Efficient removal of pharmaceutical contaminants using laccase immobilized on activated carbon derived from cashew nut shells: Thermodynamic, Kinetic and Calorimetry study

CA Guerrero-Fajardo[1] , L. Giraldo[2] , **JC Moreno-Pirajan[3*]**

[1] Facultad de Ciencias, Departamento de Química, Aprena Research Group, Carrera 30 No. 45-03, Edificio Uriel Gutiérrez, Ciudad Universitaria, Universidad Nacional de Colombia, Bogotá Sede, Bogotá, 111321, Colombia, Sur América.

[2] Facultad de Ciencias, Departamento de Química, Grupo de Calorimetría, Carrera 30 No. 45-03, Edificio Uriel Gutiérrez, Ciudad Universitaria, Universidad Nacional de Colombia, Bogotá Sede, Bogotá, 111321, Colombia, Sur América.

[3] Facultad de Ciencias, Departamento de Química, Grupo de Investigación en Sólidos Porosos y Calorimetría Aplicada, Carrera 1 No 18 A 12, Universidad de los Andes, Bogotá, 111711, Colombia, Sur América.

***Corresponding author**: jumoreno@uniandes.edu.co

1. INTRODUCTION

a. The presence of emerging compounds threatens both the environment and human health. These compounds, like pharmaceuticals, pesticides, and industrial byproducts, enter ecosystems through various pathways such as wastewater discharge.
b. Their impact, well-documented, ranges from biological process disruptions to hormonal disorders.
c. To address this, methods like biodegradation and bioadsorption gain popularity due to effectiveness and eco-friendliness. Activated carbon from cashew shells, a promising bioadsorbent, aids in organic contaminant adsorption. Immobilizing laccase on activated carbon enhances contaminant degradation, requiring extensive studies for optimization.
d. This research employs laccase-immobilized activated carbon-based bioadsorbents to target emerging compound contamination, emphasizing sustainable removal solutions.

2. METHODOLOGY AND MATERIALS

✓ **Activation methods**

The activated carbons from cashew nut shells were prepared by chemical activation using KOH (ACK), NaOH (ACN), and $ZnCl_2$ (ACZ) under the conditions suggested in the literature and by our laboratory experience.

✓ **Characterization**

✓ The characterization of the specific surface area and pore texture of the synthesized activated carbons was carried out by nitrogen adsorption-desorption analysis at 77 K, using the AutosorbiQ St equipment (Anton Paar, Boynton Beach, FL, US).

✓ The specific surface area related to micropores, S_{BET} (m^2g^{-1}), was determined using the BET (Brunauer-Emmett-Teller) method for calculation. The mesoporous pore size distribution, PSD, was estimated using the Barret-Joyner-Halenda (BJH) calculation method.

✓ To estimate the PSD of the micropores, the Nonlocal Density Functional Theory (NLDFT) calculation method was employed using the SAIEUS® software. In addition, the surface area, S_{NLDFT} (m^2 g^{-1}), the total pore volume, V_{tot} (m^3 g^{-1}), and the micropore volume, V_{mic} (m^3 g^{-1}), were also determined using the NLDFT method integrating the PSD over the entire range of pore sizes.

✓ Finally, the mesopore volume, Vmesopore (m^3g^{-1}), was calculated as the difference between V_{tot} and V_{mic} .

✓ We also utilized **FTIR analysis** to determine the chemical structure of the prepared solids. As well as, Boehm titrations were performed to evaluate laccase enzyme fixation and quantify surface functional groups. The determination of the **Point of Zero Charge (pH_{PZC})** assessed surface acidity levels. Additionally, **immersion enthalpy analysis** was conducted using a microcalorimeter to record thermal changes during solvent interaction.

Fig. 1a. Oven to prepare activated carbons from cashew nut shell waste solids, under inert atmosphere .

Fig 1b. Sortometer for measuring N_2 adsorption isotherms at 77 K

3a. RESULTS

✓ **Adsorption Studies: Isotherms, Kinetics, and Thermodynamics**

- The experimental data using the probe molecules (amoxicillin, diclofenac and carbamazepine – AMO, DCF, CBZ) on bioadsorbents (activated carbons impregnated with laccase enzyme – LAC-x; x- corresponds to the initial of the chemical agent) were analyzed using the isotherms. of Langmuir and Freundlich.
- The Langmuir model showed excellent fit (R^2 around 0.99) for all bioadsorbents, indicating monolayer formation. The LACK bioadsorbent demonstrated the highest sorption capacity, with DCF being the most adsorbed (387.7 mg g^{-1}), followed by CBZ (344.3 mg g^{-1}) and AMO (324.6 mg g^{-1}). Favorable adsorption ($0 < R_L < 1$) and strong affinity (high K_L values) were observed.
- It was found that physisorption (PFO) predominated according to the kinetic analysis, while IPD demonstrated a three-step adsorption. The thermodynamic parameters indicated spontaneous adsorption (negative ΔG^o), an endothermic process (positive ΔH^o) and stable random adsorption (positive ΔS^o).

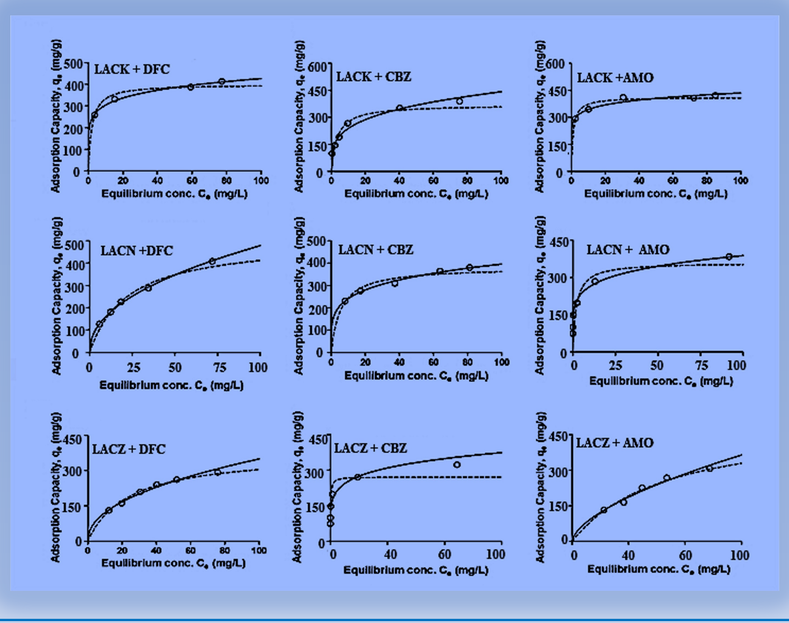

Fig. 2 Adsorption isotherms of the emerging compounds on the bioadsorbents: (a) LACK+DFC (b) LACK + CBZ (c) LACK + AMO (d) LACN+DFC (e) LACN + CBZ (f) LACN + AMO (g) LACZ+DFC (h) LACZ + CBZ (i) LACZ + AMO; The solid line represents the fit to the Langmuir model, while the dashed line represents the fit to the Freundlich model.

3b. RESULTS

✓ **Calorimetric Results Analysis**

- Calorimetric studies explored bioadsorbent-pharmaceutical interactions, analyzing immersion enthalpy as a thermodynamic parameter. LACK exhibited highest thermodynamic compatibility with pharmaceutical molecules.
- Thermograms facilitated evaluation of immersion enthalpies, predominantly negative (exothermic process). LACN showed hydrophilic behavior, attributed to -OH groups introduced during activation process and enzyme fixation.
- Interaction between LACK and pharmaceuticals primarily involved ion-dipole interactions and hydrogen bonding. LACK acid-type activated carbon demonstrated stronger interactions with pharmaceutical molecules compared to other bioadsorbents.

4. CONCLUSIONS

- This study synthesized activated carbon from cashew nut shells using KOH, NaOH, and $ZnCl_2$, with laccase enzyme immobilization to create bioadsorbents.
- Were tested for adsorption capacity of diclofenac sodium, carbamazepine, and amoxicillin, along with kinetics, thermodynamics, and calorimetric analysis.
- Results show all bioadsorbents fit Langmuir model, with LACK exhibiting highest adsorption capacity: 387.7 mg/g for DFC, 344.3 mg/g for CBZ, and 324.5 mg/g for AMO, surpassing literature values.
- Adsorption followed PFO model, with IPD analysis revealing a three-step process, likely involving chemical interaction. Thermodynamic evaluation, particularly Gibbs free energy, affirmed spontaneity. Calorimetric data confirmed exothermic enthalpy, supporting scalability.

Acknowledgments:
The authors thank you MinCiencias by economic funds approved by the projects titled "Biocatalysts supported on porous and graphenic materials for the degradation of contaminants emerging from the manufacture of plastics and the use of agrochemicals", Contract No. CT ICETEX 2023- 0814 CD 1101- 890-82458.The authors thank the Framework Agreement between Universidad de Los Andes and Universidad Nacional de Colombia—Bogotá and the act of agreement established between the Departments of Chemistry of both institutions. The Prof. Dr. Juan Carlos Moreno-Piraján also thank the grant project No. INV-2023-162- 2735 by the Facultad de Ciencias (Universidad de los Andes, Bogotá, Colombia).