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Synthesis of Guanidine and Its Deposition on Bacterial Cellulose as Green Heterogeneous Catalyst for Transesterification to Methyl Esters

Cristian Eugen Răducanu [1], Tănase Dobre [1,2,*], Dan Eduard Mihăiescu [3], Alina Moroşan [3], Roxana Jidveian [1], Doinița Roxana Cioroiu Tîrpan [4], Alexandru Dan Vasiliu [1], Cristina Ionela Gogoaşă [1], Oana Cristina Pârvulescu [1] and Bogdan Trică [5]

[1] Department of Chemical and Biochemical Engineering, National University of Science and Technology Politehnica Bucharest, 1-7 Gheorghe Polizu St., 011061 Bucharest, Romania; cristianrdcn1@yahoo.com (C.E.R.); rjidveian@gmail.com (R.J.); vasiliudanalexandru1994@yahoo.com (A.D.V.); cristina_ionela_92@yahoo.com (C.I.G.); oana.parvulescu@yahoo.com (O.C.P.)
[2] Technical Sciences Academy of Romania, 26 Dacia Blvd., 030167 Bucharest, Romania
[3] Department of Organic Chemistry, National University of Science and Technology Politehnica Bucharest, 1-7 Gheorghe Polizu St., 011061 Bucharest, Romania; dan.mihaiescu@upb.ro (D.E.M.); alina.morosan@upb.ro (A.M.)
[4] Department of Chemistry and Chemical Engineering, Ovidius University of Constanţa, 124 Mamaia Blvd., 900527 Constanţa, Romania; tirpanroxana@gmail.com
[5] National Institute for Research Development for Chemistry and Petrochemistry-ICECHIM, 202 Spl. Independentei, S6, 060021 Bucharest, Romania; bogdan.trica@icechim.ro
* Correspondence: tghdobre@gmail.com

Abstract: Green catalysts used in the transesterification reaction of biodiesel must have biodegradability and non-toxicity as their main characteristics, being thus friendly to the environment, since they perform in processes in which the content of CO_2, which is increasing from year to year, should be reduced. As a consequence, their manufacture can be extremely rigorous. This work presents the two-step construction, synthesis, and deposition of such a green heterogeneous catalyst and its testing in the catalysis of the transesterification of triglycerides with methanol, resulting in methyl esters. A CSTR-type reactor was used to perform transesterification, and the biodiesel yields obtained had values in the range of 91.7–95.7%, using 2, 3, and 4 g/g catalyst to oil, under conditions like those for obtaining commercial biodiesel in homogeneous catalysis, i.e., a 65 °C process temperature and a 4:1, 5:1 or 6:1 methanol-to-oil molar ratio.

Keywords: bacterial cellulose; superbase synthesis; synthesis of guanidine; green heterogeneous catalyst; transesterification; methyl esters

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1. Introduction

Typical diesel fuel is obtained from oil, which is responsible for a major part of the greenhouse effect and is non-renewable, so in 50–100 years it could become unexploitable [1–3]. Currently the profitable exploitation of oil is characterized by the following: (i) non-uniform availability; (ii) best availability mostly in areas of permanent conflict; (iii) financial instability in specific markets; (iv) quasi-dependence on politics; (v) huge extractive or refining facilities, whose costs (execution or maintenance and especially security today) become real barriers for some and allow only certain actors to play in this field. Biofuels, on the other hand, do not produce a greenhouse effect through use, so they are of particular interest. Biodiesel, the second most produced biofuel, has properties similar to classic diesel and features that can recommend it as an alternative: (i) it can be obtained in any quantity, so it does not necessarily require huge facilities, although production capacity is currently only governed by demand or certain environmental regulations imposed; (ii) it can be obtained

by absolutely anyone, with minimal knowledge, from quantities of tens of kg for personal use up to capacities of 100,000 t/year; (iii) it is considered a renewable resource, being a chemical compound resulting from the transesterification reaction between triglycerides (from edible/non-edible vegetable oils, animal and vegetable fats, etc.) and methanol [4–7], in the presence of a catalyst; (iv) it is not related to conflict areas, but remains politically dependent; and (v) what makes it extremely valuable and useful is its complex of properties and characteristics similar (some even more advantageous) to those of diesel 2 (D #2) for transport, from a classification that also includes D #1 and D #4 [8], with differences in sulfur content (S), different viscosities and destinations such as truck engines, locomotives or agriculture.

Catalyst selection is essential for the (trans)esterification of triglycerides, impure with free fatty acids (FFAs), to yield alkyl esters [9]. The transition from a conventional biodiesel production process to a process governed by green chemistry begins precisely with this catalyst selection, the heterogeneous catalysts standing out in terms of versatility [9]. In the case of a raw material with FFA content below 1%, it is sufficient for the catalyst basicity to be comparable to that of potassium hydroxide (KOH) or sodium hydroxide (NaOH), but this catalyst must simultaneously have environmentally friendly features. If the catalyst is biodegradable, it has reduced post-process toxicity, somewhat the opposite of NaOH or KOH from an environmental point of view, and thus the crucial characteristic for selection is achieved; if an alkaline catalyst is chemically combined with a catalytic support, it will be reusable in several production cycles and succeed in catalyzing methanolysis (or alcoholysis) of triglycerides with FFA content between 5 and 20%; the determining characteristic for a process even greener than the conventional one is thus obtained.

The supported catalyst therefore involves two key components: the catalyst, alkaline or acid, in various forms, and the catalytic support, also in many and varied forms [10–12]. As for the alternative to NaOH or KOH, organic superbases can replace them with similar end results.

Superbases are very often identified with strongly alkaline organic compounds, even though the latter are commonly limited to complex amines [13]. From Adler's proton sponge in 1968 [14], 1,8-bis(dimethylamino)naphthalene or DMAN, to Schwesinger's phosphazenes in 1985 [15] and up to Verkade's proazaphosphatranes [16] (aminophosphines—Verkade's bases), it has been demonstrated that the P-N (phosphorus–nitrogen) bond increases the basicity of these amino-phospho derivatives compared to the basicity of amino-derivatives (with only nitrogen), so the concept of organosuperbases, chemical compounds with extraordinary basicity properties, can be developed. In 1993, Caubère proposed an excellent definition of superbases, highlighting that superbases should be called compounds resulting from the mixture of two or more bases, whose properties are new and combine the characteristics of several bases [13,17].

In the category of superbases, the following can be highlighted: amidines, guanidines, phosphazenes and Verkade bases. The context in which synthetic chemistry was called upon in 1968 (the year of the presentation of the proton sponge–DMAN by Adler) to contribute to progress, in general, is different in the 2020s, in the way that, after the 2000s, synthetic chemistry adhered to the principles of green chemistry, and the evolution seems exclusively in this direction of environmentally friendly chemical synthesis. These organic superbases are characterized by easy molecular modification, reusability, biodegradability and low or no toxicity.

Guanidine can be selected as an alkaline catalyst due to its specific characteristics, being organic, biodegradable and both a product and a precursor. The basicity of guanidine, used tale quale, is 13.6, but in guanidine derivatives, such as TMGF [4,5-bis (tetramethylguanidine) fluorene]) or TBD (1,5,7-Triazabicyclo[4.4.0]dec-5-ene), the basicity (expressed in pKa_H) can reach values up to 27–28 [13].

Guanidine is an amino derivative from a class of compounds in which, with the introduction of an imino (=NH) functional group at C_1 of amines, the basicity can be increased [13]. Guanidine, having two amino (-NH_2) groups and an imino (=NH) group,

has the highest basicity among amino derivatives [13,18]. The basicity of these amino derivatives is due to the characteristic resonance phenomenon; upon protonation (under reversible conditions), the charge density at the nitrogen atom is increased by the inductive effects of the electron donors (*I*+), with electron conjugation making them more available for fixing a proton [13,19].

Compatibility between a support and a catalyst is also difficult to achieve, but certain properties of the support can facilitate the construction of a catalyst with the desired ability in terms of catalytic activity, and bacterial cellulose qualifies from this point of view.

Bacterial cellulose (BC) is cellulose bio-produced by several bacteria, fungi and even some types of algae [20–23]. Although the trend of using environmentally friendly materials is relatively new, emerging after the 2000s, BC has been present in industrial-scale applications since the 1980s (e.g., Johnson & Johnson, USA), with BC used as wound grafts [20,24,25], or in Brazil, where the company Bio Fill Produtos Biotecnologicos (Curitiba) created a new system based on BC that is also usable in the case of wound healing [20,26,27].

Cellulose is a linear macromolecular homopolysaccharide whose basic structural unit is a disaccharide, cellobiose, formed by the β-1,4 glycosidic bond between two glucose residues [28]. Cellulose is specific to the vegetable kingdom, forming over 50% of the lignocellulosic mass of trees, and in plant tissues, several cellulose macromolecules join through hydrogen bonds and form cellulose fibers.

Bacterial cellulose, on the other hand, is produced directly as a fibrous network and does not contain hemicellulose and lignin. It is, like vegetable cellulose, insoluble in water or solvents, and it has a high water adsorption capacity, an extraordinary mechanical strength (conditioned by a certain percentage of moisture) and a network of bio-cellulosic fibers about 100 times thinner in comparison with vegetable cellulose fibers, which make the structure more porous. Its production can be carried out in different ways in terms of the bioreactor medium state, each method having advantages and disadvantages. The BC obtained in our previous work in a static medium, for example, has a more rigid consistency, different from that obtained in a mobile environment (e.g., rotary drum reactor), characterized by a more gelatin-like consistency [29].

The use of BC as a catalytic support can be part of the same strategy for making biodiesel production a green process, and a specific feature of its chemical structure, the easy binding of the OH group, can help create a new matrix from the glycosidic residues [30].

The construction of a solid catalyst, which will be further used to heterogeneously catalyze the (trans)esterification reactions of waste materials, must achieve five main characteristics: (1) versatility, in terms of catalyzing conversion of waste materials with FFA in the range of 4–25%; (2) strong basicity, allowing transesterification to be performed; (3) biodegradability and (4) non-toxicity, for the aims of sustainability and durability; and (5) high catalytic activity, in terms of reusing same catalyst in multiple production cycles. Various materials used in our previous work can be used as catalytic supports, each one with properties that more or less recommend them as an element of supported catalysts: metal oxides in pure form or in combination with another chemical compound, lignocellulosic biomass, activated carbon from waste biomass sources, algae biomass or biopolymers [30]. Their functionalization has to provide, in addition to successful compatibility, a strong chemical bond as well, which would make the support very reliable. The novelty proposed in this work consists in approaching a biodegradable biopolymer, such as BC, as a versatile and viable biomaterial to be transformed into a strong heterogeneous core matrix and its subsequent functionalization for catalysis processes.

Thus, in this work, guanidine was selected as the basic catalyst due to its strong alkalinity, biodegradability and versatility, while bacterial cellulose was selected for the development of the catalytic support.

2. Materials and Methods

2.1. Materials

In this experimental research, the following raw materials were used: dimethyl sulfate (99.0% purity, Fluka Honeywell, Berlin, Germany), urea (99.5% purity, Fluka Honeywell, Berlin, Germany), methanol (99.8% purity, Fluka Honeywell, Berlin, Germany), ammonia solution 25% (Bucharest Reagent, Bucharest, Romania), distilled water (Millipore Q-Gard A2 AFS 15E laboratory distiller, Massachusetts, United States), potassium hydroxide pellets (85.0% purity, ErbaLachema, Brno, Czech Republic), glucose, yeast extract, peptone powder (Merck, Darmstadt, Germany), disodium phosphate anhydrous (99.0% purity, Merck, Darmstadt Germany), citric acid monohydrate (99.0% purity, Merck, Darmstadt, Germany), acetic acid (99.0% purity, Merck, Darmstadt, Germany), ferrous sulfate heptahydrate (99.0% purity, Merck, Darmstadt, Germany), magnesium sulfate heptahydrate (98.0% purity, Merck, Darmstadt, Germany), ethanol (99.8% purity, Fluka Honeywell, Berlin Germany), used sunflower cooking oil (domestic household).

2.2. Guanidine Synthesis

There are several paths to follow in guanidine synthesis, starting from various chemical compounds. The present work considered certain synthesis conditions (Roberts and Griffiths, 1949 [31]) as well as the further use of the main product. The synthesis of guanidine was performed in three steps, with successive reactions of methylation, ammonolysis (preceded by neutralization) and precipitation.

2.2.1. Synthesis of O-methylisourea Hydrogen Methyl Sulfate Precursor

One mole of dimethyl sulfate (A) was weighed and added to a 1 L 3-neck round-bottom flask. When the temperature of 100 °C was reached, one mole of urea (B) was added to the flask gradually over 15 min, under vigorous continuous mixing.

The exothermic reaction required continuous temperature monitoring and control to maintain it at 110–120 °C, a range which was reached in 8 min. After the entire amount of urea was added, the reaction mixture was kept at a temperature of 115 °C for 25 min to complete the reaction. The resulting product was O-methyl-isourea hydrogen methyl sulfate (C), as shown in Figure 1.

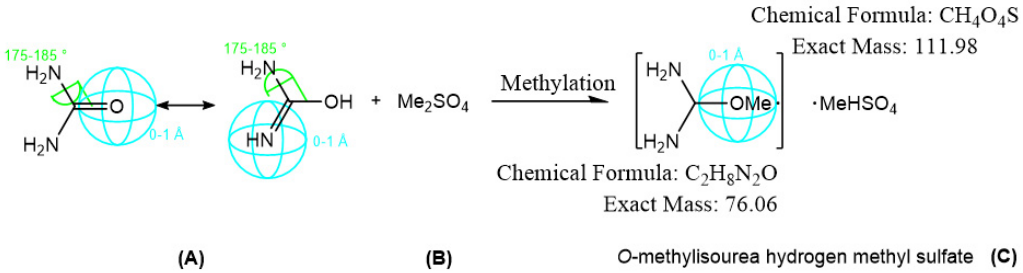


Figure 1. Stage I of guanidine synthesis, with the methylation reaction and its first intermediary chemical compound (C).

2.2.2. Synthesis of Guanidine-Methyl Hydrogen Sulfate Precursor

The solution of O-methyl isourea hydrogen methyl sulfate (C) contained basic methyl urea, as hydrogen methyl sulfate salt, being at the same time acidic, so the ammonolysis reaction, the second reaction step, was preceded by neutralization with ammonia solution (D). The neutralization reaction was initiated at 25 °C by adding 25 mL of 25% NH_3 solution, for 30 min, until the temperature of the reaction mixture reached 60 °C, when the ammonolysis reaction was initiated by adding 50 mL of 25% NH_3 solution to the reaction medium, and the reaction was maintained at this temperature for 3 h. The ammonolysis reaction led to the formation of the amino- (-NH_2) group, resulting the reaction product, guanidine-methyl sulfate acid (E), and methanol (F), as shown in Figure 2.

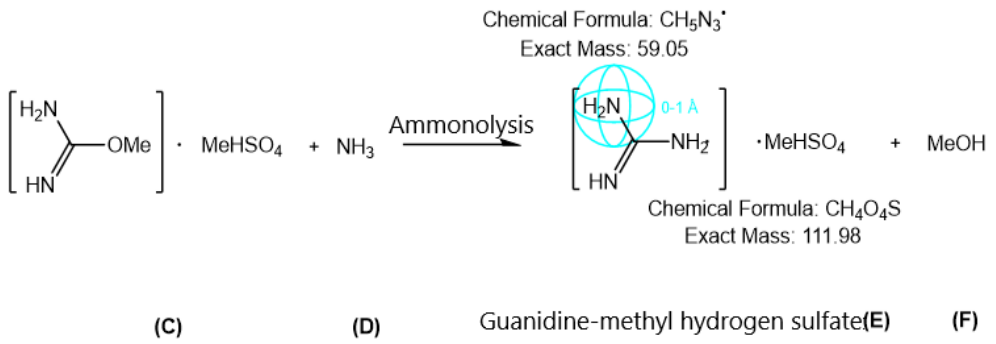


Figure 2. Stage II of guanidine synthesis, with the ammonolysis and second intermediary chemical compound (E).

Distillation was performed to remove the aqueous alcoholic solution resulting from the reaction, and this second precursor was kept at room temperature for 24 h.

2.2.3. Synthesis of Alcoholic Guanidine

Pure guanidine (H) is relatively difficult to preserve in this state, so it is preferable that it is bound to a chemical compound from which, depending on the application, it can still participate in selected chemical reactions.

For this work, it was chosen to obtain guanidine bonded to (methyl) alcohol for further use with the -CH_3 group in subsequent reactions. Thus, the distillation product was dissolved in 1000 mL of methanol by heating, and after dissolution, the reaction mixture was brought to a temperature of 25 °C.

The prepared alcoholic solution of potassium hydroxide (G) was then added, gradually, under stirring and cooling between 10 and 20 °C, and, following this treatment, potassium methyl sulfate precipitated (Figure 3).

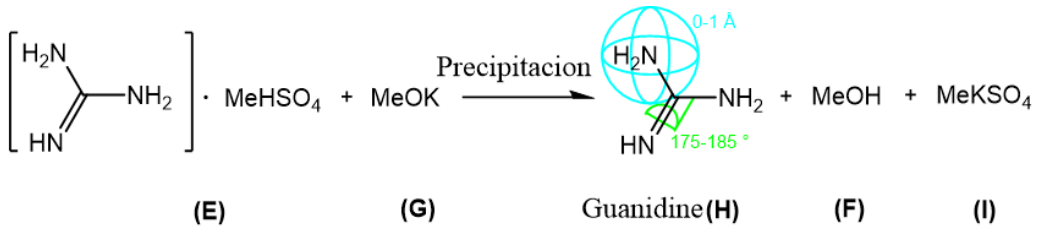


Figure 3. Stage III of guanidine synthesis, with the precipitation reaction, resulting in alcoholic guanidine compound.

The precipitate thus obtained was subsequently filtered through successive washings with methanol (cold), until the release of guanidine. The potassium methyl sulfate (I) was stored in anhydrous conditions, and the guanidine was kept in an alcoholic solution.

Figure 4 shows the evolution in terms of the visual appearance of the precursors corresponding to each reaction step.

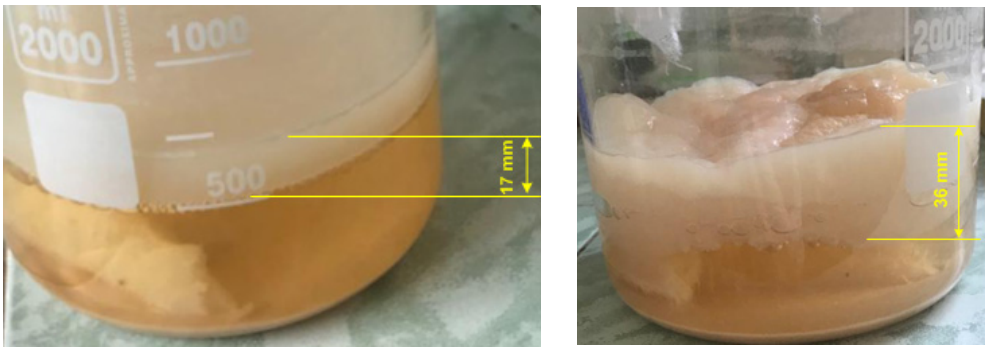
(**a**) (**b**) (**c**) (**d**) (**e**)

Figure 4. The evolution, in terms of visual appearance, of the products of the synthesis stages: (**a**) clear brown liquid, first intermediary (C) compound; (**b**,**c**) light brown wax-like solid, second intermediary (E) compound; (**d**) white (I) precipitated potassium methyl sulfate and alcoholic guanidine; (**e**) alcoholic guanidine.

2.3. Catalytic Support Preparation

BC production was performed in a static environment, using procedures from previous works [32–34] and an adapted treatment after BC production, considering the proposed goal of this work.

BC was obtained in static culture in a cylindrical 2 L Berzelius beaker covered with a gauze lid to allow aeration of the membrane forming Hestrin–Schramm (HS) culture medium (Figure 5). This medium contained 40 g/L glucose, 5 g/L yeast extract, 5 g/L peptone, 2.7 g/L anhydrous disodium phosphate, 0.003 g/L citric acid monohydrate, 0.005 g/L ferrous sulfate heptahydrate, 0.005 g/L magnesium sulfate heptahydrate, and 9 mL/L ethanol. The pH was adjusted to ~5 using 1N acetic acid solution (Schramm and Hestrin, 1954 [35]).



(**a**) (**b**)

Figure 5. Bacterial cellulose production: (**a**) BC membrane after 14 days; (**b**) BC membrane after 28 days.

A particularly important step was the chemical treatment applied immediately after removing the membrane from the reactor at the end, to remove bacteria, a treatment that usually consists of a pre-wash, followed successively by a treatment using alkaline chemical compounds, under conditions of high temperature, a wash with distilled water to neutral pH and storage by total immersion in acetic acid solution, pending further processing [32–34].

2.4. Heterogeneous Catalyst Synthesis

2.4.1. Treatment with Potassium Hydroxide

The combination of two strong bases, guanidine and KOH, aimed at obtaining new properties for the solid catalyst.

In the case of using BC as a catalytic support, two stages of catalyst development were carried out, considering the chemical composition of BC, which would allow chem-

ical bonding strategies with different compounds, with the formation of new different functional groups.

In this first step of treating the BC support, 5% and 10% KOH were used, without the support being previously dried after its production. Thus, in several cycles, 5% and 10% KOH solutions were prepared, in which BC was introduced at a ratio of 1:3 *w*/*v* dry BC/sol. KOH. BC thus treated was kept at ambient temperature for 3–5 days, for each treatment cycle, after which it was subjected to drying and storage in anhydrous conditions. The resulting BC was considered the final precursor $BC_{KOH\ 5\%}$ and $BC_{KOH\ 10\%}$ for the guanidine catalyst (Figure 6).

(**a**) (**b**)

Figure 6. BC treated with KOH: (**a**) $BC_{KOH\ 5\%}$; (**b**) $BC_{KOH\ 10\%}$.

2.4.2. Treatment of BC_{KOH} with Guanidine

This type of BC_{KOH} catalyst was prepared using the impregnation method, and it was carried out using the same technique, by immersing BC in Gu-ROH solution, and then running a reflux reaction at 65 °C temperature, for 180 min, followed by concentration by distillation. The final Gu-$_{KOH\ 5}$/BC and Gu-$_{KOH\ 10}$/BC catalysts were obtained (Figure 7).

(**a**) (**b**) (**c**)

Figure 7. BC $_{KOH}$ upon impregnation with Gu-ROH: (**a**) dried BC-KOH; (**b**) Gu-$_{KOH\ 5}$/BC; (**c**) Gu-$_{KOH\ 10}$/BC.

2.5. Catalyst Analysis

2.5.1. High-Resolution Mass Spectrometry Analysis for Guanidine

The HR-MS (high-resolution mass spectrometry) analysis performed for the quantification of chemical compounds was carried out using the HR mass spectrometer with a 15 T superconducting magnet (SolariX-XR, QqqFT-ICR HR, Bruker Daltonics), Fourier-transform-type ion cyclotron resonance (FT-ICR). Using the positive ESI ionization technique (electrospray ionization), the sample was introduced by direct infusion, using a Hamilton 250 µL pump with the sample flow set at 120 µL/h and the following parameters

for the nebulization gas (nitrogen): the pressure of 1.2 bar, temperature of 200 °C, and a flow rate of 4 L/min. The samples were dissolved in methanol (HPLC-grade, Merck Millipore). For the spectra, the low mass method was used, with the mass range in the range of low m/z 46.07 to high m/z 3000 u.a.m., with accumulation in seconds (*Accum*-0.020) and a source voltage of 4500 V.

2.5.2. Fourier Transform Infrared Spectroscopy for Guanidine

FTIR analysis was performed using the ATR technique on a Nicolet 6700-Thermo Scientific FTIR spectrometer (Thermo Fisher Scientific, Waltham, Massachusetts, United States) over a wavelength range from 4000 cm^{-1} to 500 cm^{-1}, and was performed to highlight the functional groups present in the guanidine at the end of the reaction.

2.5.3. SEM Analysis for Heterogeneous Catalyst

The morphology of the guanidine-based catalyst loading in section and on the surface was evaluated using a TM4000Plus Tabletop Scanning Electron Microscope (Hitachi, Tokyo, Japan) at 15 kV and 30 kV, in standard vacuum conditions (according to the TM4000Plus Software vacuum settings). The SEM images were obtained using both backscattered electron (BSE) and/or secondary electron (SE) detectors at multiple magnifications.

2.6. Catalyst Testing

Catalyst testing involved biodiesel synthesis and was carried out by transesterification of used sunflower oil with methanol in heterogeneous basic catalysis, in a batch reactor with perfect mixing, using Gu-$_{KOH}$/BC as a catalyst.

The methodology involved simultaneous pretreatment of the raw material, filtration and heat treatment, and activation of the catalyst, using methanol, at different levels of process temperature. Reaction conditions were set to match those of commercial biodiesel processes, 1.5–2 h reaction time, maximum 6:1 methanol/oil ratio, and 65 °C reaction temperature. Twenty experiments were carried out according to a 2^4 factorial design.

The reaction conditions are specified in Table 1.

Table 1. Dimensional process factors in a 2^4 factorial design with 4 replicates in the center of experimental plan (t = 65 °C, τ_e = 1.5 h).

Exp	z_1	z_2	z_3	z_4
1	5	2	6	75
2	5	2	4	75
3	5	4	6	75
4	5	4	4	75
5	10	2	6	75
6	10	2	4	75
7	10	4	6	75
8	10	4	4	75
9	5	2	6	105
10	5	2	4	105
11	5	4	6	105
12	5	4	4	105
13	10	2	6	105
14	10	2	4	105
15	10	4	6	105
16	10	4	4	105
17	7.5	3	5	90
18	7.5	3	5	90
19	7.5	3	5	90
20	7.5	3	5	90

z_1 = KOH concentration in Gu-$_{KOH}$/BC (%); z_2 = catalyst dose (g/100 g); z_3 = methanol/oil molar ratio; z_4 = drying temperature of BC-KOH in Gu-$_{KOH}$/BC.

The experimental investigation of the influence of process factors on the yield of biodiesel production, expressed as yield in methyl ester and yield in glycerin, followed a factorial experimental design with four factors, each at two levels. The process factors referred to the solution for obtaining the catalyst and to the conduct of the transesterification reaction. Thus, the first factor (z_1) expressed by the KOH concentration in the guanidine saturation solution and the fourth factor (z_4) specifying the temperature at which the BC saturated in KOH was dried refer to the preparation of the catalyst. The second factor (z_2), which shows the dose of the catalyst in transesterification, and the third factor (z_3), which specifies the methanol/oil molar ratio, refer to the progress of the reaction. Four experiments were performed in the center of the factorial plan (exp. 17–20), their purpose being to establish the reproducibility dispersion of the working method.

3. Results and Discussion

3.1. Analysis of Guanidine Synthesis Products

3.1.1. Fourier Transform Infrared Spectroscopy for Guanidine

Figure 8 shows the FT-IR spectrum of guanidine, and possible stretching vibrations of the N-H groups (primary amines) can be seen at the 3357 cm^{-1} wavelength, possible out-of-plane bending vibrations (asymmetric) of N-H groups (primary amines) of high intensity (strong intensity) at the 1658 cm^{-1} wavelength and possible asymmetric bending vibrations of N-H groups (secondary amines) at the 761 cm^{-1} wavelength.

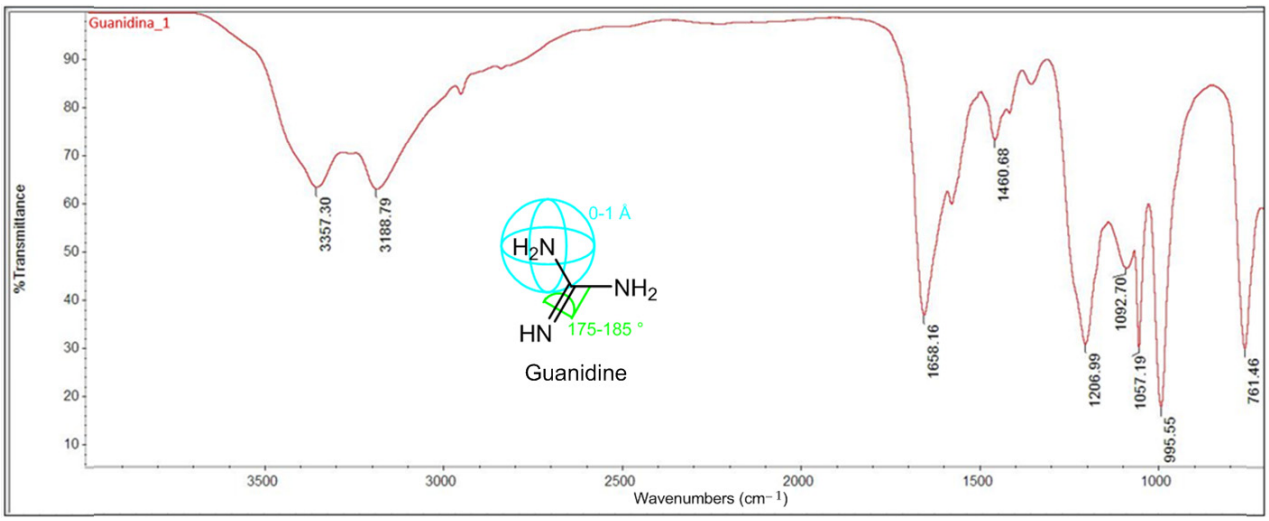


Figure 8. FT-IR spectrum of guanidine.

3.1.2. High-Resolution Mass Spectrometry for Guanidine

HR-MS analysis was performed to identify the products of each guanidine synthesis step. Figure 9 shows the first intermediate, O–methylisourea–H–methyl sulfate at m/z = 187.05, in the intensity range of I × 10^6, and Figure 10 shows the second intermediate, guanidine methyl H sulfate, in the intensity range of I × 10^8, at m/z = 172.10.

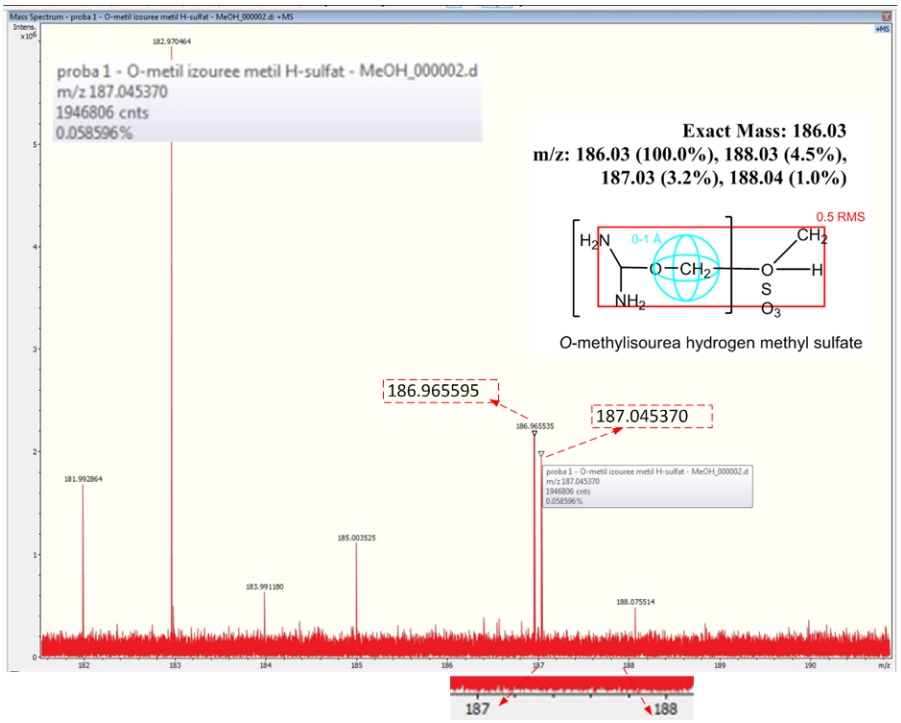


Figure 9. HR-MS spectra, to identify first stage intermediary end product of guanidine synthesis: O-methyl-isourea-H-methyl sulfate.

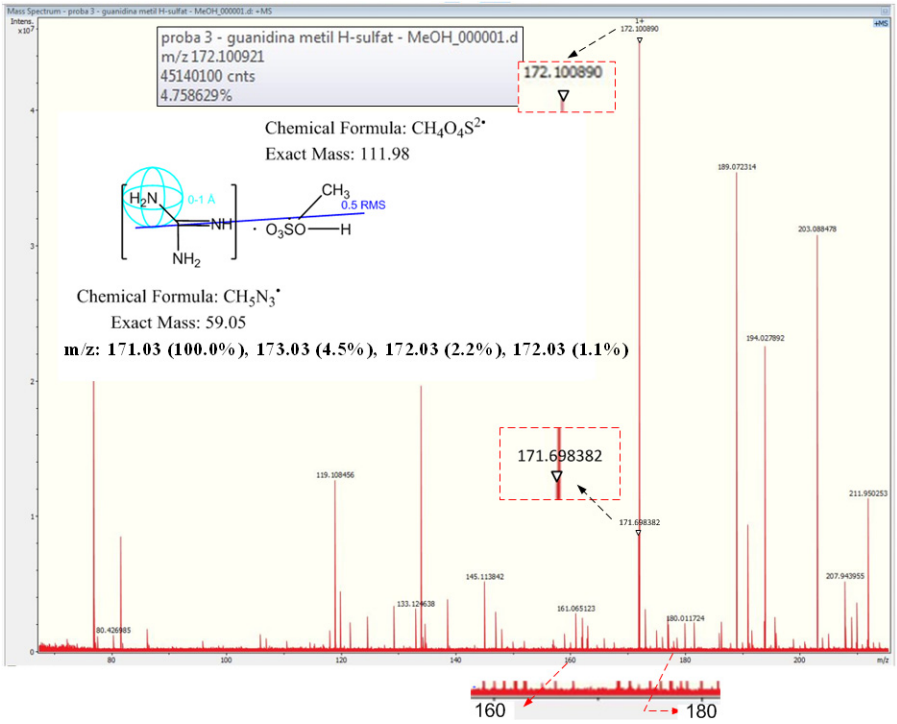


Figure 10. HR-MS spectra, to identify intermediary second stage end product of guanidine synthesis: guanidine methyl H sulfate.

Potassium methyl sulfate was identified at m/z = 151.57 (I × 10^6) (Figure 11), being considered in the reaction as the main secondary product, while in Figure 12, guanidine can be found at m/z = 60.85 (I × 10^7).

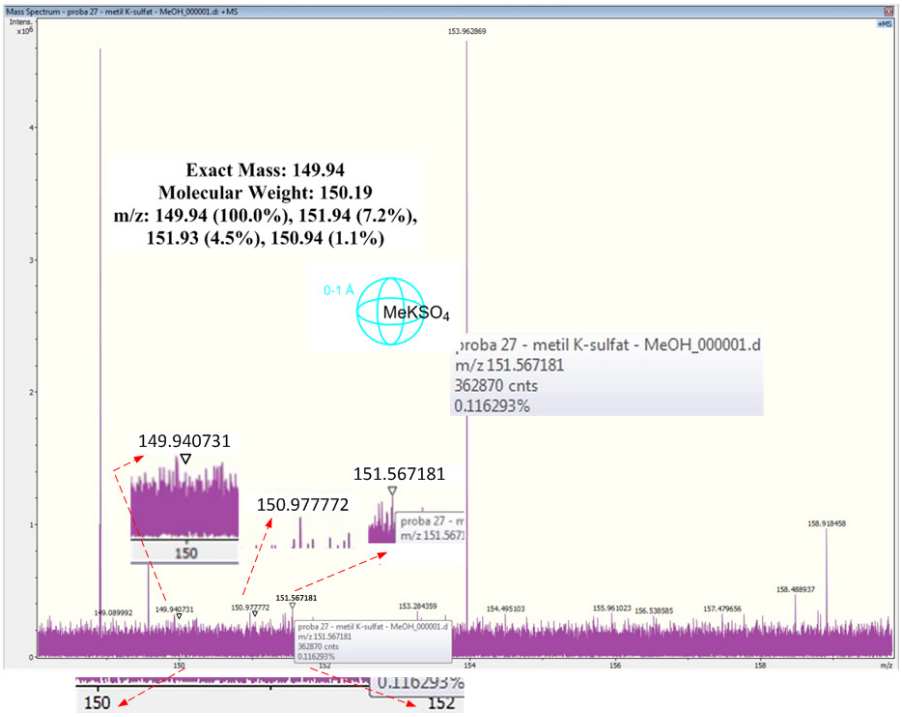


Figure 11. HR-MS spectra, to identify 3rd intermediary end product of guanidine synthesis: potassium methyl sulfate.

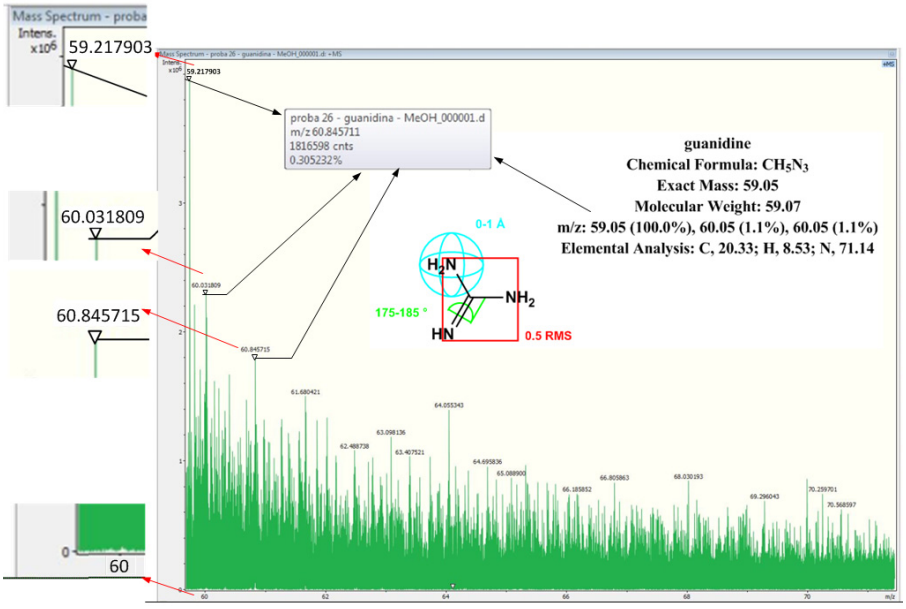


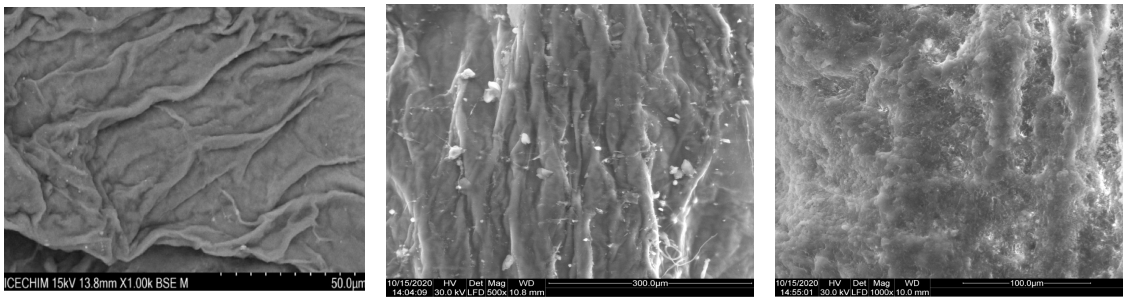
Figure 12. HR-MS spectra, to identify guanidine as the targeted synthesis product.

3.2. Heterogeneous Catalyst Analysis

SEM Analysis

The study of surface morphology for BC membranes was carried out using scanning electron spectroscopy (SEM) for each type of material. Figure 13 shows images of a plain, untreated BC membrane and the functionalized BC membrane surface. Morphological features often found in similar studies, for example, a fibrillar network of cellulose but unaltered, can be observed in Figure 13a, with whole fibers crossed, in short, a typical BC surface morphology. The three-dimensional network of BC fibrils is also visible in Figure 13b, where the presence of KOH nanoparticles can be observed. Figure 13c shows a

totally different surface compared with Figure 13a,b, as the aggregates resulting from KOH nanoparticles deposited on BC and treated with guanidine can be seen uniformly formed and dispersed.



(**a**) (**b**) (c)

Figure 13. SEM images of simple and treated BC: (**a**) non-treated BC; (**b**) presence of KOH nanoparticle after the first treatment; (**c**) superbase aggregates formed after impregnation in alcoholic guanidine solution.

The elemental analysis performed for the functionalized BC_{KOH} confirms the observations regarding the identification of the alkaline chemical compound (Figure 14) as well as superbase formation (Figure 15).

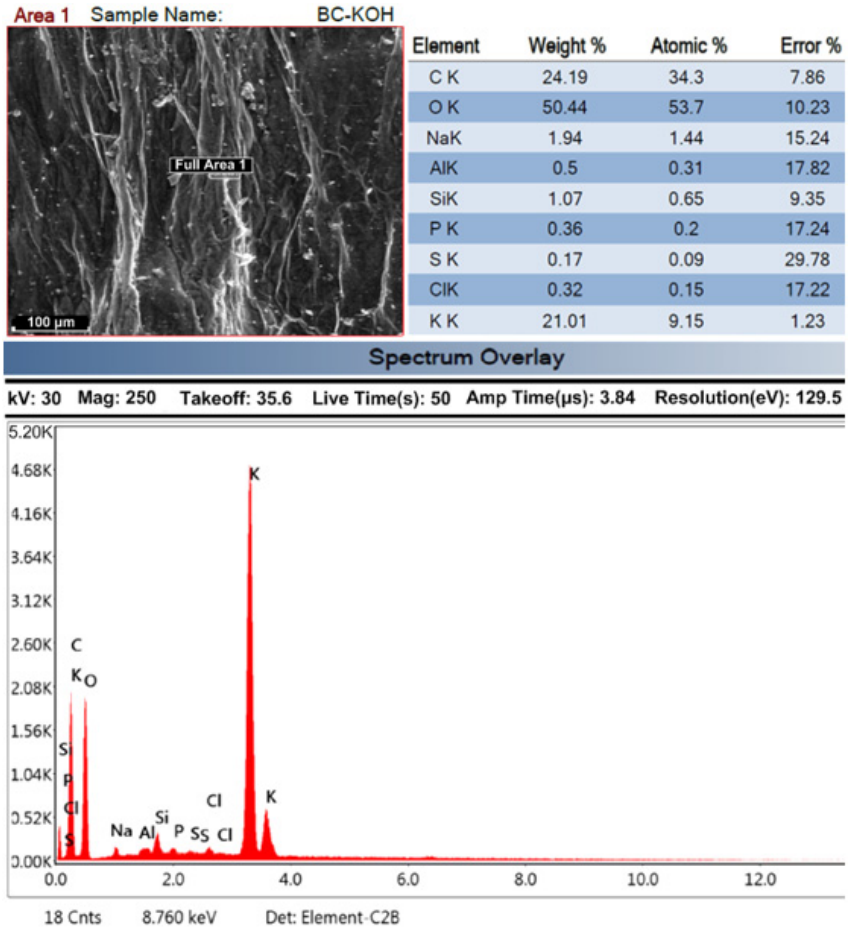


Figure 14. BC_{KOH} membrane analysis: SEM image describing the surface morphology and elemental analysis, with high peaks and mass percentage confirming the success of functionalizing chemical treatment.

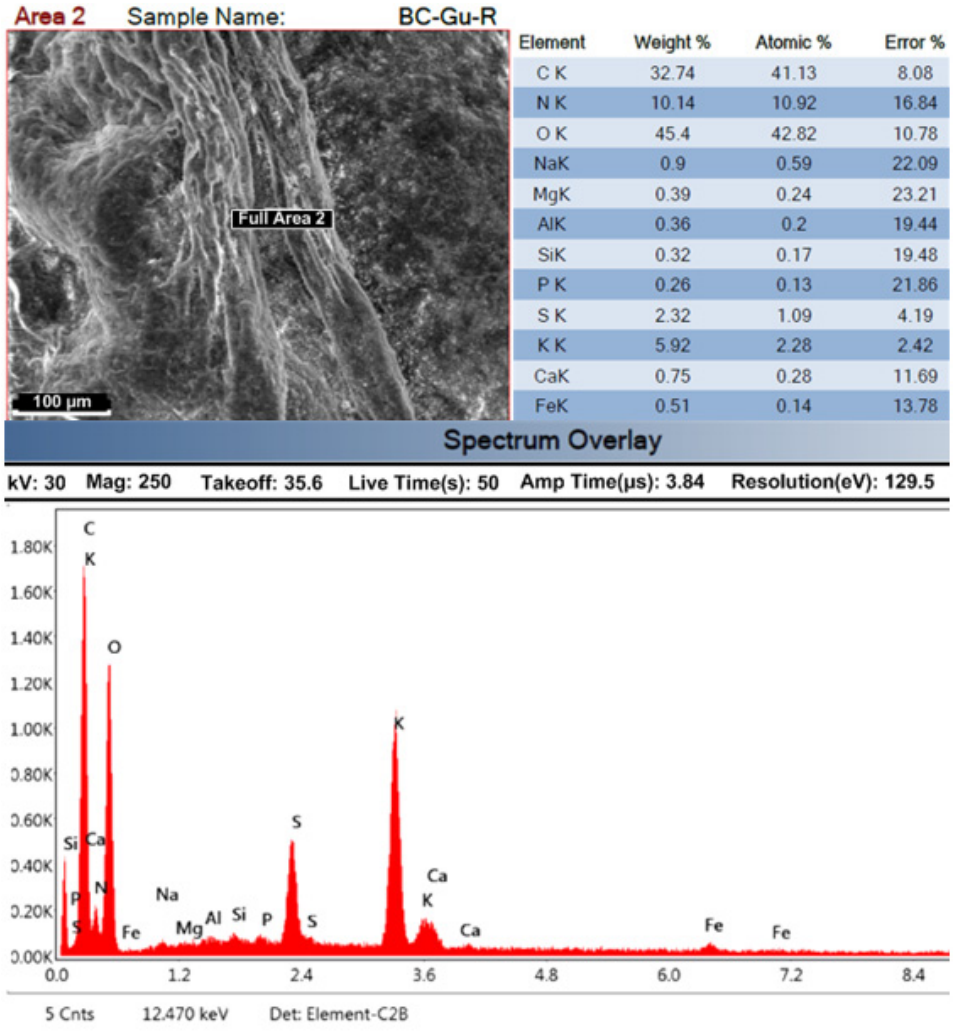


Figure 15. Gu-$_{KOH}$/BC membrane analysis: SEM image describing the surface morphology and elemental analysis, with peaks and mass percentage for guanidine corresponding nitrogen following functionalizing chemical treatment.

In the case of the Gu-$_{KOH}$/BC catalyst, in the SEM images shown in Figure 15, the deposition of the superbase on the cellulose fibrils can be observed; the superbase appears wrapped around the fibrils, coating-like, simulating a perspective of homogeneity with them. The theory of superbase formation can thus be applied, further confirmed by the nitrogen peaks corresponding to guanidine, correlated with tabulated mass percentage.

3.3. Catalyst Testing in Transesterification

For a 2^4 factorial experimental design, a response y dependent on the four factors was expressed according to Equation (1), where x_i are the dimensionless process factors. These dimensionless factors are defined by Equation (2), where they have the values +1 and −1.

$$y(x_1, x_2, x_3, x_4) = \beta_0 + \sum_{i=1}^{4} \beta_i x_i + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \beta_{ij}\, x_i x_j + \sum_{i=1}^{4} \sum_{j=i+1}^{4} \sum_{k=j+1}^{4} \beta_{ijk} x_i x_j x_k + \beta_{1234} x_1 x_2 x_3 x_4 \quad (1)$$

$$x_i = \frac{z_i - z_{iC}}{\Delta z_i} = \frac{z_i - \frac{z_{i,max} + z_{i,min}}{2}}{\frac{z_{i,max} - z_{i,min}}{2}}, \quad i = 1 \ldots 4 \quad (2)$$

For transesterification of used vegetable oils using Gu-$_{KOH}$/BC as a catalyst, the experimental investigation concerning the factors that influence the transesterification yield after esters and after glycerin is presented in Table 2. A notable difference can be observed

between the yield after esters and the yield after glycerin, the latter being able to include aspects of the separation of glycerin from the esterified mixture. This influence can be highlighted quantitatively when determining the coefficients from relation (1), where y is the yield after esters or the yield after glycerin. In Tables 1 and 2, it can be noted that, compared to the factorial plan 2^4 in the experimental investigation, four measurements were added in the center of the factorial plan (17–20), thus allowing the variance of the transesterification procedure to be appreciated in terms of reproducibility. To determine the coefficients from Equation (1), test their significance and validate the mathematical model, a specific procedure was applied [36].

Table 2. Biodiesel yield after esters (η_E) and glycerin (η_G) at different levels of dimensionless process factors in a 2^4 factorial design with 4 replicates in the center of experimental plan (t = 25 °C, τ_e = 1.5 h).

Exp	x_1	x_2	x_3	x_4	η_E	η_G
1	−1	−1	1	−1	0.9478	0.7112
2	−1	−1	−1	−1	0.9514	0.6938
3	−1	1	1	−1	0.9172	0.8013
4	−1	1	−1	−1	0.9356	0.7461
5	1	−1	1	−1	0.9411	0.8333
6	1	−1	−1	−1	0.9363	0.8915
7	1	1	1	−1	0.9347	0.8624
8	1	1	−1	−1	0.9332	0.8527
9	−1	−1	1	1	0.9521	0.9108
10	−1	−1	−1	1	0.9575	0.8817
11	−1	1	1	1	0.9405	0.9302
12	−1	1	−1	1	0.9334	0.8963
13	1	−1	1	1	0.9421	0.9835
14	1	−1	−1	1	0.9453	0.8381
15	1	1	1	1	0.9447	0.9738
16	1	1	−1	1	0.9441	0.9399
17	0	0	0	0	0.9441	0.8915
18	0	0	0	0	0.9294	0.8769
19	0	0	0	0	0.9219	0.8672
20	0	0	0	0	0.9382	0.8782

x_1 = dimensionless KOH concentration in Gu-$_{KOH}$/BC (%); x_2 = dimensionless catalyst dose (g/100 g); x_3 = dimensionless methanol/oil molar ratio; x_4 = dimensionless drying temperature of BC-KOH in Gu-$_{KOH}$/BC catalyst.

Step by step, the valorization of the statistical model (1) using the data from Table 2 is as follows:

(1) Import Table 2 as a matrix and identify the columns for independent variables $x_1 \ldots x_4$ and y in it.

(2) Considering the orthogonality of the data matrix, calculate the β coefficients in Equation (1) with Equations (3)–(7).

$$\beta_0 = \frac{\sum_{i=1}^{N} y_i}{N},\ N = 16,\ i = 1, 2 \ldots N \quad (3)$$

$$\beta_j = \frac{\sum_{i=1}^{N} y_i x_{ji}}{N},\ N = 16,\ j = 1, 2 \ldots 4,\ i = 1, 2 \ldots N \quad (4)$$

$$\beta_{jk} = \frac{\sum_{i=1}^{N} y_i x_{ji} x_{ki}}{N},\ N = 16,\ k > j,\ j = 1, 2, 3,\ k = 2, 3, 4 \quad (5)$$

$$\beta_{jkl} = \frac{\sum_{i=1}^{N} y_i x_{ji} x_{ki} x_{li}}{N},\ N = 16,\ l > k > j,\ j = 1, 2,\ k = 2, 3,\ l = 3, 4 \quad (6)$$

$$\beta_{jklm} = \frac{\sum_{i=1}^{N} y_i x_{ji} x_{ki} x_{li} x_{mi}}{N},\ N = 16,\ \ m > l > k > j,\ j = 1,\ k = 2,\ l = 3,\ m = 4 \quad (7)$$

(3) From the matrix containing experimental data, extract the matrix containing data referring to experiments characterizing the reproducibility measurements and compute their variance (Equations (8) and (9)) and the standard deviation of β coefficients (Equation (10)).

$$y_{mc} = \frac{\sum_{i=1}^{N_c} y_{ci}}{N_c} \tag{8}$$

$$s_r^2 = \frac{\sum_{i=1}^{N_c} (y_{ci} - y_{mc})^2}{N_c - 1} \tag{9}$$

$$s_\beta = \frac{s_r^{2\,0.5}}{\sqrt{N}} \tag{10}$$

(4) Establish the number of freedom degrees characterizing the experiences in the center of the experimental plan (Equation (11)), choose the confidence interval (Equation (12)) for β coefficients, and calculate the theoretical value of the Student variable ($t_{\nu\alpha}$), by solving Equation (13).

$$\nu = N_c - 1 \tag{11}$$

$$1 - \alpha = 0.95 \tag{12}$$

$$\int_{-t_{\nu\alpha}}^{t_{\nu\alpha}} \frac{\Gamma\left(\frac{\nu+1}{2}, 0\right)}{(\pi\nu)^{0.5}\Gamma\left(\frac{\nu}{2}, 0\right)} \left(1 + \frac{t^2}{\nu}\right)^{\frac{-(\nu+1)}{2}} dt = 1 - \alpha \tag{13}$$

(5) Evaluate the Student variable value associated to each β coefficient (Equations (14)–(18)).

$$t_0 = \beta_0 / s_\beta \tag{14}$$

$$t_j = \beta_j / s_\beta, j = 1, 2 \ldots 4 \tag{15}$$

$$t_{jk} = \beta_{jk} / s_\beta,\ k > j,\ j = 1, 2, 3,\ k = 2, 3, 4 \tag{16}$$

$$t_{jkl} = \beta_{jkl} / s_\beta,\ \ l > k > j,\ j = 1, 2,\ k = 2, 3,\ l = 3, 4 \tag{17}$$

$$t_{jklm} = \frac{\beta_{jklm}}{s_\beta},\ \ m > l > k > j,\ j = 1,\ k = 2,\ l = 3,\ m = 4 \tag{18}$$

(6) Verify the significance of each β coefficient by comparing its computed Student variable value with $t_{\nu\alpha}$, as follows:

$$\beta_0,\ \beta_j, \beta_{jk}, \beta_{jkl}, \beta_{jklm} = \beta_0,\ \beta_j, \beta_{jk}, \beta_{jkl}, \beta_{jklm}\ for\ t_0,\ t_j, t_{jk}, t_{jkl}, t_{jklm} \geq t_{\nu\alpha}\ ,\ \ 0\ otherwise \tag{19}$$

(7) Build the statistical model with the significant β coefficients and prove with the Fischer test that the model is adequate.

Equations (20) and (21), linking process responses to dimensionless factors, were obtained by processing the data from Table 2.

$$\eta_E = 0.941 - 0.0057x_2 + 0.0043x_1x_2 + 0.00093x_3x_4 - 0.00575x_1x_2x_3x_4 \tag{20}$$

$$\begin{aligned}\eta_G = 0.859 \quad & + 0.038x_1 + 0.016x_2 + 0.017x_3 + 0.06x_4 - 0.0058x_1x_2 - 0.023x_1x_4 + 0.014x_3x_4 - \\ & 0.0095x_1x_2x_4 - 0.0076x_1x_3x_4 - 0.0066x_2x_3x_4 - 0.015x_1x_2x_2x_4\end{aligned} \tag{21}$$

An analysis of the dependence of transesterification yield expressed after esters on process factors (Equation (20)) shows that all factors have an influence and this influence appears preponderantly as a result of factor interaction ($0.0043x_1x_2 + 0.00093x_3x_4 - 0.00575x_1x_2x_3x_4$). However, the small values of the coefficients that multiply the factors in Equation (20) may suggest that this influence is not extremely strong. It appears that for x_3 and x_4 in the center of the experimental plan (molar ratio of 5 and catalyst drying at

90 °C), the low catalyst dose and low KOH concentration in the catalyst preparation lead to methyl ester yields up to 96%. Figure 16 supports this statement.

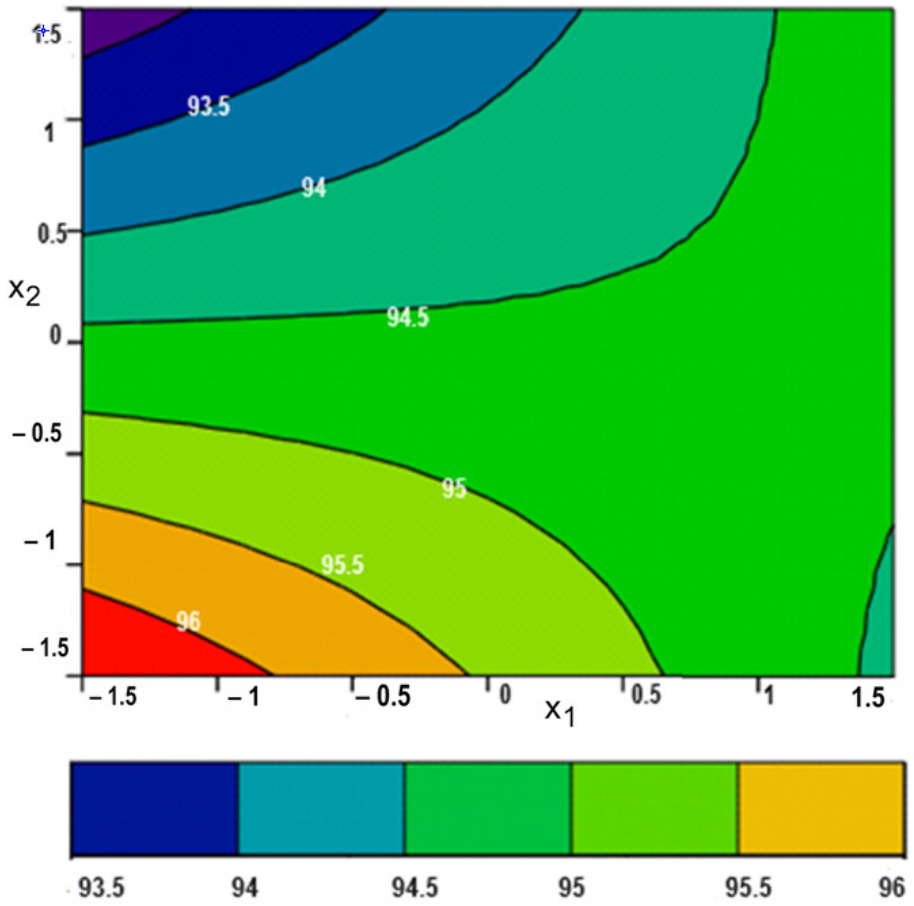


Figure 16. Dependence of transesterification yield after esters on factors x_1 and x_2 for $x_3 = x_4 = 0$.

Regarding the dependence of the transesterification yield after glycerin on the process factors, their influence is much more complex. Some coefficients with values above 0.01 in Equation (21) indicate a strong influence. The important influence of factors x_3 and x_4 on the yield of glycerin in transesterification is shown in Figure 17, where x_1 and x_2 are in the center of the experimental plan. It is observed that a yield of 0.9 is attained for a well-dried catalyst (drying at 105 °C) and a high methanol/oil molar ratio.

To maximize the transesterification yield, the system of Equations (22) and (23) was solved, where Equations (20) and (21) were used to express η_E and η_G, respectively, and the solutions are given in Table 3.

Even if some values of dimensionless variables are not close to the domain of the factors from the experimental field, Table 3 shows that if the intention is to obtain a high yield, the catalyst must be concentrated in an alkaline compound and it is necessary to use a large mass of catalyst, with a molar ratio of 6 and drying of the catalyst performed at 90 °C.

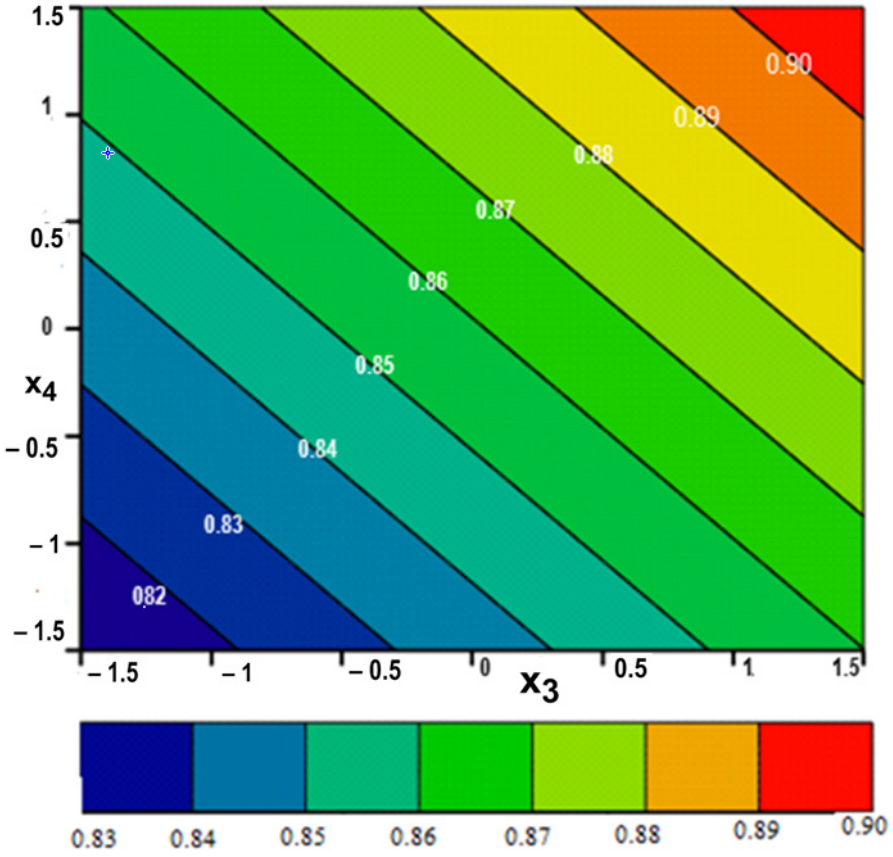


Figure 17. Dependence of transesterification yield after glycerin on factors x_3 and x_4 for $x_1 = x_2 = 0$.

$$\frac{\partial \eta_E}{\partial x_1} = \frac{\partial \eta_E}{\partial x_2} = \frac{\partial \eta_E}{\partial x_3} = \frac{\partial \eta_E}{\partial x_4} = 0 \quad (22)$$

$$\frac{\partial \eta_G}{\partial x_1} = \frac{\partial \eta_G}{\partial x_2} = \frac{\partial \eta_G}{\partial x_3} = \frac{\partial \eta_G}{\partial x_4} = 0 \quad (23)$$

Table 3. Identification of solutions for systems of Equations (22) and (23).

Equation	x_1	x_2	x_3	x_4	Max. Yield
Equation (22)	1.290	0	0.1	0	0.946
Equation (23)	1.510	2.920	1.480	0.362	0.964

4. Conclusions

In this experimental investigation, a heterogeneous catalyst was first constructed and then tested in transesterification. The heterogeneous catalyst is a supported catalyst, in the construction of which two elements participated: guanidine as a strong alkaline chemical compound and bacterial cellulose (BC) as a catalytic support.

Approaching BC from the perspective of viable and reliable materials capable of participating in reactions is not new, but from the viewpoint of viability, low-cost treatment or functionalization techniques have been implemented, which have rather paved the way for the integration of BC into the architecture of the biodiesel production process through the transesterification reaction or in any other process or reaction that requires a permissive matrix for access. The successful combination of the two elements as a supported catalyst capable of transforming triglycerides yielding over 95% methyl ester content led to the

creation of an environmentally friendly catalyst, and its reliability remains to be tested in reactions other than transesterification to methyl esters.

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