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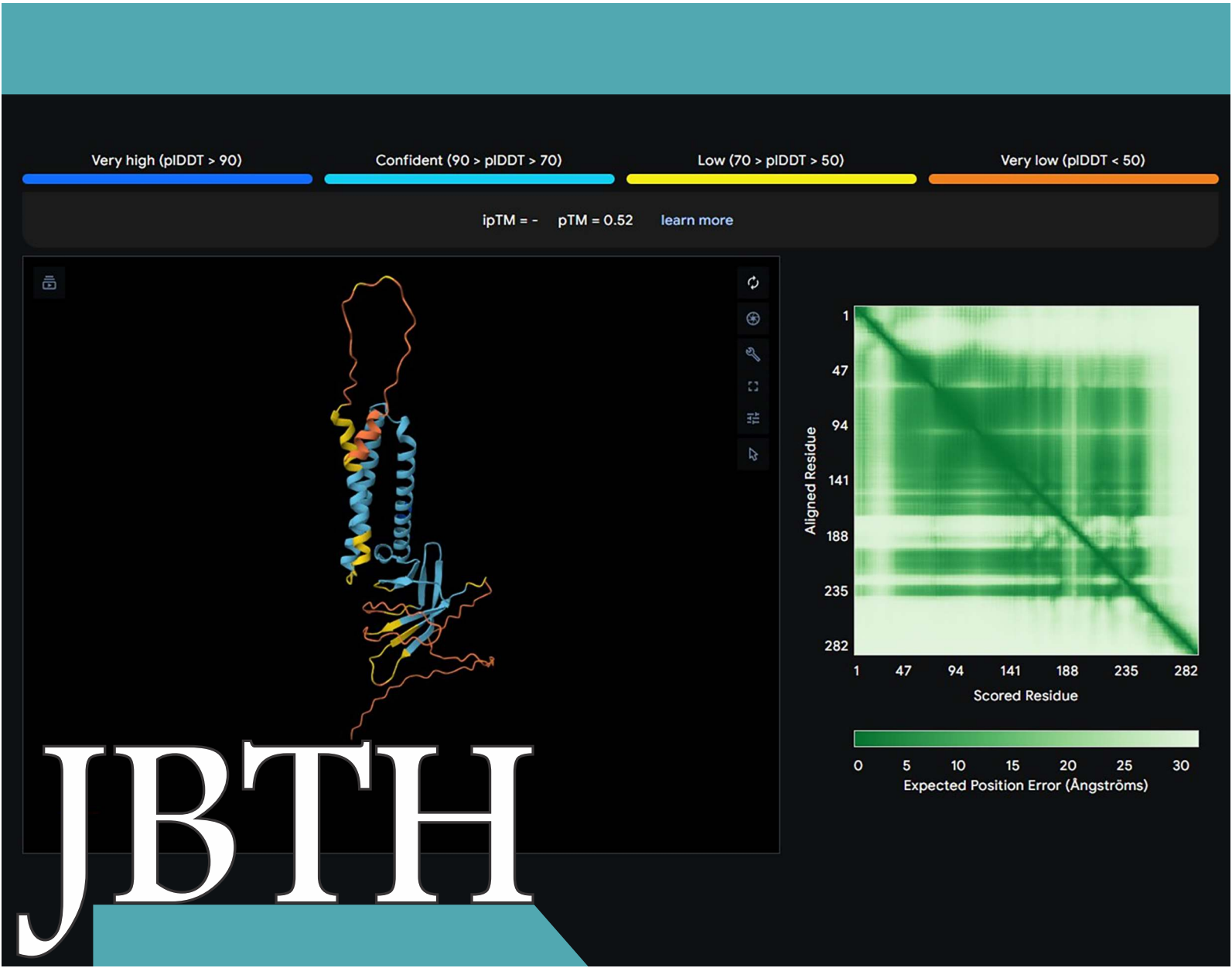

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Volume 8 • Number 3 **SUMMARY** *June 2025*

Original Articles

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COVER: Figure 1. Three-dimensional structure of the SARS-CoV-2 Spike protein sequence predicted by AlphaFold3. Application of Bioinformatics Tools in the Optimization of mRNA Sequences Focused on Vaccine and Therapeutic Development by Leo Thirso Pinto de Freitas e Souza et al. J Bioeng. Tech. Health 2025;8(3):12.

Biopolymeric Blenda Scaffolds with Incorporation of an Organic Compound with Potential Pharmacological Activity for Application as a Wound Dressing

Caio Athayde de Oliva[1]*, Arthur João Reis Lima Rodovalho[1], Willams Teles Barbosa[1], Ana Paula Bispo Gonçalves[1], Jaqueline Leite Vieira[2], Josiane Dantas Viana Barbosa[1]

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Cutaneous wounds caused by trauma, burns, or chronic diseases are challenges that tissue engineering seeks to address through innovative strategies to accelerate healing, using various resources and techniques such as hydrogels and 3D bioprinting. Among the materials used in this field, GelMA (methacrylated gelatin) stands out for its biocompatibility, biodegradability, and non-immunogenicity. Therefore, this study focused on GelMA at different concentrations (5%, 10%, and 15%) to evaluate its properties. Mechanical tests showed that higher concentrations increase the material's strength by enhancing its molecular bonds, resulting in greater tension required for rupture of the dressing. Fourier-transform infrared spectroscopy (FTIR) confirmed the methacrylation reaction. The next step of the study is to evaluate infill patterns and biological properties, aiming to enhance the effectiveness of the dressings in wound healing.

Keywords: 3D Bioprinting. Dressing. GelMA. Tissue Engineering.

Skin wounds can be caused by different factors, such as acute trauma, burns, and surgical procedures, and can be worsened by other comorbidities like diabetes. Treating these wounds depends intrinsically on the interaction between cellular factors and the surrounding extracellular matrix [1]. Tissue engineering recognizes these challenges and seeks strategies to accelerate wound treatment through various techniques, such as the use of hydrogels with different compositions or 3D bioprinting for tissue printing to provide patient-specific substitutes [2].

Gelatin is one of the most commonly used materials in tissue engineering, due to its favorable properties like biocompatibility, biodegradability, and non-immunogenicity. Gelatin-based hydrogels used in 3D bioprinting have proven important for discoveries of various chemical, biological, and mechanical functionalities in this research area [2]. It is possible to produce dressings from hydrogels like gelatin that adhere to the skin, offering protection from the external environment, and which may have antibacterial properties through the addition of other materials, while also delivering drugs that accelerate the tissue regeneration process [3].

This study aims to investigate further the mechanical and physicochemical properties of methacrylated gelatin hydrogel (GelMA) used in 3D bioprinting, by conducting mechanical, chemical, and biological tests to determine how increased concentration affects its properties and evaluate its applicability as a wound dressing.

Materials and Methods

To produce GelMA, 10 grams of gelatin were dissolved in 100 mL of ultrapure water in a magnetic stirrer for 1 hour. Then, 0.14 mL of methacrylic anhydride (MA) was added per gram of dissolved gelatin and kept under constant stirring for at least 2 hours. After this period, an additional 100 mL of ultrapure water was added to stop the reaction. The solution was then centrifuged for 10 minutes at 5000 RPM at 25 °C to remove part of the unreacted MA. Next, the solution was dialyzed in ultrapure water to remove residual MA, with daily water changes for 5 days. Afterward, the solution was removed from the membrane, frozen at -80 °C, and lyophilized for hydrogel production.

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The hydrogel was prepared at three concentrations (5%, 10%, and 15%) using lyophilized GelMA in phosphate-buffered saline (PBS), stirred magnetically at 40 °C. Lithium phenyl-2,4,6-trimethylbenzoylphosphinate (LAP) was used as a photoinitiator at 0.5% to cure the scaffold after printing. The hydrogels were printed using a 3D bioprinter, model Octopus by 3D Biotechnology Solutions, following the parameters shown in Table 1. They were stored and sealed with paraffin for 1 day to allow complete photocuring before mechanical property evaluation.

Table 1. Printing parameters.

Parameter	Value
Scaffold size	15x80x1 mm
Printing speed	150 mm/min
Layer height	1 mm
Nozzle diameter	1 mm
UV exposure time	5 min
Number of walls	5
Infill pattern	Rectilinear
Infill percentage	60%

Gelatin and GelMA were characterized by Fourier-transform infrared spectroscopy (FTIR) using attenuated total reflectance (ATR), model Nicolet Is10 (Thermo Scientific, Massachusetts, USA). The range used was 4000–500 cm^{-1} with a resolution of 4 cm^{-1}.

Mechanical behavior was analyzed by tensile testing of the stress–strain curve for each scaffold at different concentrations, using a Brookfield CT3 texture analyzer, to evaluate how increasing GelMA concentration affects mechanical resistance. The test was conducted at a speed of 0.1 mm/s.

Results and Discussion

The tensile test (Figure 1) indicated that increasing GelMA concentration is associated with a higher maximum stress sustained by the material before rupture. Although the supported tension increased, the observed strain remained similar across the three tested concentrations. The increased mechanical strength can be attributed to the greater amount of GelMA in the formulation. Higher GelMA concentrations promote stronger molecular bonding within the dressing structure, thereby requiring greater force for rupture [4].

Figure 2 shows the FTIR spectrum of pure gelatin and GelMA. In the 1200 to 1750 cm^{-1} range, band stretching associated with C=H and C=O amino groups is observed. In the 2800 to 3400 cm^{-1}

Figure 1. Stress *vs*. strain curve of GelMA at 5%, 10%, and 15% concentrations.

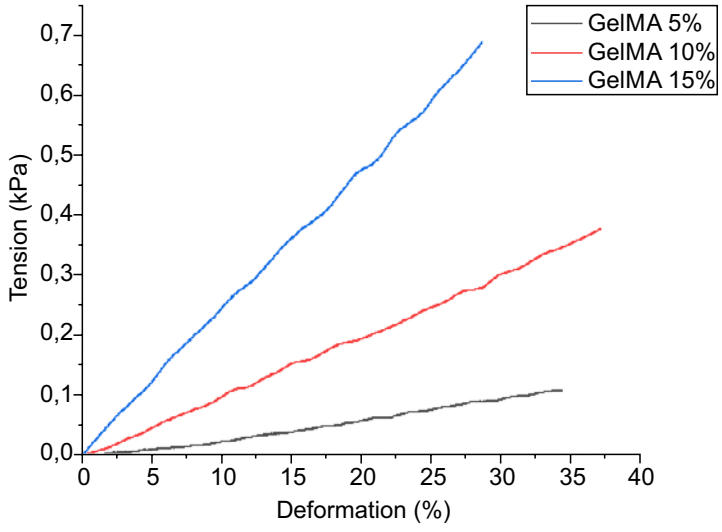
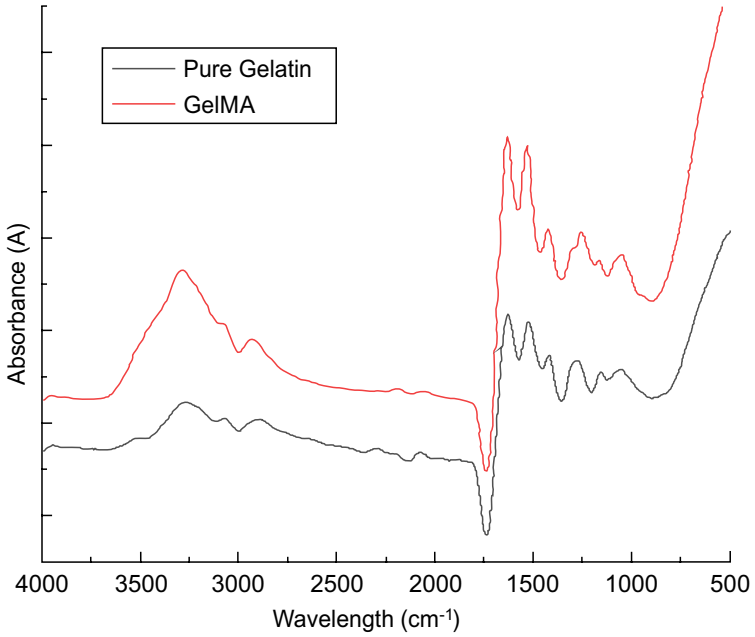


Figure 2. FTIR spectrum of pure gelatin and GelMA.



range, stretching is seen for OH groups and N-H peptide bonds [4,5].

Conclusion

According to the results, it was possible to characterize and observe stretching of certain functional group bands in GelMA compared to pure gelatin, and to verify the influence of concentration increase on the stress required to rupture the printed dressing. Future tests will be necessary to evaluate the influence of the dressing's infill pattern, as well as the incorporation of new materials to achieve additional properties. In addition to mechanical analysis, it will also be essential to investigate the biological properties, focusing on cellular compatibility and the potential for drug incorporation in the dressing to aid wound healing.

Acknowledgements

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Beyond the Lesion: How Neurobypass 1.0 Transforms Post-Stroke Rehabilitation for Motor Recovery

Isabella Camily Nato Rosa[1]*, Raphael Akira Siqueira Ishibashi[1], Atala Portella[1], Daniel Gomes Almeida Filho[1]
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Stroke is one of the leading causes of death and functional disability in Brazil, significantly impacting survivors' quality of life. The Neurobypass 1.0 project proposes an innovative approach to post-stroke motor rehabilitation through a neuro-controlled hand and wrist orthosis. Developed by SENAI CIMATEC in partnership with CEPRED, the device uses brain-computer interface (BCI), virtual reality (VR), and additive manufacturing (3D printing) to promote engagement, customization, and neuroplasticity stimulation. A systematic review identified evidence for the efficacy of BCI and AR orthoses in enhancing function and motivation in patients with hemiparesis. A randomized clinical trial protocol was designed to assess motor, emotional, and motivational outcomes of the intervention. Expected results include functional hand recovery, reduced depressive symptoms, and greater independence. The initiative highlights Brazil's potential as a reference in accessible assistive technologies.

Keywords: Stroke. Rehabilitation. Brain-Computer Interface. Virtual Reality Therapy.

Stroke, as defined by the World Health Organization (WHO), is a severe condition caused by the interruption of cerebral blood flow, which can lead to irreversible damage or death [1,2].

In Brazil, it is the leading cause of death among individuals over 50 and of early retirement, with around 200,000 hospitalizations by the Sistema Único de Saúde (SUS)in 2008 and 99,010 deaths in 2020 [3,4]. Motor sequelae, such as upper limb weakness, affect basic daily activities (eating, hygiene, dressing) and more complex occupational tasks [5].

The Neurobypass 1.0 project, developed by the SENAI CIMATEC Assistive Technologies Competence Center in partnership with the Center for the Prevention and Rehabilitation of Disabilities (CEPRED), proposes an innovative solution for post-stroke rehabilitation. The project involves a neuro-controlled hand and wrist orthosis utilizing a brain-computer interface (BCI) with electroencephalography (EEG) to capture neural signals and control the device through a mechatronic system that simulates tendon movements.Integration with augmented reality (AR) shows potential for increasing patient engagement and stimulating neuroplasticity [6,7]. Additive manufacturing (3D printing) enables customization of the device to the anatomical features of each user, improving efficacy and comfort [8]. The goal is to stimulate neuroplasticity through a technologically advanced, personalized therapeutic approach [9].

A multidisciplinary team conducts the project to ensure the development of an ergonomic and practical device that can maintain user motivation throughout the rehabilitation process.Thus, Neurobypass 1.0 not only advances motor rehabilitation but also strengthens Brazil's standing in assistive technology innovation, promoting access to cutting-edge devices and enhancing independence and quality of life for post-stroke patients.

The primary objective of developing a neuro-controlled hand exoskeleton is to facilitate motor function rehabilitation in stroke survivors. With a personalized approach tailored to each individual's needs, the device aims to restore hand function by stimulating the brain's neuroplasticity through interfaces that enable control of the device via brain activity.

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Materials and Methods

The project's first phase involved a systematic literature review across various databases, including PubMed, Scopus, Web of Science, Cochrane Library, and PEDro, using descriptors such as "Stroke rehabilitation," "Orthotic devices," "Brain-Computer Interface," and "Virtual Reality therapy." Clinical trials evaluating BCI orthoses, with or without VR, for the rehabilitation of post-stroke hemiparetic patients were selected for this review. Following the review, a comprehensive informational report was produced, covering neuroanatomy, stroke pathophysiology, post-stroke rehabilitation, the effectiveness of orthoses, and the role of BCI in neuromotor recovery. Based on the results of this review, the multidisciplinary team developed a clinical trial protocol with strict inclusion criteria (muscle strength ≥3, absence of severe cognitive deficits). Assessments will be conducted over 12 weeks using functional (dynamometer, Ashworth Scale, Barthel Index, Fugl-Meyer) and psychological (Beck Depression Inventory, Perceived Self-Efficacy Scale) tests. The assessments will follow CNS Resolution 466/12, with assessments conducted by a multidisciplinary team.

Results and Discussion

The literature review identified thirty-one studies with BCI-based orthoses and six with VR.VR has demonstrated high potential for increasing engagement, motivation, and therapeutic adherence by transforming repetitive exercises into interactive and stimulating experiences. Studies have shown that orthoses with brain-computer interfaces (BCIs) and virtual reality (VR) have a positive impact on

post-stroke rehabilitation. VR, by making exercises more interactive and immersive, increases user motivation and engagement—essential factors for better functional outcomes.

Based on these findings, a protocol was developed for a randomized, double-blind, case-control clinical trial using the Neurobypass 1.0 neuro-controlled orthosis. Assessments will be conducted at baseline and after 4, 8, and 12 weeks, including physical exams, functional assessments (grip dynamometer, Ashworth Scale, Barthel Index, Fugl-Meyer Scale), and psychological/motivational evaluations (interviews, psychological tests, cognitive and quality-of-life instruments).

The systematic review also underscored the importance of patient motivation, which directly influences therapeutic results, with motivated individuals tending to achieve greater gains [10].

Post-stroke patients frequently face emotional challenges such as depression, anxiety, and post-traumatic stress, with up to 30% developing significant depression [11]. Self-Determination Theory emphasizes that motivation during the subacute stroke phase is influenced by extrinsic factors, such as positive feedback, which can potentially evolve into intrinsic motivation through the satisfaction of basic psychological needs [12].

It was concluded that validated instruments, such as the Beck Depression Inventory (BDI-II) and the Perceived Self-Efficacy Scale, are essential for assessing emotional state and inferring motivation, especially given the lack of specific tools in Brazil.These instruments evaluate depressive symptoms and hopelessness, which directly impact motivation and engagement in rehabilitation, contributing to more effective, personalized therapeutic strategies.

Thus, combining advanced technologies like BCI and VR with robust psychological and functional assessments offers a promising path for post-stroke rehabilitation, aligning with the clinical and emotional needs of users.

Conclusion

Neurobypass 1.0 represents progress in post-stroke rehabilitation by combining brain-computer interface (BCI), additive manufacturing (3D printing), and virtual reality (VR) to develop an ergonomic, personalized neuro-controlled

hand exoskeleton. The device aims to improve motor function, stimulate neuroplasticity, and enhance therapeutic engagement, thereby aiding in the resumption of daily activities and promoting functional independence. Beyond motor benefits, the project aims to achieve scientific validation through clinical trials, thereby contributing to a deeper understanding of neuroplasticity.

In addition, Neurobypass 1.0 relies on a multidisciplinary team to adopt a biopsychosocial approach. This means the project considers not only the physical aspects of the patient, but also the psychological and social factors. The team assesses how the use of new technologies in rehabilitation impacts the user's experience and motivation, which, in turn, can directly influence the results obtained at the end of the treatment.

Neurobypass 1.0 also has the potential to position Brazil at the forefront of assistive technologies, with possible implementation in the public healthcare system (SUS), democratizing access to innovative rehabilitation and improving patient quality of life.

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Development of Novel Bioceramic Cements

Eduardo Alves Bandeira Peres[1]*, Willams Teles Barbosa[1], Ana Paula Bispo Gonçalves[1], Jaqueline Leite Vieira[2], Josiane Dantas Viana Barbosa[1]

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Bioceramic cements, composed of calcium and strontium silicates and aluminates, have gained prominence in orthopedics and dentistry due to their bioactive and biocompatible properties. These materials are effective in bone regeneration and root canal sealing, overcoming the limitations of traditional cements such as Mineral Trioxide Aggregate (MTA). The synthesis of bioceramics is performed using the solution combustion synthesis (SCS) method, which enables the production of homogeneous and highly pure powders. This process enhances control over crystalline phases and increases the materials' reactivity. The combination of different compounds and the use of this synthesis method allow for the optimization of properties such as mechanical strength and bioactivity. Thus, these materials offer a promising alternative for developing more effective and safer solutions for regenerating mineralized tissue.

Keywords: Cements. Bioceramics. SCS.

Bioceramic cements have been extensively studied for applications in orthopedics and dentistry due to their superior bioactive, biocompatible, and mechanical properties [1].

These materials have shown excellent performance in bone regeneration and root canal sealing, playing a key role in replacing traditional cements such as Mineral Trioxide Aggregate (MTA), which, despite its clinical success, has disadvantages such as long setting time, difficult handling, and potential cytotoxic effects associated with the presence of bismuth compounds [2].

In this context, new formulations based on calcium and strontium silicates and aluminates have been investigated to optimize properties such as setting time, mechanical strength, and bioactivity [3]. Calcium silicates, widely used in dentistry, exhibit excellent bioactivity and the ability to induce hydroxyapatite formation, promoting tissue regeneration [1]. However, their manipulation and setting time remain challenging. On the other hand, calcium aluminates stand out for their rapid hydration and biocompatibility, while strontium aluminates provide increased radiopacity and osteoinductive potential [4].

Recent studies suggest that combining these compounds may lead to cements with improved characteristics for biomedical applications. To prepare different cement formulations, the following parameters must be varied: the concentration of bioceramics (Calcium Silicate, Calcium Aluminate, and Strontium Aluminate); the liquid-to-solid ratio; and the incorporation of additives in the liquid phase [4]. These variations aim to optimize the physicochemical and mechanical properties of the cement, ensuring better workability, appropriate setting time, and enhanced bioactivity [2].

In this context, this project aims to develop new bioceramic cement formulations for future orthopedic and/or dental applications. It is essential to note that the project is currently in the initial phase of bioceramic synthesis, with characterization as the next step. The synthesis and characterization of these materials will be conducted with a focus on optimizing their physicochemical and biological properties, aiming for performance superior to currently available materials. Thus, the research intends to contribute to the advancement of health-applied biomaterials, offering more effective and safer solutions for mineralized tissue regeneration.

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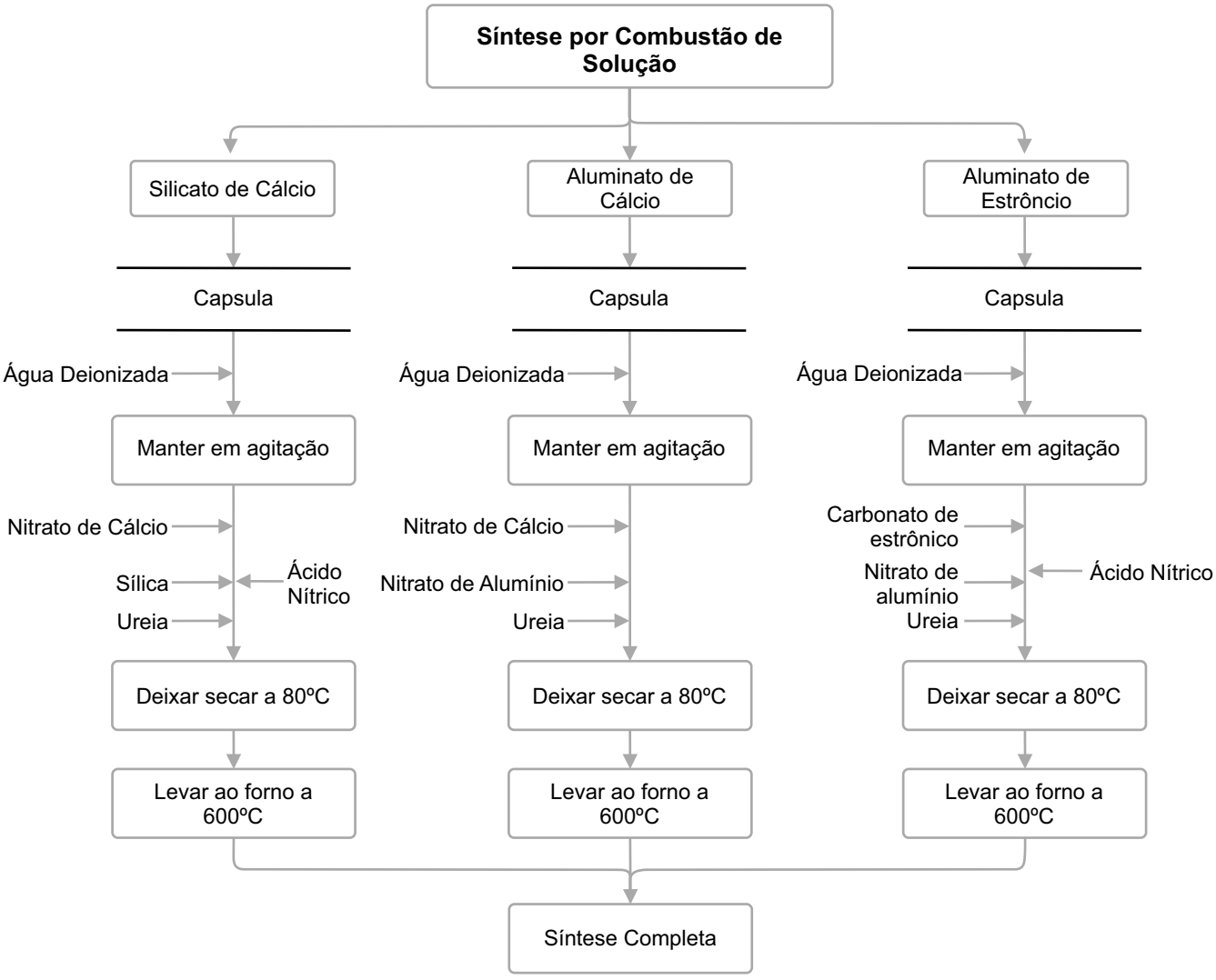
Figure 1. Methodological flowchart of bioceramic synthesis by SCS.



Materials and Methods

The method adopted for developing new bioceramic cement formulations involved two main stages: a literature review and bioceramic synthesis.

First, a search of scientific articles related to the topic was conducted in indexed databases. The keywords used were: "Bioceramics", "Endodontics", and "Synthesis". This approach allowed the identification of relevant studies on bioceramic production and their main challenges.

The literature review provided insights into the most important parameters for bioceramic production, including the influence of the liquid/solid ratio, the type of chemical precursor used, synthesis temperature, and the addition of modifying agents to control final properties.

Additionally, the main characterization techniques required to assess formulation quality were identified, including X-ray diffraction (XRD) for crystalline phase identification, scanning electron microscopy (SEM) for morphological analysis, and evaluation of radiopacity, setting time, bioactivity, biocompatibility, and mechanical strength. Based on this review, the fundamental parameters for bioceramic synthesis were defined.

Following the literature analysis, the most promising bioceramics for cement formulation

were selected, consisting of calcium and strontium silicates and aluminates. These bioceramics have already been synthesized using the SCS method. The synthesis method used for each bioceramic is detailed in Figure 1, followed by grinding and sieving.

Theoretical Framework

Bioceramics are widely studied materials in the field of materials engineering due to their applications in dentistry and orthopedics, particularly for bone regeneration and endodontic treatments. Among the most commonly used materials for these purposes are calcium and strontium silicates and aluminates, which exhibit bioactive and biocompatible properties, capable of inducing apatite formation and stimulating tissue regeneration [1].

Mineral Trioxide Aggregate (MTA) has been one of the primary bioceramic cements used in endodontics due to its excellent sealing ability and biological compatibility. However, its formulation based on Portland cement has disadvantages such as prolonged setting time and difficult handling, in addition to potential cytotoxic effects attributed to bismuth in the composition [2]. Consequently, new cements are being developed to overcome these limitations, including those based on calcium and strontium aluminates, which have better radiopacity and superior mechanical properties [3].

Aluminate-based cements offer several advantages over silicates, including more precise control over setting time and greater mechanical strength, while also promoting bioactivity through the release of ions that stimulate bone mineralization [4]. Studies suggest that the component ratios of calcium and strontium aluminates can be adjusted to optimize properties such as workability, setting time, and adhesion to bone tissue [1].

In addition to chemical formulation, the synthesis method used to produce bioceramic cements plays a crucial role in their final properties. The SCS method has been widely adopted due to its ability to produce homogeneous and high-purity powders, ensuring better control over the resulting crystalline phases and improving material reactivity [4]. This technique enables the production of bioceramics with high bioactivity and the ability to form apatite layers when in contact with simulated body fluid (SBF), an indicator of their clinical potential [2].

The theoretical framework highlights the importance of developing new bioceramic cements that overcome the limitations of current materials. The combination of different proportions of calcium and strontium aluminates and silicates, along with advanced synthesis methods, enables the design of biomaterials with tunable properties for dental and orthopedic applications. Therefore, the current research aims to contribute to the advancement of bioceramic materials by providing more effective and safer alternatives for mineralized tissue regeneration.

Conclusion

Bioceramic cements have proven to be a promising alternative for regenerating mineralized tissues, particularly in orthopedic and dental applications. The combination of calcium and strontium silicates and aluminates provides materials with excellent bioactive and biocompatible properties, surpassing the limitations of traditional cements, such as Mineral Trioxide Aggregate (MTA), particularly in terms of setting time and handling. The use of the solution combustion synthesis (SCS) method contributes to the production of homogeneous and high-purity powders, thereby enhancing the reactivity and bioactivity of cement.

The present research highlights the importance of strategically combining these compounds and optimizing synthesis conditions, such as the liquid-to-solid ratio and additive incorporation, to achieve superior mechanical and chemical properties. Furthermore, advances in material characterization—through techniques such as XRD, SEM, radiopacity, setting time, bioactivity,

and biocompatibility—are essential for ensuring formulation quality.

The development of new bioceramic cements, based on the knowledge acquired, aims not only to improve existing solutions but also to offer more effective and safer alternatives for mineralized tissue regeneration. As such, bioceramic materials are expected to enable more efficient and high-performance treatments in orthopedics and dentistry, contributing to the advancement of health-applied biomaterials technologies.

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Application of Bioinformatics Tools in the Optimization of mRNA Sequences Focused on Vaccine and Therapeutic Development

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This *in silico* study explored the optimization of therapeutic RNA modeling using AI (AlphaFold3, Boltz-1, Chai-1). An assembly method combined high-confidence regions to model complex sequences. Quality was evaluated using five standardized metrics (secondary structure, stability, translation, immunogenicity, and production). Simulations and bioinformatics analyses were used to assess the stability and function of the optimized structures. The objective was to develop more accurate and comparable modeling methodologies for therapeutic RNA design, while recognizing the need for subsequent experimental validation.
Keywords: Vaccine. Treatment. Messenger RNA. Bioinformatics.

The optimization of mRNA sequences using artificial intelligence (AI) emerges as a promising strategy for the development of effective therapies and vaccines. Recent algorithms such as AlphaFold3, Boltz-1, and Chai-1 offer the potential to predict RNA structures, paving the way for the assembly of optimized sequences with greater stability and translational efficiency. Given the lack of standardization in evaluating these algorithms, this study proposes and applies a set of five metrics to compare the performance of AlphaFold3, Boltz-1, and Chai-1 in modeling therapeutic mRNA. Our goal is to investigate the *in silico* optimization of mRNA sequences using AI by applying this standardized evaluation framework to contribute to the development of innovative molecules, while acknowledging the need for future experimental validation.

Materials and Methods

This research is configured as an exploratory and descriptive *in silico* study, conducted entirely in a computational environment. It does not involve the collection of primary data from humans or animals; thus, definitions of population in the sense of field research or ethics committee approval are not applicable. The research is ongoing, with no specific data collection period, utilizing the latest available versions of AI algorithms and RNA sequence databases at the time of analysis.

Initially, the Spike protein sequence of the SARS-CoV-2 virus was used as a model for structural predictions generated by the AI algorithms. The technique used for data collection and analysis involves applying bioinformatics methods and computational modeling.

Therapeutic or biotechnological RNA sequences was selected from public databases such as GenBank and Rfam, prioritizing those with potential for applications in immunotherapy or vaccine development, as identified in the scientific literature. Selected sequences was subjected to predictive structural analysis by three state-of-the-art AI algorithms: AlphaFold3, Boltz-1, and Chai-1. Each algorithm, using its machine learning models, generated multiple predictions of the three-dimensional structure of each RNA sequence, along with confidence metrics specific to each region of the model.

To address the limitations of these algorithms, particularly in regions with multiple MERs, a model assembly technique was implemented. This technique involves identifying and extracting the substructures with the highest confidence scores from each of the models generated for a given sequence. These substructures was

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then computationally combined to generate an optimized consensus three-dimensional model, aiming to integrate the most robust predictions from each algorithm.

The quality of the assembled models was assessed using a set of standardized criteria proposed in this study.

The five evaluation categories are:

1. **mRNA Secondary Structure Prediction Quality**, assessed by comparing predicted structures with experimental secondary structure data from databases or literature, using concordance metrics such as sensitivity and specificity;
2. **Thermodynamic Stability of mRNA**, quantified by estimating the Gibbs free energy (ΔG) of the predicted structure using bioinformatics software such as the ViennaRNA Package;
3. **Potential Translation Efficiency**, analyzed by calculating the Codon Adaptation Index (CAI) using tools like CAIcal, evaluating the frequency of unfavorable dimers using tools like EMBOSS, and analyzing ribosome binding site (RBS) accessibility using secondary structure prediction software;
4. **Immunogenicity Potential**, assessed by searching for known immunogenic sequence motifs using databases like SIDMAP and predicting dsRNA formation potential using tools such as RNAduplex from the ViennaRNA Package; and
5. **Ease of Production and Purification**, qualitatively assessed by analyzing the structural complexity of the models (presence of knots, pseudoknots) and identifying repetitive sequences that could hinder mRNA synthesis and isolation.

The data analysis involve a quantitative comparison of the evaluation metrics among the models generated by different algorithms and those optimized by the assembly method.

Descriptive statistics was used to summarize the results and identify trends in algorithm performance and the quality of optimized models. The analysis include a discussion of the implications of the results for therapeutic mRNA design, considering the limitations of the algorithms and the challenges in producing and *in vivo* applying these molecules. If molecules with high therapeutic potential are identified through *in silico* analysis, future studies may include *in vitro* experimental validation in the SENAI CIMATEC Health Technologies Institute's laboratory.

Although this research does not involve human subjects, it rigorously adheres to best practices in scientific research, including maintaining detailed records of all methodological steps, ensuring the traceability of analyses, and presenting results transparently to enable reproducibility by other researchers.

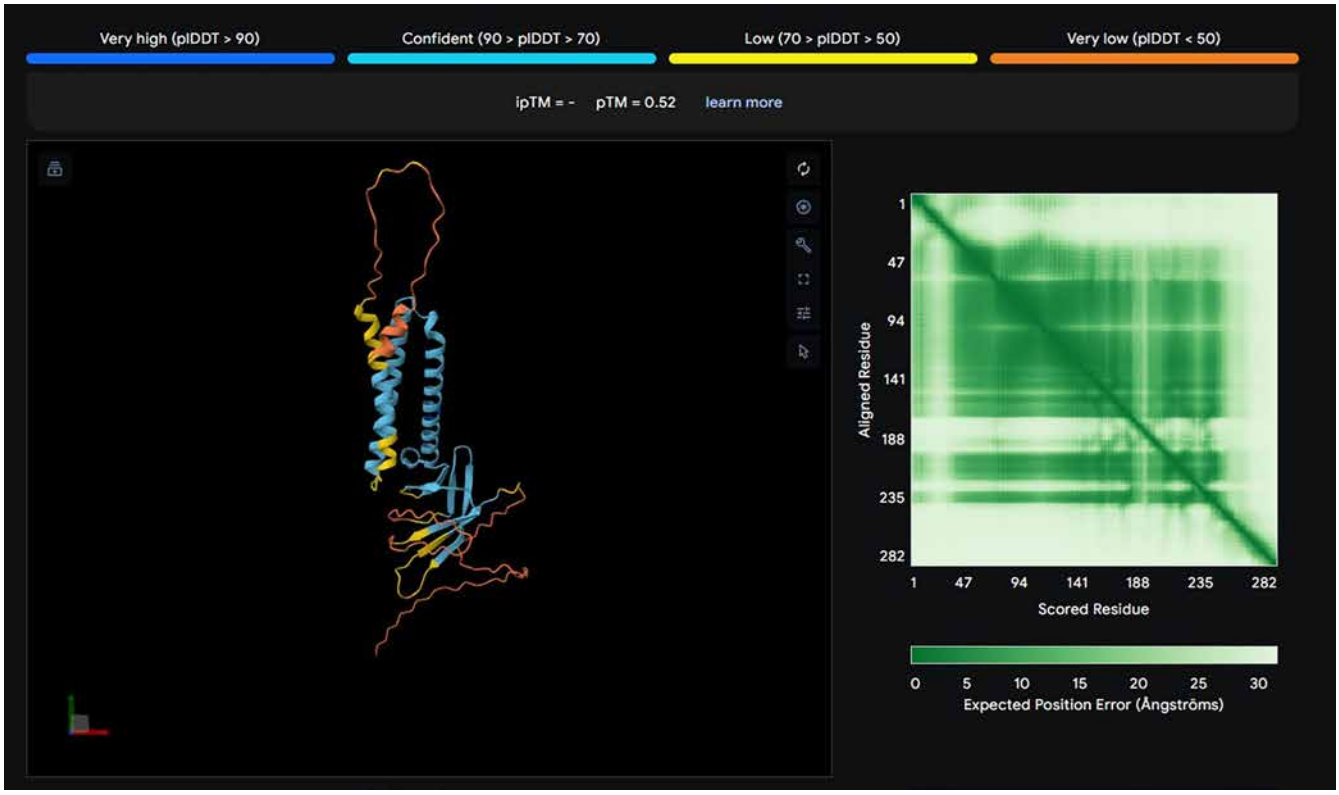
Theoretical Framework

The versatility of mRNA drives its investigation for on-demand vaccines and therapies, with rapid and safe *in vitro* production, enabling the encoding of various antigens and modulation of immune responses [3,2]. Despite its instability and challenges in *in vivo* expression, algorithms optimize sequences by considering structure and regulation [5-8]. Our laboratory [9-11] seeks to prospect and validate optimization algorithms *in vitro*, initially using the Spike protein sequence of SARS-CoV-2. The application of these tools aims to contribute to the development of more effective vaccines and treatments, with potential for future collaborations (Figure 1).

Conclusion

This *in silico* study proposed a framework to evaluate the optimization of therapeutic RNA by AI (AlphaFold3, Boltz-1, Chai-1). Despite limitations in clinical applicability and mRNA production, and the need for biological validation, the standardization of metrics and *in silico* identification of promising sequences represent a significant initial step for future RNA therapies.

Figure 1. Three-dimensional structure of the SARS-CoV-2 Spike protein sequence predicted by AlphaFold3.



Source: Data generated by AlphaFold3.

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Bioprospection of Enzyme-Producing Bacteria Applicable to the Hydrolysis of Cacao Biomass (*Theobroma cacao*)

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This article examines the potential of cocoa agro-industrial waste for biofuel production, aiming to provide a sustainable alternative to fossil fuels and promote waste reuse. It explores the bioprospecting of cellulase-producing microorganisms in cocoa-growing areas, focusing on the microbial diversity of Brazilian soil and its role in lignocellulose degradation. The method involved the induction of cellulolytic activity in a medium with CMC, isolation and characterization of the bacteria, production of an enzymatic extract, and quantification of reducing sugars via the DNSA method. The objective is to identify efficient cellulase-producing microorganisms to optimize the conversion of cocoa residues into biofuel, promoting a greener economy in the energy sector. A total of four bacterial strains (3A, 5, 6, and 8) were isolated, Gram staining was performed for each, and sugar quantification yielded the following enzymatic activity results: strain 3A – 0.42; 5 – 0.2; 6 – 0.24; and 8 – 0.27 U/mL.
Keywords: *Theobroma cacao*. Enzymes. Cellulase. Biofuel.

Brazil is a country whose economy is primarily based on agriculture, which consequently generates large amounts of waste annually. Among these, the residues from the cocoa agro-industry stand out, with 584 valid records of cocoa seedlings and seed producers integrated into the National Register of Seeds and Seedlings, with Bahia representing 13.4% of this total [1]. In 2018, this industry produced approximately 255.1 thousand tons of cocoa beans, which represent only 10% of the fruit's total mass, resulting in the generation of more than 2.5 million tons of waste [2].

Cocoa residues are lignocellulosic in nature, with lignocellulose being the main component of plant biomass. It is mainly composed of cellulose, hemicellulose, and lignin. The use of lignocellulosic materials as raw material for bioethanol production is a strategic tool for the sustainable production of fuels from renewable sources and an effective alternative for reusing organic solid waste [3].

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The project on bioprospecting enzyme-producing microorganisms aims to explore the microbial diversity present in soil, especially in cocoa cultivation areas. These bacteria have significant potential for expanding industrial applications and reducing costs, exhibiting higher growth rates than fungi, although their production potential remains underexplored [4]. Moreover, soil is a crucial biodiversity reservoir, possessing diverse functional characteristics of microbial groups [5]. The biodiversity of Brazilian soils is invaluable, benefiting the environment and enabling sustainability in various sectors. However, the degradation of this biome and desertification significantly reduce microbial diversity [6].

The present study aims to prospect for cellulase-producing microorganisms for application in sustainable biofuel production. This work aims to provide innovative solutions for the utilization of agro-industrial waste, contributing to the transition toward a greener and more sustainable energy sector.

Materials and Methods

The method employed was designed to ensure maximum reliability and precision in microbiological assays. A rigorous asepsis protocol was adopted, with the sterilization of all materials being a crucial step. Sterilization was performed

using autoclaving, a method recognized for its effectiveness in eliminating microorganisms, including bacteria, fungi, viruses, and bacterial spores. The experiments were conducted at the Microbiology and Bioprocess Laboratory "Moacyr Dunham de Moura Costa."

Cellulolytic activity was induced and monitored using carboxymethylcellulose (CMC) as a carbon source [7]. The culture medium was prepared following the protocol of Wood & Bhat (1988), with adaptations [8]. Plating was performed under laminar flow using Petri dishes that had been previously sterilized in an autoclave. Fifteen milliliters of CMC culture medium were distributed in each dish. The inoculation of cocoa samples into the CMC medium followed the method described by Alves and colleagues (2021), with adaptations [9].

Bacterial isolation involved selecting distinct colonies from primary plates and then transferring them into a saline solution to obtain a uniform suspension. Serial dilutions may be performed to reduce bacterial concentration. The suspension is then spread onto new plates, resulting in isolated colonies that can be characterized. The enzymatic extract was produced by fermenting the isolated bacterium in a liquid medium using an orbital shaker incubator for 24 hours at 27°C and 120 rpm.

The amount of reducing sugars present in the enzymatic extract was determined using 3,5-dinitrosalicylic acid (DNSA) reagent, following the DNS test protocol adapted from Miller (1959) [10]. Reducing sugars react with DNSA, reducing it to a colored compound. The intensity of the color is proportional to the amount of sugar present in the sample. The results were expressed in enzymatic activity units (U), defined as the amount of enzyme required to produce 1 µmol of glucose per mL per minute under assay conditions.

Results and Discussion

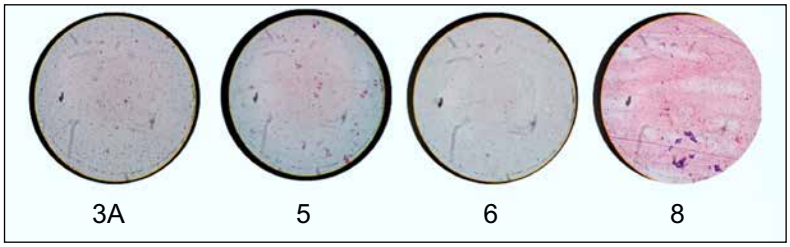
This study reports the isolation of four distinct bacterial strains, designated 3A, 5, 6, and 8, obtained from cocoa samples. Initial analysis, based on microscopic observation and Gram staining, revealed that all strains exhibit a coccoid morphology and are Gram-negative, as shown in Figure 1.

The determination of reducing sugars in the enzymatic extract yielded the results shown in Table 1. Strain 3A exhibited the highest level of free glucose in the reaction, comparable to results found in the literature, such as those reported by Malik [12], who found values between 0.2 and 0.5 U/mL under the same temperature and incubation time conditions prior to enzyme optimization. Enzymatic activity can be improved by increasing incubation time during the DNS test and avoiding freezing the enzymatic extract between fermentation and enzymatic assays. Furthermore, the cellulolytic potential can be enhanced by using multiple enzymes simultaneously.

Table 1. Results of cellulase enzymatic activity assay.

Sample	Result (U/mL)
3A	0.42
5	0.20
6	0.24
8	0.27

Figure 1. Gram-stained slides of the cellulolytic strains identified.



The analyses provide crucial information regarding the enzymatic characteristics of the isolated strains.

Thus, the findings underscore the biotechnological potential of the isolated bacterial strains, with particular emphasis on strain 3A, which exhibited the highest glucose release, suggesting noteworthy enzymatic activity. The consistent observation of Gram-negative cocci among all isolates raises the possibility of a correlation between morphological features and enzymatic performance, although further studies are required to validate this association. Comparative analysis with previous reports, such as those by Malik[12], supports the applicability of these strains in cellulose degradation processes, particularly when employing optimization strategies such as extended incubation times or the synergistic use of multiple enzymes. These preliminary results justify the continuation of this investigation, with a focus on enhancing enzymatic efficiency and assessing strain performance across various environmental and industrial conditions.

Conclusion

This project stands out due to its relevance in various aspects. It primarily addresses the urgent need to find sustainable alternatives to fossil fuels, exploring the untapped potential of agro-industrial waste as a renewable energy source. By focusing on the bioprospecting of cellulase-producing microorganisms, the study advances national biotechnology by seeking innovative and low-cost solutions for biofuel production. Additionally, it contributes to reducing the environmental impact of the cocoa agro-industry by transforming waste problems into value-generating opportunities and promoting sustainability. In summary, the project represents a significant step toward a greener economy, driving the development of clean technologies and the appreciation of Brazilian microbial biodiversity.

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Implementation of Anti-Fouling Tests with Copper Sulphate Pentahydrate Using the Coral *Favia gravida* (Cnidaria, Scleractinia)

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The use of antifouling compounds in paints on ships and oil platforms has caused environmental damage to marine species. Thus, the demand for environmentally friendly compounds and technologies has increased. The objective of this article was to perform toxicological experiments with the antifouling biocide copper sulfate pentahydrate to identify concentrations that have the least impact on the native coral *Favia gravida*. The acute test showed mortality at concentrations of 0.02 and 0.025 mg/mL, although reactions in mobility, cilia movement, and initial development were observed at concentrations above 0.01 mg/mL. The chronic test showed continued development only in the control and at a concentration of 0.005 mg/mL. The high mortality rate in the control group indicates the need for further testing. This is the first toxicological test with an antifouling agent in *F. gravida*.

Keywords: Ecotoxicology. Coral Reefs. Acute Essay. Chronic Essay.

Antifouling compounds were highly commercialized in the 1970s due to increasing demand in boat paints [1]. The importance of using antifouling compounds is linked to marine biofouling, a term used to define the action of species that establish themselves on natural and artificial surfaces, such as ships, smaller vessels, and oil platforms [2]. However, since then, several ecological consequences have been documented worldwide, including the impact on marine organisms and ecosystems [1].

Scleractinian corals are one of the organisms that can be affected by antifouling compounds. They are animals belonging to the phylum Cnidaria and are the leading builders of coral reefs [3].

These organisms secrete calcium carbonate, forming the structure of the reefs, and shelter unicellular, photosynthetic algae called zooxanthellae that live in symbiosis within the coral tissue, providing them with food [8].

However, studies on the effect of antifouling compounds on these organisms in Brazil are in their incipient stages.

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A common substance used in antifouling paints is copper sulfate. This substance is a salt formed by sulfuric acid and copper, as in Equation 1:

$$Cu^{2+} + SO_4^{2-} \leftrightarrow CuSO_4 \quad (1)$$

It has a molecular formula ($CuSO_4$) and high solubility in water [4]. Copper sulfate is one of the most widespread pollutants in the marine environment. Among its applications are its use as a fungicide, bactericide, algaecide, and fertilizer [4]. It is already used in antifouling paints.However, despite its high solubility in water, several factors, including alkalinity, hardness, and pH, influence the action of copper in water and impact marine organisms [5]. Therefore, understanding the reactions of organisms to different concentrations of copper sulfate is of great importance.

The objective of this work is to test whether the antifouling compound copper sulfate pentahydrate ($CuSO_4.5H_2O$) affects the initial development of the coral *Favia gravida*. To achieve this, acute and chronic toxicological tests were conducted at various concentrations. *Favia gravida* is a zooxanthellate coral that is highly resistant to variations in temperature, salinity, and water turbidity [6]. It forms massive, spherical, and encrusting colonies [6]. In Brazil, *F. gravida* occurs from Maranhão to Espírito Santo, being

easily found on adjacent reefs and rocky shores, and is often exposed to contamination in urbanized regions [6,8].

Materials and Methods

Coral Collection

Favia gravida colonies were collected in June 2024 on Boa Viagem beach, Salvador, Bahia. They were transported to the laboratory in thermal boxes containing seawater, aeration, and light. The larvae were released 2 days after collection and sampled with a Pasteur pipette for the experiment.

Experimental Design

To carry out the ecotoxicological tests, a control solution with seawater (without copper sulfate pentahydrate) and five treatments with different concentrations of the copper sulfate solution were used: 0.005, 0.010, 0.015, 0.020, and 0.025 mg/mL. Each treatment and control had three replications. Each one of them contained five larvae of *F. gravida*. The response variables observed were larval mobility, larval cilia mobility, larval development, settlement, and mortality.

Acute and Chronic Tests

The acute test consisted of observing the response variables every four hours over 24 hours. The chronic test consisted of analyzing the same response variables for seven days, with observations made once a day, always around 3 pm. Larvae were observed with a stereomicroscope.

Experiment Preparation

The replicas consisted of Petri dishes with saline water (35 ppm) at a range of 23-25 ºC. These were previously washed with an extra 4% and decontaminated in a muffle at 400°C. Each Petri dish had a volume of 50 mL. Initially, a stock solution of the contaminant was prepared and subsequently diluted in the seawater in the Petri dishes. The stock solution contained 15 mg of copper sulfate pentahydrate ($CuSO_4.5H_2O$) in 100 mL of seawater. Dilution calculations for the different concentrations were made according to the following Equation 2:

$$C_1 \cdot V_1 = C_2 \cdot V_2 \quad (2)$$

Where:
C1 = The concentration of the control solution;
V1 = The volume we want to find;
C2 = The concentrations delimited above;
V2 = The volume of the Petri dishes.

Results

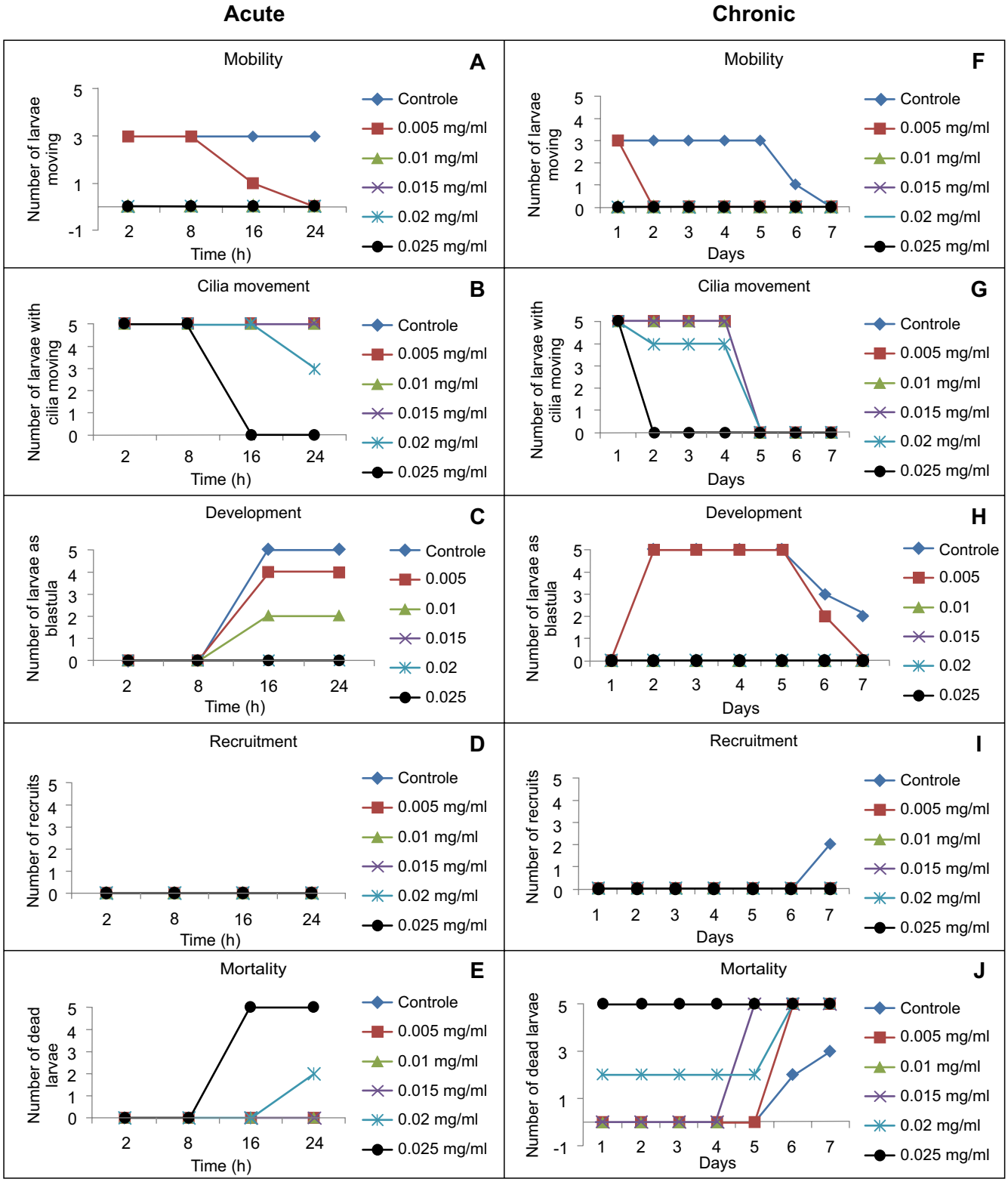
Acute Essay – 24 Hours

The acute test showed that during the first 24 hours, only the control and the concentration of 0.005 mg/mL showed movement of the larvae (Figure 1A). The beating of the larvae's cilia occurred during the first hours of almost all treatments. At the end of 24 hours, an average of two larvae from the 0.02 mg/mL treatment stopped the cilia movement, and all larvae of the 0.025 mg/mL treatment stopped the cilia movement. (Figure 1B). The initial development of the polyp occurred only in the control and at a concentration of 0.005 mg/mL (Figures 1C and 2C). No larval settlement was observed during this period (Figure 1D). Mortality was observed in all larvae treated with 0.025 mg/mL 16 hours after the start of the experiment. (Figure 1E).

Chronic Essay – 7 Days

The chronic test mainly aimed to evaluate larval development. Larvae mobility was observed in the control and 0.005 mg/mL treatment until the fifth day of the experiment. The other concentrations did not show larval displacement from the first day (Figure 1F). Cilia movement was observed in the control and at concentrations of up to 0.02

Figure 1. Response of the larvae of *Favia gravida* to different concentrations of copper sulfate pentahydrate.



A, B, C, D, E: Acute experiment; F, G, H, I, J, K: Chronic experiment.

mg/mL until the fourth day (Figure 1G). Larvae development began at the end of the first day in control and concentrations of 0.005 and 0.01 mg/mL. However, it continued to develop only in the control and 0.005 mg/mL treatment until the fifth day (Figure 1H). Recruitment occurred only in two larvae from one petri dish of the control (Figure 1I). At the end of the experiment, all larvae were dead except for the two that settled on the control (Figure 1J).

Discussion

This work presents the first toxicological test of copper sulfate pentahydrate on larvae of the coral *Favia gravida*. The results of the acute test (24 h) showed that 100% of the larvae died at a concentration of 0.025 mg/mL after one day of exposure, and that 40% of the larvae died at a concentration of 0.02 mg/mL. Mobility and blastula development only occur in the control and at a concentration of 0.005 mg/mL. The movement of the cilia is maintained at other concentrations. Regarding the results of the chronic test, the pattern remained similar. Movement and development occur in the control, and at a concentration of 0.005 mg/mL, cilia movement remains constant at other concentrations until the third and fourth day, after which recruitment occurs only in the control. We observed that the concentration of 0.025 mg/mL is highly toxic to the larvae of this species, and that only at 0.005 mg/mL does larval development occur, although they have not yet reached the recruitment stage.

Copper sulfate has already been used in toxicological tests with other organisms (Table 1). Comparing the results, it is possible to observe that the effects observed in *F. gravida* occurred at low concentrations, often lower than those reported in other studies. Chen and Lin (2000) observed mortality of the *Penaus monodon* at concentrations from 0.003 mg/mL at 15% salinity and 0.011 mg/mL at 25% salinity. Li and colleagues (2005), working with *Macrobrachium rosenbergii*, observed mortality at a concentration of 0.00042 mg/mL. Viana and Rocha (2005) showed that the diatomacea *Aulacoseira granulata* is less sensitive to copper sulfate than to atrazine, although an effect was observed at a concentration of 0.0001 mg/mL. Le Jeune and colleagues (2006) observed

Table 1. Comparison of our results with results from other articles that tested copper sulfate pentahydrate in other organisms.

Reference	Organism	Concentrations Tested (mg/mL)	Effect Concentrations (mg/mL)
Chen and Lin (2000)	Shrimp, *Penaeus monodon*	0.000, 0.001, 0.003, 0.006, 0.009, 0.012, 0.015 (15% salinity) 0.000, 0.008, 0.011, 0.014, 0.017, 0.020, 0.023 (25%salinity)	0.003, 0.006, 0.009, 0.012, 0.015 (15%salinity) 0.011, 0.014, 0.017, 0.020, 0.023 (25%salinity)
Li and colleagues (2005)	Shrimp *Macrobrachium rosenbergii*	0.00000, 0.00032, 0.00042, 0.00056, 0.00075, 0.001, 0.00135, 0.0018	0.00042, 0.00056, 0.00075, 0.001, 0.00135, 0.0018
Viana and Rocha (2005)	Diatom *Aulacoseira granulata*	0.0001, 0.00032, 0.001, 0.0032, 0.01	0.0001, 0.00032, 0.001, 0.0032, 0.01
Le Jeune and colleagues (2006)	Phytoplanktonic communities	0.00008, 0.00016	0.00008, 0.00016
Our study	*Favia gravida*	0.005, 0.010, 0.015, 0.020, 0.025	0.010, 0.015, 0.020, 0.025

changes in the phytoplankton communities with the following concentrations: 0.00008 and 0.00016 mg/mL.

Therefore, it is evident that very low concentrations can already have an impact on organisms.

Finally, the low recruitment proportion, especially in the control (with only two recruits in one petri dish), may indicate that the conditions offered in the experiment were not ideal for the larvae. The slick substrate of the petri dish may not be suitable. Some articles suggest using rougher substrates [7]. Perhaps the temperature, which had an average of 23ºC, also influenced the process. Finally, the presence of fungal contamination is a possibility, as we observed a white mucous membrane in each larva after death.

Conclusion

This is the first study to test copper sulfate pentahydrate in *Favia gravida* larvae. In a 24-hour acute assay, this substance caused mortality at concentrations of 0.02 and 0.025 mg/mL. However, reactions in the behavior of the larvae (mobility, cilia movement, and development) were observed at concentrations as low as 0.001 mg/mL. The chronic test showed continued development only in the control and at a concentration of 0.005 mg/mL, and recruitment only in the control from the fifth day onwards. The high mortality rate observed after seven days of the experiment may be related to the type of substrate and temperature used, and this relationship needs to be evaluated in future experiments.

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Development of a Side-View System for Atomic Force Microscopy (AFM)

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This study presents the development of a side-view imaging system for atomic force microscopy (AFM). The system consists of a monochromatic camera, two lenses, a light source, and image processing software. Six qualitative tests were conducted to evaluate the system's field of view and resolution. The field of view tests used a target positioned both horizontally and vertically. In contrast, the high-resolution test was conducted with the target fixed horizontally and an exposure time of 3,000 milliseconds in both tests. The results allowed the evaluation of the optical system's performance. They demonstrated its feasibility for side imaging in the AFM system under development at the Optics Laboratory, ensuring greater precision in the probe's approach to the sample.

Keywords: Atomic Force Microscopy. Imaging System. Characterization.

Atomic force microscopy (AFM) encompasses a wide range of applications, from the study of polymer surface morphology to the examination of morphological, structural, and molecular properties at the nanoscale [1]. The AFM operates based on the interaction between an excellent tip and the sample under investigation. By detecting these interactions, the equipment can map surface topography and properties at nanometric resolution. A visualization system is essential for observing the probe and sample. Thus, a dedicated imaging system is required, specifically a side-view system for the AFM under development in the Optics Laboratory. This system comprises an imaging sensor (color or monochromatic), one or more lenses for image conditioning, a light source, and image processing software. The choice of materials for the side-view system was based on the AFM system being developed in the NA@MO Project. A Basler camera was selected due to its similar technical specifications (sensor and pixel size) to the camera used in NA@MO, differing only in that it is monochromatic instead of color. Accordingly, this test aims to characterize and validate the best configuration and component selection for the AFM side-view system under development in the Optics Laboratory.

Materials and Methods

The characterization of the side-view system was performed through six experimental tests—four for field of view evaluation and two for resolution analysis, all of which were qualitative in nature. The experimental setup consisted of a Basler acA2440-35um monochromatic camera, a lens, and an illumination system (Figure 1). Additionally, an analysis was conducted regarding the distances between the sensor and the lens, and between the lens and the object. For the field of view analysis, two different lenses were tested: a 3x Mitutoyo Compact Objective coupled with a 152.5 mm extension tube, and a 3x 65 mm CompactTL Telecentric Lens. A CB760-80010 print tape was used as the target, positioned both vertically and horizontally. For resolution evaluation, both lenses were used, with the HIGHRES-1 target from Newport placed horizontally.

In all configurations, targets were exposed for 3000 ms to ensure uniformity in image acquisition.

Descriptions of each test are detailed below:

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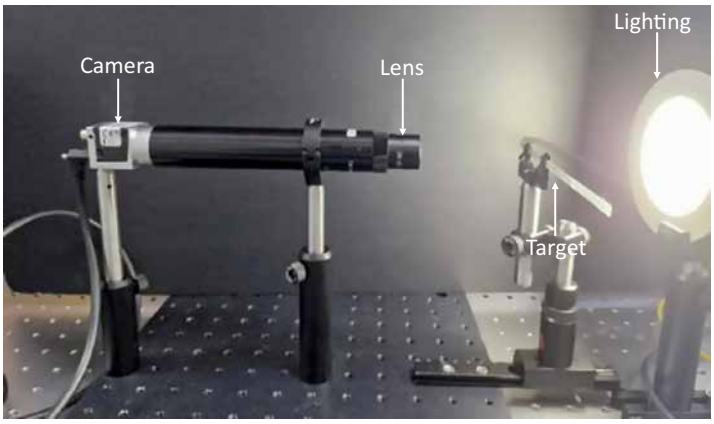
Figure 1. Experimental setup illustration.



Experiment 1: Field of view test – Basler acA2440-35um camera, 3x Mitutoyo Compact Objective, CB760-80010 target horizontally.

Experiment 2: Field of view test – Basler acA2440-35um camera, 3x Mitutoyo Compact Objective, CB760-80010 target vertically.

Experiment 3: Field of view test – Basler acA2440-35um camera, 3x 65 mm CompactTL Telecentric Lens, CB760-80010 target horizontally.

Experiment 4: Field of view test – Basler acA2440-35um camera, 3x 65 mm CompactTL Telecentric Lens, CB760-80010 target vertically.

Experiment 5: High-resolution test – Basler acA2440-35um camera, 3x 65 mm CompactTL Telecentric Lens, HIGHRES-1 target horizontally.

Experiment 6: High-resolution test – Basler acA2440-35um camera, 3x Mitutoyo Compact Objective, HIGHRES-1 target horizontally.

The tape-type target has 6 line pairs per mm (lp/mm); the horizontal and vertical field of view was calculated by dividing the number of visible line pairs by 6, resulting in the total dimension in millimeters. For resolution, the maximum value was identified by the group where the separation between lines was still distinguishable.

Results and Discussion

Figure 2 shows the results of the experiments.

After testing, it was observed that the 3x Mitutoyo Compact Objective lens achieved a field of view of 2.75 x 2.29 mm, while the 3x 65 mm CompactTL Telecentric Lens achieved 2.71 x 2.27 mm—1.59% smaller than the first lens.

Regarding resolution, the first lens resolved Group 7.3 at 161.3 lp/mm, corresponding to a pixel size of 3.10 µm, while the second lens resolved Group 7.2 at 143.7 lp/mm, representing a 10.92% improvement for the Mitutoyo lens.

As shown in Figures 3 and 4, the total distance from sensor to object was 262.52 mm for the first system and 141.53 mm for the second. Despite the 3x Mitutoyo lens's better optical performance, its larger working distance results in a bulkier setup, making focus adjustments more challenging.

Conclusion

The configuration using 3x65 mm CompactTL Telecentric Lenses was chosen, prioritizing a compact and functional side-view system. Although

Figure 2. Experiment results.

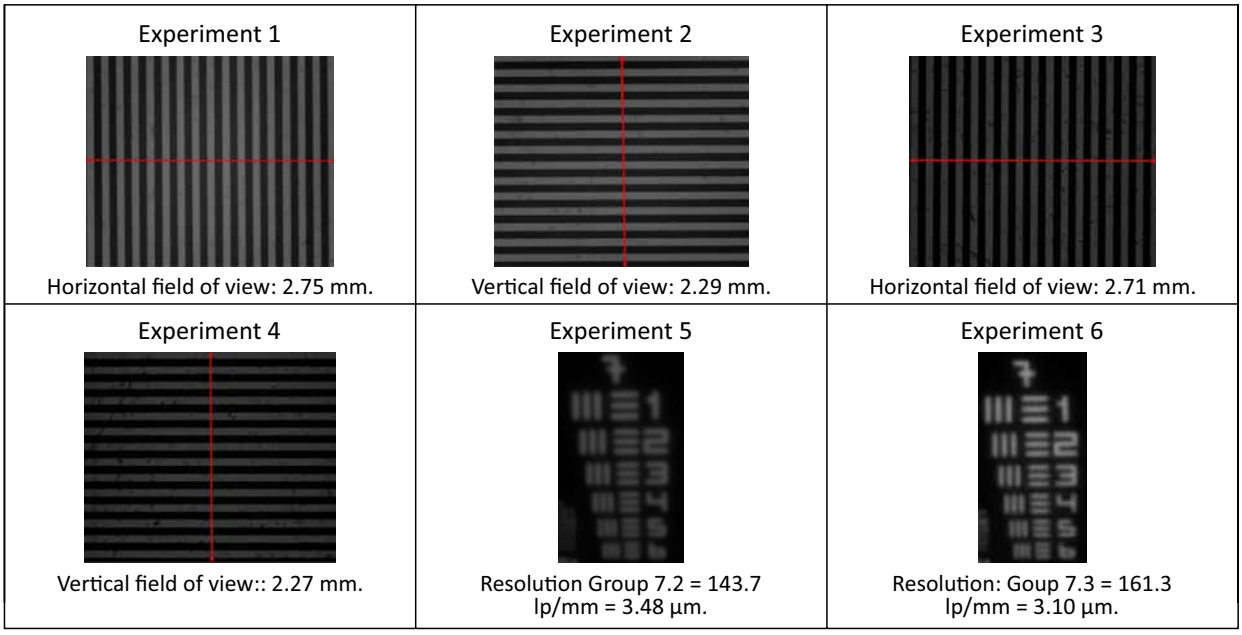


Figure 3. Mitutoyo lens measurements.

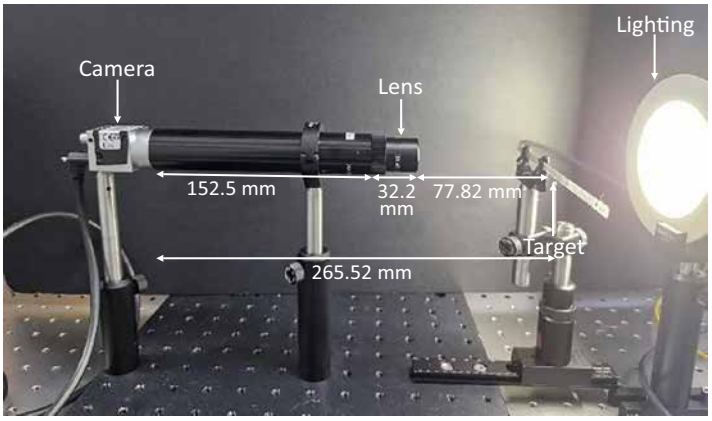
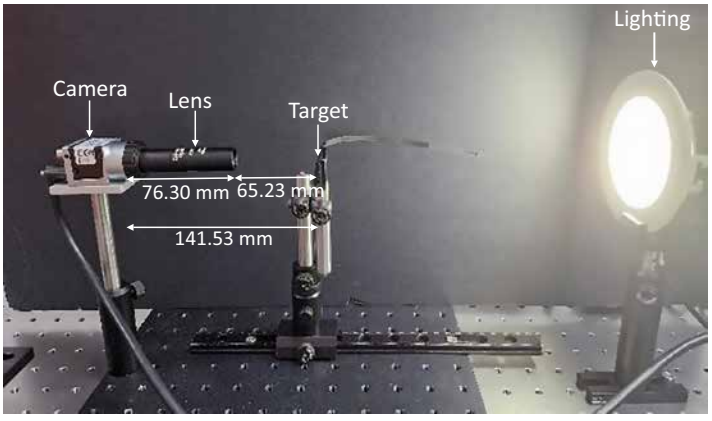


Figure 4. Telecentric lens measurements.



slightly lower in performance, this configuration meets the project requirements and offers a practical advantage in terms of compactness.

Acknowledgments

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FBG-Baesd Soft Probe for Measurement of Temperature Using Strain Decoupling Technique

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This study explores the use of Fiber Bragg Grating (FBG) sensors encapsulated in stainless steel for precise temperature measurements in a soft probe applicati on. Encapsulation enhances temperature sensitivity while minimizing strain interference, a common challenge in FBG sensor applications. Experimental results showed minimal wavelength shifts and temperature errors, demonstrating the effectiveness of the metallic capillary in decoupling strain effects from temperature measurements. The calibration curve confirmed high temperature sensitivity and low strain sensitivity. This method offers a reliable solution for precise temperature measurements in environments where strain can introduce measurement noise.

Keywords: Optical Fiber. FBG Decoupling. Sensors Packaging.

In recent years, optical fiber sensors have received significant attention due to their remarkable sensitivity, versatility, and durability. In explosive environments, where electronic sensors are often impractical or dangerous, they are a promising alternative.

Optical fibers are thin strands of glass or plastic that transmit light from one end to the other, primarily used in telecommunications. However, their unique properties also make them ideal for a wide range of sensing applications.

The use of optical fibers in sensing relies on the principle that changes in the external environment can alter the properties of light traveling through the fiber [1]. This alteration can be detected and analyzed to provide precise measurements of the desired parameters. These sensors have been utilized for monitoring sleep [2], breathing [3], and measuring various physical parameters, including vibration [4,5], strain [4], and temperature [6], making them indispensable in numerous industries. There are multiple types of optical fiber sensors, including macrobending sensors, OTDR (Optical Time-Domain Reflectometry), OFDR (Optical Frequency-Domain Reflectometry), and FBG (Fiber Bragg Grating). This work's objective is to develop a soft probe temperature sensor using a packaged FBG.

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Fiber Bragg Grating (FBG)

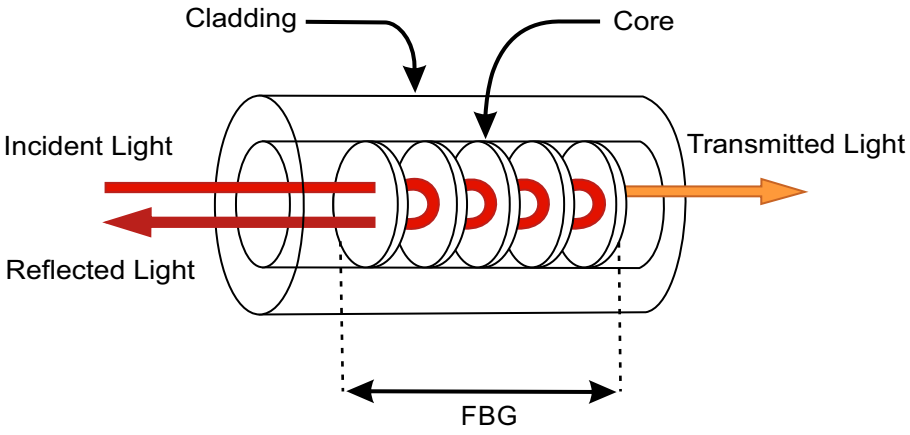
The Fiber Bragg Grating (FBG) is a type of optical fiber with a periodic modulation of the refractive index in its core, which reflects a specific wavelength of light related to the grating period. This allows for the measurement of strain and temperature when the fiber undergoes stretching or compression [7,8]. The core advantage of FBG sensors lies in their multiplexing ability, enabling the monitoring of various points along a single fiber optic cable.

Mathematically, the Bragg wavelength (λB) is determined by the equation:

$$\lambda B = 2 n_{eff} \Lambda$$

Where n_{eff} is the effective refractive index of the fiber core and Λ is the grating period, which is the distance between consecutive grating planes in the fiber. Changes in temperature and strain affect both neff and Λ, leading to a shift in the Bragg wavelength. Specifically, an increase in temperature causes thermal expansion of the fiber and changes

Figure 1. Light reflection inside the FBG optical fiber.



in the refractive index due to thermo-optic effects, increasing λB.

Similarly, applied strain stretches the fiber, increasing Λ and altering neff, which also shifts λB.

Typically, FBG sensors are used as fixed sensors for continuous monitoring of physical and chemical parameters. The development of a probe-type FBG temperature sensor is significant for applications that require point investigations and localized temperature measurements in scenarios where detailed thermal profiling is necessary, such as in medical diagnostics, materials science experiments, and specific industrial processes.

However, one of the main challenges in using FBG sensors as temperature sensors, where contact with an object or surface is required, is the coupling between strain and temperature. When FBG sensors are used in environments where both temperature and mechanical strain vary, the sensor's output can become convoluted, making it difficult to isolate the contributions of each factor. This cross-sensitivity can introduce errors and noise, undermining the sensor's reliability. To mitigate this, effective encapsulation techniques have been developed [9-12]. Packaging the FBG in a metallic capillary presents an effective solution. The metal tube, serving as housing for the FBG sensor, acts to decouple the strains due to contact with the sensing surface and the sensor structure deformations from the FBG temperature sensor. In this work, we explored a stainless-steel encapsulation technique to improve performance and reduce strain interference in an FBG temperature sensor probe.

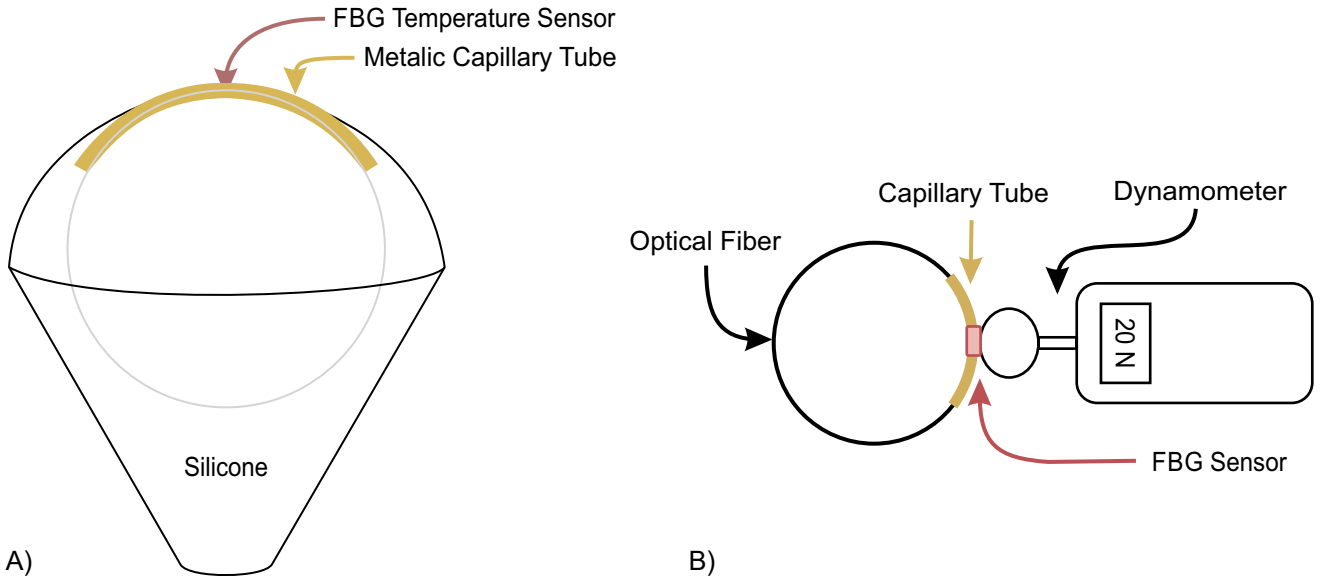
Materials and Methods

The soft probe was constructed using silicone, as depicted in Figure 2a. A stainless-steel capillary, with a 0.5 mm diameter and a length of 25 mm, was employed to encapsulate the bare FBG with the center wavelength of 1550 nm. This encapsulation served dual purposes: it protected the FBG from bending-induced strain and enhanced its thermal sensitivity.

To integrate the encapsulated FBG with the probe, a round plastic structure was used to form a fiber coil during the silicone pouring process. This configuration ensures that the silicone probe functions effectively as a contact temperature sensor, providing reliable and accurate temperature measurements in various application scenarios. To evaluate the degree of isolation of encapsulated FBG from strain, a translational stage was used to press the silicone sensor against a dynamometer with a disc at its end, as shown in Figure 2b. The reflection spectrum was monitored as the force increased from 0 to 18 N in 2 N increments; the spectra were recorded after 10 seconds of stabilization at each force step.

The temperature response of packaged FBG was obtained by placing the soft probe in contact with water on a heating table and

Figure 2. A. Diagram representation of the temperature FBG sensor. B. Schematic representation of test setup.



varying the temperature from 22°C to 100°C. The calibration curve correlating temperature with the transmitted wavelength was created using a thermocouple connected to a multimeter. The tip of the thermocouple was placed in water that had been heated to a specific temperature. The temperature was recorded using a multimeter, while the wavelength was measured with an FBG Interrogator. These measurements were then correlated to make the sensor's calibration curve.

Results and Discussion

The temperature response of the FBG sensor, illustrated in Figure 3, exhibits a linear behavior. The slope of the curve obtained through linear fitting demonstrates that the device's sensitivity is 12 pm/°C. This linearity confirms the sensor's ability to provide consistent and accurate temperature readings.

The reflection spectrum of the encapsulated FBG probe sensor was analyzed as a function of applied force within the investigated range. From this data, a graph was generated depicting the variation of the Bragg wavelength relative to the applied force (Figure 4). This graph shows that an applied force of up to 18 N causes a maximum error of 0.013 nm, which corresponds to approximately 1.1 °C of temperature error. The linear fit of the force test results indicated that the mean sensitivity of the soft probe to strain was 0.2 pm/N, demonstrating the effectiveness of our fiber packaging technique in minimizing strain interference in temperature measurements.

Comparatively, the sensitivity of our soft probe FBG temperature sensor is of the same order of magnitude as that of the sensors described in other works [11,12]. However, our packaging method offers significant advantages in terms of simplicity, asset and personnel safety, and cost-effectiveness.

Conclusion

This work demonstrated the efficacy of utilizing Fiber Bragg Grating (FBG) sensors encapsulated in stainless steel for accurate temperature measurements in a soft probe application. The results indicate that the encapsulation significantly enhances the sensor's sensitivity to temperature while effectively minimizing interference from strain.

The primary challenge addressed was the coupling between strain and temperature, which can compromise measurement accuracy. By housing

Figure 3. Calibration curve of the FBG-based temperature sensor correlating the wavelength shift variation with the temperature measured.

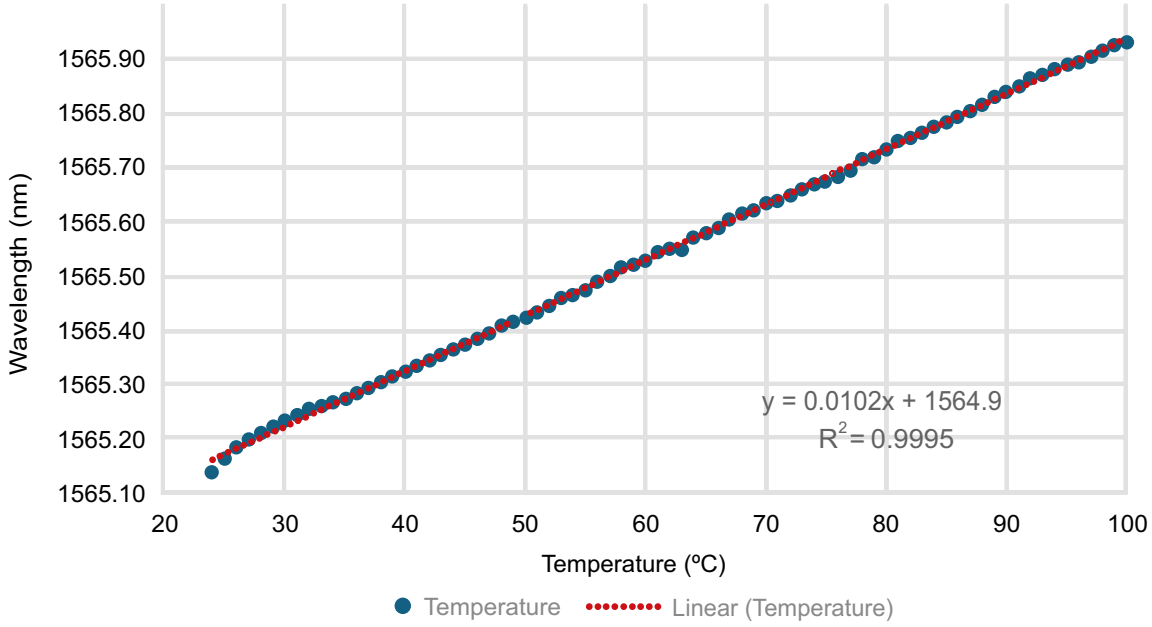
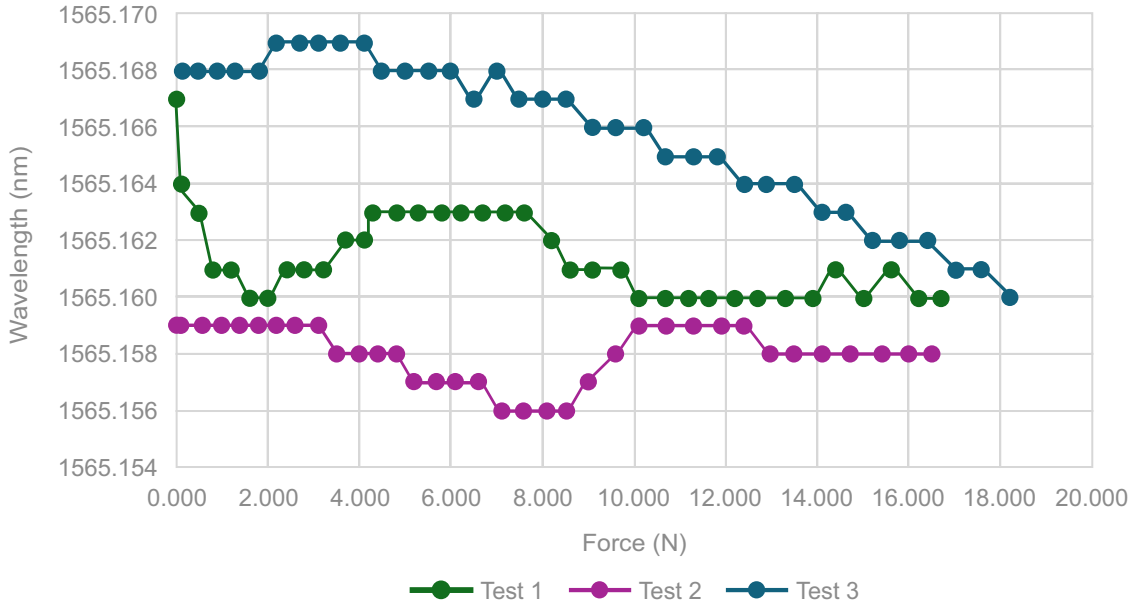


Figure 4. Wavelength shift variation as a function of applied force.



the FBG sensor within a metallic capillary, it was possible to decouple the strain effects from temperature measurements. The results showed minimal variations, with wavelength shifts of less than 1 nm and temperature errors of around 1°C, even under applied forces of up to 18 N.

The calibration curve, with an angular coefficient of 12,0pm/°C, highlighted the sensor's high temperature sensitivity. Additionally, the low strain sensitivity, indicated by an angular coefficient of 0,2pm/N, confirmed the effectiveness of the metallic capillary in decoupling strain from temperature.

These findings underscore the potential of stainless-steel encapsulation as a viable solution for enhancing the performance of soft probe FBG sensors in environments where accurate point investigations and thermal profiling are critical.

The successful decoupling of strain and temperature not only improves measurement accuracy but also broadens the applicability of FBG sensors in various scientific and industrial domains.

Acknowledgments

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Innovation in Low-Cost Sensor for Water Turbidity Assessment

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This work emphasizes the importance of monitoring water quality through a low-cost turbidity sensor to support environmental management. Using LDR sensors and an Arduino, the project determines the ideal wavelength and establishes the calibration curve. The sensor operates automatically, performing color scanning and selecting the wavelength most sensitive to variations in water turbidity. Data analysis revealed a calibration curve with a coefficient of determination (R^2) of 0.995, highlighting the sensor's high precision. Turbidity quantification is achieved through nephelometric (90°) and turbidimetric (180°) methods, optimizing sensor configuration and wavelength to obtain the best measurements.
Keywords: Turbidity Sensor. Environmental Management. Arduino. LDR.

Water is essential for life. Although two-thirds of the Earth's surface is covered by water, only 2.5% of it is freshwater. Of this fresh water, 68.9% is found in glaciers and polar ice caps, 29.9% is groundwater, 0.9% is found in soil moisture and swamps, and only 0.3% is found in rivers and lakes, available for use [1]. In this context, Brazil holds a privileged position compared to other countries, boasting the largest freshwater reserve in the world, which accounts for 12% of the global total. However, this abundance of water is not evenly distributed throughout the country. The Amazon, for example, is home to one of the largest river basins on the planet, but it is also one of the least populated areas in Brazil.

In contrast, the largest population concentrations in Brazil are found in large urban centers, which are often far from the country's main rivers [2]. The region that suffers most from water scarcity is the Northeastern Semiarid region, where the lack of water results in constant rationing, due to the irregularity of the rainfall regime, affecting both human consumption and socioeconomic development [3]. Therefore, it is essential to implement planning and management measures for available water resources to ensure a continuous supply that meets the population's needs.

Monitoring water quality is one of the main challenges for efficient water management. According to Law No. 9,433/97, this aims to ensure the availability of water with quality standards appropriate for its respective uses. The main parameters analyzed for water quality control include pH, turbidity, electrical conductivity, temperature, and dissolved oxygen. Among these, turbidity stands out as a crucial indicator, as its variation indicates an increase in suspended matter or impurities, thereby affecting the water's clarity [4-6].

The turbidity is an optical property that causes the scattering of light by particles and molecules, preventing light beams from passing through the water. In simpler terms, turbidity indicates the relative transparency of the water. There are several techniques and methods for measuring turbidity in a water sample, but the most accurate are turbidimetry and nephelometry. These methods utilize a light source that shines on the sample, capturing both transmitted and scattered light [7,8].

Turbidimetry is a method for determining turbidity based on the attenuation of a light beam that passes through the sample and reaches a detector positioned 180 ° from the radiation source. On the other hand, in nephelometry,

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turbidity is determined by the amount of scattered light that reaches the detector, located 90 ° from the emitting source [9,8].

In the literature, studies have utilized microcontrollers in the construction of turbidimeters. For example, Cardoso (2011) created a turbidity sensor to monitor several water samples, using an LED and a phototransistor that operate in the infrared region. The results indicated that the device was more effective for turbidity values above 100 NTU. In another study, Martins (2012) developed a turbidimeter capable of measuring turbidity between 16 and 4000 NTU to assess the quality of liquid effluents emitted by industries. However, these devices are not suitable for measuring the turbidity of drinking water, which should be maintained at a maximum of 1 NTU [10,11].

In this context, the present work aims to develop a low-cost turbidity sensor capable of measuring water turbidity in real-time and in continuous flow. The sensor will be able to measure levels suitable for human consumption up to higher concentrations, such as those found in industrial effluent disposal.

Materials and Methods

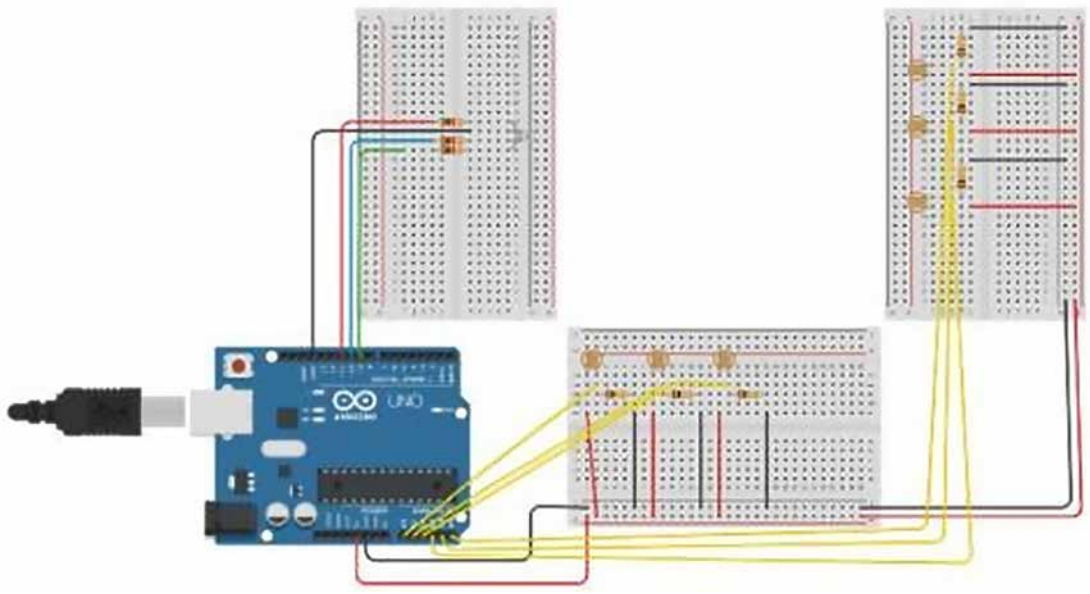
Prototype Design and Materials

The Tinkercad tool was used to design the turbidity sensor. After finalizing the sensor's architecture, the project was further developed on the Tinkercad platform (Figure 1), which streamlined the specification of materials and resources needed to build the prototype. The list of materials used is provided in Table 1.

Table 1. List of components required to assemble the prototype.

Components	Amount
LED RGB	1
LDR	6
Arduino UNO	1
Protoboard	1
Jumpers	24
Resistors de 10kΩ	6
Resistors de 220Ω	3

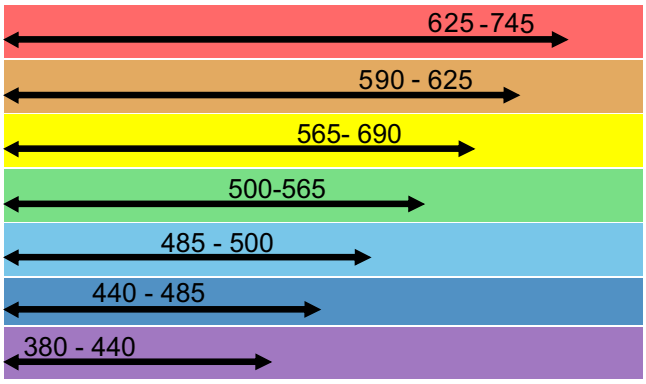
Figure 1. Turbidity sensor design developed in Tinkercad.



Data Acquisition and Analysis with Arduino

To perform data acquisition and analysis, an Arduino was used, programmed with an algorithm that scans the visible light spectrum, covering wavelengths from 380 nm to 745 nm (Figure 2). This range encompasses all colors perceptible to the human eye. The scan starts at 380 nm and gradually increases until reaching 745 nm.

Figure 2. RGB LED colors and related wavelengths.



Using RGB LED in Color Scanning

For color scanning, an RGB (Red, Green, and Blue) LED was used, which includes the three primary colors: red (R), green (G), and blue (B). By combining these colors, the LED can generate any color within the visible spectrum. The luminous intensity of each RGB component can be adjusted from 0 to 255, where 0 indicates no color and 255 represents maximum intensity. This flexibility enables the RGB LED to simulate any color required for spectrum scanning, including the primary colors depicted in Figure 3.

Plotting and Data Analysis

The data collected by the Arduino was processed and plotted using the Arduino IDE's Serial Plotter. This visualization enabled the analysis of the LDR's sensitivity to different wavelengths, offering a detailed understanding of the sensor's behavior under various lighting conditions.

Figure 3. Colors used for initial scanning of the prototype.

BLACK	(0, 0, 0)
WHITE	(255, 255, 255)
RED	(255, 0, 0)
YELLOW	(255, 255, 0)
GREEN	(0, 255, 0)
CYAN	(0, 255, 255)
BLUE	(0, 0, 255)
MAGENTA	(255, 0, 255)

Experimental Procedure

The turbidity samples were organized in ascending order, starting with the lowest NTU value and progressing to the highest. A total of 10 samples were analyzed, with each sample measured three times (in triplicate) to ensure accuracy and minimize experimental error. The sensor was programmed to have the RGB LED scan through 24 different colors, covering the entire spectrum from violet to red. LDR readings were taken at two different angles, 90° and 180°, allowing for a comparative analysis of the sensor's performance in different geometric configurations.

RESULTS AND DISCUSSION

Prototype Assembly

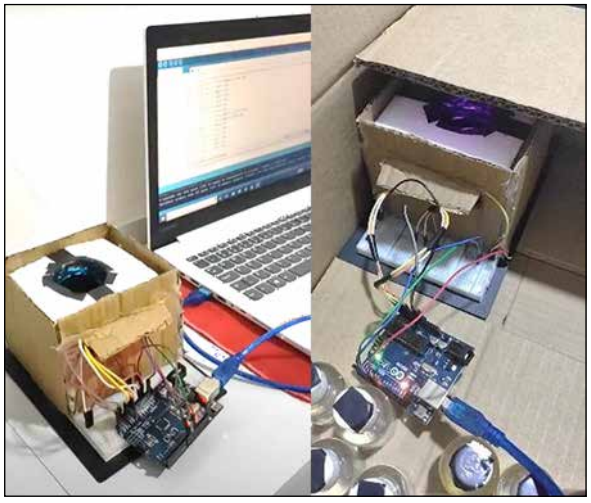
The protection against external light was meticulously designed to ensure the accuracy of the sensor measurements and to minimize interference from ambient light. Styrofoam was chosen as the primary material due to its insulating properties, with an additional layer of cardboard added to reinforce the structure. The components were secured with hot glue, providing durability and stability. Furthermore,

the interior of the cover was entirely lined with insulating tape, which was crucial for reducing internal light reflection and preventing it from impacting the sensor readings, thereby ensuring more accurate and reliable measurements. The first version of the prototype is shown in Figure 4.

Impact of Reading Angles

LDR measurements were conducted at two different angles: 90° and 180°. Data analysis revealed that the 90° angle yielded readings with greater sensitivity to wavelength variations (Figure 5, left). This increased sensitivity can be attributed to the more direct interaction between the incident light and the particles suspended in the sample, which enhances the detection of turbidity. In contrast, the 180° configuration exhibited lower sensitivity, likely due to reduced direct interaction with the particles, leading to less accurate readings (Figure 5, right). Therefore, the scan effectively demonstrated the sensor's response to various wavelengths and validated the device's ability to detect turbidity variations accurately.

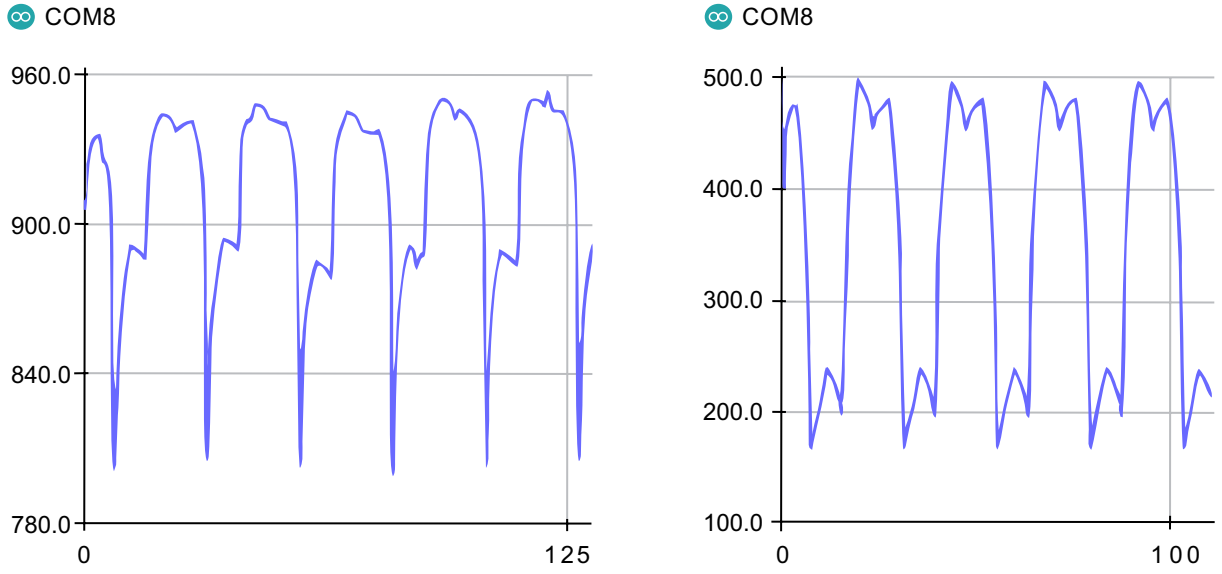
Figure 4. Prototype assembled and protected from external light interference in operation.



Turbidity Sensor Calibration

The turbidity sensor was calibrated using samples with known turbidity values provided by the Sergipe Department of Sanitation (DESO). During the tests, turbidity measurements were taken with red light at a 90° angle to the sensor. For each of the 10 samples, measurements were repeated in triplicate. The average of these readings was calculated and used to create graphs illustrating the relationship between the turbidity values (NTU) and the sensor intensity readings. Additionally, analyzing the samples arranged

Figure 5. Sensitivity reading of LDRs at 90º (left) and 180º (right) during scanning.



in order of increasing turbidity revealed a clear correlation between rising NTU values and the intensity of the sensor readings. This correlation confirms the effectiveness of the calibration method and validates the sensor's capability to deliver consistent and predictable turbidity measurements. The observed linearity in the turbidity graphs further reinforces the reliability of both the sensor and the method used for measuring turbidity variations.

Turbidity Graph Generation and Analysis

The turbidity data were plotted to visualize the relationship between NTU values and the intensity of the readings. The curve that best represented the data distribution was determined using a polynomial equation, as shown in Figure 6. The coefficient of determination (R^2) was calculated to assess the accuracy of the curve fit to the experimental data. The graphical analysis confirmed the validity of the proposed model and demonstrated the sensor's ability to measure turbidity with high accuracy. Consequently, this curve was implemented into the Arduino programming, allowing the sensor to calculate turbidity when a water sample was inserted.

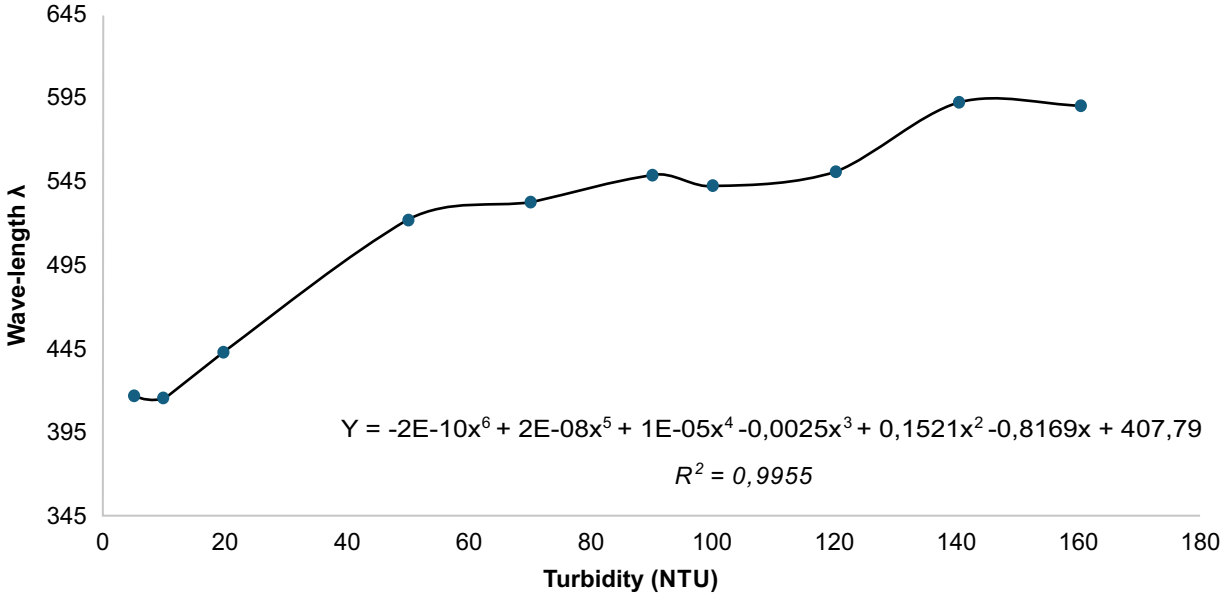
Conclusion

This work developed a low-cost turbidity sensor using Arduino, capable of measuring water turbidity in real-time and in continuous flow. The sensor proved effective in detecting various turbidity levels, achieving an R^2 value of 0.995. The 90° configuration and the use of green light yielded better results for low turbidity values, making it ideal for monitoring drinking water. The economic accessibility of the sensor is a notable advantage, enabling its use in a wide range of applications, from wastewater monitoring to drinking water quality control. Future improvements could include enhancing the color scanning algorithm and exploring the use of additional light sensors to increase measurement accuracy further.

Acknowledgments

We thank Tiradentes University for the partnership and the National Council for Scientific and Technological Development (CNPq) for the support and funding provided through the PIBITI 2019 PROGRAM (Call for Proposals No. 15/2019/DINOVE/IFS). Without the support and

Figure 6. Turbidity sensor calibration curve in red light at 90º.



trust of these institutions, this work would not have been possible.

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Meshless Simulation Software Applied to the Preliminary Design Phase of the Product Development Process

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The study involves the construction and validation of a product development method using meshless simulation software in the preliminary phase of a project, for the structural and functional evaluation of the concept selected in the conceptual phase, to obtain a more robust concept for the detailed phase. This enhances the efficiency of project development, significantly reducing costs and computational time. Two studies will be carried out using meshless software and finite elements to validate the proposed method. Through computational cost evaluation, it was observed that using the meshless software application method in the preliminary phase resulted in a reduction of approximately 19 times in the time invested in CAE analysis during the product's preliminary development phase.

Keywords: Meshless Method. Product Development. Computational Simulation. Finite Element Method.

Due to the highly competitive engineering innovation market, product development processes are being continually improved to produce and innovate in shorter periods while ensuring product quality. To reach this goal, new technologies must be integrated into these processes. In this context, CAD (Computer-Aided Design) and CAE (Computer-Aided Engineering) technologies are tools that provide significant competitive advantages by enabling the creation and validation of virtual models under real-world conditions. These tools help to significantly reduce deadlines and costs during product development [1]. In this context, applying and integrating CAD, especially CAE, into product development methodologies presents an opportunity to optimize the product development process.

Product Development Method

The area of product development is constantly evolving, leading to the creation of various methodologies to organize and systematize this process. Some methodologies are described in the literature, while others have been developed or adapted by companies. Product development models can include only the activities to be done, or they can specify procedures and methods in greater detail [1,2]. Given this, this work will follow the product development process proposed by Amaral, Silva, and Scalice (2006), whose phases are illustrated in Figure 1.

The method divides the process into three macro phases: the first is related to the strategic planning of the product, the second to development, and the third to the phase following project completion, which involves monitoring the product or service until its discontinuation.

In the development phase, the focal point of this work, there are the stages of project planning, informational design, conceptual design, detailed design, preparation, production, and, finally, the product launch. Initially, in the informational project, based on the project plan developed in the previous stage, target specifications are defined, which include product requirements and qualitative information. After this stage, the project proceeds to conceptual design, where design solutions are identified and generated, aiming for the best solution that meets the target specifications. At this stage, a set of documents is generated with the proposed solutions, resulting in a concept.

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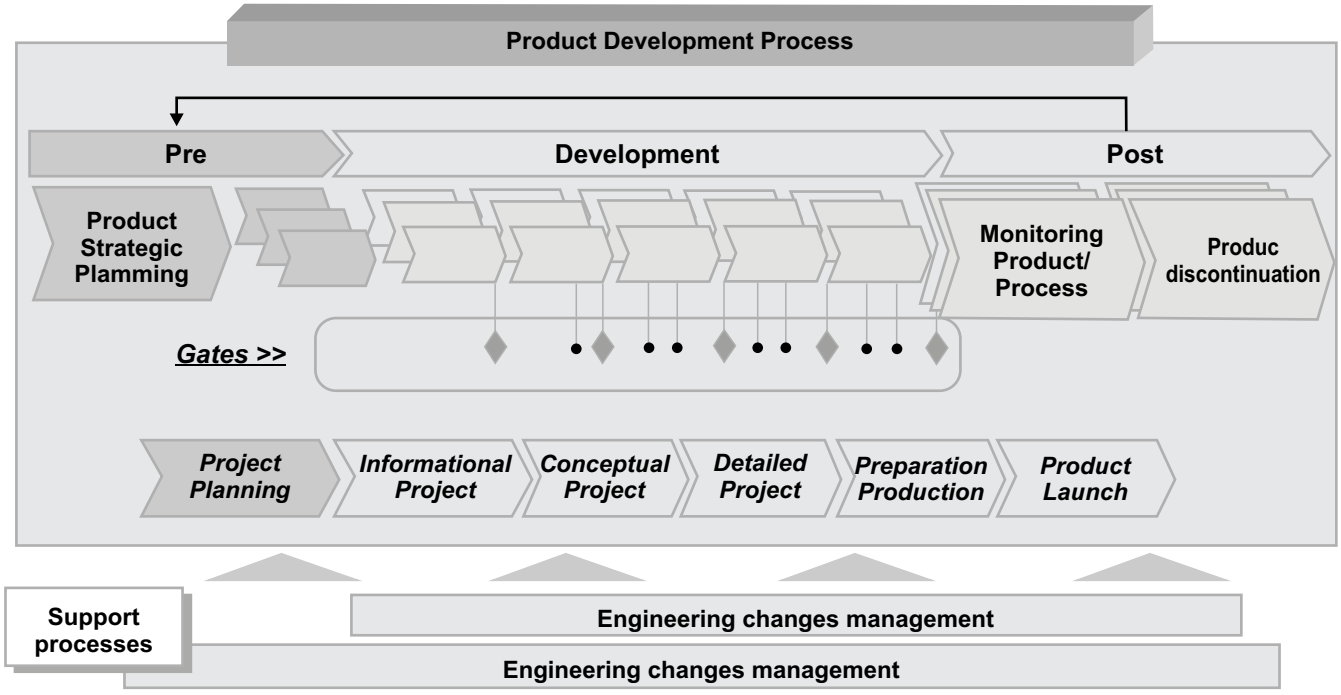
Figure 1. Steps of the product development process.



Source: Adapted from Amaral, Silva e Scalice (2006).

Before proceeding to the detailed design stage, where conceptions are converted into technical definitions, Amaral, Silva, and Scalice (2006) emphasize that other methodologies include a preliminary design stage, which is the first detailing cycle. This stage is typically included in complex projects or at the company's discretion, combining activities from both the conceptual and detailed stages. Finally, the development phase is concluded with the preparation, production, and launch of the product, and the post-development phase begins [2].

CAE: Finite Element and Meshless Methods

To analyze structures with complex boundary conditions, numerical computational methods are essential for obtaining accurate results. There are various methods with different applications, which can be based on meshing or meshless approaches. The finite element method (FEM), which is widely used and applied, provides highly accurate results but has some disadvantages, particularly in comparison to the meshless method [3].

The main disadvantages of FEM are the need to process the geometry and generate and rework the mesh, the quality of which is critical to the accuracy of the calculations and the reliability of the results, as it involves the dimensions and characteristics of the elements, among other aspects. This process is necessary to overcome element distortions, prevent errors in the simulation, and, consequently, in the results. However, this requires a longer working time, which is aggravated in complex structures with geometric discontinuities. Hence, FEM can require more time and computational cost. The meshless method emerged to overcome the difficulties associated with meshing. Using the method of dispersed nodes with points of interest eliminates the need for meshing, thereby significantly reducing the time required [4,5].

Materials and Methods

The study considered the demand for developing a support structure for a water reservoir with a capacity of 25,000 liters, featuring specified dimensions and an

extension for personnel circulation during maintenance purposes. In the project, the Product Development Process, as proposed by Amaral, Silva, and Scalice (2006), was applied, following the phases briefly, as the purpose of the work is to analyze the time spent evaluating the concept during the preliminary design stage of the development phase. Although the original method does not include this stage, it incorporates it into the conceptual phase.It was decided to add it after the conceptual phase to evaluate better the concepts developed during that phase, allowing for their refinement before they are forwarded to the detailed phase.As the project progressed, the pre-development phase was deemed complete, enabling the project to proceed to the development phase.

In the informational design stage, the project's necessities and requirements were identified, and it was determined that the structure must support the weight of a 33.5-ton water reservoir and an occupancy load of 1.5 tons, applied to the upper part of the structure. A wind load of 0.4 T/m acting on the front of the structure was also considered.

In the conceptual phase, the solution principles were identified, and it was concluded that metal beams were the most suitable solution. This led to the basic development of the concepts, which progressed to the preliminary phase. In this phase, the project proceeded with the welded concept, as shown in Figure 2, of the structure to be analyzed.

Having that in view, to select the best concept to proceed to the detailed design stage, it is necessary to apply CAE to the project. Hence, the welded concept was simulated using the finite element method (FEM) in Ansys software and the meshless method in Altair SimSolid, to evaluate the performance of the two methods and ensure the same reliability of the results. Figure 3 presents the workflows for the simulations differ; the working and simulation times in both software were timed to evaluate the computational cost and the time needed to run the simulations.

Results and Discussion

Simulation Results

After conducting simulations in the Ansys and Altair SimSolid software, following the flowchart shown in Figure 4, the results for stresses and displacements in the structure were obtained. The maximum displacement found in the Z axis was -15.467 mm in the analysis carried out in Ansys, while in SimSolid, the value found was -26.1683 mm. Both results are shown in Figure 4. Figure 5 illustrates the displacements along the Y-axis. Again, the observed displacements were greater in the meshless analysis, with a maximum displacement of -6.3230 mm, whereas in the FEM analysis, it was -4.0643 mm.

Figure 6 shows the results of the equivalent stress. For these results, the values obtained for the meshless analysis in the SimSolid software proved to be more conservative, with a maximum stress of 319.1365 MPa located in the region of one of its supports (Figure 7). In the FEM analysis using Ansys software, stress peaks were observed in the same region, resulting in higher stresses due to stress concentrators caused by geometric discontinuities arising from the shell model generation process (Figure 7).

Simulation Time and Computational Cost

The total time taken to carry out the analysis in the ANSYS software was 250 minutes, of which 135 minutes were spent processing the geometry, 90 minutes were spent adjusting the contacts and loads, and 25 minutes were spent analyzing the results and making final adjustments. In the SimSolid software, the total analysis time was 12 minutes and 28 seconds, of which 10 minutes and 55 seconds were used for configuration and 1 minute and 33 seconds for analysis execution; in meshless analysis, the geometry treatment stage is not required.

Figure 2. Welded concept.

Figure 3. Simulation work flowchart.

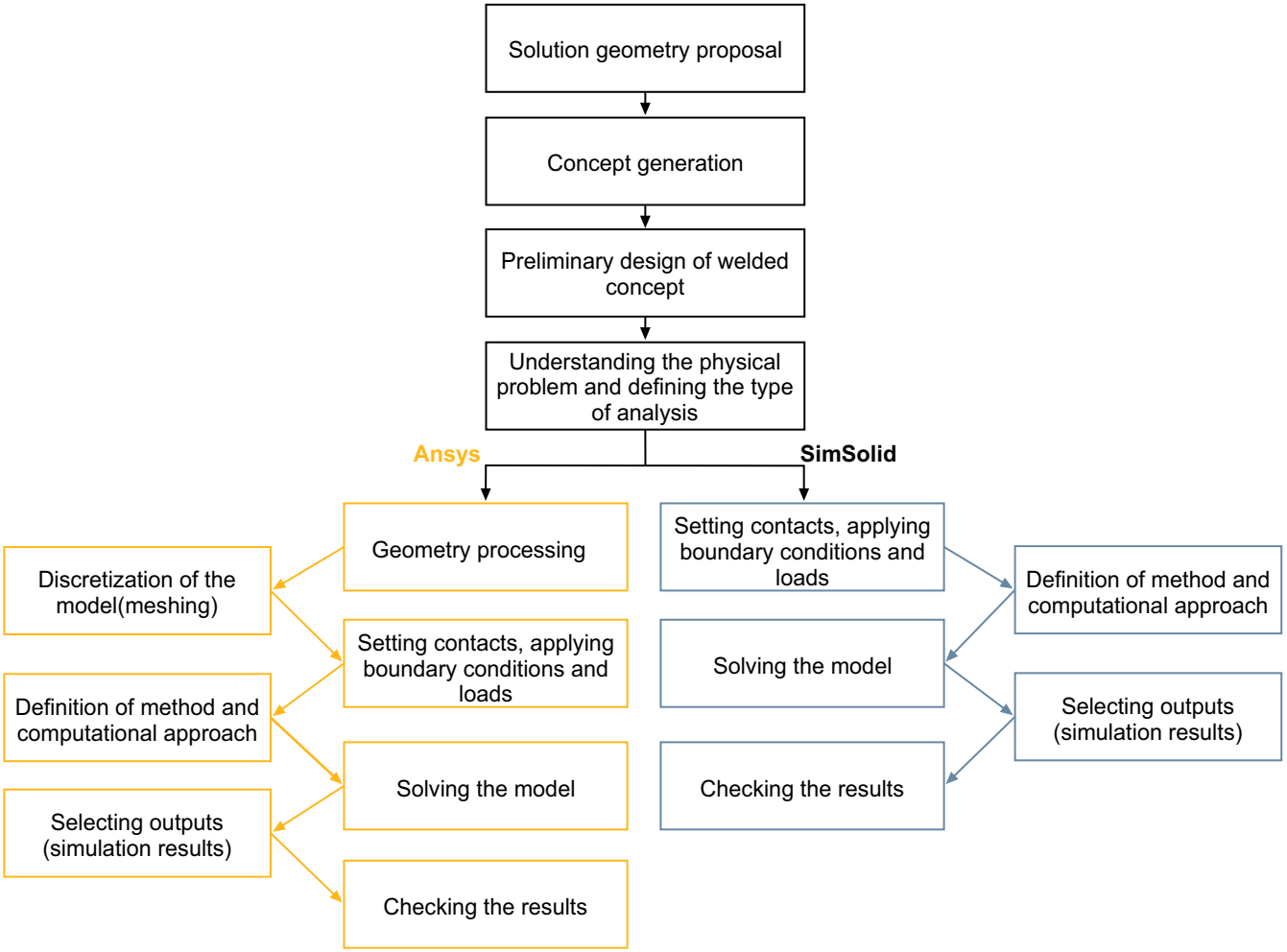


Figure 4. Z deformation results. a) Ansys, b) SimSolid.

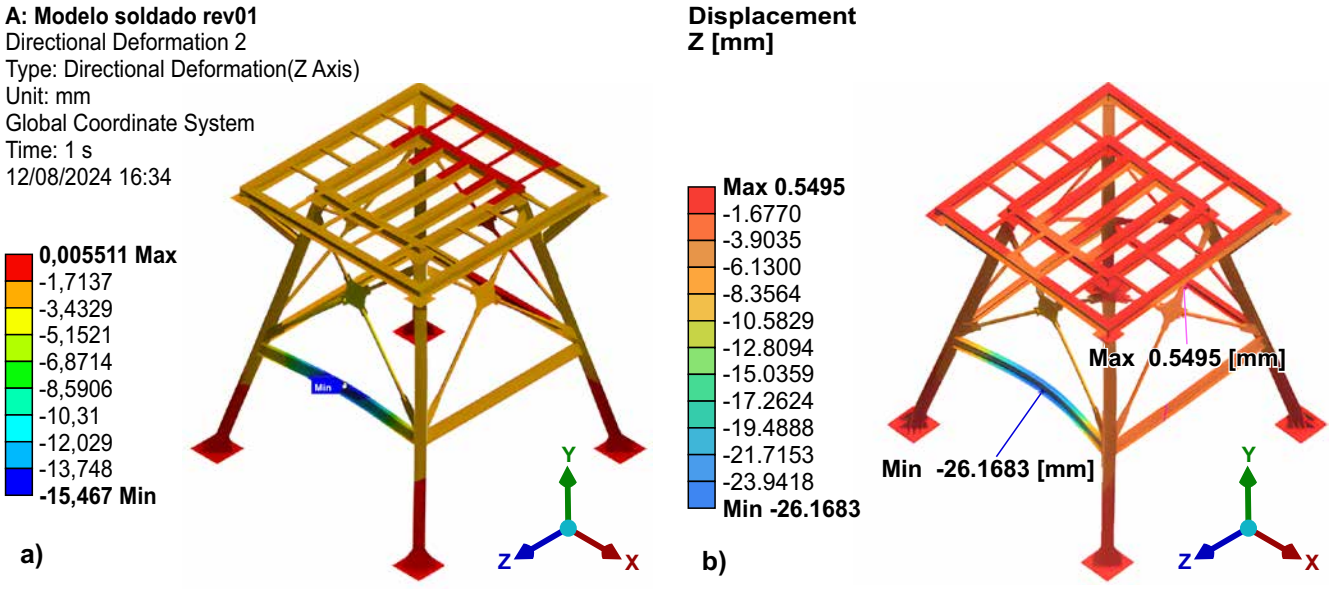
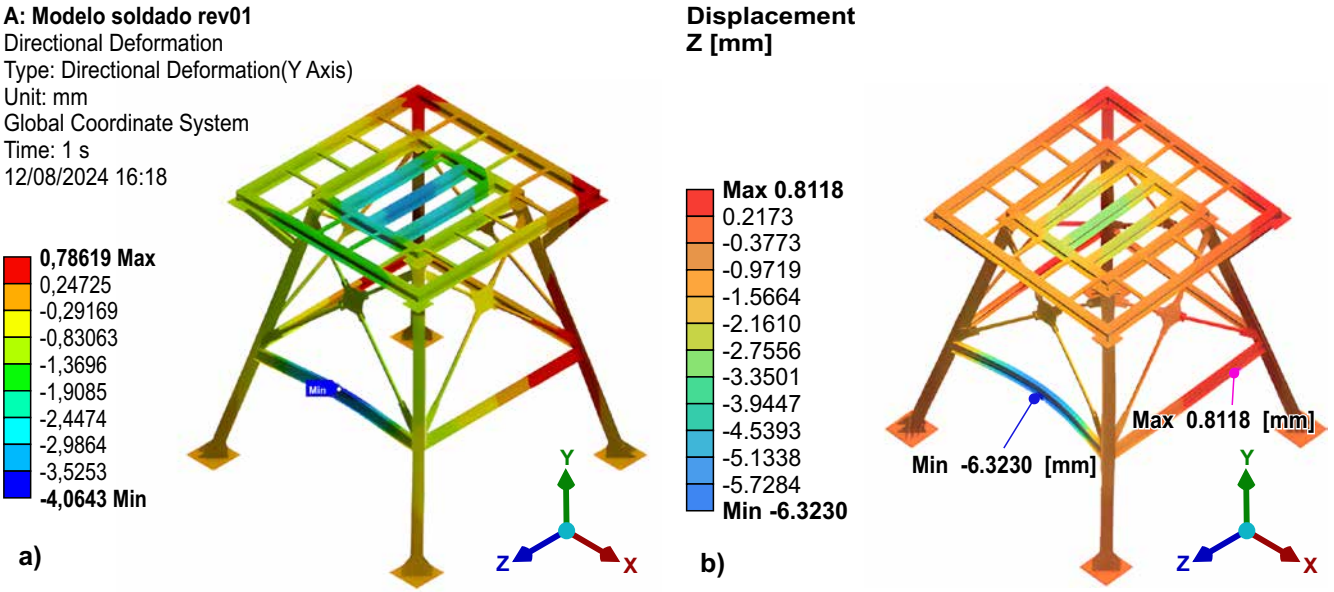


Figure 5. Y deformation results. a) Ansys, b) SimSolid.



Conclusion

Analyzing the results obtained by the Ansys and SimSolid software, it can be seen that the results from the meshless software are more conservative, with analysis times that can be 19 times shorter than those in the FEM software. It is therefore recommended to use meshless software in the preliminary design stage to obtain faster results and thus refine the concept for the following stages of design development. As the FEM software is more reliable in its results, it is recommended for use in analyses in the detailed design phase, where product specifications must be obtained. In this way, future studies can analyze multiple concepts and compare them to

Figure 6. Equivalent Stress Results. a) Ansys, b) SimSolid.

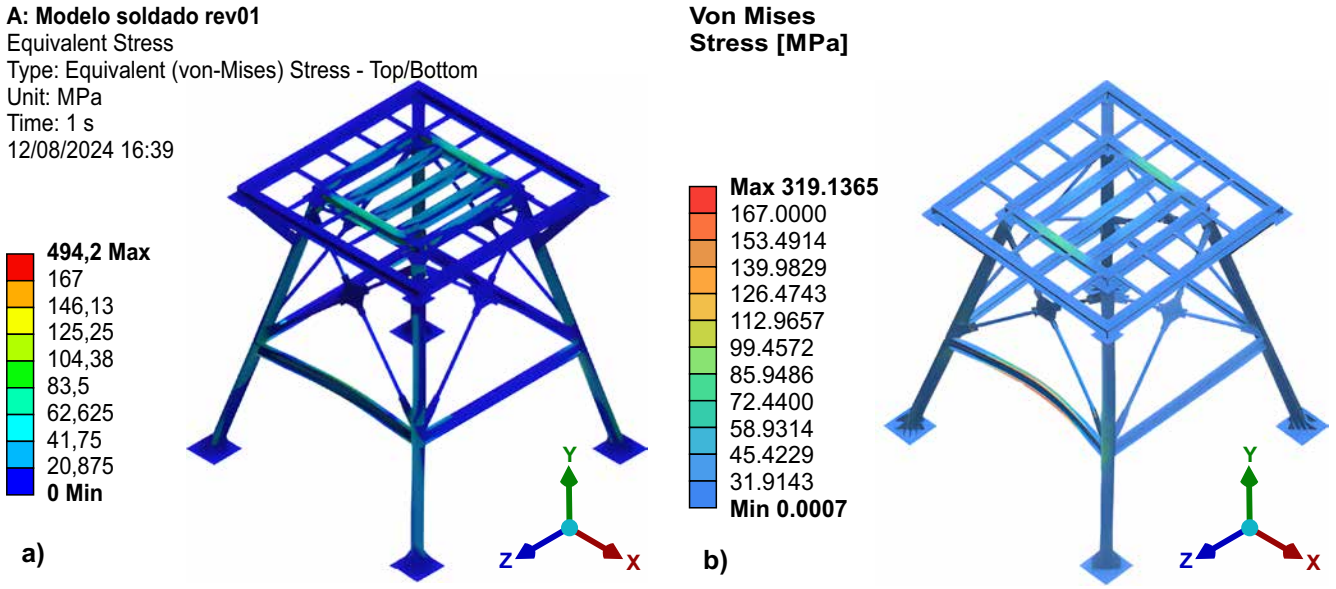
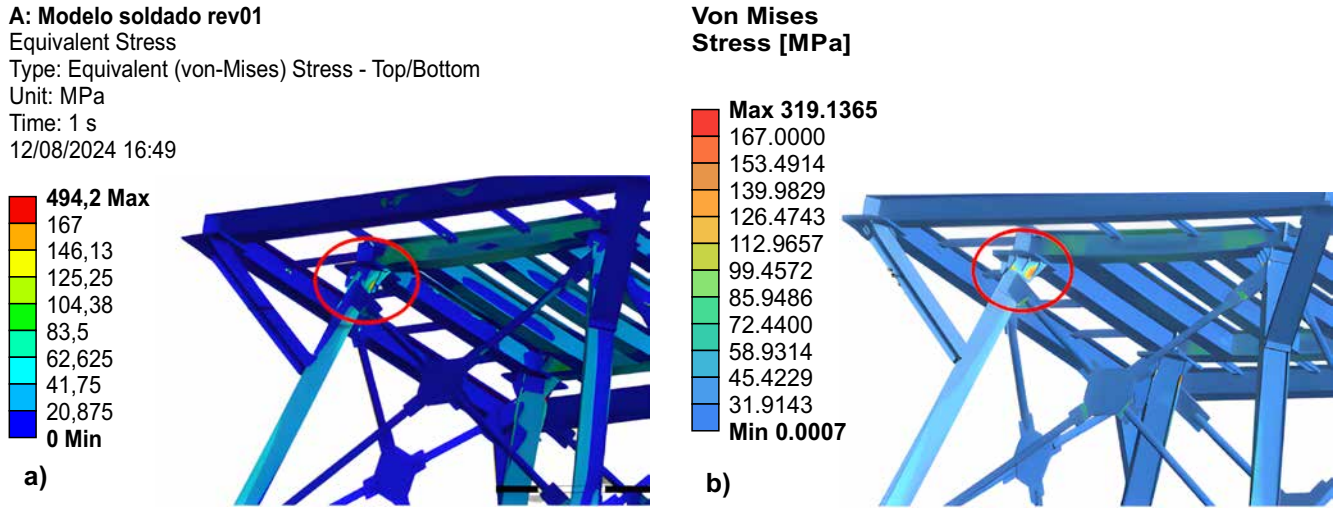


Figure 7. Region of maximum Stress Results. a) Ansys, b) SimSolid.



obtain their structural performance, and based on this, make informed design decisions to select or improve concepts.

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Technological Mapping of Fermented Cocoa Honey Beverages Using Kefir Grains with Functional Effect

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Cocoa honey is a mucilaginous and bittersweet byproduct of the chocolate industry. To increase its added value, producers and researchers have explored new applications such as fermented beverages. Water kefir grains, used in fermentation processes, exhibit functional potential due to the presence of probiotic microorganisms. This study mapped patents related to the production of functional beverages combining cocoa, honey, and kefir, using the Derwent Innovation Index (DII), accessed through Universidade SENAI CIMATEC in Salvador, Brazil. Results revealed a technological gap, with few patents combining these elements, indicating opportunities for research and development of new products. The proposal aligns with global trends in sustainable reuse of byproducts, offering a beverage with attractive organoleptic characteristics and health benefits.

Keywords: Byproduct. Functional Beverage. Fermentation.

Cocoa honey is a mucilaginous, opaque yellow liquid with a bittersweet taste, extracted from the pulp surrounding cocoa beans by cold pressing before fermentation begins. Named for its viscous and sweet characteristics reminiscent of bee honey, cocoa honey is not derived from beekeeping [1]. Considered a byproduct of the chocolate industry, producers and researchers have been seeking alternatives to valorize cocoa honey due to its physicochemical properties that resemble the fruit's original pulp [2]. Typical uses include alcoholic beverages, syrups, jams, and liqueurs, with promising potential in the pharmaceutical and cosmetic industries [3].

Water kefir grains have historically been used as starter cultures in fermentation processes, adapting to various media [4]. These gelatinous, irregularly shaped grains contain lactic acid bacteria, yeasts, and acetic acid bacteria [5]. Fermented products made with kefir grains may exhibit functional effects due to the presence of probiotic microorganisms, which help balance the gut microbiota and are associated with significant health improvements [6].

Given this context, the objective of this study was to map the patent landscape related to the production of functional fermented beverages using cocoa honey and kefir grains.

Materials and Methods

The research used the Derwent Innovation Index (DII) patent database, accessed through a license granted to SENAI CIMATEC University in Salvador, Bahia, Brazil. The search employed the keywords "cocoa honey", "kefir grain", "fermentation", and "probiotic beverage", along with the boolean operators "AND" and "OR" to refine or broaden the search results. The search was conducted between February and March 2025 to identify technologies involving the production of fermented beverages from cocoa honey using kefir grains. Graphs for analysis were generated from DII data to identify countries of origin and publication dates of patents.

Results and Discussion

Patent prospecting results (Table 1) show that although cocoa honey and kefir are individually used in beverage production, their specific combination remains underexplored. The search

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using all terms ("Honey Cocoa AND Kefir grain AND Fermentation AND Probiotic beverage") yielded zero patent records, indicating an untapped niche with high innovation potential.

Other search strategies yielded varying levels of relevance. For instance, "Honey ADJ Cocoa AND probiotic OR beverage OR kefir" returned a significant number of entries (n=187,053), reflecting the broader use of individual or less-specific term combinations. However, more targeted searches, such as "Cocoa AND Kefir" (66 patents) and "Cocoa AND Kefir AND beverage" (22 patents), revealed a smaller number of patents, reinforcing that this specific application is still in its early stages globally. Therefore, the "Cocoa AND Kefir" strategy was selected for constructing analytical graphs to explore the technological landscape and trends.

Figure 1A shows that the United States (10 patents), China (9 patents), and the European Patent Office (6 patents) lead in patent filings, indicating a more established development environment for fermented functional beverages using cocoa honey. Brazil, with only one patent, is in an early stage of this field. Figure 1B reveals that the first patent in this domain was filed in 1973. However, interest in this technology has surged more recently, with publication peaks in 2016 (9 patents), 2021 (8 patents), and 2024 (7 patents). This trend reflects growing investment and interest in technologies for developing functional beverages using fermentable substrates, such as cocoa and honey.

Conclusion

Patent analysis highlights the innovative potential of using water kefir grains to ferment cocoa honey in the development of functional beverages. The results demonstrate the technological and commercial feasibility of this approach, aligning with global trends in sustainability, the reuse of agro-industrial byproducts, and the demand for functional foods.

This strategy not only promotes more efficient use of natural resources but also adds value to regional ingredients, enabling the creation of products with unique sensory attributes and health benefits. The study highlights the significance of innovation in the food production chain, opening up new opportunities for developing sustainable, functional products in both national and international markets.

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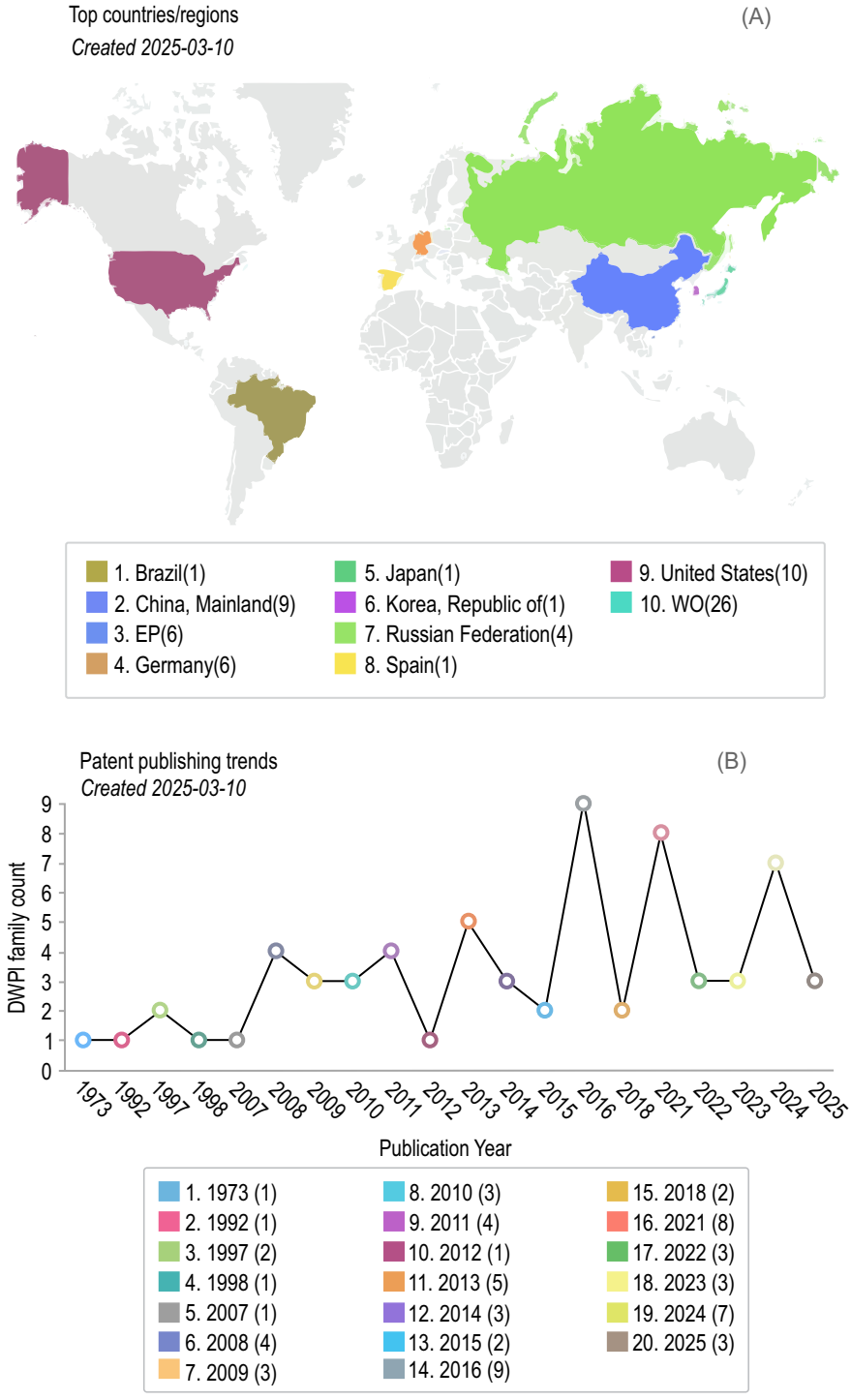
Table 1. Patent search strategies and the number of records.

Search Strategy	Number of Patents
Honey Cocoa AND Kefir grain AND Fermentation AND Probiotic beverage	0
Honey ADJ Cocoa AND Beverage	4
Honey ADJ Cocoa AND Kefir	0
Honey ADJ Cocoa AND Probiotic OR Beverage OR Kefir	187,053
Honey ADJ Cocoa OR Probiotic AND Beverage	2,248
Cocoa AND Kefir	66
Cocoa AND Kefir AND Beverage	22

Figure 1. Countries and patent publishing dates.



Source: Derwent [x].

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Energy Recovery from Municipal Solid Waste (Waste to Energy) in Salvador and Feira de Santana, Bahia, Brazil

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The generation of municipal solid waste (MSW) is closely related to the number of inhabitants and their income levels. Population growth increases MSW generation, causing economic, social, and environmental problems that the energy recovery of this biomass could mitigate. This solution is not only energetically viable but also economically attractive and environmentally beneficial, as it contributes to reducing the generation of greenhouse gases (GHGs) in landfills, thereby extending the useful life of these facilities. This work sought to identify in the literature the necessary characteristics for energy recovery from MSW and the properties of such waste in the metropolitan regions of Salvador and Feira de Santana, Bahia. Based on this analysis, new studies will be developed to investigate the composition, calorific value, and moisture content of the waste, as well as to assess the feasibility of implementation in other regions of the state. The method used included a literature review and consultation of relevant institutions in the field.

Keywords: Municipal Solid Waste. Biogas. Energy Recovery. Bahia.

Brazil generated more than 80 million tons of municipal solid waste (MSW) in 2023 [1], with various factors, including economic, social, geographic, educational, cultural, technological, and legal considerations, influencing the quantity and composition of waste [2]. Moreover, 42% of the total waste is disposed of in inappropriate sites, such as landfills and dumps [3].

The use of MSW for energy recovery, also known as waste-to-energy, involves technologies to treat waste to recover energy in the form of heat, electricity, or alternative fuels such as biogas [4]. This concept not only includes energy recovery but also helps reduce GHG emissions and decreases the volume of waste in landfills, thereby reducing final disposal, despite its high implementation cost [5].

This paper is part of a broader study on the current landscape of energy generation from MSW, focusing on the metropolitan areas of Bahia's two largest cities, Salvador and Feira de Santana. The objective is to survey the available biomass in these areas and the feasibility of energy recovery.

Materials and Methods

An extensive literature review was conducted using scientific databases such as Scielo®, Scopus®, and ScienceDirect®, as well as consultation of relevant legislation such as the National Solid Waste Policy (PNRS), the Solid Waste Outlook, and guidelines from the Energy Research Company (EPE), to understand better the regulatory and economic scenario related to energy recovery from municipal solid waste in Brazil.

Theoretical Framework

In effective waste management, it is essential to understand the quantity and type of discarded material, as this makes it possible to define more appropriate municipal policies for the waste of different regions, as well as to estimate the energy that can be generated from energy recovery, the

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amount of recyclable material, and the reduction in landfill mass [6].

Table 1 presents the main characteristics of the Municipal Solid Waste.

MSW IN FSA AND SSA

Table 2 shows the MSW data from Salvador and Feira de Santana, and Table 3 presents the generation of MSW.

Conclusion

Based on the information obtained, the conversion of municipal solid waste into energy can be a viable alternative in Feira de Santana and Salvador. Both cities show a high percentage of MSW generation compared to the state of Bahia, making this practice beneficial for reducing environmental impacts and optimizing the use of a material that would otherwise be discarded. However, this depends

Table 1. MSW characteristics.

Characteristics of Municipal Solid Waste (MSW)		
Gravimetric Composition	**Calorific Value**	**Moisure Content**
Percentage by mass of each component compared to the total mass [7], which is essential for evaluating the energy recovery potential of solid waste [8].	Related to the country or region's climatic characteristics, influenced by water presence and population income, which affect the carbon content in the solid portion of MSW [9].	It depends directly on the initial composition of the material, local climate, landfill operation processes, and the biological decomposition rate [10].

Source: Adapted from MMA, MCID, Sarker et al. 2024, Soares. 2011.

Table 2. MSW data.

MSW Material Composition	Gravimetric Composition (%)	
	Salvador	Feira de Santana
Organic	42	49
Plastic	22	19
Paper	8	20
Wood	0.3	4
Textile	5	3
Rubber	0.2	2
Glass/Ceramic	2	2
Metal	3	1
Other materials	17	-

Source: Adapted from Junqueira and colleagues, 2021 [11]; PMGIRS, 2016 [12]; Urban Cleaning Basic Plan, 2012 [13].

Table 3. MSW generation.

Location	MSW Generation (tons)	
	2000	2010
Bahia	3,743,388	4,951,225
Salvador	740,030	1,286,990
Feira de Santana	140,000	186,515

Source: Panorama dos Resíduos Sólidos no Brasil, 2003 [14] and 2010 [15], Freitas and Filho, 2009 [16].

directly on factors such as gravimetric composition, calorific value, and moisture content of the MSW, as well as the development of technologies and implementation of effective public policies.

Therefore, to adopt this perspective, it is essential to expand the collection of experimental data and enhance research on the subject, thereby contributing to more sustainable management of municipal solid waste.

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Identification and Quantification of Nitrogen Flow in the Sanitation System of the Identity Territory of Southernmost Bahia

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Sewage is a rich source of nitrogen, phosphorus, and potassium, and its efficient management can reduce pollution, promote sustainable water use, and enable its use as fertilizer. Material Flow Analysis (MFA) has been effective in managing these nutrients within the sanitation system. This study conducted a Nitrogen Flow Analysis (NFA) in the Identity Territory of the Southernmost Region of Bahia (TI07) for 2022, revealing that 46.31% of the sewage is discharged directly into the hydrosphere, possibly contaminating water bodies. Therefore, technologies such as vermifilters and green septic systems are recommended for treating sewage and recycling nitrogen for use as fertilizer or in food production. The NFA also showed that waste management accumulated 23.49% of the system's total nitrogen, highlighting the potential for composting, fertilizer supply, and strengthening local family farming.
Keywords: MFA. Nutrients. Environmental Management.

The composition of sewage is primarily feces, urine, and gray water, with urine being the primary source of nutrients (80% of nitrogen, 55% of phosphorus, and 60% of potassium) [1,2]. This means that the sanitation system is a rich source of nutrients, whose efficient management can bring benefits across a broad spectrum of applications. The recovery of nutrients from feces and urine aligns with the concept of ecological sanitation, contributing to the rational use of water, reducing pollution of water resources, and also having potential use as fertilizer.

To manage the nutrients present in the sanitation system, such as nitrogen, Material Flow Analysis (MFA) provides a comprehensive and consistent set of information on all flows and stocks within the system. Therefore, it has proven to be a highly relevant tool for assisting in decision-making, especially in environmental management and the formulation of public policies aimed at the environment [3].

Considering the increasing amount of nitrogen fertilizer imports in recent years to meet the agricultural systems in Bahia, this study aimed to conduct a Nitrogen Flow Analysis (NFA) in the sanitation system of the Identity Territory of the Southernmost Region of Bahia (TI07) for the base year of 2022. The analysis focused on identifying and quantifying primary nitrogen inflows and outflows, evaluating key opportunities for nutrient recovery, and assessing the potential environmental impacts associated with their losses, according to the types of sanitation systems used by local households.

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Materials and Methods

Study Area

The scope of the problem encompasses 13 municipalities in TI07 – Southernmost Region of Bahia, as indicated in the State Solid Waste Plan of Bahia (PERS/BA), which includes the municipalities of Alcobaça, Caravelas, Ibirapuã, Itamaraju, Itanhém, Jucuruçu, Lajedão, Medeiros Neto, Mucuri, Nova Viçosa, Prado, Teixeira de Freitas, and Vereda. The TI07 region covers an approximate area of 18,538 km² and is notable for its production of coffee, sugarcane, papaya, and cow's milk. Caravelas, Itanhém, Jucuruçu, Lajedão, Prado, and Vereda are the municipalities where agriculture and livestock are the sectors that contribute the most to the local GDP.

Flows and Processes

The NFA of TI07 corresponds to the region's sanitation system, characterized by internal processes that involve the input of nitrogen through food consumption, as well as activities related to the management of human excreta and organic solid waste. The study system involves ten processes within the sanitation system, three stocks, and 24 flows. The detailed NFA is shown in Figure 1 in the Results and Discussion section.The execution of the NFA will be carried out using the free software SubSTance Flow ANalysis (STAN), which helps create a graphical representation of the mathematical model and, as the final product, presents the Sankey diagram, facilitating the visual understanding of the substance flows.

Data Collection

For the nitrogen input flow contained in the food consumed by the population of TI07, data from the 2017 Household Budget Survey (POF) were used, supplemented with information on food consumption outside the home [4]. The nitrogen content of these foods was calculated based on the Brazilian Food Composition Table (TBCA 7.2). Sanitation data was obtained from the 2022 Census via the IBGE Automatic Recovery System (SIDRA), and information on Sewage Treatment Plants (STE) came from the 2020 Sewage Atlas (ANA). As for solid waste, the data was extracted from PERS/BA, with primary data collection carried out in the municipalities of Ibirapuã, Jucuruçu, Lajedão, and Vereda between 2020 and 2021, and secondary data from PAC 2 diagnostics, from 2018 and 2019, provided by the Urban Development Company of the State of Bahia (CONDER).

Nitrogen Content of Flows

The conversion of the protein content of foods from the POF into the amount of nitrogen was calculated by dividing the percentage of crude protein in the food by the nitrogen-to-protein conversion factor from Jones. For calculating the nitrogen content in solid waste flows, a percentage of 2.6% of the organic matter in the municipal solid waste (MSW) was adopted [5]. For determining the nitrogen content in feces and urine flows, values of 1.8 g N/ N/person/day and 11 g N/ N/ person/day were used, respectively [6].

Uncertainty Assessment

The uncertainty assessment was based on a method involving sensitivity analysis of the data and the enhancement of established techniques, such as the pedigree matrix and the HS method, for characterizing uncertainty in Material Flow Analysis (MFA) [7].

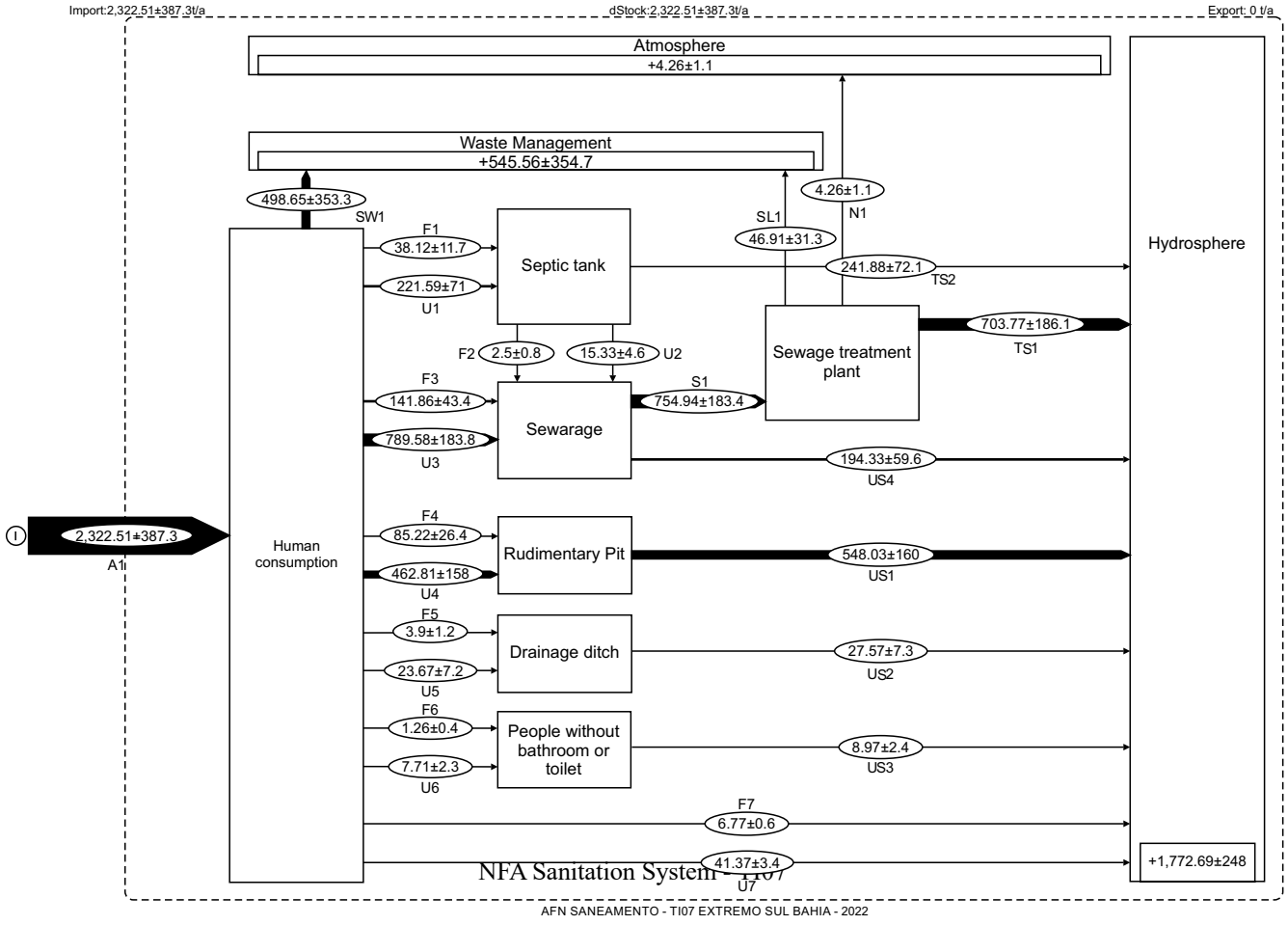
Results and Discussion

The nitrogen balance for the sanitation system of TI07 in the base year 2022 is depicted in Figure 1. This balance reveals that the total nitrogen content in the food consumed by the TI07 population amounted to 2322.51 ± 387.3 t·year^{-1}.

Out of this total, 498.65 ± 353.7 t·year^{-1} was lost as organic waste, which, under the current waste management practices in the region, is disposed of in three active landfills or open dumps. The nitrogen output from the Human Consumption process that was directed into the sanitation system corresponds to 78.53% of the remaining amount.

The flows of feces and urine were distributed according to the categories of excreta management technology adopted by each household, as per the IBGE. The types of sanitation responsible for the largest nitrogen flows into the hydrosphere are general sewerage (53.54%); rudimentary pits (30.92%); and septic tanks not connected to the sewage network (13.64%). The nitrogen fractions resulting from excreta disposed of in drainage ditches (1.56%) or directly into the hydrosphere (2.72%) are significantly smaller. The percentage of nitrogen from sewage generated by the population that reported not having a bathroom is

Figure 1. Nitrogen balance for TI07, base year 2022 - Sanitation System.



the lowest of all, corresponding to 0.51% of the total nitrogen flow discharged into the hydrosphere. Nitrogen from feces and urine flows directed to the general sewerage system is partially treated at the region's four active sewage treatment plants (STPs), located in Caravelas, Itamaraju, Mucuri, Nova Viçosa, and Teixeira de Freitas (754.94 ± 183.4 t·year-1 of N). In contrast, the remaining portion (194.33 ± 59.6 t·year-1) is discharged untreated into water bodies. The nitrogen output from the STPs follows three potential pathways: discharge into the hydrosphere after effluent treatment (703.77 ± 186.1 t·year-1 of N); final disposal of sewage sludge in waste management areas (46.91 ± 31.3 t·year-1 of N); and atmospheric emissions of N2O (4.26 ± 1.1 t·year-1 of N).

When it comes to the discharge of treated sewage into water bodies, only the municipalities of Caravelas, Itamaraju, Mucuri, Nova Viçosa, and Teixeira de Freitas treat part of their sewage through STPs, accounting for 39.70% of the nitrogen released into the hydrosphere. The Itamaraju STP channels its effluents into the Jucuruçu River, the Mucuri STP into the Mucuri River, and the Teixeira de Freitas STP into the Itanhém River. In Caravelas, effluents are directed to the Caravelas River, while in Nova Viçosa, they are sent to a tributary of the Peruípe River. The nitrogen from sewage treated by these STPs could potentially be used in irrigated agriculture, as studies in Brazil have demonstrated the feasibility and effectiveness of such applications

[8]. However, it is essential to evaluate the current conditions of these STPs before implementing any irrigation systems.

A persistent issue in municipalities is the overflow of sewage onto the streets, leading to problems such as foul odors and the proliferation of disease-carrying vectors. Furthermore, numerous points in the region discharge untreated sewage. A recent study conducted at 12 locations within the Water Planning and Management Region (RPGA), which encompasses the TI07 municipalities, found that although the Water Quality Index (IQA) is generally considered good, the Itanhém, Jucuruçu, and Peruípe river basins showed elevated levels of ammonia at some monitoring sites, according to the standards set by CONAMA Resolution 357/2005 [9]. These high ammonia levels are directly associated with sewage discharges and land use practices in the basins [10]. Runoff from fertilizers and soil leaching are among the primary sources of these pollutants. It is crucial to note that nitrogen in the form of free ammonia is highly toxic to fish [8].

To address the issue of raw sewage being discharged directly into the hydrosphere, alternative solutions to traditional septic or rudimentary pits can be considered, such as green cesspools and vermifilters. The green cesspool is a buried and waterproofed basin with filtering layers covered by soil, where plants like banana trees, taro, or lily of the valley are cultivated [11]. Vermifilters, on the other hand, function as aerobic filters that do not produce sludge and require no energy, yet they offer performance comparable to conventional treatment systems [12,13]. The humus generated by the vermifilter can serve as a nutrient source for fertilization, though it is not advisable for use in vegetable gardens. However, the food and leaves produced in green cesspools remain uncontaminated and safe for human consumption [11].

It is worth noting that a portion of the TI07 population reported not having access to a bathroom. A practical solution to this issue could be the implementation of dry toilets, which are a low-cost and easy-to-install alternative [14]. In these toilets, urine and feces are separated by a specialized toilet bowl. The feces are directed to composting for use as fertilizer. At the same time, the urine is stored for approximately a month before being utilized as a source of nitrogen and potassium for perennial, fruit, or annual crops [15]. Regarding nitrogen flows beyond excreta, food waste also accounts for a significant portion of the system's nutrients, representing 21.47%.Domestic composting of organic waste can significantly reduce the volume of urban solid waste collected, leading to cost savings, reduced material usage, and lower energy consumption for infrastructure, while also conserving landfill space and extending the operational lifespan of landfills [16,17]. The compost produced is an organic material rich in micronutrients, which can be used as fertilizer, supporting local family farming or recycling nutrients back into the soil [17].

As for the nitrogen from sewage sludge generated by the STPs, its contribution corresponds to 2.02% of the total nitrogen in the system, being directed to the regional waste management system. The chemical composition of sludge varies depending on the type of effluent, the treatment system, and seasonal factors. However, improper disposal in landfills or open dumps can lead to contamination problems and the proliferation of disease vectors [18]. After treatment and stabilization, the sludge becomes biosolid —a material rich in macronutrients (nitrogen and phosphorus), micronutrients (zinc, copper, iron, manganese, and molybdenum) [19], and organic matter, with potential applications in agriculture or the recovery of degraded areas. Like organic waste, sludge can be treated and transformed into biosolids through composting.

By combining the nitrogen flows from sewage sludge and organic waste, a nitrogen stock is formed in the waste management process. Considering an average loss of 25% of the nutrient after composting [20] and the recommendation of organic nitrogen fertilization (NOrganic = 60 kg·ha-1) for papaya cultivation [21], this stock has the potential to produce enough fertilizer to cover approximately 7,000 hectares.

Conclusion

The nitrogen flow analysis provided an overview of the nitrogen pathways in the TI07 sanitation system. The Sankey diagram presented in Figure 1 provides a visual understanding of how the system is organized in terms of flows, processes, and stocks, as well as the magnitude of the nitrogen quantities associated with each.

Through the Nitrogen Flow Analysis (NFA), it was found that a significant amount of sewage from TI07, approximately 46.31%, is being directly discharged into the hydrosphere, potentially contaminating water bodies. This situation is supported by studies indicating alarming levels of ammonia, highlighting the urgent need for interventions. The adoption of alternative technologies for decentralized sewage treatment, such as vermifilters and green pits, is a viable solution for utilizing nitrogen, either through the production of fertilizer or local food consumption.

It was also observed that the combination of nitrogen flows from food waste and sewage sludge resulted in an accumulation of 23.49% of the total nitrogen in the sanitation system. This indicates a significant potential for waste recycling, which could strengthen the still-limited local family farming.

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Development of an Artifact to Enhance the Resilience of Low-Carbon Decentralized Energy Solutions in Remote Regions

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This study aimed to assess the development of an artifact that supports the resilience of decentralized low-carbon energy generation solutions. We applied the Design Science Research (DSR) method to guide the construction of the artifact, considering the resilience of decentralized low-carbon energy generation solutions, local vulnerabilities, and social technologies. In this context, this research sought to characterize the stages of artifact construction and align them with Sustainable Development Goal (SDG) 7, which aims to ensure access to clean and affordable energy.
Keywords: Sustainability. Low-Carbon Energy. DSR. Resilience.

The energy transition has emerged to promote changes in the energy matrix by increasing the participation of renewable sources such as solar and wind [1]. One of the challenges of this movement involves expanding electricity supply infrastructure, as access to electricity is a parameter for social inclusion, especially considering that 785 million people still lack access to electricity [2,3].

Moreover, in many regions, electricity supply is precarious, especially in remote areas [4]. To ensure a continuous energy supply in these locations, it has been identified that generation systems must be resilient. According to the Intergovernmental Panel on Climate Change (IPCC), resilience corresponds to a system's capacity to meet the principles of prevention, adaptation, resistance, and recovery [5,6].

In areas where transmission networks are inefficient or nonexistent, decentralized solutions such as off-grid systems and community microgrids have emerged as viable alternatives to ensure a reliable electricity supply [7]. Redundancy in supply can also be achieved through energy storage systems that complement generation systems [8]. Despite official recognition of the potential of these decentralized systems, there is still no consensus on the best strategy to adopt [3,9].

Because generation from renewable sources occurs intermittently due to their nature, advances in energy storage technologies are necessary, primarily to address this intermittency [10,11].

In addition to ensuring supply continuity, energy storage contributes to grid stability, particularly in areas with limited infrastructure or vulnerable to extreme weather events, thus enhancing energy resilience [10,11].

The primary objective of this research was to evaluate the development of an artifact that enhances the resilience of decentralized, low-carbon energy generation solutions, considering local vulnerabilities and social technologies.

Material and Methods

The research aimed to develop an artifact that supports the resilience of decentralized, low-carbon energy generation solutions in remote regions with limited electrical infrastructure. To achieve this objective, the Design Science Research (DSR) method was applied, which guided the construction of the artifact considering the specificities of local energy chains and the intermittency of renewable sources. DSR was chosen because it is suitable for complex situations, such as this study, which involves multiple factors, including technical, social, and economic issues

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[12]. Figure 1 illustrates a schematic draft of the methodological steps for artifact construction.

Results and Discussion

The research focused on evaluating the development of an artifact to support the resilience of decentralized low-carbon energy generation solutions. The choice of this topic was motivated by its potential to contribute to the energy transition and promote social inclusion. The DSR method was chosen to guide artifact development through a sequence of steps that consider the contextual specificities.

DSR proved appropriate due to its structured approach and focus on developing practical solutions, enabling the definition of a path for artifact construction that aligns with the specific challenges of remote regions and renewable energy sources. The method enabled the integration of technical and social aspects of the solutions, facilitating the adaptation of the artifact to local communities.

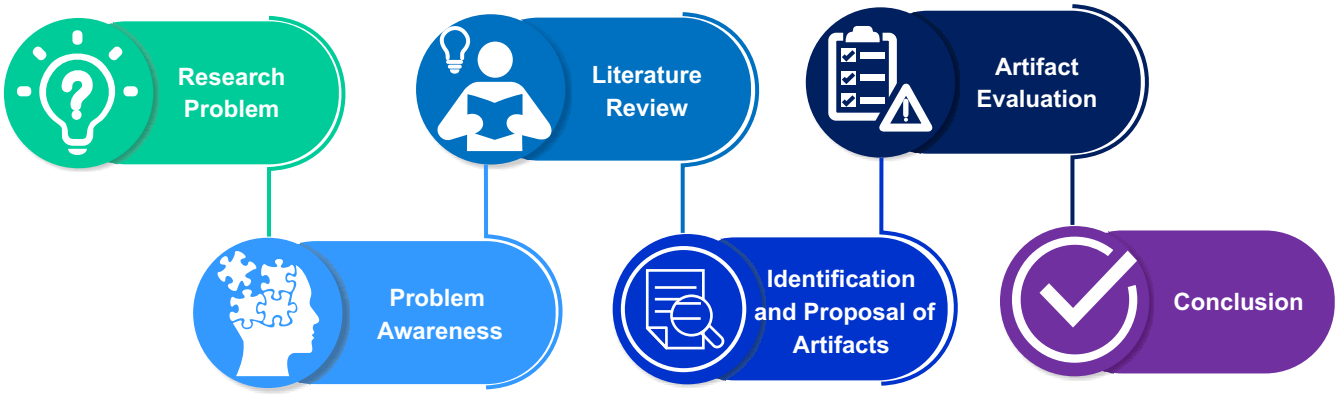
As part of the development process, a theoretical foundation is being established for the construction of an analysis platform that will support decision-making in scenarios with conflicting objectives, such as economic issues and specific topographic conditions at each location. This foundation encompasses the analysis of existing models and an examination of best practices in decentralized energy systems and renewable energy sources. The platform will be designed to suggest options and arrangements of energy generation systems tailored to regional characteristics. Thus, it will enable a dynamic evaluation of technological solutions, considering the varying conditions of each context.

However, the research has some limitations. The focus on remote areas and the simplification of certain elements, such as a detailed analysis of the intermittency of renewable energy sources and energy storage in these locations, were not addressed in depth. Additionally, the study defined the microgeneration threshold by Brazilian legislation. However, with minimal adjustments, the proposed method could be applied to other markets, provided there is knowledge of local regulations and topographical data.

Conclusion

This study developed a method to guide the construction of an artifact that supports the resilience of decentralized, low-carbon energy generation solutions. The proposed method contributes to the energy transition, social inclusion, and the creation of additional environmental and economic benefits by providing a solid foundation for building more adaptable and resilient solutions in remote regions. Although the artifact is still under development, the results obtained so far indicate that its

Figure 1. DSR methodological framework.



Source: Adapted from Dresch and colleagues and Ferreira and colleagues [13,14].

construction should consider critical factors such as the technical feasibility of the solutions, the specific characteristics of the regions where they will be implemented, and the ability to adapt to climate change. Future research should expand the analysis to other geographic areas, considering differences in local legislation and conditions, and deepen the assessment of the impact of renewable source intermittency and energy storage. Collaboration with local communities is also essential, as it will enable a deeper understanding of the specific needs and challenges of each region, facilitating the customization of solutions.

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The Importance of Validating New *in vitro* Medical Devices

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Currently, technological innovations are increasingly gaining ground in all fields, and especially in medicine, the validation process is one of the essential requirements for the approval of new medical devices (MDs) and *in vitro* diagnostic devices (IVDs). For a new device to be introduced into the market, it must meet specific requirements, and it is in this context that validation emerges. All IVDs and MDs must be approved by the National Health Surveillance Agency (ANVISA) or the relevant health authority of the country to ensure that the device is safe and effective for the population, and that its use will cause minimal harm compared to the benefits it can bring. In this context of Health Technology Assessment (HTA), this study aims to highlight, through a literature review, the importance of the validation process for IVDs and its impact on the market and the healthcare network.
Keywords: Legal Validation. Medical Devices. *In vitro* Diagnostic Devices. HTA.

An *in vitro* diagnostic device (IVD) is an instrument used outside the human body capable of analyzing human body samples, such as blood, urine, and other biological materials. They are used to store biological materials, determine diseases, or assess the condition of an organism. Therefore, they are handy for preliminary diagnoses and disease prevention [1].

New health technologies, including IVDs, must follow criteria established by ANVISA and several Normative Instructions (IN), and Collegiate Board Resolutions (RDC). Each device has different criteria depending on its functionality, the material it works with, and its operating principle. Given this wide range of possibilities, the validation process is not trivial [2].

The validation process of a new device involves testing reliability, precision, consistency, and whether it meets the requirements established by the applicable standards. One of the fundamental stages in the development of an *in vitro* diagnostic device is preclinical validation, which involves a series of laboratory tests to verify performance and safety using animal models or chemical formulations.

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In biomedical equipment innovation projects, the regulation and validation stage of new devices is noteworthy. Although these innovations require significant investment to develop and implement [3], and despite the evolution of biomedical devices through the emergence of new equipment, techniques, and algorithms aimed at improving health and well-being, various strategic management issues persist within institutions [4]. The validation process for new equipment is a frequently encountered issue and impacts the approval of new technologies and market adoption, thus deserving attention and study. In this context, this study aims to highlight the importance of validating new *in vitro* diagnostic devices, considering the significant impact that such validation has on the incorporation of new health technologies into the market. To achieve this goal, perspectives from several authors on the topic will be discussed, aiming to clarify the benefits of a structured validation method in innovation projects.

Materials and Methods

A literature review was conducted, based on exploratory research and bibliographic methods, from October 2024 to February 2025. Articles were found in Google Scholar, MDPI, and Scielo databases. They were filtered based on the year of publication (from 2018 onward) and the following keywords: biomedical devices, *in vitro* devices,

validation, and regulatory frameworks. Articles that addressed biological *in vitro* processes rather than devices were excluded, as well as those focusing on IVD maintenance. After applying the inclusion and exclusion criteria, 7 articles were selected. Some were directly related to validation, while others were included for their conceptual contributions.

Table 1 below presents a systematization of the content extracted from each article for qualitative analysis on the importance of *in vitro* medical device validation.

Theoretical Framework

As theoretical knowledge does not always translate into practical results, strict control is necessary over which devices can be introduced into the market and public health networks. Regulatory agencies aim to protect patients and society from unsafe devices, striking a balance between protection and market needs. Their primary goal is to promote public health [4].

Moreover, ANVISA defines the criteria for risk classification of medical devices and health products

Table 1. Selected articles.

Author	Title	Year	Source	Summary
Murilo Contó and Luciene Bonan	Legal framework for the incorporation and access to medical devices in Brazil: structure, types of evaluation, and opportunities for progress	2020	Google Scholar	Provides relevant information on testing and classifications.
Tina Morrison and colleagues	Modeling and simulation in biomedical engineering: regulatory science and innovation for advancing public health	2023	Google Scholar	Contextualizes the field, highlighting the importance of regulation.
Rosa Mayelin Orcid and colleagues	Impact of regulations on innovation in the field of medical devices	2018	Sciel Reviews	Conflicting issues in the evaluation of biomaterials and devices, and stresses the need for a method.
Lei Wang and colleagues	Methods and Advances in the Design, Testing and Development of *in vitro* Diagnostic Instruments	2023	MDPI	Summarizes related technologies and key R&D aspects.
Pernille Fauskanger and colleagues	Quantification of Difference in Nonselectivity Between In Vitro Diagnostic Medical Devices	2025	Google Scholar	Proposes a metric for quantifying non-selectivity differences in IVD-MDs.
Joana Freitas	Organization and management of biomedical equipment maintenance focusing on patient risk	2018	Google Scholar	Summarizes biomedical equipment management issues.
José Silva	Biomedical Engineering Center of the Brazilian Air Force: support and development	2019	Google Scholar	Discusses challenges in biomedical device development.

through INs and RDCs. These are mandatory prerequisites for IVD registration. ANVISA works with laboratories and Product Certification Bodies (OCPs) accredited by INMETRO [1]. Access to health technology is often limited by the difficulty in validating and regulating new devices, due to the lack of specific policies and literature on regulation. Each device has specific legislation and requirements that must be carefully analyzed and fulfilled [1].

Validation is also essential in the module planning stage, as it is used to develop product prototypes. IVD projects integrate several fields of engineering and technology: mechanical, electronic, optical, inspection, computer science, and control [2].

MDs and IVDs must maintain high precision even under external factors, justifying the need for rigorous validation before market acceptance [5]. Selectivity is a critical parameter that defines the device's capacity to accurately measure the target analyte without interference from other substances [5].

A crucial point is that, despite advances in human physiology research, applying this knowledge presents challenges, resulting in a treatment device approval rate below 50%. Increasing this rate improves quality of life and supports healthcare systems. Model-based development gathers diverse data to reduce uncertainty and failure rates, providing insights that would otherwise be unattainable. Computational methods thus offer viable alternatives to ease validation [6]. Each device has unique features that make validation a complex process. Nonetheless, sufficient clinical evaluations must be conducted to ensure safety [7].

Conclusion

This study examines the complexity and importance of the validation process for *in vitro* medical devices. It highlights the importance of validating new IVDs, as validation determines the acceptance of new proposals. Validation ensures the efficacy, reliability, and safety of treatments for patients and professionals. Structuring validation from the product's conception increases the likelihood of regulatory approval and optimizes development time. This research continues with a literature review on innovation in IVD equipment and regulatory frameworks for validation.

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A Review of Different Methods for Superhydrophobic Textiles and Fabrics Using Nanotechnology

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Recent developments in superhydrophobic textiles involve many technologies and fields of knowledge, such as textile engineering, surface treatments, and nanomaterials. Several methods can be employed to achieve the superhydrophobicity of fabrics, including dip coating, *in situ* growth, the sol-gel approach, and immersion. This review presents each method and investigates its peculiarities, correlating it with reagents and results. A brief conclusion is also presented with future perspectives. The P.R.I.S.M.A. (Preferred Reporting Items for Systematic Review and Meta-Analysis) protocol was followed, aiming to select the most relevant papers for the area and to evaluate the results accordingly. Selected keywords were superhydrophobic AND [textile OR fabric OR cloth] AND [coating OR immersion OR gel]. Furthermore, only articles published between 2019 and 2024, in the English language, were selected.
Keywords: Systematic Review. Superhydrophobic Textile. Nanotechnology. Advanced Methods.

Superhydrophobic textiles are components with many applications in the engineering field [1]. Some examples include antivirus and antibacterial fabrics, oil-water separation, self-cleaning properties, and the facile removal of surface impurities. Applications of superhydrophobic textiles can range from outdoor clothing and sportswear (to keep athletes dry and comfortable), to medical textiles (for water-repellent bandages and surgical gowns), and even in everyday items like furniture upholstery and automotive interiors. They offer benefits such as self-cleaning properties, stain resistance, and improved durability in wet conditions [2].

These textiles are designed to exhibit extremely high contact angles with water, typically exceeding 150°. This means that when water comes into contact with the surface of a superhydrophobic textile, it forms spherical droplets that roll off easily, carrying dirt and debris away with them.

The superhydrophobic properties of these textiles are typically achieved through surface modification techniques, such as coating the fabric with hydrophobic substances or altering the surface structure at the nanoscale level. This modification creates a rough or textured surface that minimizes contact between the water droplets and the fabric, leading to the repelling effect [3].

In a recent review report, the most recent materials used for manufacturing hydrophobic textiles were presented in studies from the past five years. Many studies using natural fibers, such as cotton, have been the focus in the last decade.

However, some disadvantages of these types of fibers are known, such as the difficulty of incorporating nanomaterials to improve mechanical response. In this regard, synthetic fibers may be used. Several studies have reported on the use of PET polyester fabrics as the primary fiber for producing superhydrophobic textiles.

Some advantages include excellent mechanical response, high reliability, and commercially available textiles in various forms [4].

Among the nanofillers used for modifying those textiles, silane treatment and silica use are the most notable. Additionally, poly (dimethylsiloxane) is the most commonly used additive for increasing the contact angle of textiles, a crucial parameter for measuring hydrophobicity. As a conclusion to such a study [4], many techniques can be used for modifying textiles. Figure 1 highlights the most commonly employed methodologies for fabricating superhydrophobic textiles when

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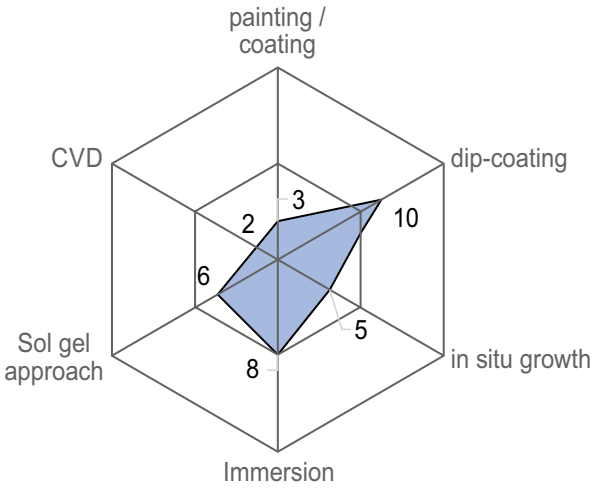
nanotechnology is utilized. Dip-coating is the most commonly employed method, as well as immersion, due to the ease and low cost of these processes.

Figure 1. Main methods applied, using nanotechnology for hydrophobic textiles.



Despite the excellent results for hydrophobicity from such studies, no correlations have been reported between the used methodologies and their influence on the superhydrophobicity of these textiles elsewhere. The primary objective of this study is to present the methodologies employed for improving the hydrophobicity of textiles and fabrics.

Materials and Methods

The research method, papers' organization, and results presentation of this research were based on previous work of the group [5–8]. Additionally, to support this systematic review, the online software "Parsifal" and its associated tools (parsif.al) were utilized. "Scopus" (scopus.com), "Web of Science" (webofknowledge.com), and "Multidisciplinary Digital Publishing Institute (MDPI)" (mdpi.com) were used as databases to search for articles. For that, "superhydrophobic AND [textile OR fabric OR cloth] AND [coating OR immersion OR gel]" were used as keywords. Review articles, book chapters, and conference papers were excluded, and only papers published in English within the last 5 years (2019-2024) were selected. Then, some articles were obtained, as shown in Figure 2. Subsequently, duplicated records were removed, leaving 1083 articles for selection of the relevant ones.

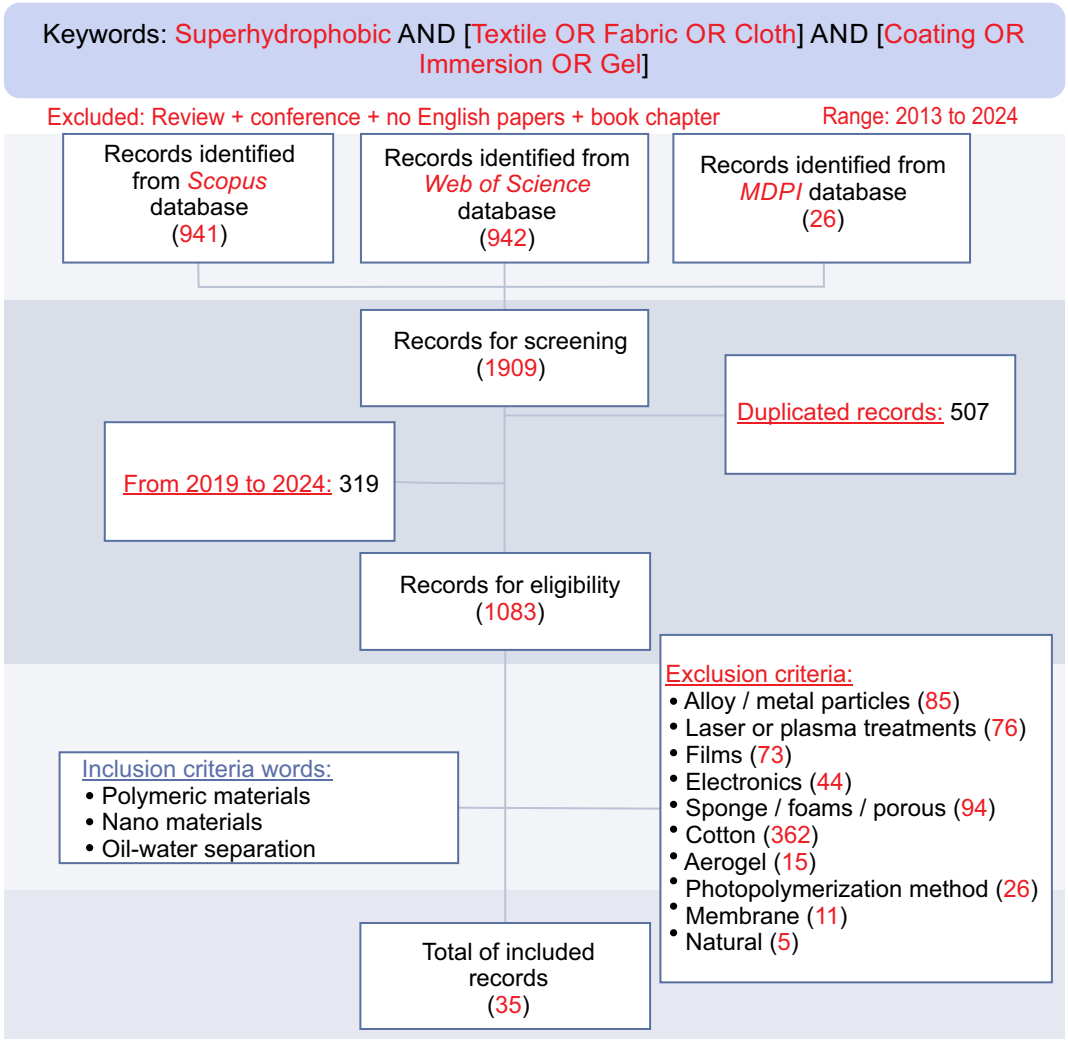
As exclusion criteria, no textile OR cloth OR fabric, development of coatings (or when only the coating was analyzed), laser/plasma surface treatments, metal/alloy particle surface treatment, when no nanomaterials were employed. Additionally, certain materials, including films, sponges/foams, aerogels, and membranes, were excluded. Textiles for electronics were excluded, as were those that used photo treatments. Additionally, inclusion criteria were selected when only polymeric textiles were used, when nanomaterials or nanotechnology were applied, and when the textile was developed explicitly for oil-water separation. At the end of the papers' screening, 35 articles were selected and fully read, and used for data acquisition and comparison of results.

Results and Discussion

Figure 3 shows the evolution of papers published in the last 5 years. A clear trend of increasing the number of publications is observed, primarily on the Web of Science platform. A trend of maintaining a constant publication rate over time is observed on the Scopus platform, as well as in MDPI, with only one paper published per year.

Figure 4A shows the main results from those 35 papers selected, using the inclusion and exclusion criteria. As can be seen, the coating process is the most highlighted, with 28 of the 35 papers selected. This result may be attributed to the ease, affordability, and high-quality finished parts produced when this method is employed [9]. In addition to the coating method, some studies also applied Ultraviolet light (UV), aiming to improve the surface's quality and adhesion between the coating and the textile surface [4]. Additionally,

Figure 2. Systematic review diagram, showing the screening steps for the paper's selection.



UV is used for curing the coating in some cases, aiming to speed up the curing process and also improve the process's productivity [4, 10].

Immersion is one of the most used methods. This is perhaps due to the ease of such a process even when a non-uniform distribution of the coat occurs [11]. Chemical Vapor Deposition (CVD), as well as condensation, have been explored as methods for producing superhydrophobic cloths, which are expected to have low commercial viability due to the high cost and difficulty of producing a soft surface [10]. Finally, the sol-gel approach yielded a relatively low number of publications, whereas dip-coating is a technique used to create coatings on various substrates. Dip-coating is a technique used to apply a coating to a material by immersing it in a solution or suspension of the coating substance. It is widely used in various industries, including manufacturing, electronics, and pharmaceuticals, for creating uniform coatings on objects. Briefly, dip-coating consists of six steps: preparation (cleaning) of the surface, coating of the solution, immersion, withdrawal, drying/curing, and inspection. Some advantages include uniform coating, cost-effectiveness, versatility, and scalability. Dip-coating is utilized in various applications, including electronics, manufacturing, pharmaceuticals, and textiles [12]. This step is crucial for enhancing the durability and stability of the superhydrophobic coating [13]. Post-treatment

steps may also involve chemical modification or functionalization of the surface to enhance its superhydrophobic properties. This could include using silanes or fluorinated compounds to lower surface energy and increase water repellency [14].

Figure 4B presents the most used nanomaterials and additives for the improvement of the superhydrophobicity of textiles and cloths. Silica and silane-based treatments were the most commonly used, which may be related to the non-polar nature of silanes and their weak interactions with water molecules. Similarly, Poly(dimethylsiloxane) (PDMS) was the second most commonly applied treatment. PDMS is known for its flexibility, low glass transition temperature (typically around -125°C), and high thermal stability. It is resistant to heat, water, oxidation, and various chemicals, with some of its advantages presented in Figure 5B.

Additionally, it offers easy processability and is biocompatible. PDMS has numerous applications in various fields, including mold making (due to its ability to reproduce intricate details and easy mold release properties), microfluidics (characterized

Figure 3. Evolution of papers' publication with time, during the last 5 years.

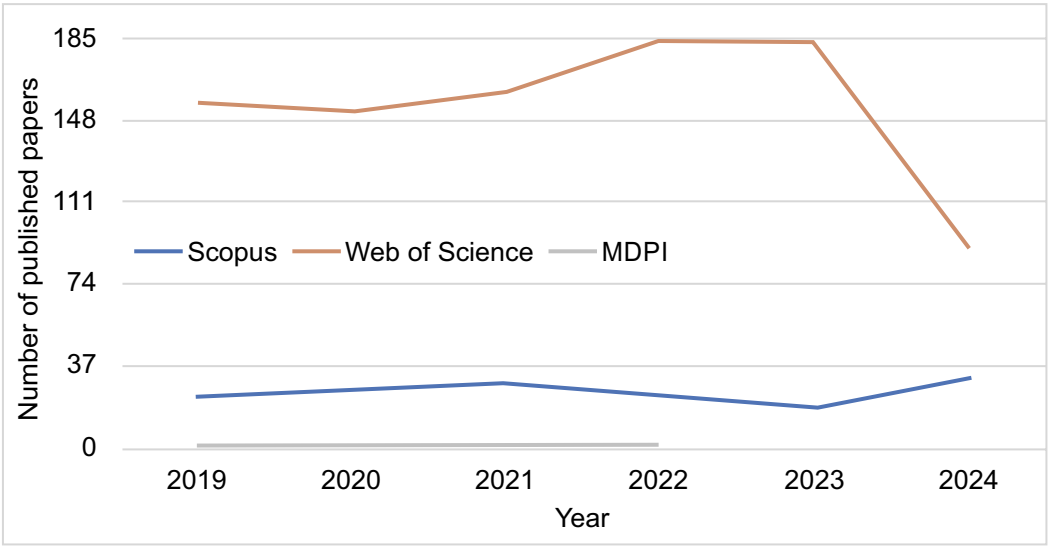


Figure 4. Type of modification process used for the fabrication of superhydrophobic textiles (A) and the type of nanomaterial and/or additive used for the superhydrophobic textile manufacturing (B).

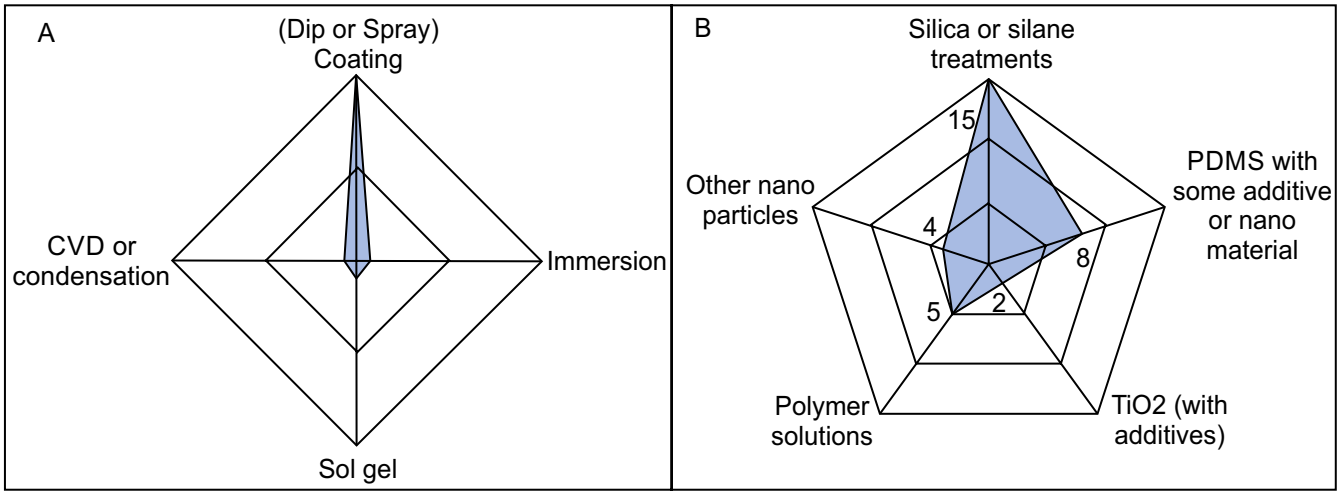
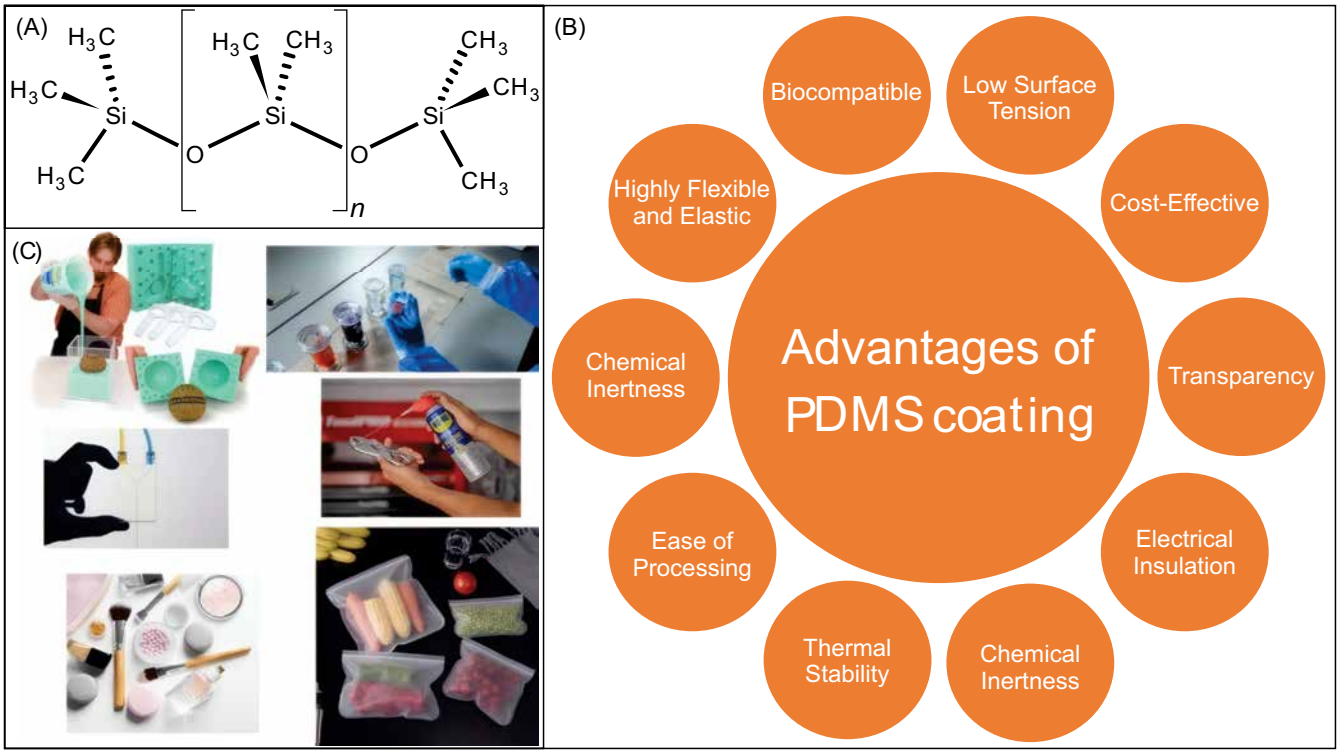


Figure 5. Molecule structure of PDMS (A), Advantages of PDMS coating (B), and Applications of PDMS in many fields (C).



by transparency, biocompatibility, and ease of fabrication), biomedical applications (such as catheters, prosthetics, and components in contact lenses), lubricants, cosmetics, and food packaging. Figure 5C presents some fields of application for PDMS and some advantages of the additive.

Finally, other nanomaterials have also been used to increase the hydrophobicity of textiles, including calcium nanoparticles, metal oxide nanoparticles, zinc oxide nanoparticles, and cellulose nanocrystals. Additionally, polymeric solutions were also identified, highlighting fluorinated polymers such as polytetrafluoroethylene and poly(perfluorohexyl ethyl acrylate-co-isocyanate ethyl methacrylate).

Conclusion

This paper showed the main results of a systematic review of manufacturing techniques used for superhydrophobic textiles and fabrics. The results showed that many papers have been published in the field over the last 5 years, utilizing nanotechnology, and it is expected that more papers will be published in the future. Additionally, the development of nanotechnology is also expected to utilize as-received textiles. The highlighted techniques include dip-coating and immersion, with some studies using CVD and the sol-gel approach. The main filler used was silica or silicate treatments, followed by PDMS treatments. PDMS is the leading modifier agent used for textiles when superhydrophobicity is required, offering numerous advantages and applications in various industries, including biomedicine, cosmetics, and packaging. Future research papers are expected to utilize nanotechnology, with a focus on silica, silicates, and innovative methodologies.

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Manuscript	Original	Review	Birief Comunication	Case Report	Editorial ; Letter to the Editor; Editor' s Corner	Innovative Medical Products	State-of-the-Art	Health Innovation Initiatives
Font Type	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial	Times or Arial
Number of Words – Title	120	90	95	85	70	60	120	90
Font Size/Space-Title	12; double space	12; double space	12; double space	12; double space	12; double space	12; double space	12; double space	12; double space
Font Size/Space-Abstracts/Key Words and Abbreviations	10; single space	10; single space	10; single space	10; single space	-	-	10; single space	10; single space
Number of Words – Abstracts/Key Words	300/5	300/5	200/5	250/5	-	-	300/5	300/5
Font Size/Space-Text	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space	12; Double space
Number of Words – Text	5,000 including spaces	5,500 including spaces	2,500 including spaces	1,000 including spaces	1,000 including spaces	550 including spaces	5,000 including spaces	5,500 including spaces
Number of Figures	8 (title font size 12, double space)	3 (title font size 12, double space)	2 (title font size 12, double space)	2 (title font size 12, double space)	-	2 (title font size 12, double space)	8 (title font size 12, double space)	8 (title font size 12, double space)
Number of Tables/Graphic	7 title font size 12, double space	2 title font size 12, double space	2(title font size 12, double space)	1(title font size 12, double space)	-	-	7 title font size 12, double space	4 title font size 12, double space
Number of Authors and Co-authors*	15	10	5	10	3	3	15	10
References	20 (font size 10,single space	30(font size 10,single space	15 (font size 10,single space)	10 (font size 10,single space)	10 (font size 10,single space	5(font size 10,single space	20 (font size 10,single space	20

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❑3. The entire manuscript (including tables and references) must be typed according to the guidelines instructions.

❑4. The order of appearance of material in all manuscripts should be as follows: title page, abstract, text, acknowledgements, references, tables, figures/graphics/diagrams with the respective legends.

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