

Research Article

Synthesis and Physicochemical Characterization of Anion Exchanger Based on Green Modified Bottle Gourd Shell

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The structural performance and biosorption behavior of a new cationic biosorbent (CALV) prepared from bottle gourd (*Lagenaria vulgaris*) shell (LVS) as a potentially valuable agrowaste have been established. The biosorbent with anion exchangeable function was synthesized from a modified lignocellulosic biomass (MLV) by a quaternary ammonium agent, N-(3-chloro-2-hydroxypropyl)trimethylammonium chloride. Preparation of the appropriate MLV precursor was firstly carried out by the process of alkaline pretreatment of LVS biomass using a green carbonate solution. In this way, partial delignification, removal of extractive substances, and increased porosity of the cellulose-enriched MLV biomass, as well as the activation of available cellulosic microfibrils to alkaline-cellulose have been achieved. The optimization of synthesis conditions was carried out by direct estimation of the CALV biosorption efficiency to phosphate (using ICP-OES) and nitrate (using UV-VIS) from the aqueous solutions. The structural changes of MLV precursors during green modification and the success of synthesis and biosorbent performances before and after the removal of anions from contaminated solutions have been registered and clarified by physicochemical methods, which include elemental analysis, chemical constitution, morphological characterization, and FTIR spectroscopy. In accordance with the FTIR spectroscopic analysis and isotherm studies, a structural model of the CALV biosorbent and its ion exchange mechanism are proposed.

1. Introduction

The constant need for the supply of herbicides, nutrients, and artificial fertilizers to agriculture or detergents to industry has led to undesirable effects of overloading phosphates and nitrates in sensitive ecosystems. One of these effects is the occurrence of eutrophication [1]. Therefore, it was necessary to develop methods of treatment that facilitate the removal of phosphates and nitrates from wastewater before release into natural water. In the technology of wastewater treatment, various methods, including chemical and biological processes, have been successfully applied [2]. Among these methods, the anion exchange is well known as one of the simplest and ecologically acceptable methods for removing pollutants from wastewater [3]. The advantage of

this method is the simultaneous removal of various ionic pollutants, such as heavy metals and many anion species (primarily nitrates and phosphates), while the effectiveness of treatment is relatively constant [4].

On the other hand, large amounts of unused agricultural residues, widespread throughout the world, are waiting for effective use due to low-cost, availability, and biodegradability. Over time, many attempts have been made to find cheap and effective anion exchangers based on agricultural residues [2, 5]. Various studies have shown that many agricultural materials, such as lignocellulosic biomass, can be modified into anion exchangers and used for this purpose [2, 6]. Anion exchangers based on the biomasses represent a potential alternative to commercial resins due to their specific properties, such as chemical stability and high

reactivity due to the presence of reactive hydroxyl groups in the lignocellulosic matrix. Thus, natural lignocellulosic biomasses have increased usage in low-cost water purification technologies for the removal of toxic metals [7] or some other cationic pollutants such as dyes [8], herbicides, or pharmaceuticals [9] from water systems. However, these biomasses must be modified before being used for the removal of different anionic pollutants from aqueous solution. Modification reactions including functionalization of biomass surfaces (primarily quaternization) are commonly applied to enhance biosorption capacity and stability of the components present in the biomasses. In principle, most of the biomasses are composed of polysaccharides (cellulose and hemicelluloses) and lignins. In this respect, there are different modification methods, and these can be done using various organic agents (epichlorohydrin, N, N-dimethylformamide, formaldehyde, ethylenediamine, diethylamine, and trimethylamine) [2] or some quaternary ammonium compounds [4]. The chemical modification influences the creation of a large number of active sites for the binding of anions to biomass.

Similar to other biomasses according to its composition and properties, the bottle gourd (*Lagenaria vulgaris*) shell (LVS), as a potentially valuable agrowaste, has not been investigated so far to remove anionic pollutants from aqueous solutions. Therefore, this study examines the possibility of obtaining a cationic biosorbent based on the bottle gourd shell (CALV) and applying it as an original anion exchanger. The synthesis of CALV biosorbent with the ion exchange properties has been performed by quaternizing the alkaline-modified biomass (MLV) as a precursor. Regarding this, preparing the precursor meant activating the cellulose component of biomass to the alkaline-cellulose form, by translating the surface $-OH$ groups of glucopyranose units into a more reactive $-ONa$ groups using an alkaline agent. These groups are further functionalized with a quaternary ammonium agent, N-(3-chloro-2-hydroxypropyl)trimethylammonium chloride (CHMAC), to the cationic form ($LV-N^+R_3$) of the final CALV product. This modification method significantly influences the increase in positive surface charge, which is a prerequisite for effectively removing negative anionic species from the solution. In order to obtain a more productive biosorbent (a better affinity for anionic pollutants and greater sorption capacity), the synthesis conditions (such as reagents concentrations, biomass/reagent molar ratio, temperature, pH, and contact time) were optimized. The obtained product was characterized by physicochemical methods, primarily using the spectroscopic FTIR analysis. In terms of potential application in the process of wastewater treatment, the efficiency of cationic biosorbent has been tested in the case of phosphate and nitrate anions' removal from contaminated aqueous solutions.

2. Materials and Methods

2.1. Reagents and Materials. All reagents used in this study were of analytical grade (Sigma-Aldrich Chemie GmbH). The solutions for modification and synthesis processes, as well as to rinse obtained products, were prepared with

deionized water (18 m Ω). In this experiment, the matured fruit of *L. vulgaris* from southern area of Serbia (near Leskovac) was used as the starting lignocellulosic material, grown at an altitude of 250 m. The naturally dried shell of *L. vulgaris* was manually emptied and crushed into 1 to 2 cm pieces. The raw material was soaked and washed with deionized water to remove dust and soluble impurities. The washed material was dried in an oven for 24 h at 60°C, milled in a crusher mill (Waring 8010 ES, Germany), and sieved to separate a size between 400 and 800 μm using successive sieving through octagon sieves (Oct-Digital 4527-01).

2.2. Pretreatment of Biomass. In order to prepare the MLV precursor, dried and crushed (400–800 μm) *L. vulgaris* shell (5 g) was treated with 100 cm³ of weak alkaline solution (mixture of 1% Na₂CO₃ and 1% NaCl) for 4 h at a temperature of 25 \pm 1°C and atmospheric pressure, with constant mixing on a magnetic stirrer (150 rpm). The suspension was filtered (by 0.45 μm of microfiltration membrane, Agilent Technologies, Germany) and washed with deionized water until discoloration. The residue was then dried at 80 \pm 1°C to a constant weight. The prepared MLV biomass was subjected to physical-chemical and spectroscopic characterization and then to the analysis of the phosphate anion biosorption efficiency.

2.3. Biosorbent Synthesis. After alkaline pretreatment, the MLV precursor (1 g) was treated with NaOH solution of the desired concentration (5 to 40 mmol/g precursor) at a temperature of 25 \pm 1°C to activate the primary and secondary $-OH$ groups of the glucopyranose unit and to translate the cellulose into more reactive alkali-cellulose with characteristic $-ONa$ groups. After 60 min, a specific volume of the original (60 wt.%) aqueous CHMAC solution was added to the reaction mixture gently in drops, with a mild increase in temperature to 80°C. The reaction mixture was further treated at 80 \pm 1°C for a specified time (1–16 h), with constant stirring (150 rpm). The synthesis reaction was performed in a specific three-neck round-bottom flask (with reflux), intended for these purposes (Figure 1), to prevent the ammonium agents' loss as well as the appearance of increased pressure due to the emergence of aggressive vapors (unpleasant odor) at the synthesis temperature. The resulting cationic product, after filtering and washing, was dried for 12 hours at 60 \pm 1°C (to constant weight) and then subjected to characterization as a biosorbent.

2.4. Characterization Methods. We followed the methods of Marković-Nikolić et al. [10]. The constituents and physical properties of LVS were determined according to the standard Association of Official Analytical Chemists procedures [11]. Elemental analysis (CHNS/O) of unmodified LVS, precursor MLV, and CALV biosorbent was performed using an Elemental Analyzer (Vario EL III CHNS/O systeme GmbH), to assess add functional groups in biosorbent. Scanning electron microscopy (SEM, JEOL JSM-6610LV)

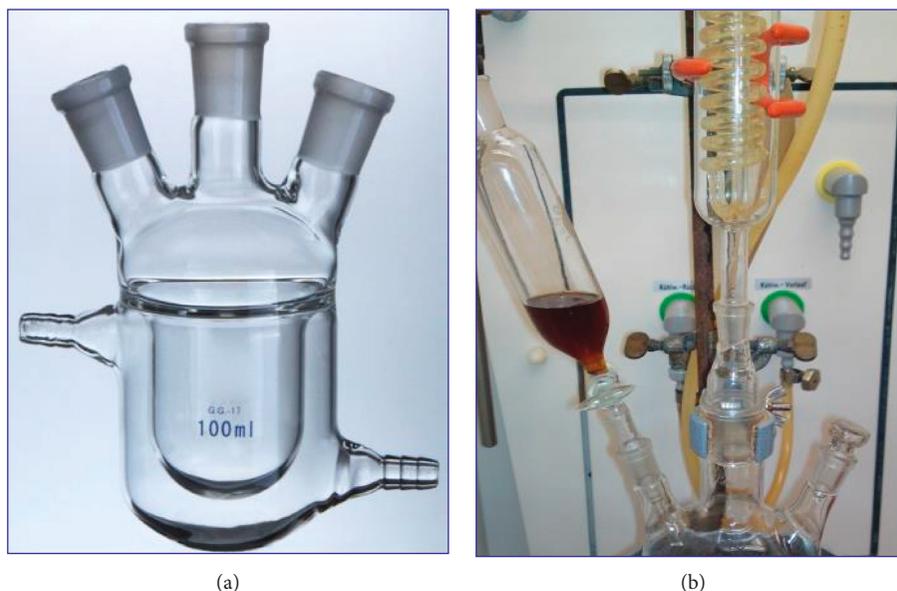


FIGURE 1: Glass jacketed flask reactor vessel round bottom flask beaker double layer three necks and the complete synthesis apparatus.

was used to examine the surface morphology of the biomass and CALV biosorbent before and after anion loading. The samples were attached to copper-based mounts (10 mm) using a double-sided tape and then sputter-coated with chrome, under vacuum in an argon atmosphere. An accelerating voltage of 20 keV for primary electrons, as well as working distance of 10 mm proved to be satisfactory. Spot sizes varied depending on the applied magnification. The infrared spectra of the all investigated samples were recorded using a Bomem MB-100 (Hartmann & Braun, Canada) Fourier transform infrared spectrometer (FTIR), in the range of 4000–400 cm^{-1} , at resolution of 2 cm^{-1} and with 16 scans. The samples were compressed in a potassium bromide (KBr) pellet prior to spectroscopy. The obtained FTIR spectra were analyzed using Win Bomem Easy software.

2.5. Batch Biosorption Experiments. The biosorption experiments were carried out under the batch conditions, using 50 cm^3 of the anion (phosphate or nitrate) solution. The solution was stirred at 150 rpm (using a magnetic stirrer) for 5 min in order to ensure a good mixing without vortex effect. Temperature was maintained at $20 \pm 1^\circ\text{C}$ using a temperature-controlled water bath. The initial pH of the solution was adjusted to the pH value of 6.5 ± 0.1 with HCl (0.1 M) or NaOH (0.1 M). Then, the biosorbent ($2 \text{ g}\cdot\text{dm}^{-3}$) was added in the working solution. Aliquots of the solutions (3 cm^3) were withdrawn at appropriate time intervals (up to 60 min) and filtered by $0.45 \mu\text{m}$ microfiltration membrane. The phosphate filtrates were analyzed for residual phosphate concentration (expressed over $\text{P}\cdot\text{PO}_4$), axially at 213.618 nm by inductively coupled plasma-optical emission spectrometry (ICP-OES) on the instrument ARCOS FHE12 (Spectro, Germany), according to the manufacturer's instructions. The nitrate filtrates were analyzed for residual nitrate concentration (expressed over $\text{N}\cdot\text{NO}_3$), at 220/275 nm by

a UV-VIS spectrophotometer (Cary-100 Conc., Varian), according to the Standard 4500- NO_3 methods [12]. Anion concentrations on the solid phase were calculated based on the initial and final aqueous concentrations. The equilibrium concentration was calculated as follows:

$$Q_e = \frac{(c_o - c_e)V}{m}, \quad (1)$$

where Q_e ($\text{mg}\cdot\text{g}^{-1}$) is the amount of $\text{P}\cdot\text{PO}_4$ or $\text{N}\cdot\text{NO}_3$ biosorption per gram biosorbent at equilibrium, c_o and c_e ($\text{mg}\cdot\text{dm}^{-3}$) are the concentrations of $\text{P}\cdot\text{PO}_4$ or $\text{N}\cdot\text{NO}_3$ at original and equilibrium, respectively, V (dm^{-3}) is the volume of solution, and m (g) is the dry mass of biosorbent. Following the experiments, the postbiosorption materials were collected, rinsed with deionized water, and dried at 60°C in an oven for further characterizations.

3. Results and Discussion

3.1. Design of Pretreatment Process. The efficiency of the alkaline pretreatment process of biomass depends on several factors, which influence the change in the macroscopic and microscopic properties of the lignocellulosic material, including the dimensions, specific surface, structure, and chemical composition of the particles (primarily lignin and hemicellulose content) [13]. The following effects of pretreatment are extremely important for the preparation of the optimal MLV precursor: breakdown of ester bonds between lignin and other constituents of the cross-linked lignocellulosic structure, partial destruction of the lignin matrix to smaller fragments, decrease in the polymerization degree of hemicellulose, reduction in the proportion of crystalline cellulosic form in favor of microfibril amorphism, causal swelling of the lignocellulosic skeleton, and extraction of released inorganic matter from biomass. Bearing in mind that the lignin polymer represents a structural barrier to

TABLE 1: Biochemical properties of the initial LVS biomass and MLV precursor after alkaline pretreatment (1% Na₂CO₃ and 1% NaCl, 4 h, rpm 150, normal conditions).

Parameter	LVS biomass	Loss (%)	MLV precursor
Mass	5.00 ± 0.01 g	-15.2	4.22 ± 0.02 g
Cellulose	39.58 ± 0.42%	0.0	47.12 ± 0.26%
Hemicellulose	18.22 ± 0.14%	-71.4	6.01 ± 0.13%
Lignin	41.90 ± 0.51%	-5.2	46.87 ± 0.34%

these effects, first it was necessary to perform the delignification of LVS biomass. In this sense, the design of the alkaline pretreatment process involved the optimization of parameters such as particle size, contact time, temperature, type and concentration of alkali, and amount of water and biomass/alkali volume ratio.

For the process, optimum particle size of biomass is <10 mm (preferably about 1 mm), and for that reason, the milled and sieved LVS biomass with particle size in the range of 400–800 μm was used. Unlike the nonoxidative method, the oxidative process is more efficient regarding the lignin degradation. Air is more desirable than oxygen because the application of oxygen requires special equipment due to high pressure and corrosion of equipment. The temperature for the oxidative process (in the presence of air) usually ranges from 20 to 50°C. Accordingly, the experiment was conducted at a temperature of 25 ± 1°C and atmospheric pressure, with constant mixing on a magnetic stirrer (150 rpm). The necessary pretreatment time ranges from several hours to several days, depending on the applied temperature and type of biomass [13]. Higher temperature reduces the pretreatment duration, but at the same time, it affects the increased consumption of alkali, which is important from the economic and ecological aspect. Therefore, normal conditions (room temperature and atmospheric pressure) are applied, which is one of the process advantages.

Unlike fast acidic pretreatment [14], used mainly for targeted sugar degradation, the optimal process of alkaline pretreatment requires a longer time (several hours). The alkaline pretreatment is the most acceptable in the case of LVS biomass modification. The advantage of this process is the partial destruction of the lignin due to ester bond hydrolysis in the associated lignin and hemicellulose [15]. Depending on the alkaline reagent, there are several ways of biomass pretreatment (by hydroxides, carbonate, and ammonia). However, great interest in recent times has caused the use of green liquid (Na₂CO₃ and NaCl) for the pulp treatment or the conversion of lignocellulosic biomass [16]. The application of green liquid as weak alkaline reagent results in mild degradation of the lignocellulosic LVS biomass, with better retention of polysaccharides and increased delignification selectivity (Table 1).

After delignification, the exempted hemicellulose is dissolved in water and subjected to hydrolysis by alkali, which further leads to the conversion into water-soluble monomers. Removal of hemicellulose molecules leads to increased biomass porosity, which makes activated cellulose available to chemical N-agents during the biosorbent synthesis. Additionally, the pretreatment allows the removal of

uronic and acetic acid caused by degradation of hemicellulose, which may affect further process and outcome of the synthesis reaction [17]. Unused alkali, acid, and degradation products, as well as released mineral matters from the cell walls, are converted during the pretreatment process into nonrenewable salts and must be removed from the solution. In order not to incorporate these salts into biomass, filtration and detailed washing of biomass with deionized water were performed.

3.2. Synthesis Optimization of Cationic Biosorbent. The lignocellulosic LVS biomass, like other agricultural residues, provides a good platform for making cationic biosorbent with the ion exchange properties. The synthesis of cationic CALV biosorbent based on MLV precursor was performed in accordance with the procedure described in the experimental part. Reaction pathway for the biosorbent synthesis is presented in Figure 2.

In order to achieve the maximum degree of biosorbent cationization, as well as better capacity for more efficient biosorption of the investigated anion species, the optimization of reaction conditions was carried out. The problem of defining the optimal conditions for the synthesis of cationic biosorbents using CHMAC agents was investigated in many studies [2, 6], through alternatively changing one of the important parameters, such as temperature (60–100°C), time (2–24 h), medium (benzene, ethanol, water, and DMF), and a molar ratio of NaOH/CHMAC (0.5–2.0). These studies have shown very diverse results, suggesting that the reaction conditions of the synthesis are still inherent of the starting biomass nature. The different quality of used agents, as well as the concentration of their commercially available solutions, supports this consideration. From these reasons, these investigations included the analysis of the following synthesis parameters: the amount of alkali (NaOH), the amount of quaternary agent (CHMAC), pH value, temperature, and reaction time. The optimization of reaction conditions of the synthesis was carried out by direct estimation of the biosorption efficiency of synthesized cationic biosorbents to phosphate anions. A series of biosorption experiments using synthesized CALV was performed on phosphate solution with a concentration of 30 mg P·dm⁻³ as a model. The reaction conditions of the biosorption process (mixing speed 150 rpm, contact time 60 min, temperature 20°C, and pH 6.5) were selected in accordance with the literature data [2, 5], which proved as optimal for these purposes. In accordance with the biosorption capacities determined using (1), the graphic dependence in the function of varied parameter for the optimal value estimation was constructed.

3.2.1. Influence of NaOH Amount. The influence of alkali amount on the quaternary biosorbent synthesis by varying the NaOH solution concentration (in the range of 0.25 to 2.0 mol·dm⁻³) was analyzed, whereby the values of other reaction conditions were kept constant. In this regard, the MLV precursor (1 g) was treated with 20 cm³ of prepared solution with desired NaOH concentration during 60 min, at

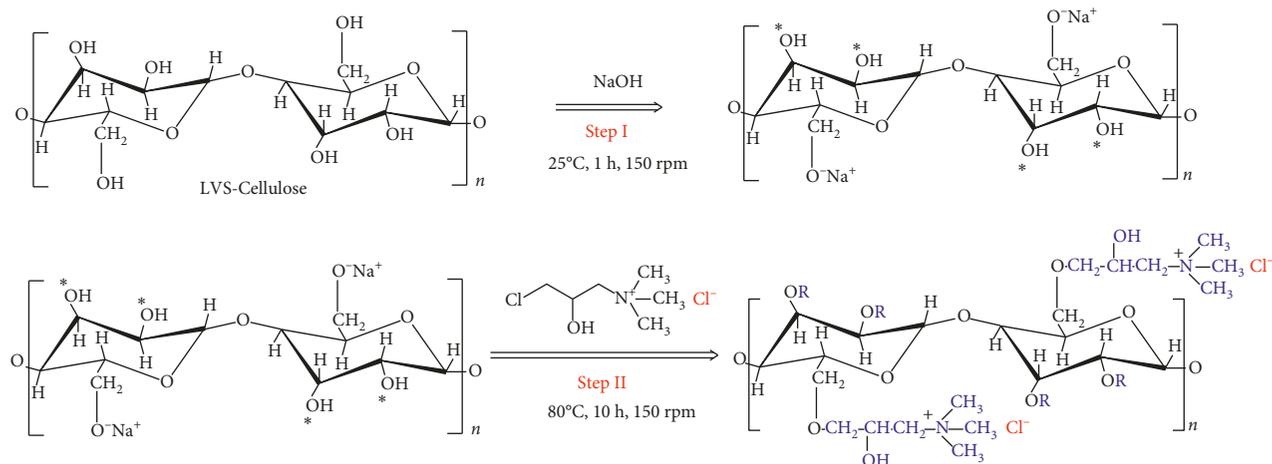
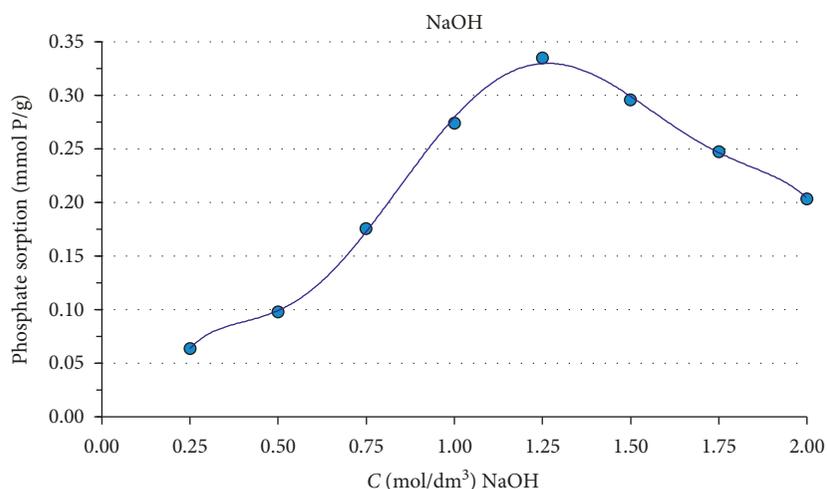


FIGURE 2: Reaction synthesis pathway of cationic CALV biosorbent.

FIGURE 3: Phosphate sorption (expressed as P) by prepared CALV biosorbents in the function of different NaOH solution concentrations (0.25–2.0 mol·dm⁻³).

a temperature of $25 \pm 1^\circ\text{C}$, under stirring (150 rpm). Then, in the series of reaction mixtures (8 samples), 7 cm^3 of the CHMAC solution (25.8 mmol/g MLV) was added. The synthesis at 80°C for 15 h was carried out, according to the method described in the experimental part. The success of the CALV biosorbent synthesis was monitored in the function of phosphate anion sorption from aqueous solution of $30\text{ mg P}\cdot\text{dm}^{-3}$ concentration, with a biosorbent dose of $2\text{ g}\cdot\text{dm}^{-3}$, at pH 6.5 and temperature of $20 \pm 1^\circ\text{C}$ (Figure 3).

The maximum sorption capacity of phosphate of $0.335\text{ mmol P}\cdot\text{g}^{-1}$ (69.2%) was achieved with CALV biosorbent, which was obtained in the presence of $1.25\text{ mol}\cdot\text{dm}^{-3}$ of NaOH solution. When lower or higher concentrations of NaOH solution were used in the synthesis, a significant reduction in the content of sorbed P on the obtained CALV biosorbent could be observed (Figure 3). For this reason, the amount of 25 mmol NaOH per gram of MLV precursor was selected as the optimal value for efficient biosorbent synthesis.

3.2.2. Influence of CHMAC Amount. In order to optimize the amount of quaternary agent required for the synthesis of

effective biosorbent, at a selected alkali concentration of $1.25\text{ mol}\cdot\text{dm}^{-3}$ (25 mmol NaOH/g MLV), the influence of variation in the volume of 60 wt.% CHMAC solution, from 1.4 cm^3 (5 mmol/g MLV) to 8.2 cm^3 (30 mmol/g MLV), was investigated. The volume of the agent was adjusted so that the mass ratio of NaOH/CHMAC was in range from 1.0:0.0 (without CHMAC) to 1.0:1.2 mmol/g MLV. The efficacy of obtained biosorbents was tested on the phosphate solution model at the concentration of $30\text{ mg P}\cdot\text{dm}^{-3}$, with a biosorbent dose of $2\text{ g}\cdot\text{dm}^{-3}$, at pH 6.5, and a temperature of $20 \pm 1^\circ\text{C}$, for 15 h. The sorption capacity of the synthesized CALV samples in the function of NaOH/CHMAC mass ratio is shown in Figure 4.

The experiments of varying the mass ratios of alkali and quaternary agent during the biosorbent synthesis showed that the ratio of 1.0:0.8 mmol/g of MLV was optimal for obtaining the CALV biosorbent with the highest phosphate sorption effect ($0.335\text{ mmol P}\cdot\text{g}^{-1}$). In the absence of quaternary agent, the MLV precursor (treated with NaOH solution only) exhibits a very low tendency to remove phosphate from the solution ($\approx 0.5\%$). However, the addition

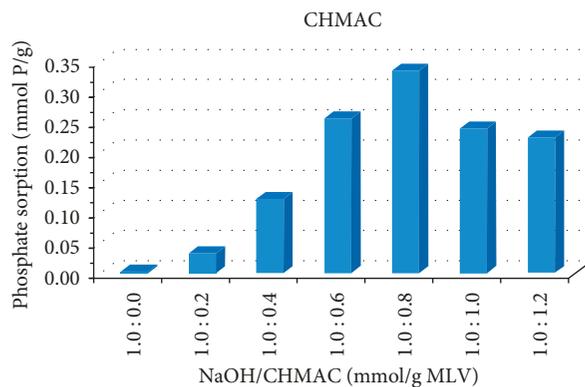


FIGURE 4: Phosphate sorption (expressed as P) by CALV biosorbent in function of the NaOH/CHMAC ratio (1.0:0.0 to 1.0:1.2 mmol/g MLV).

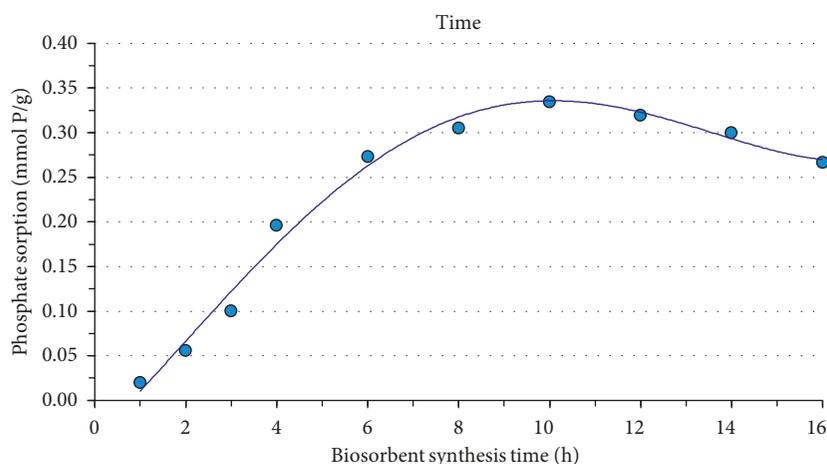


FIGURE 5: Phosphate sorption (expressed as P) by CALV biosorbent in the function of the synthesis time (1–16 h).

of CHMAC agent into the alkaline reaction mixture during synthesis, with a NaOH/CHMAC ratio of 1.0:0.4, shows a significant effect of phosphate sorption (25.4%) on CALV. Adding the larger amount of quaternary agent till the ratio of 1.0:0.8 in the mixture (25 mmol NaOH:20 mmol CHMAC/g MLV) improves the removal of phosphate anions (up to 69.2%) by CALV, where the maximum sorption was observed. Characteristically, the degree of quaternization of MLV precursors decreases with increasing amounts of CHMAC agent, leading to the lower efficiency of phosphate sorption (<50%). It can be noted that each amount of CHMAC agent in the reaction mixture out of the ratio 1.0:0.8 results in the formation of a lower sorption capacity product for phosphates. Hence, an amount of 20 mmol CHMAC per gram of MLV precursor was selected as the optimal value.

3.2.3. Influence of Reaction Time and Temperature. In addition to the amount of alkali and the quaternary agent used, the reaction time and temperature significantly affect the CALV biosorbent synthesis outcome. From these reasons, a series of experiments on the synthesis reaction was carried out by varying the reaction time and temperature, under the previously determined optimal conditions. The efficiency of the obtained biosorbent was firstly tested in the function

of the synthesis time (1–16 h) at a temperature of 80°C. Experiments have shown that maximum phosphate sorption ($0.335 \text{ mmol P}\cdot\text{g}^{-1}$) is achieved using the CALV biosorbent obtained by synthesis after 10 h (Figure 5). It has been noticed that phosphate sorption is rapidly increasing in samples obtained by increasing the reaction time from 1 h to 9 h. In the next 120 minutes, the biosorption capacity did not change significantly. However, a longer synthesis time of 12 h influenced the reduction of the phosphate sorption efficiency by CALV biosorbent.

The optimum temperature value of the biosorbent synthesis was assessed by performing the reaction in the presence of air, under previously defined optimal conditions, by varying the temperature parameter in the range of 20 to 90°C (Figure 6). This series of experiments showed that the maximum degree of phosphate sorption ($0.335 \text{ mmol P}\cdot\text{g}^{-1}$) is achieved with the CALV biosorbent obtained by synthesis at the temperature of 80°C. Therefore, this value was chosen as optimal.

Based on experimentally obtained data from the investigations of the effect of reaction conditions on the outcome of CALV biosorbent synthesis, in the function of phosphate sorption capacity, the optimal reaction parameters for MLV precursor modification by using a quaternary agent were determined. In this sense, the process of cationic CALV biosorbent

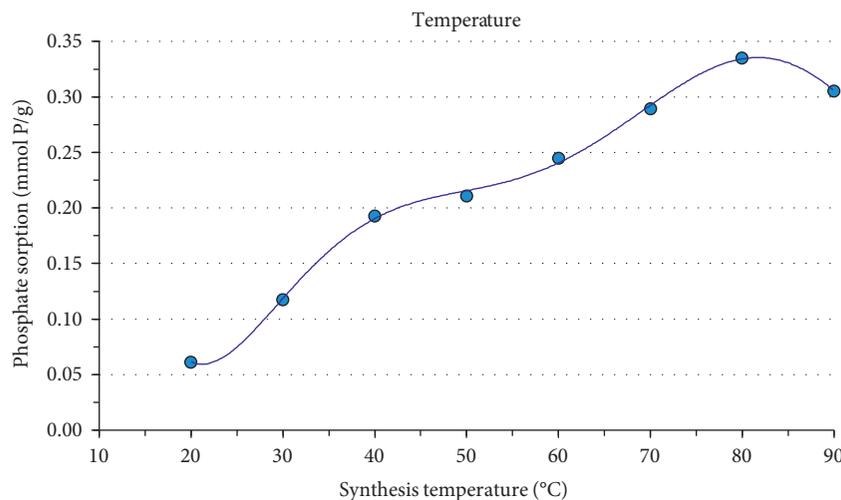


FIGURE 6: Phosphate sorption (expressed as P) by CALV biosorbent in the function of the synthesis temperature (20–90°C).

TABLE 2: Elemental analysis and reaction efficiency of synthesized CALV and CARLVS biosorbents compared to the raw LVS biomass and MLV precursor.

Sample	Elements (%)				N_{add} (mmol N·g ⁻¹)	RE (%)	DS	PY (%)
	C	H	O	N				
LVS biomass	45.46	5.98	48.55	0.01	—	—	—	—
MLV precursor	45.51	5.84	48.65	0.01	—	—	—	84.4
CARLVS biosorbent	46.17	6.25	46.34	1.23	0.87	4.35	0.17	89.7
CALV biosorbent	47.22	6.12	44.71	1.95	1.39	6.95	0.31	98.8

production was defined, expecting the effective removal of not only phosphate but also other anions from contaminated aqueous solutions. The synthetic method involves first alkaline treatment of 1 g of MLV precursor with 20 cm³ of 1.25 M NaOH solution (25 mmol/g MLV) for 60 min, at a temperature of 80 ± 1°C, to ensure the activation of biomass to alkali-cellulose. The second step of the synthesis (Figure 2) refers to the chemical reaction, which is performed by adding 5.43 cm³ 60 wt.% CHMAC solution (20 mmol/g MLV) in the reaction mixture, gently dropping, with a slight heating to 80°C. Further synthesis flow was carried out by treating the reaction mixture at 80°C for 10 h with the condenser. The resulting CALV product was subjected to filtration and rinsing, as was described in the experimental part.

To compare the biosorption efficiency, and primarily to determine the significance of the alkaline pretreatment of the starting biomass in order to prepare the precursor of synthesis, under the same optimal conditions, the synthesis of the second biosorbent (CARLVS) was carried out on the basis of raw (unmodified) LVS biomass.

3.3. Physicochemical Characterization of Biosorbent. CALV (based on MLV precursors) and CARLVS (based on raw LVS) biosorbents, obtained by an identical synthetic method under optimum conditions, were characterized by elemental analysis and biosorption behavior. The aim of these analyses was to confirm the introduction of quaternary cationic groups into the structure of LVS biomass, or MLV precursor prepared by alkaline pretreatment. Simultaneously, the

efficiency of the synthesized biosorbents was estimated by using the biosorption capacity values for different anion species (phosphates and nitrates).

3.3.1. Elemental Analysis and Reaction Efficiency. Elemental analysis (CHNS/O) was used to estimate the amount of added N-functional groups in biosorbents during synthesis. Changes in the content and type of elements of synthesized biosorbents, compared to the initial LVS biomass and MLV precursor, are shown in Table 2. In the function of nitrogen content (N%), other characteristic parameters including reaction efficiency (RE), degree of substitution (DS), amount of added cationic groups (N_{add}), and product yield (PY), were also determined:

$$N_{\text{add}} (\text{mmol N} \cdot \text{g}^{-1}) = (N_{\text{BS}} - N_{\text{BM}}) \cdot 0.714, \quad (2)$$

$$\text{DS} = 162 \cdot \frac{N_{\text{BS}}}{(14 \cdot 100 - M \cdot N_{\text{BS}})}, \quad (3)$$

$$\text{RE} (\%) = \left(\frac{N_{\text{add}}}{Q} \right) \cdot 100, \quad (4)$$

$$\text{PY} (\%) = \left(\frac{m_{\text{BS}}}{m_{\text{BM}}} \right) \cdot 100, \quad (5)$$

where N_{BS} and N_{BM} represent nitrogen content (%) in biosorbent and biomass, respectively, estimated from the elemental analysis, factor 0.714 converts units (% N to mmol N·g⁻¹), Q is the amount of cationic CHMAC agent added to

the reaction mixture per gram of biomass (20 mmol/g LVS), M is the molecular weight of cationic CHMAC agent (188.1 g·mol⁻¹), 162 is the molecular weight of the anhydrous glucose unit (g·mol⁻¹), while 14 is the relative atomic mass of the nitrogen, and m_{BS} and m_{BM} are the mass (g) of biosorbent and biomass dry samples, respectively.

Based on the elemental analysis (Table 2), it was found that the values of CHO content in the raw LVS biomass (H/C ratio 0.13) were very similar to the results of elemental analysis of other studies, which report the content of 42–59% for C, 3–7% for H, and 32–50% for O in various agricultural lignocellulosic by-products [4, 7]. The presence of sulfur has not been identified in the tested samples. The alkaline pretreatment of LVS biomass did not significantly change the content of these elements, considering that the decomposition of MLV precursor retains the proportionality of the observed elements (H/C ratio 0.13). Significant changes in the nitrogen content were recorded in both biosorbent samples, confirming the cationization efficiency of LVS biomass and MLV precursor. Higher nitrogen content (1.95%) in CALV biosorbent confirms that the alkaline pretreatment significantly contributes to the introduction of cationic N-groups into the biomass structure. Using (2), it was calculated that 12.18 mg N·g⁻¹ and 19.46 mg N·g⁻¹ (in the form of the cationic -N⁺R₃ group) were bound on the raw LVS biomass surface and in the structure of the alkaline-modified MLV precursor, respectively. These data indicate the higher theoretical value of ion exchange capacity of CALV biosorbent (1.39 mEq·g⁻¹). In this regard, maximum biosorption capacity for phosphates of 43.09 mg P·g⁻¹ should be expected.

However, taking into consideration better results for reaction efficiency (6.95%) and degree of substitution (0.31), it can be concluded that the reaction between biomass and ammonium agent was not particularly effective. This can be very important in the semi-industrial (scale-up) process because it is one of the factors determining the cost of the process [4]. Namely, the synthesis of CALV biosorbent was carried out in the excess of ammonium agent (20 mmol·g⁻¹) to 3.4 mmol·g⁻¹ glucopyranose units of precursor. This represents a theoretically required ratio for maximum binding of 3 molecules of the CHMAC agent to one primary and two secondary -OH groups of glucopyranose unit and the same number of agent molecules to the phenolic -OH groups of lignin monomers. Therefore, it can be assumed that used amount of ammonium agent was not a limiting factor for the synthesis outcome. On the other hand, based on the calculated DS value, the impression is that the substitution takes place on every third glucopyranose unit in the cellulose chain. Considering the assumption that the reaction efficiency is a property of biomass rather than an amount of ammonium agent, the problem of process economy can be resolved in a positive sense by simply reducing the starting concentration of the cationic N-agent. This would significantly contribute to the increase in reaction efficiency (i.e., reducing the consumption of chemicals), but the same degree of substitution would be retained. However, as previously found, a smaller amount of ammonium agent directly affects the reduction of the sorption capacity (Figure 4).

These assumptions can be better explained by the fact that the availability of biomass-reactive sites is most likely the limiting factor for the reaction efficiency. In this sense, the biomass density (function of the material composition) and the amount of lignin in the material could represent the reaction barriers. Thus, a relatively large bulk density (0.46 g·cm⁻³) in the case of LVS biomass could be a result of a high lignification degree (41.9%, Table 1). Namely, it has been established that the bulk density of biomass shows inverse linear correspondence with the amount of added cationic groups [4]. Accordingly, it is believed that diffusion limits of the reaction between the cationic N-agent and the reactive cellulose could exist, as a result of high bulk density. Similarly, the small values for reaction efficiency were also found in other quaternized agricultural by-products tested for the removal of phosphate [4]. Thus, RE values varied from 1.70% for high-density shell of walnut (0.64 g·cm⁻³) to 7.26% for rice husk with less density (0.27 g·cm⁻³). For the same reason, highly lignified LVS biomass (41.9%) does not react so well with the CHMAC agent, due to limited access to cellulose in the cross-linked lignocellulosic structure. Also, many authors have confirmed that the delignification of lignocellulosic materials can increase the quaternization efficiency [18–21]. Accordingly, the applied alkaline pretreatment of LVS biomass contributed to delignification of 5.2% (Table 1), which led to an increase in reaction efficiency for 2.6% only (Table 2). Obviously, the mild alkaline modification of LVS biomass with green carbonate agent was not sufficient for complete delignification of biomass. On the other hand, this was not the aim of this study since modification with strong alkali can lead to depolymerization of cellulose and thus to a decrease in the biosorption ability of the product.

Considerable variation in the CHO content as well as yield values lower than 100% in all observed cases could be due to the binding of N-agent on the biomass surface on one side, as well as biomass destruction (i.e., extraction of the depolymerized hemicellulose and lignin fragments) on the other, which was confirmed by the FTIR analysis as well. Namely, the lower product yield was based on a significant loss of product weight due to alkaline pretreatment in comparison with the contribution of mass derived from inclusion of additional cationic -N⁺R₃ groups. A similar trend of lower yield was also observed in other biomasses reach in hemicelluloses [4]. In general, the results of the elemental analysis suggest that a significant amount of quaternary N-agents was involved in the lignocellulosic structure of biomass during synthesis, which has led to an increase in N content in synthesized biosorbents. On the other hand, the obtained results validate the superiority of MLV precursor for cationic modification in comparison with numerous agricultural by-products, as well as commercial ion-exchange resins tested for phosphate [2, 4] and nitrate [5, 22] removal from aqueous solutions.

3.3.2. The Efficiency of Anion Biosorption. The efficiency of synthesized CALV and CARLVS biosorbents was assessed through investigating the biosorption capacity for different anion species (phosphates and nitrates). In this sense,

TABLE 3: Biosorption efficiency of phosphate and nitrate by cationic biosorbents based on raw (LVS) and alkaline-modified (MLV) bottle gourd shell.

Biosorbent	Phosphates (10 mg P·dm ⁻³)		Nitrates (10 mg N·dm ⁻³)	
	Q _e (mg P·g ⁻¹)*	(%)	Q _e (mg N·g ⁻¹)*	(%)
LVS biomass	0.04 ± 0.02	0.8	0.03 ± 0.01	0.6
MLV precursor	0.05 ± 0.03	1.0	0.04 ± 0.02	0.8
CARLVS	3.27 ± 0.11	65.4	2.77 ± 0.06	55.4
CALV	4.41 ± 0.13	88.2	3.95 ± 0.10	79.0

*Q_e: equilibrium biosorption capacity (mean of three consecutive determinations).

a series of experiments for the phosphate removal from the solution of 10 mg P·dm⁻³ initial concentration and removal of nitrate from the solution of initial concentration of 10 mg N·dm⁻³, with biosorbent dose of 2 g·dm⁻³, were carried out in the batch system. These initial concentrations were selected according to the effluent quality standards for wastewater treatment in eutrophication-sensitive regions (Council Directive 91/271/EEC). For comparison, the reaction conditions of the biosorption process (mixing speed 150 rpm, contact time 60 min, temperature 20°C, pH 6.5) were selected according to the literature data that proved to be optimal for these purposes [2, 5]. Comparative results of the biosorbent efficacy testing, regarding the initial LVS biomass and the MLV precursor, are presented in Table 3.

The results of biosorption efficiency, under the same reaction conditions, confirmed the success of chemical modification of the starting biomass by the N-agent. By comparing the obtained results (Table 3), it was found that the raw LVS biomass does not show biosorption properties for investigated anions (<1%). Also, alkaline pretreatment did not have an effect on improving the ability of MLV precursor to remove the tested anionic species from the solution. However, alkaline pretreatment significantly contributed to an increase in the equilibrium biosorbent capacity of CALV biosorbent. Thus, in the case of CARLVS biosorbent testing (based on the raw LVS biomass), the degree of phosphate removal from the solution of 65.4% or nitrate of 55.4% was determined. More efficient biosorption of both types of anion (for 23–24%) was recorded with CALV biosorbent (based on the MLV precursor). This fact also confirms the importance of applied alkaline pretreatment of LVS biomass. Better reaction efficiency and higher sorption capacity of CALV biosorbent are probably due to the creation of a number of active centers (–N⁺R₃) on the biomass surface. In addition, the experiment found that CALV biosorbent has a higher affinity for phosphate (4.41 mg P·g⁻¹) than for nitrate anions (3.95 mg N⁻¹). Since it showed better properties as an anion exchanger, the cationic CALV biosorbent was selected for further structural test.

3.3.3. Isotherm Studies. The isotherms for the phosphate and nitrate biosorption by CALV were analyzed according to the nonlinear forms of Langmuir [23] and Freundlich models [24], using OriginPro 8.0 software. Figure 7 shows the fit of the isotherm models to the experimental data for the anion

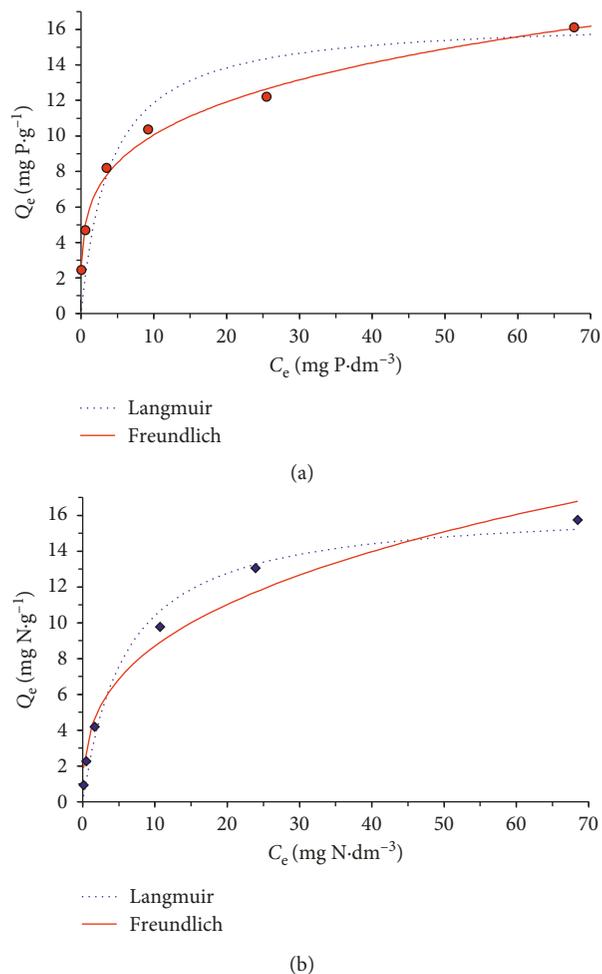


FIGURE 7: Biosorption isotherms of the nonlinear forms for the phosphate (a) and nitrate (b) biosorption onto CALV ($C_0 = 5\text{--}100\text{ mg}\cdot\text{dm}^{-3}$; pH 6.5; $T = 20^\circ\text{C}$; 60 min; 150 rpm).

biosorption under optimal conditions (20°C, pH 6.5, agitation speed of 150 rpm for 60 min) and initial anion concentration in the range of 5–100 mg·dm⁻³. The results of nonlinear regression are presented in Table 4. It can be seen (Figure 7(a)) that the isotherms are characterized by a large increase in the Q_e values at low phosphate concentrations. The Freundlich model was in good agreement with the experimental data ($R^2 > 0.99$). The relatively poor determination coefficients ($R^2 < 0.89$) and very high level of the chi-squared distribution ($\chi^2 > 4.2$) observed for the Langmuir model may be attributed to the unequal distribution of the biosorption surface area and active sites into CALV biosorbent. It can be concluded that the Freundlich model is the most appropriate to describe the biosorption of phosphate onto CALV as a heterogeneous system. According to this model, phosphate saturation is not predicted [24]. The estimated Freundlich parameters (Table 4) reflect heterogeneous surface and a multilayer biosorption on the surface. The values of constants related to biosorption capacity (K_F) and intensity of biosorption ($1/n_F < 1$) suggest that the biosorption of phosphate by the cationic CALV biosorbent was favorable.

In other case (Figure 7(b)), the Langmuir isotherm model shows a satisfactory determination coefficient ($R^2 > 0.98$) for

TABLE 4: Expressions of isotherm models and nonlinear regression parameters for phosphate and nitrate biosorption onto CALV.

Models*	Expressions and isotherm parameters		Statistics	
			R^2	χ^2
<i>Phosphate biosorption</i>				
Langmuir	$Q_e = Q_{\max} K_L C_e / (1 + K_L C_e)$	$Q_{\max} (\text{mg}\cdot\text{g}^{-1}) = 16.608$ $K_L (\text{dm}^3\cdot\text{mg}^{-1}) = 0.249$	0.8899	4.1923
Freundlich	$Q_e = K_F C_e^{(1/n_F)}$	$K_F (\text{mg}\cdot\text{g}^{-1}) (\text{mg}\cdot\text{dm}^{-3})^{1/n} = 5.728$ $1/n_F = 0.244$	0.9955	0.1693
<i>Nitrate biosorption</i>				
Langmuir	$Q_e = Q_{\max} K_L C_e / (1 + K_L C_e)$ $R_L = 1 / (1 + K_L C_0)$	$Q_{\max} (\text{mg}\cdot\text{g}^{-1}) = 16.532$ $K_L (\text{dm}^3\cdot\text{mg}^{-1}) = 0.169$ $R_L = 0.05-0.54$	0.9839	0.7641
Freundlich	$Q_e = K_F C_e^{(1/n_F)}$	$K_F (\text{mg}\cdot\text{g}^{-1}) (\text{mg}\cdot\text{dm}^{-3})^{1/n} = 3.957$ $1/n_F = 0.341$	0.9712	1.3327

* Q_e : equilibrium solid phase concentration ($\text{mg}\cdot\text{g}^{-1}$); C_e : equilibrium liquid phase concentration ($\text{mg}\cdot\text{dm}^{-3}$); Q_{\max} : maximum biosorption capacity; K_L : Langmuir constant related to the energy of biosorption ($\text{dm}^3\cdot\text{mg}^{-1}$); R_L : Langmuir separation factor; K_F : Freundlich constant characterizing biosorption capacity ($(\text{mg}\cdot\text{g}^{-1}) (\text{mg}\cdot\text{dm}^{-3})^{1/n}$); n_F : Freundlich parameter related to energy or intensity ($1/n_F$) of biosorption.

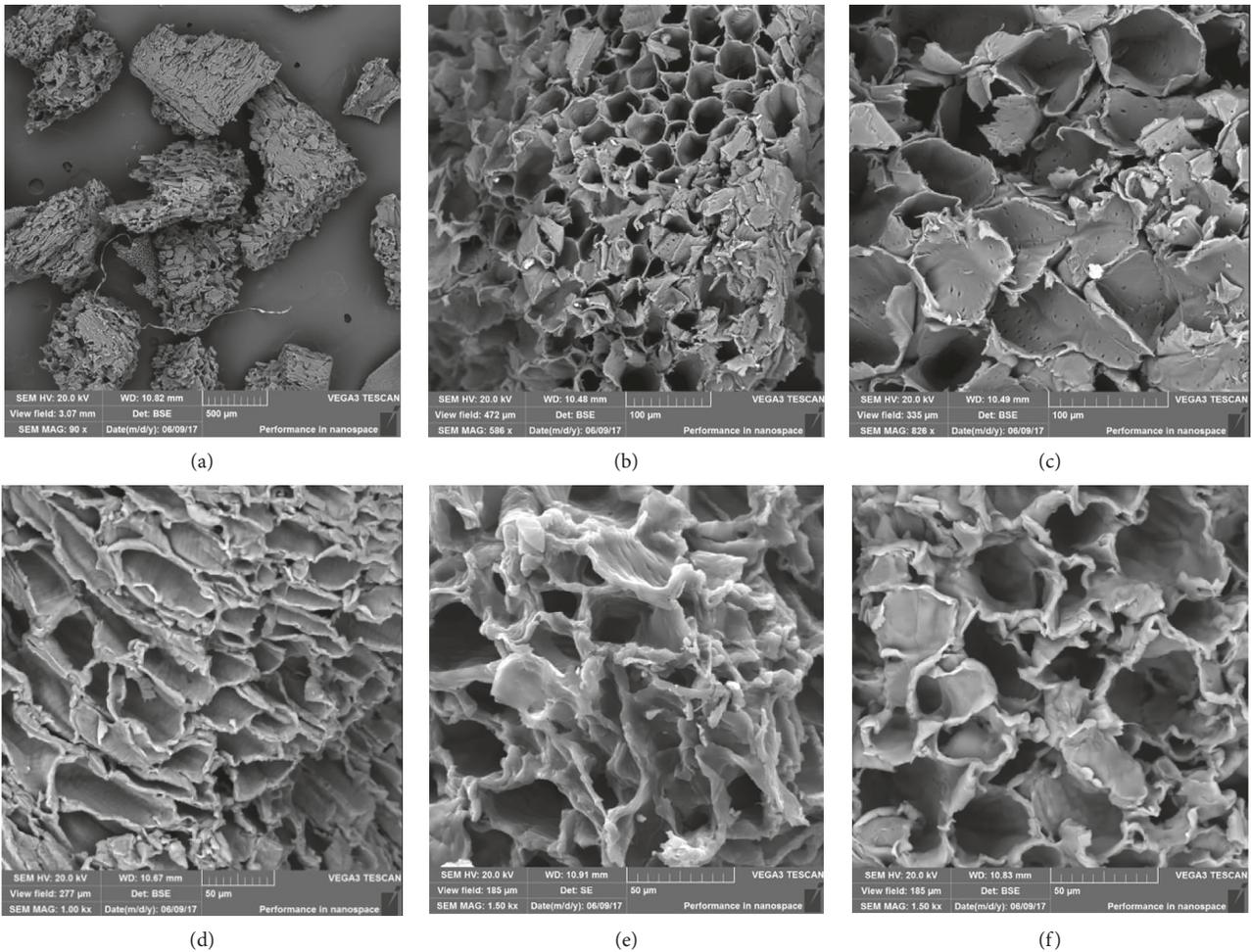


FIGURE 8: SEM micrographs of LVS biomass (a, b), MLV precursor (c), and CALV biosorbent before (d) and after phosphate (e) and nitrate (f) biosorption.

describing the nitrate biosorption by CALV. This model assumes the phenomenon of monolayer biosorption on a homogeneous surface [23]. The maximum biosorption

capacity (Q_{\max}) for nitrate from the Langmuir model was $16.53 \text{ mg N}\cdot\text{g}^{-1}$. Based on a relatively low value of biosorption energy (K_L), we can assume a high affinity of CALV

TABLE 5: Absorption IR bands assignation for the lignocellulosic LVS biomass and MLV precursor.

Band position (cm ⁻¹)		Functional group	Vibration	Assignment		
LVS	MLV			Cellulose	Hemicellulose	Lignin
3400	3400	O-H	Valence	•	Δ	■
2921	2925	C-H	Valence	•	Δ	■
2874*	2870*↓	C-H	Valence			■
2857	2858	C-H	Valence	•	Δ	■
1738*	1735*↓	C=O	Valence		Δ	
—	1650*	C=O + C=C	Valence			■
1637	1635	H-O-H	Deformation	•		
1601*	1600*↓	C=C aromatic	Valence			■
—	1557*	C=C aromatic	Skeletal			■
1510	1508	C=C aromatic	Skeletal			■
1460	1458	C-H	Deformation	•	Δ	■
1425	1423	O-H	Deformation	•	Δ	■
1376	1375	C-H aliphatic	Deformation	•	Δ	■
—	1336*	C-H + O-H	Deformation	•	Δ	■
1327*	—	Ring + C-O	Deformation			Syringyl
—	1320*	CH ₂	Deformation	•	Δ	
1260	1260	C-H	Deformation	Crystalline		
1162	1160	C-O-C	asym. Val.	•	Δ	
1106	1109	C-OH	Valence sec.	•	Δ	
1086	1086	C-OH	Deform. sec.	•	Δ	■
1042*	1049*	C-O + C-C-O	Valence	•	Δ	■
990*	—	C-O	Valence	•		
—	960*	H-C=C-H	Deformation			■
896	897	C-H	β-glycosid.	•	Δ	
830*	814*	C-H + C=C	Deformation		Δ	■
<800	<800	C-H + O-H	Deformation	•	Δ	■

Characteristic changes are emphasized by star and arrow.

biosorbent for nitrate (Table 4). The calculated values of Langmuir separation factor ($R_L < 1$) in the function of initial nitrate concentration (between 5 and 100 mg·dm⁻³), which denotes the biosorption nature, indicated that the biosorption process was favorable. Spontaneity of the process increases with an increase in initial nitrate concentration.

3.4. Morphological Characterization of Biosorbent. The morphological characteristics of LVS biomass, MLV precursor, CALV biosorbent, and the biosorbent surface after phosphate and nitrate biosorption were examined by scanning electron microscopy (Figure 8). SEM micrograph of LVS biomass shows a larger particle (about of 500 μm) of highly porous structure, with pores of different shapes and sizes (Figure 8(a)). The biomass is mainly composed of tiny disorganized plant vessels of various dimensions (20–40 μm) and diverse morphology (Figure 8(b)). This micrograph indicates the channels' existence of horizontal and/or vertical orientation and a variety of cavities on the external surface. Besides, the SEM image of MLV precursor (Figure 8(c)) reveals that the biomass has macropores with no uniform size (0.5–1.0 μm). These may contribute to the relatively high surface area of the biomass [7]. However, regardless of this surface morphology, the MLV biomass does not show the ability of anion biosorption. The morphological aspect of CALV (Figure 8(d)) shows that the

solid is in the form of tunnel-/flake-like part. Namely, in case of the CALV synthesis, the chemical treatment partially degraded lignin and hemicelluloses and spaced the fibrils, probably due to sodium hydroxide [25], which could contribute to the changes in morphology. Irregular particles of the CALV biosorbent with porous surface show sharp edges (Figure 8(d)). Sparse round holes are visible on the surface at magnification of 1000 times. Such holes are openings of the large pores which are significant for diffusion of anions through the structure of the solid.

Certain morphological differences were observed between the SEM micrographs of the CALV biosorbent surfaces taken before and after anion biosorption (Figures 8(e)–8(f)). At one side, shape and pore arrangements are comparable in both samples, suggesting that the biosorption has no impact on the uniformity of the biomass structure. However, the effect of phosphate or nitrate binding to the biosorbent surface has nevertheless caused some changes in the surface morphology. Thus, the lateral ends of the cell wall are visibly thicker, while the dimensions of the macropores are smaller compared to those before the phosphate biosorption. Obviously, biosorption of anions onto the CALV surface leads to a constriction of the pore channels, as a result of anions attachment to the internal framework surfaces. This suggests that the phosphate or nitrate biosorption is closely related to the existence of the represented chemical groups (quaternary ammonium) on the solid surface, which is additionally

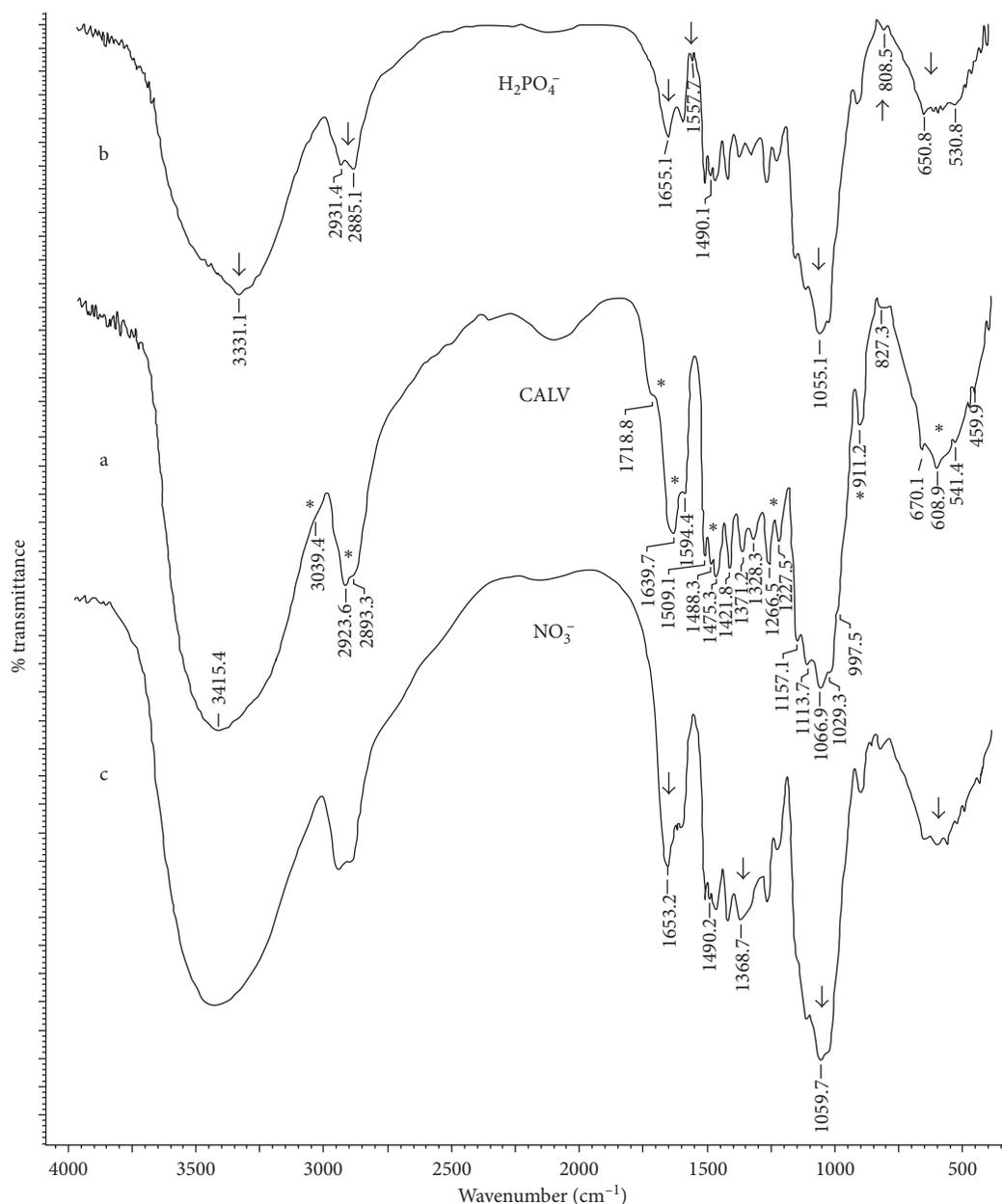


FIGURE 9: FTIR spectra of CALV biosorbent (a), after phosphate sorption (b), and after sorption of nitrate (c).

confirmed by FTIR and elemental analysis. Also, it seems that anions can easily penetrate into the interior of the biosorbent through pore channels and further be biosorbed at active centers. This is in accordance with a number of authors who have identified surface morphological changes of different biosorbents before and after phosphate loading through scanning electron microscopy [26, 27].

3.5. Structural Characterization of Anion Exchanger.

In order to analyze the chemical structure of LVS biomass and MLV precursor, as well as behavior of CALV biosorbent as an anion exchanger, the FTIR spectroscopy was used. As a rapid analytical technique, FTIR is ideal for initial classification of characteristic biomass functional groups [28, 29]. The spectroscopic characterization of raw LVS and

MLV precursor has been used to identify changes in the lignocellulosic structure during alkaline pretreatment (Table 5). A comparative spectroscopic analysis of MLV precursor and the synthesized CALV biosorbent should be able to confirm the success of the biomass chemical modification by the quaternary CHMAC agent. By analyzing the FTIR spectra of the CALV biosorbent before and after the phosphate and nitrate biosorption (Figure 9), the functionality of the cationic biosorbent and the mechanism for removing anionic species from aqueous solutions were assessed.

Spectral FTIR data of both analyzed LVS and MLV samples (Table 5) are typical for the lignocellulosic biomass. Due to the complex nature of LVS biomass, no significant changes were observed in the region of 4000–2000 cm⁻¹ after alkaline pretreatment. The characteristic band of C–H

stretching vibration at 2870 cm^{-1} is in correlation with the present lignin, so it can be preliminarily used for behavior analysis during alkaline pretreatment. The band intensity decrease indicates possible degradation and partial loss of lignin from the LVS sample during alkaline pretreatment. This phenomenon was confirmed by the band intensity reduction of the lignin aromatic C=C vibration at 1600 cm^{-1} , as well as the appearance of new bands at 1650 and 1557 cm^{-1} typical for lignin-conjugated rings. The band at 1738 cm^{-1} is derived from saturated ester C=O and is correlated with the presence of hemicellulose in LVS biomass. The band intensity reduction suggests the hydrolysis of glycosidic C–O–C bonds, depolymerization of released hemicellulose, and its partial removal from biomass as a result of alkaline pretreatment. The deformation C–H vibration characteristic of the anomeric β -glycosidic (C–O–CH) cellulose bond at 896 cm^{-1} remain unchanged during treatment, which indicates the structural stability of the cellulose chain to adequately applied reaction conditions.

The corresponding FTIR spectra of CALV biosorbent before and after the anion (phosphate and nitrate) biosorption are presented in Figure 9. The FTIR spectrum of CALV biosorbent (Figure 9(a)) with numerous absorption bands indicates a complex lignocellulosic structure, which derives from the complex nature of LVS biomass. Namely, a wide band at about 3415 cm^{-1} could be attributed to the stretching O–H vibrations of glucopyranose unit (cellulose), overlapped with the vibrations of the phenolic O–H groups (lignin). This asymmetric band, in its position and shape, indicates a pronounced presence of hydrogen bonds in the biomass structure [30, 31]. Bands at 2923 and 2893 cm^{-1} correspond to asymmetric and symmetric stretching C–H vibrations of aliphatic methylene groups in cellulose side chains, as well as aromatic lignin methoxy groups [32]. The medium intensity band at 1718 cm^{-1} in the biosorbent spectrum is derived from the ester O–C=O group of hemicellulose, which is more intensive in the spectrum of LVS biomass at 1738 cm^{-1} [33]. The centroid of complex band at about 1640 cm^{-1} is typical for the deformation O–H vibration of crystallohydrate (i.e., water molecule) in a lignocellulosic structure. The bands at 1594 and 1509 cm^{-1} belong to aromatic C=C skeletal vibrations, which confirms the presence of the phenolic ring of lignin. Absorption at 1475 cm^{-1} (in the LVS biomass spectrum at 1462 cm^{-1}) is characteristic of the deformation vibrations of lignin methoxy (O–CH₃) and cellulosic methylene (–CH₂) groups [34]. The band at 1421 cm^{-1} is attributed to the deformation O–H vibration of the glucopyranose ring of cellulose, as well as the phenolic C–O valence vibrations of lignin [33]. Absorption at 1371 cm^{-1} derives from deformation C–H vibrations. The band of deformation O–H vibration at 1328 cm^{-1} is characteristic of the siringil ring in nonwood lignin [31]. The bands at 1266 and 1030 cm^{-1} are assigned to deformation O–H and stretching C–O vibrations that stand out in primary alcohol, while the band at 1113 cm^{-1} originates from the stretching C–O vibration of secondary alcohol. These centroids were not clearly visible in the spectrum of unmodified LVS biomass since they are characterized by complex bands at 1260 and 1040 cm^{-1} . The

appearance of a new band at 911 cm^{-1} is assigned to C–O deformation vibration (from a hydroxypropyl group) and is correlated with the presence of CHMAC agent in the CALV biosorbent [35]. This band confirms the chemical modification of MLV biomass into the corresponding cationic biosorbent. The weak band at 897 cm^{-1} is characteristic of the deformation C–H vibration of the β -glucoside linkage [32]. An even smaller intensity absorption band at about 830 cm^{-1} can be assigned as a lignin C=C deformation vibration (out of the plane). The spectral region below 800 cm^{-1} corresponds to the deformation vibrations of the C–H and O–H partners.

Based on assigned FTIR bands, it is clear that the synthesized CALV biosorbent (Figure 9(a)) contains functional groups that are characteristic for cellulose (900 – 1300 cm^{-1}), hemicellulose, and lignin (1200 – 1750 cm^{-1}). The preserved lignocellulosic structure in the biosorbent base after synthesis confirms the presence of peaks identified in the spectrum of the raw LVS biomass (Table 5). Thus, peaks at 1030 , 1066 , 1113 , and 1157 cm^{-1} are typical of O–H, C–OH, C–O, and C–H vibrations of OH and CH₂ groups in glucopyranose units of cellulose [32]. The main peaks at higher wave numbers (1266 , 1328 , 1371 , 1421 , 1475 , 1509 , and 1594 cm^{-1}) correspond to the vibrations of OH, CH₂, CH₃, C–O functional groups typical of lignin, and C=O vibration (1718 cm^{-1}) for hemicellulose. The identified absorption bands were used to detect the structural changes that occur, both during the chemical treatment of the MLV biomass to the cationic biosorbent and after the anion (phosphate or nitrate) biosorption from aqueous solutions.

By comparative analysis of the FTIR spectral data of CALV biosorbents (Figure 9(a)) and MLV biomass (Table 5), some significant changes in the number, position, and intensity of the absorption bands were revealed. It is obvious that these changes were induced by a chemical reaction, that is, modification of MLV biomass with the used CHMAC agent. For example, a decrease in the intensity of IR bands derived from vibration of OH and CH groups (to 2858 , 1371 , 1328 , 1266 , and 420 cm^{-1}) as well as IR band at 1718 cm^{-1} derived from ester C=O group of hemicellulose can be easily noticed. On the contrary, the intensities of IR bands at 2893 , 1640 , 1066 , and 608 cm^{-1} are much more pronounced. Also, typical are the shifts of the IR bands at 1718 cm^{-1} (from 1738 cm^{-1}), 1640 cm^{-1} (from 1650 cm^{-1}), 1594 cm^{-1} (from 1601 cm^{-1}), 1475 cm^{-1} (from 1458 cm^{-1}), 1266 cm^{-1} (from 1260 cm^{-1}), and at 1066 cm^{-1} (from 1050 cm^{-1}), suggesting the interaction of representative groups. These changes clearly indicate that during the chemical modification of biomass, the breakdown of the established hydrogen bonds, a partial disturbance of the lignin structure, as well as a significant destruction of the glycosidic bonds of hemicellulose could take place. The change in the band intensity at 1601 cm^{-1} (aromatic C=C), which simultaneously shifts to 1594 cm^{-1} , and the change in the band position from 1458 to 1475 cm^{-1} (derived from lignin –O–CH₃ group) confirm partial delignification during synthesis [34]. The change in the band intensity at 1735 cm^{-1} , along with simultaneously shifting to 1718 cm^{-1} , indicates depolymerization of hemicellulose to soluble oligosaccharides.

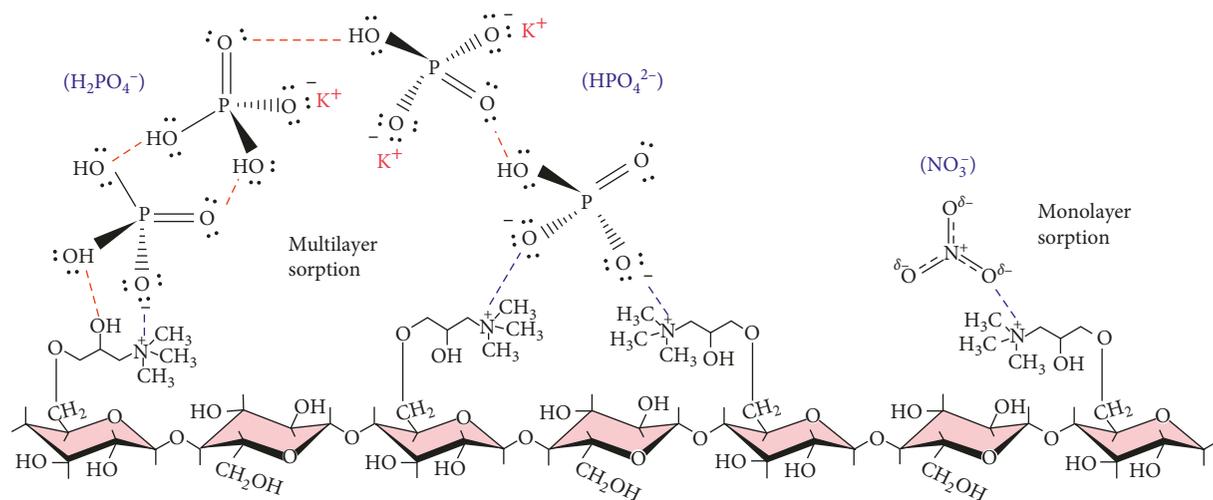


FIGURE 10: Structural model of the biosorbent after the multilayer sorption of phosphate ions and monolayer sorption of nitrate ions from the aqueous solution.

Reduction of the bands' intensity resulting from O–H vibration indicates a decrease in the content of hydroxyl groups, verifying that these cellulosic groups are involved in the reaction with the quaternary ammonium agent [30].

On the other hand, it is characteristic that the band at about 1260 cm^{-1} (derived from C–OH vibration) is doubled into two new bands (at 1266 and 1227 cm^{-1}). This fact indicates the appearance of functionally different types of OH groups, probably one from the glucopyranose unit of cellulose and the other from the coupled hydroxypropyl fragment of the CHMAC agent. A similar phenomenon was observed in the area of stretching C–O vibrations, where the band at 1050 cm^{-1} is doubled to two bands (1066 and 1029 cm^{-1}), which confirms the previous assumption. The most important proof of a successful synthesis of biosorbent is the emergence of a new band (lower intensity due to overlap) at about 1490 cm^{-1} (Figure 9(a)) corresponding to the asymmetric C–H vibration of the introduced quaternary $-\text{N}^+(\text{CH}_3)_3$ functional group [36]. The partner of this band at about 980 cm^{-1} (from asymmetric N–C₄ vibration) suggests the presence of quaternary $-\text{CH}_3$ groups, but in this case, it cannot be clearly seen due to overlapping with deformation C–OH vibrations. However, the appearance of a new band at 2893 cm^{-1} confirms the presence of new $-\text{CH}_3$ groups. These observations clearly indicate the incorporation of the cationic $-\text{N}^+\text{R}_3$ groups into the ligno-cellulosic structure of the biosorbent during chemical modification of biomass [20, 30, 33].

The FTIR analysis of CALV biosorbent after phosphate biosorption showed small but important differences of significance for the assessment of the outcome and the biosorption mechanism (Figure 9(b)). An asymmetric intense band with a typical centroid at 3331 cm^{-1} represents the sum of valent vibrations of all hydrogen-bonded OH groups. In addition to the unreacted phenolic and other $-\text{OH}$ groups of glucopyranose unit, the part of this sum corresponds to phosphate $-\text{OH}$ groups (from H_2PO_4^-) present in the CALV biosorbent. The band of the ester C=O

vibration, which previously appeared at 1735 cm^{-1} (MLV biomass) or at 1718 cm^{-1} with reduced intensity (CALV biosorbent), was not detected in the FTIR spectrum (Figure 9(b)). It is characteristic that after the phosphate biosorption, the intensity of the band at 670 and 608 cm^{-1} derived from deformation vibrations of C–H and O–H groups of cellulose was reduced, while new bands at about 650 and 530 cm^{-1} of phosphate P–OH groups appeared. Furthermore, the centroid of band at about 1030 cm^{-1} characteristic for the P–O vibration mode of the phosphate (PO_4^{3-} group), as well as the bands at 603 cm^{-1} and 564 cm^{-1} corresponding to the deformation O–P–O mode of phosphate anions [32], could not be clearly observed in this case. Also, the band of P=O vibration (which usually appears at 1140 cm^{-1}) cannot be clearly noticed due to overlap with the complex band of C–OH vibration (which appears in the range $1200\text{--}1100\text{ cm}^{-1}$). Probably, there are other phosphate species (such as H_2PO_4^- and HPO_4^{2-}) present in the biosorbent instead of orthophosphate PO_4^{3-} groups (dominant in the alkaline medium). It is in agreement with the stability of various phosphate forms in acidic aqueous solutions, from which their removal is done [30]. These facts, together with clear shifting of some bands (at 1331 , 2885 , 1655 , 1055 , and 808 cm^{-1}) that correspond to the vibrations of OH and CH groups, play an important role in the interpretation of phosphate biosorption on the biosorbent surface.

FTIR spectra of biosorbent before and after nitrate sorption are very similar (Figure 9(c)) since the NO_3^- ions incorporated in the modified biomass do not form chemical bonds, but electrostatically interact with the active $-\text{N}^+\text{R}_3$ centers in the monolayer. An absorption IR band at about 1370 cm^{-1} , characteristic for the N–O vibration, indicates the presence of sorbed NO_3^- ions [5].

Considering that there is no evidence of chemical bonding typical of phosphate or nitrate chemisorptions, the ion exchange mechanism (Cl^- ions from active $\text{R}_4\text{N}^+\text{Cl}^-$ groups with H_2PO_4^- or NO_3^- ions from the solution) could

be assumed. The expected type of multilayer phosphate sorption on the energy nonhomogeneous solid surface occurs; thanks to the interaction between the sorbed anions [28]. In accordance with the morphological characterization, the FTIR spectroscopic analysis and isotherm studies, a structural biosorbent model after the biosorption of the dominant phosphate anions (H_2PO_4^- and HPO_4^{2-}), as well as nitrate anions (NO_3^-) from the solution, was proposed and is presented in Figure 10.

4. Conclusion

The constant development of renewable and sustainable resource technologies is of great importance in terms of environmental problems. Research in this field provides a scientific contribution to a better understanding of the anion biosorption mechanism at the surface of natural materials, as well as a practical contribution to the improvement of the product sorption characteristics in terms of their further application in the process of anion purification from contaminated water and techniques of preventing the occurrence of eutrophication. This study confirms that an efficient anion exchanger can be generated from low-cost agricultural residue such as bottle gourd shell, as starting material which contains significant amounts of cellulose and lignin. The introduction of N-quaternary groups through the green modification of lignocellulosic materials to produce cationic biosorbent represents a potential alternative to commercially available anion exchange resins. The results of applied physicochemical tests confirmed that alkaline pretreatment leads to the partial release of certain constituents from the lignocellulosic matrix and the removal of their hydrolyzed fragments from the system. After the pretreatment process, it was found that approximately 78% of the total polysaccharides are retained in the biomass. This is important for the successful synthesis of cationic biosorbent, as confirmed by the FTIR spectroscopic analysis. The maximum exchange capacity ($1.39 \text{ mEq}\cdot\text{g}^{-1}$) of CALV biosorbent has been achieved under optimal synthesis conditions, such as a temperature of $80 \pm 1^\circ\text{C}$, contact time of 10 h, and a molar NaOH/CHMAC ratio of 1 : 0.8. The anion biosorption by CALV was found to be spontaneous, favorable, and ion exchange process. The Freundlich isotherm model provided the best description of the phosphate biosorption process, revealing the favorable multilayer phosphate sorption. The nitrate biosorption followed the Langmuir isotherm model with the maximum biosorption capacity of $16.53 \text{ mg N}\cdot\text{g}^{-1}$.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

- [1] L. E. De-Bashan and Y. Bashan, "Recent advances in removing phosphorus from wastewater and its future use as fertilizer (1997–2003)," *Water Research*, vol. 38, no. 19, pp. 4222–4246, 2004.
- [2] T. A. H. Nguyen, H. H. Ngo, W. Guo, and T. V. Nguyen, "Phosphorous removal from aqueous solutions by agricultural by-products: a critical review," *Journal of Water Sustainability*, vol. 2, no. 3, pp. 193–207, 2012.
- [3] X. Xu, B.-Y. Gao, Q.-Y. Yue, and Q.-Q. Zhong, "Preparation of agricultural by-product based anion exchanger and its utilization for nitrate and phosphate removal," *Bioresource Technology*, vol. 101, no. 22, pp. 8558–8564, 2010.
- [4] L. H. Wartelle and W. E. Marshall, "Quaternized agricultural by-products as anion exchange resins," *Journal of Environmental Management*, vol. 78, no. 2, pp. 157–162, 2006.
- [5] P. Loganathan, S. Vigneswaran, and J. Kandasamy, "Enhanced removal of nitrate from water using surface modification of adsorbents—A review," *Journal of Environmental Management*, vol. 131, pp. 363–374, 2013.
- [6] A. Keränen, T. Leiviskä, B. Y. Gao, O. Hormi, and J. Tanskanen, "Preparation of novel anion exchangers from pine sawdust and bark, spruce bark, birch bark and peat for the removal of nitrate," *Chemical Engineering Science*, vol. 98, pp. 59–68, 2013.
- [7] D. L. Mitic-Stojanovic, A. Zarubica, M. Purenovic, D. Bojic, T. Andjelkovic, and A. Bojic, "Biosorptive removal of Pb^{2+} , Cd^{2+} and Zn^{2+} ions from water by *Lagenaria vulgaris* shell," *Water SA*, vol. 37, no. 3, pp. 303–312, 2011.
- [8] D. Bojić, M. Ranđelović, A. Zarubica et al., "Comparison of new biosorbents based on chemically modified *Lagenaria vulgaris* shell," *Desalination and Water Treatment*, vol. 51, no. 34–36, pp. 6871–6881, 2013.
- [9] D. Bojic, M. Momcilovic, D. Milenkovic et al., "Characterization of a low-cost *Lagenaria vulgaris* based carbon for ranitidine removal from aqueous solutions," *Arabian Journal of Chemistry*, vol. 10, no. 7, pp. 956–964, 2017.
- [10] D. Z. Marković-Nikolić, A. Bojić, G. Petković, N. Ristić, M. Cakić, and G. S. Nikolić, "The preparation and utilization of the cationic sorbent based on the surfactant modified bottle gourd shell," *Advanced Technologies*, vol. 6, no. 2, pp. 38–50, 2017.
- [11] AOAC, *Official Methods of Analysis*, AOAC International, 20th edition, 2016.
- [12] APHA, *Standard Methods for the Examination of Water and Wastewater*, American Public Health Association, Washington, DC, USA, 20th edition, 2000.
- [13] S. Kim and M. T. Holtzaple, "Delignification kinetics of corn stover in lime pretreatment," *Bioresource Technology*, vol. 97, no. 5, pp. 778–785, 2005.
- [14] V. Chaturvedi and P. Verma, "An overview of key pretreatment processes employed for bioconversion of lignocellulosic biomass into biofuels and value added products," *Biotechnology*, vol. 3, no. 5, pp. 415–431, 2013.
- [15] Y. Sun and J. Cheng, "Hydrolysis of lignocellulosic materials for ethanol production: a review," *Bioresource Technology*, vol. 83, no. 1, pp. 1–11, 2002.
- [16] L. Yang, J. Cao, Y. Jin et al., "Effects of sodium carbonate pretreatment on the chemical compositions and enzymatic saccharification of rice straw," *Bioresource Technology*, vol. 124, pp. 283–291, 2012.
- [17] N. Mosier, R. Hendrickson, N. Ho, M. Sedlak, and M. R. Ladisch, "Optimization of pH controlled liquid hot

- water pretreatment of corn stover," *Bioresource Technology*, vol. 96, no. 18, pp. 1986–1993, 2005.
- [18] J. A. Laszlo, "Preparing an ion exchange resin from sugarcane bagasse to remove reactive dye from wastewater," *Textile Chemists and Colorists*, vol. 28, pp. 13–17, 1996.
- [19] U. S. Orlando, A. U. Baes, W. Nishijima, and M. Okada, "A new procedure to produce lignocellulosic anion exchangers from agricultural waste materials," *Bioresource Technology*, vol. 83, no. 3, pp. 195–198, 2002.
- [20] F. Xu, J. Yu, T. Tesso, F. Dowell, and D. Wang, "Qualitative and quantitative analysis of lignocellulosic biomass using infrared techniques: a mini-review," *Applied Energy*, vol. 104, pp. 801–809, 2013.
- [21] X. Li, J. Pan, J. Dai et al., "Surface molecular imprinting onto magnetic yeast composites via atom transfer polymerization for selective recognition of cephalixin," *Chemical Engineering Journal*, vol. 198–199, pp. 503–511, 2012.
- [22] V. K. Gupta, J. M. Carrott, M. M. Carrott, and L. R. Subas, "Low-cost adsorbents: growing approach to wastewater treatment—A review," *Critical Reviews in Environmental Science and Technology*, vol. 39, no. 10, pp. 783–842, 2009.
- [23] I. Langmuir, "The adsorption of gases on plane surfaces of glass, mica and platinum," *Journal of the American Chemical Society*, vol. 40, no. 9, pp. 1361–1403, 1918.
- [24] H. M. F. Freundlich, "Over the adsorption in solution," *Journal of Physical Chemistry*, vol. 57, pp. 385–471, 1906.
- [25] A. C. A. de Lima, R. F. Nascimento, F. F. de Sousa, J. M. Filho, and A. C. Oliveira, "Modified coconut shell fibers: a green and economical sorbent for the removal anions from aqueous solutions," *Chemical Engineering Journal*, vol. 185, pp. 274–284, 2012.
- [26] S. Mor, K. Chhoden, and K. Ravindra, "Application of agro-waste rice husk ash for the removal of phosphate from the wastewater," *Journal of Cleaner Production*, vol. 129, pp. 673–680, 2016.
- [27] S. Yaghoobi-Rahni, B. Rezaei, and N. Mirghaffari, "Bentonite surface modification and characterization for high selective phosphate adsorption from aqueous media and its application for wastewater treatments," *Journal of Water Reuse and Desalination*, vol. 7, no. 2, pp. 175–186, 2017.
- [28] K. Riahi, B. B. Thayer, A. B. Mammou, A. B. Ammar, and M. H. Jaafoura, "Biosorption characteristics of phosphates from aqueous solution onto *Phoenix dactylifera* L. date palm fibers," *Journal of Hazardous Materials*, vol. 170, no. 2–3, pp. 511–519, 2009.
- [29] Z. Ismail, "Kinetic study for phosphate removal from water by recycled date-palm wastes as agricultural by-products," *International Journal of Environmental Studies*, vol. 69, no. 1, pp. 135–149, 2012.
- [30] T. S. Anirudhan, F. Noeline, and D. M. Manohar, "Phosphate removal from wastewaters using a weak anion exchanger prepared from a lignocellulosic residue," *Environmental Science and Technology*, vol. 40, no. 8, pp. 2740–2745, 2006.
- [31] M. Stanković, N. Krstić, I. Slipper et al., "Chemically modified *Lagenaria vulgaris* as a biosorbent for the removal of Cu(II) from water," *Australian Journal of Chemistry*, vol. 66, no. 2, pp. 227–236, 2013.
- [32] R. M. Silverstein, G. C. Bressler, and T. C. Morrill, *Spectrometric Identification of Organic Compounds*, John Wiley & Sons, Hoboken, NJ, USA, 5th edition, 2005.
- [33] K. A. Krishnan and A. Haridas, "Removal of phosphate from aqueous solutions and sewage using natural and surface modified coir pith," *Journal of Hazardous Materials*, vol. 152, no. 2, pp. 527–535, 2008.
- [34] M. A. Khan, S. M. Ashraf, and V. P. Malhotra, "Development and characterization of a wood adhesive using bagasse lignin," *International Journal of Adhesion and Adhesives*, vol. 24, no. 6, pp. 485–493, 2004.
- [35] G. Nikolic, S. Zlatkovic, M. Cacic, S. Cacic, C. Lacnjevac, and Z. Rajic, "Fast Fourier transform IR characterization of epoxy GY systems crosslinked with aliphatic and cycloaliphatic EH polyamine adducts," *Sensors*, vol. 10, no. 1, pp. 684–696, 2010.
- [36] A. S. Landim, R. M. N. de Assuncao, and D. A. Cerqueira, "Application of cationic hemicelluloses produced from corn husk as polyelectrolytes in sewage treatment," *Polimeros*, vol. 23, no. 4, pp. 468–472, 2013.

