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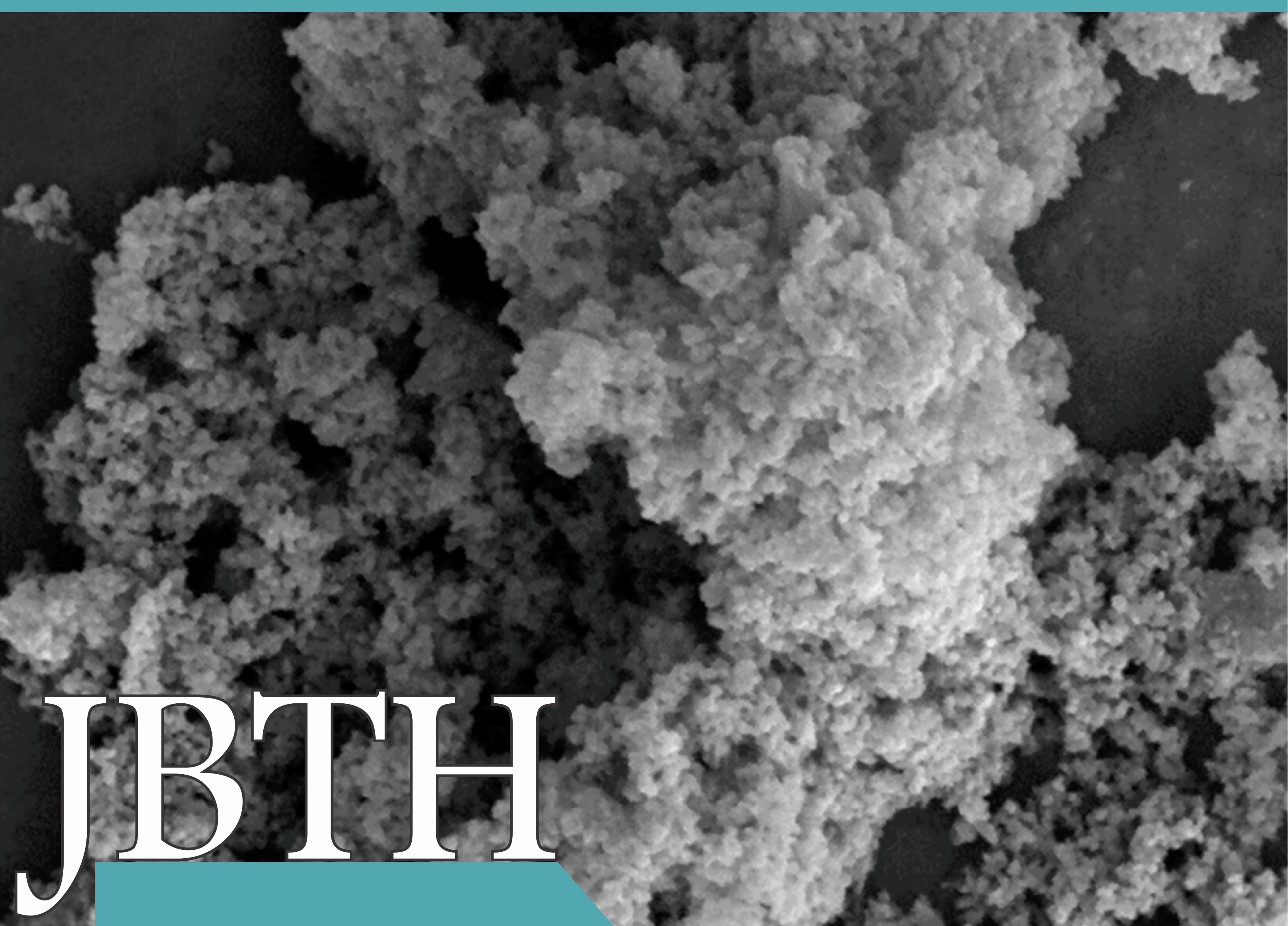

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SUMMARY

Original Articles

Systematic Review / Review Articles

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Prediction of Pancreatic Cancer Through Biomarkers Using Machine Learning Techniques: An Approach for Early Diagnosis

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This article explores the prediction of pancreatic cancer using CA 19-9 and CA 125 biomarkers with three machine learning models: Gradient Boosting, Random Forest, and Logistic Regression. The study evaluates their effectiveness through 10-fold cross-validation. Results showed competitive performance, with the Logistic Regression model displaying the highest accuracy, precision, and F1-score, indicating its potential for early diagnosis. Integrating biomarkers and machine learning promises for improving pancreatic cancer prediction and patient outcomes.
Keywords: Pancreatic Cancer. Machine Learning. Cancer Prediction.

Introduction

Pancreatic cancer is a highly challenging and devastating disease that poses a severe public health problem. Its aggressive nature and the absence of distinctive symptoms in the early stages make early diagnosis difficult, resulting in significantly elevated mortality rates [1]. Moreover, pancreatic cancer has the lowest survival rate compared to other cancers, with approximately 80% of cases being inoperable, and 74% of patients succumbing within the first year [2]. In this context, it is crucial to investigate effective approaches for predicting and detecting this type of cancer to improve prognoses and increase patients' chances of survival.

One promising line of research in this field involves the use of biomarkers. Biomarkers are biological substances that can be measured and evaluated as indicators of normal or pathological biological processes [3], including cancer development [4]. Two tumor markers commonly associated with pancreatic cancer are CA 19-9 and CA 125. The presence of these proteins at elevated levels in the blood may indicate the existence of malignant tumors in the pancreas, making them potential candidates for early disease detection [5,6].

Significant advancements have occurred in machine learning, greatly benefiting many medical applications. Machine learning allows algorithms to learn complex patterns in data and make accurate predictions. Integrating biomarker information with machine learning models can be a promising approach to enhance the prediction and diagnosis of pancreatic cancer, increasing the sensitivity and specificity of the detection process [7].

Machine Learning Models

Machine learning is a subfield of artificial intelligence that focuses on developing algorithms and models capable of learning patterns and making decisions from data without being explicitly programmed to perform specific tasks. These models are trained on previously collected datasets, allowing them to recognize relevant features and make predictions or classify new data based on this learning [9].

The models proposed in this study are three popular machine-learning approaches applied to pancreatic cancer prediction.

Gradient Boosting Model

Gradient Boosting is a machine learning technique based on decision trees, in which several weak trees are combined to form a robust and more

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accurate model. This method works in sequential steps, that each tree is adjusted to correct the errors of the previous model. The result is a weighted combination of predictions from all the trees, which tends to be more robust and accurate in predicting cases of pancreatic cancer based on biomarkers [10].

Random Forest Model

Random Forest is a technique based on decision trees but with a different approach. In this model, multiple decision trees are constructed from random subsets of the original dataset, and their predictions are combined through voting to reach a final decision. This approach helps to reduce the probability of overfitting (excessive fitting to the training data). It increases the model's generalization to unseen data, making it a viable option for predicting pancreatic cancer based on biomarkers [11].

Logistic Regression Model

Unlike tree-based models, Logistic Regression is a machine learning method that aims to predict a binary categorical variable (in this case, pancreatic cancer or not). It uses a logistic function to calculate the probability of belonging to a specific class based on the values of biomarkers. The model is trained to adjust coefficients that weigh the influence of each biomarker on the probability of pancreatic cancer occurrence. From this, predictions can be made, and patients with a higher risk of developing the disease can be identified. When combined with biomarkers CA 19-9 and CA 125, these models can offer a promising approach to improve pancreatic cancer prediction and early diagnosis, enabling more effective treatment and increasing the chances of survival for affected patients [12].

Materials and Methods

The data used in this research were obtained from a dataset by Wieand and colleagues [13], containing information about patients with a history of pancreatic cancer. The dataset includes records of the biomarkers CA 19-9 and CA 125 and the classification of patients into positive and negative cases for pancreatic cancer.

Data Preprocessing

Before proceeding with the analysis, a data preprocessing step was performed. In this phase, possible missing or inconsistent values were handled, and normalization techniques were applied to standardize the scale of the biomarkers. The objective was to ensure data integrity and reliability for subsequent experiments and avoid the disproportionate influence of higher numerical values on the models' outcomes. Data normalization was executed to adjust the values of the biomarkers CA 19-9 and CA 125 to the exact numerical scale. This way, significant differences between the magnitudes of the biomarkers were avoided, preventing them from unduly influencing the performance of the machine learning models. Normalization allowed the algorithms to focus on analyzing relationships and patterns within the data, contributing to more accurate and consistent results.

Cross-Validation

The technique of 10-fold cross-validation was used to evaluate the models' performance robustly and avoid training bias. The dataset was divided into 10 equal parts, where each model was trained on 9 folds and tested on the remaining fold. This process was repeated 10 times, alternating the test folds. Performance metrics were recorded at each iteration, and at the end, the averages were calculated to obtain more accurate estimates of the model's performance.

Metrics of Evaluation

The metrics used to assess the models' performance were accuracy, precision, recall, F1-

score, and the area under the curve (AUC). Accuracy measured the proportion of correct predictions out of the total predictions made. Precision evaluated the models' ability to avoid false positives, meaning the proportion of correctly identified positive cases among those predicted as positive. Recall measured the models' ability to correctly find all positive cases of pancreatic cancer. The F1 score provided a measure of the balance between precision and recall. The AUC metric estimated the models' discriminative capacity.

Results and Discussion

This section presents the evaluation results of the three machine learning models (Gradient Boosting - GB, Random Forest - RF, and Logistic Regression - LR) applied to pancreatic cancer prediction based on the biomarkers CA 19-9 and CA 125.

The experiments used a 10-fold cross-validation to ensure robust results and minimize training bias. Before discussing the model performance metrics, we analyzed the importance of each feature (Figure 1). For the Gradient Boosting Model, both biomarkers, CA 19-9 and CA 125, played a significant role in the prediction, with the relative importance of 71.1% and 28.9%, respectively. The Random Forest Model also showed considerable relevance for both biomarkers, with values of 62.2% for CA 19-9 and 37.8% for CA 125. On the other hand, the Logistic Regression Model attributed higher importance to CA 19-9 (71.9% of the total) compared to CA 125 (28.1%).

The results of the 10-fold cross-validation revealed that all three models showed a solid overall performance in predicting pancreatic cancer. The average accuracy (Figure 2A) obtained was 75.1% for the GB and RF models and slightly higher at 79.4% for the LR model. Around 75% to 79% of the predictions were correct. Furthermore, the precision metric (Figure 2B) demonstrated the models' ability to avoid false positives, meaning their capacity to correctly identify actual positive cases of pancreatic cancer. The LR model achieved the highest precision, reaching 89.9%, followed by the RF model with 83.3% and the GB model with 82.5%. Regarding the recall metric (Figure 2C), which indicates the models' ability to find all positive cases of pancreatic cancer, the LR model achieved 77.8%. In comparison, the GB and RF models obtained a slightly lower rate of 78.9%. It indicates that all three models performed similarly concerning this metric. The F1-score (Figure 3A), which presents a

Figure 1. Feature importance graph.

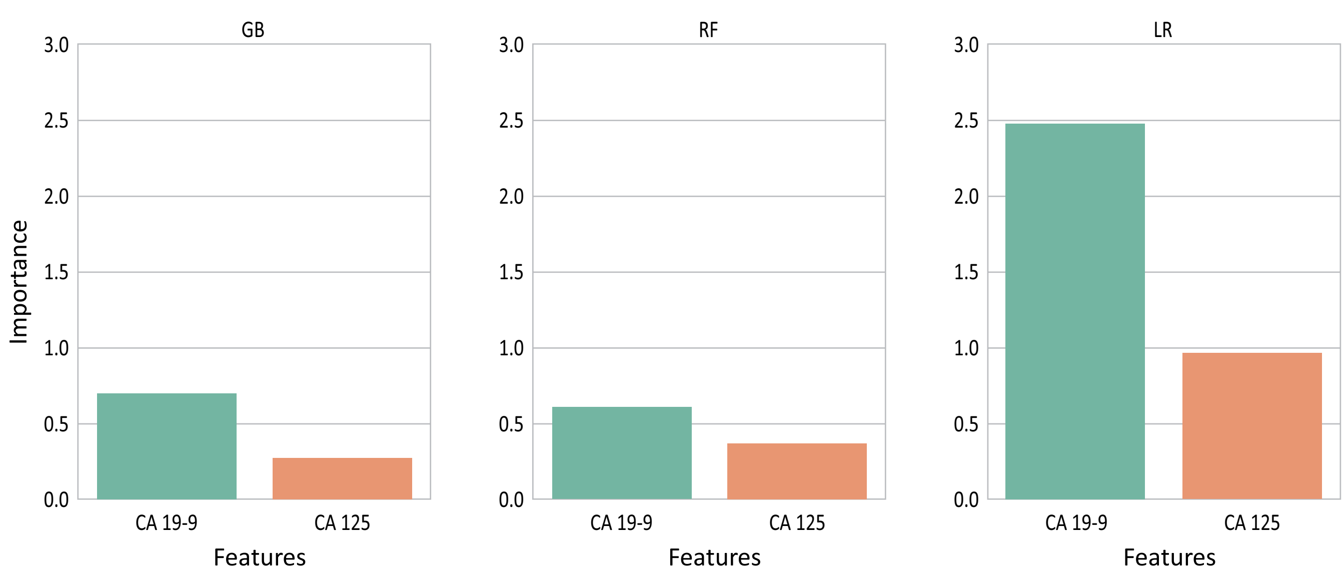


Figure 2. Performance metrics: A) accuracy; B) precision; C) recall.

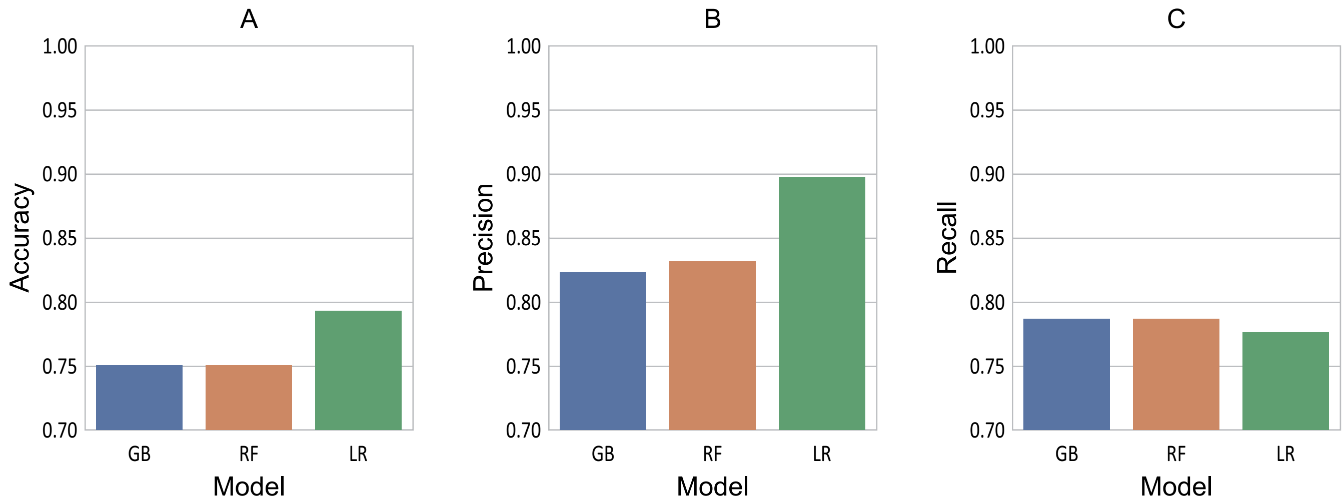
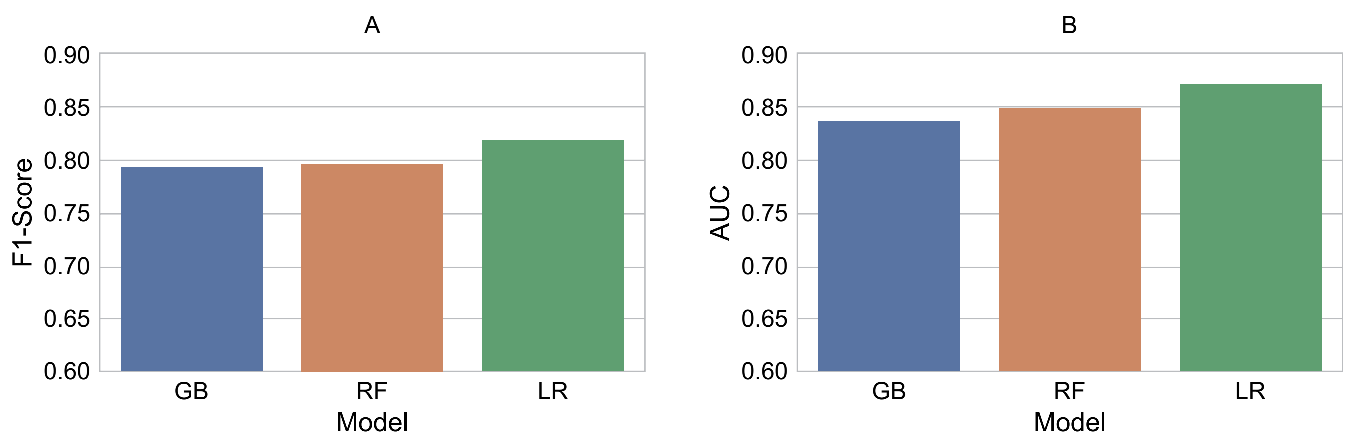


Figure 3. Performance metrics: A) F1-score B) AUC.



harmonic mean between precision and recall, revealed that the LR model achieved the best balance between the two measures, reaching 81.9%. The GB and RF models also performed satisfactorily, with F1 scores of 79.4% and 79.7%, respectively. Finally, we evaluated the AUC (Area Under the Curve) metric (Figure 3B), which represents the area under the Receiver Operating Characteristic (ROC) curve and measures the models' discriminative capacity. Once again, the LR model performed the best, at 87.3%. The GB and RF models also showed promising results, with AUCs of 83.8% and 84.9%, respectively.

The results obtained in this study demonstrate that the three machine learning models, when integrated with the biomarkers CA 19-9 and CA 125, show promising performance in predicting pancreatic cancer. The Logistic Regression model was the most accurate approach, offering a solid balance between precision and recall. However, the Gradient Boosting and Random Forest models also demonstrated efficacy in early disease identification. These results suggest that the machine learning approach based on biomarkers can be a valuable ally in the diagnosis and timely treatment of pancreatic cancer, significantly improving human health.

Conclusion

This study investigated three machine learning models (Gradient Boosting, Random Forest, and

Logistic Regression) for predicting pancreatic cancer based on CA 19-9 and CA 125 biomarkers. The results of the 10-fold cross-validation demonstrated that the models showed solid performance in predicting the disease. The Logistic Regression Model achieved the highest precision, avoiding false positives. The Gradient Boosting and Random Forest models showed promising results with good F1-scores. CA 19-9 and CA 125 biomarkers were identified as essential factors in the prediction. These findings indicate that the machine learning approach integrated with biomarkers can be a valuable tool for early diagnosis of pancreatic cancer, contributing to timely medical interventions and potentially improving the survival of patients affected by the disease. Future studies and clinical validation are recommended to consolidate these findings and enhance diagnostic tools in medical practice.

Acknowledgments

The authors thank Wieand S. and colleagues for providing the data on pancreatic cancer diagnostic markers CA 19-9 and CA 125. We also appreciate the reference to the Biometrika paper (Wieand S, Gail MH, James BR, and James KL. Biometrika 1989;76(3):585-92), where the dataset was cited. This dataset was instrumental in our research on applying machine learning models for early pancreatic cancer detection. We sincerely appreciate the valuable contribution of this dataset to our study.

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Knowledge Translation in One Health: Actions for Strengthening the Health System

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This study aims to investigate and characterize the current knowledge on research initiatives associated with One Health, involving the formation and integration of research networks at Fiocruz, and to analyze the possibilities and limitations of using these coordination mechanisms to enhance research in One Health. An investigation was carried out on the institutional initiatives, mapping the researchers working on One Health. A low level of connections in the scientific collaboration network was observed, demonstrating the need to strengthen research actions. On the other hand, the notice released by the institution configures an action to mobilize and integrate researchers into the theme.
Keywords: Knowledge Translation. One Health. Research Networks.

Introduction

The constant changes in the world related to social, political, economic, and environmental determinants have caused changes in the way of life and, consequently, in the population's health conditions [1]. Therefore, integrating, mobilizing, and cooperating to share technical-scientific discoveries and the application of knowledge is essential to accelerate innovation, strengthen health systems, and improve the population's health. In this scenario of knowledge production, the translation of knowledge (TK) is a broad concept, encompassing all phases from knowledge creation to its application to produce beneficial results for society [2]. TK refers to transforming knowledge from research into practice for disseminating and implementing results favoring society [3]. In the transformation and interaction of knowledge, Unified Health (One Health) encompasses all the interdependency between human health, animal health, and the environment. This scientifically established definition arose from the connection of zoonosis studies involving multiple actors [4,5]. For the United Nations (UN), interdisciplinarity and the vision of One Health are fundamental to achieving the Sustainable Development Goals (SDGs) of the UN's 2023 Agenda [6]. Thereby, public health policies must connect sectors using interdisciplinary collaboration within a complex approach to One Health [5].

In this context, network-shaped work environments characterized by collaborative production are stimulated and incentivized. The network approach can be used to analyze the constitution of production spaces formed by institutional, public, or private actors, government actors, and individual actors, researchers, scholars, managers, or social actors [7]. In these environments, coordinating goals, communication, and resource optimization are crucial to guarantee the efficacy of the network [8,9].

We emphasize that the Oswaldo Cruz Foundation (Fiocruz), a research institution in Health, has been working on different fronts to strengthen network collaborative research. One of its actions for this purpose was the creation of the One Health Translational Research Program (Fio-Saúde Única) [10]. The initiative aims to strengthen the work of professionals from the institution, promote their articulation and integration, and mobilize resources to establish internal and external partnerships with researchers and research groups.

The goal of the present work was to characterize current knowledge on the formation and integration

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of research networks in Fiocruz and to analyze the possibilities and limitations of the formation and integration of these networks to increase research on One Health using the translation of scientific knowledge.

Since its appearance around the year 2000, the One Health approach has been working to advance its operationalization on global, regional, and national levels using commitments made between governments, academic institutions, and non-governmental organizations to strengthen intersectoral collaborations by using platforms, networks, director committees, and task forces [11]. The concept of One Health has been present in interdisciplinary and multisector discussions for several years, but there is increasing interest in applying and translating this approach into action [12].

An essential action in this context has been the quadripartite effort of the Food and Agriculture Organization of the United Nations (FAO), the UN's Environmental Programme (UNEP), the World Organization for Animal Health (WOAH), and the World Health Organization (WHO) to collectively act in order to prevent future pandemics and to promote Health sustainably using the One Health approach [13]. This joint effort resulted in the One Health Joint Plan of Action (2022-2026), which aims to strengthen the ability to face complex and multidimensional health risks and create more resilient global, regional, and national health systems. The plan was structured to consider six action lines that help establish sustainable health and food systems, reduce global health threats, and improve ecosystem management [13].

The operationalization process of One Health, characterized as a transdisciplinary, multisectoral, multiprofessional approach that focuses on society, must be carried out in a coordinated and articulated fashion to establish structuring cooperations that strengthen collective work and avoid the duplication of efforts [14] always aiming for healthcare in human, animal, and environmental healthcare. A coordinated action for this operationalization is structuring research networks that focus on collaboration and sharing of resources and knowledge, the quality of the relations among their participants, and collective goals and results [15].

In this context, Fiocruz has been working on the strengthening of collaborative network research by instituting the translational research program and the Fio-Saúde Única, which have been making an effort to integrate researchers who somehow develop studies on One Health and boast more than one hundred researchers registered in the program. Fio-Saúde Única has the goal of subsidizing future joint actions of different Fiocruz areas of operation, promoting transversality and considering the interdependence between human health, animal health, and the environment, all the while promoting and strengthening articulation and integration with professionals from partner institutions, government agencies, and society as a whole.

Translation of Knowledge in Research

Translation of Knowledge (TK) refers to applying scientific research and academic knowledge discoveries and breakthroughs, solving real problems to meet the demands of society. The term, a very commonly used term in Health, first appeared in the 1970s and began to be widely used early in the 1990s [16,17]. TK has various meanings. In Europe, the terms used are the science of implementation or use of research. In the United States, the terms dissemination, diffusion, use of research, transfer, and absorption of knowledge are frequently used [18]. We emphasizes that this is a vast area that still requires further studies, and it makes it possible to identify and use about ninety adjacent terms.

Differentiating TK from other theories is fundamental to ensuring that interactions between knowledge producers and users of knowledge are robust [16]. There are five "knowledge in action" theories, and their textual content differs. They all differ from TK in their disciplinary roots and types. These are the use of knowledge, diffusion of knowledge, implementation of knowledge, transfer of knowledge, and TK itself [19]. According to Davison (2009) [16], TK has fundamental characteristics related to multidimensionality, involving interaction between the different actors, their target public, and their context, and with information often reported by the researcher about their work and the knowledge regarding the processes and products of the research. These characteristics can either be facilitators or create barriers to knowledge translation.

TK incorporates the steps between the production of new knowledge and their application in practical terms to produce results for society, which involves communication, interaction, sharing, management, and ethics and research as guidelines and a summary of results in a global context [20]. Fiocruz has therefore focused on research that aims to promote the union between scientific production and technological advancements, generating solutions for the population's health problems. It has been possible thanks to the participation of scientists and health professionals engaged in creating innovative methods to meet the demands of society.

Materials and Methods

The method developed was of the exploratory type, which uses a qualitative approach. The data collection strategy involved bibliographic research on existing databases such as PubMed and Scielo. Additionally, other contents were used, such as those made available by the World Health Organization (WHO), the Food and Drug Administration (FDA), and public documents on institutional and governmental websites. The analysis tool was Fiocruz's e-Lattes, a technological device based on web architecture. It can be accessed through https://elattes.fiocruz.br/ using a computer, a tablet, or a cell phone [20]. The databases used for the prospection of the tool are the Lattes platform, the Directory of Research Groups (DGP), the Brazilian Digital Library of Theses and Dissertations (BDTD), international scientific bases of the Web of Science already treated (CWTS), and the database of altmetric.com [21]. The study also mapped the results of the public call launched by Fiocruz through the Research Support Foundation of Rio Grande do Sul (FAPERGS). The research investigated and characterized current knowledge on the initiatives of research lines associated with One Health, which involve forming and integrating research networks at Fiocruz. The collected data attempted to identify punctual or collective institutional actions that aim to strengthen the One Health strategy in Fiocruz. Researcher mapping was done within the One Health Translational Research Program - Fio-Saúde Única. This network environment aims to articulate, integrate, and mobilize resources to establish internal and external partnerships with researchers and research groups. The tool generates information and indicators that allow for the measurement of scientific production and its relevance, whether it refers to an author in terms of productivity criteria (total number of articles), to an article using its relevance, to its influence on other researchers (total number of citations), or to the impact factor of the journal which published the articles [21].

Results and Discussion

The analysis generated by e-Lattes based on these data includes important metrics for the context of this research. Figure 1 shows indicators of the scientific production of Fio-Saúde Única researchers related to the number of publications per year and the stratification of the quality of these publications. Figure 2 presents the collaboration network of Fio-Saúde Única, in which dots represent researchers and colors represent groups of researchers formed according to their relations. Larger dots represent researchers with more publications. Figure 3 represents the distribution of the researchers participating in Fio-Saúde Única per area of operation; one researcher may be active in more than one area of knowledge. Based on the information extracted from the analysis generated by e-Lattes, we observed a higher number

Figure 1. Indicators of scientific production related to the One Health Network (between 2019 and 2023).

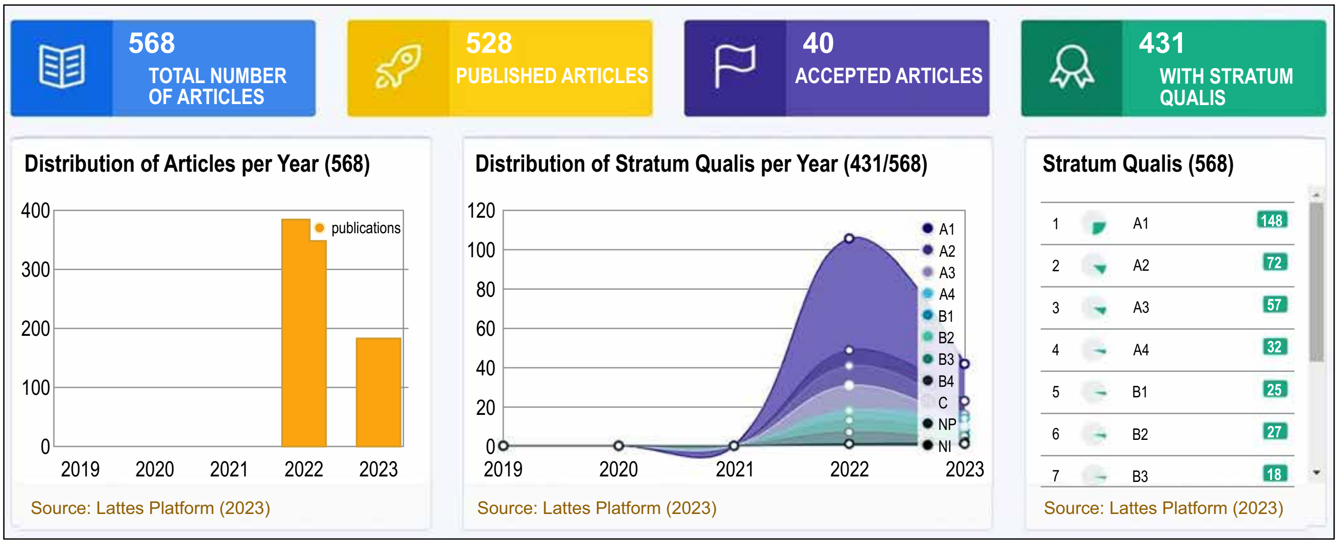
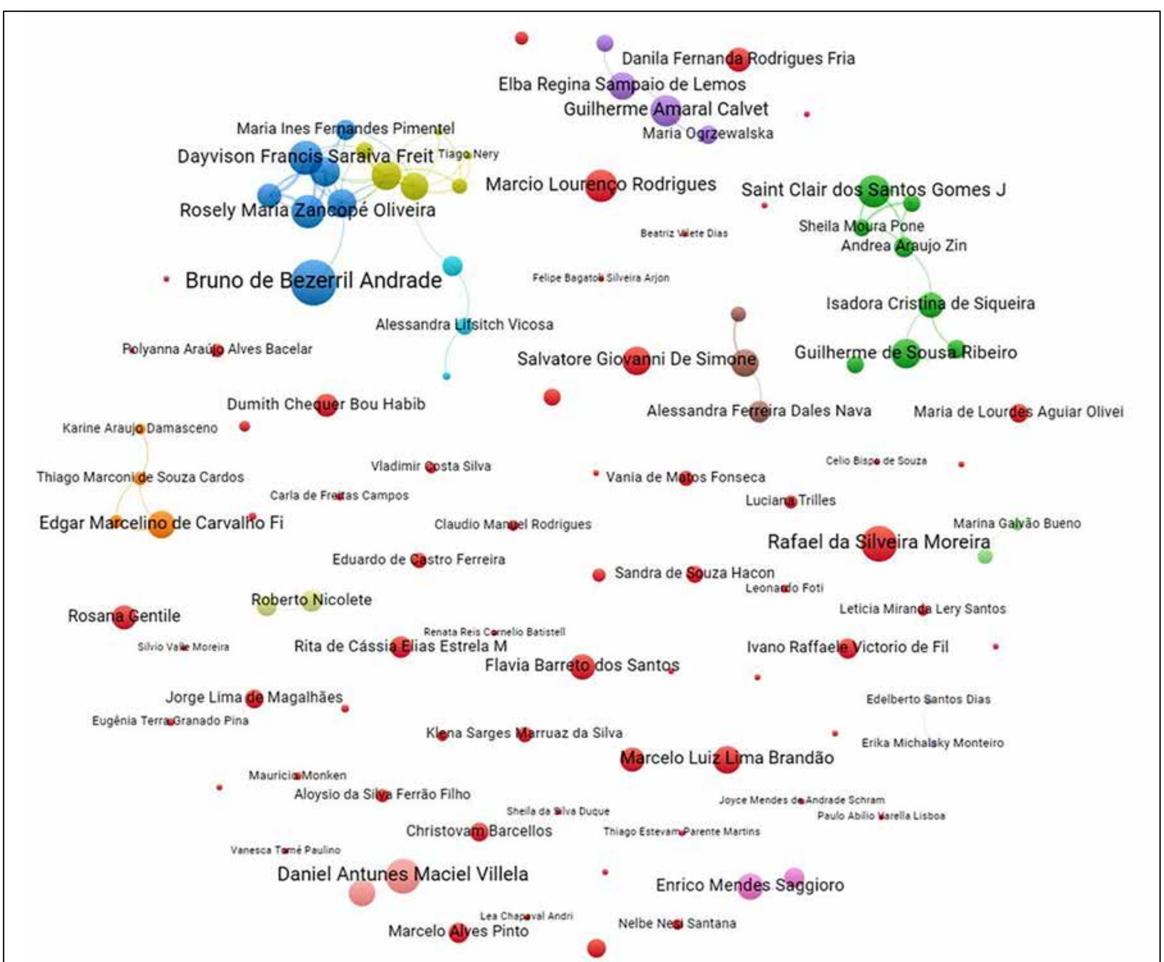
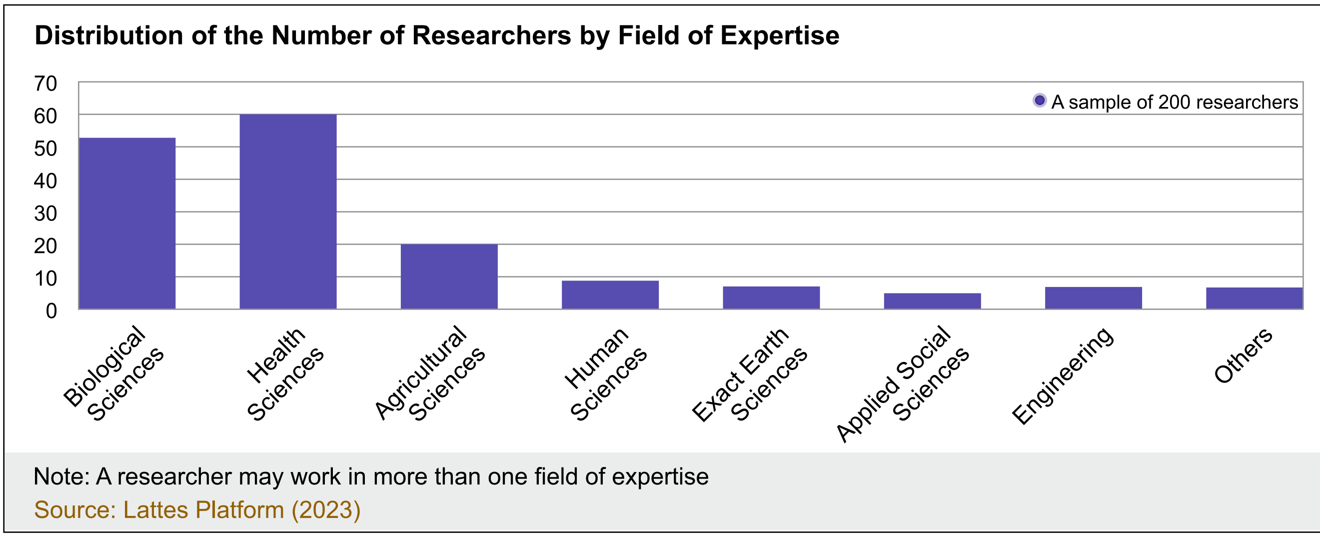


Figure 2. Scientific collaboration network - One Health, Fiocruz.



Source: Lattes platform.

Figure 3. Overlapping of fields of operation of Fio-Saúde Única researchers.



of publications for the 106 researchers registered in the network in 2022 (528 articles published and 40 accepted for publishing). However, we also observed a low level of connections in the scientific collaboration network, with the formation of small research groups, which shows the need to strengthen actions for collaborative network research in the field of One Health [14] within the institution by activating and mobilizing resources that promote a higher interaction between the actors and ensure the sustainability of the network. Another critical piece of information is the distribution of the fields of operation of the researchers registered in the network, as well as their institutional and sectional distribution. We observed at least 7 significant areas where these researchers work: biological and health sciences, agricultural sciences, exact and earth sciences, applied human and social sciences, and engineering. In this sense, this information illustrates the expectation of great potential to develop research on One Health, characterized by the transdisciplinary character, multisectoral cooperation, and inter-institutional work [11]. Other initiative mapped in this research was the public call launched by Fiocruz via the Research Support Foundation of Rio Grande do Sul (FAPERGS) [22], in a model structured between the two institutions to strengthen collaborative projects of multiple disciplines among the members of the Health Network and expand research on One Health in the state of Rio Grande do Sul (RS), focusing on the needs of the Unified Health System (SUS). In this work, 12 scientific projects were selected considering the One Health approach within the axis "Scientific research with disruptive or incremental innovation in One Health, with repercussions on human health." The institutions that participated in the public call in Rio Grande do Sul (universities, science and technology institutions, and institutions related to the state government) and in Fiocruz (technical-scientific units on a national level) whose projects were approved addressed these priority subjects: (i) antimicrobial resistance, (ii) pollution, (iii) disasters and the environment, (iv) social inequalities and vulnerable populations, (v) integrated surveillance in health, (vi) zoonosis and vectors; and (vii) medical microbiology. These themes are related to the internal and external networks of Fiocruz and have been working as complements to the different expertise distributed among the institutions. It is essential to highlight that the research lines of the projects approved in this public call directly relate to the action lines elaborated in the quadripartite joint plan of FAO, UNEP, WOAH, and WHO [13]. We emphasize that the proposal of the public call makes it possible to integrate projects using multisectoral collaboration, inter-institutional articulation, and transdisciplinary actuation, which are important characteristics of the One Health approach [12, 14].

Conclusion

The challenge of One Health is to look at research from a wide perspective articulated in the ecosystem in a transdisciplinary, multisectoral, multiprofessional, and inter-institutional manner to integrate knowledge. In this context, Fiocruz has been implementing integrative actions: the public call launched by Fiocruz through the Research Support Foundation of Rio Grande do Sul (FAPERGS) [22] and the structuring of the One Health Network using Fio-Saúde Única. These actions aim to strengthen research on One Health, stimulating a higher interaction between researchers and better integrating projects to expand collaborative research. We observed that even with the activation of a research network, collaboration and integration between these actors are still incipient and, therefore, need structuring actions for their strengthening. For this purpose, the process of coordinated mobilization of this network would be crucial.

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Production of Polycaprolactone/Hidroxyapatite Scaffold for Application in Tissue Engineering

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The study aims to produce fibrous polycaprolactone (PCL) scaffolds with hydroxyapatite (HA) incorporation using the Solution Blown Spinning (SBS) technique for application in tissue engineering. The scaffolds were characterized by scanning electron microscopy (SEM), Fourier transform infrared spectroscopy (FTIR), contact angle, and thermogravimetric analysis (TG). The images obtained by SEM showed that the fibers have a diameter on a micrometer scale. FTIR spectra indicated physical and chemical interactions between PCL and HA. TG analysis showed the presence of HA, which provided an anticipation of the PCL degradation temperature. We obtained micrometric fibers of PCL and PCL/HA with different orientations. The technique used is promising for making scaffolds.
Keywords: Tissue Engineering. Composites. Scaffolds.

Introduction

Tissue engineering seeks to create functional tissues from biocompatible materials and living cells to improve the quality of life of people who suffer bone fractures or trauma. The development of three-dimensional porous scaffolds has gained attention since they can promote support for cells to adhere, grow, and proliferate to form the neo-tissue [1].

The development of hybrid systems of biodegradable polymers, such as polycaprolactone (PCL), and ceramics, such as hydroxyapatite (HA), is an approach commonly used in bone tissue engineering to assist bone recovery [2]. PCL has advantageous mechanical properties, while HA is biocompatible and promotes osseointegration. Combining PCL and HA makes it possible to obtain a material with mechanical resistance and bioactive properties, favoring the formation of bone tissue [3,4].

Despite the number of techniques available to manufacture 3D scaffolds, solution-blown spinning (SBS) has attracted the attention of researchers because of its simplicity and advantages, as high production rate and low cost. The SBS uses a high-speed air stream to promote fiber production from a precursor solution. Therefore, the study's objective was to develop and optimize the preparation of PCL and PCL/HA scaffolds by SBS, seeking to obtain homogeneous fibers efficiently and economically.

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Materials and Methods

Perstorpo supplied PCL (Mw = 80,000 g/mol) as pellets. Nanosized HA was synthesized and kindly donated by the biomaterials laboratory (LABIOMAT) of the Brazilian Center for Research in Physics (CBPF) - UFRGS. The solvent used was glacial acetic acid (GAA), with a molar mass of 60.052 g/mol supplied by Exodus Scientific.

Preparation of Scaffolds

PCL and PCL/HA scaffolds were produced by preparing PCL solutions in 6 mL GAA at different polymer/solvent ratios (see Table 01). The solutions were vigorously stirred at 40 °C for 1 h. Further, the solutions were stirred for 2 h at room temperature. For the PCL/HA scaffold, the nanosized HA powder was added into the PCL acid solution (after the first hour) and then sonicated for 10 min. The solution was further mixed for 2 h. Finally, the precursor solutions were spun into fibers by SBS to produce the hybrid scaffolds, employing the following conditions: 6 mL/h feed rate, 20 - 30 Psi spinning pressure (Table 1), and a working distance of 25 cm. It was used a static collector.

Characterization

X-ray diffraction analysis was carried out using the Empyrean PANalytical instrument. Angular range from 2-75 (2θ) with 0.01 step/time. 40 kV and 40 mA. The diffraction intensities of maxima of hydroxyapatite were used to measure the crystallinity ratio of the samples and were defined according to Annex D of ABNT NBR ISO 13779-3:2020. FTIR analysis was carried out in a Perkin Elmer Spectrum device, version 10.4.2, in the 400 to 4000 cm^{-1} range, using KBr disks. A HITACHI Scanning Electron Microscope, model TM3000, assessed the nanostructured hydroxyapatite's morphology and the scaffolds produced. Measurements of the diameter of the fibers were performed with the help of the ImageJ software, taking 25 measurements per sample. Contact angle measurement was performed by the sessile drop procedure using 5 x 5 cm samples for 3 minutes. A 3 μL sessile drop of water was deposited on each specimen's surface with a micropipette. The drop was captured with the Kruss Goniometer equipment, model DSA-25. Five measurements were taken and averaged to obtain the final contact angle value. The thermal stability of the scaffolds was evaluated by thermogravimetric analyses (TA Instruments DSC model Q3 was used instruments, New Castle, USA) using a thermal protocol in the range 25 - 800 °C, a heating rate of 10 °C /min, and in a nitrogen atmosphere.

Results and Discussion

Characterization of Sample Hydroxyapatite

Figure 1 shows the XRD of the nanosized HA calcinated at 1000° for 15 h. The diffraction pattern corresponds to hydroxyapatite of chemical formula $Ca_5OH(PO_4)^3$, as the ICDD 01-084-1998 card corroborates. Also, the nanosized HA is of high purity and with a degree of crystallinity of 82.5%. No peaks related to calcium oxide or α- or β-tricalcium phosphate were detected. Therefore, the hydroxyapatite can be considered stoichiometric, with the Ca:P ratio at 1.667.

Figure 1. X-ray diffraction analysis of HA.

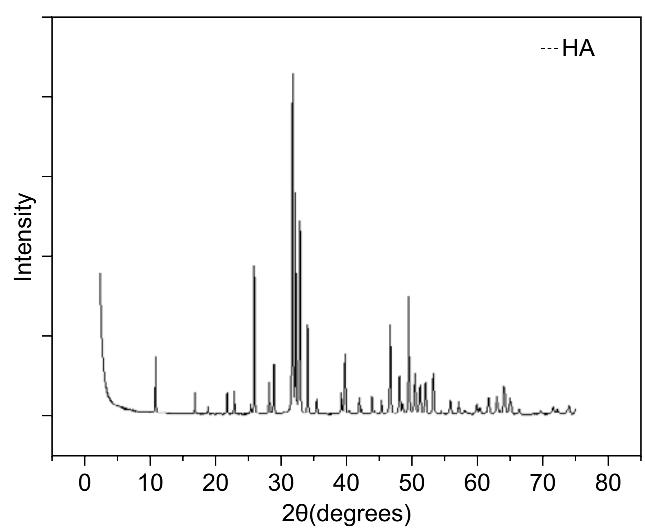


Table 1. Studied variations used to produce the scaffolds.

Sample	Concentration (%)	Pressure (PSI)	Polymer mass (g)	HA (g)
PCLA	18	20	1.0865	-
PCLB	22	20	1.327	-
PCLC	18	30	1.0891	-
PCLD	22	30	1.3244	-
PCLHA	22	20	1.3134	0.0395

Figure 2 shows the FT-IR analysis for the nanosized HA. The bands at 3571 cm^{-1} and 632 cm^{-1} were attributed to the stretching and bending of the hydroxyl group (OH^-) constituting the crystalline structure. The vibrations related to the phosphate group (PO_4^{3-}) were assigned to the bands at 1089 cm^{-1} and 1046 cm^{-1} (asymmetric stretching), 962 cm^{-1} (symmetric stretching), 602 cm^{-1} and 572 cm^{-1} (asymmetric stretching P-O) and 474 cm^{-1} (O-P-O flexion). The bands mentioned above are characteristic and confirm the chemical composition of HA. The absorption bands at 3454 cm^{-1} and 1636 cm^{-1} are characteristic of hydroxyl vibrations relative to adsorbed water [5-7].

In Figure 3, we observe the HA morphology evaluated by SEM. The image shows that the HA

Figure 2. Fourier transform infrared spectrum of the nanosized HA.

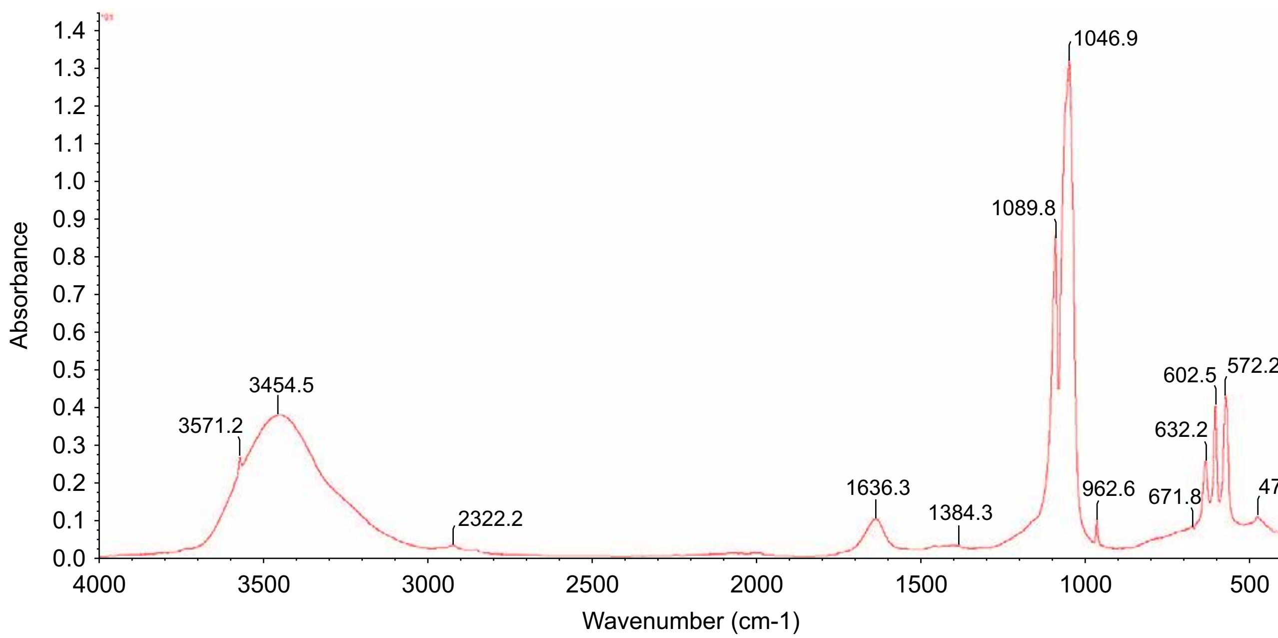
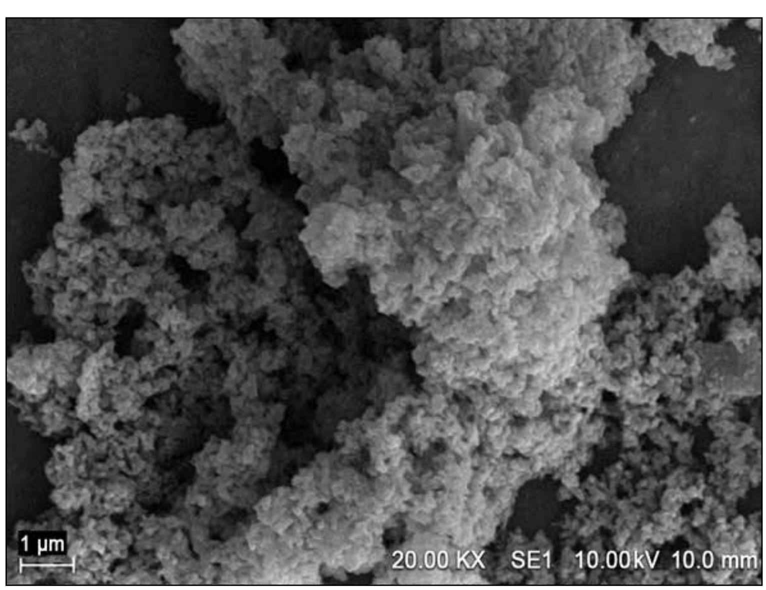


Figure 3. SEM from hydroxyapatite.



is formed by agglomerates of smaller particles, on the order of nanometers, with an elongated shape.

Figure 4 shows the infrared spectra of the obtained PCL and PCL/HA samples and their characteristic bands. The samples show a similar profile with slight differences, suggesting the combination of pure polymer and PCL/HA, causing some overlapping bands. These bands show slight variations in intensity and small shifts. The characteristic bands of PCL are observed at 2943 cm^{-1} (asymmetrical axial strain of the CH_2 group) and 2864 cm^{-1} (symmetrical axial strain of the CH_2 group). Additionally, the bands at 1720 cm^{-1} and 1161 cm^{-1} correspond to the elongation of the absorbance group, representing the symmetric vibration of O-C-O [8]. The band located at 601 cm^{-1}, along with the bands at 631 cm^{-1} and 574 cm^{-1}, correspond to the vibrational modes of the PO_4^{3-} group attributed to HA, indicating physical and chemical interactions between the PCL carboxyl groups and the HA [8,9].

Figure 5 presents micrographs of the scaffolds obtained. The macro appearance after the spinning process is similar to a cotton-wool-like structure. However, micrographs with 2000x magnification reveal the presence of many "beads" (beads) in all samples, which may be attributed to the incomplete solvent evaporation. According to SEM histograms, fibers (a) and (c) have mean diameters of approximately 0.6 µm and 0.3 µm, respectively, due to the application of different pressures. The same pattern was observed in samples (b) and (d), with mean diameters of 0.78 µm and 0.45 µm, respectively. The diameter variation can also be observed when comparing samples (a), (b), (c), and (d) due to the change in the solution concentration. The incorporation of bioceramics results in an increase in the average diameter of the fibers due to the increase in the viscosity of the solution.

Figure 6 shows that most of the samples have contact angles greater than 90°, indicating a hydrophobic character of the scaffolds thanks to the PCL matrix. HA in the fibers increases the surface roughness, resulting in contact angles of around 128°, compared to 119° for the sample without HA [10].

Figure 7 demonstrates the thermal stability of samples. For the PCL, the mass loss was around 90.31%, with the maximum degradation rate at a temperature of 396°C. Char is formed as a residue. In the case of the PCL/HA hybrid, the mass loss

Figure 4. FTIR of PCL and PCL/HA fibers.

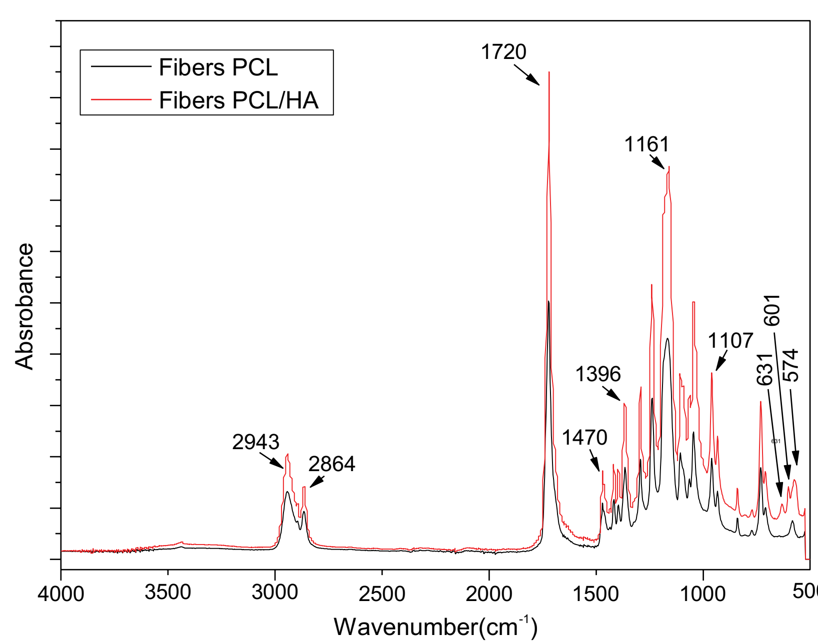


Figure 5. SEM of PCL and PCL/HA fibers with different concentrations.

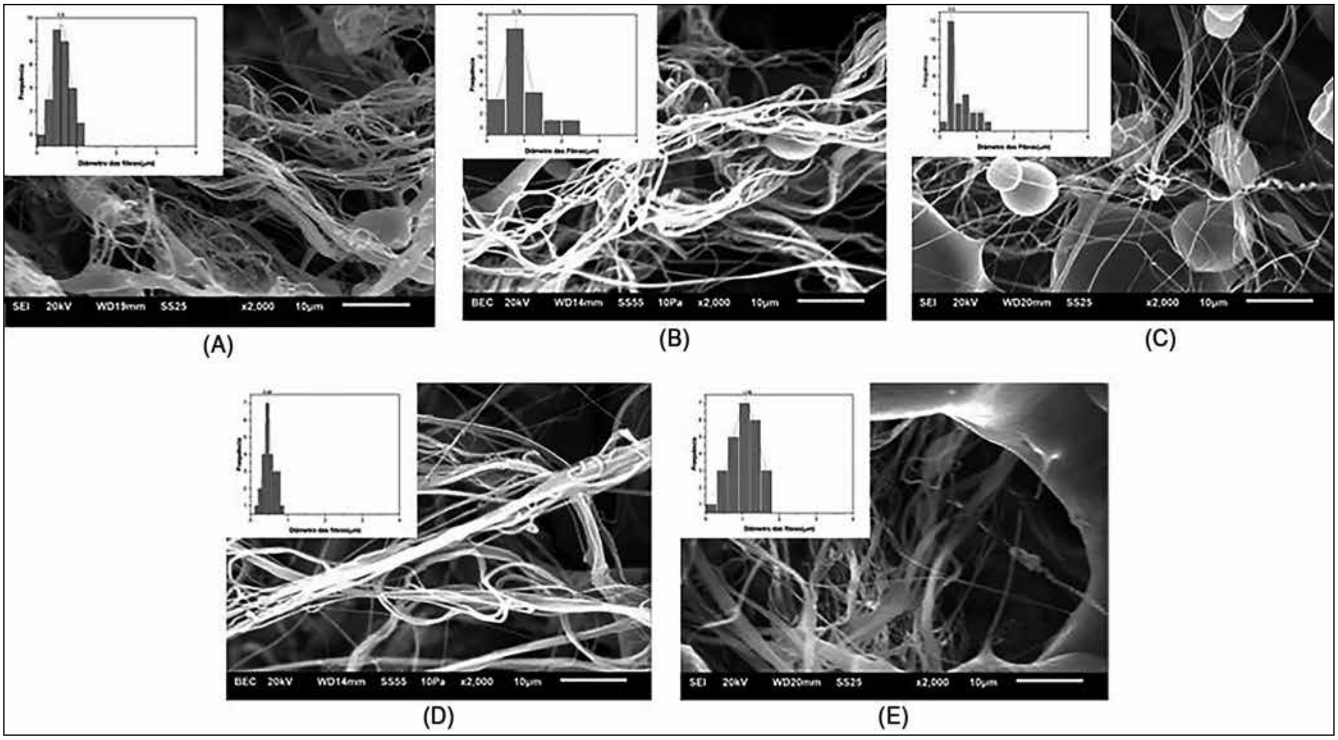


Figure 6. The contact angle of PCL and PCL/HA fibers.

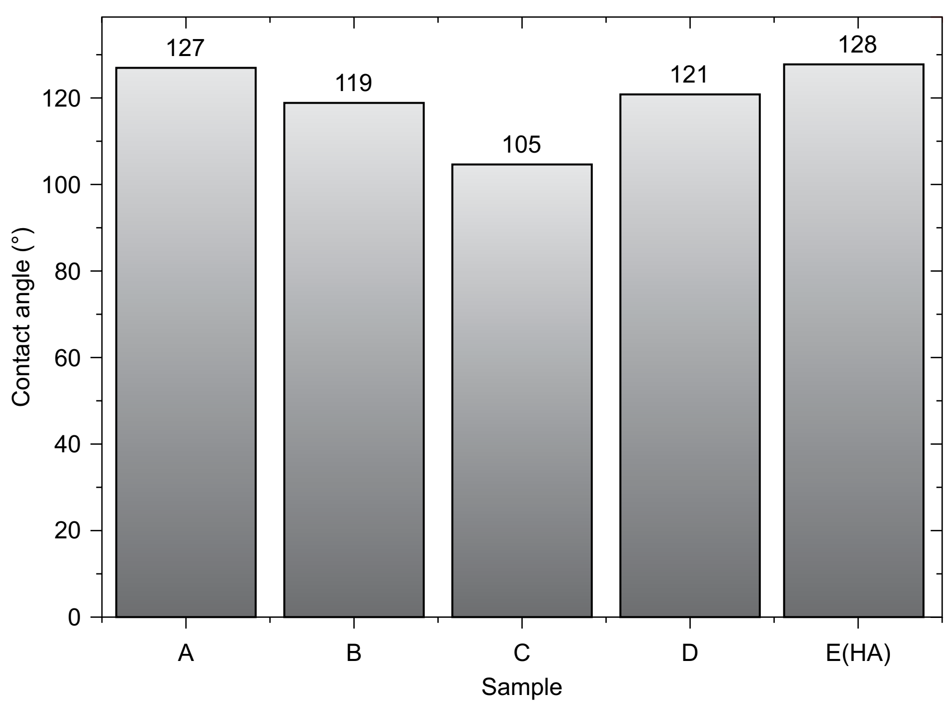
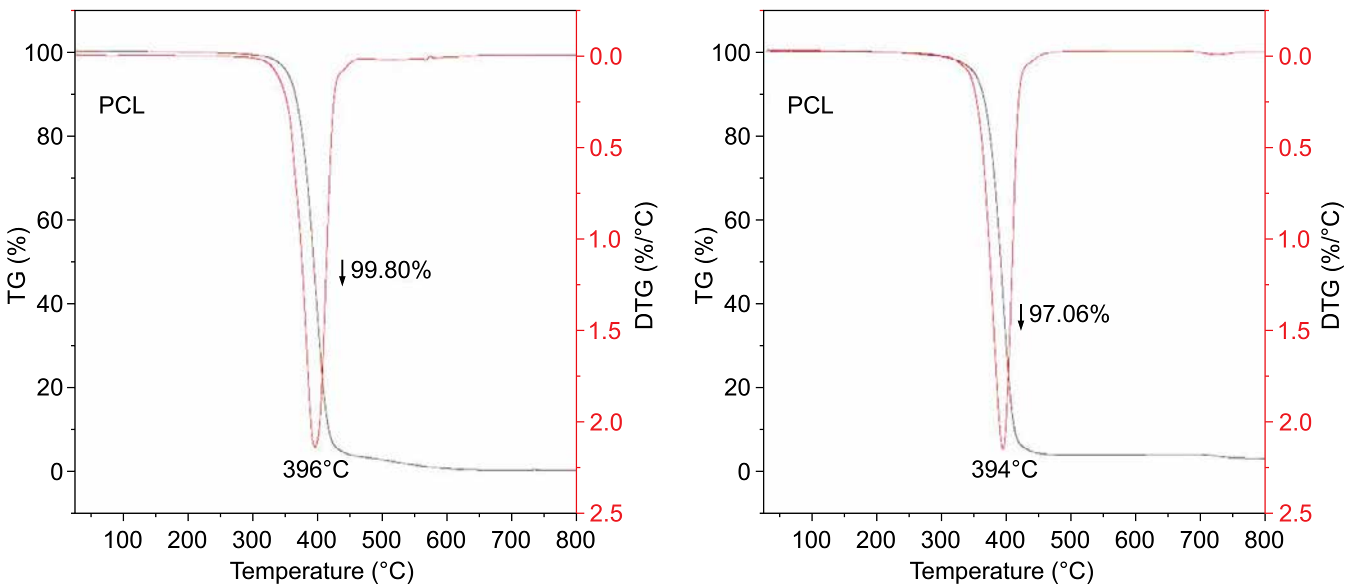


Figure 7. Thermographic analysis of PCL and PCL/HA fibers.



was approximately 75.81%, and the temperature for maximum degradation rate was shifted to 385°C. The addition of HA resulted in anticipation of the polymer thermal degradation. Also, the residue formed can be attributed to inorganic HA and char formed.

Conclusion

In this work, fibers of different polycaprolactone (PCL) orientations with hydroxyapatite (HA) incorporation were produced using the blow-spinning technique, a promising approach for scaffold fabrication. HA, as an inorganic component, is advantageous due to its chemical properties. SEM analyzes observed the presence of "beads" due to incomplete evaporation of the solvent. FTIR spectra indicated possible physical and chemical experiments accommodated between PCL and HA. X-ray diffraction analysis confirmed the crystalline structure of HA. These results suggest that the SBS technique combined with HA can contribute to developing high-quality and high-performance biomedical implants. However, further studies are needed to improve processing conditions and evaluate the tissue response to the scaffolds produced.

Acknowledgments

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Benefits of Using Information and Communication Technology in the Control of Residues in Medicine Production Within Public Organizations: A Sustainability Practice

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This case study aims to elucidate the advantages of employing communication and information technology tools for disposing of pharmaceutical residues in a public production laboratory, highlighting its significance as a sustainable practice. The study employs a qualitative approach, incorporating applied methodology, technical procedures, bibliographic document research, and a case study with an exploratory objective. The primary outcomes reveal enhancements in optimization processes, reflecting paradigm shifts and technologies within laboratory operations. The study concludes that substantial impacts have occurred in work processes, fostering the dissemination of sustainability practices within the organization.
Keywords: Residues Management. Sustainability. Pharmaceutical Industry. Information and Communication Technology. Public Laboratory.

Introduction

The evolution of industrial processes in the pharmaceutical industry has led to the development of new technologies and health inputs, impacting the environment through the increased generation of residues often disposed of inadequately [1]. A Syndicate of Pharmaceutical Industries study in Brazil reported that the pharmaceutical industry moved approximately BRL 76.98 billion in 2020, creating 90,025 thousand direct jobs at the beginning of the same year in companies manufacturing drugs for human use [2].

While this intense production benefits the economy by creating jobs and enhancing access to medicines, it poses a significant environmental challenge. Despite economic, labor, and social advances, the environmental impact must be transparently discussed with society [1]. Environmental preservation has been a subject of discussion in Brazil since the 1990s, with sustainability gaining traction in technical, scientific, and political spheres [3].

The Sustainable Development Goals (ODS) urge governments to devise strategies for socio-environmental action [4]. Waste disposal in the pharmaceutical industry becomes a focal point in aligning actions with these goals. The industry, representing a substantial portion of the global health-productive market, generates waste through the production of medicines. The complexities of the drug production process result in various wastes, necessitating proper treatment and disposal [5].

We emphasize that specific rules and procedures governed by applicable laws manage industry residues. Addressing SDG objectives, particularly Objective 6, which emphasizes ensuring the availability and sustainable management of water and sanitation for all, and Objective 9, promoting resilient infrastructures, inclusive and sustainable industrialization, and fostering innovation [4], becomes imperative for the pharmaceutical industry. Improving internal processes to reinforce the sustainability cycle aligns with these objectives, aiming to minimize adverse environmental impacts and achieve the indicators of the 2030 agenda.

The industry's commitment to social responsibility is paramount in managing residues,

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necessitating innovative management practices [6]. The pharmaceutical industry has explored adopting information and communication technology tools (ICT) to enhance process controls, especially in managing residues [7-9]. This work presents a case study highlighting the benefits of ICT in controlling waste generated during drug production. It emphasizes its role as a sustainability practice through a systemic solution for managing pharmaceutical residues within a public laboratory warehouse.

Materials and Methods

Sample Case Study

The study was conducted in a public Pharmaceutical Laboratory, a technical-scientific unit linked to Brazil's Ministry of Health (MS). Established in 1976, the laboratory serves as a market regulator in the pharmaceutical sector, occupying a 40,000 m² facility in Jacarepaguá, Rio de Janeiro, Brazil. The laboratory, certified with ISO 14001 (Environmental Management System) since 2015, operates under the pillars of socio-environmental sustainability, adhering to the 5R concept – Rethink, Reduce, Refuse, Reuse, and Recycle [4].

Results and Discussion

Warehouse Waste Disposal Process

In 2014, the laboratory acquired the SAP® integrated Information and Management System to optimize and integrate its processes. This system addressed data security concerns, complied with sanitary regulatory requirements, and ensured traceability throughout the production flow and product life cycle. The Logistics Department managed warehouses storing raw materials, packaging materials, and medicines. The department handled inventory control, storage conditions defined by the manufacturer, internal expeditions for production and research, and distribution to State and Municipal Health Secretariats based on the Ministry of Health's strategic programs. Responsibilities also included collecting medicines from customer complaints, managing deviations in quality, and disposing of expired, disapproved, and returned items from customers.

Item disposal emerged as a crucial activity among defined internal procedures, impacting all process stages. Figure 1 illustrates flaws in the warehouse waste disposal process due to inadequacies in the existing computerized system. Consequently, a new system was acquired, necessitating the definition of new activities and routines in its programming. The entire process was programmed into computerized routines within the purchased software, offering advantages such as greater control of item validity, correct disposal of residues, issuance of reports for control bodies, participation of waste-generating areas, accounting control for accountability to control bodies, and reproducibility of actions in SIAFI, the Financial System of the Brazilian Federal Government.

The pharmaceutical industry's high demand has resulted in substantial consumption, leading to numerous consequences related to the improper disposal of medicines. Globalization and the Third Technological Revolution have exacerbated the disposal of drug waste, impacting citizens' lives [4]. Studies reveal that certain substances in drugs resist treatment processes, negatively affecting the environment for extended periods and causing environmental and socioeconomic problems [12]. Changes were implemented in the waste disposal flow to address disposal challenges (Figure 2). The implementation of ICT introduced opportunities for mitigating disposal challenges.

Sustainability has gained increased attention in the pharmaceutical industry, making residue disposal a prominent subject in academic studies [13]. Environmental sustainability, particularly in terms of cleaner production, green supply chains, green materials, and sustainable human resources management, has become a central

Figure 1. Failures and opportunities in the warehouse waste disposal process.

Failures Diagnosed in the Use of the Own Computerized System	Opportunities Diagnosed in the Use of ICT
a. There was no write-off of discarded items; b. The generating areas did not paricipate in the disposal process, leaving all activity under the responsibility of the Warehouse Manager; c. Lack of accounting control, as the system did not have accounting logic; d. There was no differentiation between the stock of inputs or expired drugs, from the regulated waste classifications; e. The activities were time-consuming, due to the processing of authorizations for disposals being carried out through memorandums, causing an increase in stock in the rejected areas; f. Time-consuming flow, as it contains several steps to be controlled.	a. Creation of Systemic Disposal Flow; b. Disposal Approval Flow by item characteristics; c. Flow initiated and controlled by the Logistics Manager; d. Approval flow, with justification of the reason for disposal, being attached to the system; e. Easy-to-understand flow; f. Paricipation of the Accounting Sector, in the accounting items' classification; g. Systemic action in real time, through the integrated system; h. Information sharig at all stages of the process; i Transformation of items into classified waste in the system; j. Blocking of disapproved stock, preventing movement; k. Control of quantity and final value of the disposal; l. Systemic write-off and automatic transformation into classified waste, according to the waste legislation; m. Issuance of tax documents; n. Projection of annual quantity, for the elaboration of the Term of Reference for contracting a company specialized in the final destination; o. Issuance of reports containing all information from the approval of the disposal to the issuance of the INEA Manifest; p. Elaboration of indicators; q. Search by Transfer Order number, document generated for transfer between virtual stock and waste deposits; r. Search by Waste Manifest number; s. Search by DANFE number, as inserted in the Manifest; t. Search for Original Material Transfer document used in the Disposal Approval Flow; u. Search by Source Material / Batch; v. Name of the disposal approver, resposible for the disposal approval information; w. Annual search criteria for disposal documents; x. Description of the source material; y. Return date of the Manifest, as a Workflow closing step.

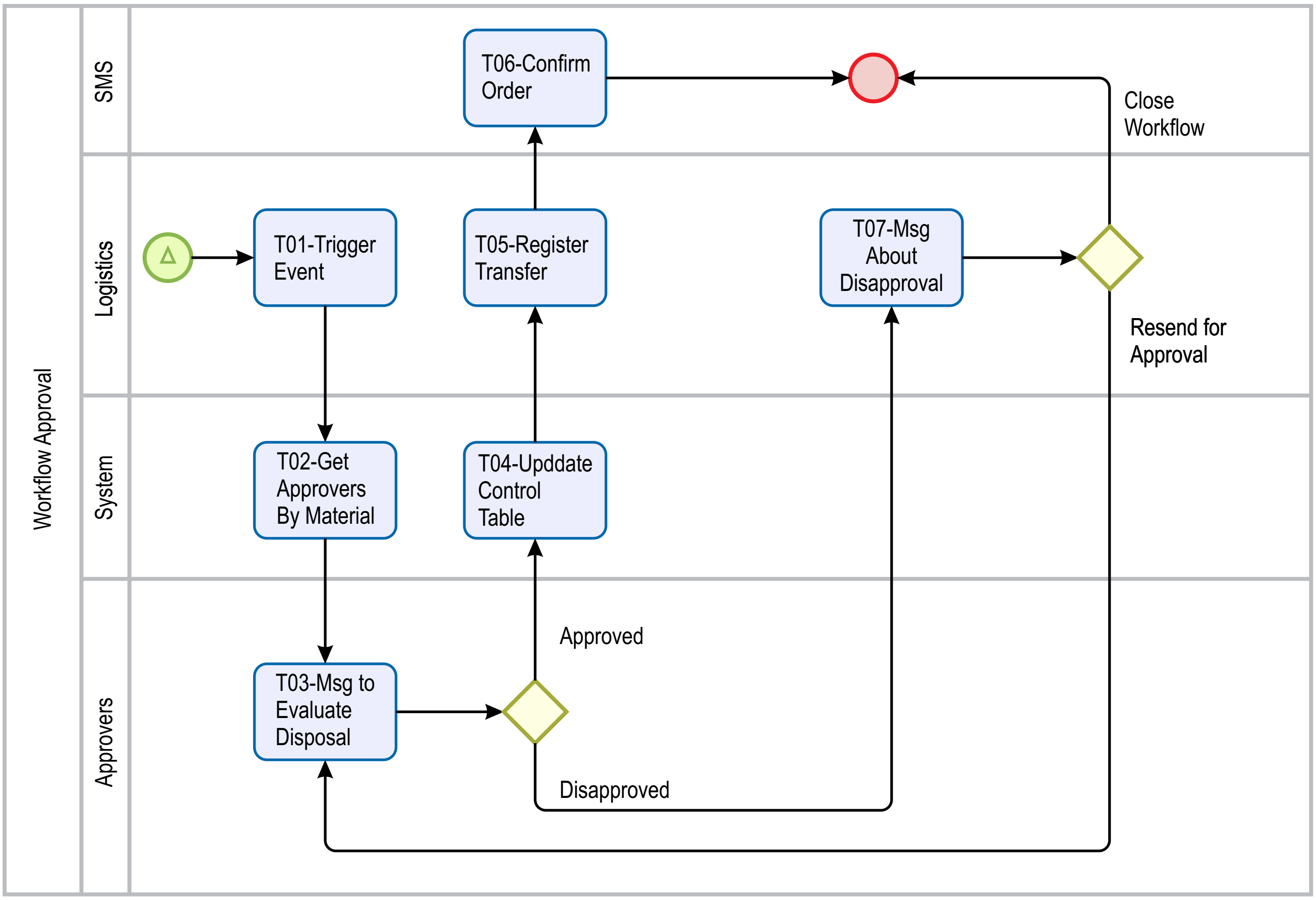
Source: Authors by internal management report (2012).

focus of management studies [3]. The regulation of waste management in the pharmaceutical industry underwent multiple changes from 1981 to 2022, reflecting legislative efforts to address environmental concerns (Figure 3).

Conclusion

ICT has significantly advanced responsible waste disposal in the pharmaceutical laboratory studied. The impacts on work processes were notable, including a reduction in the storage time of disapproved or expired items, the transformation of manual processes to computerized systems, faster processes, enhanced reliability of records with control bodies, real-time traceability, provision of disposal indicators, issuance of reports by waste classification/quantity/sector, reduced use of paper and office materials, and the introduction of recyclable disposal bins. ICT provided these advantages and facilitated better management and integration between

Figure 2. Warehouse disposal approval flow.



Source: Disposal Workflow Project Elaboration Report (2016).

involved areas, enhancing the reliability of generated data. On the other hand, it allows for more qualified analyses by managers during decision-making processes, contributing to disseminating a culture of sustainability practices within the organization.

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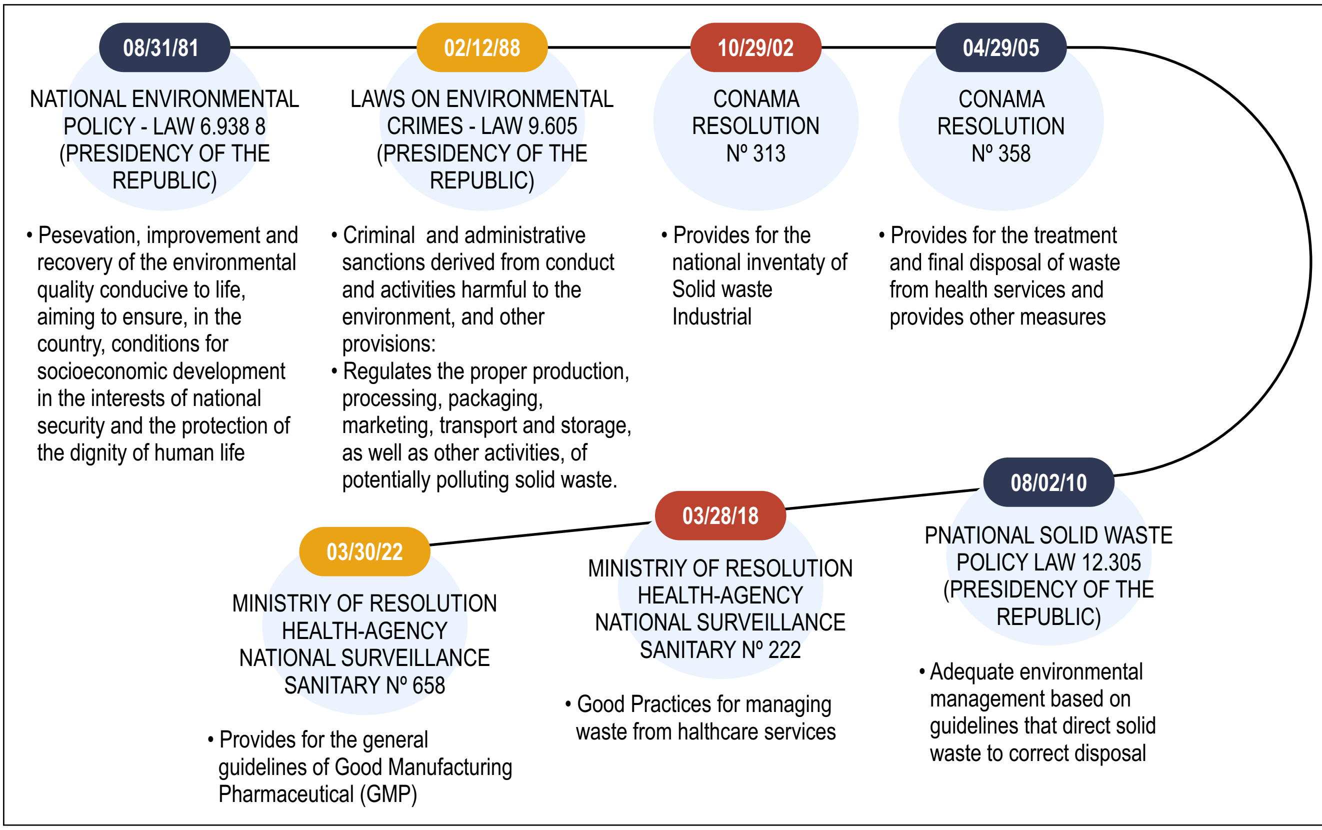
Figure 3. Regulation of waste management in the pharmaceutical industry.



Source: Authors.

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Development of an Educational Alarm System: Study of IEC 60601-1-8:2022 - Alarm Systems in Electromedical Equipments

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This article addresses the development of an educational alarm center applied to the study of ABNT NBR IEC 60601-1-8:2022 - general guidelines, tests and guidelines for alarm system requirements in electrical medical equipment and electrical medical systems. The project covers prioritization conditions, frequency, and aspects related to the functional structure of a prototype. The process was divided into three stages: general and specific research, schematic and organizational assembly, and circuit validations. The perspective is to develop it with the performance characteristics of an electromedical device.
Keywords: Alarm Center. Automatic Electromedical Equipment. Alarm Issuing System.

Introduction

The advancement of technologies applied to the healthcare field has significantly transformed how services related to this sector are delivered. With this advancement, alarms are crucial in assisting healthcare professionals in monitoring patients. However, in many cases, there is an observed saturation in the number of alarms present in hospital beds, which can lead to desensitization of the hospital staff, potentially resulting in dangerous situations. This scenario emphasizes the need for the study of alarms and their specific characteristics.

In Brazil, all technologies encompassing these devices are based on the provisions of NBR IEC 60601-1-8:2022, which standardizes Alarm Systems in electromagnetic equipment and systems, determining their aspects.

There is no literature similar to the educational alarm kit device, both in concept and in purpose. Consequently, educational projects that prepare students in the biomedical and electromedical sectors for the characteristics of alarms in hospital equipment are limited and need to be more widely disseminated.

This article aims to show the elements that make up the regulatory framework related to alarm generation and instruct students on standardization in electromedical equipment. The prototype aims to facilitate the student's teaching and learning process through interaction with the device. According to Pagel and colleagues [6], the student's interaction with equipment during practical classes enables a better understanding of the subject of study, allowing them to establish cognitive connections inherent to their environment. With the kit, students can understand the functional logic structure implemented in a hospital alarm device. It is important to emphasize that understanding this operation also means comprehending the regulatory structure presented by ABNT – Associação Brasileira de Normas Técnicas. Finally, the inspiration for this development was derived from the many applications of educational robotics concepts as a tool for technology integration [1,2].

Materials and Methods

This project developed a prototype that should indicate alarm levels by NBR IEC 60601-1-8:2022 for each type of urgency (low, medium, and high). The project was divided into three stages:

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a) Planning: Researching the standard and the components and characteristics of the electronic circuit and software.

b) Schematic and Organizational: The circuit diagram was constructed using the KiCad software. Subsequently, the circuit was assembled with the appropriate electronic components. The PlatformIO platform, an extension of the Visual Studio Code source code editor, was used to upload the algorithm.

c) Testing and Validation: Functional tests were performed on the proposed circuit. Additionally, we observed whether the alarm signal was by NBR IEC 60601-1-8:2022.

Definitions

The Educational Kit corresponds to a benchtop prototype developed with materials applied to robotics. It is divided into three parts: the first contains the normative parameter, followed by the logical operating principle, the sequence of operation, and finally, the electronic circuit with the programming code. Firstly, the criteria applied to the system's operation correspond to specific topics of the NBR IEC 60601-1-8:2022 standard. The features were incorporated by analyzing the sound emission, the text on the screen, and the emitted color. The most important topics of the ABNT NBR IEC 60601-1-8 standard for the scope of the project were the 6.1.2, which determines the alarm conditions and priority assignments according to the patient's needs; the 6.3.2.2, which characterizes visual alarm signals for high, medium, and low priority; and the 6.3.3, which describes auditory alarm signals, their frequency, and activation time according to priority conditions. Additionally, the standard includes the pulse frequency (150 to 1000 Hz) in its annexes and melodies to avoid confusion between alarms (Annex F of the NBR IEC 60601-1-8:2022 standard). The standard also stipulates that in the event of a power supply failure, the system may generate an auditory alarm that does not comply with the requirements of Annex F. Table 1 detailed all applied topics.

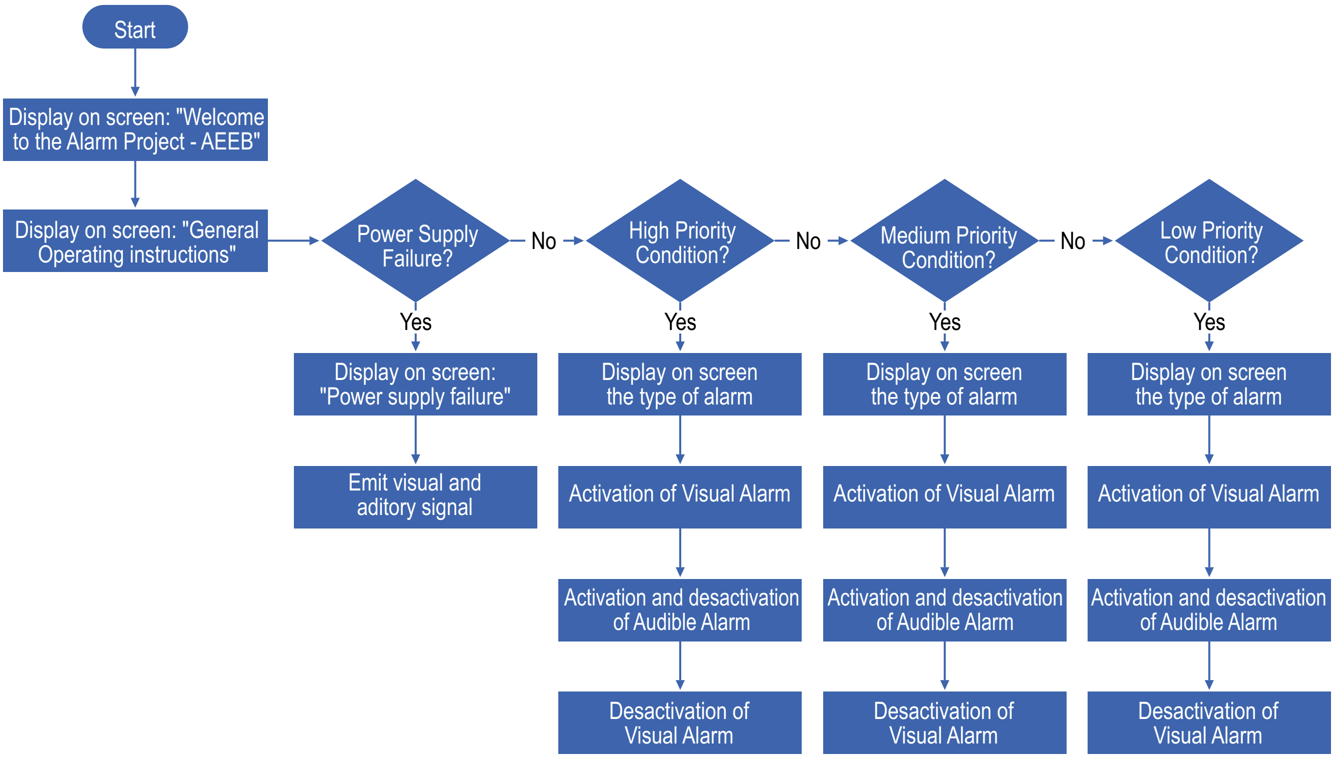
Based on these topics, we can establish the logical operating principle (Figure 1), and the programming structure.

Table 1. Topics of Standard 60601-1-8:2022.

Specifications	High priority	Medium priority	Low priority
Color	Red	Yellow	Cyan or Yellow
Frequency	1.4 Hz at 2.8 Hz	0.4 Hz at 0.8 Hz	Constant
Number of save pulses	10	3	1 or 2
Pulse interval (milliseconds)	Between 12 and 50	Between 50 and 100	Between 50 and 100
Interval between saves (milliseconds)	Between 2.5 and 15	Between 2.5 and 30	>15 or without repetition
Pulse difference	10 dB	10 dB	10 dB
Effective pulse duration (milliseconds)	Between 25 and 75	Between 90 and 200	Between 400 and 600
Melodic condition (Cardiac cause)	C E G, Pause, G C*	C E G	E C

*One octave higher.

Figure 1. Operating logic.



The programming structure is available on the drive and can be accessed through a link [3].

The alarm center should display text messages to the user on the screen based on the operator's action by pressing the buttons, enabling the demonstration. A sequence of decisions determines the priority conditions and the alarm emission involved, and the operation time of the devices is applied to these decisions according to the type of priority. For the LEDs, it is between a constant regime and a period of 1.25 seconds, and for the Buzzer, it is an interval between 12 milliseconds and 30 seconds. We have to consider the pulse time (pulse the emission of a frequency), the interval between pulses, and the interval between bursts (being the bursts a sequence of repetitions) for auditory emissions.

Figure 2 reveals the circuit's sequence of operation. The screen displays the information of the command coming from the operator through the button press, and, after that, the system emits the alarms.

Figure 3 presents the electronic circuit and Table 2 shows the devices of the educational alarm.

Results and Discussion

In the benchtop prototype, powered via USB, all four emission formats were achieved: Power-on failure, low priority condition, medium priority condition, and high priority condition (Figures 4 and 5). For each concept, the emission had two essential characteristics: the frequency and activation period. Despite the standard not specifying the frequency of the emitter set, in the case of Power-on failure, we introduced the frequency. The emitted melody sequence is one octave higher C, C minor, and C minor, with the LEDs having a constant frequency.

Moreover, an amplifier circuit (transistor, forward-biased diode, 100Ω resistor) was adopted in the project to increase the intensity of the sound emission from the buzzer (Figure 3) to improve the system' functioning. The most challenging factors in the tests' validation process were:

Figure 2. Sequence of operation.

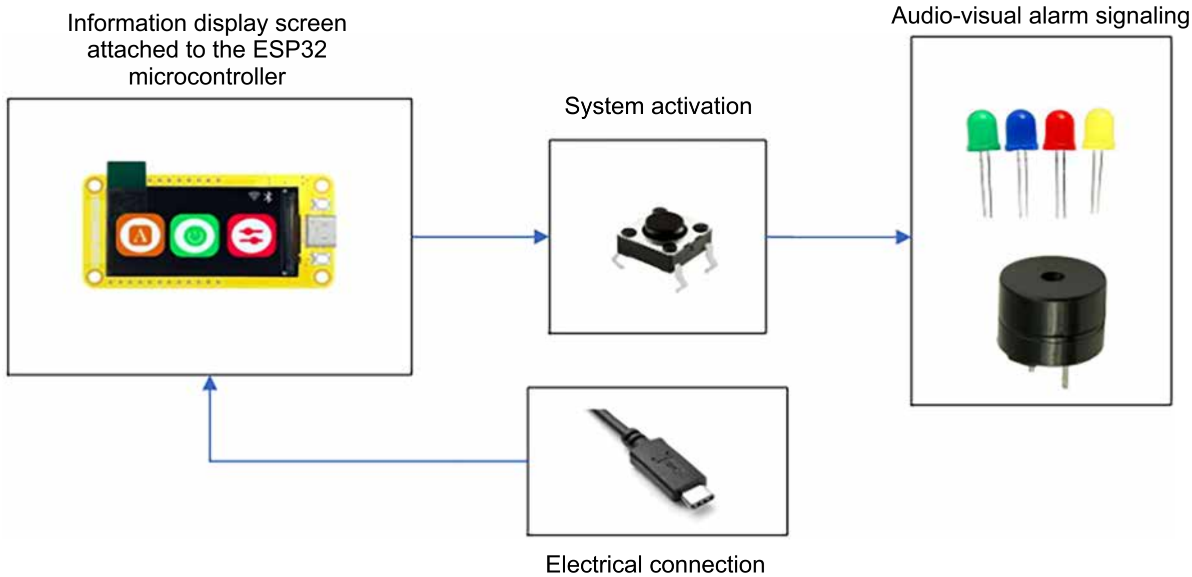


Table 2. Devices of the educational alarm center.

Device	Quantity	Functionality	Specificity
LED	2	Visual emission of light	Colors: red and yellow
Resistors	6	Apply electrical resistance	470Ω, 100Ω e 1000Ω
Button	3	Circuit actuator	Push button
Buzzer	1	Audio emission at specific frequencies	5-volt passive buzzer
Microcontroller	1	Visual information display	ESP32-S3-R8N16
Protoboard	1	Circuit construction and activation board	Model: ZY-204
Transistor	1	Amplifier	Model: BC546
Diode	1	Conduct electric current	Model: 1N4148
Jumpers	16	Perform a connection	Use of many colors

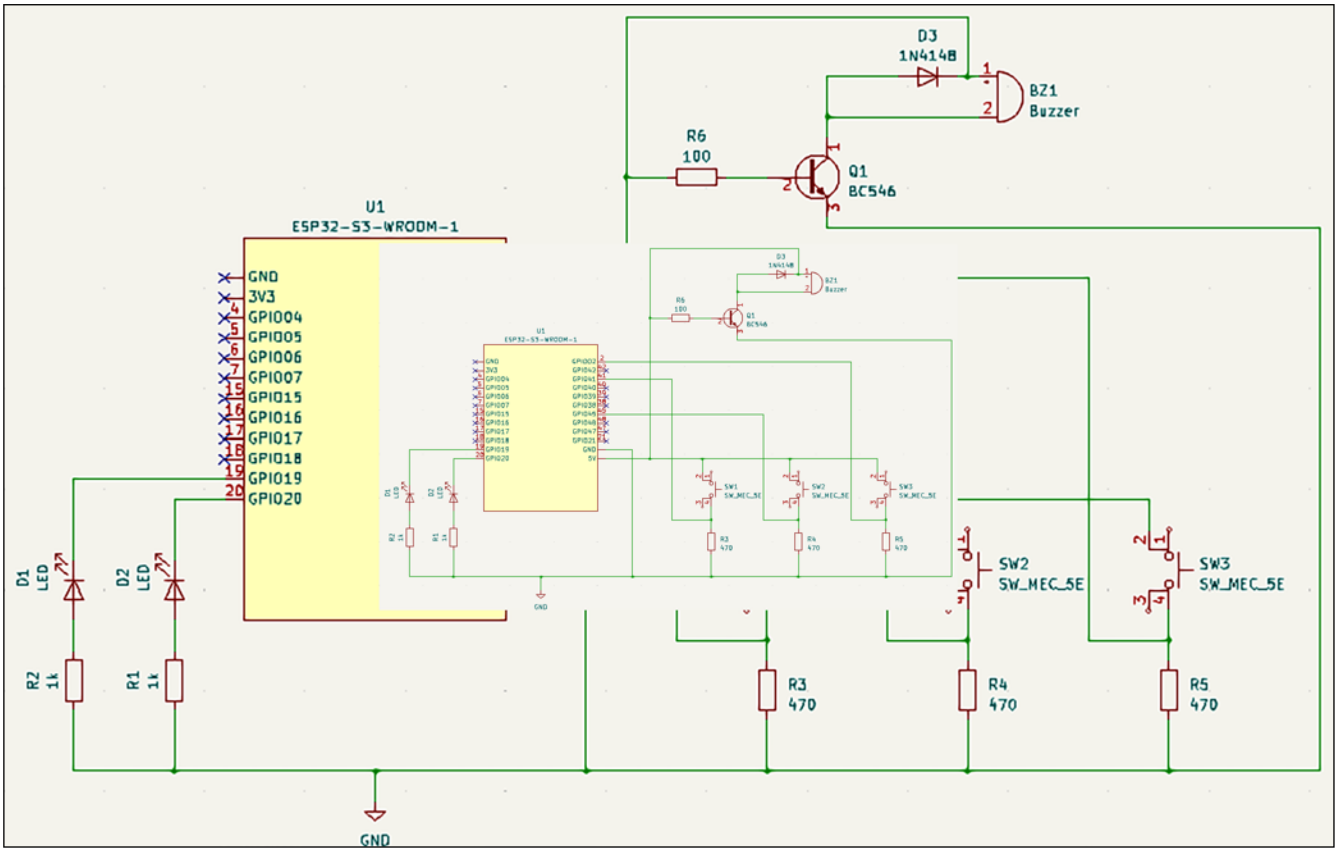
- The perception of the difference between the active and passive buzzers, i.e., the operation based on the frequency determined by the programmer, which the passive buzzer has, and the operation of a single frequency, not determined and already associated with the device, which the active buzzer has.

- The activation of the LEDs while there was the sound activation, meaning to start the LED and sound almost at equivalent moments.

Initially, the entire structure in this aspect was placed sequentially. However, the possibility of using time timers in the system became apparent after in-depth analysis. Thus, using the built-in timer of the microcontroller system, through two timers for medium and high-priority conditions, it was possible to create an impressive performance context.

In general, the proposed concept was achieved, the entire structure was tested and validated,

Figure 3. Electronic circuit of the alarm center.



and a comparison of emission with the device [4] associated with the equipment [5] in the Biomedical Equipment Engineering Area at SENAI CIMATEC was conducted. Both systems observed the following signal characteristics: comparison of activation time, synchrony, and sound and LED frequencies.

Conclusion

This article describes developing an electronic alarm emission project inspired by normative requirements. The main characteristic is that it enables immersion in the standard NBR IEC 60601-1-8:2022 and thus creates a context of practical learning for students in the medical field. For the following steps, the educational alarm system could have several applications, such as using an SD module to add images to the screen, measuring and comparing the emitted frequency with another device, manufacturing printed circuit boards, incorporating and parameterizing a medical signal, and building an integrated system with an alarm communicator.

Acknowledgments

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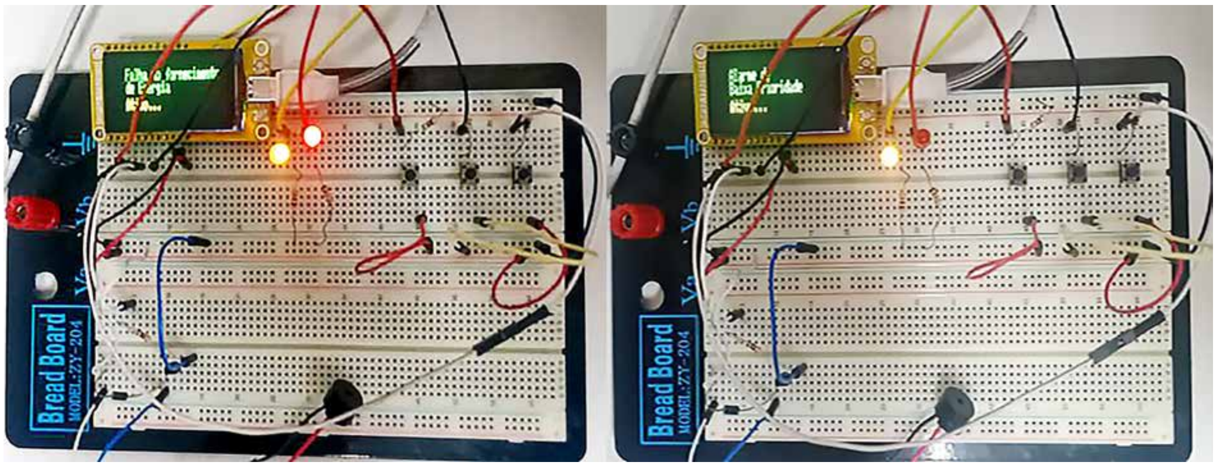
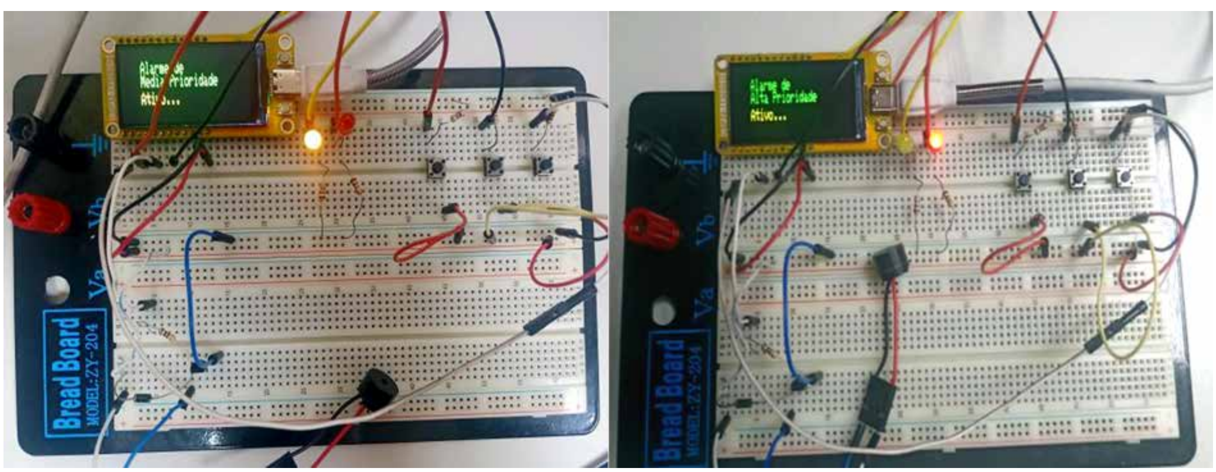
Figure 4. Power supply failure (a) and low priority condition (b).



(a) (b)

Figure 5. Medium priority condition (a) and high priority condition (b).



(a) (b)

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The Use of Artificial Intelligence (AI) is Customer Service Process in the Pharmaceutical Industry: A Case Study in Public Laboratories

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The Consumer Service (SAC) is an important communication and relationship channel with the customer. This study sought automated data collection and analysis techniques to trace profiles and strategies that are part of the SAC 4.0 model in official laboratories A and B. The method consisted of document analysis of standardized procedures and database research using the keywords SAC, artificial intelligence, and pharmaceutical industry. The results showed that laboratories A and B use the SAC 2.0 model and do not apply AI techniques. Evidence shows that pharmaceutical industries in Brazil use the SAC 4.0 model. It is concluded that the AI tools in the SAC contribute to improvements and efficiency in the process.
Keywords: SAC. Artificial Intelligence. Pharmaceutical Industry. Public Laboratory.

Introduction

Brazil has the Strategic Component of Pharmaceutical Assistance (CESAF) through its National Medicines Policy [1], which guarantees equitable access to medicines and supplies financed and acquired by the Ministry of Health (MS) in its strategic programs of the Unified Health System (SUS) [2].

Brazil has two large official laboratories located in Rio de Janeiro, which, for the purposes of this study, we called A and B. In addition to drug manufacturers, these laboratories are instruments for promoting scientific and technological development and innovation in health, acting through research activities and development of new medicines [3].

In the pharmaceutical industry, the leading players in the pharmacovigilance systems of the Consumer Service (SAC) are responsible for collecting relevant data on the safety of medicines available on the consumer market. The information provided by SAC helps to increase patient safety, prevent business problems, and reduce costs for new drug treatment options [4]. Customer service can be carried out through several integrated channels of regulated service providers in order to deal with consumer demands, such as information, doubts, complaints, disputes, suspension, or cancellation of contracts and services [5]. SAC emerged with Law No. 8.078 of September 11, 1990, of the Consumer Protection Code, but was only regulated with guidelines and standards on April 5, 2022, by Decree No. 11.034. Over time, the functionality of the SAC increased, and today, it works in the form of e-mail, online chat, and self-service [5,6]. In addition to being an important communication channel and improving customer relationships, the SAC helps companies to organize and optimize time for increase their results [6].

Laboratory B replaced the term "consumer" in the acronym SAC with "citizen," as the drugs supplied are from MS and are distributed free of charge to the population, and the term consumer is associated with a commercial relationship of products, which does not exist in the SUS. In laboratory A, the terms "consumer" and "customer" are used in the SAC, even without having a commercial relationship with the products. Despite the peculiarities, the rules applied in the public laboratory are the same for a private one.

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Official laboratories A and B use a SAC 2.0 model without applying advanced AI techniques and are completely manual; processes are rigid and prioritize the "dialogue with the customer". There is also SAC 3.0, which provides personalized service via omnichannel and works with integration between the various service channels. With the Industrial Revolution 4.0, where automation and data are used for decision-making, several technologies have been used to achieve the same result, as in the SAC 4.0 model [7]. Among these technologies, Artificial Intelligence (AI) [8] stands out, which came to optimize processes through algorithms and predictive tools such as Neural Networks through Machine Learning and Deep Learning.

This research becomes relevant as it aims to contribute to improvements in the SAC processes of the pharmaceutical industries, allowing the analysis of data and responses to citizens to be faster and more effective. In this work, automated data collection and analysis techniques were addressed, and the information results outline profiles and strategies that are part of the SAC 4.0 model in official laboratories A and B.

Materials and Methods

This work is based on a case study in two public pharmaceutical laboratories. One of them, called A, was founded in 1976 and currently has 41.722 m² of built area in total. However, a new campus is being built in Rio de Janeiro, on an area of 580.000 m², and will have the capacity for 120 million vials of vaccines and biopharmaceuticals per year. Laboratory B, also founded in 1976, occupies a strategic position as the most recognized official pharmaceutical laboratory linked to the MS, with an installed production capacity of more than 2,5 billion medication units per year. It is an observational, exploratory, and descriptive documentary study, where the applied methodology was divided into document analysis of the Standard Operating Procedures (SOP) used in the SAC of laboratories A and B, and qualitative research in the databases of the CAPES journal portal, Scopus, WOS and cases found on Google's open platform, using the keywords: SAC, artificial intelligence and pharmaceutical industry.

Analysis of SOP from laboratories A and B, available in PDF format and accessed through the query drive, to which only authorized employees have access, was performed. The SAC procedure consists of registering the contact received in the software of each laboratory for planning and execution of actions until final analysis and response to the citizen (Figure 1).

Finally, qualitative research was carried out in the journal portal databases of CAPES, Scopus, WOS, and Google through the keywords SAC, artificial intelligence, and pharmaceutical industry to search for AI tools and cases of pharmaceutical industries to apply AI in their companies' SAC.

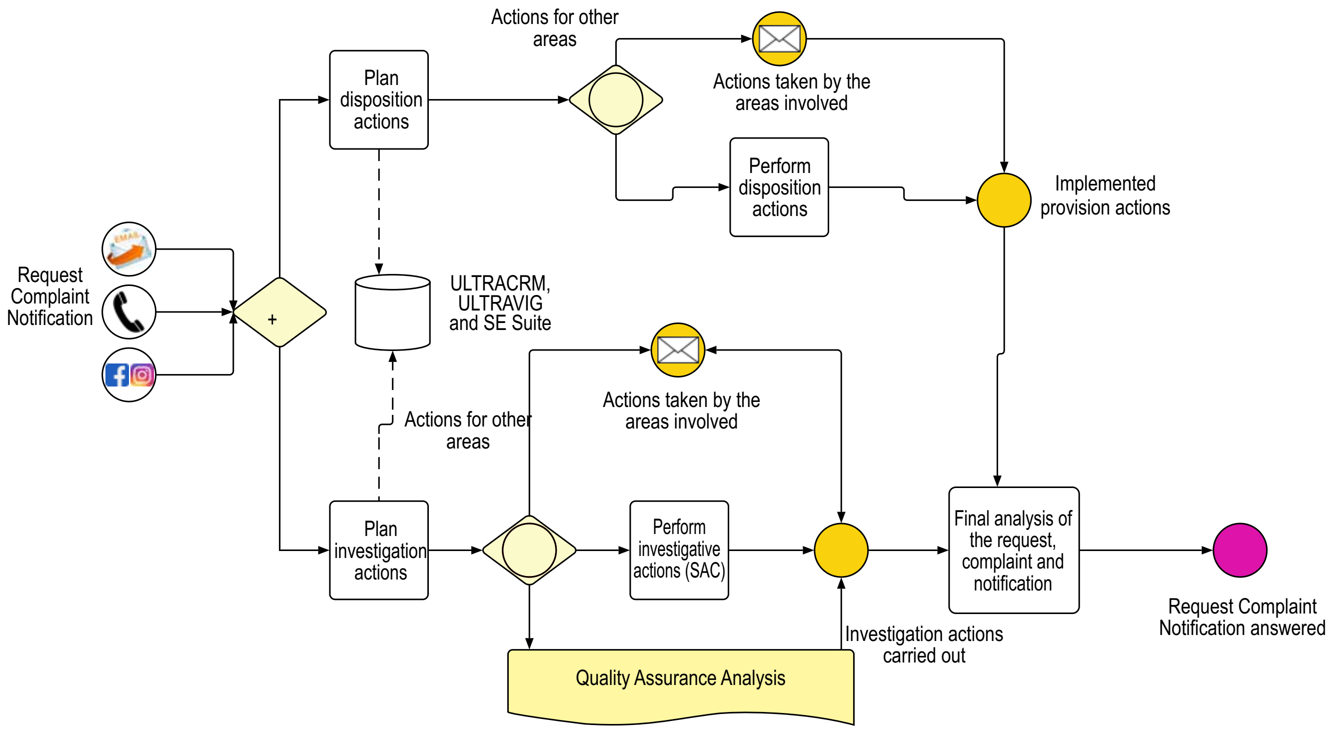
Results and Discussion

The SAC of laboratories A and B aims to answer all citizens' contacts in a coherent and standardized way in its internal procedures in order to clarify doubts about all products and activities of the laboratories. Contact with the SAC of laboratories A and B can be made by e-mail or phone from Monday to Friday, from 8 am to 5 pm, in addition to the Facebook or Instagram channels for laboratory A. The SAC service of these laboratories is carried out by a pharmacist, who must ensure a database with as much information as possible at the time of his service so that all areas involved in the industry can plan their actions, making the service possible for citizen demands. In this way, all doubts/complaints or suspected adverse events received by the SAC of laboratory A are registered in the ULTRACRM and ULTRAVIG Systems, respectively, and those of laboratory B are registered in the SoftExpert Excellence Suite (SE Suite) software, to be later carefully investigated.

Service and Occurrence Registration in the Software

The SAC attendant must identify the reason for the contact as: "Information," "Request,"

Figure 1. Mapped SAC process of official laboratories A and B.



"Compliment," "Thank you," "Suggestion," "Complaint," "Suspected Adverse Event," or "Other." In case of doubt/complaint, the attendant must classify it according to the software options of each laboratory, such as failure in the packaging, different appearance/coloring/flavor of the product, lack of package leaflet, adverse events, and so on, always collecting the highest number of possible data on the object of doubt/complaint, such as product name, concentration, suspected deviation, storage conditions, batch number, manufacturing date, expiration date, notifier's full name, complete address, telephone number DDD, among other information.

Action Planning and Research

The SAC pharmacist must define and plan the actions in the software, such as communicating the areas involved, informing what happened and requesting measures, contacting the citizen to request additional information, or identifying possible adverse events and forwarding them to the Pharmacovigilance area that will the investigation in this case. The SAC professional must define which investigation action(s) should be adopted and manage the deadlines for these actions, which are defined in an internal procedure. Upon completion of the investigation, the SAC professional must approve the conclusion of the actions and perform a causal analysis of the doubt/complaint in the software.

Satisfaction Survey and Reporting

At the end of the service, the SAC professional asks the citizen to respond to the Satisfaction Survey, where the data will be used to prepare technical reports. From the detailing of the SAC processes of laboratories A and B, we observed that these laboratories only use manual techniques such as those of SAC 2.0 in assisting citizens with their products.

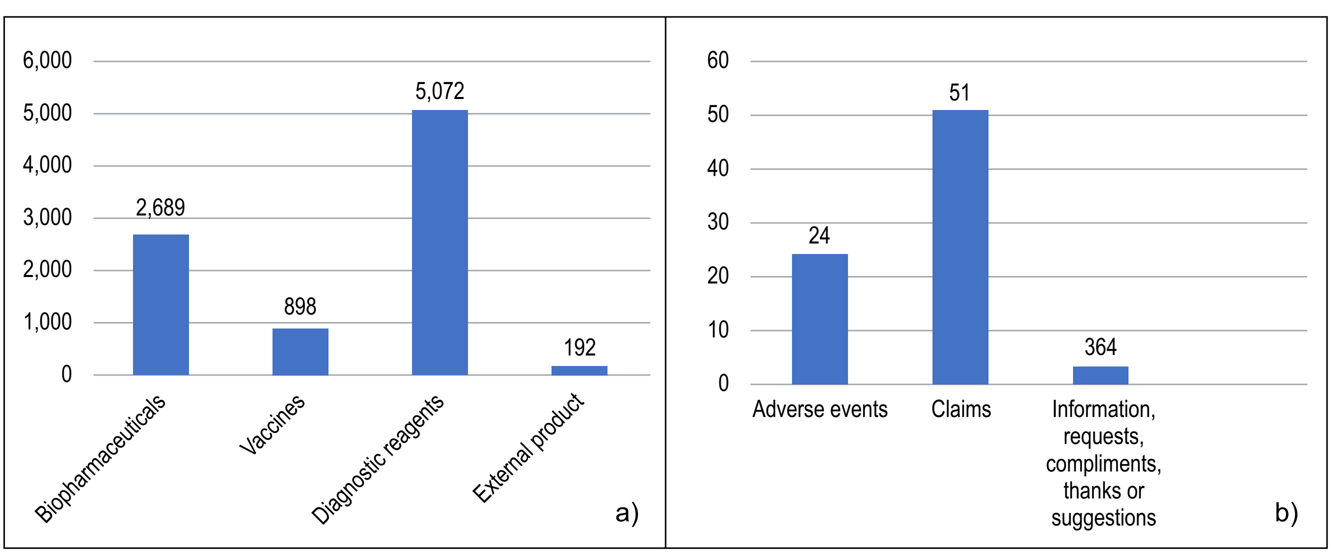
Number of Calls from the SAC

In 2020, laboratory A's SAC carried out 8.851 calls, 2.036 of which were through customer calls and e-mails, where records were distributed among the laboratory's three product lines. The high number of consultations for diagnostic reactions is justified since about 50% of the calls were related to the SARS-CoV-2 test. In the same year, the SAC of laboratory B carried out 439 calls in total, through calls and e-mails from citizens, where the records were distributed according to the classification of the service (Figure 2).

Use of Artificial Intelligence in the SAC of the Pharmaceutical Industries

Online virtual agents increasingly replace human agents in companies' SAC [9,10]. A chatbot is a computer program that uses AI and natural language processing (NLP) to understand customer questions and automate responses, simulating a human conversation. These technologies rely on machine and deep learning to develop an increasingly granular knowledge base of questions and answers based on user interactions [11]. A challenge for companies' SAC is always meeting unpredictable demands and training the team to provide consistent responses. Today, chatbots can manage customer interactions at any time, continuously improving the quality of responses and keeping costs low [12,13]. A chatbot eliminates long wait times for phone, e-mail, chat, and web-based customer support because they are simultaneously immediately available to any number of users [9]. In 2021, the Union of Pharmaceutical Industries of São Paulo (SINDUSFARMA) created a Working Group SAC to discuss using AI and digital marketing tools [15]. In 2022, in Austria, the 26th Congress of the European Association of Hospital Pharmacists took place, with representatives from Brazil, where the main trends for the pharmaceutical market were discussed, with emphasis on chatbots that should revolutionize areas such as the SAC of the pharmaceutical industries [14]. União Química, a national pharmaceutical company, began studies on using bots in its customer service channels. The company has already introduced a QR code [16,17] on its medicine packaging, where it is possible to access an interactive menu with different types of media to read, listen to, access the leaflet, call the SAC, and obtain information on packaging disposal. Another AI tool that is being applied is the voice command instructions within the virtual assistant Alexa [18]. The national pharmaceutical company Libbs joined Omnichannel after integrating all its current service platforms: 0800, e-mail, social networks, and AI-based service via Whatsapp [14,19]. Due to various regulatory requirements, pharmaceutical companies have slowly adopted

Figure 2. Number of calls from the SAC of laboratories A and B in 2020.



digital assistants. Although chatbots have been around for more than two decades, pharmaceutical company Pfizer leads the way with the recent launch of three chatbots worldwide: Medibot in the US, Maibo in Japan, and Fabi in Brazil. MediBot was the first chatbot deployed by Pfizer to help answer specific questions related to the stability of temperature-sensitive biologics and vaccines. Maibo was recently launched in Japan to serve healthcare professionals seeking medical information [20]. Fabi is the first digital assistant in the pharmaceutical industry, answering questions related to the entire portfolio of Pfizer Brasil products. It is a more advanced chatbot with AI abilities, such as natural language processing capabilities, that allow users to enter text and be guided by prompts (commands) to receive a response [20].

Conclusion

AI can empower humans and aid decision-making as it manages data much faster but is not intended to replace humans completely. Information on the use of drugs, vaccines, and biopharmaceuticals will serve in healthcare decision-making; in this way, AI cannot make mistakes and must ensure that systems meet ethical, legal, and regulatory standards. The experience of using AI in the pharmaceutical industry has been positive and a market trend, especially with the case of the chatbot implemented in Pfizer, Brazil. Official laboratories A and B, with their great demands for production and assistance to the population, should invest in AI tools in the SAC in order to seek constant improvement in their processes and meet the needs of the public, as well as other companies in the field pharmacist, especially in emerging countries, where there is a great need for information about medicines.

Acknowledgments

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Object Identification for Visually Impaired

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In computer vision, several algorithms carry out the process of object recognition through machine learning. Among them, You Only Look Once (YOLO) stands out, a Darknet algorithm that had its most significant relevance in the YoloV4 version for presenting an outstanding performance on both personal computers and mobile devices. This article presents a feasibility study of this tool for the possibility of helping visually impaired people detect objects through their mobile devices and thus help in everyday tasks.
Keywords: Visual Impairment. Android Studio. Convolutional Neural Network. YOLOv4.

Introduction

Computer engineering is an area that covers many aspects related to information technology, including hardware, software, and communication systems. Automation engineering deals with the automation of industrial processes involving the control of automated equipment and systems. Both areas aim to create and improve systems that simplify and streamline complex tasks.

A relevant topic with significant social impact is the development of applications aimed at helping people with visual impairments. According to Souza and Oliveira (2018) [1], the lack of accessibility to applications can limit the independence and participation of these people in society since many services and information are available exclusively through mobile devices. Among these applications, the ones that stand out the most are those that use machine learning and image segmentation techniques to recognize objects and people in the environment.

According to Pires and Lima (2017) [2], object recognition technology has been increasingly used in creating applications that help visually impaired people identify objects around them. There are several computer vision training models, and one presenting high performance in computational cost is the YOLO algorithm (you only look once). Through neighborhood recognition, this algorithm analyzes a group of pixels on the screen and verifies the possible correlated outputs through a Convolutional Neural Network (CNN), thus detecting an object class.

This work aims to develop an application for mobile devices that uses image processing and machine learning techniques for object recognition and description for people with visual impairments. In addition, object recognition technology has been widely used in different areas, such as robotics, security, and electronic commerce [3]. Tools and technologies in mobile application development, such as Android Studio and frameworks like TensorFlow Lite and Yolov4, will provide a practical and accessible solution for this specific audience.

Materials and Methods

The training used Python to create the convolutional neural network through Colab and the YOLOv4 learning algorithm. At the end, a report containing the accuracy metrics obtained during training was generated. To confirm these metrics and ensure the operation and usability of the application in real scenarios, images of practical scenarios were used in different conditions to analyze the results more comprehensively.

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Following a resolution configuration of 416 x 416 pixels in the JPG format with the most varied characteristics of luminosity, position, and distance, it is possible to create a dataset of images that represents different situations of the objects (Figure 1). The labeling process is carried out from these images, in which each image is carefully labeled using the Labellmg program. After obtaining and preparing the dataset, the next step involved training and testing the Convolutional Neural Network (CNN). The implementation of the CCR was carried out using the Python programming language and the YoloV4 algorithm in conjunction with the Darknet framework. Furthermore, Colab provides a GPU integrated into the virtual environment, which is needed to increase the training speed due to the CUDA feature being available only on NVIDIA graphics cards. A set of 1300 images was used, 1040 for training and 260 for testing the model. These images covered different situations, lighting characteristics, positions, and distances, comprehensively representing the study objects: person, car, and fan. During the training of the RNC, adjustments were made to the parameters to achieve the best possible performance. The training step consisted of feeding the labeled data set. A cross-validation strategy was adopted to assess the model's generalizability, dividing the data set into training and validation subsets. In total, 6,000 epochs were performed, representing cycles in which all images were submitted to training, following the guidelines of AlexeyAB (2021) [4]. The batch size was set to 64 with 16 subdivisions for training efficiency.

Results and Discussion

After completing the training, the Darknet framework provides a report to analyze the precision and accuracy of the model in detecting the objects of interest (Figure 2). Performance metrics, such as average precision (mAP), precision in each class (recall), F1-score, and average IoU (Intersection Index over Average Union), were used to measure how well the Convolutional Neural Network (CNN) was able to correctly identify and locate the study elements, such as person, car, and fan.

However, if more images are introduced into the dataset, there is room for improvement to increase the model's ability to recognize and locate objects of interest more precisely. The analysis of the results, according to the image provided by the framework, revealed that the average precision for the person class is 58.45%, for the car is 73.60%, and for the fan is 100% (Figure 2). The high precision obtained in the fan

Figure 1. Class labeling.

class is due to the number of images available in the database and its geometric nature, which generally presents recurrent characteristics, such as propeller blades. The person class, on the other hand, obtained a lower average precision due to the significant variability of characteristics and appearances present in the data set. After epoch 1400, values are more constant, resulting in insignificant changes in machine learning. However, it is still possible to see a relationship between the number of epochs used and the percentage of success, becoming clear when reaching epoch 4800, reaching 78% accuracy (Figure 3).

Using the GitHub of user hunglc007 (2020) [5], where it is possible to find a specific Android project for neural network models trained by YoloV4, the training performance was verified practically, in which there would be some adversities, such as different levels lighting and distance to objects. Figure 4 presents the results of the practical tests in which it is possible to notice that even with low luminosity, a high precision value was obtained.

The tests revealed a very high recognition capacity in the three classes used, even when the luminosity changes (Figure 4). They demonstrated that the trained model is not biased; it is not limited to identifying only the images with which it was trained. The precision and quality of the model are directly related to the amount of information available, also highlighting that the person class was the one that presented the lowest detection reliability due to its several distinct characteristics. The application showed satisfactory results in detecting and obtaining reasonable reliability in the average detection of the proposed objects, with values above 50%. It is essential to have a comprehensive and diversified dataset to train the model to obtain more consistent and reliable results. These results show the effectiveness of the developed application in object recognition and identification. They confirm the application's potential to improve the independence and quality of life of people with visual impairments, providing an accessible and reliable tool for identifying objects in their surroundings.

Conclusion

Using YOLOv4 with the Darknet framework, it was possible to develop an RNC to detect the objects proposed in this article. It was found that the quality and reliability of the tested images showed more significant variability in classes where the characteristics were adverse. Finally, the results were satisfactory, detecting the required classes and informing the user, through a sound message, informing the class of the objects in front of him. However, it is possible to increase

Figure 2. Performance data.

```
 calculation mAP (mean average precision)...
 Detection layer: 30 - type = 28
 Detection layer: 37 - type = 28
60
 detections_count = 609, unique_truth_count = 223
class_id = 0, name = pessoa, ap = 58.45%        (TP = 54, FP = 34)
class_id = 1, name = carro, ap = 73.60%         (TP = 84, FP = 28)
class_id = 2, name = ventilador, ap = 100.00%           (TP = 16, FP = 0)

 for conf_thresh = 0.25, precision = 0.71, recall = 0.69, F1-score = 0.70
 for conf_thresh = 0.25, TP = 154, FP = 62, FN = 69, average IoU = 52.94 %

 IoU threshold = 50 %, used Area-Under-Curve for each unique Recall
 mean average precision (mAP@0.50) = 0.773498, or 77.35 %
Total Detection Time: 1 Seconds
```

Figure 3. Season training.

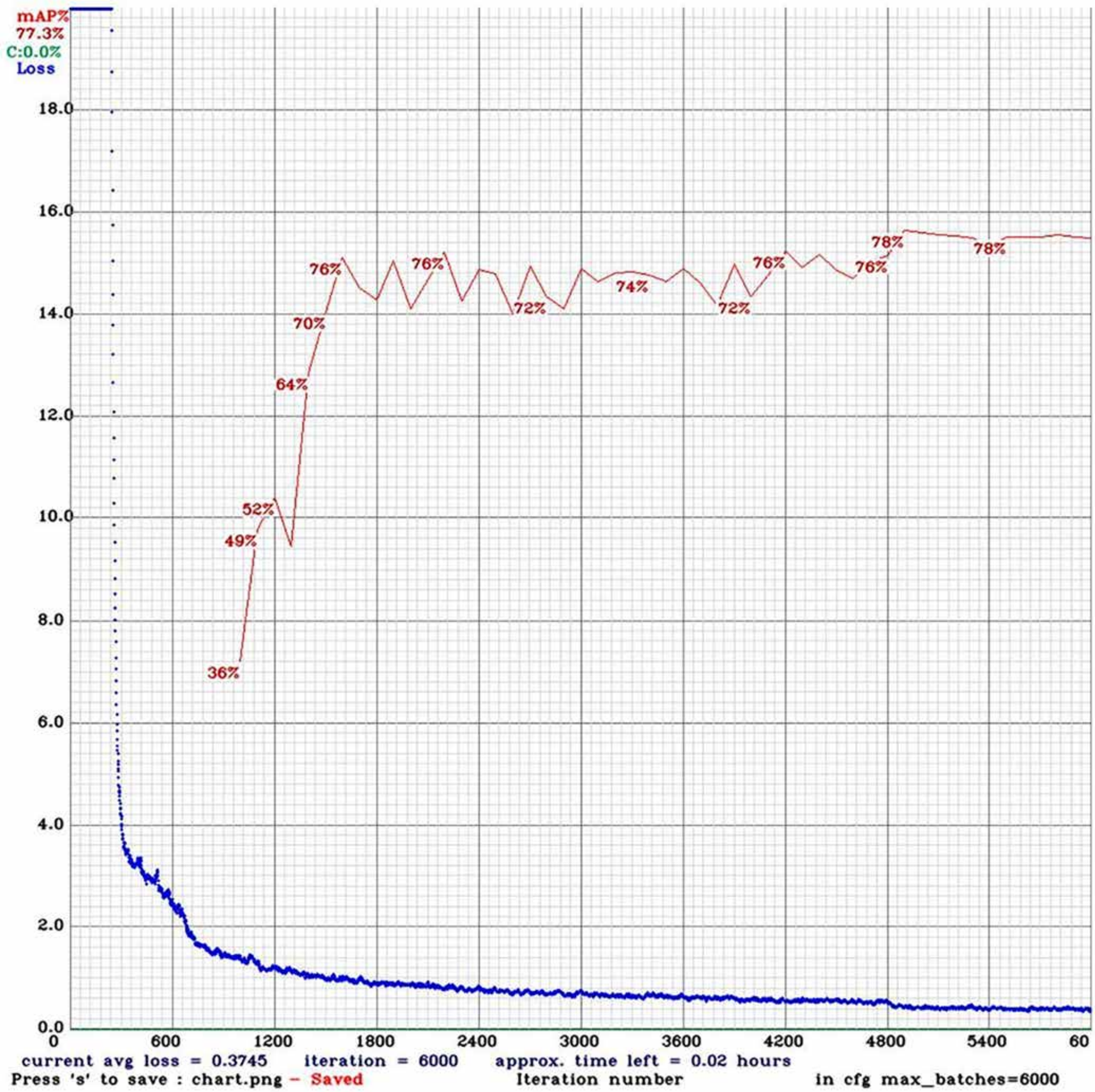
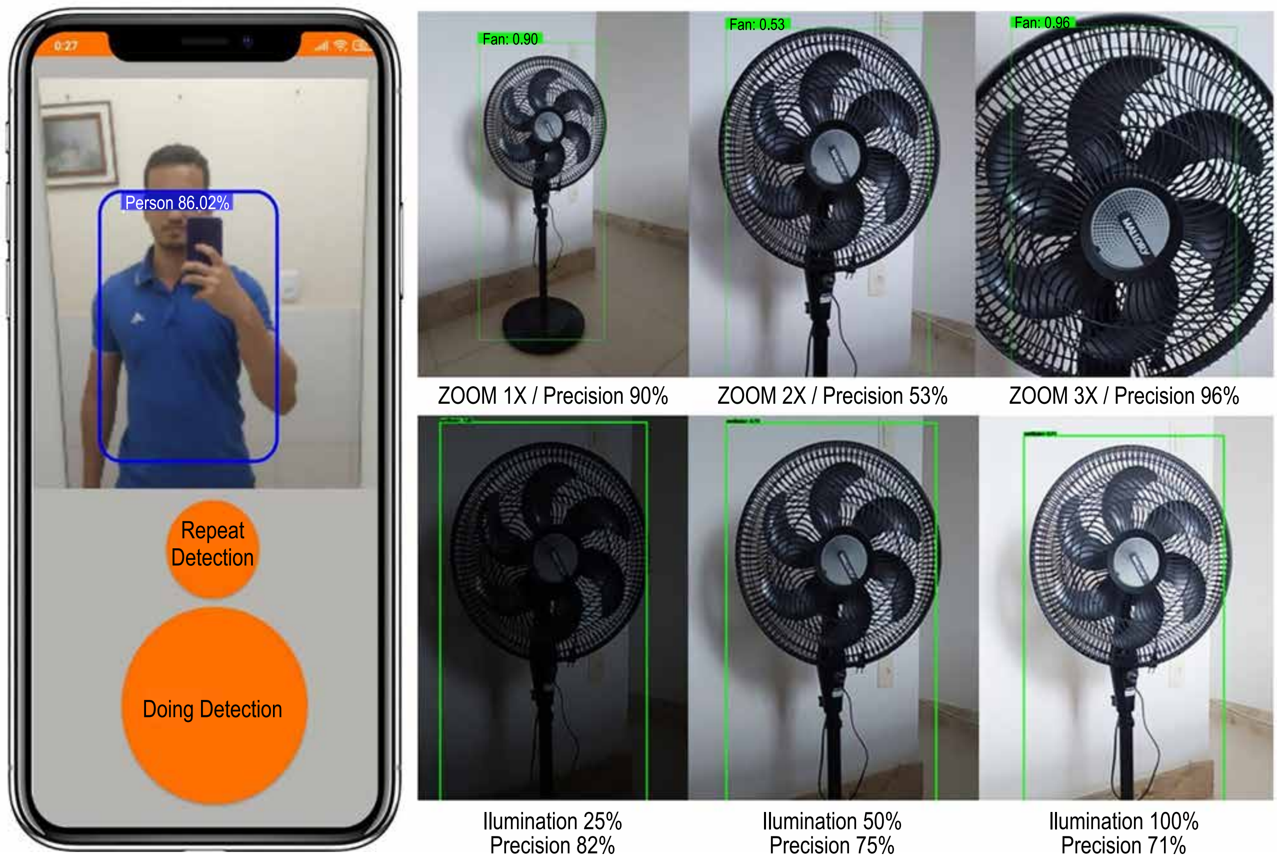


Figure 4. Reliability test.



more classes in the proposed RCC model from a new training, as long as enough images are available to create an image bank for the new desired class and thus perform reliable training.

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Automatised Bioreactor to Produce Biogas from Biomass: Development and Evaluation

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Brazil's abundant biomass from agricultural and industrial activities offers significant potential for biogas production, aligning with the ONU Sustainable Development Goals. This study aims to explore anaerobic digestion for biogas production from biomass, investigating key factors influencing the process and advancing efficient technologies. A literature review utilizing biogas as a sustainable energy source was conducted, and an Arduino-controlled bioreactor was built to automatically monitor and control crucial variables in anaerobic digestion, ensuring high-quality and efficient biogas production. The developed bioreactor efficiently enabled continuous tracking of pressure, temperature, hydrogen, and methane in the bioreactor, contributing to energy transition and decarbonization.
Keywords: Biogas. Bioreactor. Efficiency. Automation. Sustainability.

Introduction

World energy policies are shifting towards renewable energy sources, and Brazil's energy matrix stands out, with 44.5% sourced from renewables, as reported by EPE [1]. With its rich cultural heritage and agricultural trade, Brazil has abundant biomass resources for energy production. Anaerobic digestion offers an efficient means to convert biomass into biogas. In 2021, the country had 755 operational biogas plants, producing 2.3 billion cubic meters of biogas [2]. A study on biogas as an alternative energy source [3] revealed that 1 mL^3 of biogas equals 0.613 liters of gasoline, 0.579 liters of kerosene, or 0.553 liters of diesel. Brazil's biogas production could replace 1.4 billion liters of gasoline, 1.3 billion liters of kerosene, or 1.27 billion liters of diesel. Research and development of sustainable and environmentally friendly energy sources has become a global priority amid challenges related to climate change and natural resource scarcity. In this context, biogas production through anaerobic digestion from biomass has emerged as a promising and renewable alternative to meet the energy demand. Anaerobic digestion is a biological process that converts organic materials, such as biomass, into biogas, a renewable energy source composed mainly of methane (CH_4) and carbon dioxide (CO_2) [4-6]. This environmentally friendly technology is crucial in waste management and renewable energy production. The anaerobic digestion process involves four key stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis. In hydrolysis, hydrolytic enzymes break down complex organic matter into simpler compounds, such as lipases and proteases. This stage prepares the biomass for further degradation. During the acidogenesis stage, acid-forming bacteria convert the simpler compounds into volatile fatty acids (VFAs), alcohols, and other intermediate products. In the subsequent acetogenesis stage, acetogenic bacteria metabolize these intermediate products, producing acetic acid, hydrogen (H_2), and carbon dioxide (CO_2). Finally, methanogenic archaea carry out methanogenesis, where methane-producing microorganisms utilize the acetate, H_2, and CO_2 produced in the previous stages to generate methane [5-14]. The overall reactions can be represented as follows:

a. Hydrolysis: Complex organic matter → Simple compounds
b. Acidogenesis: Simple compounds → VFAs, alcohols, and other intermediates
c. Acetogenesis: VFAs, alcohols, and intermediates → Acetic acid, H_2, and CO_2

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d. Methanogenesis: Acetic acid, H_2, and CO_2 → Methane (CH_4) and CO_2 Different types of biomass can be used as feedstock for anaerobic digestion.

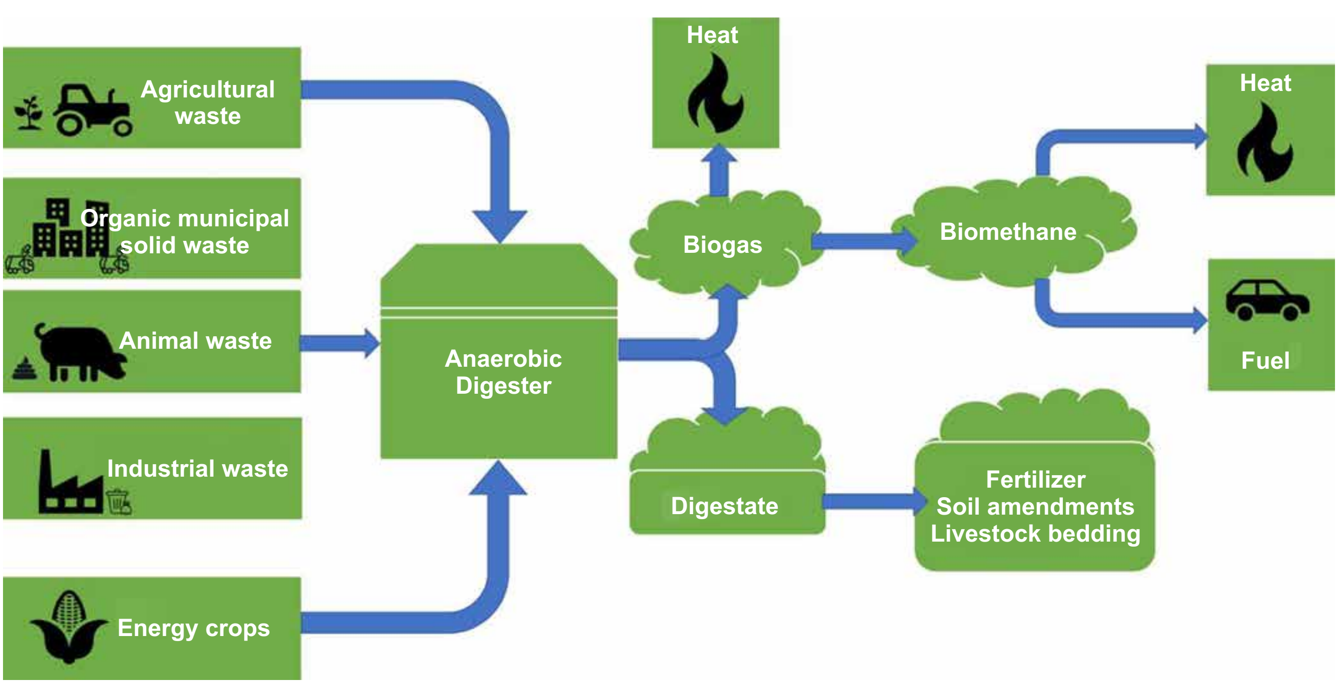
Each type of biomass has its unique composition, which influences the efficiency and performance of the anaerobic digestion process [14]. The most common biomass used are:

1. Agricultural waste: Crop residues, manure, and other agricultural byproducts are commonly used as biomass for biogas production. These materials are readily available and can help recycle waste [10,12].
2. Organic municipal solid waste (MSW): Food waste and green waste from households, restaurants, and food industries are potential biomass sources for anaerobic digestion [6,13].
3. Animal waste: Livestock manure, such as from cattle, pigs, and poultry, is a valuable source of biomass for biogas production. Anaerobic digestion of animal waste generates biogas and helps manage and reduce greenhouse gas emissions.
4. Industrial waste: Organic waste generated by industries, such as breweries, food processing plants, and distilleries, can be utilized as biomass for anaerobic digestion [10].
5. Energy crops: Dedicated energy crops like corn, sugarcane, and switchgrass can also be grown specifically for biogas production, providing a sustainable source of biomass [11].

Proper management and optimization of the anaerobic digestion system, tailored to the specific biomass characteristics, are essential to achieve optimal biogas production. Overall, anaerobic digestion is a versatile and environmentally beneficial technology that can convert various biomasses into biogas, contributing to renewable energy generation and waste management and reducing greenhouse gas emissions (Figure 1).

Brazil is known for its rich biodiversity and abundant biomass from agricultural and industrial activities. The country holds significant potential for biogas production. The efficient utilization of biomass through anaerobic digestion can contribute significantly to the country's sustainable development, aligning with the ONU Sustainable Development Goals. In this context, the present work aims to study and evaluate the anaerobic

Figure 1. Schematic of anaerobic digestion applications.



digestion reaction for biogas production from biomass, seeking to comprehend the key factors influencing the process and develop more efficient technologies for biogas production. Through a comprehensive literature review and analysis of experimental data, valuable insights are expected to be provided for advancing the utilization of biogas as a clean and sustainable energy source.

This work has built an Arduino-controlled bioreactor for automatic monitoring and control of essential variables in the anaerobic digestion of biomass for biogas production to produce high-quality and efficient biogas.

Materials and Methods

Anaerobic digestion is a complex biological process involving a consortium of microorganisms, including archaea methanogens, responsible for producing methane gas. Several key factors, including temperature, pH, and the composition of methanogenic archaea communities, influence the efficiency of methane production in anaerobic digestion. This literature review aimed to provide insights into how these parameters impact methane production in anaerobic digestion processes.

Temperature

Temperature is a critical factor affecting the rate and efficiency of anaerobic digestion. Different groups of methanogenic archaea have distinct temperature optima. According to the literature, anaerobic digestion at around 25-35°C promotes higher methane production due to lower energy requirements and better process stability [15,16].

pH

The pH level of the anaerobic digestion reactor is another critical parameter influencing methane production. Methanogenic archaea are sensitive to pH changes, and their optimal growth typically occurs in the range of 6.5 to 7.5. Acidic conditions ($pH < 6$) can inhibit methanogenic activity, reducing methane production and process instability. On the other hand, overly alkaline conditions ($pH > 8$) can affect the structure and activity of the microbial community, disrupting the overall digestion process [17,18].

Methanogenic Archaea

The composition and diversity of methanogenic archaea communities significantly impact methane production. Various species of methanogens have different substrate preferences and tolerances to environmental conditions. Understanding the dynamics of methanogenic archaea can help optimize anaerobic digestion processes to enhance methane production. Advanced molecular techniques, such as high-throughput sequencing and quantitative polymerase chain reaction (qPCR), have enabled researchers to identify and quantify methanogenic archaea and their abundance in different anaerobic digesters [19,20].

The construction of the bioreactor was developed following the following steps:

Materials Selection

1 Arduino uno R3, 1 digital pressure sensor module 0- 40kpa, 1 hydrogen gas sensor mq-8, 1 methane gas sensor MQ-4, 1 temperature sensor ds18b20 waterproof, 1 relay module 4 channels 5v with optocoupler, 1 2l glass bottle with hermetic lid, 2 tire valves, 2 20mm pvc tee, 20mm PVC tube and 2 gas pressure valves.

Arduino System Selection

Arduino is an open-source single-board electronics prototyping platform designed with a microcontroller and uses a user-friendly standard programming language. This system was chosen for this work because it is an efficient and low-cost process. With the built control platform, it was possible to monitor the reaction temperature in real-time through the DS18B20 Waterproof

Temperature Sensor, the pressure through the Digital Pressure Sensor Module (0-40 kPa), and quantify the production of methane (CH_4) with the Methane Gas Sensor MQ-4 and hydrogen (H_2) with the Hydrogen Gas Sensor MQ-8.

<u>Excel program for Monitoring</u>

The chosen Temperature Sensor (DS18B20) is digital and performs measurements in the range of -55 ° to 125 °C, in a dry, humid, or submerged environment. The digital pressure sensor (0-40 KPa) allows measuring the pressure of dry and non-corrosive gases between 0 kPa and 40 kPa. The MQ-8 sensor detects hydrogen gas concentrations in the air in the 100-10,000 ppm range, and the MQ-4 sensor detects methane in the 300-10,000 ppm concentration range. The set also has a relay module that activates an electrical resistance if the temperature of the bioreactor reaches values below the established value (T=35 °C) or activates a cold-water pump to decrease the temperature of the bioreactor when the sensor identifies temperatures above 35 °C. An Excel program was developed (Figure 2) that allows data collection every second, storing the data in a spreadsheet for later analysis. Additionally, an interface was created in Excel that allows a real-time visualization of the bioreactor's operational conditions.

Results and Discussion

According to the literature review studied in this work, anaerobic digestion for biogas production is a promising and sustainable process with great potential to contribute to renewable energy generation and reduce the environmental impact of organic waste. Understanding the main reaction parameters, such as organic load, C/N ratio, temperature, pH, and hydraulic retention time (HRT), is essential to optimize the process and maximize biogas production. Furthermore, using various biomass provides flexibility and economic viability, making this process attractive for transitioning to a more sustainable and clean energy matrix [15-20]. Table 1 shows the most critical parameters to optimize the anaerobic digestion reactions for biogas production.

Figure 2. Excel program developed.

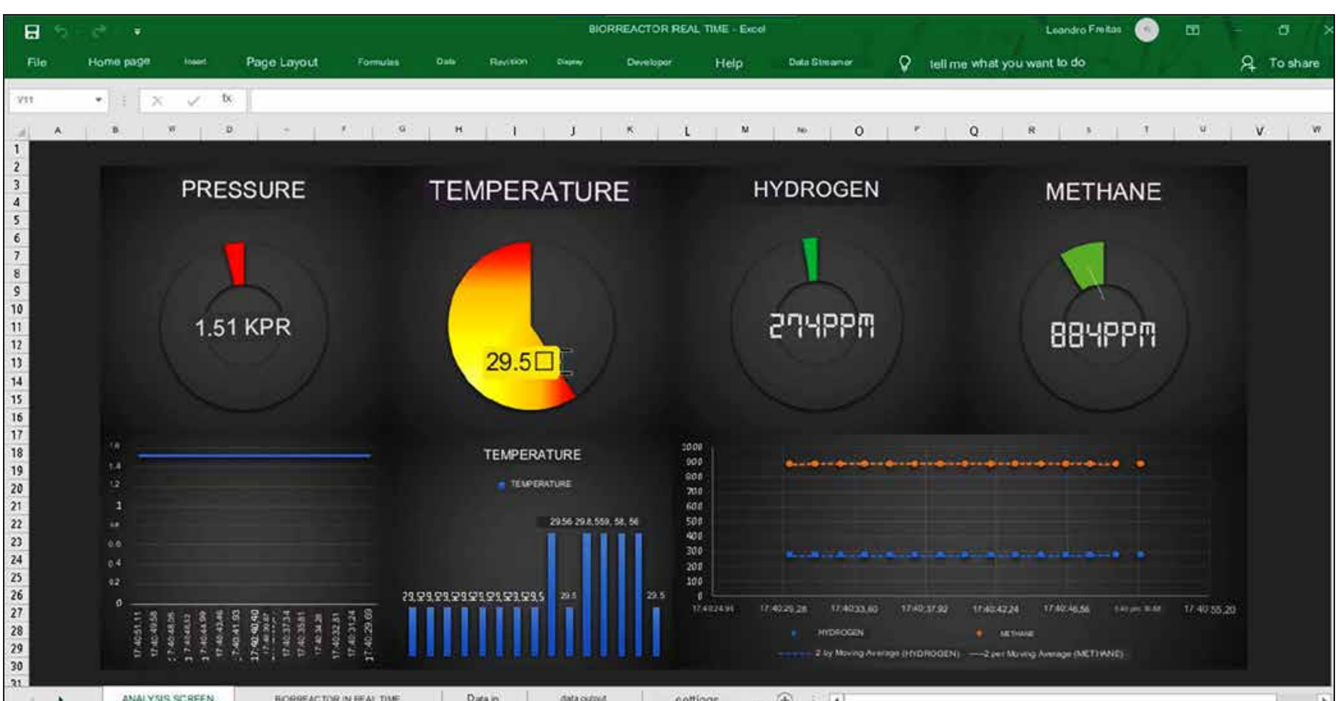


Table 1. Main reaction parameters of anaerobic digestion [15-20]

Organic Load (Feeding Rate)	The organic load is a critical parameter that determines the amount of biomass added to the anaerobic reactor within a specific period. An appropriate balance between the organic load and the microorganisms' capacity to degrade the biomass is essential to avoid process inhibition.
Carbon/Nitrogen Ratio (C/N)	The ratio of carbon to nitrogen in biomass is significant for microbial activity. An ideal C/N ratio typically ranges from 20:1 to 30:1. When the ratio is too high, toxic ammonia accumulation may occur in the reactor, affecting the efficiency of anaerobic digestion.
Temperature	Anaerobic digestion is temperature-sensitive and can be classified into three ranges: mesophilic (30-40°C), thermophilic (50-60°C), and hyper mesophilic (above 60°C). Temperature directly affects the activity of microorganisms and, consequently, the biogas production rate. Temperature control is crucial for maximizing process performance.
pH (Hydrogen Ion Potential)	pH is crucial for the functioning of microorganisms involved in anaerobic digestion. The optimal pH range is generally between 6.5 and 7.5, varying depending on the type of biomass and the dominant group of microorganisms in the reactor. hydraulic
Retention Time (HRT)	HRT is the average period that biomass remains in the reactor, determined by the total reactor volume and the biomass feeding rate. An appropriate HRT is essential to provide microorganisms with sufficient time to degrade organic matter.

The experiments used fermented beans as inoculum and sugar as a substrate. The Excel program satisfactorily identified the formed products (H_2 with 274 ppm and CH_4 with 884 ppm), as well as the temperature and pressure (respectively 1.51 KPa and the reaction temperature without activating the heater was 29.5 °C) (Figure 3). The Excel program was developed to accurately and continuously monitor the internal pressure of the bioreactor, temperature, and the precise amount of hydrogen and methane produced (3 hours).

Conclusion

In conclusion, temperature, pH, and the composition of methanogenic archaea communities are critical parameters that significantly influence methane production in anaerobic digestion processes. Understanding and optimizing these parameters are essential for achieving higher biogas yields, increased process stability, and overall efficiency in sustainable biogas production. Further research in this area can lead to advancements in biogas technology and contribute to developing environmentally friendly renewable energy sources. The realization of this work proved to be positive since the expected objectives were achieved; the prototype of the bioreactor fulfilled its function of helping researchers to identify changes in real-time and thus know what measures to take so that the reactions occur more efficiently, with the controlled temperature monitoring made it possible to keep the most suitable organisms active for the process. The prototype is efficient and can be improved by including

Figure 3. Bioreactor.

other sensors, such as pH and CO_2 concentration, and adding more automatic control functions.

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Evaluation of Lignocellulosic Biomasses for Pyrolysis Product Generation

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This study aimed to assess agroindustrial residues, namely acerola seeds, poultry litter, bean pods, corn cobs, coconut fiber, pine nut shells, peanut shells, pine, and passion fruit, for the production of pyrolysis products, thereby adding value to these environmental assets. The characterization of these biomasses was carried out through elemental analysis (CHN), thermogravimetry (TG), higher heating value (HHV), ash content, and protein content determination. The samples exhibited low ash content (1.04% to 11.92%), protein content (1.22% to 15.06%), and moisture content (7.82% to 15.31%). Thermogravimetric analysis revealed that compound degradation occurred between 115°C and 500°C, with higher heating values (16.73% to 19.94 MJ.kg^{-1}), indicating strong applicability in pyrolysis processes.
Keywords: Characterization. Pyrolysis. Agroindustrial Residue.

Introduction

Environmental pollution and the energy crisis resulting from using fossil fuels have driven the development of clean and renewable energy alternatives [1-5]. One such alternative is the utilization of biomass, which encompasses any renewable resource derived from organic matter of animal or plant origin found in nature or generated by humans and/or animals [1]. Lignocellulosic waste has been identified as an attractive feedstock for fuel production due to its potential for zero CO_2 emissions, abundant availability, and low cost. Biofuel is considered carbon-neutral and has garnered attention as a potential renewable energy source, alongside its capacity to mitigate greenhouse gas emissions [6-8]. Consequently, the biomass composition is crucial in assessing the generated products and their properties.

Lignocellulosic biomass resources can be utilized cleanly and efficiently through appropriate conversion techniques. One such technique is pyrolysis, which is employed for the efficient transformation of biomass, resulting in the production of numerous products (Table 1).

Depending on the characteristics of the samples, the pyrolysis products can be gas, liquid, or char. Thus, this study aims to characterize 9 different biomass sources.

Materials and Methods

Sample Preparation

Nine different biomass sources were employed in this study: acerola seeds (ACE), poultry litter (CF), bean pods (FEI), corn cobs (Mi.S), pine nut shells (PIN), peanut shells (AMD), pine bark (PNU), passion fruit peels (Ma.C) and coconut fiber (COC) (Table 2).

Method

Ash Content

The determination was performed by gravimetry in a muffle furnace, following the NREL/TP-510-42622 standard [18]. Porcelain crucibles were pre-weighed, and approximately 2 g of moisture-free samples were added. Triplicates were placed in the SPLABOR muffle furnace, model SP-1200, and heated to a temperature of 575 ± 25 °C for 4 hours. The samples were removed and transferred to a desiccator until they reached room temperature.

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Table 1. Applications of different types of pyrolysis.

Type	Process	Product	Reference
Animal fat	Catalytic Pyrolysis	Gasoline and diesel	[9]
Leather	Microwave-Assisted Pyrolysis	Liquid fractions and biochar	[10]
Bone	Pyrolysis and Co-pyrolysis	Biochar	[11]
Chicken feathers	Slow Pyrolysis	Biochar	[12]
Bovine manure	*In-situ* Catalytic Pyrolysis	Biogas	[13]
Tree leaves	Slow, fast, and microwave pyrolyzes	Biogas and biochar	[14]
Tree trunks and leaves	Intermediate Pyrolysis	Bio-oil and biochar	[15]
Tree branches	Co-pyrolysis	Biochar	[16]
Plant roots	Catalytic Pyrolysis	Biogasoline	[17]

Note: The references are listed with the author's names followed by the publication year.

Table 2. Biomass sources studied in this work.

Biomass	Acronym	Collection Location	Origin	Preparation
Acerola seeds	ACE	Aracaju/SE	Plant	Milling
Poultry litter*	CF	São Cristóvão/SE	Animal	Sorting
Bean pods	FEI	São Cristóvão/SE	Plant	Food Processor
Corn cobs	Mi, S	Vitória da Conquista/BA	Plant	Grater
Pine nut shells	PIN	Maringá/PR	Plant	Grater
Peanut shells	AMD	Malhador/SE	Plant	Milling
Pine bark	PNU	Commercial Sample	Plant	Milling
Passion fruit peels	Ma, C	Nossa Senhora do Socorro/SE	Plant	Grater
Coconut fiber	COC	Nossa Senhora do Socorro/SE	Plant	Grater

*With a usage batch (40 days in contact with the animals plus 15 days of sanitation gap), it is composed initially of wood shavings.

The calculation of the ash content was obtained using Equation 1 as follows:

$$\text{Ash Content (\%)} = \left(\frac{\text{Mass of Crucible with Ash} - \text{Mass of Crucible}}{\text{Sample Mass}}\right) \times 100 \qquad (1)$$

Elemental Composition (C, H, N, and O)

Elemental composition analysis was conducted using a Leco analyzer, Model CHN628, with helium (99.995%) and oxygen (99.99%) as gases and a furnace temperature of 950 °C. The equipment was calibrated using an EDTA standard (41.0% C, 5.5% H, and 9.5% N) with mass ranges between 10 and 150 mg to prepare the calibration curve. Approximately 50 mg of the sample was used for analysis in both cases. Tin foil was employed as a sample support for subsequent analysis. The percentage of oxygen was determined according to Equation 2 below [19]:

$$\%O = 100 - (\%C + \%H + \%N + Ash\ Content\ (\%)) \quad (2)$$

in which: $\%C$ = carbon content; $\%H$ = hydrogen content; $\%N$ = nitrogen content.

The percentages of these atoms in the biomass can determine the molar ratios H/C and O/C, where the first parameter forms the abscissa axis in the Van Krevelen diagram, and the second parameter forms the ordinate axis.

Higher Heating Value (HHV) and Protein Content

HHV was calculated according to the following equation [20]:

$$HHV\ (MJ\ kg\text{-}1) = -1.3675 + (0.3137xC) + (0.7009xH) + 0.0318xO) \quad (3)$$

in which HHV = Higher Heating Value; C = carbon content; H = hydrogen content; N = nitrogen content.

Protein content was determined following the NREL/TP-510-42625 methodology [21], and the equation below was applied:

$$Protein\ Content\ (\%) = \%N\ x\ 6.25 \quad (4)$$

in which $\%N$ is equal to the nitrogen content.

Thermogravimetric Analysis (TG)

TG analyses were conducted using the Simultaneous DTA-TG Apparatus, model DTA-50, manufactured by Shimadzu. The temperature range was set from 50 to 1000°C with a heating rate of 10°C/min under an inert atmosphere of N_2 at a 100 mL/min flow rate. A platinum crucible containing 5 to 10 mg of biomass was used for the analysis. The results were elucidated through the dTG curve, corresponding to the first derivative of mass change concerning temperature in the TG curve (dm/dT). This representation allows for easier visualization of information. Thus, the area under the peak corresponds to the mass variation [22].

Results and Discussion

Biomass Characterization

The samples exhibited less than 10% ash content, except for CF (11.92%) (Table 3). This higher value is attributed to the type of material used and the number of batches, leading to varying mineral concentrations being deposited [2,3]. Depending on the composition, ashes can function as catalysts, considering the target product [6]. This particular sample also displayed a higher heating value (HHV) of 16.73 MJ/kg, surpassing biomass sources like wood chips and eucalyptus bark, which are widely used in industrial boilers and have values of 16.46 and 13.92 MJ/kg, respectively [4].

CF also stands out for having the highest protein content in the dataset (15.06%). However, under these conditions, this biomass is unsuitable for industrial use due to the emission of toxic gases such as HCN, NOx, and NH_3. However, for the chemical and pharmaceutical industries, there is significant value depending on the compounds present, such as pyrrole, pyridine, and índole [5]. The calorific value can be assessed through the atomic ratio of O/C and H/C in the biomass using the van Krevelen diagram (Figure 2).

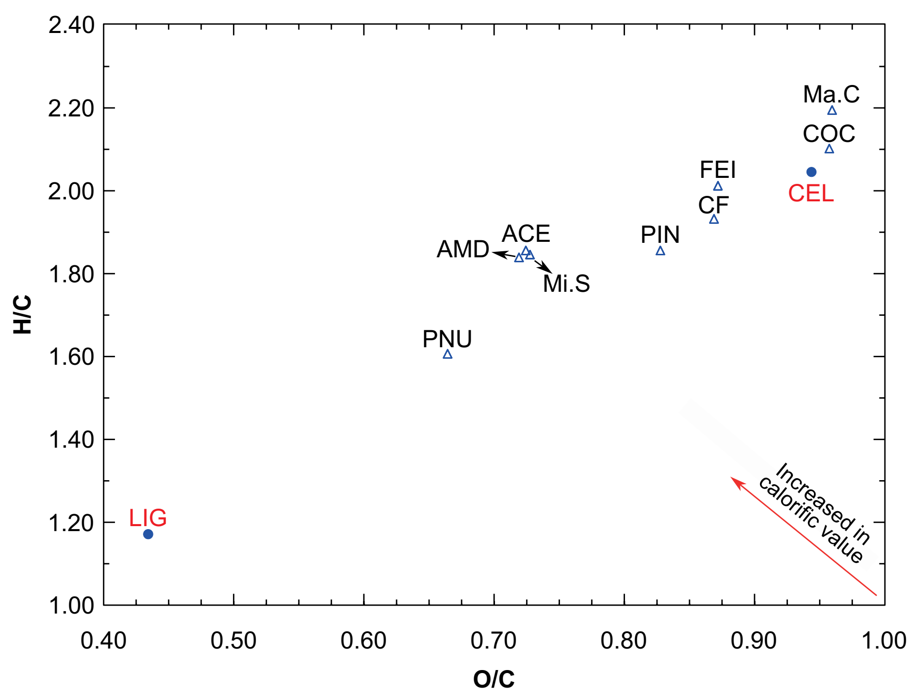
The ACE, Mi.S, AMD, and PNU samples exhibited high H/C and low O/C ratios, granting them the highest calorific value within the dataset. These samples also possess the lowest ash content, enhancing the thermal efficiency of the process and preventing reactor fouling [3]. The ACE, AMD, and Mi.S samples possess very close H/C ratios (1.86, 1.84, and 1.85, respectively). The same applies to the O/C ratio of these biomasses (0.72, 0.72,

Table 3. Higher heating value, protein content, and ash content of the biomasses.

Biomass	Acronym	Ash (%)	HHV (MJ/kg)	Protein (%)
Acerola seeds	ACE	2.71 ± 0.01	19.11	9.12
Poultry Litter	CF	11.92 ± 0.74	16.73	15.06
Bean pods	FEI	4.38 ± 0.06	17.60	7.91
Corn cobs	Mi.S	1.37 ± 0.13	19.44	5.86
Pine nut shells	PIN	3.88 ± 0.22	17.98	3.48
Peanut shells	AMD	2.76 ± 0.08	19.33	3.01
Pine bark	PNU	1.04 ± 0.13	19.94	1.22
Passion fruit peels	Ma.C	6.10 ± 0.32	16.79	8.03
Coconut fiber	COC	3.96 ± 0.11	17.22	3.29

Note: The values presented are mean values with associated standard deviations.

Figure 2. Van Krevelen diagram of the studied biomasses.



and 0.73, respectively), justifying the proximity of these data points on the graph. The remaining biomasses feature elevated molar H/C and O/C ratios, resulting in higher yields of volatiles and liquids and decreased energy conversion efficiency due to more significant CO_2 emissions from the higher O/C ratio [23]. High H/C ratios enhance the potential of using biomass for biofuel production, making it advantageous to incorporate feedstocks with these characteristics in thermochemical processes, thereby improving product quality [6].

Regarding the thermogravimetric analyses, when examining the events highlighted in Table 4, it is evident that the passion fruit peel biomass

Table 4. Temperature ranges and percentage mass loss of the samples.

Samples	Moisture ΔT (°C)	Moisture Mass Loss (%)	Hemicellulose ΔT (°C)	Hemicellulose Mass Loss (%)	Cellulose ΔT (°C)	Cellulose Mass Loss (%)
ACE	27.00 – 114.00	7.82	221.00 – 298.00	14.05	305.00 – 403.00	27.15
Mi.S	31.68 – 77.78	7.94	259.06 – 304.29	29.36	346.42 – 379.89	28.51
AMD	29.00 – 110.00	8.01	250.00 – 409.00	50.11*	-	-
PNU	34.91 – 85.38	11.99	295.51 – 392.86	36.77*	-	-
Ma.C	32.19 – 76.94	15.31	208.77 – 257.85	21.62	298.59 – 331.67	18.60
COC	26.01 – 111.67	10.06	235.13 – 308.82	17.35	310.73 – 384.90	20.89
PIN	37.22 – 100.88	12.56	251.34 – 290.78	16.77	319.66 – 349.56	10.04
FEI	28.56 – 105.00	11.89	216.00 – 263.00	7.76	277.00 – 388.00	39.94
CF	39.94 – 115.96	10.59	267.68 – 353.27	42.00	437.67 – 486.51	6.41

Note: ΔT represents the temperature range in Celsius, and mass loss is expressed in percentage. *Demonstrated only an area corresponding to the temperature range of holocellulose.

exhibits the highest moisture content among the samples (15.31%). It also possesses the highest O/C ratio and the second-highest ash content (6.10%), which impacts its higher heating value (16.79 MJ/kg). However, this moisture percentage is considered low, and conditions, to the contrary, are not conducive to pyrolysis. Adequate thermal pre-treatment is recommended in such cases [7]. Each sample exhibited distinct temperature ranges for the degradation of lignocellulosic components (hemicellulose, cellulose, and lignin), considering their specific characteristics. In general, the degradation of compounds commences at around 115°C, concluding below 500°C. Above this temperature, no significant mass loss events are observed, enabling the assessment of pyrolysis of these biomasses for the production of bio-oil, biochar, or biogas.

Conclusion

A high O/C ratio and low protein, ash, and moisture content primarily characterize the analyzed biomasses. Thermogravimetric and elemental composition analyses reveal a higher calorific value than some biomasses already employed in combustion systems, showcasing their viability for pyrolysis processes to produce bio-oils. Characterizing these biomasses through GC-MS enables an assessment of their individual and/or combined applicability, contingent upon their chemical properties. This consideration encompasses a diverse range of industrial applications, including the food, pharmaceutical, cosmetic, fuel, and fine chemical industries.

Acknowledgments

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Production and Characterization of Bio-Oil from Cassava Peel

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Agroindustrial residues are biomass of interest as they are energy sources and bioproducts with lower environmental impacts than traditional fossil fuels. Pyrolysis has been used to convert residual biomass into higher value-added products such as bio-oil. This work aimed to pyrolyze cassava peels to produce bio-oil and characterization using chromatographic techniques to obtain essential compounds for the chemical industry, adding value to this residue. The pyrolytic process generated 17.5% of bio-oil, with a high concentration of phenols and alcohols. Thus, this residual biomass has potential for industrial use, reducing the environmental impact of its deposit in landfills and offering industrial and economic benefits.

Keywords: Biomass. Bio-Oils. Chromatography Analysis. Pyrolysis.

Introduction

The global demand for energy and industrial inputs from renewable sources has led to a sustainable production and energy chain to replace non-renewable sources, such as fossil fuels [1].

Biomass stands out as a promising source of energy and bioproducts, considering its easy access and availability, low environmental impact with lower emissions of greenhouse gases, lower cost, and the reuse of various residues in their raw form [2,3]. Additionally, it positively impacts the generation of chemical products such as food and pharmaceutical inputs, surfactants, organic solvents, and fertilizers [4,5].

In developing countries, biomass is more abundant and consists of various materials, mainly agricultural residues. In Brazil, for example, tons of agroindustrial waste are generated annually [6].

The utilization of these residues is a highly relevant topic due to the large quantity of organic waste generated worldwide and the negative environmental impact of improper handling of these materials [7,8].

Cassava (*Manihot esculenta*) is a biomass of great interest and is one of the most popular crops in Brazil, cultivated in all regions of the country. One of its primary uses is to produce flour, and one of the central residues obtained during the beginning of this process is the peel [9,10].

According to the systematic survey of production (LSPA) from January 2022, conducted by the Brazilian Institute of Geography and Statistics (IBGE, 2022), the estimated production in Brazil is 18.03 million tons per year, cultivated on a total area of 1.24 million hectares [11].

Agroindustrial processes that use roots to produce flour generate large quantities of solid and liquid waste, including peels and pomace [12]. After pre-cleaning the peels, the leading industrial residue is produced, which usually lacks a sustainable destination and is disposed of in landfills [13].

The volume generated from this waste can reach up to 7.8% of the total harvested. If properly used, this waste can minimize environmental issues in the industry and, if commercialized, contribute to the production chain of cassava starch or flour and add profit to the sector [14].

One of the routes for reutilizing this waste is the pyrolysis process. Pyrolysis is a thermochemical conversion process that converts biomass into

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organic products of lower molecular complexity through thermal decomposition in an inert atmosphere, intending to generate higher-value products [15,16]. The process yields biochar (solid), non-condensable gases, and a condensable liquid fraction (bio-oil). The efficiency of the pyrolysis process depends on physicochemical parameters and the understanding of the properties of the biomass to be used in the process [17,16].

Bio-oil utilization is directly related to its physicochemical properties, particularly its chemical composition. Based on this composition, the produced bio-oil can have various applications in different areas, such as diverse chemical products like asphalt, pesticides, fertilizers, and pharmaceuticals, or directly as fuel in industrial boilers and gas turbines [18,19].

The most used analytical technique for characterizing bio-oil is gas chromatography (GC), typically coupled with spectroscopic methods such as mass spectrometry (MS) to provide further information about the chemical structure of the bio-oil components. A commonly used mass analyzer is the quadrupole, which is accessible and compatible with mass spectral libraries [20,21]. The work focuses on producing and characterizing bio-oil derived from cassava peel biomass. This biomass is an essential source of raw material.

Materials and Methods

Biomass Characterization

According to ASTM standard methods, biomass was characterized by proximate analysis [22,23]. Moisture was determined using a moisture analyzer (SHIMADZU, model MOC63u) at 140°C. The elemental analysis of the biomass was performed using a FlashEA 1112 Nitrogen and Carbon analyzer (Thermo Scientific), and the results were processed with CHN-628 v. 1.3 software. These results were then used to determine the biomass's High Heating Value (HHV). The determinations were performed by Sheng and Azevedo [24] according to the equation:

$$HHV \text{ (MJ kg-1)} = — 1.3675 + 0.3137.C + 0.7009.H + 0.0318$$

in which C, H, and O represent the percent weight of each element.

The thermal decomposition of the biomass was determined using a thermal analysis system (STA200RV, Hitachi, Japan), using a heating rate of 20 ºC min^{-1} till 900 °C under a flow of nitrogen gas. The weight loss of the sample was recorded as a function of temperature. The TGA results can be used to determine the different stages of the thermal decomposition of biomass.

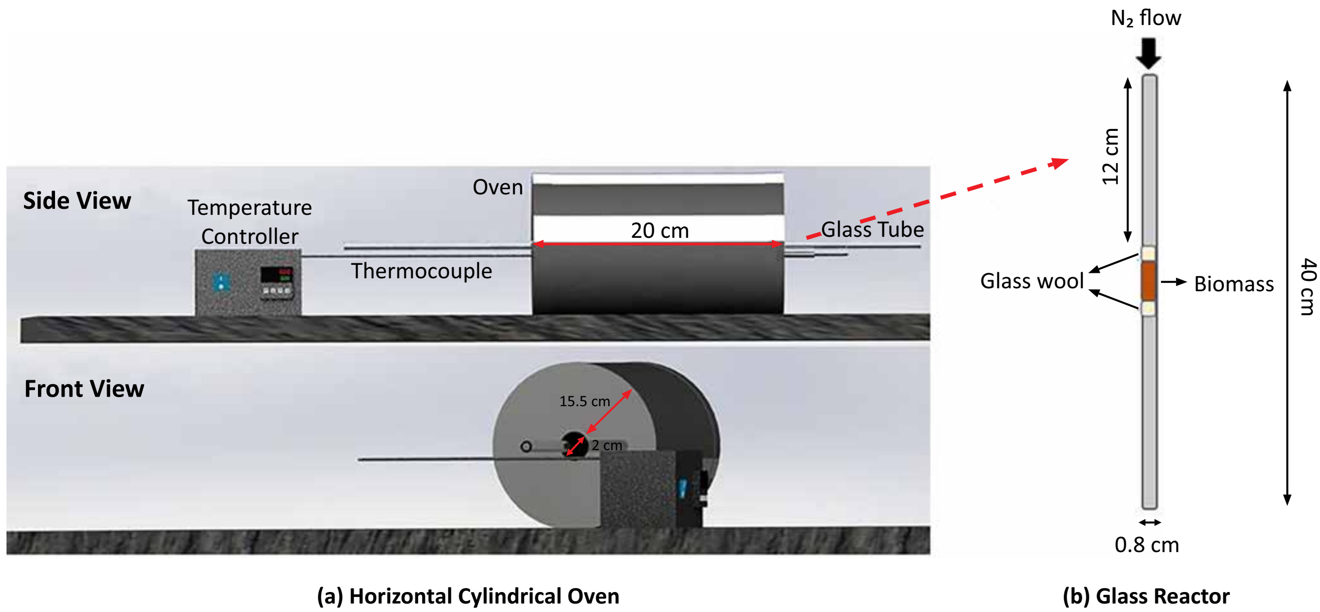
Pyrolysis Conditions

Fast pyrolysis experiments were conducted using a system consisting of a circular horizontal furnace with a diameter of 25 cm and a length of 20 cm. [25] The oven has an internal refractory layer with 11.5 cm of thickness and a central orifice with a diameter of 2 cm for introducing the glass reactor. The glass reactor has an inlet for inert gas (N_2). A thermocouple controls the temperature. Figure 1 (a, b) schematically illustrates the complete system and its detailed parts. The pyrolysis experiments were conducted at 600 °C, with a nitrogen flow rate of 2 mL min^{-1} and 0.1 g of biomass. The biomass was placed inside the reactor using glass wool at both ends [Figure 1 (b)]. The reactor containing the biomass was inserted into the preheated oven, ensuring that the biomass was centrally positioned for more uniform heating. The reactor was connected to the N_2 flow. The temperature was controlled so that the biomass inside the reactor reached 600 °C and remained at this temperature for 1 minute. Subsequently, the reactor was removed from the furnace, cooled to room temperature, and the bio-oil was eluted with 15 mL of acetone. Both bio-oil and biochar after solvent evaporation were weighted for mass yield determination.

Chromatographic Analysis – GC/qMS

Chromatographic analyses were performed using the GCMS-QP2010-Ultra model equipment

Figure 1. Pyrolysis system. (a) Oven, (b) reactor.



(Shimadzu, Japan). The capillary column was a DB-5, 60 m long, 0.25 mm internal diameter, and 0.25 µm stationary phase thickness. Helium was used as a carrier gas with a flow of 1 mL min^{-1}. In the splitless mode, sample injection (1 µL at a concentration of 5000 ppm) was performed using the AOC-20i automatic injector (Shimadzu, Japan). The system operated in SCAN mode. The oven was heated according to the following schedule: from 40 °C to 300 °C at 3 °C min^{-1}, remaining at this temperature for 10 min. Data treatment was performed using the GCMS solution software. The compounds were identified by retention index and mass spectra.

Results and Discussion

Biomass Characterization and Pyrolysis

Figure 2 presents the thermogravimetric profile of the biomass used in this study. The initial weight loss of 10.4% refers to the liberation of CO_2 and H_2O. The second significant event is the decomposition of cellulose and hemicellulose, which can not be differentiated. This weight loss begins at 160 °C (likely hemicellulose) and ends at 480 °C (probably cellulose), accounting for approximately 44,6% of total mass loss. Afterward, lignin decomposition occurs, corresponding to a loss of 14.1%.

Figure 3 displays the results of the preliminary biomass analysis and the pyrolysis mass yield. The sample shows a low moisture content, which is suitable for pyrolysis (< 10%). The volatile content was high (95.4%), while the ash and fixed carbon content were low. These results indicate the potential of this biomass to produce a higher content of bio-oil. The elemental composition highlighted the high percentage of oxygen (42.3%) related to bio-oil quality, which will probably be prosperous in oxygenated compounds like phenols, aldehydes, and ketones. Calculating the atomic ratios H/C (1.5) and the High Heating Value (HHV= 19.72 MJ kg^{-1}) are important energetic parameters for Cassava peel. H/C can be considered high for biomasses and indicates the presence of hydrocarbon-saturated chains due to the predominance of cellulose over lignin (which is more aromatic). HHV found is in accord with the literature [26].

The pyrolysis results for residual biomass show that bio-oil yield was 17.5%, but the amount of gases and the losses are very high. In a closed system, these gases could be reused to preheat

Figure 2. Profile obtained from the thermogravimetric analysis of the biomass.

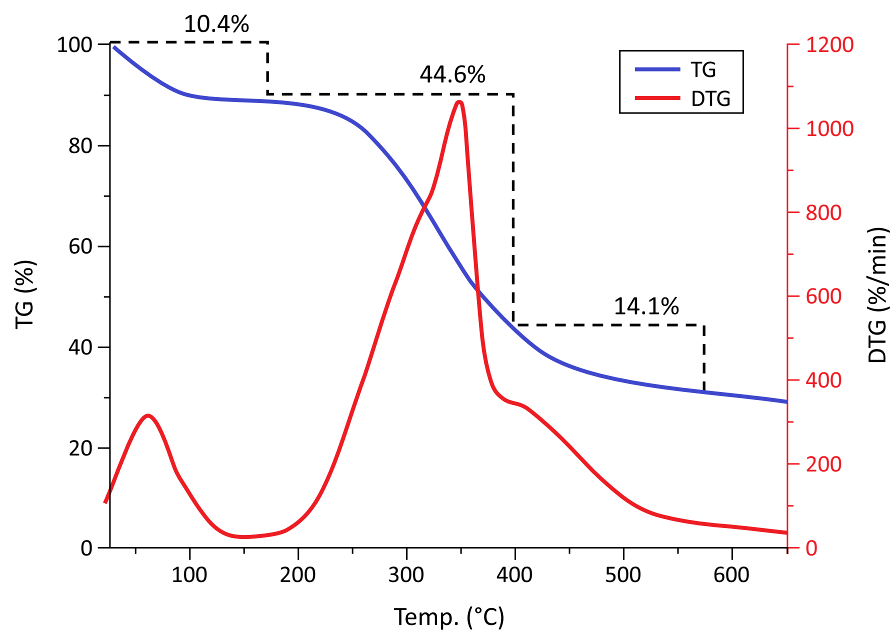
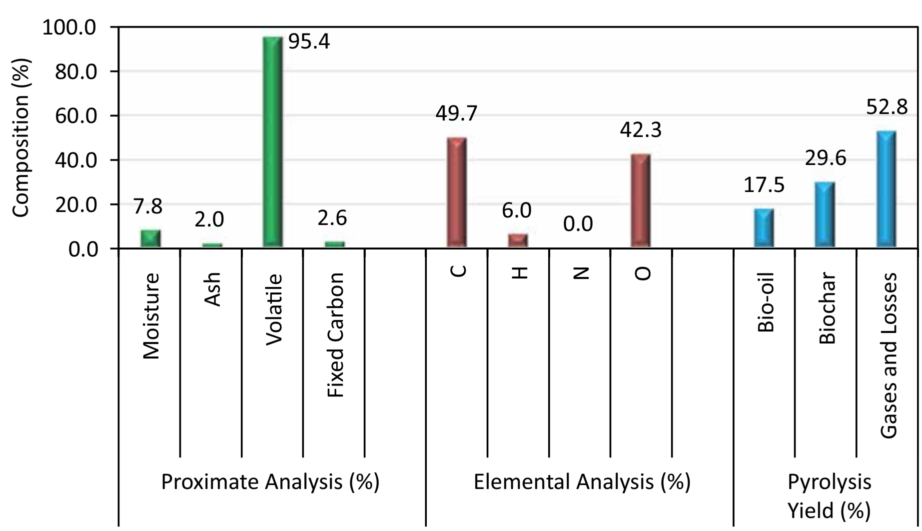


Figure 3. Preliminary analysis of the biomass and mass yield of the pyrolysis.



the system, which would not be considered a process loss. The loss of water and burning of gases leads to a substantial reduction in the residual volume of biomass (forming biochar with 29.6 % of the initial mass), which is an advantage of the pyrolytic process if considering the environmental impact of the significant number of residues deposited in landfills.

Bio-Oils Gas Chromatographic Analysis

Figure 4 shows the number of peaks and the percentage composition (related to the percentage area of each peak) of each class of chemical compounds found in the sample bio-oil. Table 1 shows the significant compounds in the analyzed cassava peel bio-oil.

Figure 4. Distribution of chemical classes found in the sample concerning percentage areas and peak numbers.

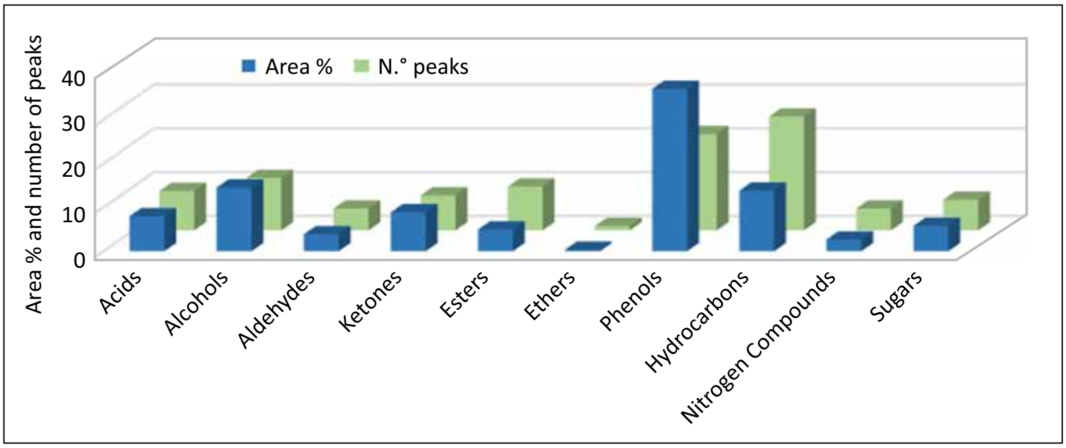


Table 1. Main compounds found in the analyzed bio-oil (area % ≥ 2.0%).

Chemical Classes	Compound Name	Area %
Acid	Oleic acid	2.00
Alcohols	1-heptacosanol	2.66
	1-octacosanol	2.2
Ketone	Acetovanillone	3.73
Sugar	Anhydrous sugar	2.06
Phenols	4-methyl catechol	4.20
	5-tert Butyl pirogalol	2.54
	4-methyl-syringol	2.43
	Isoeugenol	2.46
	4(1-propenyl)-syringol	2.4
	syringol	5.74

Phenols were the predominant class in terms of percentage area, followed by alcohol. Hydrocarbons and phenols presented the highest number of compounds (peaks). The presence of phenolic compounds, such as catechol and 4-methyl catechol, is appreciable, and they have a variety of applications, including photographic developer, dye developer for skins, intermediate for antioxidants in rubber and lubricating oils, polymerization inhibitors, and pharmaceutical products [27].

Phenolic compounds also have industrial and medical applications, replacing petroleum-derived compounds in producing phenolic resins. Compounds from other classes also have significant and diverse applications.

Conclusion

Cassava peel biomass exhibited expected results from elemental and proximate analyses, with yields viable for bio-oil production through pyrolysis. Chromatographic analysis has determined biomass to be highly suitable for producing bio-oil, which holds great potential for industrial applications. This promising solution helps minimize waste generation and reduces dependence on fossil fuels, thereby contributing to reducing carbon emissions in the atmosphere.

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Evaluation of Biosurfactant Production by *Bacillus subtilis* Using Glycerol

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There is a constant concern about the environmental impacts due to the increase in industrial activity. The leading causes of these impacts come from chemicals, especially organic compounds such as hydrocarbons and their derivatives, which companies often use. In search of long-term solutions, biosurfactants have gained significant prominence regarding their cost-benefit. It is favorable when compared to surfactants of petroleum origin. In order to lower costs and reduce the impacts on the environment caused by industries, this work aims to evaluate the production of biosurfactants by *Bacillus subtilis* using glycerol in different concentrations.
Keywords: Biosurfactant. Industrial Waste. Glycerol. *Bacillus subtilis*.

Introduction

Developing eco-friendly and economically competitive substances has been gaining prominence for socio-environmental issues. It is necessary to invest in bioproducts to reduce the concerns and expenses associated with pollution prevention, waste management, and elimination or reduction of consumption of industrial products that are harmful to the environment. It is worth noting that agricultural or agroindustrial waste from various origins is an excellent sustainable raw material for various bioprocesses in producing bioenergy and other bioproducts. The need to convert the waste obtained into sustainable products is essential to minimize the need to use sources from oil. Initially, glycerol has stood out as a highly accessible raw material for manufacturing biomolecules of industrial importance due to its high consumption in biodiesel production. Glycerin is one of the main by-products linked to the biodiesel manufacturing chain. The biodiesel production process includes the combination of a triglyceride with short-chain alcohol (whether methanol or ethanol The companies involved in this industry assume that the increase in the availability of this co-product in the domestic market is related to the manufacture of biodiesel. For every 1,000L of biodiesel produced, approximately 100 kg of glycerol is generated; having said that, this material is of paramount importance since its excess production is harmful to the environment [1]. Surface tensions between immiscible liquids are minimized by creating a molecular layer that communicates between the interfaces of complex substances to homogenize. Biosurfactants undergo biotransformations of renewable materials, thus contributing little to adverse environmental effects and favoring the disuse of chemicals.

The production of biosurfactants faces many technical challenges that must be overcome to ensure an efficient and high-throughput process. The selection of the producing microorganism is a crucial step, requiring the choice of a species capable of synthesizing the desired molecules in significant quantities and with robustness to withstand adverse conditions. In addition, it is necessary to optimize cultivation conditions, such as pH, temperature, and carbon sources, to stimulate the synthesis of biosurfactants [2-4].

An approach that is successful for the production of biosurfactants, also known as surfactants of biological origin, is the use of glycerol as a substrate using bacteria as BS producers. The fact that agroindustrial residues are generated in tropical or temperate climate areas and are abundant makes them advantageous as an organic substrate for industrial fermentations. This study aimed to evaluate biosurfactants' production using glycerol

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in different concentrations in the synthetic mineral medium by *B. subtilis*.

Materials and Methods

Cultivation of the Microorganism

For this work, the strain of *Bacillus subtilis* from the Federal University of Bahia (UFBA) was used in the laboratory of Microbiology and Bioprocesses Moacyr Durham de Moura-Costa, being used by the advisor Tatiana Vale in the doctoral thesis. The bacterium was isolated in waste from the oil and gas industry and produced water. The culture was maintained in the solid medium in plates with Tryptic Soy Broth (TSB) culture medium. The conservation of the microorganism is of great value because it prevents contamination, the loss of viability, and against possible mutations.

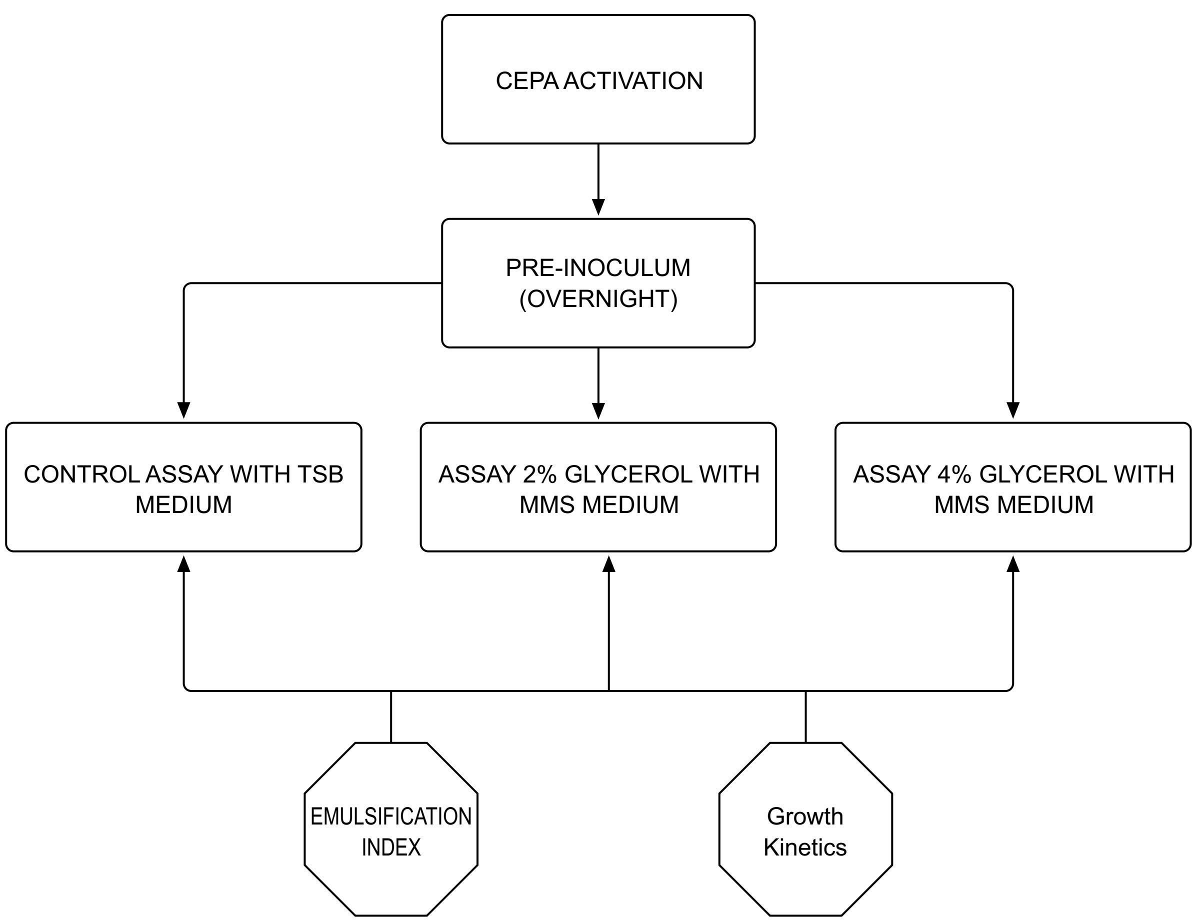
Flowchart and Organization of Bioassays (Figure 1)

The crops were developed in a TSB medium to determine the growth kinetics and maintenance of the strain. This culture underwent an activation (growth for 24 hours) and a pre-inoculum before the assays to ensure its adequate bacterial concentration for the assay. According to Vale, to maintain the conservation of the culture and its growth in ideal conditions, reaching a standard number of living and viable cells, a pre-inoculate (overnight) is performed using the activation previously performed containing 90mL ofmedium, 10mL of activation for 12-16 hours at 130 rpm and 37°C before the start of the assays [3].

Tests and Production of Biosurfactant

Simultaneous processes were carried out in duplicates of two different concentrations of

Figure 1. Flowchart of procedures performed until the development of the tests.



substrate-glycerol PA (20g/L and 40g/L) under 37ºC and 180 rpm for 72 hours on an agitator table. For each replicate was prepared in an Erlenmeyer of 250mL, 95 mL of synthetic mineral medium (MMS), proposed by Sant'Anna (2001), containing $Na_2HPO_4.12H_2O$ (4.2g/L); KH_2PO_4 (1g/L); $Ca(NO_3)_2.4H_2O$ (0.072g/L); (NH_4) The fermentation process for the production of biosurfactant was carried out with the aid of an agitating table, with pre-fixed agitation and keeping the temperature constant at 37°C.

Emulsion Index (Figure 2)

According to Alvarez and colleagues, the emulsifying activity is evaluated by mixing 2 mL of mineral oil and 2 mL of cell-free broth supernatant in a test tube. Each tube was shaken for 2 minutes and kept at 25°C for 24 hours. The index (E24%) was calculated using the formula shown below:

$$E24\% = (Hfe / Htotal) \times 100 \quad (1)$$

in which Hfe = Height of the emulsified phase, and Htotal = Total liquid height.

Aliquots were removed for cell growth analysis (verified through optical density) and E24% (Emulsification Index) in pre-established times. The tests were carried out to monitor the production of biosurfactants with different substrate concentrations under fixed process conditions. The emulsion index and optical density (600 nm) analyses were performed in duplicates throughout the test [5].

Results and Discussion

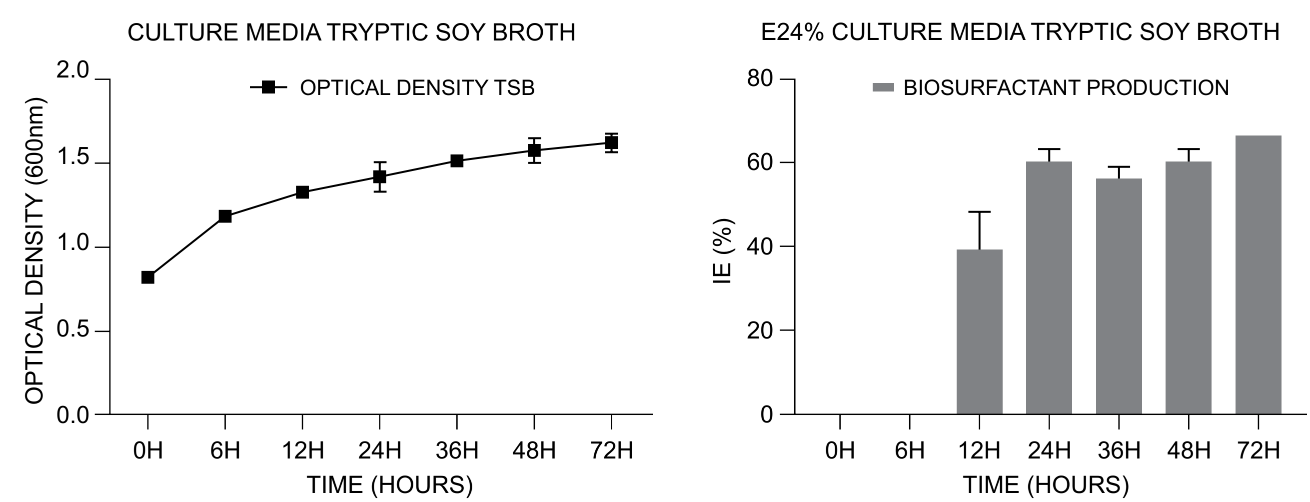
Tryptic Soy Broth Medium Test

Initially, a test was carried out in the TSB culture to recognize the bacterium's growth kinetics under ideal conditions. This trial was performed with 12 250 mL Erlenmeyers containing 95 mL of TSB and 5 mL of pre-inoculum. Each test point was collected in duplicate, representing its result as an average of the replicates.

The effectiveness of the produced biosurfactant can also be evaluated by its ability to emulsify mixtures of hydrocarbons in oil, resulting in a significant increase in the degradation of these organic compounds. Furthermore, the biosurfactant's capacity to influence the emulsions' stability or instability is a relevant indicator [6].

Based on these considerations, it is notable that this trial demonstrated the highest production of both biomass and biosurfactant. In a similar study, Vale associated *Bacillus subtilis* with the TSB synthetic culture medium as the most suitable commercially

Figure 2. Growth kinetics of the *Bacillus subtilis* bacterium in TSB culture medium and E24% emulsification index analysis of biosurfactant production with TSB medium.



producing biosurfactants [3]. These results reinforce the importance of the culture medium in obtaining high-efficiency biosurfactants and highlight the potential of this specific assay as a promising candidate for future industrial applications.

MMS Assay 2% Glycerol (Figure 3)

Therefore, a test was carried out with 12 Erlenmeyers containing 95 mL of MMS medium, 5 mL of pre-inoculum, and 1 mL of trace metal solution. Glycerol (20g/L) and yeast extract (5g/L) were added as carbon sources for the MMS.

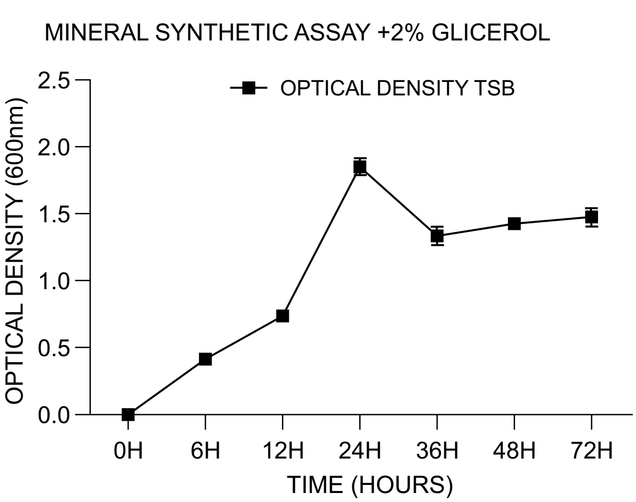
In this specific assay, we observed slower bacterial growth. However, the production of biosurfactants occurred in quantities comparable to the use of the TSB medium as a reference. This phenomenon can be evidenced at the 24-hour and 72-hour time points, at which optical density (O.D.) values of 1.853 and 1.477 were obtained, together with emulsification rates (E24%) of 54.16% and 60.41 %, respectively. It is crucial to point out that the choice of the ideal culture medium for the production of biosurfactants by *B. subtilis* must be weighed in terms of economic efficiency, especially when compared to the use of an MMS medium. Sousa and colleagues also investigated biosurfactant production using the MMS medium, observing a gradual increase after 48 hours of incubation at 30°C under agitation at 180 rpm, culminating in a maximum peak after 70 hours of cultivation [7]. In an additional study, de Faria and colleagues performed an assay involving *B. subtilis* using a Tryptic Soy Agar (TSA) medium with glycerol as substrate. They obtained similar results in their optical density analyses (1022, 1036, 1044, and 1058) [8]. These findings reinforce the importance of choosing a suitable culture medium to produce biosurfactants by *B. subtilis* [8,9].

Assay in MMS 4% Glycerol

A trial was conducted with 12 Erlenmeyers containing 95 mL of MMS medium, 5 mL of pre-inoculum, and 1 mL of trace solution. Glycerol (40g/L) and yeast extract (5g/L) were added as carbon sources for the MMS.

In the present test, it is observed that the bacterial concentration reached significantly higher values in a substantially shorter period (24 hours) compared to the control test that used Tryptic Soy Broth (TSB) as a culture medium, where this bacterial growth led to 72 hours to complete. However, it is essential to highlight that the production of biosurfactants was significantly attenuated in the context of this experiment. It is essential to mention that Sousa and

Figure 3. Optical density analysis by spectrophotometry of bacterial growth in MMS 2% and E24% emulsification index analysis of biosurfactant production in MMS 2%.



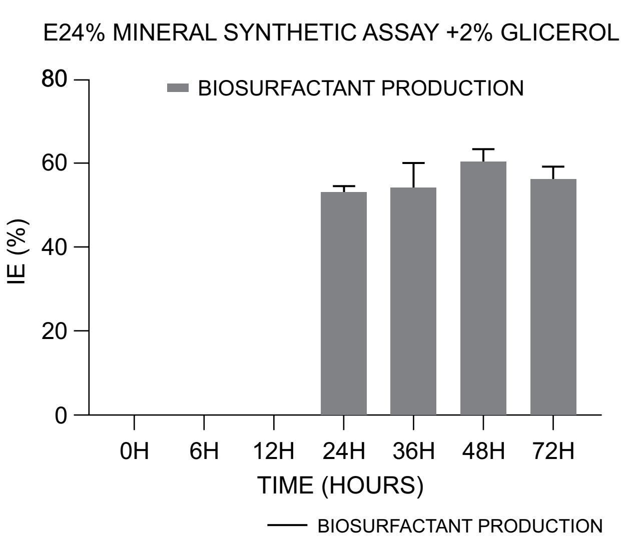
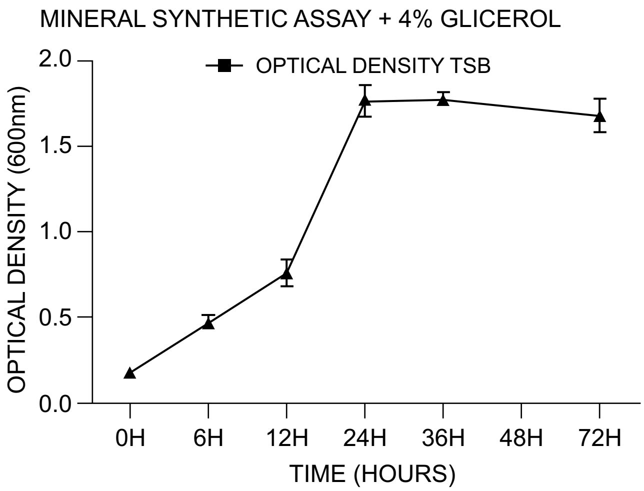
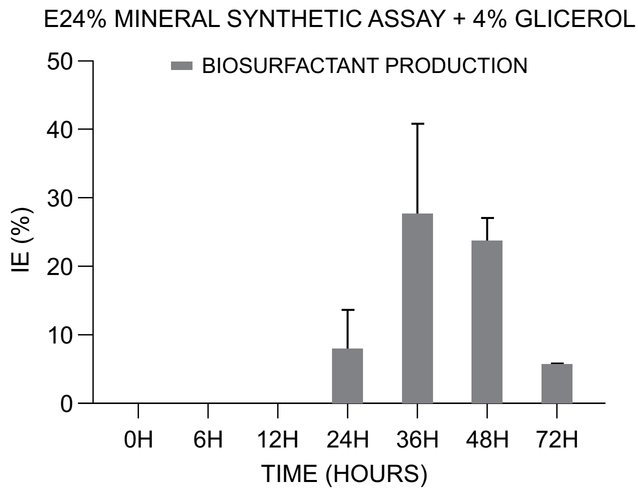


Figure 4. Optical density analysis by spectrophotometry of bacterial growth in MMS 4% and E24% emulsification index analysis of biosurfactant production in MMS 4%.





colleagues also elucidated the formation of surfactin through the application of glycerol in substrates containing *B. subtilis*, observing a maximum peak of 263.64 mg L^{-1} of surfactin after 30 hours of cultivation, corroborating our results—findings in this research context [7].

Conclusion

Among the two analysis tests carried out, it is notable that the test using glycerol at a concentration of 2% stood out significantly in terms of biosurfactant production when compared to the test using glycerol at 4%. On the other hand, the assay with 4% glycerol revealed a more robust bacterial development. It is essential to highlight that the test with TSB showed superior performance to the other tests due to its ideal intrinsic characteristics for the growth and production of biosurfactants. However, the test with 2% glycerol revealed that, when using a synthetic minimal medium (MMS) with glycerol as a carbon source, biosurfactant production reached comparable values in a substantially shorter period compared to the medium traditionally considered ideal for *Bacillus subtilis* cultures. This analysis provides many considerations, particularly concerning cost-effectiveness and lead time. Therefore, this study presents data of great relevance that can serve as a basis for future research, highlighting the need for more in-depth and refined investigations in this promising biotechnology field.

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Potential Application of Bacterial Lipase from *Bacillus subtilis* in the Production of Biodisel

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This work aims to evaluate the potential of bacterial lipase of *Bacillus subtilis* in biodiesel production. Assays were performed to obtain, purify, and immobilize the enzyme and compare chemical and enzymatic transesterification tests. The Tryptic Soy Broth (TSB) culture medium without supplementation showed the highest enzymatic activity and purification yields when using 60% of $(NH_4)_2SO_4$. Citral was the most efficient agent for lipase immobilization. Even though the free enzymes showed a higher activity rate, the immobilized enzymes are associated with a better-quality product. In conclusion, the lipase enzyme showed positive potential in biodiesel production.
Keywords: *Bacillus subtilis*. Lipase. Biodiesel. Immobilization.

Introduction

The demand for sustainable fuel sources is a growing requirement for economic growth. Alternative sources of fuels are being researched to replace high-impact fossil fuels with greener options.

Biodiesel is a renewable fuel made from transesterification of biologically produced lipids. It is often carried out by a chemical process where triglycerides react with an alcohol, usually ethanol or methanol, generating two products: esters (biodiesel itself) and glycerin [1]. However, various catalysts can also be employed in the transesterification process, including alkaline, acid, and biological catalysts [2].

Lipases are triacylglycerol-acyl hydrolases that are used to replace the chemical transesterification process. Lipases have broad specificity to the substrate and their functional groups or enantioselectivity [3]. The enzymatic-driven process generates fewer byproducts or chemical wastes. However, the performance of enzymatic transesterification is limited by the need for knowledge of different enzyme potentials and methodological approaches.

Immobilization is an easy process that can be applied to improve enzyme transesterification performance. It can increase the number of reacting agents (enzymes) and possibly use them several times. It is possible because immobilization sustains the conformational structure of the enzyme [4]. This work aims to evaluate the potential of lipase bacteria from *Bacillus subtilis*, free and immobilized, in the conversion of oils into biodiesel.

Materials and Methods

The Figure 1 presents the experimental steps of this study.

Obtaining and activating the *Bacillus subtilis* strain

The strain of *Bacillus subtilis* came from the isolation carried out in the doctoral thesis of Tatiana Oliveira do Vale, obtained at the Microbiology and Bioprocesses Laboratory Moacyr Durham de Moura-Costa - Federal University of Bahia (UFBA).

A loop of the petri dish was removed with the microorganism and sown in Erlenmeyer of 250 mL containing 50 mL of TSB medium. The culture medium was kept at 37°C under agitation of 130 rpm for 24 hours. Then, 12-16h overnight

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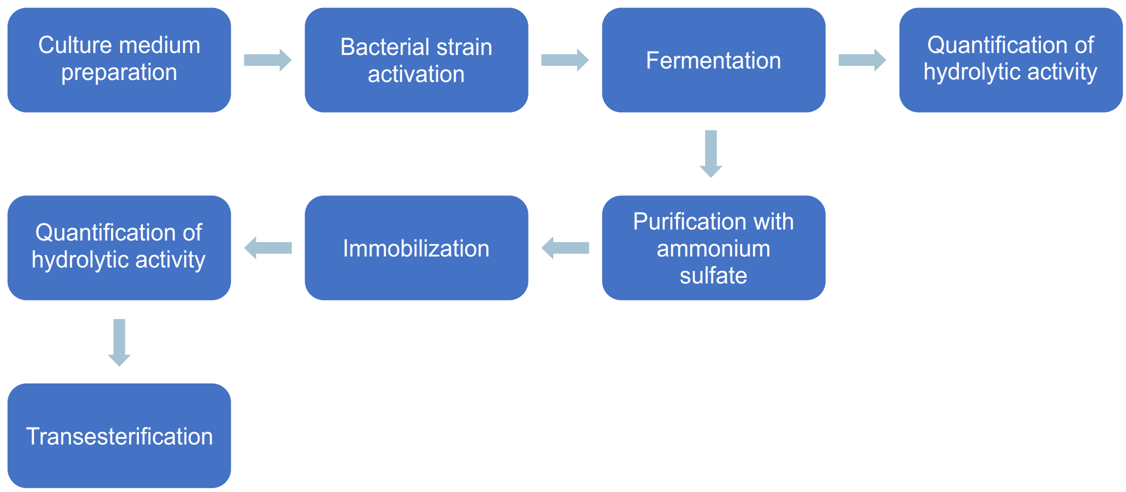
Figure 1. Flowchart of experimental steps performed in the study.



was performed to standardize the number of microorganisms.

Culture Medium Preparation and Fermentation

Three culture media were used for the growth of the bacteria: TSB, TSB 10x diluted and with 1% olive oil, and SMM (Synthetic Mineral Medium) with 1mL of trace element solution and supplemented with 1% olive oil; the media were sterilized in autoclave 121°C for 20 minutes.

For fermentation, 10% of inoculum was added in each fermentative media, growth was carried out for 72h, under agitation of 180 rpm at 37°C, and aliquots were removed for 0h and 72h. These aliquots were centrifuged at 4,000 rpm for 20 minutes at 25ºC to obtain the cell-free broth. This stage and the following were performed in triplicate.

Purification of the Enzyme

In the purification step, 15 mL of the enzymatic extract was removed, then 6.354g of ammonium sulfate was added for 60% saturation and 4.236g of ammonium sulfate for 40% saturation. The reaction medium was placed in a magnetic stirrer until the salt was dissolved entirely [5].

Immobilization in Chitosan

For the immobilization, 0.18 g of chitosan was solubilized in 6 mL of 1% acetic acid and precipitated in 1M NaOH solution (12 mL). The solution of the acid medium was dripped with a syringe in the alkaline solution and kept under gentle agitation of 90 rpm in an orbital agitator for 24 hours at room temperature [6].

Then, two substances were tested for activation, one with Quaternary ammonium and the other with Citral; the activation occurred for 1 hour at 90 rpm. After activation, 10 mL of enzymatic solution was added. The solution was kept in agitation at 90 rpm for 4 hours at 4°C so that the enzyme could bind to the support.

Quantification of Hydrolytic Lipase Activity by Titration

The reaction mixture, composed of gum arabic 6% and tributyrin, along with the enzymes (free or immobilized), was incubated at 37º C for 30 min at 160 rpm. Then, the reaction was interrupted with a 1:1:1 (v/v/v) solution of acetone:ethanol: water and titrated with a standardized 0.05 M NaOH solution. The white reaction was prepared using the same method described above, except for adding the enzyme. [7]

Transesterification

For transesterification, 50 g of soybean oil and 20 g of methanol (25.25 mL) were used, having as chemical catalysts 0.5 g of NaOH and as biological catalysts 0.1 mL and 0.5 mL of free enzyme and 0.1g and 0.5g of immobilized enzyme [8].

For both, it was counting the time of 1 hour after the change of color of the reaction; after the determined time, the solution was placed in a separation funnel so that the separation by the density of biodiesel and glycerin occurred, washing steps, and finally,y the biodiesel was filtered with filter paper.

Results and Discussion

Fermentation

Among the culture media tested was the one that obtained the highest enzymatic activity with 1.11 U/mL. In contrast, the TSB medium obtained a deficient activity, being considered as zero, comparing the media with the addition of oil (MMS and TSB 1%); the synthetic mineral medium showed the highest enzymatic activity (0.72 U/mL) (Figure 2).

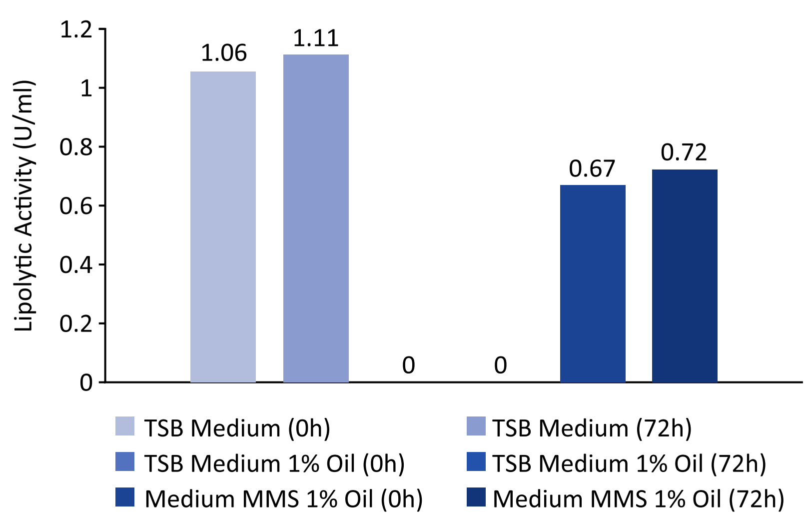
The production of lipase by other species of *Bacillus* is reported in the literature, as the use of *Bacillus licheniformis* that using as a carbon source orange flour obtained a variation of 1.19 to 4.44 IU/mL in its enzymatic activity [5], while the cultivation of *Bacillus megaterium* in modified LB medium obtained a maximum activity of 0.07 IU/mL [6]. These results show that the production of lipases can be improved depending on the growing conditions, allowing more space for research in the area.

Precipitation of Lipase

Precipitation with ammonium sulfate is based on differential precipitation in high concentrations of ionic salts. Two concentrations of ammonium sulfate saturation in the enzyme, 60% and 40%, were tested. They presented better purification to saturation with a 60% concentration of $(NH4)_2SO_4$ (Figure 3).

Similar results are found in other studies, where the use of an 80% concentration of ammonium sulfate obtained an increase in the specific enzymatic activity of the crude extract after purification, going from 0.29 to 17.74U/mg of

Figure 2. Comparison between the enzymatic activity of TSB Medium, TSB Medium 1% oil, and Synthetic Mineral Medium.



specific activity from vegetable lipase of soybean oil [7], in another research used ammonium sulfate for purification of the enzyme *Bacillus* sp. lipase having an increase in enzymatic activity from 6.531 U/mL to 67.899 U/mL [8].

Enzyme Immobilisation

Two reagents were tested for chitosan activation: quaternary ammonium (NH_4^+) with two concentrations, 1% and 0.5%, and Citral. The activation with ammonium quaternary was ineffective, while the activation with Citral was successful, presenting an average activity of 3.89 U/g, with a yield of 74.5% (Figure 4).

The activation of chitosan occurs through a nucleophilic attack between the amino group of chitosan and the carbonyl group of compounds such as glutaraldehyde, formaldehyde, and methane [9]. The presence of the aldehyde group in Citral indicates the positive result of immobilization due to the possible interaction between this compound's carbonyl group and chitosan's amino group.

Figure 3. Lipolytic activity precipitation with ammonium sulfate.

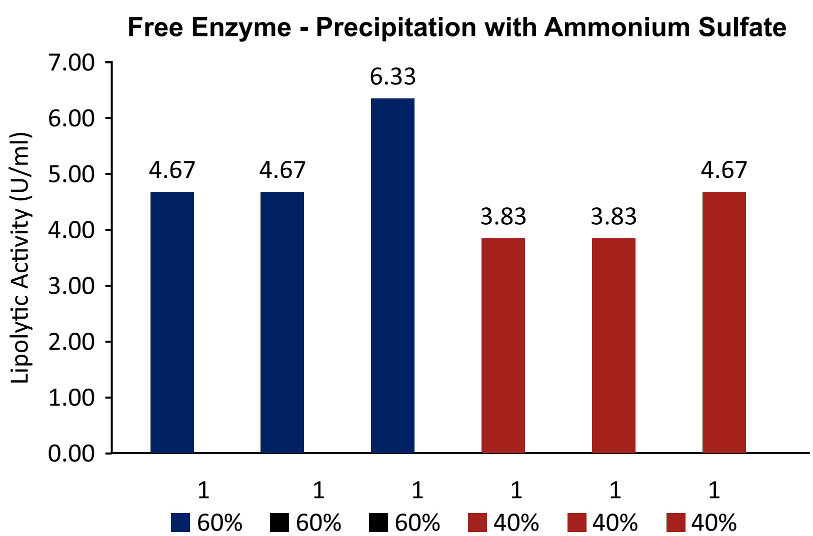
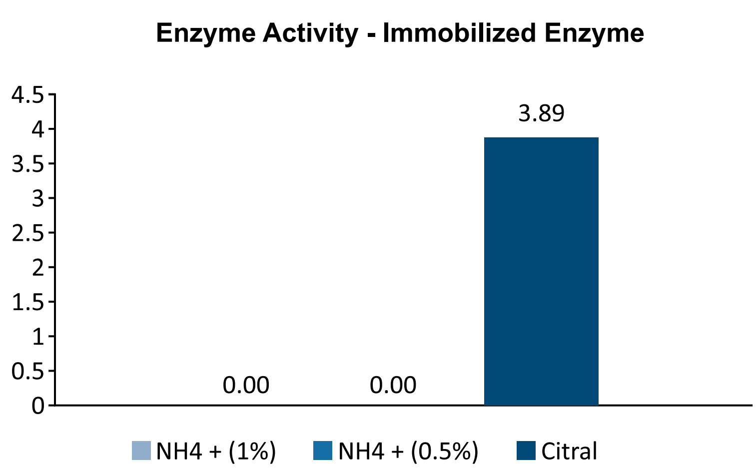


Figure 4. Comparison between the lipolytic activity of NH_4^+ and citral.



Quantification of Lipolytic Activity

After purification, the extract with a 60% concentration showed the highest activity, having an average of 5.22 U/mL, while with 40%, it presented only 4.11 U/mL. The enzyme immobilized with NH_4^+ presented a shallow activity, considered in this work as zero, for the concentration of 60% of ammonium sulfate; the enzyme immobilized with Citral presented an average activity of 3.89 U/mL (Table 1).

Biodiesel Synthesis

The density of biodiesel is linked to its overall molecular structure; biodiesel shows higher density if most lipids have long carbon chai; biodiesel combustion quality is also linked to the number of unsaturated lipids comprising the mixture [10]. Therefore, titration, acidity index, free fatty acids, and ester content were carried out to estimate the quality of the biodiesel generated with enzymatic transesterification (Table 2).

As none of the produced samples reached 96.5% of esters described by the ANP, it is impossible to classify them correctly as biodiesel. However, it is possible to analyze the processes that obtained the values most similar to the standard requested. The results show that the biodiesel produced using immobilized enzymes is close to reaching the standard parameters described by the ANP. When using 0.1 g of immobilized enzymes, the product showed the best acidity index, density, and the highest levels of esters.

Conclusion

TSB without supplementation was identified as the best medium for the growth of *Bacillus subtilis*. We observed that purification with ammonium sulfate with 60% concentration helped recover the enzymes and retained the highest enzymatic activity. Citral was the most efficient agent for lipase immobilization. Free enzymes showed a higher enzymatic activity than the immobilized ones. However,

Table 1. Data enzymatic activities: free enzyme and immobilized enzyme.

Immobilized Enzyme (U/g)			**Free Enzyme (U/mL)**	
NH_4^+		Citral	60%	40%
1%	5%	3.89	5.22	4.11
0	0			

Table 2. Biodiesel quality analysis.

Quality Parameters	**Values Found**					**ANP**
	1*	2*	3*	4*	5*	
Acidity Index (mgKOH/g)	1.40	0.67	0.61	0.53	0.44	0.50
Free Fatty Acids	0.70	0.33	0.31	0.26	0.22	NC*
Ester Content (%)	2.8	1.4	2.8	11.2	12.6	96,5
Density (g/mL)	0.87	0.80	0.95	0.85	0.98	0.85 a 0.90

1* = Biodiesel with chemical catalyst; 2* = Biodiesel with enzymatic catalyst (0.1mL); 3* = Biodiesel with enzymatic catalyst (0.5); 4* = Biodiesel with immobilized enzymatic catalyst (0.1g); 5* = Biodiesel with immobilized enzymatic catalyst (0.5g); NC* = Not listed.

the transesterification product was closer to ANP standards when using 0.1g of immobilized enzymes. Therefore, the lipase enzyme obtained from the *Bacillus subtilis* could undergo transesterification during biodiesel production. The present study demonstrated the potential of applying enzymes to replace the chemical transesterification process during biodiesel production. It is a valuable contribution to the biofuel industry and should receive special attention or further research to improve yields and large-scale applications.

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Preliminary Assessment of Fuel Spill Effects Utilizing *Daphnia similis* as a Test Organism

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Human interference in the aquatic ecosystem has created an unideal environment fpo-ooor aquatic creatures, emphasizing accident fuel and oil leaks from offshore cargo ships and offshore oil drilling, respectively. In this context, a preliminary study was conducted to analyze further the effects that a fuel spill causes in a water environment. The methodology used the standard method of conducting ecotoxicological essays using *Daphnia similis* but was adapted for a preliminary context, using fewer resources to obtain a primary analysis. The results indicate that fuel spills are still dangerous to underwater life, even in low concentrations. This study showcases the immediate effects of fuel spills and is still open for further experimentation.
Keywords: *Daphnia*. Ecotoxicology. Fuel. Assessment.

Introduction

There is a crescent number of studies utilizing *Daphnia* as a stakeholder for their essays in Ecotoxicology. The organisms are more sensitive and allow for a finer analysis of chemical compounds and effluents [1,2]. In this fashion, *Daphnia* are the organisms used to monitor the quality of freshwater ecosystems. It has become a staple, and its application has grown exponentially in ecotoxicology studies. The *Daphnia* used in this essay belongs to *Daphnia similis*. Its anatomy is very similar to other *Daphnia* species, such as *Daphnia magna* and *Daphnia pulex*. In tropical climates, it is more common the use of *Daphnia similis* because these species are more adapted to living in tropical conditions [3].

Daphnia Anatomy and Life Cycle

The anatomy of *Daphnia similis* (Figure 1) is very similar to those of other *Daphnia* species, although other species may present more eggs per organism and a higher sensibility [4-6]. The organism has a pretty simple structure and is easy to visualize due to its transparent body. Also, they are microscopic organisms (about 0.5 to 5.0 nm), being easy to manipulate in a laboratory setting.

A *Daphnia*'s life cycle is concise. For laboratory uses, the cycle goes for 28 days. Up until the first 24 hours of life, the organisms are called neonates or newborns; from the first day to the seventh day, they become younglings, and from the seventh day forward, they enter the reproductive stage, ending on the 28th day, when the organisms are discarded [8]. Although every organism cultivated in a lab is a female, there is some chance of males appearing in the environment. It is commonly found in nonformidable settings for cultivating *Daphnia* species or when there is any stress caused by external means to the organisms, such as loud noises or shaking. All of this will result in deceased and/or paralyzed organisms, together with ephippium (Figure 2), that are always discarded.

Fuel Contamination Context

The aquatic ecosystem has been suffering from the impacts caused by human intervention in nature, which increases the amount of spill from numerous chemical compounds. This causes a decrease in the quality of life of the species that inhabit the water [9]. Water contamination through accidents of cargo transport ships leaking

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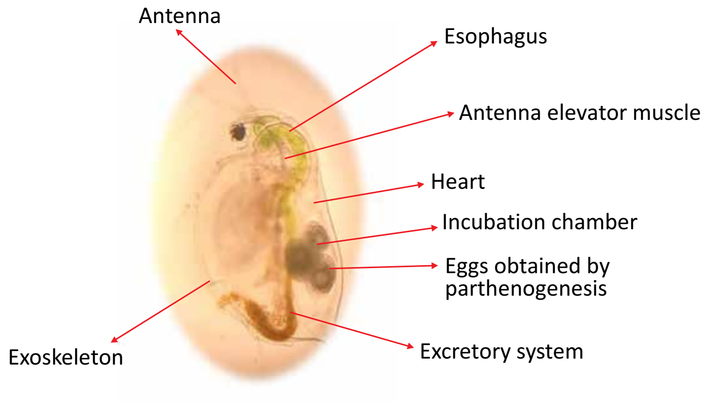
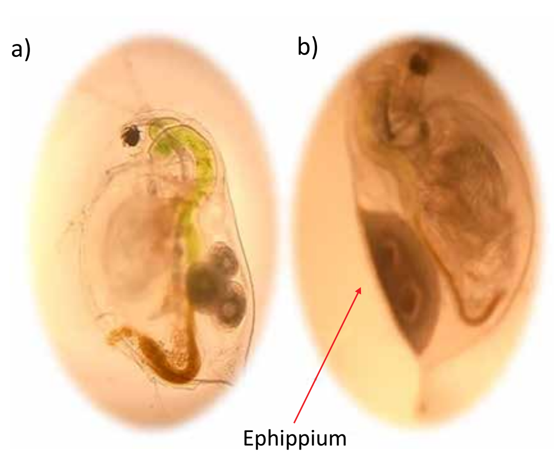
Figure 1. *Daphnia similis* anatomy.



Source: adapted from Oliveira and colleagues [7].

Figure 2. Comparison between a healthy organism with parthenogenesis eggs and an ephippium.



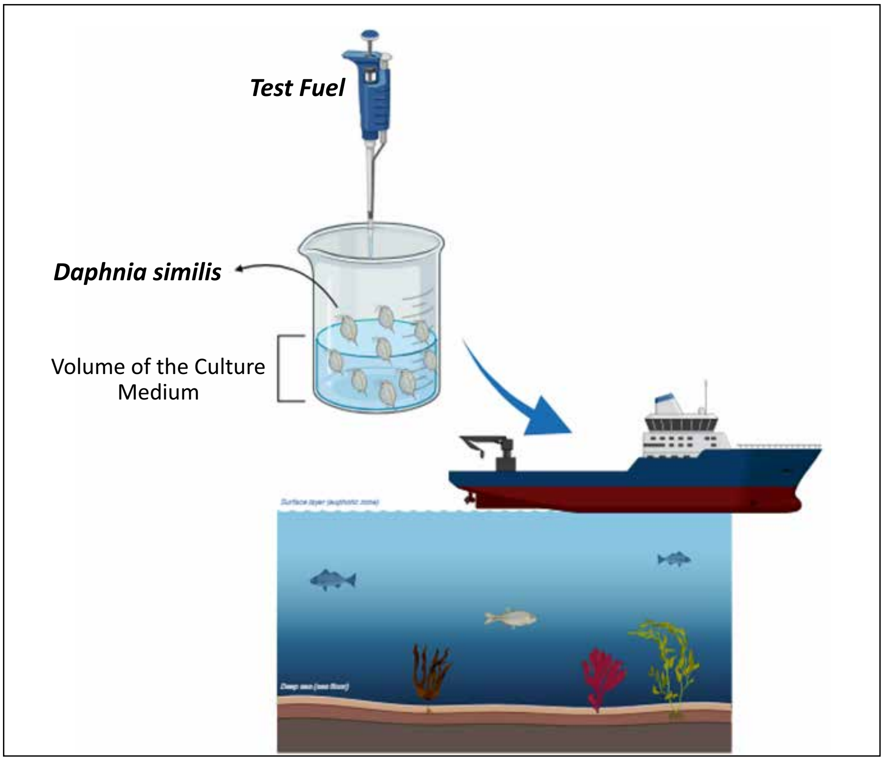
or offshore oil drilling has aroused some concern in the world [10]. In this manner, this study aims to show the immediate preliminary effect of a fuel spill in a freshwater environment using four different compounds and *Daphnia similis* as a test organism.

Materials and Methods

The tests aimed to simulate a fuel spill, with 10 organisms (*Daphnia similis*) per replica mixed with fuel in 50 mL of culture medium (Figure 3). The volume of fuel needed for each replica was the dilution factor, which consists of dividing the total volume by a certain number to obtain a percentage that determines the fuel concentration in correlation to the total volume of the test solution. In this fashion, the percentages chosen for the essay were 0.1%, 0.05%, 0.024%, 0.012%, and 0.006%. Nevertheless, as this was only a preliminary test to evaluate the instant effects, only the most negligible concentration (0.006%) was used.

The organisms were distributed in 5 replicas (10 organisms in each one), one replica for each fuel, and a control (containing only the culture medium).

Figure 3. Illustration representing the method used for the preliminary tests.



The fuels used for this study are showcased in the following table (Table 1) and their specifications.

Results and Discussion

After adding the fuel in each replica, it was noted that the replicas containing both the HVO and the S10 diesel had an immediate effect of immobilization at the surface of the test solution with 100% efficiency. However, some organisms were still moving with restrictions in the S10 replica, but in the HVO, there was complete paralysis. E1G and E2G had the opposite effect; the organisms were not immobilized nor paralyzed and kept moving like normal. More tests must be conducted to establish a final result using the higher fuel concentrations chosen for the final essay. The volume of the test solution could also be changed for a more realistic approach since there is more water than animals in freshwater and saltwater ecosystems.

Table 1. Fuels used for this essay.

Test Fuels Used	Specific Mass (Kg/m³)	Water Content (%/M/m)	Viscosity (40°C)
S10 Diesel	835.7	0.11	3.48
HVO	774.07	0.10	3.89
Ethanol 1 G (E1G)	809.8	7.24	Not detected
Ethanol 2 G (E2G)	795.4	1.83	Not detected

Conclusion

This study is open for future work. Those tests must be done with higher concentrations to measure the full effects of contamination through fuel spill in a water environment, emphasizing S10 diesel and HVO since those had the more immediate effects in the *Daphnia*.

Acknowledgments

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Use of Biomass from Beached Algae of the Genus *Caulerpa* to Obtain the Alkaloid Caulerpin

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Caulerpin is an alkaloid with various biological activities, such as antinociceptive, anti-inflammatory, and antitumoral. This natural product is the majority in algae of the genus *Caulerpa*, which are found fixed to rocks on the seabed and beached to the sands. The objective of the research was to evaluate the possibility of using the biomass of these marginalized algae to obtain the alkaloid. Using ultrasound extraction and analysis by gas chromatography coupled with mass spectrometry, the results showed that it was possible to identify caulerpin in the beached algae. Furthermore, the alkaloid showed good resistance to photodegradation since caulerpin remained practically unchanged after 7 days of exposure to sunlight.
Keywords: Biological Activities. Marginalized Algae. Extraction, Analysis. Photodegradation.

Introduction

The seas and oceans are vital resources for the global economy, which justifies the growth of their productive activities in recent decades [1]. The bioprospecting of natural products in the seas has been increasingly valuable for the pharmaceutical industry since they stand out as a source and inspiration for several drugs currently marketed [2]. Macroalgae play an essential role in the market since they are one of the largest producers of chemically active metabolites with valuable cytotoxic properties, which can treat diseases like cancer [3]. Thus, regarding biological activities in marine natural products found in algae, caulerpin is a molecule that stands out [4].

Caulerpin is a bisindolic alkaloid, reddish-orange in color and molecular formula $C_{24}H_{18}O_4N_2$ (Figure 1) [5]. This molecule plays a crucial role in medicinal chemistry and as a chemical agent, possessing several therapeutic potentials such as antidiabetic, antinociceptive, anti-inflammatory, antitumoral, anti larvicidal, antiherpes, antitubercular, antimicrobial and immunostimulant, among other activities [4]. It is found in macroalgae of the genus *Caulerpa* [5], especially in the species *Caulerpa racemosa*, found on the Brazilian coast [6]. In addition to being attached to rocks and even associated with other algae on the ocean floor, algae of the genus *Caulerpa* may also be beached to the sand due to a natural process in which tidal activity eventually causes the algae to detach from the surface, where they are attached, leading them to the beach [7].

These residual algae can undergo various degradation processes, mainly due to heat and ultraviolet light, because of the high exposure to sunlight on the shores of beaches.

However, how some molecules respond to light exposure may differ, they do not undergo as much change and are more stable than others algae [8,9]. The utilization of the biomass of these algae emerges as an extraordinarily sustainable and highly productive initiative for giving utility to these resources, given as dysfunctional, by obtaining powerful natural products with biological actions.

This work aimed to extract the caulerpin of the biomass of the beached algae genus *Caulerpa*. In addition, the photostability of caulerpin was evaluated for more economical and sustainable extraction.

Materials and Methods

The collection of live algae of the species *Caulerpa racemosa* occurred on the ocean floor,

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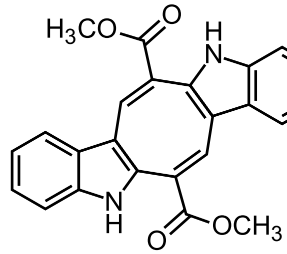
Figure 1. Caulerpin.



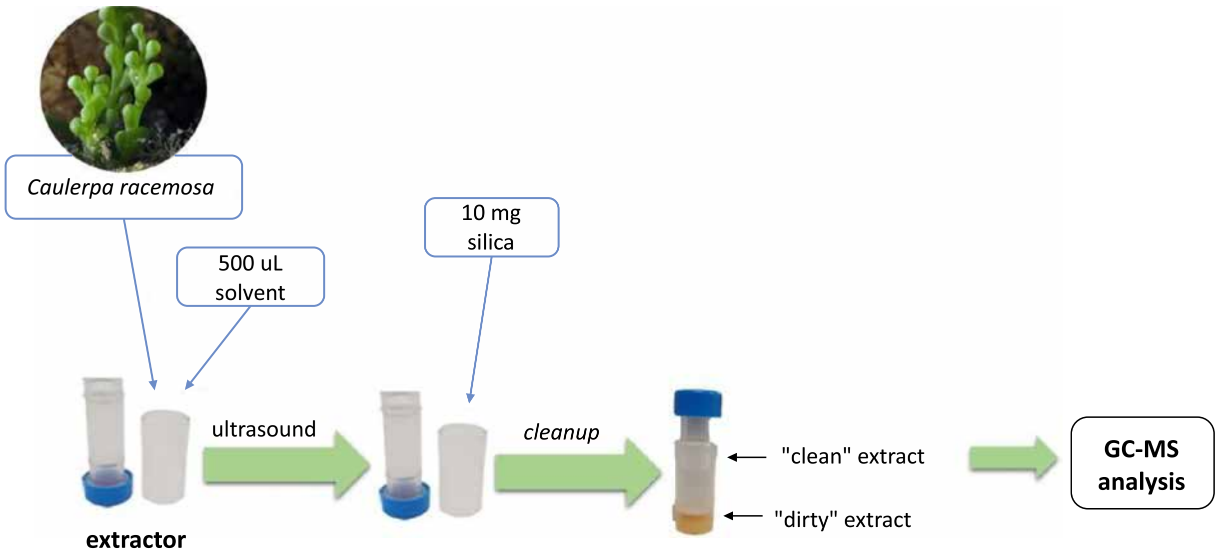
on the coral reef via scuba diving at a depth of 8-16 meters in the Baía de Todos os Santos. After this step, the algae were transported in a thermal box containing ice bags to the Federal University of Bahia (UFBA), where they were sorted and freeze-dried in an SL-404 Solab lyophilizer (Piracicaba, Brazil) for 42 hours and then ground in a ball mill Cienlab (Campinas, Brazil). The ground samples were transferred to clean 50 mL glass flasks, covered by aluminum foil, and transported to the Integrated Laboratory of Applied Research in Chemistry (LIPAQ) of SENAI CIMATEC, where the procedures continued. The Caulerpin standard was obtained according to Cantarino and colleagues [10]. After isolation, caulerpin was analyzed in a Shimatzu gas chromatography coupled to a mass spectrometer (GCMS-QP2020 SE) equipped with OAC-20i autosampler and DB-5 column (30 m × 0.25 mm DI, 0.25 μm film, Agilent, Waldbronn, Germany) into the SCAN mode. The chromatographic conditions were: after 1 min at 220 °C the temperature was increased by 5 °C min^{-1} to 300 °C and held at 300 °C for 20 min. A 1 μL sample aliquot was injected in splitless mode at 290 °C. High-purity helium (99.999%, White Martins, Brazil) was used as carrier gas at a column flow rate of 1.40 mL min^{-1}. The mass spectrometer was operated in electron ionization mode (EI, 70 eV), starting at 40 m/z and ending at 400 m/z. The ion source and transfer line temperature were maintained at 250 ◦C and 280 ◦C, respectively. To extract the caulerpin from the live algae collected, they were crushed with sanitized scissors to begin the ultrasonic extraction, adapted from Cantarino and colleagues [10]. This method was chosen due to the extraction capacity in a short period with the use of a small amount of sample mass. The extraction occurred in a micro extractor flask (Whatman Mini-Uniprep Syringeless Filters, Cytiva, USA), adding 10 mg of crushed algae and 500 μL of ethyl acetate as solvent. The flask was directed to the ultrasonic bath (Nova Instruments, model NI 1208, 220 Volts), which remained for 20 minutes. Then, 10 mg of silica was added as a cleanup step to the extract, which was manually stirred and filtered in the micro extractor, which has a filtering membrane. After the extraction, the sample was analyzed by gas chromatography coupled to mass spectrometry under the same conditions and method as the standard was analyzed. After performing on the algae collected alive, the same methodology of extraction and analysis of caulerpin (Figure 2) was applied to the beached algae collected in Praia do Forte, a region located in the municipality of Mata de São João, Bahia. However, unlike those collected alive, these algae underwent a natural drying process with sunlight instead of being lyophilized.

For the caulerpin photostability assays, a freeze-dried algae sample collected alive was placed in a watch glass and exposed to sunlight for 7 days in a ventilated, covered environment with solar solid incidence. After that, the sample was crushed with scissors and subjected to a granulometric sieve of 80 mesh, where the granules were homogenized. Finally, the algae sample exposed to sunlight underwent the extraction and analysis process, together with an algae sample that was not exposed to the sun (Figure 1), for comparative purposes, in which the latter underwent the same grinding and homogenization process as the sample exposed to the sun.

Results and Discussion

Figure 3a presents the GC-MS chromatogram for the isolated standard caulerpin from the algae

Figure 2. Microextraction of the algae *Caulerpa racemosa*.



collected alive, with a retention time of 33.09 minutes. Moreover, the mass spectrum (Figure 3b) generated by the peak referring to caulerpin corresponded with the literature data, in which it is possible to observe the molecular ion peak as the base peak of the spectrum of m/z 398. The fragments referring to successive losses of methanol (MeOH) and carbon monoxide (CO), of m/z 366 (M - MeOH), m/z 338 (366 - CO), m/z 306 (338 - MeOH) and m/z 278 (306 - CO), reveal the breakdown of the caulerpin molecule [11].

According to the method, caulerpin was extracted and analyzed from 10 mg of live algae collected. The extraction process was initially carried out with algae that were not beached to ensure that caulerpin would be obtained. In the total ion chromatogram, it was possible to observe the peak referring to the alkaloid (Figure 4), confirmed by comparing the retention time and mass spectrum with the standard (Figure 3b) and with data from the literature [11].

Once the caulerpin was confirmed and the extraction and analysis methodology were optimized, the same process was carried out on 10 mg of beached algae. Once this procedure was carried out, a total ion chromatogram was generated, whose peak referring to caulerpin was also identified (Figure 5), with a mass spectrum generated identically to that found previously.

When analyzing the chromatogram (Figure 5), it was noted that the peaks not referring to caulerpin were less intense than that of the alkaloid, i.e., the extract was purer than that evidenced in Figure 4. As previously presented, the algae marginalized in the beach sand suffer from the incidence of sunlight, in addition to the fact that the beached algae sample was dried naturally with sunlight instead of freeze-dried. Thus, these results served as a basis for studying the photostability of caulerpin. In this sense, a fraction of algae collected alive and lyophilized was exposed to sunlight for 7 days to show the difference in the total ion chromatogram. Thus, when performing the extraction and analysis of this sample, simultaneously with a fraction of the same algae without having been exposed to the sun, to generate a more reliable comparison, it was found that the peak referring to caulerpin practically did not change after 1 week of exposure to the sunlight (Figure 6).

The results from Figure 6 and Figure 7 allowed a qualitative analysis that the peak referring to caulerpin remained practically unchanged, and the extract was cleaner after 7 days of exposure to sunlight. It suggests that caulerpin has a high resistance to photodegradation.

Figure 3. Results of the analysis of the caulerpin standard. (a) Total ion chromatogram of the standard. (b) Mass spectrum of caulerpin.

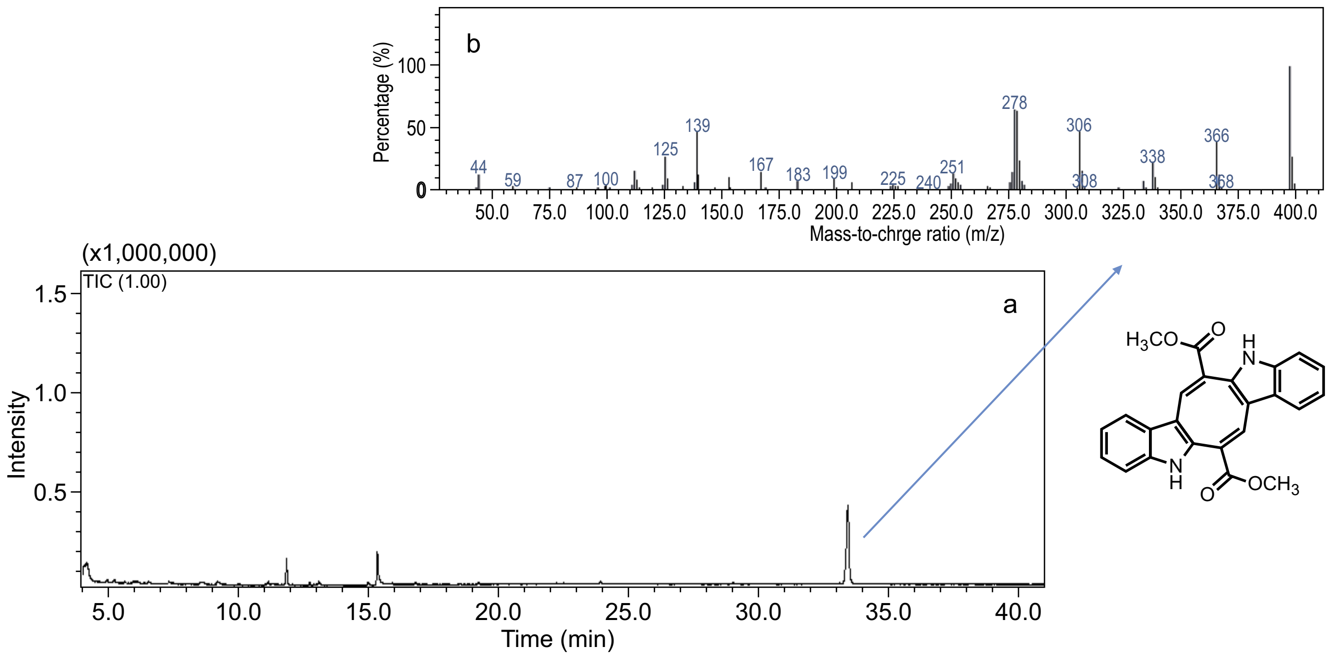


Figure 4. Total ion chromatogram of the algae sample collected alive.

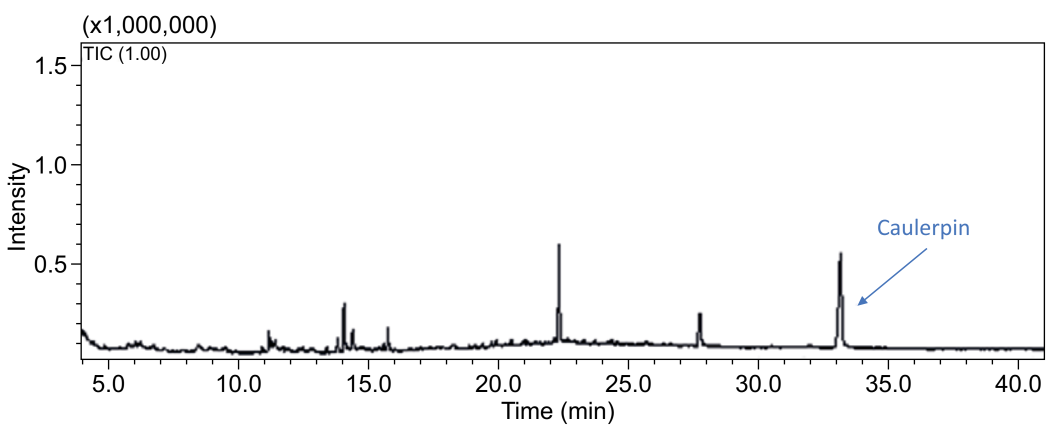


Figure 5. Total ion chromatogram of the beached algae sample.

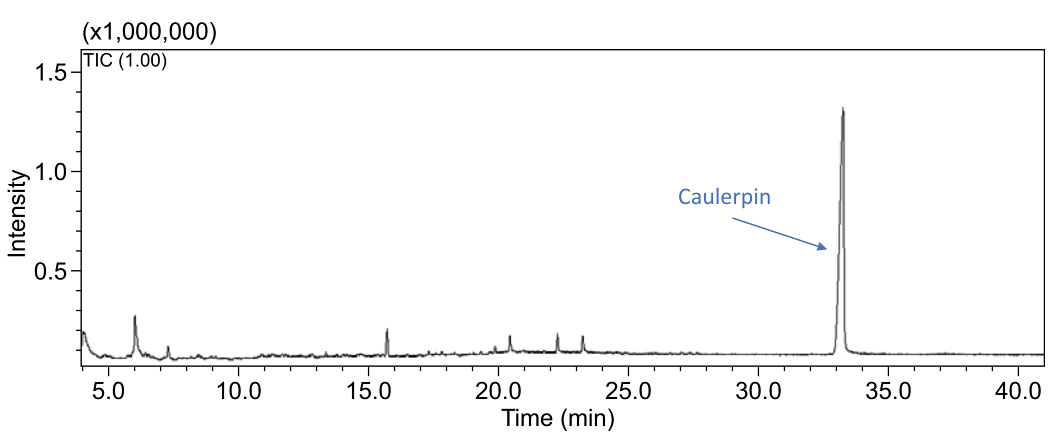


Figure 6. Total ion chromatogram of the freeze-dried live algae sample.

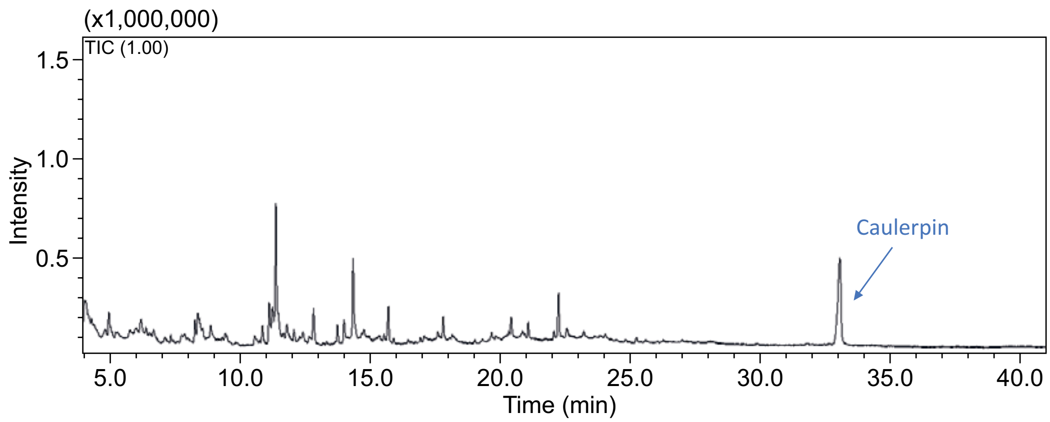
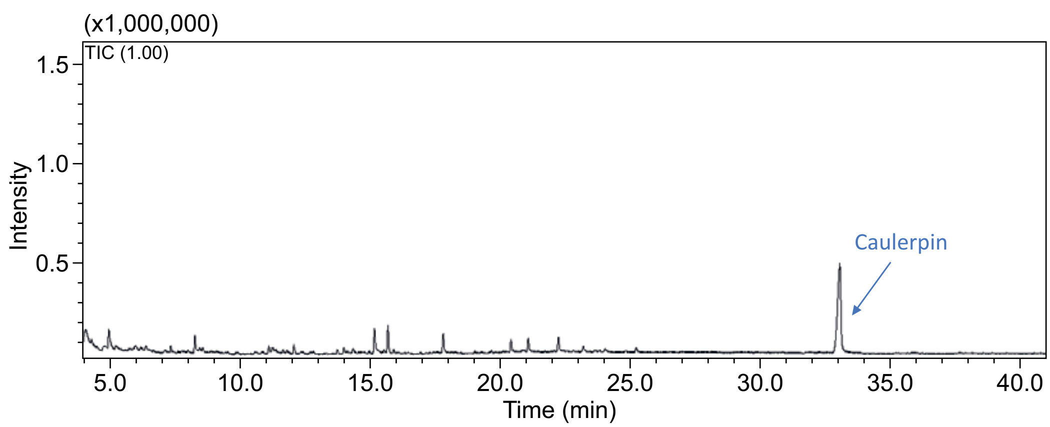


Figure 7. Total ion chromatogram of the freeze-dried live algae sample exposed to the sun for 7 days.



Conclusion

Finally, it was possible to verify that the residual algae, although degraded, can be helpful since, by applying the extraction and analysis methodology on beached algae, the presence of caulerpin was confirmed by identifying the corresponding peak and its mass spectrum. This result prompted photostability studies of the alkaloid, which proved remarkably sunlight resistant. Therefore, the results presented propose a much more dignified destination for the large biomass of algae harbored in the sands of the beaches since these can contain essential natural products with various biological activities, which can be helpful for the most diverse areas of the industry. Hence, using beached algae of the genus *Caulerpa* is a very sustainable and intelligent alternative.

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Technological Prospection and Development of Smart Film with Phytochemical Actives such *Curcuma longa L.*

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Biodegradable plastics are in evidence regarding packaging, especially for food. Innovative products have their stages better targeted when understanding what has been protected by patents and identifying unexplored trends. This work makes technological prospection and does a film based on PVA added with a dry extract of *Curcuma longa*, evaluating its function as a barrier to light transmission in five concentrations. The results showed that films with different colorings were rigid and slightly malleable. As a barrier to light transmission, the product proved efficient as the concentration of *Curcuma longa L.* increased. The films have been presented as an excellent option for the light barrier; improvements are necessary regarding the solubility of the active in the filmogenic solution.
Keywords: Active Packaging. *Curcuma longa*. Biodegradable Film.

Introduction

Since the most remote time in history, the various species of humans inhabited the earth have lived self-sufficiently and without worrying about storing food [1,2]. When that need arose, nature provided gourds, shells, and leaves. "Later, containers were manufactured with natural materials, such as excavated logs, grasses, and animal organs" [1,2].

With the evolution of society, there was an increasing need to pack food and other goods, whether for storage and conservation or transportation. With the advancement of discoveries with the brilliance of the Industrial Revolution, it began implementing an innovative packaging discovery: plastic [2].

An article of use as diverse as plastic soon followed, applied in almost all branches of society. It led to problems such as the accumulation of plastic waste and its disposal. It is estimated that around 8 billion kilograms of plastic have been produced by 2018 and that the same amount is floating through the oceans yearly. The problems caused by this accumulation are immense, directly affecting marine and human life [3,4].

The demand for increasingly resistant packages (which support even more weight and withstand variables such as temperature and pressure), efficient (which also contribute to the physical and chemical stability of the products they involve) has had a significant increase with the advancement of plastic, a result of population growth and an exponential increase in consumption. Also, over the years and with the recurring problems related to its excessive use, it became necessary to search for alternatives for ecologically correct plastic packaging, understood as bioplastics or biobased plastics, since they are biodegradable, minimizing the problem of disposal [3-5].

Therefore, this work consists of studying current technologies protected by patents on the biodegradable film with antimicrobial and/or antioxidant activity and developing a biodegradable PVA film with *Curcuma longa L.* as an active and evaluation of specular light transmission.

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Active Packaging

For packaging to be considered active, it must present other barriers to protecting and conserving food besides the physical and inert ones to external influences. It must be composed of assets that interact with the person and the environment to provide more safety and durability to food. Thus, it is possible to obtain, for example, plastic films with antimicrobial and/or antioxidant action, plastic sachets with humidity control, and films with temperature-sensitive permeability, among others [6-8].

Several studies are emerging regarding the development of active packaging, the main focus being the production of antimicrobial films, oxygen absorbers, ethylene absorbers, humidity regulators, releasers, and/or absorbers of heat and odors.

The *Curcuma longa L*.

Even in a time of high technology, there is an increasing search for vegetable raw materials (especially essential oils and extracts) or even synthetic ones (polymers with a shorter life cycle and biodegradable, even if they come from petroleum) with low environmental impact. A plant that has long been recognized for its antioxidant and antimicrobial properties and has been incorporated as another option for the production of active packaging is *Curcuma longa L*. [9].Polyvinyl alcohol (PVA) has been an essential polymer for the commercial production of active films because of its excellent characteristics, soluble in water, and biodegradability [10]. *Curcuma longa L*. is a plant belonging to the Zingiberaceae family. They are popularly known as saffron, saffron, Indian saffron, and other nomenclatures, depending on location. It is an herbaceous plant with large and long leaves with rhizomes that can reach 10 cm in length. When cut, they have a reddish-yellow color [9,10].

Curcuma longa L. is a plant native to India and Southeast Asia. It was introduced in Brazil in the 80s. Its use in India dates back to around 4,000 years BC. It was initially used for its nutritional value and later for its various ethnomedicinal properties, including antioxidant, antimicrobial, hypoglycemic, gastroprotective, and anti-inflammatory activity [9-11].

Materials and Methods

Technological Prospection

The database used in this work was collected on September 11, 2021, from the Espacenet platform, the European patent search office, with access to more than 130 million documents. The search was carried out in a space delimited as code C08L, with the following matched terms: packaging, biodegradable, antimicrobial, and antioxidant. From the data collected, it was possible to carry out a quantitative and qualitative analysis, aiming to map generated technologies and possible trends for new technologies involving bioplastics and their uses as packaging.

Materials

Polyvinyl alcohol polymer, also known as PVA (Dinâmica), solvent deionized water (*), and rhizome powder of *Curcuma longa L*. standardized in 95% of curcuminoids obtained commercially with certification were used for the production of the films.

Production of Film

The films were produced using the casting technique of pouring a filmogenic solution into small plates or molds. This technique is best adapted on a laboratory scale for producing plastic films [12].

Five samples were produced with concentrations between the range of 2 and 40 g/L (named A1 to A5) of *Curcuma longa L*.

The filmogenic solutions contain Polyvinyl Alcohol and of solvent deionized water. The polyvinyl alcohol was mixed with the rhizome powder of *Curcuma longa L.* and dissolved in distilled water, under agitation and heating, between 40 and 90 ºC in a Shaker Bath. The filmogenic solution was transferred to Petri dishes, where they dried for 6 days at room temperature.

Specular Light Transmission

The five samples of PVA films with the addition of the active *Curcuma longa L.* were prepared to evaluate specular light transmission and to know their barriers in the range of UV-visible light. Three samples of each film concentration were used. The methodology used was an adaptation of Bucci [13], and the equipment used was a Shimadzu UV-1800 UV-visible spectrophotometer adjusted to a scanning range of 250-750 nm. Readings were recorded at wavelengths 250, 350, 450, 550, 650, and 750 nm, and transmittance results were noted for each.

Results and Discussion

Technological Prospection

Figure 1 explains the number of patents resulting from technological prospecting, comparing their development from 1996 to 2021 and their cumulative result.

After analyzing the data obtained from the Espacenet database, it was possible to identify that works involving antimicrobial, antioxidant, and biodegradable biodegradable packaging, which generated patents, began to appear only in 1996. From 2009 to 2019, there was an increase in the number of deposits.

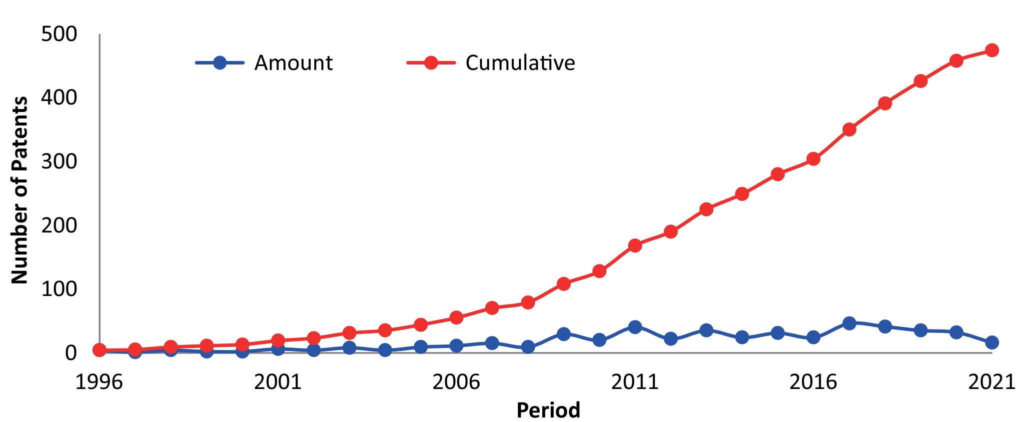
Figure 2 shows the total number of patents filed by country.

The most extensive records of patent deposits are from developed or developing countries that want to expand their technologies, such as the United States and China. Despite being one of the world's largest consumers of plastic and one of the largest generators of plastic waste [14], Brazil held only eight patents out of the 598 available in the database.

The Film of *Curcuma longa L.*

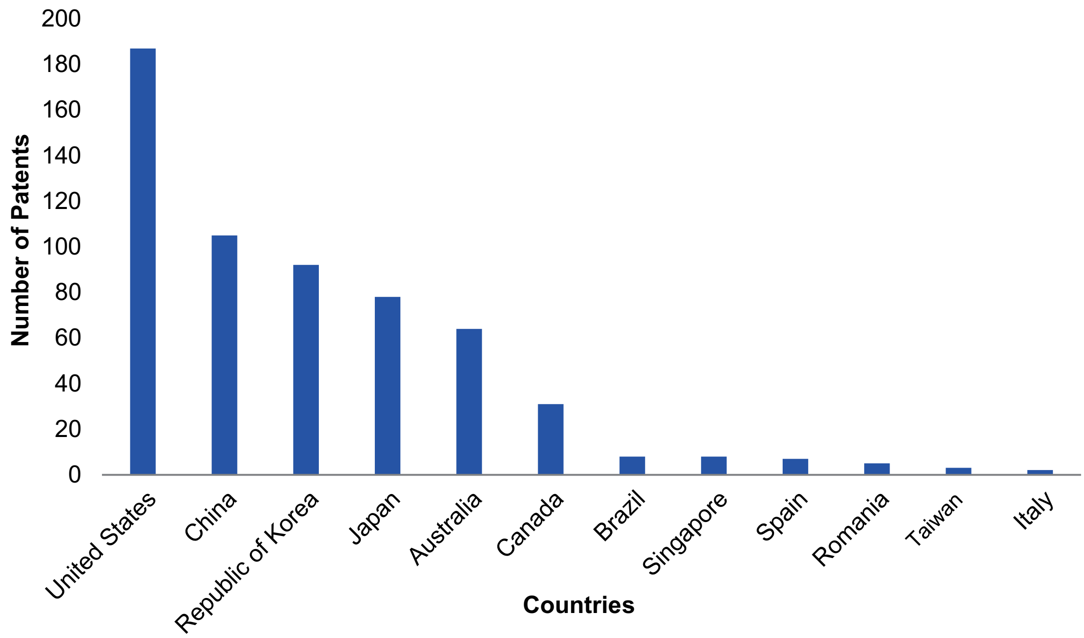
We considered the articcle from Niamsa and Sittiwet [15] to find the best concentration of active [15], was taken into account. The study concludes that the active Minimum Inhibitory Concentration (MIC) would be 4-16 g L^{-1}, while the active Minimum Bactericidal Concentration (MBC) would be 16-32 g L^{-1}. Tests were performed on five samples prepared. The resulting films did not have satisfactory aspects, as the high concentrations of the active did not completely solubilize in the

Figure 1. Ratio number of patents x initial year of application.



Source: Own authorship.

Figure 2. Depositor countries.



Source: Own authorship.

solution, leaving evident small lumps in various parts of the same and even regions where portions of the dry extract of *Curcuma longa*.

Due to the inability of the solvent to solubilize the active, the concentration had to be reduced, hoping to achieve a uniform film with excellent analytical reliability. In this sense, the methodology of [12] was adapted, which understood that when the concentration was reduced by 12,5 times from the MBC, the weight of the filmogenic solution would be adequate. Then, the concentration between the 2 and 40 g/L range (A1 to A5) was defined. Adaptation resulted in more uniform films with good apparent solubility despite still having insoluble microdots (especially at higher concentrations). The images in Figure 3 show the finished films at the five concentrations plus non-solubility points.

The results showed that more studies need to be conducted to find the ideal conditions to prepare the film with this performance using this method. Some studies suggest using surfactants to solubilize *Curcuma longa* and analyze the effective antimicrobial action of this product.

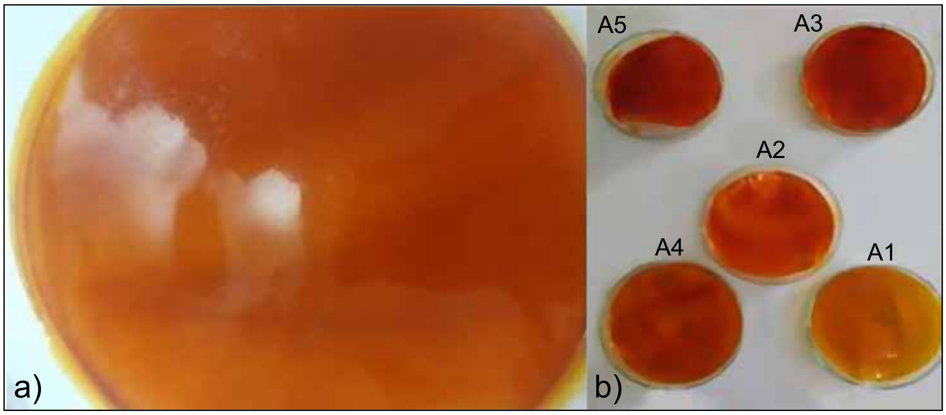
Specular Light Transmission

The results obtained from the light transmission analysis for the five different concentrations of *Curcuma longa L.* change according to the concentration. The readings were taken in triplicate, and an average was taken for each wavelength. From then on, an average of the wavelength averages was made, thus considering only one average per concentration, which was used to plot the graph shown in Figure 4.

Conclusion

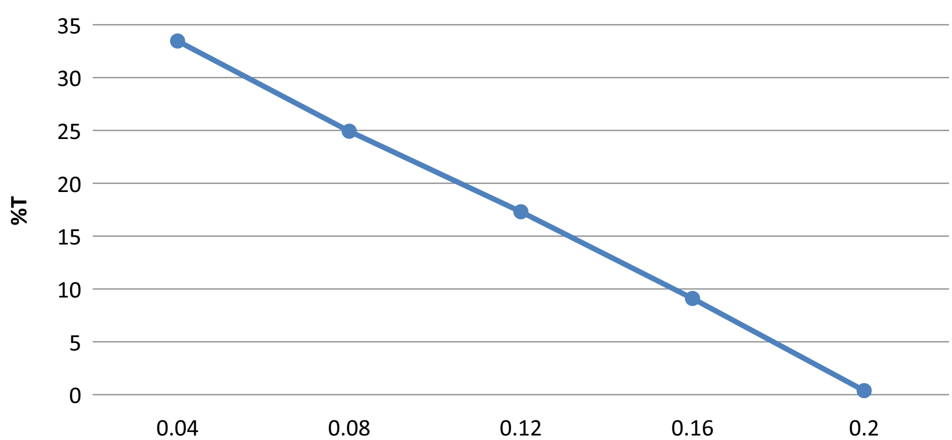
As for prospecting, the data show that from 2009, searches for research related to the development of biofilms with antioxidant, biodegradable, and antimicrobial characteristics took a turn, with their apex of records in 2017. Since then, it has been an active market and ascending. The leading countries in these records are the United States and China. Despite its recognized potential with natural products, Brazil has little expressive numbers in patent deposits,

Figure 3. PVA film (a) uniform appearance.



(a): Obtaining a film based on *Curcuma longa* with a uniform appearance; (b): Obtaining dry films with different concentrations of *Curcuma longa*.

Figure 4. Graphic representation of the 250-750 nm scan of *Curcuma longa L.*



perhaps requiring investment in the area. As for the development of the films, using the casting technique, which is the most used on a laboratory scale, the applied concentrations needed to be improved to reach the total solubilization of the active in deionized water. It suggests decreasing the concentration, changing the solvent, or even opting for prior preparation of a *Curcuma longa L.* solution with a higher affinity solvent. Based on the methodology used and the results obtained through the analysis of specular transmittance, it is possible to understand that the films developed have an excellent light barrier when made at high concentrations, extending their protection in the regions between 350-750nm, covering the ultraviolet and the UV-visible.

Acknowledgments

The authors thank the State University of Southwest Bahia for supporting this work.

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Study and Characterization of Vanilla Pods *Tahitensis* Species for Characterization for Future Application in Products Derived from Vanilla *in natura*

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The growing search for natural and healthier foods is a determining factor for studying and applying new routes for developing sustainable products. Therefore, this work aims to study and characterize the *V.tahitensis* vanilla bean for future application *in natura* vanilla extract. The physical-chemical and centesimal characterizations were carried out according to the methods described in the Adolfo Lutz Institute manual. The results obtained from the analyses were auspicious, and the intention is to develop the vanilla extract through a removal method that presents the best percentage of vanillin to be applied in the market.
Keywords: Vanilla Pods. Natural. Centesimal. Physical Chemistry. Characterization.

Introduction

Vanilla is a plant belonging to the *Orchidaceae* family; it originates from a flower, the orchid. Among its species, the most famous are *Vanilla planifolia* and *Vanilla tahitiensis*. On the world stage, vanilla pods are produced in tropical and subtropical regions, mainly in India, Indonesia, Mexico, Uganda, and Congo. Madagascar is currently the world's largest producer. In Brazil, Vanilla is cultivated south of the state of Bahia [1].

After harvest in the field, the pods go through a long process of beneficiation, drying, and cure to obtain the characteristic aroma, with vanillin primarily responsible for vanilla's aromatic composition, where its content varies according to the species and origin of the pod. Among the products derived from vanilla, one of the most prominent in the market is the commercial extract. The global vanilla extract market was valued at US$5.26 billion in 2021 and is projected to grow from US$4.94 billion in 2022 to US$6.30 billion by 2029 [1,2]. Commercial vanilla extract can be obtained in several ways. One is the percolation method, which consists of a circulating mixture of ethanol and water containing 35-50% alcohol for 30 days with constant agitation. Another known extraction technique is Soxhlet, in which an organic solvent, usually hexane, is used in an extraction chamber coupled to a condenser, with a temperature close to the solvent's boiling point and a time varying from 3 to 8 hours. Another method used is ultrasound, which consists of immersion in an ultrasonic bath at a frequency of 60khz and ethanol/water in different proportions as solvent [3-5]

Aiming to develop sustainable practices, mainly about sustainable production and consumption, companies have sought to create strategies and technological routes for sustainable development. Therefore, seeking to meet the Sustainable Development Goals (SDGs) of the United Nations (UN) is a significant differential, as it helps guarantee sustainable food production systems, in addition to helping maintain ecosystems [6]

Faced with the demand for healthier foods and natural sources to produce safer and more attractive products, the present work aims to develop a study and characterization of vanilla pods of the *tahitensis* species to carry out future application in products derived from natural vanilla based on analysis for centesimal and physical-chemical characterization, in addition to evaluating global market.

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Materials and Methods

Vanilla pods (*V. tahitensis*), *in natura*, and dehydrated after the extraction process, and the extract were supplied by Vanilla Brasil. Vanilla pods were received and underwent a pre-treatment stage, which consisted of drying the beans in a dehydrator (Figure 1A and 1B) for 36 hours at 40 °C. After drying, the beans were ground with a blender until reaching a granulometry of 28 mesh. After the pre-treatment, the samples were stored in glass jars at room temperature. Subsequently, physical-chemical characterization and centesimal composition analyses were carried out. The procedure performed with vanilla pods *in natura* and dehydrated after the extraction process (Figure 2).

The centesimal and physical-chemical characterization of the samples will be carried out following the methodologies described in the manual of physical-chemical methods for food analysis from the Institute Adolfo Lutz. [7] Some of the analyses will be carried out with equipment available in the laboratory (Table 1).

As for the analysis of vanillin determination in the vanilla extract, by the thin layer chromatography method, chromatoplates of aluminum, and hexane and ethyl acetate 1:1 were used. The extract, previously diluted in three concentrations (100, 50, and 25%), was applied to the chromatoplate approximately 0.5 cm from its lower base. Then, the TLC was introduced into a glass containing hexane and ethyl acetate, igniting and dragging the less adsorbed compounds into the stationary phase, separating them from the more adsorbed compounds. Then, the plate was dried with a hot air dryer, and the chromatoplates were submitted to the development process using UV (254 nm) and sublimated iodine. [8] In addition to the characterization, a market study

Figure 1. Vanilla pods *in natura* (a) Vanilla pods dried after the extraction process (b).

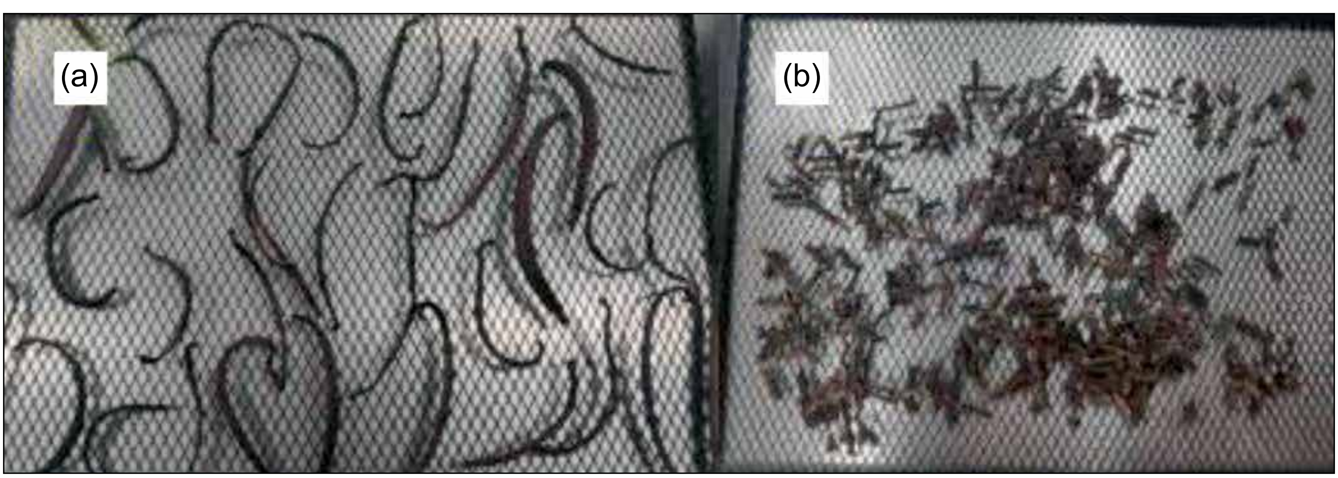


Figure 2. The procedure was carried out with vanilla pods.

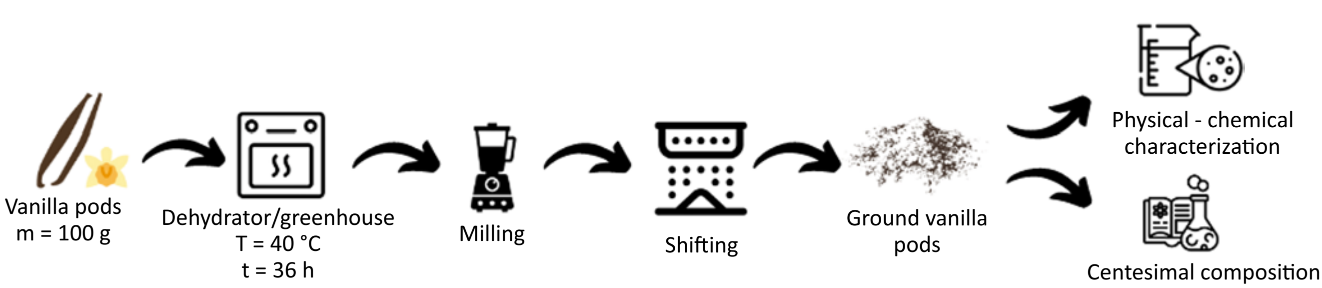


Table 1. Methods for physical-chemical characterization and centesimal composition.

Analyses	Experimental Methods
Physical-Chemical Characterization	
pH	pH content in the Bel Engineering PHS3BW digital pH meter
Total soluble solids (°Brix)	Content of soluble solids in the portable digital refractometer (SPINLAB/SPIN-104-D)
Water activity	Water Activity Content (LabMaster-Aw TECNAL)
Humidity	Humidity Content (Shimadzu Moc 120 H)
Color	Color Content in the KONICA MINOLTA Handheld Colorimeter (CR-400)
Centesimal Characterization	
Ashes	Total ash content by muffle incineration at 550 °C [7]
Lipids	Total lipid content by Bligh Dyer [7]
Proteins	Protein content by Kjeldahl digestion method [7]
Carbohydrates	Total carbohydrate content will be calculated by the difference: 100 - (% moisture + % ash + % protein + % lipids)

was carried out using the Mordor Intelligence platform to obtain an overview of the vanilla market. In addition to checking the leading players and the main trends in the market.

Results and Discussion

The vanilla pod samples, *in natura* and after the extraction process, after a pre-treatment step, were assigned to conduct the analyses to obtain results regarding physical-chemical characteristics (Table 2) and centesimal composition (Table 3).

According to the results obtained from the physical-chemical analyses, the pH values are very close, which indicates that the beans maintained their acidity after the extraction process; these values are above 4.5, that is, they have low acidity, and there may be a proliferation of fungi, yeasts, and pathogenic bacteria.[9] As for the brix degree, the values are very consistent, in which there is a reduction in the total soluble solids of the pods after extraction, which means that part of these components were consumed in the process. The water activity is a parameter that relates to the water availability of the product, with values found between 0.4 and 0.7, values that configure the low and medium aw, that is, the beans are in a qualitatively acceptable range, indicating that the samples have a satisfactory level of perishability [10]. Regarding moisture, pods *in natura* are higher than post-extraction beans, as the pods used in the extraction undergo a pre-treatment, in which the raw material is dried before being introduced into the process. As for color, the values of L* (Brightness) are below 100, compared to white, which has a brightness of 100. As for chromatic coordinates, a*(red/green coordinate) and b* (yellow/blue coordinate), the a* and b* of the beans are positive, indicating a region close to red and yellow, respectively. [11]

Regarding the results of the centesimal composition, the ash and lipid content of both broad beans are very close, with a slight variation. As for protein content, fava beans *in natura* contain a smaller amount; however, in the study by Barrientos and colleagues [12], the percentage found in the bean of the species V. Planifolia was 4.58, that is, very close to the value obtained in

Table 2. Results of physical-chemical characterization.

Vanilla pods *in natura*		Vanilla pods dried after the extraction process	
pH	4.527 ± 0.006	pH	4.790 ± 0.036
Total soluble solids (°Brix)	2.300 ± 0.001	Total soluble solids (°Brix)	0.767 ± 0.058
Water activity	0.721 ± 0.002	Water activity	0.414 ± 0.006
Humidity (%)	37.713 ± 0.150	Humidity (%)	7.763 ± 0.365
Color	L*= 36.323 ± 0.218 a*= 17.833 ± 0.172 b*= 6.143 ± 0.208	Color	L*= 26.673 ± 0.025 a*= 12.380 ± 0.173 b*= 5.243 ± 0.085

Table 3. Results of centesimal composition.

Vanilla Pods *in natura*		Vanilla Pods Dried After the Extraction Process	
Ashes (%)	5.495 ± 0.112	Ashes (%)	5.935 ± 0.070
Lipids by Bligh Dyer (%)	50.701 ± 0.618	Lipids by Bligh Dyer (%)	50.184 ± 1.143
Proteins (%)	4.359 ± 0.117	Proteins (%)	8.087 ± 0.179
Carbohydrates (%)	5.7	Carbohydrates (%)	28.2

the present work. As for the carbohydrate content, found by calculating the difference, it is noticed that the vanilla pod *in natura* has a lower content; this can be explained due to the significant difference between the values obtained for moisture of the pods. As for the results of the analysis of vanillin determination by the TLC method (Figure 3), we observed that this method presented initially positive results for high concentrations of vanillin (from 0.1 %). The commercial vanilla extract and the standard vanillin solution showed the same retention time. However, regarding spot intensity, the commercial alcoholic extract was visible, while the dot of the other standard extract did not even appear on the plate. Therefore, carrying out new tests to validate the results is interesting.

According to the market analysis conducted on Mordor Intelligence, the major players in the market are Mc Cormick, Sensient, Nielsen-Massey, Symrise, and Givaudan (Figure 4), with Mc Cormick & Company Inc. being the leader in the field, as it sought to create more efficient market strategies to expand its products and gain more visibility.

These companies gain prominence in the market, as they have natural vanilla-based products such as vanilla extract and paste. In addition to assessing market players, the survey analyzed how the demand for natural foods is going (Figure 5), with a growing consumer consensus in perceiving food products as healthy if derived from natural ingredients, such as vanilla.

After the analysis, we observed that the trend towards natural products is growing, both in the food market and in the cosmetics and personal care sector, making it increasingly important for companies to consider the inclusion of natural ingredients in their products.

Conclusion

Due to the results presented, the physical-chemical characterization and the centesimal

Figure 3. Results of the analysis of the vanillin content of vanilla by the TLC method.

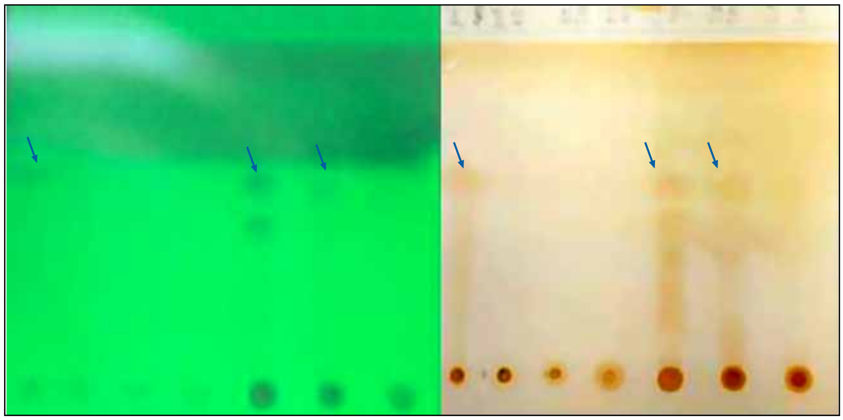
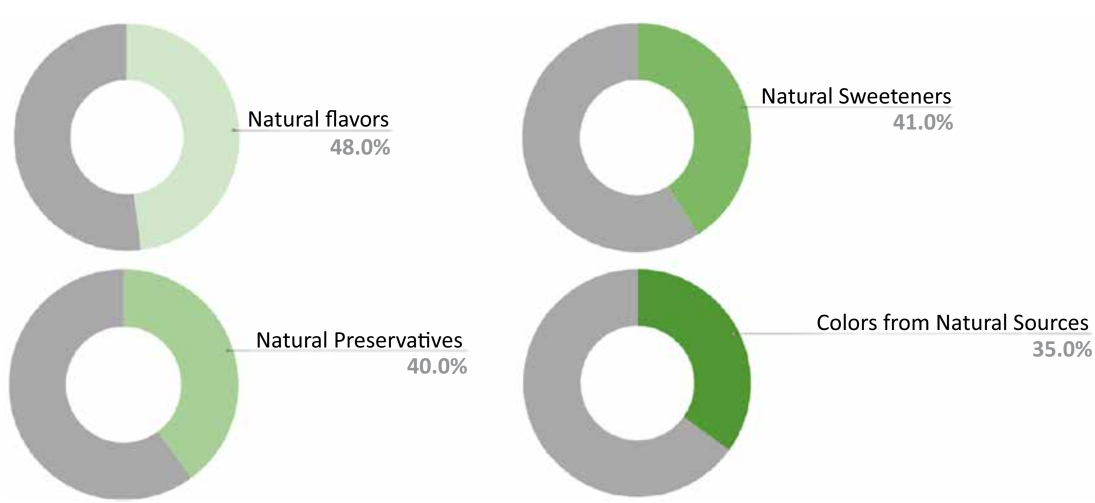


Figure 4. Significant players in vanilla's products.

Source: Mordor Intelligence (2020).

Figure 5. Vanilla Market: Consumer Preference for Food Products in %, US, 2021.



Source: Adapted from Mordor Intelligence (2020).

composition of the vanilla pods *in natura* and after the extraction process, presented auspicious results, where it was possible to perceive essential points and the differences in the characteristics between the pods. From the results of the determination of vanillin in the commercial extract, it is interesting to carry out new tests to validate the results and use a more robust analysis such as High-Performance Liquid Chromatography (HPLC). Thus, from these results, it is intended to develop the vanilla extract with an exciting percentage of vanillin to be applied in the market, testing three extraction methods, with modifications in the process variables such as temperature, time, solvent, and equipment, to obtain the product through an optimized process.

Acknowledgments

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Evaluation of Torrefaction on the Quality of Bio-Oil Obtained by Pyrolisis of Green Coconut Wastes

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Pyrolysis of green coconut residues is considered a promising source of biomass for transforming energy and generating value-added products from thermochemical processes such as pyrolysis, mainly due to the high volume of this material in the Northeast region. For better pyrolytic results, some pre-treatments of the biomass can be used. In this work, the pre-treatment technique of oxidative and non-oxidative torrefaction was applied, using temperatures of 200 and 250 °C. These torrefied biomasses were subsequently subjected to fast pyrolysis at 700 °C. GC/qMS characterized the bio-oil obtained, and an increase in the content of phenolic compounds was observed in the torrefied biomass samples when compared to the raw biomass, indicating that the process is viable and adds value to the bio-oil obtained by pyrolysis.

Keywords: Torrefaction. Pyrolysis. Bio-Oil. Phenolic Compounds. Chromatography Analyses.

Introduction

The global economy is currently based on the use of fossil sources for both energy generation and products for the chemical industry. However, this source generates polluting gases that disperse into the environment, contributing to increased pollution and climate change impacts. Consequently, there is a need for new sustainable sources of energy generation and products [1].

In this scenario, biomass stands out, defined as any organic matter of animal or plant origin that can generate energy. When biomass is derived from agroindustrial waste, its use helps minimize inappropriate disposal of these materials, reducing environmental impacts [2].

Brazil is one of the world's largest agroindustrial producers, resulting in significant volumes of organic waste. Among the biomass sources derived from these agroindustrial wastes, fiber, and husks of coconut can be highlighted. Brazil ranks fifth in global coconut production, with 1.6 billion fruits produced. The Northeast region concentrates 80.9% of the harvested coconut area and stands out with approximately 73.5% of the national production [3].

One way to utilize these waste materials is through pyrolysis, which is defined as a thermochemical process of biomass degradation under an inert atmosphere, resulting in three products: non-condensable gases, biochar, and bio-oil [4]. Bio-oil, the liquid product of pyrolysis, is a complex mixture of water and organic compounds, including hundreds of oxygenated compounds that give bio-oil acidic characteristics, high viscosity, low calorific value, and high instability [5].

Biomass, mainly derived from agroindustrial waste, presents some disadvantages in its application to pyrolysis, such as high water and oxygen content, which negatively impact thermochemical conversion processes. Therefore, pre-treatment processes aim to improve biomass quality before it is subjected to pyrolysis [6]. Pre-treatment methods such as torrefaction release volatile compounds and modify biomass's physicochemical and structural properties, increasing its energy density and improving its characteristics [7]. Torrefaction occurs at moderate temperatures of 200 to 300 °C under an oxygen-

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free atmosphere. This process can be classified according to the operating temperature ranges: mild (200-235 °C), moderate (235-275 °C), and severe (275-300 °C). Furthermore, it effectively reduces the moisture content in biomass and eliminates volatile oxygenated compounds with lower molecular weight [8,9].

Only hemicellulose is degraded in mild torrefaction, while cellulose and lignin remain unchanged, retaining more biomass mass and energy. Moderate torrefaction leads to partial decomposition of cellulose, resulting in a higher loss of mass and energy than mild conditions. Severe torrefaction causes the depolymerization of lignin, cellulose, and any remaining hemicellulose. This condition results in a greater mass and energy loss but guarantees higher energy density for the biomass [10]. This pre-treatment type destroys biomass's fibrous nature and increases its calorific value. Additionally, it makes the biomass more resistant to water adsorption, thereby increasing its resistance to fungal and bacterial proliferation [11].

Oxidative torrefaction is a process that occurs in an atmosphere containing oxygen at the same temperature range as non-oxidative torrefaction (200-300 °C). The oxidative reactions in this process are generally exothermic, generating heat and reducing the heating demand in torrefaction reactions. Moreover, some studies have suggested that increased oxygen concentration could lead to more significant biomass decomposition, resulting in shorter torrefaction or milder temperatures [12].

Once this biomass undergoes pre-treatment techniques and subsequently undergoes pyrolysis, the generated bio-oil needs to be characterized to propose better applications for it, widely used techniques in various studies include gas chromatography coupled with mass spectrometry (GC/MS).

This study aimed to evaluate a pre-treatment method (torrefaction) for residual coconut biomass to improve the quality of bio-oil produced in pyrolysis, enriching its phenolic compound content. Furthermore, the produced bio-oils were characterized by GC/qMS to assess the potential industrial applications of the identified compounds.

Materials and Methods

Torrefaction Conditions

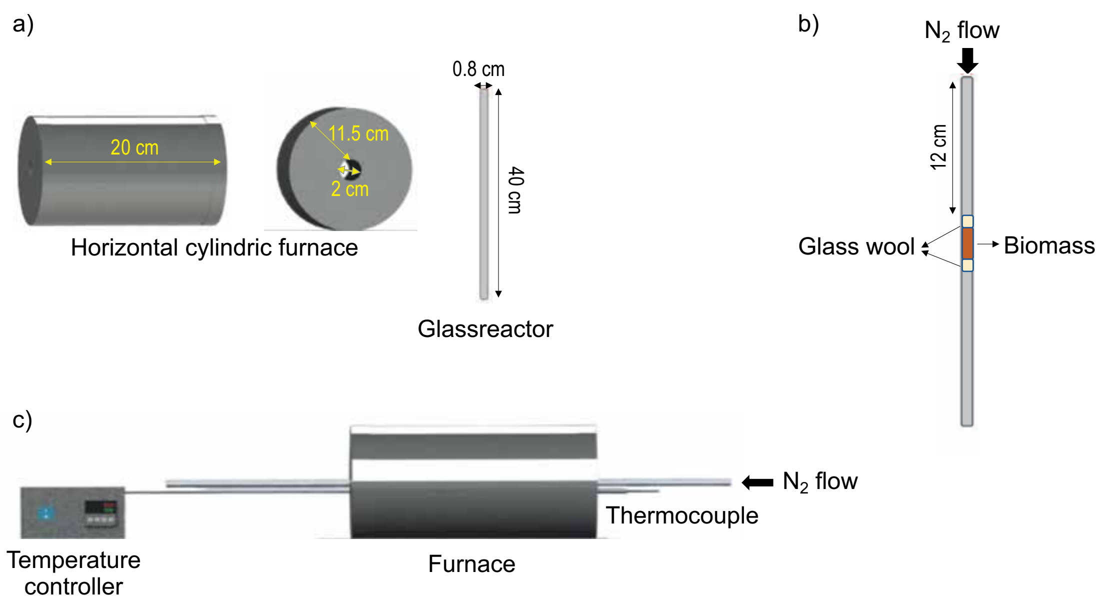
Two types of torrefactions were performed: oxidative and non-oxidative. For non-oxidative torrefaction, a stainless-steel pyrolysis reactor with a diameter of 60 mm and a length of 260 mm was used. It had an upper inlet for biomass addition, a side inlet for injecting inert gas (N_2), and an outlet for removing the gases generated. The detailed system is described in the study by Santos and colleagues, 2020 [13]. The experiment was conducted at 250 °C for 15 minutes, with a nitrogen flow rate of 100 mL min^{-1}, and 3 g of biomass. For oxidative torrefaction, the experiments were conducted in a muffle furnace with dimensions of 81 cm x 76 cm x 64 cm. 3g of biomass were placed in ceramic containers, and the experiments were conducted at 200 °C for 15 minutes. All experiments were performed in triplicate.

Pyrolysis Conditions

Fast pyrolysis experiments were conducted using a system consisting of a circular horizontal furnace with a diameter of 25 cm and a length of 20 cm. The furnace had an internal refractory layer with a thickness of 11.5 cm and a central orifice with a diameter of 2 cm, into which a 40 cm glass reactor was inserted. The glass reactor had an inlet for inert gas (N_2) and a 12.5 cm thermocouple for temperature control. Figure 1 (a, b, c) schematically illustrates the complete system and its detailed parts.

Torrefied and raw biomass samples were pyrolyzed under the same conditions, with a temperature of 700 °C, nitrogen flow rate of 2 mL min^{-1}, and 0.1 g of biomass. The biomass was placed inside the reactor using glass wool at both ends [Figure 1 (b)]. The reactor containing the biomass

Figure 1. Pyrolysis system.



was inserted into the preheated furnace, ensuring that the biomass was centrally positioned for more uniform heating. The reactor was connected to the N_2 flow. The temperature was controlled so that the biomass inside the reactor reached 700 °C and remained at this temperature for 1 minute. Subsequently, the reactor was removed from the furnace, cooled to room temperature, and the formed bio-oil was eluted with 15 mL of acetone.

Chromatographic Analysis – GC/qMS

Chromatographic analyses were performed using the GC/MS-QP-2010-Ultra model equipment (Shimadzu, Japan). The capillary column was a ZB-5, 30 m long, 0.25 mm internal diameter, and 0.25 µm stationary phase thickness. Helium was used as a carrier gas with a flow of 1 mL min^{-1}. In the splitless mode, sample injection (1 µL at a concentration of 5000 ppm) was performed using the AOC-20i automatic injector (Shimadzu, Japan). Injector, interface, and ion source temperatures were kept at 300 °C, and electron impact ionization energy was 70 eV. The system operated in SCAN mode, ranging from 45 to 450 Da. The oven was heated according to the following schedule: from 40 °C to 300 °C at 3 °C min^{-1}, remaining at this temperature for 10 min. Data treatment was performed using the GCMS solution software. The compounds were identified by retention index and mass spectra.

Results and Discussion

The yield obtained from the torrefaction of fresh coconut was 43.5 ± 7.3% for oxidative torrefaction and 70.2 ± 0.3% for non-oxidative torrefaction. Oxygen in the torrefaction atmosphere reduces the mass yield of the process due to biomass carbonization, resulting in rapid mass loss [14]. Table 1 presents the mass yields obtained from pyrolysis of raw and torrefied biomass.

A reduction in bio-oil yield from the raw biomass to the torrefied samples can be observed, especially for the oxidative torrefaction process. In contrast, a considerable increase in the biochar content is observed. Table 2 shows the percentage areas of the compounds identified in each sample.

Figure 2 illustrates the relative areas (%) and number of peaks of the identified compounds in each sample. The data analysis shows that the major

Table 1. Yields of pyrolysis products.

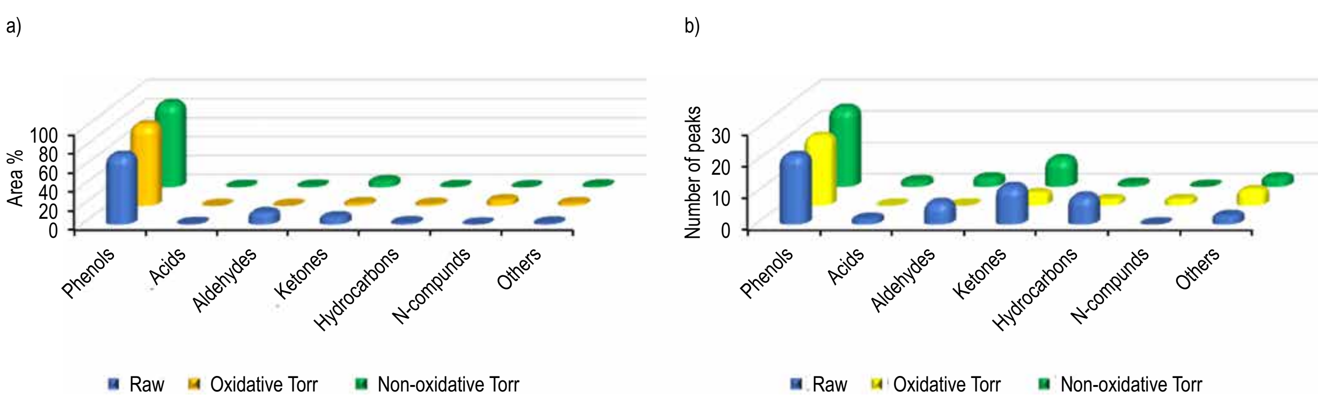
Sample	Bio-oil (%)	Biochar (%)	Gases and Losses
Raw Biomass	21.87	23.06	55.07
Oxidative Torr	4.46	51.63	43.91
Non-oxidative Torr	15.49	37.57	46.94

Table 2. Identification of compound classes regarding the number of peaks and their percentage areas for bio-oil samples.

	Area %			Number of Peaks		
Chemical classes	**Raw**	**Oxidative Torr**	**Non-oxidative Torr**	**Raw**	**Oxidative Torr**	**Non-oxidative Torr**
Acids	1.17	n.d.	0.90	2	n.d.	2
Sugars	n.d	0.87	n.d.	n.d	1	n.d
Alcohols	0.74	n.d.	0.48	1	n.d.	1
Aldehydes	13.22	n.d.	1.19	7	n.d.	3
Ketones	8.49	2.81	7.28	12	4	9
Esters	0.29	0.41	0.52	1	1	1
Ethers	0.73	1.80	0.66	1	3	1
Phenols	73.17	85.50	88.28	22	22	25
Hydrocarbons	2.19	2.00	0.70	9	2	1
N-Compounds	n.d.	6.61	n.d.	n.d.	2	n.d.
Total	100	100	100	55	35	43

n.d. = not detected.

Figure 2. Distribution of percentage areas (a) and total number of peaks (b) of the compounds identified in the samples.



chemical classes found in the raw biomass bio-oil sample were phenols, followed by aldehydes, ketones, and, to a lesser extent, hydrocarbons. Compared to the raw biomass, an increase in the phenolic compound content is also noted in the bio-oils from torrefied biomass. These phenolic compounds are derived from lignin degradation and aromatization reactions of the fragments formed from cellulose decomposition. This behavior can be explained by the degradation of hemicellulose and part of the cellulose during the torrefaction process, increasing the lignin content in the sample, leading to the formation of more aromatic precursors during the pyrolysis process, thus increasing the content of phenolic compounds [14]. The presence of critical phenolic compounds suggests their purification for the acquisition of various high-value chemical products. Phenols are widely used in chemical, pharmaceutical, food, and other industries. Phenol, the primary compound found in all analyzed samples, has well-established industrial applications, high production costs, and is obtained from crude oil.

Conclusion

Based on the results, the implementation of pre-treatment processes on the samples before subjecting them to pyrolysis was justified. This technique improved the bio-oil's quality by increasing higher-value phenolic compounds that can be applied in the chemical industry.

Acknowledgments

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Extraction of Antioxidant Compounds from *Eugenia uniflora L.* Leaves by Energized Dispersive Guided (EDGE) System

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The leaves of Pitanga (*Eugenia uniflora L.*) present recognized antioxidant potential that has practical applications in pharmaceuticals, food, perfume, agrochemical, and cosmetic industries. The Energized Dispersive Guided Extraction (EDGE) system, which combines dispersive solid-phase extraction with the Accelerated Solvent Extraction system, was employed in "combined mode" with extraction cycles to obtain antioxidant compounds from Pitanga leaves. The results showed that extraction cycles with different solvents produce similar extraction yields. At the same time, their ability to scavenge free DPPH radicals exhibited variations according to the polarity of the extraction solvent. Thus, acetone was the optimal solvent for extracting antioxidant compounds from *E. uniflora* leaves in the combined mode.
Keywords: Antioxidants. *Eugenia uniflora L.* EDGE.

Introduction

The species of *Eugenia uniflora L.*, also known as pitanga, Brazilian cherry, or purple cherry, is a plant native to the Amazon rainforest in South America, with high economic and pharmacological potential evidenced by various scientific studies. Its extracts are rich in bioactive compounds such as terpenoids, tannins, and flavonoids with recognized antioxidant potential [1,2]. Based on this assumption, using the genus *Eugenia* in traditional medicine has been encouraged by several studies emphasizing its bioactive compounds and biological potential associated with antioxidant activity related to this species [3,4].

In general, antioxidant compounds can interact with free radicals before vital molecules are damaged, playing a crucial role against various diseases, including chronic inflammation, atherosclerosis, cancer, and cardiovascular diseases, as well as slowing biochemical aging processes [5,6]. Such constituents exhibit biological properties that the pharmaceuticals, food, perfume, agrochemical, and cosmetic industries can use. However, choosing the ideal extraction method for these compounds represents a significant analytical challenge [7,8].

The extraction of bioactive compounds can be carried out through traditional techniques, including liquid-liquid and solid-liquid extraction, as well as modern methods like ultrasound-assisted extraction (UAE), microwave-assisted extraction (MAE), subcritical fluid extraction (SFE), and accelerated solvent extraction (ASE). However, most extraction protocols utilizing these techniques have drawbacks, such as large amounts of organic solvents, multiple operational steps, extended analysis time, and potential thermal degradation, which can impact the yield and quality of the obtained extracts [9,10].

Recently, novel techniques have been developed to efficiently extract bioactive compounds from natural sources. These technologies are environmentally friendly due to reduced consumption of organic solvents, shorter operation times, and improved yield and extract quality [11].

The Energized Dispersive Guided Extraction (EDGE) system, introduced by CEM Corporation in October 2017, was developed to combine

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dispersive solid-phase extraction with the ASE system, being considered "faster than Soxhlet, more automated than QuEChERS, and simpler than other solvent extractions" [12].

The EDGE uses a two-piece open sample vessel called "Q-Cup" to hold the sample. During an extraction, the solvent is added to the sample in the Q-Cup, and the sample and solvent are pressurized and heated to the selected temperature and time. When the extraction is finished, the extract passes through the bottom of the Q-Cup through the filter and moves through the fluidic pathway of the system, including a cooling coil, to be dispensed into the collection vial. The final extract is at room temperature, filtered, and ready for analysis [13].

This system has some successful applications for extracting pesticides from natural sources, such as strawberries, cucumbers, tomatoes, green peppers, phthalates and additives from plastics, or the recovery of fats from foods [14]. Since then, some authors have shown the excellent potential of this system to extract target compounds from fruits and drugs from feeding stuff [15,16]. This study aimed to extract antioxidant compounds from Pitanga leaves using the EDGE system in "combined mode". The process can be divided into multiple cycles collected in the same vial for each solvent in this extraction mode. Each cycle involves extracting Pitanga leaves using 10 mL of solvent to maximize the extraction of desired compounds.

Materials and Methods

Collection, Identification, and Preparation of Plant Material

Leaves of *Eugenia uniflora L.* (Pitanga) were collected on private property in the city of Aracaju, Sergipe, Brazil, according to the geographical coordinates: latitude 10°59'15.8" S and longitude 37°04'03.8 "W. The plant material was herborized, preserved, and identified in the plant collection of the Biology Department at the Federal University of Sergipe at the number 145362-82 in the UFS-AJU Herbarium. The plant material was cleaned with distilled water to remove impurities and dried at 40 ºC using a circulating air oven for 4 days. The leaves were ground using a knife mill, sieved to achieve a particle size between 32 and 60 mesh, and stored in a dry place, protected from light and heat.

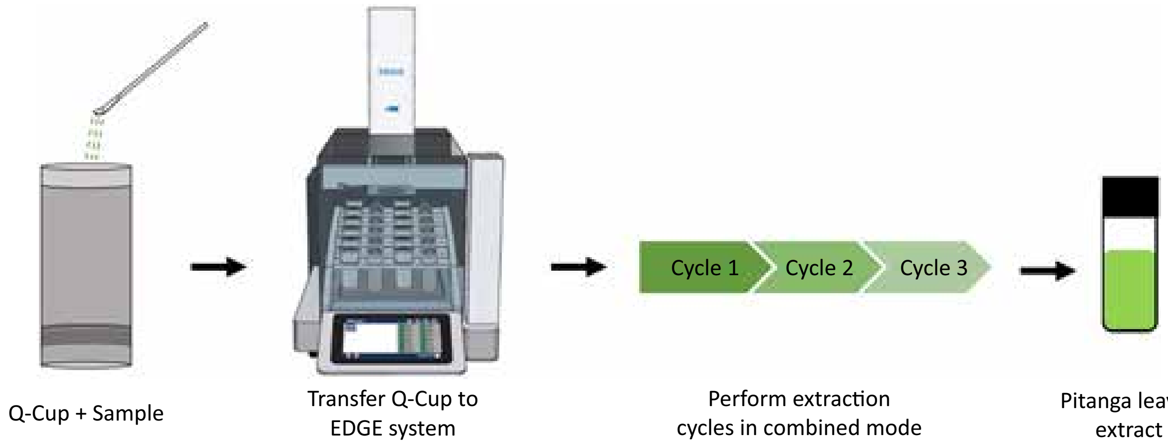
EDGE Extraction

Figure 1 schematized the extraction process. Firstly, dry leaves (1g) were directly weighed into a Q-Cup containing S1 filters, and the Q-Cups were placed on the removable rack of the EDGE system, accompanied by collection vials with a maximum volume of 40 mL. The extracts were obtained in combined mode, extracting Pitanga leaves with three extraction cycles with 10 mL of hexane, dichloromethane, or acetone added from the top of the Q-Cup and heated at 75 ºC for 120 seconds. After each extraction, a washing step was performed by passing 10 mL of the respective extracting solvent in the system at 100 °C for 30 seconds, preparing the EDGE instrument for the following sample. The percentage yields (weight to weight) were calculated in triplicate according to the extract dry weight basis.

DPPH Radical Scavenging Assay

The antioxidant activity was measured in a UV–vis spectrophotometer (Hach DR 5000) using a quartz cuvette (45 mm × 12.5 mm × 12.5 mm) with a 1.00 cm optical path at room temperature (25 °C), according to modified DPPH free radical scavenging method developed by Brand- Williams and colleagues [17]. Around 100 µL of methanolic solutions at different concentrations of the extracts (200, 400, and 600 ppm) were diluted in 3.9 mL of a methanolic solution of DPPH (0.06 mM). Methanol was used as a blank solution. After 1 h of incubation at 25 °C, the absorbance was measured at 515 nm. Free radical DPPH inhibition in percentage (I %) was calculated as follows

Figure 1. Extraction process in combined mode with EDGE system.



[Equation (1)]:

$$I\,\% = \left(\frac{A\ control\ -\ A\ sample}{A\ control}\right) \times 100$$

in which: a control is the absorbance of the control solution and A sample is the absorbance of the tested extract.

Results and Discussion

The EDGE process starts with the automated transfer of the Q-Cup into a sealed extracting chamber, where a smaller amount of solvent (0 - 10 mL) can be added between the extraction cell and the chamber walls or from the top of the cell, where a more considerable amount of solvent (0 - 30 mL) can also be added. The chamber walls are heated (Tmin: 25°C - Tmax: 200°C), causing the solvent to expand and disperse into the sample through the holes at the bottom of the Q-Cup. As the system is sealed, temperatures above the solvent's boiling point can be applied, and the volatilized product does not escape from the cell (Pmax: 200 psi) [12]. Thus, the extraction efficiency is governed by several critical parameters, including solvent type, temperature, pressure, time, and, obviously, extraction technique [13]. In this experimental study, extraction time, temperature, and solvent volume were kept constant for all cycles with the organic solvents tested, aiming to achieve lower IC50 values by DPPH assay. The results showed that extraction cycles with different solvents produce similar yields (Acetone: 3.76 % > Dichloromethane: 3.27 % > Hexane: 3.07 %) (Figure 2). The similarity observed in the yields obtained using different organic solvents at the same extraction conditions can be attributed to the fact that the EDGE is a fully automated system, which reduces human error associated with the extraction process. However, despite the similar extraction yields obtained by applying the EDGE system with extraction cycles in "combined mode", its capacity to scavenge free DPPH radicals exhibited variations depending on the utilized solvent (Figure 3).

The average IC50 values were obtained by DPPH scavenging assay with acetone. (0.47 mg L^{-1}), dichloromethane (9.77 mg L^{-1}) and hexane (24.52 mg L^{-1}) correspond to the same order of the dielectric constant of the solvents (εacetone = 20.7 > εdichloromethane = 8.93 > εhexane = 1.88), indicating that the polarity of extraction solvent highly influenced the extraction yields and recovery of antioxidant compounds from Pitanga leaves. In the EDGE system, extraction cycles represent the number of extractions performed with the same sample. In this context, prolonged exposure of the matrix to the extracting solvent improves the solvent's penetration into the sample's interstice, facilitating contact with the analytes. Various techniques have been employed to obtain antioxidant compounds from plant leaves. However, this work showed higher DPPH scavenge capability than

Figure 2. Extraction yields were obtained by the EDGE system with Pitanga leaves.

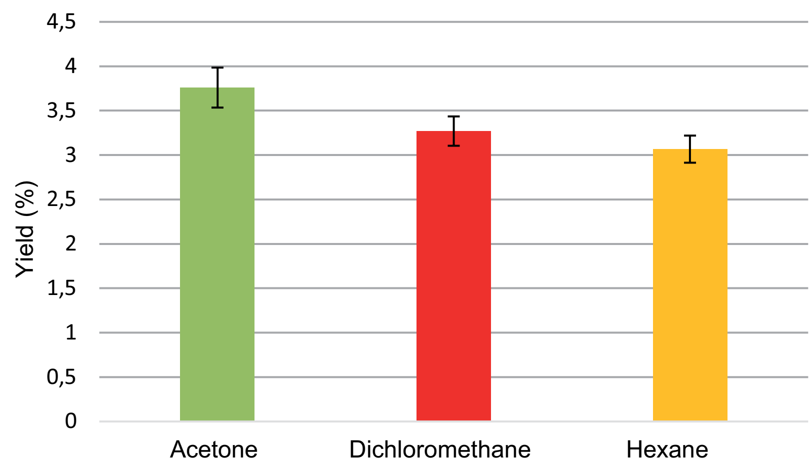
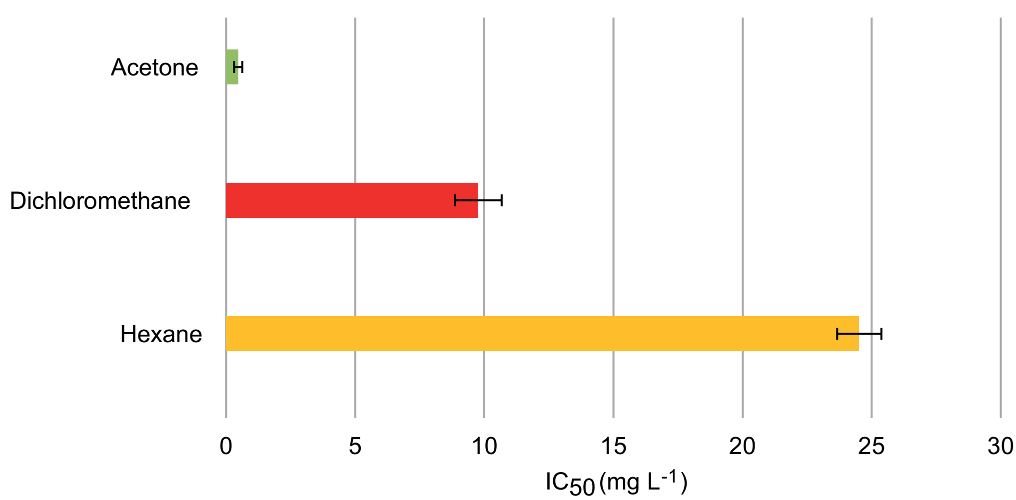


Figure 3. IC50 (mg L^{-1}) for Pitanga leaf extract with different solvents.



that described in the literature by the same assay. Zhu and colleagues [18] reported that the antioxidant activity of the aqueous extract of *S. oleracea* leaves obtained by MAE is 365 mg L^{-1}. In comparison, the aqueous extract of A. sativum leaves reported by Liu and colleagues [19] showed 421 mg L^{-1}. Chang and colleagues [20] reported that the aqueous extract of C. sativa leaves obtained by maceration had IC50 values of 361 mg L^{-1}, attributed to various phytochemicals acting synergistically to neutralize free radicals. Based on these results, the EDGE system emerges as a new, fully automated technique for recovering antioxidant compounds from plants, offering advantages such as reduced solvent and time consumption and enhanced reproducibility compared to techniques commonly described in the literature.

Conclusion

In this study, the innovative EDGE system was applied in a combined mode to extract antioxidant compounds from Pitanga leaves. The extraction time, temperature, and solvent volume (acetone, dichloromethane, or hexane) were kept constant for all cycles with the organic solvents tested, aiming to achieve lower IC50 values on the DPPH assay. The results obtained showed that extraction cycles produce similar extraction yields (acetone: 3.76 % > dichloromethane: 3.27 % > hexane: 3.07 %); moreover, despite the similar extraction yields, its capacity to scavenge free DPPH radicals exhibited variations according to the solvent utilized (acetone: 0.47 mg L^{-1}, dichloromethane: 9.77 mg L^{-1} and hexane: 24.52 mg L^{-1}), indicating that the polarity of extraction solvent highly influenced the process. Among the tested solvents, acetone was the most suitable to extract antioxidant compounds from E. uniflora leaves. The EDGE has proven to be an efficient, fully automated technique for recovering antioxidant compounds, offering several advantages such as lower solvent and time consumption and good reproducibility.

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Priority Areas Evaluation for Green Hydrogen Production Implementation in Bahia

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This study aims to assess different priority scenarios for developing Green Hydrogen (GH2) projects in Bahia, considering different input conditions in a mathematical model. This analysis supports the decision-making process regarding implementing GH2 production units, especially those utilizing the water electrolysis technique and electricity from renewable sources. Using the Analytic Hierarchy Process (AHP) method, eight hypothetical scenarios were modeled, considering the use of on-grid and off-grid renewable energy, production intended to meet local or external demands, and water availability for the electrolysis process. Due to the abundance of water resources and renewable energy sources, the Bacia do Rio Grande region emerged as the most suitable for the implementation of GH2 projects in the majority of the analyzed scenarios. However, the Metropolitano de Salvador region is the top option when factors such as development, infrastructure, and GH2 demand (internal and external) are more significant.

Keywords: Green Hydrogen. Scenarios. Priority Areas.

Introduction

The energy transition involves decarbonizing the energy matrix by replacing fossil fuel sources with clean energy alternatives. Brazil takes center stage in this discourse, given that its national energy matrix is predominantly composed of renewable sources, holding immense potential for mitigating Greenhouse Gas (GHG) emissions. Due to its unique geographic characteristics, the State of Bahia presents excellent potential for generating a clean and renewable energy source, such as green hydrogen (GH2).

Bahia is the fifth-largest Brazilian state in land area, with the longest coastline in the country (extension of 1,183 km) [1]. The geographical, environmental, historical, social, cultural, and economic diversity throughout the Bahian territory brings challenges, advantages, disadvantages, restrictions, and opportunities [2] for developing the GH2 production chain in the different state regions.

Then, evaluating the potential location for constructing renewable hydrogen production plants is essential. AHP is a multi-criteria decision-making method applied to determine the weights of criteria and priorities of alternatives in a structured and hierarchical manner based on comparing pairs [3] and can be used for this purpose.

This work aims to evaluate different scenarios for priority areas for the development of the GH2 projects in Bahia using a mathematical model to help in the decision-making process. In this study, we assumed that GH2 is produced from water electrolysis using electricity generated by renewable sources.

Materials and Methods

The present study used the Analytic Hierarchy Process (AHP) method to design the mathematical model. To build the AHP Model, 28 indicators (sub-criteria) were defined at different hierarchical levels and grouped into four criteria: social and economic development, availability of infrastructure, environmental aspects, and availability of resources. Most of the data used in the calculation of the indicators and, consequently, in the construction of

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the AHP Model, are in the public domain, accessed from government repositories, or provided directly by the Environment Secretariat - SEMA (data related to the state's Protected Areas), Infrastructure Secretariat - SEINFRA (data related to the state's Electric System, Transmission and Distribution Lines) and the Economic Development Secretariat of Bahia - SDE (socioeconomic data of the state [2].

The AHP model was weighted based on the degree of influence exerted by the criteria and sub-criteria among themselves, based on the comparative analysis of the relevance of these indicators in the GH2 production chain. The method adopted to carry out the dynamics with the experts was Quantity, Quality, and Systematic Management (QQS), from which paired comparisons were conducted at the same hierarchical level. This methodology is detailed in GH2 Bahia Atlas [2].

Eight different hypothetical scenarios were proposed from the combination of three specific conditions, as described below:

Condition 1. Off-Grid or On-Grid Renewable Energy

Off-grid: GH2 production plant operates directly linked to a renewable energy generation system.

On-grid:GH2 production plant is connected to the national electricity grid (National Interconnected System - SIN). It uses renewable energy certificates to ensure that the hydrogen produced is classified as green.

Condition 2. Consumption of Local or External GH2

Local: GH2 consumption is local, occurring in the surroundings of the production unit.

External: GH2 consumption requires long-range transportation and depends on the existing transportation logistics infrastructure.

Condition 3. Water Resources Availability

Relevant: water availability can be a limiting resource for GH2 production in specific regions and, therefore, has significant relevance in the analysis.

Irrelevant: water is an abundant resource in the state and does not represent a limiting factor for GH2 production, making it of little relevance to the analysis.

Table 1 shows the combination of three specific conditions. These hypothetical scenarios allow specific analysis within a broad spectrum of possibilities for developing the green hydrogen economy in Bahia State.

Based on these scenarios, values were calculated for the 27 Territories of Identity of Bahia State, posteriorly ranked according to their suitability for GH2 production.

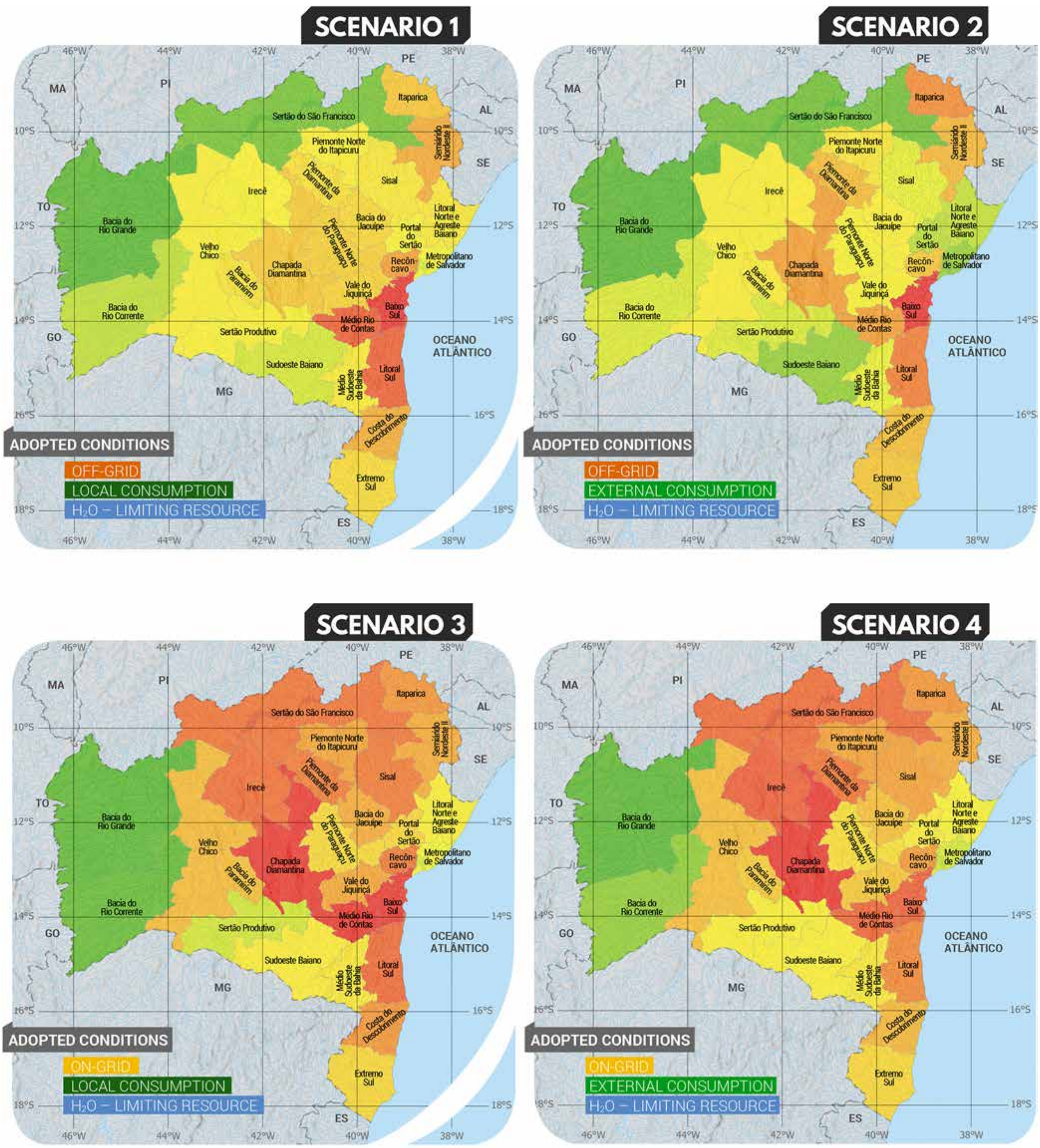
Table 1. Hypothetical scenarios.

Scenario	Condition 1	Condition 2	Condition 3
1	Off-grid	Local Consumption	H_2O - Limiting Resource
2	Off-grid	External Consumption	H_2O - Limiting Resource
3	On-grid	Local Consumption	H_2O - Limiting Resource
4	On-grid	External Consumption	H_2O - Limiting Resource
5	Off-grid	Local Consumption	H_2O - Abundant Resource
6	Off-grid	External Consumption	H_2O - Abundant Resource
7	On-grid	Local Consumption	H_2O - Abundant Resource
8	On-grid	External Consumption	H_2O - Abundant Resource

Results and Discussion

The heterogeneity of Bahian territory allows different approaches to developing the GH2 economy in Bahia, especially adapted to the particularities of each region. In this context, Figure 1 displays the eight hypothetical scenarios that indicate the suitability for GH2 production based on the combination of specific conditions representing different possible strategies for developing the GH2

Figure 1. Maps of priority areas for hypothetical scenarios.



economy in Bahia. Table 2 presents the numerical values that detail the ranking of each of the Identity Territories in the eight evaluated scenarios.

The rank of priority areas changes significantly if the GH2 production plant operates directly linked to a renewable energy generation system (off-grid) or if it is connected to the national electricity grid (National Interconnected System - SIN) and uses renewable energy certificates to ensure that the hydrogen produced is classified as green (on-

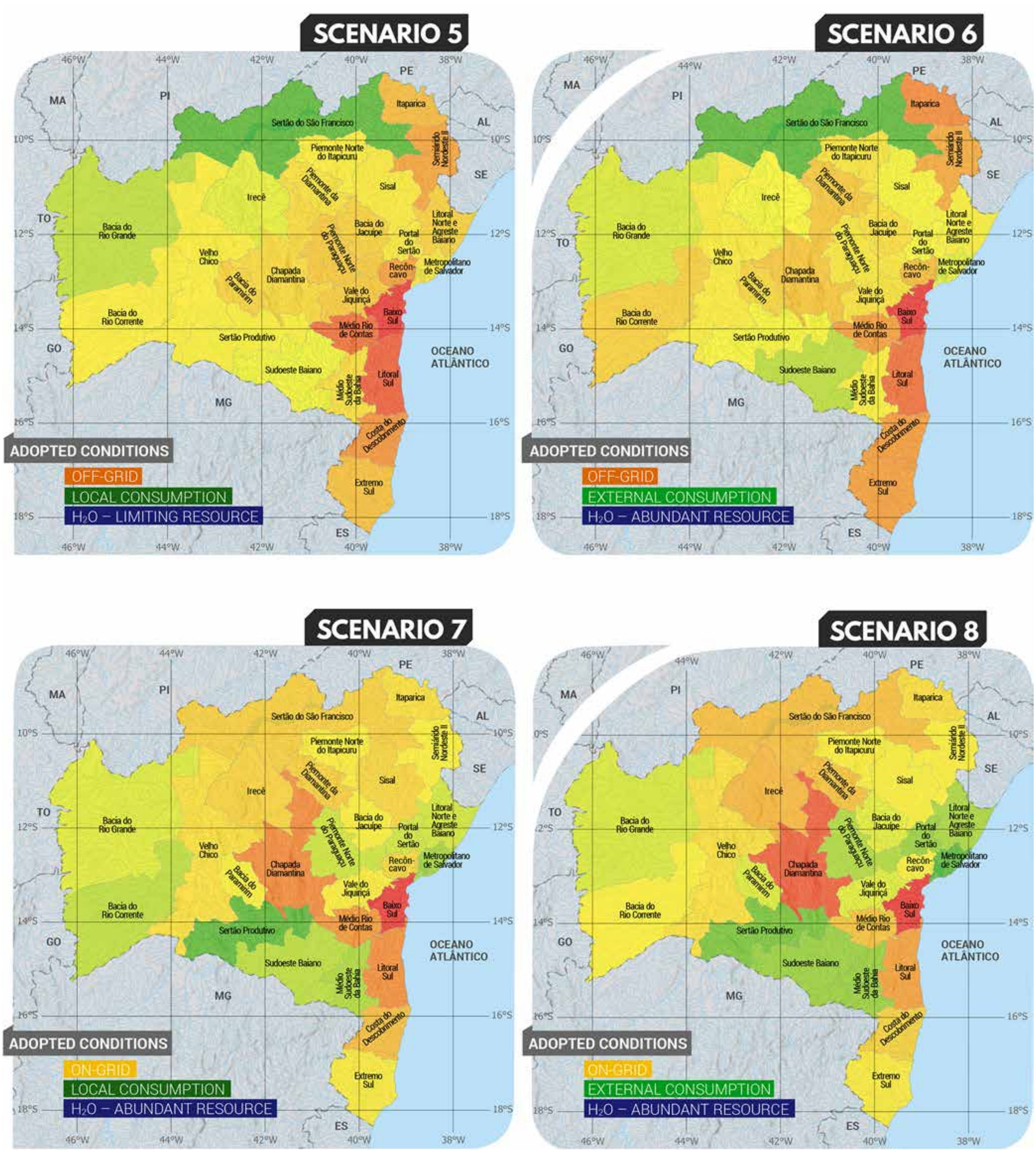


Table 2. Numerical ranking values for the different Identity Territories generated through the AHP Model.

	Territory of Identity	Hypothetical scenarios							
		SC1	SC2	SC3	SC4	SC5	SC6	SC7	SC8
1	Irecê	5.10	4.52	0.97	0.65	5.69	5.25	3.17	2.37
2	Velho Chico	4.65	4.61	2.48	2.56	4.35	4.15	3.59	3.59
3	Chapada Diamantina	2.95	1.96	0.05	0.00	3.48	2.79	1.49	0.67
4	Sisal	4.01	5.62	1.24	1.98	4.10	5.11	3.35	4.55
5	Litoral Sul	0.73	1.33	0.89	1.48	0.65	1.02	1.59	1.92
6	Baixo Sul	0.00	0.00	0.04	0.72	0.00	0.00	0.00	0.00
7	Extremo Sul	3.51	2.83	3.43	3.44	2.58	1.87	4.10	4.10
8	Médio Sudoeste da Bahia	4.16	5.48	4.04	4.00	3.64	4.35	7.25	8.25
9	Vale do Jiquiriçá	3.25	4.80	2.08	2.83	2.89	3.81	4.23	5.73
10	Sertão do São Francisco	9.20	9.37	1.09	0.93	10.00	10.00	3.09	2.56
11	Bacia do Rio Grande	10.00	10.00	10.00	10.00	6.70	5.90	5.82	5.98
12	Bacia do Paramirim	3.83	4.21	2.60	2.98	3.28	3.35	4.81	5.78
13	Sertão Produtivo	4.87	5.84	6.02	4.58	4.71	5.22	10.00	9.26
14	Piemonte do Paraguaçu	3.19	5.02	3.48	3.64	2.85	3.96	6.20	7.21
15	Bacia do Jacuípe	3.46	4.34	2.14	2.64	3.12	3.58	4.57	5.71
16	Piemonte da Diamantina	3.70	2.54	1.28	1.15	3.73	2.84	3.07	2.60
17	Semiárido Nordeste II	2.15	2.40	2.17	2.17	2.01	2.11	4.34	4.46
18	Litoral Norte e Agreste Baiano	4.05	6.42	4.60	5.07	3.12	4.49	6.06	7.53
19	Portal do Sertão	4.65	6.82	3.33	4.12	3.97	5.22	6.12	8.29
20	Sudoeste Baiano	5.95	8.30	4.20	4.49	5.44	6.81	6.81	8.24
21	Recôncavo	1.97	2.88	1.24	1.83	1.90	2.45	3.42	4.37
22	Médio Rio de Contas	0.70	1.95	0.00	0.82	0.99	1.88	1.77	2.66
23	Bacia do Rio Corrente	6.71	5.88	9.76	8.51	4.27	3.13	6.78	4.99
24	Itaparica	2.79	1.59	1.71	1.73	2.44	1.48	3.57	3.51
25	Piemonte Norte do Itapicuru	4.44	3.98	2.04	2.00	4.29	3.84	4.21	4.25
26	Metropolitano de Salvador	5.07	8.06	5.33	5.94	3.89	5.62	7.73	10.00
27	Costa do Descobrimento	2.22	2.63	1.75	2.41	1.71	1.85	2.51	3.23

grid). The classification also varies depending on local or external consumption, which requires transportation over long distances and depends on the existing transportation logistics infrastructure. The water and renewable energy resource availability analysis highlights the mesoregions Vale São Francisco, north-central and south-central of the state, mainly driven by the sub-criteria Wind & Solar Complementarity. The Bacia do Rio Grande territory is the most suitable for green hydrogen projects in most evaluated scenarios due to water and of-grid renewable energy resources. However, when development aspects, infrastructure, and demand (internal and external) are more significant, the Metropolitan Region of Salvador is ranked first (Scenario 8 in Figure 1). Due to the specificities of Bahia state, there are no data to compare to the results obtained in this work. It is essential to emphasize that the results obtained in this study represent a macro analysis of highly complex factors interconnected to the production chain associated with GH2. These factors may change over time, requiring the consideration of new premises, criteria, and sub-criteria, as well as the adaptation of the approach based on new technologies (e.g., the use of biomass for GH2 production) and other factors relevant to the state of Bahia. Therefore, it is necessary to update the mathematical model from time to reflect the conditions of the local reality as accurately as possible.

Conclusion

Considering the 27 Territories of Identity of the state, mapping the indicators inserted in the AHP Model pointed out the diversity of characteristics (geographical, environmental, historical, social, cultural, and economic) present throughout the Bahian territory. Water and renewable energy resources, infrastructure, and GH2 demand are essential to indicate priority areas for hydrogen production. By determining specific conditions (e.g., SC1: Off-grid, Local Consumption, H_2O - Limiting Resource), we can identify the Bacia do Rio Grande region as a preferential area for GH2 production. Another defined condition (e.g., SC8: On-grid, External Consumption, H_2O - Abundant Resource) may highlight the Metropolitano de Salvador region as the most prominent for GH2 production. In this regard, the business model and the set of prioritized conditions must be carefully analyzed when defining priority areas for GH2 production.

Acknowledgments

The authors thank the Government of the State of Bahia for their technical cooperation and support in developing the GH2 Atlas Bahia.Table 2: Numerical ranking values for the different Identity Territories generated through the AHP Model.

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Thermodynamic Modeling and Performance Evaluation of Absorption Refrigeration System with Graphene Nanofluid

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The study presents a comprehensive computational analysis of an absorption refrigeration system that employs graphene nanofluids as the secondary fluid. The system's tube-type evaporator, utilizing copper tubes, was optimized and analyzed under varying nanofluid concentrations through thermodynamic modeling. Key thermal and design parameters were thoroughly examined, including evaporation area, heat flux, conductive and convective coefficients, and overall heat transfer coefficient. The findings demonstrate notable enhancements in the analyzed parameters when employing nanofluids in the system, with improvements of 5.2% in the pumping power of the secondary system and 0.8% in the evaporation area. These outcomes highlight the potential benefits of incorporating graphene nanofluids in absorption refrigeration systems, paving the way for more efficient and sustainable cooling solutions.

Keywords: Refrigeration. Absorption. LiBr. Nanofluids. Graphene.

Introduction

In recent decades, the improvement of efficiency rates of thermal systems has become of great interest to academia and industry. This fact is justified by the social and governmental requirement to reduce emissions in production processes and the transport sector. Such aspirations, associated with the variability of the hydrocarbon market, have driven the search for alternative and optimized systems from an energy point of view due to this sector's vital importance for maintaining countries' economies. An example of this search is presented by Zhang [1], in which methodologies are discussed that make it possible to increase the efficiency levels of thermal systems applied to electricity production, focusing on the efficiency of the energy transition and its determinants during each stage of the process productive. Currently, several countries have increased their investments in the optimization and diversification of their energy sources. This decision is also associated with environmental concerns arising from the social charging process, accelerated by the intensification of environmental catastrophes that affect today's societies. A point of most significant concern for government entities is the NET ZERO 2050 report by the International Energy Agency (IEA), which aims to achieve total decarbonization by the year 2050. In this sense, initiatives such as the one in Dubey [2], characterized by the search for increasing efficiency gains in a 210 MW thermoelectric plant in operation in India through the use of regeneration processes, are vital for meeting the established goals since the analyzes presented are characterized by the demonstration of the viability of this type of process by demonstrating increased efficiency in the production plant.

In a scenario of energy transition, thermal systems, especially refrigeration, have been gaining prominence due to their vital importance for industrial processes and guaranteeing thermal comfort, which is crucial for regions with severe climates. In this sense, the search for the optimization of this typology of a system under the emphasis on its efficiency through parameters such as the coefficient of performance (COP) has

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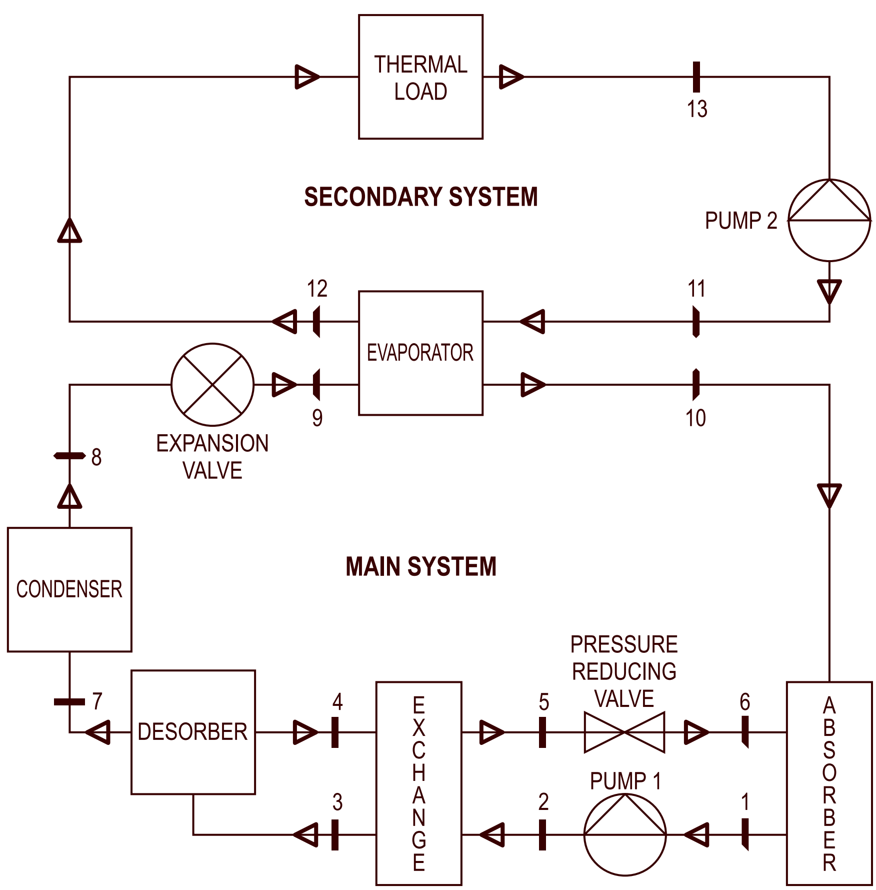
been encouraging the development of several studies and technologies, with emphasis on the use of nanoparticles as performance optimizing agents, especially under the emphasis of working fluids, as can be seen in Almeida, Barbosa, and Fontes [3] and Monteiro [4]. According to Loiaza [5], introducing these particles in thermal fluids improves the energy exchange capacity compared to conventional fluids due, among other possible reasons, to the more significant order of magnitude of the thermal conductivity of the solids used compared to the liquids.

In advancing refrigeration systems' efficiency and reducing their environmental impact, gaining a deeper understanding of the behavior of thermal fluids when integrating new particle types becomes paramount. This study aimed to establish a comprehensive computational model for analyzing the influence of graphene nanoparticles in a secondary absorption refrigeration system. By investigating the impacts of such nanoparticles, we strive to identify configurations that can significantly enhance the system's efficiency, ensuring greater competitiveness and sustainability in refrigeration technology. The present work contributes insights into optimizing absorption refrigeration systems, leveraging the unique properties of graphene nanoparticles for improved performance and environmental stewardship.

Materials and Methods

A computational model was developed using EES software to analyze the impacts of graphene nanoparticles operating in the secondary system of an absorption refrigeration system. The system consists of a single-effect absorption refrigeration system operating with the LiBr-H_2O pair and a secondary system whose thermal fluid is graphene nanofluid (Figure 1).

Figure 1. Refrigeration system.



The investigation entails a comprehensive approach, encompassing mathematical modeling, meticulous mass and energy balances, precise parameterizations, and successive system simulations. It involves operating the system while varying the concentrations of graphene nanoparticles within the range of 1% to 5%. By systematically analyzing these variations, the proposed modeling aims to gain valuable insights into the system's performance and efficiency when employing different concentrations of graphene nanoparticles.The maximum and minimum operating pressures were defined as 7.4kPa and 0.7kPa, respectively. These definitions are essential for characterizing the pressures at each point of the system and determining temperatures at some points whose iteration process is necessary. Such values were chosen based on the indications of Ashrae [6]. It also indicates that high-pressure operation requires thick-walled equipment and significant electrical power, which may be required to pump fluids from the low-pressure side to the high-pressure side. A vacuum requires large-volume equipment and unique means to reduce the pressure drop in the refrigerant vapor paths. It should be noted that in the proposed model, the pressure drops in the system are neglected.

The definition of the characteristics of each point of the system was adopted to enable the optimization and operability of the system. Two sections demand attention: The generation-absorption circuit and the evaporator points 1 to 6 and 9 and 10, respectively.

Determining the temperature at point 4 serves the purpose of providing the required heat to the evaporator. According to Herold, Radermacher, and Klein's research [7], in a typical single-effect machine using aqueous lithium bromide, the generator must supply heat above approximately 90 °C (this value serves as a rule of thumb, considering actual application-specific requirements). When heat is introduced to the solution, the volatile component, in this case, the refrigerant (water for aqueous lithium bromide), undergoes evaporation. It is crucial to consider the temperatures and concentrations during the definition of operating conditions to prevent approaching the crystallization line of the LiBr-H_2O solution. This precautionary measure safeguards against any potential system performance and stability issues. For Point 10, the evaporator outlet, it was defined that the fluid is saturated vapor. This attitude is justified by sending the most considerable steam to the absorber. Another critical point in the data definition process is the temperatures. Meanwhile, the temperature T[1] = 33° C was set for the pump inlet. Experiments shown in the literature indicate that the region of the absorption cycle using the LiBr-H_2O pair where there is the most significant risk of crystallization of the solution occurring is in the piping between the heat exchanger outlet and the absorber inlet due to the high concentration of the solution in the region. It is necessary to calculate a minimum enthalpy at the output of the solution heat exchanger to avoid crystallization and a consequent interruption in the cycle [8].

The system's operating conditions are well defined to analyze the effects generated using graphene nanofluid on the system; emphasis is given to the process of analysis and sizing of the evaporator. The choice of this equipment as the focus is justified by the fact that it interfaces with the primary and secondary systems (Figure 1).

The analyses based on the mass and energy conservation apply Equation 1 for mass balance.

$$\left.\frac{dm}{dt}\right|_{vc} = \sum \dot{m}_{entra} - \sum \dot{m}_{sai} \tag{1}$$

In turn, the analysis takes place in the energy balance through the first law of thermodynamics, given as a function of mass flow rates and enthalpies. Equation 2 below makes it possible to evaluate state changes in a system.

$$\left.\frac{dE}{dt}\right|_{vc} = \dot{Q}_{vc} - \dot{W}_{vc} + \sum \dot{m}_{entra}\, h_{entra} - \sum \dot{m}_{sai} h_{sai} \tag{2}$$

Based on Equations 1 and 2 and respecting the due considerations, the mass and energy balances for the evaporator are obtained through equations 3 and 4.

$$\sum \dot{m_{entra}} = \sum \dot{m_{sai}} \quad (3)$$

$$\dot{Q_{evap}} = \dot{m_9}(h_{10} - h_9) \quad (4)$$

An essential point of this process is establishing the evaporator heat equivalent to the secondary system thermal load. For this, a load referring to 12,000 Btu/h was adopted. For the secondary system, the temperatures at the inlet were adopted as being T[11] = 10°C and T[12] = 5°C and at the outlet of the evaporator, and T[12] was adopted to seek to reduce the risk of crystallization, as recommended by Loiaza [5].

It was adopted as a design assumption that the equipment is a double tube heat exchanger operating in countercurrent. Another critical point is the definition of the fluid in the central system at the evaporator outlet in the condition of saturated steam (Q[10] = 1), eliminating the need to dimension the superheating region. Regarding the geometric aspects, the selection was made to utilize copper piping of type M, featuring an annulus dimension of 1 ½ inches and a tube dimension of ¾ inches. As for the arrangement of fluids, the hydraulic criterion was adopted, which dictates that the fluid with the higher flow rate is routed through the annulus, while the one with the lower flow rate goes through the tube. In this case, the secondary fluid is directed through the annulus, following the hydraulic criterion for optimum performance and efficiency. In the definition of nanoparticles, the data referring to this material used by Flores [9] were used; in this sense, the following characteristics are presented:

1. Specific heat - 0.710 kJ/kg K
2. Volumetric density - 2100 kg/m³
3. Conductive Coefficient - 5000 W/mK

Results and Discussion

Table 1 presented the results of imputing a required thermal load of 3.52 kW.

The non-alteration of these values due to changes in mass flow concentrations indicates system coupling. Table 2 shows the values of mass flow rates, pressures, temperatures, and enthalpies for each state,

Table 1. General indicators.

Parameter	Value (unit)
Qcondenser	3.73 kW
Qevaporator	3.52 kW
Qgenerator	4.82 kW
Qheatexchanger	0.95 kW
Wpump1	0.063 kW

Table 3 presents the values obtained for the thermophysical parameters of the graphene nanofluid in the range of 1 to 5% of concentration marked by zero concentration.

After analyzing the presented results, we inferred that:

- The specific mass was characterized by a linear behavior, given by the 11 kg/m³ increase for each 1% nanoparticle added to the fluid. The observed linearity results from the contribution given by the uniform dispersion of the nanoparticle in the base fluid since the graphene nanofluid has a higher practical thermal conductivity than the base fluid alone, thus implying an improved heat transfer capacity.
- The specific heat was notable for a gradual reduction of its values with the increase in the concentration. Despite its apparent linearity, there is a downward trend in the fluid's heat reduction capacity from 4% graphene oxide.
- The conductive coefficient was characterized by the increase of its values in terms of the progressive increase of the percentage of graphene. When comparing the growth indices, it is noted that there is a tendency for growth from 3%.
- The convective coefficient presented a behavior similar to the conductive coefficient. It highlights the existence of growth stability for the concentrations of 2% and 4%, initiating a reduction process in the quantitative increase of the coefficient values.

Table 2. General system properties.

State	Mass flow [kg/s]	Pressure [kPa]	Temperature [°C]	Enthalpy [J/g]
1	0.01542	0.700	33.00	87.73
2	0.01542	7.400	33.00	87.73
3	0.01542	7.400	63.69	149.56
4	0.01391	7.400	90.00	225.92
5	0.01391	7.400	53.52	157.39
6	0.01391	0.700	46.07	157.39
7	0.00151	7.400	76.44	2642.55
8	0.00151	7.400	40.05	167.70
9	0.00151	0.700	1.88	167.70
10	0.00151	0.700	1.88	2503.98
11	0.16789	3.000	10.00	41.99
12	0.16789	3.000	5.00	21.02
13	0.16789	1.000	10.00	41.99

Table 3. Thermophysical properties of graphene oxide nanofluid (GO).

% Wt graphene	0	1	2	3	4	5
Specific mass [kg/m³]	1000	1011	1022	1033	1044	1055
Specific heat [kJ/kgC]	4,193	4,120	4,050	3,980	3,912	3,846
Conductive coefficient [W/mC]	0,563	0,580	0,597	0,615	0,633	0,651
Convective coefficient [W/m²C]	28,460	28,790	29,120	29,440	29,760	30,070
Dynamic viscosity [kg/ms]	0,001406	0,001435	0,001464	0,001495	0,001526	0,001559
Reynolds number	3094	3067	3038	3008	2977	2945
Prandtl number	0,010480	0,010200	0,009933	0,009678	0,009436	0,009206
Global Coefficient [kW/m²C]	1,3630	1,3630	1,3640	1,3650	1,3660	1,3660
Heat flow [kW/m²]	5,7890	5,7930	5,7960	5,7990	5,8020	5,8050
Evaporation area [m²]	0,6080	0,6077	0,6073	0,6070	0,6067	0,6064
Pump Power 2 [W]	0,3358	0,3322	0,3286	0,3251	0,3217	0,3183

- In dynamic viscosity, it is possible to notice the viscosity growth pattern in terms of the addition of nanoparticles, with the lowest viscosity growth rates observed for concentrations of up to 2%.
- When analyzing the Reynolds number, it is clear that the system is under a turbulent regime. However, the addition of the nanoparticle has reduced indices; such behavior is due, among other factors, to the impacts caused by the increase in viscosity.
- The Prandtl number shows a downward trend for its values, showing that heat tends to diffuse more quickly than velocity. Between 3% and 4% showed the best indicators of parameter reduction.
- The effect of nanoparticle concentration on the global coefficient showed an interesting behavior (Figure 13). The impact on the system was null for concentrations of up to 1%, with the same behavior visible when comparing the values in the 4% and 5% range.
- Having as a parameter the study of the trends presented by the heat flux x concentration ratio, it is observable that the heat flux tends to increase as a function of the larger quantity of nanoparticles, thus remarkable the impact of the nanofluid on the system.
- An interesting point to be highlighted is the general impact of the nanofluid on the area. Note that the application of 5% translates into 0.0016m², which translates into an area gain of 0.3%.
- The purpose of pumping power is to define the values the secondary system pump requires. Their values indicate the drop in pumping power with the addition of graphene nanoparticles. When analyzing the concentration of 5% and comparing it with the absence of nanoparticles, a gain of 5.2% is observed.

Conclusion

The application of graphene nanofluid demonstrated notable enhancements in the system's overall performance, particularly in terms of the conductive and convective coefficients, which saw improvements to varying degrees. Systematic analysis of the thermophysical properties of the nanofluid revealed promising gains in values, significantly impacting system performance. Global heat transfer coefficients increased linearly with higher graphene concentrations, enhancing gains. Additionally, the concentration of nanoparticles exhibited an inverse relationship with the Reynolds number, indicating a reduction in the turbulent regime as nanoparticle concentration increased. Geometric indicators also played a crucial role in assessing system gains, with the evaporation area showing the biggest improvement, at 0.8%, compared to the system operating without nanofluid. Moreover, the pumping power of the secondary system experienced a substantial efficiency increase of 5.2%. These findings underscore the potential benefits of employing graphene nanofluid in absorption refrigeration systems, contributing to improved thermal parameters and overall system performance.

Acknowledgments

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The Role of Social Skills in Work Engagement and Burnout: A Systematic Review

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This study aims to investigate the role of social skills (SS) in work engagement (WE) and burnout through a Systematic Review (SR). The SR was performed in Scopus and Web of Science databases using the PRISMA-ScR approach. Eighteen research were selected, and the findings show a direct and indirect correlation between SS, positive with WE, and negative with burnout. It is concluded that SS acts as a protective factor for workers' health and, consequently, for better institutional results, and interventions should be implemented with a focus on developing workers' SS to promote engagement and prevent burnout.

Keywords: Social Skills. Work Engagement. Burnout. Job Demands-Resources Theory. JD-R Model.

Introduction

The incorporation of new technologies, combined with increased uncertainty and ambiguity, has resulted in more volatile professional performance, with the social space of work being exercised in the same physical space as individuals' private lives, forcing them to constantly change their mode of social action when they are required, which can have an influence on their health and performance [1].Positive psychology research emphasizes the relevance of psychosocial protection in ensuring health throughout one's working life [2]. Organizations should include elements that mitigate possible risks and harms to worker health and increase motivation, engagement, and well-being [1].

Work engagement (WE) results from the motivational nature of resources [4]. It can be defined as a positive motivational state toward work that manifests itself in feelings of vigor, dedication, and absorption [3], according to the Job Demands-Resources (JD-R) model, which assumes that all characteristics of the work context can be modeled by job demands and resources [3]. It is desirable for organizations and workers because it promotes well-being and raises performance [4].

Burnout, on the other hand, is defined as a state of exhaustion related to the work environment caused by the presence of high demands and a lack of resources [5], which causes individuals to be unable to cope with the negative influences of demands and, as a result, fail to achieve their goals and are frustrated [6].

Social Skills (SS) constitute a descriptive construct of social classes and performances in a person's behavioral repertoire in managing interpersonal situations [7]. Communication, civility, making and maintaining friendships, empathy, assertiveness, expressing solidarity, resolving conflicts and interpersonal problems, expressing affection and intimacy in sexual-affective relationships, coordinating groups, and speaking in public constitute a general portfolio of relevant SS [7,8]. SS studies demonstrate that they operate as protective factors and that their absence is a risk factor [8].

Little research has looked at SS as a driver of WE and burnout prevention. The association between SS and burnout [10] and engagement [9,10] has been investigated, but no worker samples were used. Furthermore, the perspective of WE is still limited by the lack of studies that address its antecedents and behavioral consequences [4].

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Given the positive effects of engagement and the negative consequences of burnout, understanding their interrelationships with SS can aid in developing interventions focused on drivers of engagement and mental health. Thus, this study aims to conduct a literature review to investigate the role of SS in WE and burnout.

Materials and Methods

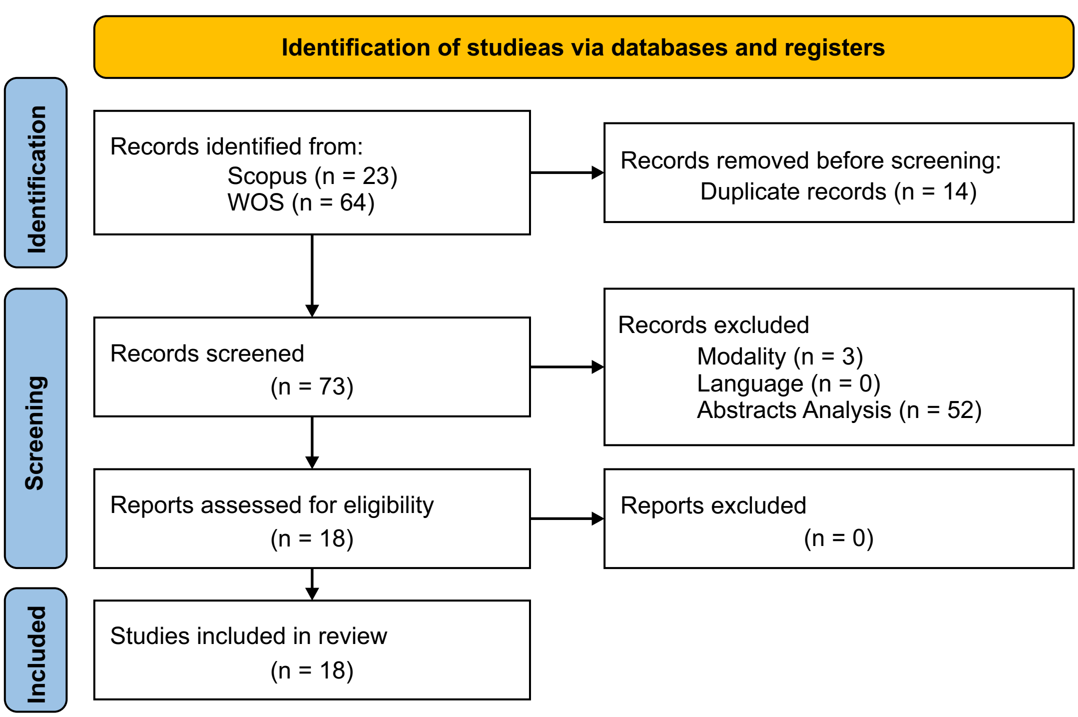
This study was conducted through a Systematic Review (SR) using the PRISMA method [11], with the following guiding question: What are the possible interrelationships of social skills with work engagement and burnout?

The search used strings in the Scopus and Web of Science (WOS) databases. For the definition of specific terms for SS, the classes of SS were considered [7,8]. Furthermore, tests and adjustments were conducted until more qualified results were obtained. The following string was employed: "("social skills" OR communication OR civility OR friendship OR empathy OR assertiveness OR solidarity OR "resolving (conflicts or interpersonal problems)" OR affection OR "coordinating groups" OR "speaking in public" OR "social intelligence" OR "teamwork" OR "emotional intelligence" OR "leadership")" + "work engagement" + "burnout" + "("Job demands-resources" OR "Job demand-resource" OR "JD-R")".

The search was conducted in March 2022, and the preliminary screening retrieved 87 articles. The following inclusion and exclusion criteria were applied: removal of duplicates; inclusion of scientific articles, excluding books, book chapters, and other textual forms; and articles in English, Spanish, and Portuguese.

As a result, the abstracts were analyzed to identify articles that addressed some association between at least one of the SS classes or subclasses with WE and burnout within the scope of the JD-R model. It resulted in the exclusion of 52 texts. Figure 1 illustrates the study selection procedure.

Figure 1. SR flow diagram. Identification of studies via databases and registers.



Source: Adapted from PRISMA 2020 [11].

Results and Discussion

Table 1 lists the articles retrieved as a result of the execution of this SR.

The interpersonal interactions addressed were classified considering the SS classes to identify the SS discussed in the articles [7]. The framework was based on the theoretical references to characterize the concepts addressed. In overlapping concepts with many classes, the class with the most similarity to the concept was chosen. It should be highlighted that the studies indirectly addressed SS by examining how other constructs involving interpersonal interactions, such as leadership and social support, influence engagement or burnout. The SS "coordinating groups" were addressed in studies that examined the impact of various leadership styles, and they are directly or indirectly positively associated with WE and negatively associated with burnout.

Table 1. List of selected articles.

Article Title
Emotional intelligence, work, and psychological outcomes in a public service context [12]
Empowering leaders optimize working conditions for engagement: A multilevel study [13]
Drivers of work engagement: An examination of core self-evaluations and psychological climate among hotel employees [14]
Engaging leadership in the job demands-resources model [15]
Associations of occupational stressors, perceived organizational support, and psychological capital with work engagement among chinese female nurses [16]
Organizational goal ambiguity and senior public managers' engagement: does organizational social capital make a difference? [17]
The hardier you are, the healthier you become. May hardiness and engagement explain the relationship between leadership and employees' health? [18]
How does emotional intelligence help teachers to stay engaged? Cross-validation of a moderated mediation model [19]
Engaging leader - Engaged employees? A cross-lagged study on employee engagement [20]
Drivers of employee engagement in global virtual teams [21]
Authentic leadership and psychological capital in job demands-resources model among Pakistani university teachers [22]
The influence of job and individual resources on work engagement among chinese police officers: A moderated mediation model [23]
The home care work environment's relationships with work engagement and burnout: A cross-sectional multi-centre study in Switzerland [24]
How engaging leaders foster employees' work engagement [25]
Job demands-resources model, transformational leadership and organizational performance: a multilevel study [26]
A dual path model of work-related well-being in healthcare and social work settings: the interweaving between trait emotional intelligence, end-user job demands, coworkers related job resources, Burnout, and work engagement [27]
Do digital competencies and social support boost work engagement during the COVID-19 pandemic? [28]
Gain or loss: the double-edged effect of empowering leadership on employees' innovative behaviours [29]

Engaged leadership facilitates the increase of resources while decreasing demands, resulting in an indirect relationship between burnout and WE via demands and resources [15]. It has a beneficial impact on two resources (social support and autonomy) [20], as well as directly impacting WE through interactions with subordinates and indirectly through work resources [25]. Transformational leadership acts as a resource that can increase personal resources (resistance and resilience) and WE [18]. It also balances resources and demands, which helps to reduce burnout [26]. Empowering leadership has an indirect positive influence on WE and burnout, which is mediated by workers' trust in their leaders [29], as well as mobilizing and optimizing resources, which strengthens the effects of WE [13]. Finally, authentic leadership directly affects engagement and indirectly influences burnout (through self-efficacy, hope, optimism, and resilience) [22]. "Empathy" was studied in studies that evaluated leaders' support for their subordinates and support among members of work teams. It has a direct positive [14,16,17,23,24] and an indirect positive correlation with engagement [23], is directly and negatively related to burnout [24], or does not have an interrelationship with engagement [28]. Social support [14,16,17,23,24] and interpersonal trust [17] are resources that have the potential to improve engagement [14,17], which can be mediated by job satisfaction [23]. Social support and exhaustion have a considerable negative association [24]. The SS "resolving conflicts and interpersonal problems" was investigated in studies that examined the relationship between emotional self-efficacy [23] and emotional intelligence (EI) [12,19,27], both of which are capacities that allow people to deal with their emotions and cushion their effects on interpersonal interactions. The former influences engagement positively [23]. In contrast, EI is a personal resource that influences engagement positively [12,19,27] while also moderating the effects of the work context on psychological outcomes [12], buffering intrapersonal and interpersonal processes, and assisting professionals in coping with the harmful effects of stress [19]. EI protects against burnout by mitigating the probability of harming relationships, and it drives engagement by allowing the development of positive relationships [27]. Finally, "communication" is also directly and positively associated with WE [14,21,24], as well as directly and negatively related to burnout [24]. In this regard, the culture of information sharing can collaborate with the positive psychological climate [14], and formal and informal communication skills are critical for workers to positively adapt to remote work as predictors of engagement in virtual teams [21]. Managers who use task-oriented feedback procedures contribute to increased engagement and reduce the impacts of burnout [24].

Conclusion

The findings of this study allowed us to identify the role of certain SS classes in engagement and burnout. They act as protective factors for workers' health, directly and indirectly related to engagement (positive effect) and burnout (negative effect) via resource mobilization and as work demand buffers. For better institutional results, interventions should focus on developing workers' SS to promote engagement and prevent burnout. It also provided an overview of the current literature, focusing on research investigating how interpersonal relationships between leaders and subordinates affect WE and burnout. The findings indicate that scientific studies addressing the relationship of SS as a theoretical-practical field with WE and burnout are still incipient and, as such, should be part of a future research agenda.

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Organizational Culture and Emotionality in Knowledge-Intensive Organizations in Health: A Systematic Literature Review

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This study aims to characterize the knowledge about how emotionality is approached in the context of knowledge-intensive organizations (KIO) organizational culture using the PRISMA – ScR methodology). A search for studies published between 2017 and 2021 was carried out in the ScienceDirect, Google Scholar, and Scielo databases after evaluations of 6 articles were identified. The results revealed a tendency towards horizontalizing structures and how organizational culture guides the sense of reality. It was concluded that the relationship between KIO organizational culture and emotionality is still little explored. However, we observed in the articles the importance of leadership in conducting KIOs and how the leader can influence the construction of organizational culture.
Keywords: Knowledge-Intensive Organizations. Organizational Culture. Emotionality. PRISMA-ScR.

Introduction

In the current business environment, where the economy is based on knowledge, sustaining a competitive advantage and adapting to complex environments and situations across organizations depends on the capability to use and generate new knowledge [1]. Although all productive activities require knowledge to be carried out, KIOs differ from other organizations in that their activities are predominantly intellectual, and most of their workforce comprises skilled workers [2]. These knowledge-intensive workers have sophisticated intellectual and social capital and technologically literate, global, and operationally agile skills [1] to achieve exceptionally above-average results [2]. As a socially constituted phenomenon, knowledge does not exist by itself but is dependent on social recognition that requires support or even being institutionalized by others; in this sense, it is strongly dependent on social relationships and the formation of social ties; thus, interaction processes are the basis for carrying out intensive knowledge work [2].

We can say that KIO is "people-intensive" because systems, structures, technology, and products matter less than people. Most KIOs rely on a corporate ideology – a set of guiding ideas, beliefs, emotions, and values often more influential than formal structures in controlling people [2]. Knowledge production and management issues are closely intertwined with organizational culture [3], as culture is significant for understanding how knowledge is created, shared, maintained, and used [4]. However, unlike the individual's knowledge, the organizational culture conforms to a collective phenomenon, built-in social interactions, which form a system of meanings and symbols. Meanings refer to how an object or statement is interpreted. It is the meaning given subjectively and is related to an expectation, which, in the case of organizational culture, is shared by others [4]. A symbol is defined as an object, a word, a statement, a type of action, or a material phenomenon that ambiguously represents something else or more than the meaning itself [4].

Alvesson and Sveningsson [5] observe that organizational culture is complex, confusing, and challenging to understand and understand, as it involves a web of meanings, emotions, values,

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behaviors, technologies, systems, and structures, as well as knowledge, objectives, strategies, vocabularies, systems, identities, social relations, networks, and power relations. Thus, managing KIO implies managing social relationships in which individuals' feelings and emotions connect [1], which demands emotionality from organizations and leaders in these social interactions.

In his research on Vygotsky, Abreu [6] presents emotionality as a mental function that is interrelated with other human functions in a complex and uncontrollable way. Emotionality is a driving force that moves the subject to action that results in emotions. Thus, emotionality has a character of systematicity inherent to the human mind, and emotions result from emotionality. In organizations, emotionality is presented as the ability to generate emotional connections between individuals, thus inducing their actions [7].

Bounded emotionality is presented by Mumby and Putnam [8] as an organizational alternative in which nurturance, care, community, support, and interrelationships merge with individual responsibility for shaping organizational experiences.

Limited emotionality admits that there are intersubjective limitations because it is a mental function or even a complex and uncontrollable capacity. For this reason, there is a need for tolerance to ambiguity to preserve people's self-identity through support between individuals [9].

In this context, this research aims to characterize the current knowledge about how emotionality is approached in the context of the organizational culture of knowledge-intensive organizations through a systematic literature review.

Materials and Methods

For this research, we followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA - ScR) model, which aims to improve methodological quality, make SRL reports and research more transparent and more complete by systematizing and proposing a minimum set of items that should compose the research [10,11].

As the PRISMA – ScR method [10,11] guides, we identified the research information bases among the available bases. The databases selected for this SRL were ScienceDirect, Scielo, and Google Scholar.

The databases were chosen based on their international scope, as they have citation indexing criteria, in addition to accessibility by any researcher, even independent researchers who are not linked to postgraduate institutions. The main research questions that guided searches in the databases were:

- Q1: In the cultural context of knowledge-intensive organizations, how do people express emotionality?
- Q2: What are the implications of emotionality for constructing organizational culture in knowledge-intensive organizations?

The guiding questions and the themes presented in the questions were the basis for identifying the string descriptors. If related terms identify the same theme, we use the "Boolean" "OR" to expand the search within the same theme. To identify the articles that presented the correlation between descriptors of different themes, we used the "Boolean" "AND" to restrict and search only articles that correlated the themes. Terms were searched in English, and initially, we used the string ("knowledge-intensive organizations" OR "knowledge-intensive firms") AND ("emotionality") AND ("organizational culture") to search for descriptors only in titles, abstracts, and keywords. However, no results were found, even without establishing a period for the search.

The research considered the entirety of each document and not just the titles, abstracts, or keywords.

The search was conducted in the Science Direct, Google Scholar, and Scielo databases in March 2022. The initial survey identified 36 results in Science Direct and 55 in Google Scholar, making 91 articles. No articles with the string were found in the Scielo database. A selection by period was carried out; in this way, articles published between 2017 and 2021 were selected to select current works since the activity of the ICO is strongly dependent on current knowledge and impacted by technological advances. This screening identified 3 articles in the Science Direct database and 14 in the Google Scholar database. Among the SRL stages using the PRISMA-ScR model [10,11], the evaluation consists of identifying the eligibility criteria of the articles that will be analyzed in the research. The following criteria were adopted to select the most relevant articles for the research (Table 1).

Articles in which the themes appear only in the bibliographic references were excluded.

At the end of the article eligibility process, we obtained 5 articles (Table 2). However, three articles were selected for analysis. They were read in full, seeking to understand the relationships between the presented results and identify patterns, divergences, and research opportunities to answer the guiding questions.

Results and Discussion

The article by Alvesson and Blom [12] is a theoretical article that presents 6 ways in which an organization operates: 3 verticals that concern the hierarchy (whether formal or not) and 3 horizontals that concern the equality and influence of the actors. For the authors, there is a utopia in seeking that all managers be leaders in their respective areas because what will determine the best mode of organization will be the situations faced and the internal or external environment of the organization. The article by Al-Kurdi, El Haddaedh, and Eldabi

Table 1. Filters and justifications applied to the selection of SRL articles.

Order	Filter	Justificative	Nº of Exclusions
1	Duplicates	Duplicate articles between databases were excluded.	1
2	Modality	Only scientific articles published in full in peer-reviewed journals were selected.	7
3	Accessibility	Only open access articles were deliberately included to allow the dissemination of knowledge and replication of the study to the greatest number of researchers.	2
4	Article analysis	Articles in which the themes appear only in the bibliographic references were excluded.	1

Table 2. Papers selected after eligibility criteria.

References	Title
[12]	Beyond leadership and followership: Working with a variety of modes of organizing
[13]	The role of organizational climate in managing knowledge sharing among academics in higher education
[14]	Exploring linkages between organizational culture and gender equality work—An ethnography of a multinational engineering company
[15]	Influence mechanism of jobsatisfaction and positive affect on knowledge sharing among project members: Moderator role of organizational commitment.
[16]	A holistic approach to knowledge risk

[13] is a quantitative research that evaluates the factors that may contribute to or inhibit the practice of knowledge sharing among academics from higher education institutions in the United Kingdom, Saudi Arabia, United Arab Emirates, Bahrain, Kuwait, Qatar, Oman, Jordan, and Egypt. The article by Utoft [14] is an action research that presents how cultural traits of the organizational culture of a Danish engineering company influenced the implementation of a gender equality program. Although they use different methodologies, it is possible to draw a parallel between the articles based on the keywords used in the string: Knowledge-Intensive Organizations, Emotionality and Organizational Culture. The three articles present us with the characteristics of knowledge-intensive professionals. The articles present characteristics such as a tendency to horizontalize hierarchies in knowledge-intensive organizations [12] and the knowledge worker prefers autonomous work [12,13]. In the three articles, the theme of knowledge-intensive organizations only touches on the research, and the theme needs to be deepened in any of the articles. Emotionality is a theme that only touches the articles and is not deepened. In the articles by Alvesson and Blom [12] and Al-Kurdi, El Haddaedh, and Eldabi [13], emotionality touches on the role of the leader in emotionally inspiring his followers. In Utoft's article [14], emotionality is addressed in the methodology as an aspect of the researcher's narrative. Organizational culture was the theme that was best worked on in the three articles. The objective of the articles was not to deepen concepts of organizational culture but to present how organizational culture can shape the sense of reality of individuals and organizations. In the different research, we observed how organizational culture impacts the organization of work; it can define the style of leadership or non-leadership, how culture impacts the success or failure of projects, and how culture impacts knowledge sharing. We observe a convergence between the articles in working on leadership, and although the methodologies are different, there is a certain complementarity between them. The theoretical article by Alvesson and Blom [12] makes it possible to analyze the contexts in which each of the modes of organization can be applied and even combined when leadership should be exercised as a tool to achieve an expected result and which profile is most appropriate for an organization intensive in knowledge. Quantitative research by Al-Kurdi, El Haddaedh, and Eldabi [13] identifies a professional segment in different countries, academics from higher education institutions, when leadership is an essential tool to foster desired behaviors, such as knowledge sharing, even in knowledge-intensive organizations that present a more autonomous and less hierarchical work characteristic. Finally, Utoft's [14] action research looks at a knowledge-intensive organization with a magnifying glass. It identifies how outstanding leadership can reverberate the organization's behavior and how leadership commitment can impact project implementation.

Conclusion

The relationship between the organizational culture of Knowledge-Intensive Organizations and emotionality still needs to be explored. However, the importance of leadership in the conduction of Knowledge-Intensive Organizations is perceived as how the leader can influence the construction of organizational culture and the need to develop, together with leaders, emotional intelligence to deal with their own emotions and the emotions of their subordinates. As future research, it is suggested to deepen research on the role of leadership and how the emotional intelligence of leaders enables a better result in the context of Intensive Organizations in Knowledge. Still, as future research, we suggest further research on how training leaders in Knowledge-Intensive Organizations takes place, where learning for generating and using knowledge is the primary interest of knowledge-intensive professionals.

However, these professionals have little interest in following the career of leaders in this segment.

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Assistive Technologies for the Deaf: A Study of the Search for Indexed Patents at the Brazilian National Institute of Industrial Property

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This article presents a patent search study of 'Assistive Technologies for Deaf People.' The aim is to investigate patented technologies in Brazil, deposited with the National Institute of Industrial Property, to identify the tools developed for deaf people and to identify market opportunities for the advancement and development of new technologies that benefit the information and communication of deaf people. The methodological approach is a systematic review followed by a prospective study. Nineteen documents with extensive coverage deposited in the INPI's patent database were selected. Finally, the prospection showed a significant indication of assistive technologies, the vast majority of which favor the communication of deaf people, prioritizing Sign Language, thus ensuring linguistic accessibility, autonomy, and social inclusion.
Keywords. Patents. Assistive Technology. Communication. Deaf. Social Inclusion.

Introduction

In contemporary society, technology and its advances have enabled humanity's quality of life, influencing the ways of living in society in various social spaces, whether at home, at school, at work, or church, among other areas. In this sense, technological innovations have a premise to develop and/or expand ideas to meet demands, whether of products or processes, so the importance of producing new technologies that meet new demands is essential.

The Brazilian Inclusion Law - LBI (2015) [1] corroborates what is defined in the Convention on the Rights of Persons with Disabilities of the United Nations - UN (2007) [2]. Article 2 of Law 13.146, of July 6, 2015, presents the concept of a person with a disability with long-term impairment of a physical, mental, intellectual, or sensory nature. The law above refers to several instruments that aim to guarantee the rights of people with disabilities so that they are enforced and respected.

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Also, in article 3 in III, the section of the BII, assistive technology is defined as: "products, equipment, devices, resources, methodologies, strategies, practices and services which aim at promoting the functionality and participation of the person with a disability, aiming at his/her autonomy, independence, quality of life and social inclusion". According to the Technical Help Committee – CAT, from the Republic's Human Rights Secretariat, assistive technology consists of an area of knowledge of interdisciplinary characteristics, which encompasses products, resources, methodologies, strategies, practices, and services that aim at promoting the functionality, related to the activity and participation, of people with disabilities, aiming at their autonomy, independence, quality of life and social inclusion. Thus, the legal devices, through public policies, aim to ensure rights. Because of the above, it is essential to emphasize the need to develop technologies that help the autonomous lives of people with disabilities. In this study, hearing impairment/deafness was prioritized to weave a dialog about the assistive technologies explicitly developed for Deaf people, using a search for patents indexed at the National Institute of Industrial Property – INPI.

According to Marchesi (1996) [3], deafness is, therefore, characterized by a greater or lesser loss of normal perception of sounds, and there are several categories of hearing impairment, generally classified according to the degree of hearing loss. In contemporary times, the term hearing impaired is used in the clinical/medical environment. Thus, a person with a hearing deficit cannot acquire the oral/auditory language. However, the word Deaf is used by the deaf community, whose goal is to base the construction of a linguistic and cultural identity anchored in the Brazilian Sign Language - Libras, recognized and regulated by laws and federal decree. According to Skliar (1999) [4], sign language cancels the disability and allows deaf people to constitute a different minority language community, not a deviation from "normality". Thus, to think of deafness as a linguistic difference, not as a pathology and not as a disability that attributes to the Deaf person a condition of inferiority for being users of a language of gestural mode.

Thus, the main objective of this study is to prospect patents filed with the INPI and thus identify assistive technologies developed for deaf people as accessible tools for communication and identify market opportunities for the advancement and development of new technologies that benefit information and communication for deaf people.

Assistive Technology for the Deaf

According to research by the Brazilian Institute of Geography and Statistics - IBGE (2019) [5], it is estimated that 5% of the Brazilian population comprises deaf people. Around 10 million citizens, of which 2.7 million have profound deafness. Thus, it is necessary to understand that assistive technology is a powerful tool that enables accessibility for the Deaf, including them autonomously. Therefore, it is significant to highlight those assistive technologies for the Deaf as a principle to ensure equity, thus promoting communicative, educational, and social accessibility, which ensures their participation in a society mainly composed of hearing people. In this sense, the technologies aimed at Deaf people are essential tools that can create several spaces for interaction, teaching, and learning, providing autonomy and making information and communication accessible to everyone in the democratic process of contemporary society. Nowadays, with the inventiveness of the Internet, as well as technological advances and innovation, information arrives more and more quickly to people through the use of several tools that provide information, communication, and learning. These technical resources are intended to promote the accessibility of people with disabilities, which aims to provide conditions for using social spaces, various services, media, and education.

Materials and Methods

A systematic exploratory literature review was carried out to broaden knowledge on the subject. A qualitative approach was used, followed by a descriptive prospective study of technological solutions that offer accessibility to deaf people. A survey was carried out of patent application processes on the National Institute of Industrial Property (INPI) platform, indexed nationwide from 2000 to 2023 [6]. The period chosen is justified by the "Accessibility Law" enacted by Law 10.098 on December 19, 2000 [7]. The National Institute of Industrial Property is a government body that works with patent and trademark registration. It is a federal autarchy linked to the Ministry of Economy, according to Decree No. 9660 of January 1, 2019. The INPI was chosen because its main objective is to enforce the rules regulating industrial property nationally. The INPI is a free platform for online access to the patent search and analysis system with information on patent applications filed in Brazil (INPI, 2023) [6].

The search strategy used was advanced search - Keyword, Title, and Abstract for the following descriptors in Portuguese - Assistive Technology, Deaf, Hearing Impairment, Deafness, and Technology. Data was collected in May 2023 (Table 1). The exclusion criteria were patents

Table 1. Search descriptors.

Palavra Língua Portuguesa	Tradução Inglês
Tecnologia Assistiva	Assistive Technology
Surdos	Deaf
Deficiência Auditiva	Hearing Impairment
Surdez	Deafness
Tecnologia	Technology

that did not focus on the research topic and those filed before the accessibility law.

Results and Discussion

Forty-one patent documents were found. After reading and analyzing the documents, nineteen were selected, focusing on the study of Assistive Technologies developed for deaf people found in the INPI database (2023) [6]. With the results obtained, a table was drawn up describing the names of the patents, a graph showing the annual evolution of patent filings over the years surveyed, and a table describing the institutions that safeguarded assistive technologies designed for deaf people, with the number of patents listed according to the name of those institutions. It is important to note that the research aimed to identify possible technologies that help deaf people communicate, be autonomous, and be socially included. Table 2 lists the names and titles of the patents applied for at the INPI between 2000 and 2023, in descending order. After reading the applications requested by developers of specific technologies for deaf people, it was possible to see the diversity of innovative projects aimed at helping these people in various social spaces in their daily lives.

The graph shows the annual evolution of patents filed between 2000 and 2023. Figure 1 shows the annual evolution of patent applications filed, correlated to the various technological areas, between 2000 and 2023. The number of patents registered remained the same from 2000 to 2007, with a pattern of growth in 2010, when there was a more significant occurrence in the number of patent registrations with (04) filings. It was noted that from 2020 to November 2021, no number of patents were filed in the area researched in the INPI database. Thus, it is essential to note that there are also no records between December 2021 and May 2023. However, the reason why we did not see any new patents can be attributed to secrecy, which, according to the rules, usually lasts 18 months.

Table 3 shows the list of applicants separated by column with the classification by the name of the university, company, and individual who protected these assistive technologies designed for the Deaf. It is worth noting that nineteen patents were selected, and the table shows eighteen applicants, as the Federal Center for Technological Education of Minas Gerais has two applications.

Conclusion

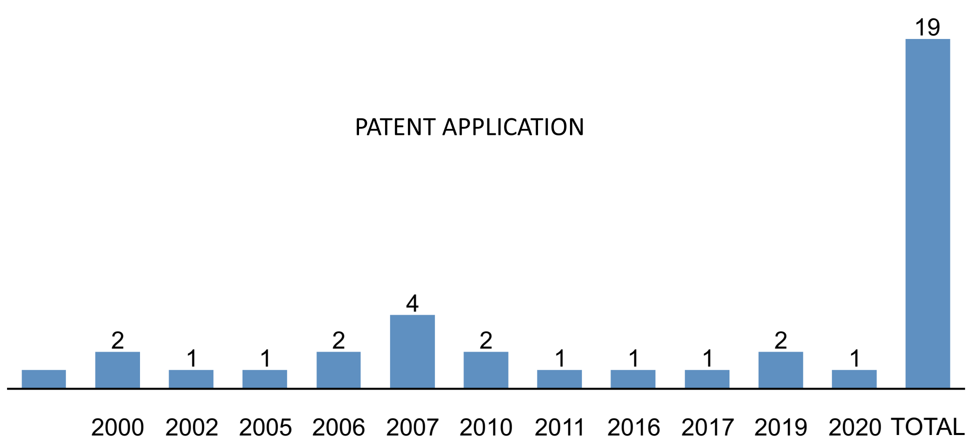
This research allowed us to map the existence of patented technological innovations considered "Assistive Technologies for the Deaf," defined as resources or services to benefit the lives of deaf people in society. The search revealed that there are (19) technologies registered with the INPI, which are considered assistive technologies, as they help deaf people mainly by favoring information and communication, thus guaranteeing linguistic accessibility, as provided for in Brazilian legislation through laws and federal decrees, which consider Libras to be the first language. Thus, it was found that patent registration is an essential indicator of the development of technologies that

Table 2. Patent titles.

Real-Time Sign Language Conversion for Communication in a Contact Center
Method for the Concatenative Synthesis of Sign Languages for the Generation of Realistic Three-Dimensional Signaling Avatars
Electronic System for Monitoring Babies and Children by Hearing Impaired Caregivers
System and Methods for Generating, Preserving and Signing Terminological Neologisms in Sign Languages
Electromechanical Vibrational Wearable Device for Musical Initiation of People with Hearing Impairment
Tactile/Visual Stimulator for Deaf Vehicle Drivers
Obstacle Signalizer
Sound Monitoring Device for the Deaf and Hard of Hearing
Tactile Stimulator for the Deaf
Automatic Bidirectional Translator System between Sign Languages and Hearing Languages
Obstacle Signalizer
Telecommunication Device and Other Functions for Deaf and Hearing People.
Rybena: Communication Method and System that Uses Text, Voice and Libras to Enable Accessibility for People with Special Needs
Portable Equipment for the Hearing Impaired
Telephone Training for Communication between Deaf and Dumb Listeners
Agenda in Libras
Closed Captioning Method, Sponsor of Visual Information Broadcasting for Accessibility for People with Hearing Impairment.
Device for People with Hearing Loss
Digital Inclusion System with a Focus on Accessibility in Brazilian Sign Language and Operated by Augmented Reality Application

Source: Adapted by authors from INPI (2023).

Figure 1. Annual trend in patent filings over the years.



Source: Adapted by authors from INPI (2023)..

Table 3. Identification of the depositors.

Depositor	University	Company	Citizens
AVAYA INC. (US)	State University of Campinas (BR/SP)	Paranaense Association of Culture - APC (BR/PR)	Paulo Marcelo Freitas de Barros (BR/PE) / Ana Maria dos Anjos Carneiro Leão (BR/PE)
State University of Campinas (BR/SP)	Federal Technological Education Center of Minas Gerais (BR/MG) / Foundation for the Support of the Research of the State of Minas Gerais - FAPEMIG (BR/MG)	J.J Fantin Pereira - ME (BR/SP)	Carlos Vicente Sgarbi (BR/GO)
Paranaense Association of Culture - APC (BR/PR)	Federal Technological Education Center of Minas Gerais (BR/MG) / Foundation for the Support of the Research of the State of Minas Gerais - FAPEMIG (BR/MG	Rof Empreendimentos Inteligentes Ltda.	Paulo Marcelo Freitas de Barros (BR/PE)
Federal Technological Education Center of Minas Gerais (BR/MG) / Foundation for the Support of the Research of the State of Minas Gerais - FAPEMIG (BR/MG)	Federal University of Itajubá - UNIFEI (BR/MG)	Paranaense Association of Culture - APC (BR/PR)	João Elison da Rosa Tavares (BR/RS)
J.J. Fantin Pereira - ME (BR/SP)	Institute for Research and Development in Software Technology (BR/DF)		André Miguel de Souza e Silva (BR/PR)
Paulo Marcelo Freitas de Barros (BR/PE) / Ana Maria dos Anjos Carneiro Leão (BR/PE)			Paulo Roberto de Oliveira Noernberg (BR/PR)
Carlos Vicente Sgarbi (BR/GO)			Claudio Roberto Sindicic (BR/SP)
Federal University of Itajubá - UNIFEI (BR/MG)			Jean Gleison Florêncio de Miranda (BR/SC) / Rodrigo Oscar Braga de Godoy (BR/SC)
Paulo Marcelo Freitas de Barros (BR/PE)			Manuel de Souza Araújo (BR/RJ)
João Elison da Rosa Tavares (BR/RS)			Eduardo Felipe Loesch (BR/RS)
Rof Empreendimentos Inteligentes Ltda.			
André Miguel de Souza e Silva (BR/PR)			
Institute for Research and Software Technology Development (BR/DF)			
Paulo Roberto de Oliveira Noemberg (BR/PR)			
Claudio Roberto Sindicic (BR/SP)			
Jean Gleison Florêncio de Miranda (BR/SC) / Rodrigo Oscar Braga de Godoy (BR/SC)			

Source: Adapted by authors from INPI (2023).

enable information and communication for the Deaf, indicating that of the nineteen assistive technologies selected that favor communication, (07) deal with Libras, which is considered to be the mother tongue of the Deaf, consisting of a linguistic system of a visual-motor nature and (04) devices for subtitles and (08) vibrational devices, which allow the Deaf person to perceive sounds, enabling safety and autonomy for life in a society made up of a majority of hearing people. Because of the above, we emphasize that assistive technologies aim to enable autonomy, independence, and social inclusion. The results of this patent study show that there is a market demand to be explored for the development of assistive technologies that help deaf people to be included in society. Therefore, there are possibilities for these technologies to be expanded, as well as the emergence of other technological innovations that guarantee linguistic accessibility and autonomy for deaf people, given the significant number of deaf people in Brazil.

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Analysis of Inter-institucional Cooperation Practices for Science, Technology, and Innovation in Health

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This study aimed to analyze practices of inter-institutional cooperation for science, technology, and innovation in health in different countries and institutions available in scientific journals. Eight articles were chosen for discussion in this study. This work also introduced a method for constructing a platform model that might guide performance improvement actions in inter-institutional cooperation activities in science, technology, and innovation in health. We observed that this subject is evolving and that there is an opportunity to perform additional studies to develop this scientific area. This work enhanced the discussion on this progressing scientific and practical area.
Keywords: Science, Technology, and Innovation. Health Institutions. Inter-institutional Cooperation. Practices. Platform.

Introduction

In recent decades, health has been a central concern of global governance, and diplomacy has played a significant role in building a global health governance system, as shown by the importance of global health diplomacy in keeping countries jointly committed to improving health for all [1,2]. Thus, diplomacy is a field evolving fast with the changing world of globalization [3].

Nevertheless, whether and how the policies developed and the integration between organizations meet the expectations to achieve practical cooperation in global health is unclear. To accomplish that, it is necessary not only political will but also a better understanding of the institutions, interests, and environments of ideas, which can ease or hinder global health diplomacy efforts [4].

If the efforts to bring about change do not persist, there is a high probability that poor outcomes and inequalities in health will worsen [5]. Thus, it is vital to consider the broader goals of improving global health rather than focusing solely on individual issues [6]

Global Health Diplomacy acknowledges the interplay between health and foreign policy, emphasizing negotiation's significant role in achieving goals and entails negotiations among various actors, including state and non-state entities. This fact involves integrating health concepts into policy strategies for broader political, economic, or social aims [7]

Inter-institutional cooperation (IIC) is a widely studied issue, but there is still much to learn. IIC allows different organizations to work together to achieve common goals. It is a complex process that requires a variety of typical collaborative practices. Some standard practices are aggregation of sectoral actions, mutual consultation, establishing units, and confirming committees and subcommittees. These practices could make IIC more effective and efficient. A more complex understanding of the conditions that could promote and facilitate inter-institutional cooperation could stimulate collaborative behavior, and it would be easier to define a typology of tools adjusted to different contexts [8].

This study aimed to analyze practices of inter-institutional cooperation for science, technology, and innovation in health in different countries and institutions available in scientific journals.

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Materials and Methods

Scientific journal articles (original research papers and reviews) were searched at Scielo, PubMed, Scopus, and Web of Science databases from January 2019 to August 2023 to gather the most recent contributions to the theme. The research was structured so that the records to assess might contain the descriptors for a defined search string in the Title, Abstract, and Keywords fields. The search string applied combined term variations of the main categories: "Inter-institutional cooperation,"; "health," "science, technology, innovation," "platform," and "practices."

From the spectrum of fifty-four documents, the most pertinent ones were selected. Additional ones were then identified and extracted via snowballing (consulting the first selected articles' references). Documents were included based on their contents (papers describing theories, policies, frameworks, and guidelines in the public sector, focusing on the public health sector). As a result, 8 (eight) articles were chosen and fully read for the discussion of this study.

This article also intended to introduce a method for constructing a platform model to guide performance improvement actions in inter-institutional cooperation activities in science, technology, and innovation (STI) in health. The authors conceived the mentioned method (described in the 3.1 section) and will be performed as doctoral thesis research shortly.

Results and Discussion

In this section, we portrayed the findings from the 8 (eight) final chosen articles to address the aim of this research (Table 1). The theme seemed to be regaining attention more recently. Studies in different countries and continents showed a worldwide interest in the matter. The primary type of research verified was qualitative, and the scientific methods applied varied (interviews and focal groups).

In general, the findings and results of the selected papers pointed out the positive potential of inter-institutional cooperation approaches and the need to deepen the knowledge of this emerging subject. Those encompassed a range of inter-institutional collaboration models, capacity-building strategies, and policy analyses in public health and research. The results highlighted the need for trust, communication, and coordination to overcome collaboration challenges (such as the need for shared vision, resources, and commitment) and realize its benefits (increased efficiency, effectiveness, and sustainability). The review of the articles provided a comprehensive overview of the models, challenges, importance, and benefits of inter-institutional collaboration for public health. They also called for further research on the most effective collaboration models and the obstacles that must be addressed. However, they did not approach any optimal models, specific promotion strategies, or factors that influence inter-institutional collaboration's success and performance. The possible reasons for that might be because the articles were published in different years and journals, they focused on different aspects, and might reflect diverse perspectives on inter-institutional collaboration. They might report issues based on different times, data, and methods; their findings might not be directly comparable, but they showed trends and clues for future research paths.

The Proposed Method

The findings of the eight articles have highlighted essential implications for public health practice. They suggested that inter-institutional collaboration is essential for addressing complex public health problems. As the findings also emphasized the need to develop and implement effective models of inter-institutional collaboration that tackle the challenges of trust, communication, and coordination, it corroborated the proposition of building a model of the platform that may guide

Table 1. Papers' findings/results.

#	Title/ Reference	Year	Findings/results, from the Papers´ authors
1	Models of inter-institutional collaboration to build research capacity for reducing health disparities. [9]	2008	"Three collaboration models – traditional, consultant, and mentoring – have arisen from the Yale-Howard Partnership Center. While the focus here is on these models, ongoing assessment of their effectiveness is acknowledged. Each model presents distinct attributes, advantages, and limitations. Notably, the traditional and mentoring models have demonstrated optimal efficacy within the Yale-Howard Partnership Center. However, the consultant model necessitated discerning selection to ensure project completion. These models collectively fostered augmented competencies and research environments in both institutions, notably in the realm of health disparities elimination."
2	The Guatemala-Penn partners: An innovative inter-institutional model for scientific capacity-building, healthcare education, and public health. [10]	2017	"The Guatemala-Penn Partners represents a collaboration between Guatemalan public and private universities and Penn that is founded on the principles of university-to-university connections, dual autonomies with locally led capacity building, and mutually beneficial exchange. Its ongoing initiatives in the domains of science, health-care education, and public health strive to fulfill the World Health Organization's Global Health Workforce Alliance strategies of partnerships and education. The goal of both describing and analyzing the success and limitations of these initiatives is to supply insight into strategies that can be adapted to other contexts to promote and strengthen similarly oriented global partnerships."
3	Inter-sectorial and inter-institutional cooperation and coordination in public health within the market model of the Colombian Health Care System, 2012- 2016. [11]	2018	"Inter-institutional cooperation and articulation are recognized as essential for the development of the public health. Some specific and short-term experiences have been reported. The market and competition model of the Colombian Health Care System does not allow for strengthening the values required to achieve sustainable development in public health. Given the wide range of actors involved in public health actions, inter-institutional and intersectoral coordination and articulation is imperative to rationalize resources, improve efficiency and effectiveness, and build values and social fabric as a scaffolding for the development of public health."
4	The institutional building of science and innovation diplomacy in Latin America: toward a comprehensive analytical typology. [12]	2021	"We understand Science and Innovation Diplomacy (S&ID) in Latin America (LA) as a tentative re-organization of different states and subnational actors around the study and institutionalization of the governance of contemporary transformations on the systems of Science, Technology, and Innovation (ST&I). The main contribution here is to supply a simple typology regarding the varieties of S&ID initiatives and how its institutional building is being influenced by state and non-state actors, regionally and globally. The main finding is the necessity for better articulation between S&ID initiatives in and among LA countries, as well as a wider understanding of the dynamics of ST&I in Global South countries, which brings challenges but also possibilities of open agendas."
5	The interface of multisectoral and multilateral dimensions of public health policy: what is new in the 21st century? [13]	2022	"As the developments prove, the interface between multisectoral and multilateral dimensions of health policy has substantially diversified and enriched in the 21st century. The two dimensions tend to, increasingly, interact, inform, and reinforce each other. Such interaction would, alongside the profound intersectoral potential embedded in Health in All Policies and the SDGs, be one of important drivers of 21st century intersectoral policy—and international cooperation—for health."
6	R&D and innovation efforts during the COVID-19 pandemic:The role of universities. [14]	2022	"One of the main contributions of this study is the understanding of the Research and Development and Innovation (RDI) potential of the region and the relevance of setting up inter-institutional and business cooperation networks at national and international levels. The study shows that during the pandemic universities showed high RDI potential to quickly react to critical needs, offered open innovations, open licensing, showed collaborative abilities and effective use of their academic and student resources."
7	Brazil's foreign policy and health (1995-2010): A policy analysis of the Brazilian health diplomacy – from AIDS to 'Zero Hunger'. [15]	2023	"The main argument of this study is that national and international policies are intertwined in this process and that domestic dynamics and societal engagement are essential, but more is needed: governmental choices are also determinant. Institutional arrangements and policies shift in different conjunctures and are constantly prone to conflicts and change. Therefore, we emphasized the importance of more systematic and rigorous studies on the possibilities and limits of the links between health and international relations, as on the so-called health diplomacy."
8	Strengthening national public health institutes: a systematic review on institution building in the public sector. [16]	2023	"The overriding result is the identification and definition of six domains of institution building in the health sector: "governance," "knowledge and innovation," "inter-institutional cooperation," "monitoring and control," "participation," and "sustainability and context-specific adaptability." Our results show that the described domains are highly relevant to the public health sector, and that managers and the scientific community recognize their importance. Still, they are often not applied consistently when creating or developing National Public Health Institutes (NPHIs). We conclude that organizations engaged in institution building of NPHIs, including International Association of National Public Health Institutes, may benefit from state-of-the-art research on institution building as presented in this study."

performance improvement actions in Inter-institutional cooperation activities in STI in health.

Table 2 shows the technical procedures designed for the proposed method for constructing the platform conceived by the authors. The proposed method, supported by this literature review, will be the basis for constructing the inter-institutional cooperation platform, which is the object of the first author's thesis. This review will also sustain future deepening studies by the author's research group on the subject.

Conclusion

The studied subject is evolving; therefore, there is an opportunity to perform studies to improve this scientific field. The research suggested that inter-institutional collaboration can be a valuable tool for improving public health. However, it is crucial to be aware of the challenges and take steps to mitigate them. This study had some limitations associated with its method and defined scope. The limitations were related to using only four different databases and needing to apply strict systematic review protocols and checklists such as Preferred Reporting Items for Systematic Reviews and Meta-Analyses - PRISMA. Further and complimentary research agenda may include the development of scoping and/or systematic literature reviews that could be beneficial to deepen the main concepts involved in the proposed method. The evidence collected pointed out that the proposed method for constructing a platform model to guide performance improvement actions in inter-institutional cooperation activities in STI in health might fill an existing gap. This work also enhanced the discussion on this progressing scientific and practical area.

Table 2. Proposed method technical procedures.

#	Technical Procedures	Detailed Description
1	Critical factors prioritization	Prioritize, based on the scientific literature and through a survey with national and international experts, the critical success factors for the implementation of inter-institutional cooperation and the challenges faced in the process.
2	Challenges identification	Identify the challenges faced by specific cases in a Brazilian health science, technology, and innovation institution (HSTII), through documentary research and statements collected from managers, researchers and operators involved in HSTII cooperation activities.
3	Focal groups holding	Hold representative focus groups to validate the critical success factors raised from the literature and with experts.
4	Platform model proposition	Propose a model of an inter-institutional cooperation platform for science, technology, and innovation in health.
5	Platform model validation	Validate the proposed model through an expanded survey research with HSTII managers from Brazil and abroad.

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Modernizing Occupational Safety in Industry 4.0: A Review of State-of-the-Art Safety Wearables

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Industry 4.0 signifies a paradigmatic transformation in occupational safety, paving the way for the safety wearables (SWs) market, which is still in its early stages in Brazil. This study aims to comprehensively review the global state of the art of safety wearables, compiling pertinent technological trends for the country's reindustrialization debate. Articles were sourced from the Scopus platform, and VosViewer was utilized for keyword tracking. A global race of SWs research is evident, with the construction industry at the forefront, emphasizing monitoring employees' mental states. The emergence of local startups in Brazil within this theme is expected in the forthcoming years, potentially contributing to reducing the high rate of work-related accident mortality in the country.
Keywords: Digital Transformation. Wearable Safety Devices. Workplace Safety.

Introduction

Intending to gather development strategies to assist the modernization of Brazilian industry in the face of 21st-century challenges, the National Confederation of Industry published the document entitled "Industrial Revitalization Plan" in May 2023 [1]. Proposal 57 of this Plan mentions implementing new occupational health and safety regulatory standards (NRs) and initiating a monitoring process to achieve greater effectiveness in reducing occupational risks and accidents. The goal of this proposal is commendable, especially when considering the national context of occupational accidents. According to the International Labour Organization (ILO), Brazil ranked second among the G20 countries in work-related mortality in 2020 [2].

An issue that the occupational safety area still encounters in industries operating in large areas and/or with a large number of individuals is individual worker supervision. This monitoring is paramount since, in an industrial setting, unsafe behavior can lead to an unsafe condition for other workers (reduced reliability process). In this context, the concept of human error emerges. According to Sanders and Moray (1991), "human error is a decision or behavior that reduces or has the potential to reduce the safety or performance of a system" [3]. This panorama is similar to activities carried out by machines since they were previously programmed by humans and, in operation, they work in a man-robot collaboration. Thus, the need arises for measures not only of prevention (e.g., personal protective equipment, occupational health and safety regulatory standards, and training) and monitoring and interpretation of patterns (of use and execution). Therefore, having a database regarding worker behavior allows for the creation of patterns and their categorization, that is, screening, as a filter, whether the individual is fit for work. The technological advancement capable of performing this function is through safety wearables (i.e., intelligent wearable devices capable of collecting and analyzing data and providing feedback to the user) [4]. The growth of research related to SW in the global scenario is evident. The individual monitoring of workers and interpreting their results are becoming increasingly necessary to ensure safe working conditions in line with this new paradigm of industrial digitalization. Therefore, this article aims to conduct a state-of-the-art review of SWs, mapping the principal

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countries, emerging technologies in the market, promising areas, and critical authors. The goal is to compile these trends and analyze them in the context of Brazil. Thus, this work seeks to contribute to discussing the modernization of the occupational health and safety field in the context of the new Brazilian industry.

Materials and Methods

The articles analyzed in this study, extracted from the Scopus platform, resulted from a search using the keywords "safety and wearables*" in the abstract, keywords, and title. The inclusion criteria for this research encompassed documents in English and of the type "article." The search was conducted in May 2023 and yielded 2,027 results; however, only the documents from the top five authors were individually examined.

The articles were read and categorized in an Excel spreadsheet (Main Spreadsheet) according to their respective application areas. The purpose of this spreadsheet was to compile patterns, classifying the information extracted from reading the abstracts and the full articles into nine categories (e.g., author, title, area, technology, classification, device, sensor, and biological systems). The criterion for the "classification" category was based on the work of Jabelli and colleagues (2019) [5].

The VOSviewer platform was used in this study to conduct a broader review, encompassing all the results from the search of the main terms mentioned in the 2,027 publications. For the compilation of the graph, a co-occurrence analysis was chosen using the fractional counting method, and the inclusion criterion was a minimum of 30 repetitions of keywords. Additionally, the platform was used to verify if the patterns found in the Excel spreadsheet (Main Spreadsheet) covered the keywords and trends of the entire dataset. In other words, to assess whether the author's microanalysis was consistent with the results of the macro-level analysis.

Results and Discussion

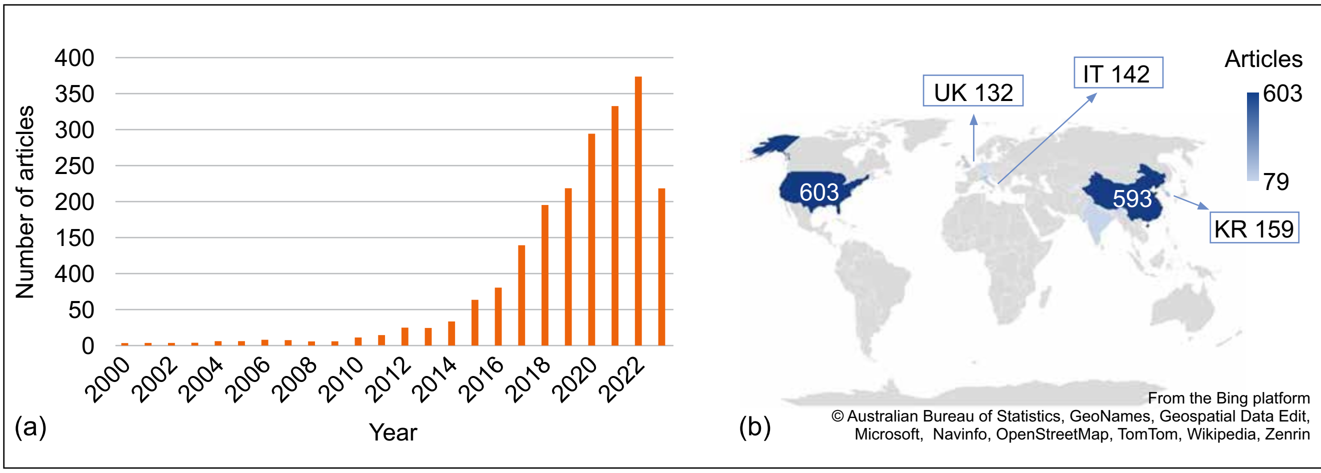
During the study of articles from the top 5 authors, a notable emphasis was observed on chemistry with 22% of the publications (batteries), and 78% in the construction industry. Consequently, a more specific criterion was chosen for further analysis. Therefore, the publications related to the construction industry were compiled in the Excel spreadsheet (Main Spreadsheet). This Excel data and Scopus platform information generated graphs for temporal evolution, main classifications, and top publishing countries.

Temporal Evolution and Key Players

The temporal evolution of published articles from 2000 to 2023 was obtained from the research results. The average publication, with 5-year intervals, ranges from 3 publications (i.e., from 2000 to 2004) to 139 (i.e., from 2015 to 2019). Analyzing the Figure 1, we evidenced that the set of Industry 4.0 incentive programs reflects the advancement of the scientific community. Since 2015, countries such as China, South Korea, the United States, and the United Kingdom have invested approximately R$1.2 trillion in policies focused on digital transformation, science, technology, innovation, decarbonization, and more [1].

On the other hand, it is observed that Brazil currently does not occupy a prominent position in this field and still demonstrates very incipient research efforts. However, given three key factors, the country presents fertile potential for developing this area: domestic investment, abundant raw materials, and an innovation ecosystem. Firstly, the resumption of public policy discussions focused on the domestic industry is paramount. Secondly, Brazil's expertise in silicon extraction and purification processes generates a more stable and appealing context for the global economy, especially when combined with the fact that the country holds approximately 95% of the world's

Figure 1. Number of publications per year (a) and key publishing players (b).



quartz deposits [6]. Lastly, the stakeholders in this ecosystem, including research centers such as universities and federal institutes, as well as companies like the National Center for Electronic Technology (CEITEC), play a crucial role. CEITEC, a state-owned company specializing in sensor and integrated circuit development, is a potential player for SW (Silicon Wafer) projects. CEITEC boasts extensive market experience, with semiconductor applications focused on cryptography, wireless communication, and security, among others [7].

Classifications and Main Devices

Figures 2 and 3 demonstrate the results obtained when analyzing the classification groups and the primary devices mentioned in the articles. Notably, the monitoring of workers' mental state received significant attention within the category of articles. It occurs due to the association between brain activity and stress, concentration, or enthusiasm levels and how its analysis yields accurate data. The worker's mental state is essential even in human-robot collaboration activities [8]. In line with this finding, the primary device mentioned is the electroencephalogram (EEG), capable of reading cerebral electrical activities.

VOSviewer (Keyword Clusters)

The Figure 4 generated in the VOSviewer platform [9] demonstrates that the trends found in the macro-level analysis confirm the trends of the micro-level analysis. In other words, even when analyzing a subset of 39 works, the patterns found in this research also stand out when viewed as a whole. The construction industry field obtained an average of 48.5 occurrences, with the main keywords being "construction workers" and "construction safety." Among other significant results, there is the appearance of the words "fatigue" (30 occurrences), "ergonomics" (54 occurrences), "risk assessment" (76 occurrences), "walking" (41 occurrences), and "gait" (49 occurrences), all related to the classification graph (Figure 2). As for the occurrences in the graphs of the devices, "textiles" (56 occurrences) and "electroencephalography" (48 occurrences) stand out.

Conclusion

In conclusion, there is a global race in research on wearable safety devices, as workplace safety is increasingly perceived as an investment. This perspective goes beyond the social aspect, emphasizing a more humane approach toward workers while recognizing the potential of these

Figure 2. Classifications.

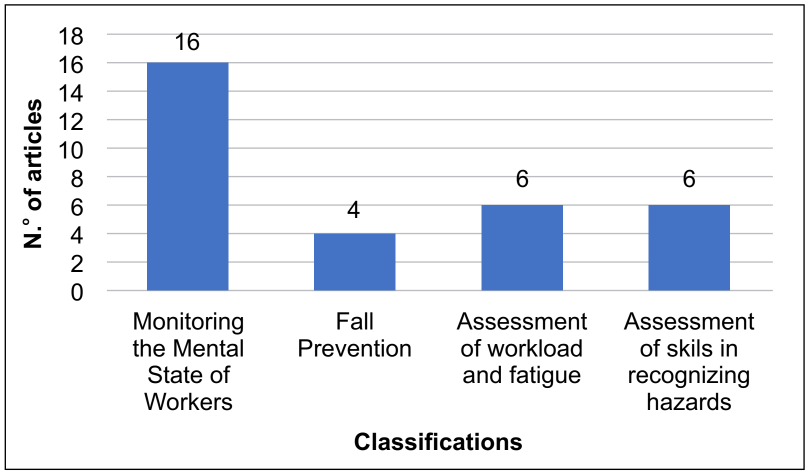
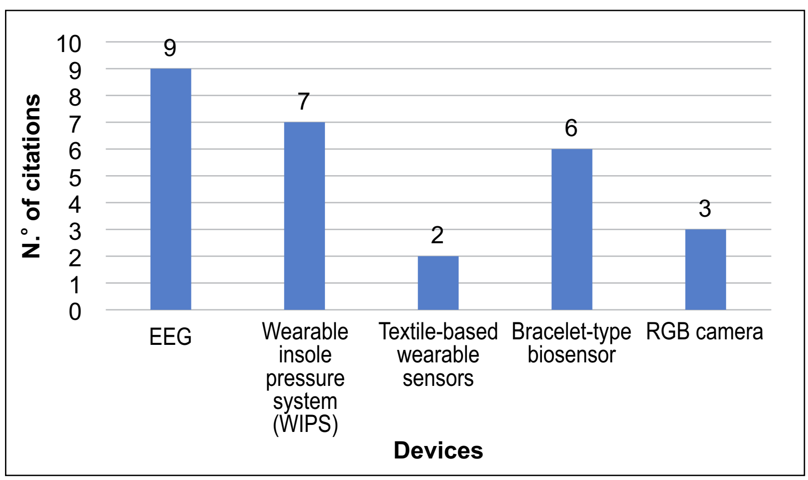
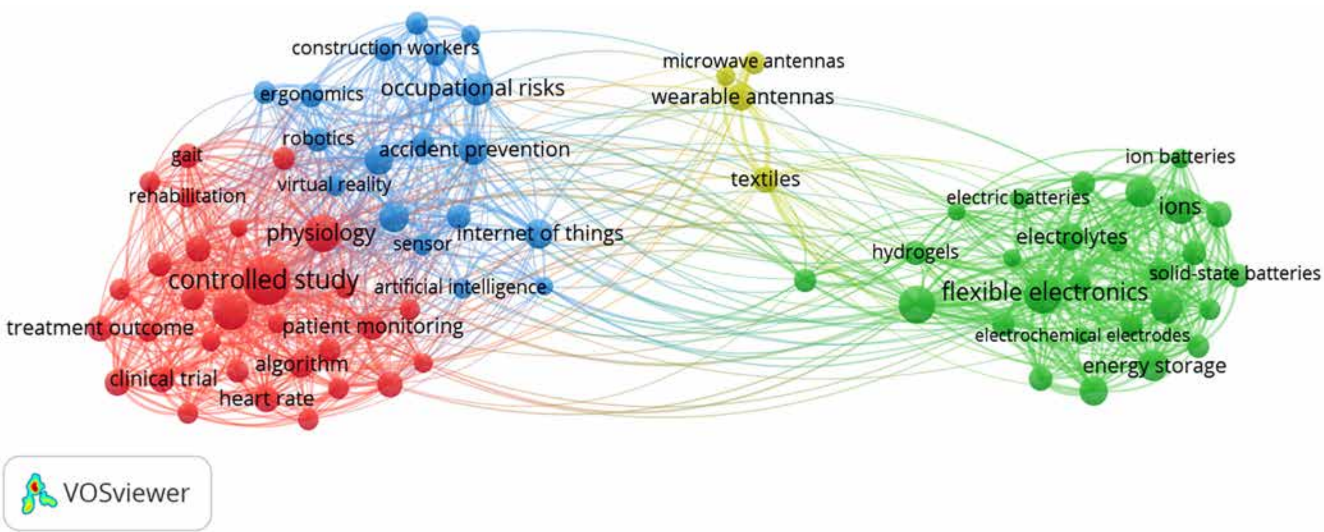


Figure 3. Devices.



devices to reduce occupational risks, misuse of machinery, and unnecessary maintenance, enhance technical operations, and create new markets for SW innovations. While SWs currently excel in the construction industry, expanding these devices to other sectors is viable to address similar safety gaps. It is particularly relevant and feasible in the national context, where incentives for industrial development, such as the Growth Acceleration Program, are increasing, and the internal scenario of accidents calls for restructuring. The four categories discussed, Monitoring the mental state of workers, Fall prevention, Assessment of workload and fatigue, and Assessment of skills in recognizing hazards, represent a latent opportunity for analyzing applications of SWs in companies, facilitating their integration, and potentially mitigating workplace accident rates. In addition, it was possible, through this research, to map the main types of devices (EEG, WIPS, Textile-based wearable sensors, Bracelet-type biosensors, RGB cameras) already used in the field of SW. Ultimately, this article provided insights into the emerging global field of SW in Brazil, especially regarding the emergence of new companies that can operate synergistically with CEITEC, fostering an innovation ecosystem to support Brazil's reindustrialization in light of the Industry 4.0 paradigm of production.

Figure 4. Map from the VOSviewer platform.



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Study of the Main Factors Responsible for the Phenomenon of Self-Combustion Observed in Solid Biomass Waste Piles

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This article discusses the causes of wood-derived biomass self-combustion. We researched technical and academic literature on chemical processes at SENAI CIMATEC. The factors influencing spontaneous combustion are microbial activity, temperature, humidity, and the concentration of O_2 and CO_2. Fungus and bacteria play a leading role in microbial activity, degrading organic matter and releasing toxic and flammable gases. The temperature is crucial for monitoring this phenomenon.
Keywords: Self-Combustion. Biomass. Energy. Wood. Microorganisms.

Introduction

During these last two decades, considerable efforts to reduce greenhouse gas emissions and implement the circular economy model have led to the establishment of a chain of processes, ranging from developing environmentally friendly technologies to the need for waste treatment and/or mitigation [1]. Various industrial sector companies, aiming to comply with current environmental regulations, have proposed replacing non-renewable energy sources with clean energy, such as substituting mineral/vegetable coal and natural gas with solid biomass derived from wood processing waste [2]. The physical treatment applied to biomass, such as that used with different types of wood, results in a significant amount of bark, chips, and sawdust, which, due to their relatively lower thermal efficiency compared to pellets used in boilers, have been discarded in landfills or even piled up in warehouses without proper safety precautions. This use of this new fuel source in larger-scale processes also leads to increased demand for waste storage. Self-combustion occurs when storing this material, which is often done in piles or spaces with insufficient ventilation, leading to accidents and even fatal incidents [3]. This article aims to shed light on the causes of the self-combustion phenomenon, presenting an analysis of the contributing factors based on a review of academic and technical literature. The article also presents some real-life incident cases where the phenomenon of self-combustion or the generation of toxic gases resulted in fatal accidents.

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Materials and Methods

This article was built based on the technical expertise of the research team in the field of chemical processes at SENAI CIMATEC, as well as on the review of relevant academic and technical literature from leading scientific journal platforms, such as Scopus and Elsevier.

The strategy used in the search for literature about that matter was based on separating keywords into groups such as 'material," 'agent," 'effect," and "impact on the system" (Table 1). We did not restrict the research concerning the publication date or journal.

Results and Discussion

Spontaneous combustion, also known as self-combustion, is an event that occurs when a

Table 1. Bibliographic search strategy.

Material	Agent	Effect	Impact on the System
Biomass pile	Fungus	Gas emissions	Self-combustion
	Bacteria	Gas emissions	
	Temperature	-	
	-	Incidents	

specific body or surface emits certain gases/vapors and comes into contact with oxygen, resulting in an exothermic reaction, leading to combustion without an external heat source. In the storage of wood chips, especially in piles, spontaneous combustion is frequently observed due to certain factors that promote an increase in temperature and emission of gases capable of undergoing combustion, thereby enhancing autoignition. One of the significant contributors to this process is microbial activity. Due to its chemical composition (hemicellulose, cellulose, and lignin), wood is generally highly susceptible to organic decomposition, involving the action of fungi and bacteria [4-6]. Fungi are microorganisms with the most significant ability to decompose biomass material. They are classified as decayers, mildews, and stain-causers (Table 2).

Associated with microbial activity, which can attack the woods cell structure, leaving it more exposed to oxidative processes, self-heating is also a significant factor in spontaneous combustion, leading to the formation of hotspots, reaching temperatures of up to 140°C [7,8]. The formation of hotspots is directly related to the height of the storage piles of the material, which increases the potential for combustion due to reduced air circulation, hindering heat dissipation. Additionally, the moisture content present in the material contributes to self-heating because the heating of water caused by organic matter decomposition can increase the temperature of the wood chip pile, leading to evaporation and subsequent shifts in chemical equilibrium, favoring combustion reactions and replenishment of evaporated water, [9]. Moreover, moisture levels above 16% create favorable conditions for the proliferation of fungi and bacteria, increasing microbial activity, which can initiate system heating in aerobic respiration processes and eventually lead to spontaneous ignition [7,10].

The degradation process of organic matter causes uncontrolled emissions of greenhouse

Table 2. The action of each class of fungus in the decomposition of organic matter [5,6].

	Fungus Class		
	Decayers	**Mildews**	**Stain-Causers**
Action on Organic Matter	Responsible for decay (white, brown, and soft rot) resulting from the decomposition of cellulose, hemicellulose, and lignin Causes loss of mass and color change	Induces surface alterations Does not affect the thermal properties of the material May degrade the cell wall, leading to physical and mechanical changes	Secrete colored substances into the albumen Cause surface or deep stains Reduce density, hardness, flexural strength, and impact resistance

gases, posing risks to the health of people in the vicinity, as well as operators working in enclosed or semi-open environments. The deterioration of organic matter can also occur in anaerobic processes, resulting from the depletion of oxygen in the environment, known as off-gassing [11].

Relevant Scale Experiments

Large-scale experiments were conducted to simulate industrial-scale storage or practical pile setups to investigate the self-combustion process and understand how the storage conditions of the material are related to its physicochemical properties, potentially leading to spontaneous ignition. These experiments allowed for the observation of thermal dynamics, moisture content evolution, degassing, and microbial communities [12,13]. The investigated biomass from wood consists of wood chips from forest residues, wood bark, and wood pellets, with moisture content ranging from 5% to 10%, stored in small piles of tens of cubic meters, with residence times varying from one month to over a year [13-15]. The experimental factors and variables include biomass species (fresh or aged), moisture content, particle size, storage height, shape, density, and volume of the pile, storage strategy (internal vs. external, with or without covering), storage time and duration, preservatives, among others.

Microbial Activity

During the investigation, it was evident that microbial activity and heat release in the initial phase increased the pile temperature, leading to a transition from mesophilic to thermophilic microbial communities and finally back to mesophilic communities at lower temperatures. It was identified that temperature, moisture, nutrient supply, concentrations of O_2 and CO_2, and pH are factors that directly influence the colony's development [16].

Temperature

Temperature is one of the most critical parameters, as it is a reliable indicator of microbial activity, material degradation, and storage performance. Microbial degradation can significantly increase the system's temperature due to the exothermic nature of degradation reactions, balanced with heat transfer within the pile [17].

In the study conducted by Anerud and colleagues, the temperature behavior over the storage time of a pile measuring 15 m in width, 35 m in length, and 6.5 m in height, representing an estimated volume of 1450 m^3, was described. The pile consisted of wood chips ranging from 8 to 45 mm in size. The pile used in this study contained a mixture of the following plant species: Picea abies (in larger quantities), Pinus sylvestris, and Betula spp, harvested in the region of Skinnskatteberg, Sweden. This material was segmented into four regions, with two consisting of material collected in September 2013 (residing for 3 months) and the other two regions in January 2014 (residing for 7 months). Each region (regions A and B) was exposed to precipitation (Figure 1) [17].

The pile exposed for three months had its temperature data measured every two weeks. Figure 2 displayed the acquired sensor data.

We observed that there is a temperature gradient in the studied pile, with the maximum temperature reaching around 67°C in the northern region of the pile. Notably, in the first month, all points reached a temperature of 60°C. Additionally, a 1.4% loss in mass was identified during the experimental period. These findings indicate significant microbial activity, especially in the initial storage stages.

Concentration of CO_2 and O_2

The concentration of CO_2 and O_2 is a parameter capable of indicating microbial activity, which is dominated by aerobic processes, thereby reducing the concentration of O_2 and increasing the concentration of CO_2. The emergence of CO indicates chemical oxidation occurring at

Figure 1. Organization of the pile for the experiment [17].

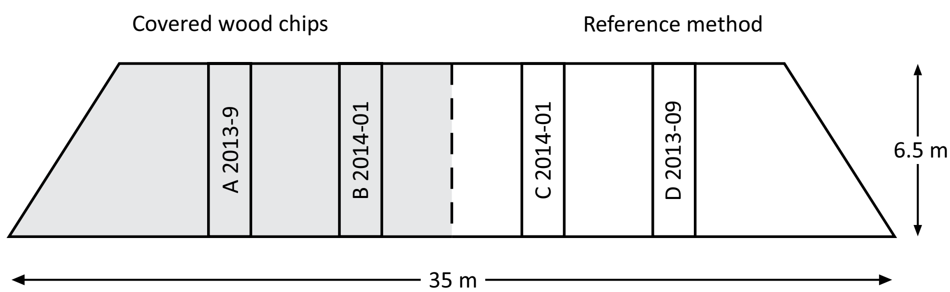
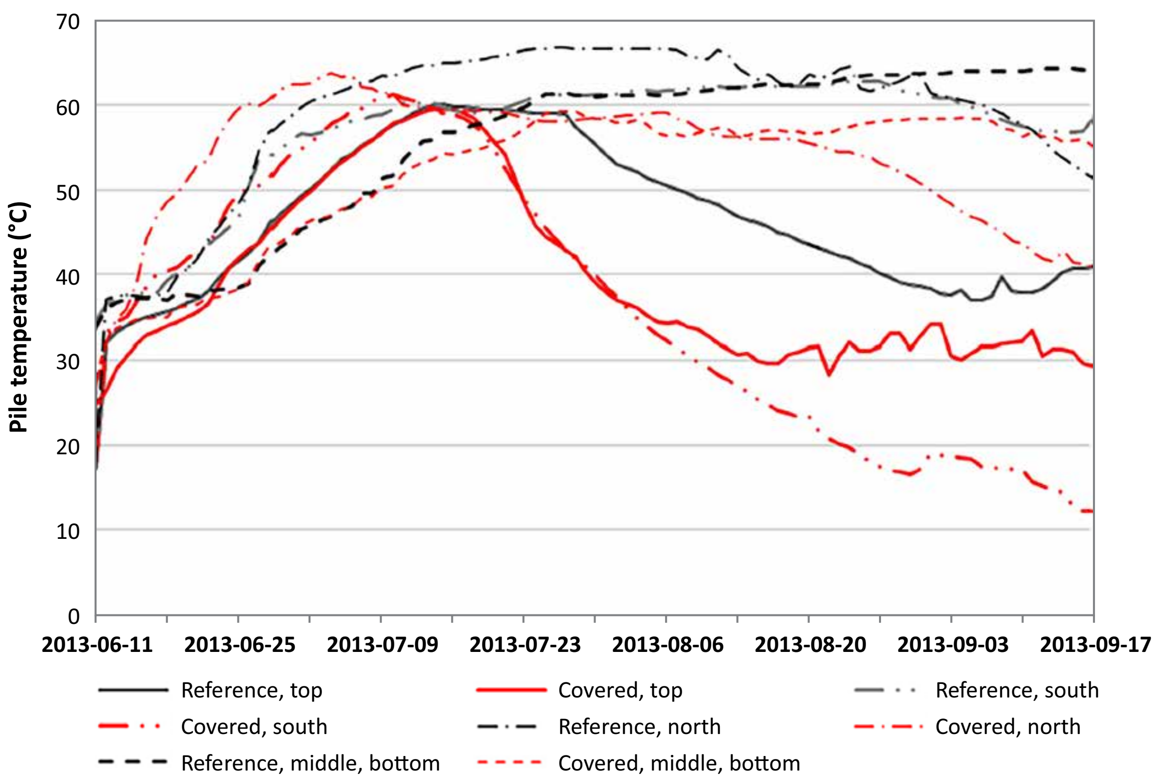


Figure 2. Evolution and temperature distribution in the regions of the pile over 3 months [17].



relatively higher temperatures caused by microbial heating. It is worth noting that the presence of CH_4 indicates the anaerobic activity of microorganisms, and depending on the stage of decomposition, its emission can also be detected [13,18].

Accident Involving Lignocellulosic Biomass Storage

In this context, addressing the accident on the commercial ship AMIRANTE (IMO 7425334) on July 15, 2009, is crucial. The vessel departed from Riga (Latvia). It was loaded with 2600 tons of wood pellets destined for the Amagerværket power plant, owned by Wattenfall, which had recently converted one of its coal blocks to solid biomass [19]. It was reported that two crew members were missing on the ship and were later found dead at the bottom of the stairwell leading to the bow compartment. Surviving crew members mentioned that they experienced great difficulty in breathing and strong weakness while trying to remain in that area.

During the investigation, it was identified in the wood pellet sample that the concentration of CO in the environment was above 150 ppm, information that, together with the autopsies, confirmed death by CO poisoning, indicating levels of COHb saturation in the blood at 52% and 60% for each of the crew members. The inhalation occurred due to a gap in the door's opening, through which gases traveled from the hold to the bow [19].

Conclusion

It is understood that spontaneous combustion is directly linked to certain factors related to storage conditions, such as temperature, light, time, humidity, and gas concentration, mainly CO_2 and O_2. These factors, in turn, impact microbial development and the action of fungi and bacteria in the deterioration of organic matter, further influencing temperature, humidity, and gas concentration parameters. It is also possible to produce some flammable gases, increasing the chance of spontaneous ignition. Linked to this is the health risk to people located in the vicinity of areas susceptible to spontaneous combustion due to the strong inhalation of toxic gases, as occurred in the AMIRANTE ship accident, where operators in a confined space lost their lives due to CO poisoning. Therefore, spontaneous combustion is a phenomenon that should be further investigated to fully understand the factors that contribute to its occurrence, enabling the development of solutions to reduce accidents and greenhouse gas emissions in the atmosphere.

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Study of Oil and Gas Prospecting Techniques: A Review

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Consumption of petroleum products continues to grow steadily. In 2020, oil consumption numbers surpassed exploration's, indicating a possible market imbalance. That said, the demand for new oil reserves becomes essential. Prospecting techniques for these commodities are used to have greater accuracy before drilling a well. Different Geological, Geophysical, and Geochemical methods are applied with different principles in their search, with Seismic being the most widely used technique. Sniffers have gained ground among geochemical methods, and new technologies are emerging. Despite many methods, none is conclusive and should be used in conjunction with others, whether in the prospecting area or for leak monitoring.
Keywords: Oil and Gas Prospecting. Geochemical. Geophysical. Hydrocarbon Anomaly.

Introduction

The search for oil and gas continues to grow yearly, and it is not without reason. According to data from the statistical yearbook of ANP (National Agency of Petroleum, Natural Gas, and Biofuels of Brazil) for 2022, the volume of oil production increased by 1.6% from 2020 to 2021, totaling an average of 89.9 million barrels per day. As for the consumption of this commodity, it rose by 6% during the same period, reaching 94.1 million barrels per day. Regarding Natural Gas, the production and consumption equaled absolute numbers of 4 trillion cubic meters in 2021, but in percentage terms, the growth was 4.5% and 5%, respectively [1].

In other words, the pursuit of acquiring oil and gas has been greater than the quest for exploration. Despite significant reserves being exploited, paying attention to studies searching for new deposits is essential to maintaining a balanced market.

The prospecting of oil and gas areas and the involved techniques are paramount and constitute the first and most crucial part of their exploration. Through prospecting, it becomes possible to determine qualitatively and even quantitatively the presence or absence of hydrocarbon chains, thereby eliminating high drilling costs in unpromising areas. Numerous methods are used, and many have emerged or been refined over the years to increase reliability, reduce environmental impact, and mitigate exploration failures, as the absolute certainty of the amount of oil present in a well is only obtained when it is drilled. This paper aims to mention the methods and techniques already used and consolidated in the prospecting of oil and gas areas while providing a brief overview of some new strategies and methodologies under study that are being developed for the subject at hand.

Materials and Methods

Three main methods are used for oil and gas prospecting, namely Geological, Geophysical, and Geochemical [2]. Each method involves specialized techniques to obtain specific information when searching for hydrocarbon chains. Each technique alone cannot predict the occurrence of oil in the region. However, combining the results obtained from each method makes it possible to achieve a more accurate prediction. Generally, Geological and Geophysical methods are the first to be performed, followed by Geochemical methods to complement and validate the entire study [2].

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We used the "Web of Science" database based on two systematic reviews to identify the most established techniques and those being tested in recent years in the field of oil and gas exploration,

In the first and most comprehensive review, the Boolean operator "and" was used with the keywords "prospecting techniques," "oil and gas," and "methods of prospect" for the last 10 years. After gaining a broader understanding of the subject, the second review aimed to delve deeper into the techniques, and the following keywords were used with the "and" operator: "methods of prospecting of oil and gas", "exploration of oil and gas" and "hydrocarbon anomaly."

Geological Methods

The first step in prospecting a new region is to study its geology, aiming to understand all the conditions of hydrocarbon formation and accumulation. Geologists' main tools in this stage are called surface geology maps and data [2]. The construction of the surface geology map is carried out by explorations using techniques such as aerial photogrammetry and photogeology to indicate potential areas. By analyzing the rocks that emerge on the surface, it is possible to understand the sedimentary basins in the region comprehensively and search for hydrocarbon accumulations [2].

In summary, the techniques employed in geological methods involve studying samples of collected rocks and the presence of fossils within them. Based on this, geologists can identify the most favorable structures for oil and gas accumulation and collect data on the permeability and porosity of the basin, as these parameters can pose challenges in the exploration process [2].

Geophysical Methods

After applying geological methods, indirect or geophysical methods are employed to enrich the analysis and enhance the prospecting process. Geophysics involves studying the composition of rocks and their structures [2].

Seismic reflection is still the most widely used method for oil and gas prospecting in the industry. Due to its relatively low cost compared to other techniques and its ability to provide high-quality subsurface descriptions, this geophysical method is extensively employed as an initial study to locate hydrocarbon accumulations [2].

Its methodology involves generating elastic waves within the Earth that, as they propagate and reflect off petrophysical rock layers, return to surface sensors, providing images of the local geology and its layers, allowing for the analysis of the presence or absence of oil reserves [4]. Figure 1 demonstrates this phenomenon.

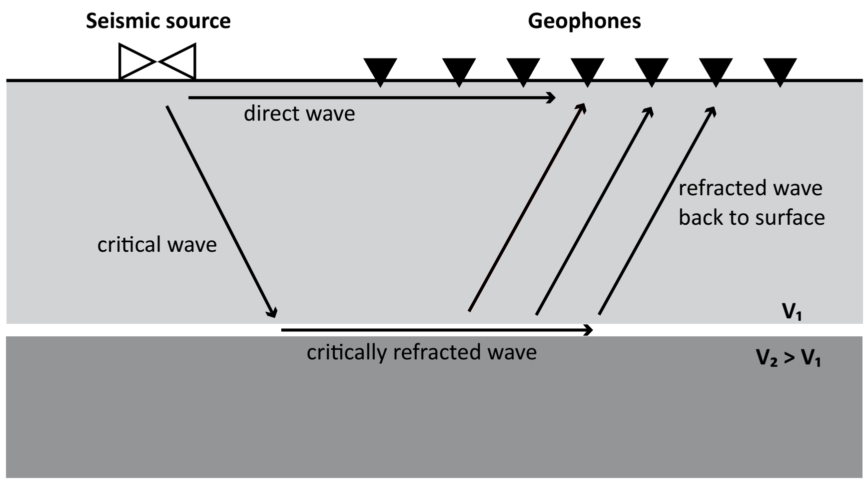
Explosives and vibratory equipment are commonly used in land prospecting to generate these elastic waves, while compressed air guns are utilized in marine prospecting. The pulses generated by these disturbances propagate in all directions and are then captured by electromagnetic receivers onshore and pressure receivers offshore, known as geophones and hydrophones, respectively. After processing the acquired data, images are generated, and location analysis can be conducted [2].

However, every prospecting methodology has limitations, so professionals must combine other methods for a higher success rate and reliability. Other techniques can also complement seismic studies, such as magnetometry, which is based on detecting magnetic anomalies that may be present on the seafloor.

Rocks that can provide information about hydrocarbons commonly contain magnetic minerals in their composition. In the offshore industry, this detection can be performed using a remotely operated underwater vehicle (ROV) or an autonomous underwater vehicle (AUV). However, the downside of magnetometry is that anomalies may not be detected for various reasons, such as sediment accumulation in the area, or it could mistakenly detect a magnetic field generated by any other source [5].

Electromagnetic methods function similarly to magnetometry, where transmitters are placed

Figure 1. Seismic data survey scheme [4].



on the seafloor and send electrical currents into the rocks. Analyzing the difference between the transmitted and received electrical currents makes it possible to assess the presence or absence of petroleum, as the layers may exhibit different electrical resistivity compared to the rocks. One advantage of this method is its ability to be used in profound layers. However, its disadvantages include the influence of salt presence on the seafloor readings and higher costs and complexity of the equipment involved [5].

Geochemical methods serve as complementary approaches to the geophysical methods already employed. As locating oil reserves becomes increasingly challenging, geochemical methods gain more and more significance due to their accuracy in directly indicating the presence of petroleum. Prospecting for oil via the geochemical method involves searching for chemical activity to detect the accumulation of hydrocarbons at the surface. It is possible because oil reserves are not completely impermeable, causing volatile components in the reservoirs to escape through diffusion to the atmosphere and oceans' surface [3].

Gas chromatography techniques are commonly used to analyze the presence of hydrocarbons, both in onshore and offshore situations [3].Methane is not a suitable parameter for indicating the presence of oil, as it is produced in large quantities by other natural processes. The hydrocarbons most used as indicators are Ethane, Propane, N-butane, and iso-butane [3].

One of the earliest methods used was the analysis of pore gas, where at the site to be analyzed, a hole is drilled to a depth of 1 to 2 meters, sealed, and then a sample of air is extracted for analysis. The technique involves reducing the pressure in the hole and collecting the geo-gas. If the ratio between the concentration of light to heavy hydrocarbons is high, it indicates the presence of a gas reservoir, while a low ratio indicates an oil deposit. However, this method is not always conclusive as it heavily depends on the geological characteristics of the area, such as faults and fractures. Therefore, this method has been improved over time to enhance its accuracy [3].

One of these improvements was using gas chromatographic analysis of adsorbed gas. A soil sample is collected at 5 to 10 meters deep and then treated with acid and heat to release the hydrocarbons. Subsequently, a gas chromatographic analysis is conducted [6]. These methods are more commonly used in the onshore industry and, in comparison, to offshore operations. In marine environments, the influence of fluid zones is much less impactful than on land, and the temperature and pressure at the ocean floor are more constant, facilitating the analysis of hydrocarbon anomalies in the area [3,6] (Table 1).

The geochemical methods for oil and gas prospecting in oceans have similarities, with the main differences in the way samples are collected and where and when they are analyzed. Older and less efficient techniques, such as collecting gas bubbles, may be used to store these gases in glass vessels for subsequent analysis of carbon compound concentrations and isotopes. Another method involves phase equilibrium analysis of water samples along with a sample of equal volume of Helium [3], which, being an inert gas, does not react with hydrocarbon compounds and can be inserted into a chromatograph to measure the concentrations of molecules.

Newer and more efficient methods are being studied and continuously improved, utilizing devices that conduct remote analysis, referred to by some researchers as "sniffers," which collect samples of dissolved gases and perform the analysis. This method shows a strong tendency to evolve and become increasingly influential, as accurate on-site analysis saves significant time and enhances productivity in the process [6].

However, these sniffers have specific sensors for predetermined compounds, usually light hydrocarbons, which means they can still not capture all present hydrocarbon chains. Similar studies are being conducted in the mining prospecting industry, where a robot known as "PMGRA - Portable Multicomponent Gas Rapid Analyzer" [7] is used to analyze hard-to-reach areas and provide solutions to overcome limitations in terms of sample transportation, analysis, and the number of human resources required. These areas' geological and physical characteristics favor the diffusion and vertical migration of gases, which can carry information about deep mineralization in the region based on their composition. In other words, by analyzing geo gases (gases in the soil) that reach the surface, conducting both mineral and oil prospecting in those areas is possible. In other words, the main objective of geochemical techniques is to determine the presence of hydrocarbons in a particular location, not only

Table 1. Prospecting techniques and their place of application.

Method	Technique	Application
Geological	Aerophotogrammetry	On-shore/Off-shore
	Photogeology	On-shore/Off-shore
Geophysical	Seismic	On-shore/Off-shore
	Seismic 3D	On-shore/Off-shore
	Seismic 4D	On-shore/Off-shore
	Magnetometry	On-shore/Off-shore
	Electromagnetism	On-shore/Off-shore
	Gravimetry	On-shore/Off-shore
Geochemical	Pore gas analysis	On-shore
	Adsorbed gas analysis	On-shore
	Dissolved gas analysis	On-shore/Off-shore
	Phase equilibrium (Helium)	Off-shore
	Microbiological analysis	Off-shore
	Sniffers	On-shore/Off-shore
	Isotope analysis	Off-shore

qualitatively but also quantitatively, assessing their distribution in space. The choice of technique will depend on each specific case and the research objectives. However, operating with more than one methodology is crucial to ensure excellent reliability in the final results. Combining multiple methods provides a more comprehensive and accurate investigation.

New Prospecting Methods

Due to the most methodologies has pros and cons, new techniques are being studied and tested in relevant environments to complement these studies. This article will briefly mention some new methods that go beyond the traditional approaches, bringing innovation and fresh perspectives to oil and gas prospecting.

One of the techniques that is gaining space and relevance and yielding highly positive results is the use of suggested gamma-ray spectrometric data. Although this method was developed in the late 20th century, it is currently being utilized on a larger scale due to advancements in surface and aerial spectrometry prospecting technologies [8]. The methodology involves the normalization of Thorium to reduce the influence of lithological and environmental factors, where Potassium and Uranium values are regulated based on Thorium [8]. Experiments and tests show that low measurements of these two normalized components can indicate the presence of nearby hydrocarbon deposits [9].

A study conducted in the Egyptian desert by Nigm and colleagues (2018) exemplifies this method well, where airborne gamma-ray spectrometry was employed, and several maps of the region with radioelement compositions were generated and analyzed. The result identified nine large, well-defined areas with high confidence in the presence of hydrocarbons.

Another technique targeted for studies in the last decade to prospect oil areas involves the quantitative and qualitative analysis of hydrocarbon-degrading bacteria in the soil. Lighter gases such as methane, ethanol, propane, and butane migrate from petroleum reservoirs to the soil through diffusion [10], and various microorganisms, including these bacteria, take advantage of these components for metabolic activities. The soil collection is performed, and the sample is stored in sterilized backpacks, then rapidly cooled to temperatures between 2 – 4°C until they arrive at the laboratories to analyze the bacterial colonies. The results are plotted as a function of the population density of these microorganisms [10]. Like other prospecting methods, this technique is used with other techniques. Its significant advantage is that it can be applied in any area where soil samples can be collected, including those where the seismic method (initial information) cannot be used due to the geography of particular locations.

Another technique refined over the past 20 years is the indirect analysis of hydrocarbon presence based on heavy metal ion measurements in regions close to oil basins. Metals as Cu, Pb, Ni, Ti, V, Co, Mg, and so on are expected to be observed. Nickel, lead, and mercury are present in heavier oil fractions, such as asphaltenes [11]. Therefore, using selective electrodes capable of detecting the presence of heavy metals in potential areas is necessary, especially in the offshore sector, where water near oil basins becomes enriched with heavy metals. Studies conducted in Russia in 2020 [11] used an apparatus that performed seismic measurements, geo-electrical mapping of heavy metal ions, and sonar for methane detection. In other words, it was a hardware device that incorporated at least three different prospecting methods, complementing each other to provide more significant reliability in the final results. Lead and copper were the heavy metals measured using a potentiometer, and the device could be used at distances between 200-400m below the watercraft's level [11].

Conclusion

Many methods and techniques concerning oil and gas prospecting can be observed. Some of them are already well-established, like the seismic method, while others, such as Sniffers, show high prospects for improvements and applicability. However, more recent methodologies are proving to be increasingly refined and innovative, gaining prominence in research and development. It is worth noting that no technique is 100% effective and will not provide reliability if used alone. Combinations of techniques are necessary to ensure the investment in drilling the well. In addition to this, these technologies can not only be used for oil prospecting but also for monitoring hydrocarbon leaks in platforms.

Acknowledgments

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Hydrophobic Polymer/Graphene and Derivatives Based Nanocomposites: A Systematic Rieview

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Graphene presents exceptional properties. When this nanomaterial is present in nanocomposites, even at low concentrations, their properties are drastically changed and can be tuned according to the desired final nanocomposite characteristics. Graphene-based materials, such as graphene oxide (GO) and reduced GO, can be functionalized with different chemical species, enhancing their versatility. Furthermore, these materials can provide hydrophobic properties for nanocomposites, being useful for applications in anticorrosive coatings, membranes, and packaging. This review analyzed different methodologies/functionalizations reported in the literature to obtain graphene-based nanocomposites with hydrophobic properties.

Keywords: Graphene. Hydrophobic. Nanocomposite.

Introduction

Graphene is a lamellar (2D) nanomaterial with outstanding mechanical, chemical, electrical, and thermal properties. Graphene's structure is based on a single-atom-thick sheet of carbon atoms bonded by sp2-hybridized bonds and arranged in a hexagonal honeycomb lattice. The exceptional mechanical properties of graphene are attributed to the C-C bonds, the most robust connection found in nature [1]. Graphene oxide (GO) is a derivative material from graphene, generally obtained by chemical exfoliation of graphene nanosheets. Unlike graphene, GO presents abundant functional groups bonded to the central carbon sheet, including hydroxyl, epoxide, and carboxylic groups [2].

Consequently, while graphene is considered a hydrophobic material, graphene oxide is hydrophilic, and this property can be tailored depending on the application demand. Functionalization of the GO with different species can produce an extensive range of materials for the most diverse applications. To cite a few examples, graphene and its derivatives can be used on electronics, sensors, batteries, screens, textiles, coatings, packaging, and biomaterials [1,2].

The hydrophobicity effect of graphene and its derivatives is significant for some applications, such as coatings and packaging. A surface can be considered: I. Hydrophilic if the contact angle (q) between the surface and a water droplet on this surface is $10° \leq \theta < 90°$; II. Hydrophobic if $90° \leq \theta < 150°$; III. Super hydrophilic, if $\theta < 10°$; and IV. Superhydrophobic if $\theta \geq 150°$ [1,3].

The superhydrophobic effect results from combining chemical water-repellent species and hierarchical rugosity on the surface. A hydrophobic surface can become superhydrophobic if rugosity is produced on the surface. A natural example of this effect is in the lotus flower, whose leaves are repellent to water drops, which roll over the surface with a contact angle higher than 150°. The leaves present a micrometric rugosity and a nanometric film of wax, which act synergistically to reduce the surface's wettability (Figure 1a) [3].This effect is a consequence of the low energy of the wax on the surface, together with the entrapment of air pockets between the water and the surface due to recesses and projections (micro-structured texture) of the surface [2,3]. For example, biomimetic strategies are studied to achieve this superhydrophobic effect for self-cleaning surfaces (Figure 1b) and anticorrosive coatings. The addition of graphene nanoparticles as fillers in nanocomposites can contribute to the

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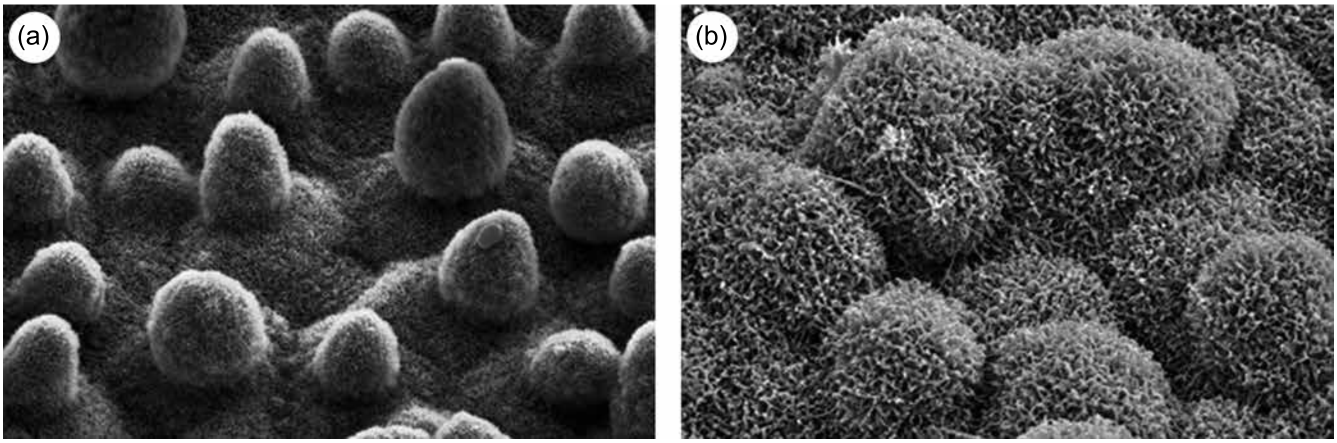
Figure 1. SEM images of the (a) biological model of a lotus (*Nelumbo*) surface and (b) biomimetic copy of an electrochemically hierarchically structured copper foil in the same magnification.



two factors for the superhydrophobicity effect: The intrinsic hydrophobicity of graphene sheets as well as the addition of irregular rugosity on surfaces due to the presence of the nanoparticles [1].

The exceptional properties of graphene have attracted many researchers and the interest of a wide range of industries. This study's main objective was to develop a systematic review to understand the use of graphene or its derivatives as a hydrophobic filler on nanocomposites, selecting reports from 2017 to 2023. We focused on studies demonstrating the transition of nanocomposite surfaces from hydrophilic to hydrophobic after the addition of graphene and on the graphene or graphene oxide functionalization to achieve more hydrophobic properties.

Materials and Methods

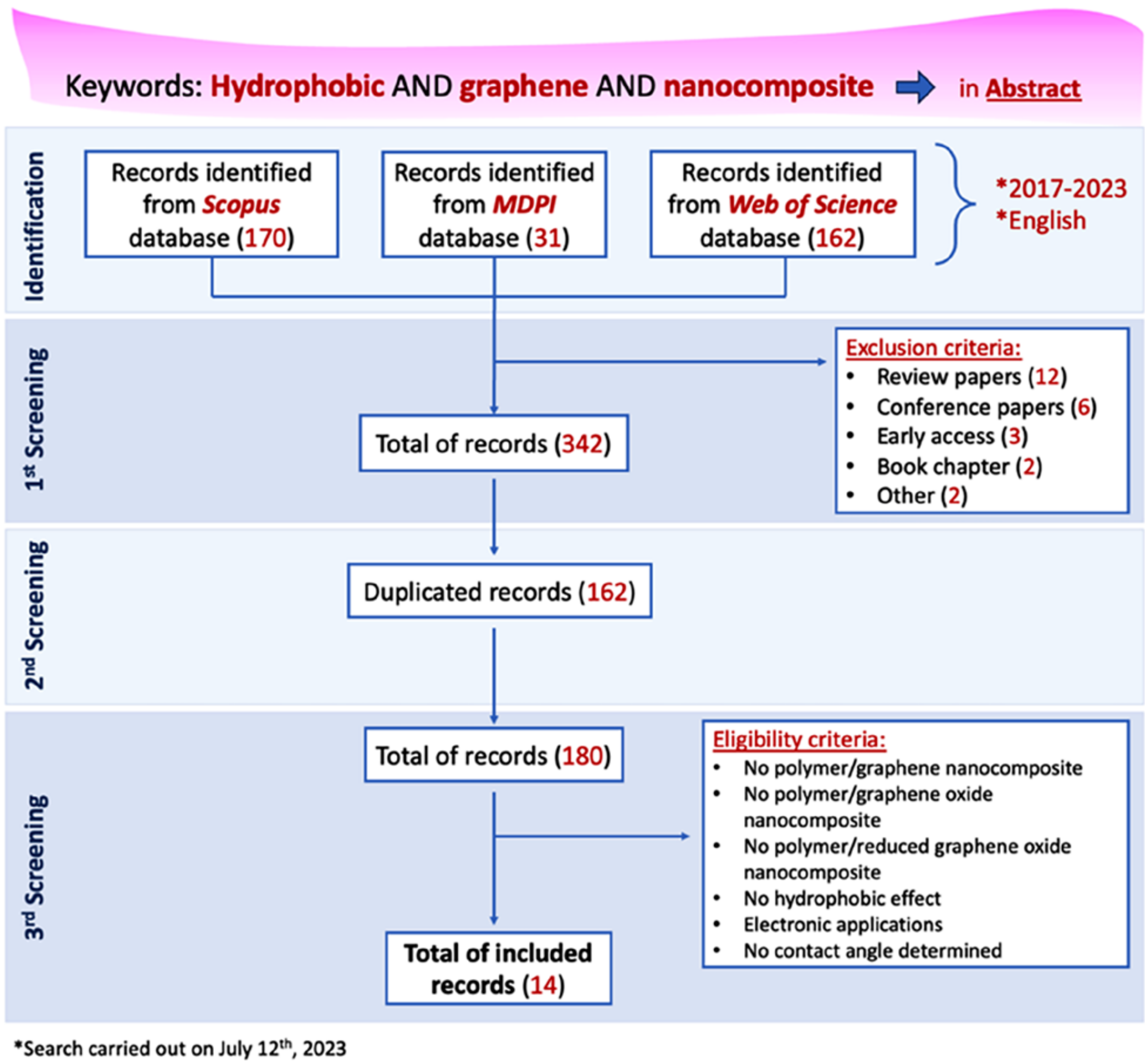
This systematic review followed the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) protocol. Three different literature databases were used to search the papers: Scopus (www.scopus.com, Accessed on: July 12th, 2023), Web of Science (www.webofknowledge.com, Accessed on: July 12th, 2023), and MDPI (www.mdpi.com, Accessed on: July 12th, 2023). The keywords and Boolean operators used for the search were "hydrophobic," AND "graphene" AND "nanocomposite". These three cited keywords had to be present in the paper abstract, and the search included only papers in the English language published from 2017 to 2023.

Figure 2 shows the papers' search and selection process flowchart based on PRISMA requirements. The search in the Scopus, MDPI, and Web of Science databases resulted in 342 papers, including all research articles and excluding review papers (12), conference papers (6), early access articles (3), book chapters (2), and others (2). The next step was to exclude the duplicated papers from the three used databases, leaving 180 documents. At the 3rd screening, due to a large number of studies in various fields, we considered and maintained only the papers concerning polymer/graphene (or derivatives) nanocomposites, related to hydrophobic films or membranes production, presenting oil or water "contact angle" essays, and excluding electronic applications. Finally, 14 papers were considered relevant to be presented in this review.

Data Collection Outcomes

Table 1 summarizes all the papers selected using the systematic review protocol (PRISMA) (Figure 2).

Figure 2. Systematic review flowchart, following PRISMA protocol.



Zhou and collaborators (2017) [5] obtained a superhydrophobic property by coating a PET film with GO functionalized with octadecyl amine (GO-ODA). While the PET film coated with only GO coating (2 mg/mL) presented a water contact angle (WCA) of 21° (hydrophilic due to the hydroxyl and carboxyl groups of GO), the GO-ODA coating (2 mg/mL) exhibited a very high superhydrophobic effect, presenting WCA of 164°, attributed to the long carbonic chains of ODA. Similarly, Xu and colleagues (2019) [16] used octadecylamine (ODA) functionalized GO (GO-ODA) as filler in regenerated cellulose films to enhance the water vapor barrier performance of the nanocomposite films. Pure GO presented WCA=38°, while GO-ODA increased the contact angle to 126° due to the hydrophobic carbonic chains of ODA, which contributes to the water repellence.

Yadav and colleagues (2023) [12] produced hydrophobic polyurethane foams with different GO concentrations (0%, 1%, 2%, 3%, 4%, and 5%) for application as oil sorbent apparatus for water decontamination and for recovering oil and organic solvents from polluted natural waters.

Table 1. The polymeric matrix, graphene type, functionalization species, nanoparticles (NP) concentration, and water contact angle of the surfaces with and without graphene or functionalized graphene of the selected papers.

Reference	Polymeric Matrix	Graphene Type	Functionalization Species	Water Contact Angle (Without NP)	Water Contact Angle (With NP)	NP Conc. (wt.%)
Bera and colleagues (2020) [4]	Thermoplastic Polyurethane (TPU)	GO	p-phenylenediamine (GO-PPD); hexamethylenediamine (GO-HMD); ammonia (GO-NH_3)	87° (TPU); 84°(TPU/GO)	96,7° (TPU/GO-PPD); 105,3° (TPU/GO-HMD); 90,7° (TPU/GO-NH_3)	0.1
Zhou and colleagues (2017) [5]	Polylactide (PLA)	GO	Octadecylamine (GO-ODA)	21° (PET/GO)	164° (PET/GO-ODA)	0.2
Ravi and colleagues (2021) [6]	Polyvinylidene difluoride (PVDF)	GO	Perfluorodecyltriethoxysilane (PFDTES)	85° (PVDF)	100° (PVDF/1%GO-PFDTES) 110° (PVDF/2%GO-PFDTES)	1.0 2.0
Paseta and colleagues (2020) [7]	Polyamide (PA)	rGO	Octadecylamine (ODA)	70° (PA)	81° (PA-rGO-ODA-0.03) 84° (PA-rGO-ODA-0.06)	0.03 0.06
Zhang and colleagues (2023) [8]	Epoxy (EP)	rGO	Polyaniline (PANI) + $CePO_4$	51° (Epoxy)	92° (EP-rGO-PANI/$CePO_4$)	1.0
Wang & Lin (2021) [9]	Epoxy (EP)	G	--	52° (Epoxy)*	110° (EP-Graphene + Silica)*	1.0
Xavier & Vinodhini (2022) [10]	Epoxy (EP)	GO	Nano Zr_2C + 3-aminopropyl tris[2-(2-methoxy ethoxy) ethoxy] silane (APTMEES),	68°(Epoxy)	161° (EP-GO/APTMEES-Zr_2C)	2.0
Ramirez-Soria and colleagues (2021) [11]	Epoxy (EP)	GO	NH_2 and NH_3+ (BFGO)	62° (Epoxy)	105° (EP-BFGO)	0.5
Yadav and colleagues (2023) [12]	Polyurethane (PU) foam	GO	--	124° (PU foam)	135° (PU-4-GO)	4.0
Ly and colleagues (2021) [13]	Poly(acrylic acid) (PAA) + Poly(ethylene imine) (PEI) + Poly(sodium 4-styrene sulfonate) (PSS) + Poly(allylamine hydrochloride) (PAH)	rGO	Poly(sodium 4-styrene sulfonate) (GPSS) and Poly(allylamine hydrochloride) (GPAH)	130° $(PEI/PAA)_{60}$ Layer-by-Layer film	136° $(GPAH\text{-}PEI/GPSS\text{-}PAA)_{60}$ Layer-by-Layer film	--
Ramirez-Soria and colleagues (2022) [14]	Epoxy (EP)	GO, rGO	Propyl-GO and Propyl-rGO	62° (Epoxy)	95° (EP/propyl-GO) 100° (EP/propyl-rGO)	0.5
Rajitha & Mohana (2020) [15]	Epoxy (EP)	GO	2-Aminothiazole (GO-AT) and 2-amino-4-(1-Naphthyl) Thiazole (GO-ANT)	86° (Epoxy)	92° (EP/GO) 95° (EP/GO-AT) 97° (EP/GO-ANT)	0.2
Xu and colleagues (2019) [16]	Regenerated cellulose	GO	Octadecylamine (GO-ODA)	38° (GO)	126° (GO-ODA)	0.5 1.0 2.0 5.0
Abakah and colleagues (2021) [17]	Epoxy (EP)	Commercial G (X50, M15,C750)	--	64° (Epoxy)	72° (EP/X50) 81° (EP/M15) 102° (EP/C750)	1.0

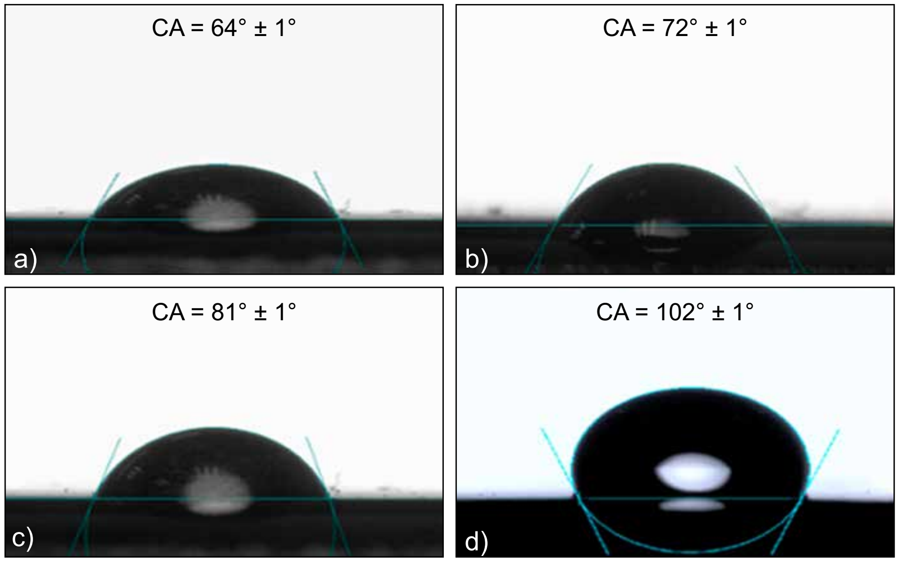
*Data obtained after abrasion test.

The authors evidenced the excellent oil absorption capacity of the foams, which is super-lipophilic and, therefore, highly hydrophobic. The authors reported that the PU foam without GO exhibited a water contact angle (WCA) of 124°, and even after the 500th reuse cycle, the WCA was maintained hydrophobic, with WCA=105°. This effect can be due to the hydrophobic polymeric chains of PU and the high rugous foam surface. Furthermore, Yadav and coauthors found that among the concentrations of GO studied, 4 wt.% of GO in the PU foam increased its hydrophobic effect, presenting initial WCA of 135° and 111° after the 500th reuse cycle. Ly and colleagues (2021) [13] reported the development of a hydrophobic film composed of multilayers of polyelectrolytes with opposite charges using the Layer-by-Layer assembly technique. The authors used the positively charged polyelectrolytes Poly(ethylene imine) (PEI) and Poly(allylamine hydrochloride) (PAH). In contrast, Poly(acrylic acid) (PAA) and Poly(sodium 4-styrene sulfonate) (PSS) were used as the negatively charged polyelectrolytes. The hydrophobic film was produced by depositing alternatively positively (PEI+PAH+rGO mixture) and negatively (PAA+PSS+rGO mixture) charged nanocomposites on a glass plate surface, repeated 60 times. The authors noted only a slight increase in the WCA after adding the rGO nanoparticles, from 130° for the $(PEI/PAA)_{60}$ film (without rGO) to WCA=136° for the $(GPAH\text{-}PEI/GPSS\text{-}PAA)_{60}$ film (with rGO). AFM analyses revealed a rougher surface for the (GPAH-PEI/GPSS-PAA)60 nanocomposite than for the (PEI/PAA)60 film due to the presence of the nanoparticles, creating a hierarchical structure that increases the surface hydrophobicity. In 2022, Ramirez-Soria and collaborators [14] demonstrated a hydrophilic-hydrophobic transition of epoxy coating by adding only 0.5 wt.% of propyl-functionalized GO or rGO. While the pure epoxy coating presented a hydrophilic WCA of 62°, the epoxy coating loaded with propyl-GO increased the water repellence, exhibiting WCA=94°. Since propyl-rGO presents a smaller number of hydrophilic groups than propyl-GO, adding propyl-rGO to the epoxy coating increased the hydrophobic behavior to WCA=100°. Abakah and colleagues (2021) [17] tested the effect of three different commercial graphene types (X50, M15, C750) on epoxy nanocomposite properties. C750 graphene presents a medium diameter of up to 2 mm, M15 has a medium diameter of up to 15 mm, and X50 exhibits nanoparticles with a diameter of about 150 mm. The WCA of the neat epoxy coating was 64°, demonstrating a hydrophilic nature. The epoxy nanocomposite with 1 wt.% of X50 showed a WCA of 72°, while the nanocomposite with the graphene M15 presented WCA=81°, and the EP/C750 nanocomposite presented WCA=102°, demonstrating a hydrophobic behavior (Figure 3). Considering that the three graphene types do not have hydrophilic functional groups, the differences in the wettability properties can be related to the diameter of the graphene nanoparticles and their respective surface area, consequently producing rough surfaces on the nanocomposites. The C750 graphene nanoparticles, which have the most minor diameter among the three types of graphene used (up to 2 mm), probably produced the roughest surface with a hierarchical structure. Furthermore, the nanocomposite EP/C750 presented the best corrosion protection performance.

Conclusion and Future Perspectives

Graphene presents fascinating properties for strategic and commercial applications. Many scientific researchers and industrial interests involving graphene synthesis and functionalization to diverse applications demonstrate how this 2D nanomaterial has excellent potential to be present in the most diverse recent technologies. Hydrophobicity is only one of the properties that graphene and derivatives can provide to graphene-based nanocomposites, and superhydrophobicity can be achieved by using functionalization methods and producing hierarchical structured surfaces with at least two different scale levels. As described in this review, many studies report the hydrophobic effect of graphene-based materials

Figure 3. Surface contact angle of (a) pure epoxy (EP), (b) EP/X50, (c) EP/M15, and (d) EP/C750 coatings.



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in nanocomposites, but the superhydrophobicity effect is still scarce in the literature. Exploring new chemical species and functionalization methods and producing biomimetic surfaces can be the next step to achieving more water-repellent and self-cleaning surfaces.

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