23	2.79; 3.31	4.28	0.12	4.14; 4.42
40	2.03	0.01	2.02; 2.04	52.78	0.37	52.36; 53.2	22.84	2.05	20.52; 25.16	12.31	1.59	10.51; 14.1	9.59	0.08	9.5; 9.68

95% CI—95% confidence intervals, STRR—surface tension reduction rate, EI_{24}—emulsification index. There are significant differences ($p < 0.05$) between corncob and sunflower stalk liquors for all the means.

Regarding cell concentration, maximum values (2.84 g/L using CC liquor and 4.57 g/L with SS liquor) were observed in fermentations containing 20% liquor; however, Table 4 also shows a decrease when 40% liquor is used. This decrease was quite accentuated; thus, these results were very similar to those obtained in the absence of liquor, reinforcing how the excessive use of carbon can unbalance the microorganism metabolism and its ability to excrete biosurfactant. Despite carbon sources being used for cell growth and product formation, Shu [67], affirmed that the production of secondary metabolites usually occurs when carbon sources are limited. In other words, the presence of carbon sources would repress the formation of secondary metabolites. When no CC liquor was added, a 1.34 g/L cell concentration was measured, while at 40%, this value was 2.52 g/L. On the other hand, the values obtained for the hemicellulose liquor extracted from SS were 2.31 g/L (with no SS liquor) versus 2.03 g/L (with 40% liquor).

Comparing Tables 3 and 4, it is noticeable that the STRR results obtained by using liquor concentrations at 20% were very similar, indicating good repeatability during experimental tests. As already expected for this condition, the liquor positively influenced the STRR and EI_{24} results, but, as previously discussed, it was not beneficial when used in excess. The values corresponding to the EI_{24} reinforced the affinity for toluene and kerosene when the biosurfactant was synthesized in CC media, different from the stability and interaction observed for hexane by producing biosurfactant from SS.

4. Conclusions

The alkaline pretreatment was effective for hemicellulose extraction, whose content was determined at 48.8% in CC and 65.7% in SS. Quantitative analyses showed that the insertion of SS liquor allied with glucose in the fermentative media induced a significant growth of the *B. subtilis* strain, reaching a cell concentration equivalent to 4.56 g/L. Although the medium containing CC liquor and glucose is associated with the emulsification index's maximum values (56.90% in hexane, 65.63% in toluene, and 64.86% in kerosene), the most expressive STRR (56.88%) was maintained by the biosurfactant obtained using SS liquor as a partial substitute for glucose. All these results were observed at 2.5% glucose, 20% liquor (from CC or SS), and 1% mineral salts, indicating the strong potential of both liquors to be used in fermentation processes focused on biosurfactant production. Future investigations will evaluate quantitative parameters and characterization techniques to identify and classify the obtained bioproducts.

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References

1. Akbari, S.; Abdurahman, N.H.; Yunus, R.M.; Fayaz, F.; Alara, O.R. Biosurfactants—A new frontier for social and environmental safety: A mini review. *Biotechnol. Res. Innov.* **2018**, *2*, 81–90. [CrossRef]
2. Sałek, K.; Euston, S.R. Sustainable microbial biosurfactants and bioemulsifiers for commercial exploitation. *Process Biochem.* **2019**, *85*, 143–155. [CrossRef]
3. Jahan, R.; Bodratti, A.M.; Tsianou, M.; Alexandridis, P. Biosurfactants, natural alternatives to synthetic surfactants: Physicochemical properties and applications. *Adv. Colloid Interface Sci.* **2020**, *275*, 102061. [CrossRef] [PubMed]
4. Borghi, C.C.; Fabbri, M.; Fiorini, M.; Mancini, M.; Ribani, P.L. Magnetic removal of surfactants from wastewater using micrometric iron oxide powders. *Sep. Purif. Technol.* **2011**, *83*, 180–188. [CrossRef]
5. Badmus, S.O.; Amusa, H.K.; Oyehan, T.A.; Saleh, T.A. Environmental risks and toxicity of surfactants: Overview of analysis, assessment, and remediation techniques. *Environ. Sci. Pollut. Res.* **2021**, *28*, 62085–62104. [CrossRef]
6. Cserháti, T.; Forgács, E.; Oros, G. Biological activity and environmental impact of anionic surfactants. *Environ. Int.* **2002**, *28*, 337–348. [CrossRef]
7. Johnson, P.; Trybala, A.; Starov, V.; Pinfield, V.J. Effect of synthetic surfactants on the environment and the potential for substitution by biosurfactants. *Adv. Colloid Interface Sci.* **2021**, *288*, 102340. [CrossRef]
8. Cotou, E.; Castritsi-Catharios, I.; Moraitou-Apostolopoulou, M. Surfactant-based oil dispersant toxicity to developing nauplii of Artemia: Effects on ATPase enzymatic system. *Chemosphere* **2001**, *42*, 959–964. [CrossRef]

9. Olkowska, E.; Ruman, M.; Polkowska, Ż. Occurrence of Surface Active Agents in the Environment. *J. Anal. Methods Chem.* **2014**, *2014*, 769708. [CrossRef]
10. Sobrinho, H.B.; Luna, J.M.; Rufino, R.D.; Porto, A.L.; Sarubbo, L.A. Biosurfactants: Classification, properties and environmental applications. *Recent Dev. Biotechnol.* **2013**, *11*, 1–29.
11. Nikolova, C.; Gutierrez, T. Biosurfactants and Their Applications in the Oil and Gas Industry: Current State of Knowledge and Future Perspectives. *Front. Bioeng. Biotechnol.* **2021**, *9*, 626639. [CrossRef]
12. Kumar, A.; Singh, S.K.; Kant, C.; Verma, H.; Kumar, D.; Singh, P.P.; Modi, A.; Droby, S.; Kesawat, M.S.; Alavilli, H.; et al. Microbial Biosurfactant: A New Frontier for Sustainable Agriculture and Pharmaceutical Industries. *Antioxidants* **2021**, *10*, 1472. [CrossRef]
13. Bezerra, K.G.; Silva, I.G.; Almeida, F.C.; Rufino, R.D.; Sarubbo, L.A. Plant-derived biosurfactants: Extraction, characteristics and properties for application in cosmetics. *Biocatal. Agric. Biotechnol.* **2021**, *34*, 102036. [CrossRef]
14. Mouafo, H.T.; Sokamte, A.T.; Mbawala, A.; Ndjouenkeu, R.; Devappa, S. Biosurfactants from lactic acid bacteria: A critical review on production, extraction, structural characterization and food application. *Food Biosci.* **2022**, *46*, 101598. [CrossRef]
15. Evonik Biosurfactants by Evonik—Entering a New Era of Surfactants. Available online: https://household-care.evonik.com/en/products/biosurfactants (accessed on 4 April 2023).
16. Lipofabrik Accueil—Eléphant Vert. Available online: https://www.elephant-vert.com/ (accessed on 4 April 2023).
17. Biosurfactants | Jeneil Biotech. Available online: https://www.jeneilbiotech.com/biosurfactants (accessed on 4 April 2023).
18. Banat, I.M.; Satpute, S.K.; Cameotra, S.S.; Patil, R.; Nyayanit, N.V. Cost effective technologies and renewable substrates for biosurfactants' production. *Front. Microbiol.* **2014**, *5*, 697. [CrossRef]
19. Das, A.J.; Kumar, R. Utilization of agro-industrial waste for biosurfactant production under submerged fermentation and its application in oil recovery from sand matrix. *Bioresour. Technol.* **2018**, *260*, 233–240. [CrossRef]
20. Nalini, S.; Parthasarathi, R. Production and characterization of rhamnolipids produced by Serratia rubidaea SNAU02 under solid-state fermentation and its application as biocontrol agent. *Bioresour. Technol.* **2014**, *173*, 231–238. [CrossRef]
21. Ji, F.; Li, L.; Ma, S.; Wang, J.; Bao, Y. Production of rhamnolipids with a high specificity by Pseudomonas aeruginosa M408 isolated from petroleum-contaminated soil using olive oil as sole carbon source. *Ann. Microbiol.* **2016**, *66*, 1145–1156. [CrossRef]
22. Jadhav, J.V.; Pratap, A.P.; Kale, S.B. Evaluation of sunflower oil refinery waste as feedstock for production of sophorolipid. *Process Biochem.* **2019**, *78*, 15–24. [CrossRef]
23. Vecino, X.; Rodríguez-López, L.; Gudiña, E.; Cruz, J.; Moldes, A.; Rodrigues, L. Vineyard pruning waste as an alternative carbon source to produce novel biosurfactants by Lactobacillus paracasei. *J. Ind. Eng. Chem.* **2017**, *55*, 40–49. [CrossRef]
24. Vieira, W.T.; de Farias, M.B.; Spaolonzi, M.P.; da Silva, M.G.C.; Vieira, M.G.A. Endocrine-disrupting compounds: Occurrence, detection methods, effects and promising treatment pathways—A critical review. *J. Environ. Chem. Eng.* **2021**, *9*, 104558. [CrossRef]
25. Makkar, R.S.; Cameotra, S.S. Biosurfactant production by microorganisms on unconventional carbon sources. *J. Surfactants Deterg.* **1999**, *2*, 237–241. [CrossRef]
26. Ruzene, D.S.; Silva, D.P.; Vicente, A.A.; Gonçalves, A.R.; Teixeira, J.A. An Alternative Application to the Portuguese Agroindustrial Residue: Wheat Straw. *Appl. Biochem. Biotechnol.* **2008**, *147*, 85–96. [CrossRef] [PubMed]
27. Chen, H. Applications of Lignocellulose Biotechnology in Bioenergy. In *Biotechnology of Lignocellulose*; Springer: Berlin/Heidelberg, Germany, 2014; pp. 213–245. [CrossRef]
28. Sharma, L.; Alam, N.M.; Roy, S.; Satya, P.; Kar, G.; Ghosh, S.; Goswami, T.; Majumdar, B. Optimization of alkali pretreatment and enzymatic saccharification of jute (*Corchorus olitorius* L.) biomass using response surface methodology. *Bioresour. Technol.* **2023**, *368*, 128318. [CrossRef] [PubMed]
29. Amin, F.R.; Khalid, H.; Zhang, H.; Rahman, S.U.; Zhang, R.; Liu, G.; Chen, C. Pretreatment methods of lignocellulosic biomass for anaerobic digestion. *AMB Express* **2017**, *7*, 72. [CrossRef]
30. Bhatia, S.K.; Jagtap, S.S.; Bedekar, A.A.; Bhatia, R.K.; Patel, A.K.; Pant, D.; Banu, J.R.; Rao, C.V.; Kim, Y.-G.; Yang, Y.-H. Recent developments in pretreatment technologies on lignocellulosic biomass: Effect of key parameters, technological improvements, and challenges. *Bioresour. Technol.* **2020**, *300*, 122724. [CrossRef]
31. Wyman, C.E. BIOMASS ETHANOL: Technical Progress, Opportunities, and Commercial Challenges. *Annu. Rev. Energy Environ.* **1999**, *24*, 189–226. [CrossRef]
32. *FAO World Food and Agriculture—Statistical Yearbook 2021*; FAO: Rome, Italy, 2021; ISBN 978-92-5-134332-6.
33. Da Silva, J.C.; De Oliveira, R.C.; Neto, A.D.S.; Pimentel, V.C.; Santos, A.D.A.D. Extraction, Addition and Characterization of Hemicelluloses from Corn Cobs to Development of Paper Properties. *Procedia Mater. Sci.* **2015**, *8*, 793–801. [CrossRef]
34. Binici, H.; Eken, M.; Kara, M.; Dolaz, M. An environment-friendly thermal insulation material from sunflower stalk, textile waste and stubble fibers. In Proceedings of the 2013 International Conference on Renewable Energy Research and Applications, ICRERA, Madrid, Spain, 20–23 October 2013; pp. 833–846. [CrossRef]
35. D'Adamo, I.; Gastaldi, M.; Morone, P.; Rosa, P.; Sassanelli, C.; Settembre-Blundo, D.; Shen, Y. Bioeconomy of Sustainability: Drivers, Opportunities and Policy Implications. *Sustainability* **2021**, *14*, 200. [CrossRef]
36. Paraszkiewicz, K.; Bernat, P.; Kuśmierska, A.; Chojniak, J.; Płaza, G. Structural identification of lipopeptide biosurfactants produced by Bacillus subtilis strains grown on the media obtained from renewable natural resources. *J. Environ. Manag.* **2018**, *209*, 65–70. [CrossRef]

37. Cooper, D.G.; Macdonald, C.R.; Duff, S.J.B.; Kosaric, N. Enhanced Production of Surfactin from *Bacillus subtilis* by Continuous Product Removal and Metal Cation Additions. *Appl. Environ. Microbiol.* **1981**, *42*, 408–412. [CrossRef]
38. Sheppard, J.; Cooper, D. The response of Bacillus subtilis ATCC 21332 to manganese during continuous-phased growth. *Appl. Microbiol. Biotechnol.* **1991**, *35*, 180639. [CrossRef]
39. Marin, C.P.; Kaschuk, J.J.; Frollini, E.; Nitschke, M. Potential use of the liquor from sisal pulp hydrolysis as substrate for surfactin production. *Ind. Crop. Prod.* **2015**, *66*, 239–245. [CrossRef]
40. ASTM Standard Test Method for Acid-Insoluble Lignin in Wood. Available online: https://www.astm.org/d1106-21.html (accessed on 27 February 2023).
41. Sluiter, A.; Hames, B.; Ruiz, R.; Scarlata, C.; Sluiter, J.; Templeton, D.; Crocker, D. *NREL/TP-510-42618 Analytical Procedure—Determination of Structural Carbohydrates and Lignin in Biomass*; Technical Report; National Renewable Energy Laboratory: Golden, CO, USA, 2012.
42. NREL: Biomass Research—What Is a Biorefinery? What Is a Biorefinery? NREL: Biomass Research—What Is a Biorefinery? Available online: http://www.nrel.gov/biomass/biorefinery.html?print (accessed on 14 January 2016).
43. Cooper, D.G.; Goldenberg, B.G. Surface-Active Agents from Two *Bacillus* Species. *Appl. Environ. Microbiol.* **1987**, *53*, 224–229. [CrossRef]
44. Jesus, M.; Mata, F.; Batista, R.A.; Ruzene, D.S.; Albuquerque-Júnior, R.; Cardoso, J.C.; Vaz-Velho, M.; Pires, P.; Padilha, F.F.; Silva, D.P. Corncob as Carbon Source in the Production of Xanthan Gum in Different Strains *Xanthomonas* sp. *Sustainability* **2023**, *15*, 2287. [CrossRef]
45. Jesus, M.S.; Romaní, A.; Genisheva, Z.; Teixeira, J.A.; Domingues, L. Integral valorization of vine pruning residue by sequential autohydrolysis stages. *J. Clean. Prod.* **2017**, *168*, 74–86. [CrossRef]
46. Jesus, M.; Romaní, A.; Mata, F.; Domingues, L. Current Options in the Valorisation of Vine Pruning Residue for the Production of Biofuels, Biopolymers, Antioxidants, and Bio-Composites following the Concept of Biorefinery: A Review. *Polymers* **2022**, *14*, 1640. [CrossRef]
47. Marrugo, G.; Valdés, C.F.; Chejne, F. Characterization of Colombian Agroindustrial Biomass Residues as Energy Resources. *Energy Fuels* **2016**, *30*, 8386–8398. [CrossRef]
48. Pang, C.H.; Lester, E.; Wu, T. Influence of lignocellulose and plant cell walls on biomass char morphology and combustion reactivity. *Biomass Bioenergy* **2018**, *119*, 480–491. [CrossRef]
49. Almeida, J.R.M.; Bertilsson, M.; Gorwa-Grauslund, M.F.; Gorsich, S.; Lidén, G. Metabolic effects of furaldehydes and impacts on biotechnological processes. *Appl. Microbiol. Biotechnol.* **2009**, *82*, 625–638. [CrossRef]
50. He, Y.; Wu, T.; Wang, X.; Chen, B.; Chen, F. Cost-effective biodiesel production from wet microalgal biomass by a novel two-step enzymatic process. *Bioresour. Technol.* **2018**, *268*, 583–591. [CrossRef] [PubMed]
51. Mosier, N.; Wyman, C.; Dale, B.; Elander, R.; Lee, Y.Y.; Holtzapple, M.; Ladisch, M. Features of promising technologies for pretreatment of lignocellulosic biomass. *Bioresour. Technol.* **2005**, *96*, 673–686. [CrossRef] [PubMed]
52. Chen, C.; Lin, J.; Wang, W.; Huang, H.; Li, S. Cost-Effective Production of Surfactin from Xylose-Rich Corncob Hydrolysate Using Bacillus subtilis BS-37. *Waste Biomass-Valorization* **2019**, *10*, 341–347. [CrossRef]
53. He, X.-F.; Yang, L.; Wu, H.-J.; Liu, N.; Zhang, Y.-G.; Zhou, A.-N. Characterization and pyrolysis behaviors of sunflower stalk and its hydrolysis residue. *Asia-Pac. J. Chem. Eng.* **2016**, *11*, 803–811. [CrossRef]
54. Sharma, S.K.; Kalra, K.L.; Grewal, H.S. Enzymatic saccharification of pretreated sunflower stalks. *Biomass Bioenergy* **2002**, *23*, 237–243. [CrossRef]
55. Du, R.; Su, R.; Qi, W.; He, Z. Enhanced enzymatic hydrolysis of corncob by ultrasound-assisted soaking in aqueous ammonia pretreatment. *3 Biotech* **2018**, *8*, 166. [CrossRef]
56. Su, Y.; Du, R.; Guo, H.; Cao, M.; Wu, Q.; Su, R.; Qi, W.; He, Z. Fractional pretreatment of lignocellulose by alkaline hydrogen peroxide: Characterization of its major components. *Food Bioprod. Process.* **2015**, *94*, 322–330. [CrossRef]
57. Yu, H.; Guo, J.; Chen, Y.; Fu, G.; Li, B.; Guo, X.; Xiao, D. Efficient utilization of hemicellulose and cellulose in alkali liquor-pretreated corncob for bioethanol production at high solid loading by *Spathaspora passalidarum* U1-58. *Bioresour. Technol.* **2017**, *232*, 168–175. [CrossRef]
58. Sarubbo, L.A.; Silva, M.D.G.C.; Durval, I.J.B.; Bezerra, K.G.O.; Ribeiro, B.G.; Silva, I.A.; Twigg, M.S.; Banat, I.M. Biosurfactants: Production, properties, applications, trends, and general perspectives. *Biochem. Eng. J.* **2022**, *181*, 108377. [CrossRef]
59. Jimoh, A.A.; Lin, J. Production and characterization of lipopeptide biosurfactant producing *Paenibacillus* sp. D9 and its biodegradation of diesel fuel. *Int. J. Environ. Sci. Technol.* **2019**, *16*, 4143–4158. [CrossRef]
60. Santos, D.K.F.; Rufino, R.D.; Luna, J.M.; Santos, V.A.; Sarubbo, L.A. Biosurfactants: Multifunctional Biomolecules of the 21st Century. *Int. J. Mol. Sci.* **2016**, *17*, 401. [CrossRef]
61. Phulpoto, I.A.; Yu, Z.; Hu, B.; Wang, Y.; Ndayisenga, F.; Li, J.; Liang, H.; Qazi, M.A. Production and characterization of surfactin-like biosurfactant produced by novel strain *Bacillus nealsonii* S2MT and it's potential for oil contaminated soil remediation. *Microb. Cell Factories* **2020**, *19*, 145. [CrossRef]
62. Vieira, I.M.M.; Santos, B.L.P.; Silva, L.S.; Ramos, L.C.; de Souza, R.R.; Ruzene, D.S.; Silva, D.P. Potential of pineapple peel in the alternative composition of culture media for biosurfactant production. *Environ. Sci. Pollut. Res.* **2021**, *28*, 68957–68971. [CrossRef]
63. Sharma, J.; Kapley, A.; Sundar, D.; Srivastava, P. Characterization of a potent biosurfactant produced from *Franconibacter* sp. IITDAS19 and its application in enhanced oil recovery. *Colloids Surf. B Biointerfaces* **2022**, *214*, 112453. [CrossRef]

64. Pathania, A.S.; Jana, A.K.; Jana, M.M. Valorization of waste frying oil to lipopeptide biosurfactant by indigenous *Bacillus licheniformis* through co-utilization in mixed substrate fermentation. *Braz. J. Chem. Eng.* **2021**, *39*, 369–385. [CrossRef]
65. Singh, V. Assessment of biosurfactant as an emulsifier produced from *Bacillus aryabhattai* SPS1001 grown on industrial waste coal tar for recovery of bitumen from oil sands. *Biomass-Convers. Biorefinery* **2022**, 1–12. [CrossRef]
66. Meena, K.R.; Dhiman, R.; Singh, K.; Kumar, S.; Sharma, A.; Kanwar, S.S.; Mondal, R.; Das, S.; Franco, O.L.; Mandal, A.K. Purification and identification of a surfactin biosurfactant and engine oil degradation by *Bacillus velezensis* KLP2016. *Microb. Cell Factories* **2021**, *20*, 26. [CrossRef]
67. Shu, C.-H. Fungal Fermentation for Medicinal Products. In *Bioprocessing for Value-Added Products from Renewable Resources*; Elsevier: Amsterdam, The Netherlands, 2007; pp. 447–463.