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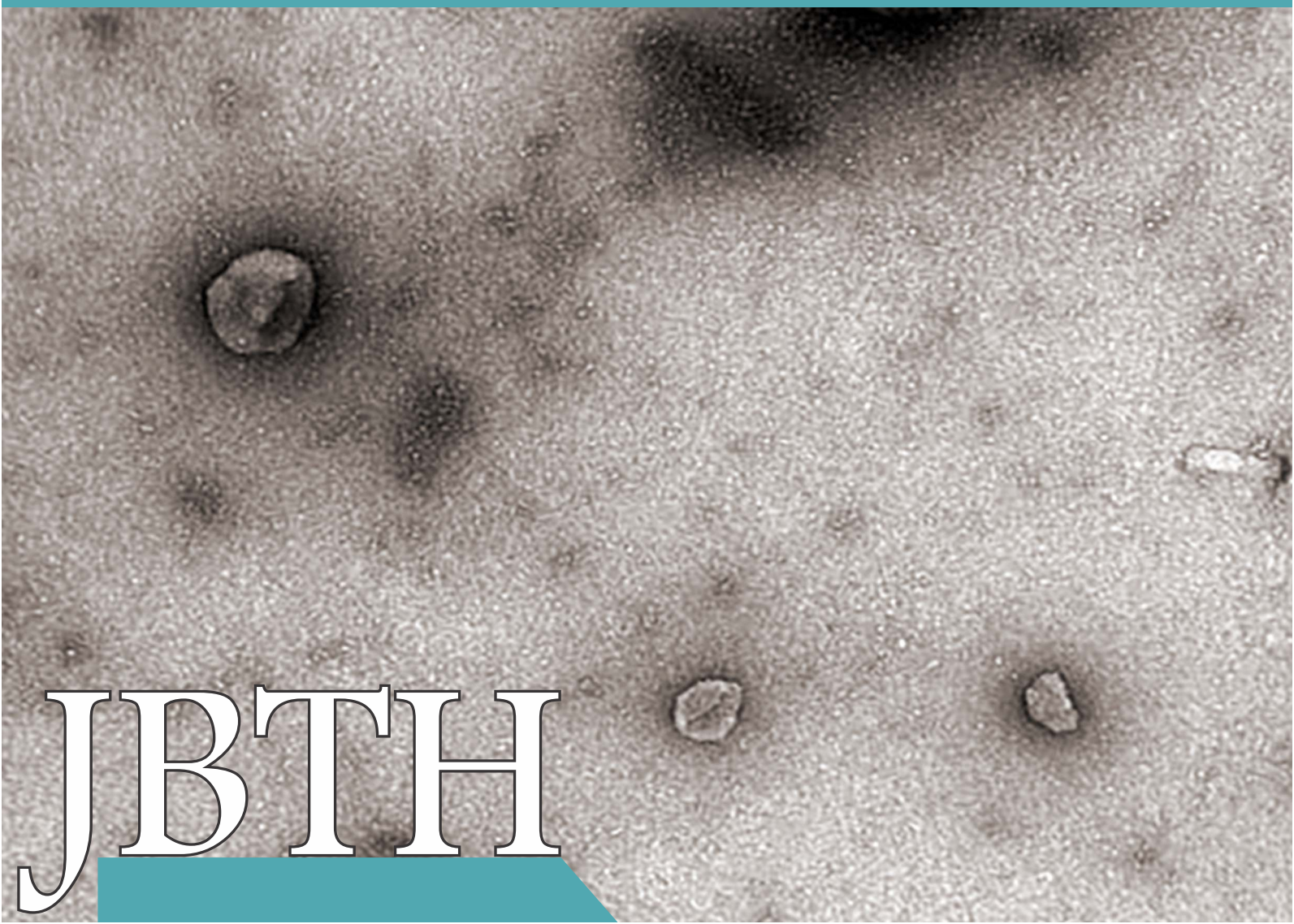

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SUMMARY

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The Journal of Bioengineering, Technologies and Health (JBTH) is an official publication of the SENAI CIMATEC (Serviço Nacional de Aprendizagem Industrial - Centro Integrado de Manufatura e Tecnologia). It is published quarterly (March - June - September - December) in English by SENAI CIMATEC – Avenida Orlando Gomes, 1845, Piatã, Zip Code: 41650-010, Salvador-Bahia-Brazil; phone: (55 71) 3879-5501. The editorial offices are at SENAI CIMATEC.

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COVER: Morphological characterization of extracellular vesicles obtained by electronic transmission microscopy. Figure 2 from the article Extracellular Vesicles Derived From Mesenchymal Stem Cells with Therapeutic Potential: Isolation and Characterization by Laura Maria Mota do Rio Bamar et al. J Bioeng Tech Health 2023;6(2):114.

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Extracellular Vesicles Derived From Mesenchymal Stem Cells with Therapeutic Potential: Isolation and Characterization

Laura Maria Mota do Rio Bamar[1*], Danielle Devequi Gomes Nunes[1], Milena Botelho Pereira Soares[1,2]
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Extracellular vesicles are a heterogeneous population of cell-derived vesicles that are spherical and bounded by phospholipids. In addition, vesicles derived from mesenchymal stem cells carry aspects of their parent cell, such as migration capacity, ability to repair damaged tissues, and immunomodulation capacity. These properties give the vesicles great therapeutic potential to treat relevant chronic diseases. Therefore, the present project aims to isolate and characterize extracellular vesicles from mesenchymal stem cells genetically modified to overexpress Flagellin, preliminarily assessing their potential for activating the immune system. Hence, we isolated extracellular vesicles and characterized them concerning particle size and polydispersion index, morphology, and induction of an essential cytokine for activating the immune system.
Keywords: Mesenchymal Stem Cells. Extracellular Vesicles. Microvesicles.

Introduction

Mesenchymal stem cells (MSCs) are adult stem cells found in different tissues, including bone marrow, fat, umbilical cord blood, and placenta. MSCs are capable of self-renewal, in addition to having immunomodulatory properties, reducing inflammation and helping to repair damaged tissues, and activating the immune system [1]. Studies aim to improve the therapeutic properties of MSCs through genetic modification in order to increase the production of specific proteins and factors, like cytokines that are capable of stimulating the immune system, overexpressing the flagellin protein (flag-s).

Flagellin, for instance, is a protein that makes up the bacterial flagellum and can activate the host's immune system, triggering an adaptive immune response [2]. MSCs secrete bioactive mediators, such as extracellular vesicles (EVs), which have immunosuppressive effects, anti-apoptotic, anti-inflammatory, angiogenic, and anti-fibroti [3]. EVs are small membranous structures secreted by many types of cells, including mesenchymal stem cells, cancer cells, immune cells, carriers of DNA, various types of RNA, proteins, lipids, and metabolites. There are two main types of EVs: exosomes and microvesicles. These reproduce the same characteristics and biological function as their mother cell. However, they have unique properties, like their small size, low immunogenicity, acting as transporters of molecules, with several therapeutic properties [4,5].

The present study aims to isolate and characterize extracellular vesicles from mesenchymal stem cells, genetically modified overexpressing the flagellin protein, to understand their potential for modulating the immune system response.

Materials and Methods

Cell Culture and Isolation of Extracellular Vesicles

The mesenchymal stem cells used in this study were previously genetically modified for

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overexpression of flag-s (EVs-flag-s) and are part of the cell bank available in our laboratory. Cells were cultured *in vitro* using DMEM High Glucose medium. After three days of supernatant collection, it was initially centrifuged twice in a benchtop centrifuge and finally ultracentrifuged at 100,000 x g for 1 hour and 20 minutes at 4°C (Ultracentrifuge Himac CP 80wx – Hitach). After ultracentrifugation, the supernatant was discarded, and the vesicle pellet was resuspended with 100 uL of phosphate-buffered saline solution (PBS), where, every 100 uL, the pellet was vortexed to provide maximum recovery of the vesicles, which were stored in a freezer at –80°C.

Characterization of Extracellular Vesicles

The vesicles were characterized for their protein content using the NanoDrop Lite (ThermoFisher). Vesicles were also characterized for size, polydispersion index (PDI), and concentration using the NanoSight NS300 and Zetasizer Nano ZS DLS (Malvern) equipment. Finally, the vesicles were observed by the electronic transmission microscope JEM-1230 JEOL through the partnership SENAI CIMATEC and Fiocruz Bahia.

Cytotoxic and Immunomodulatory Activity

The cytotoxic capacity of s-flag-EVs was evaluated against peritoneal macrophages using the MTT colorimetry technique. In addition, the evaluation of the potential immunomodulatory activity was performed by Sandwich ELISA to identify the induction of the cytokine IL-6 in peritoneal macrophages treated with EVs-flag-s.

Results and Discussion

EVs derived from MSCs genetically modified for overexpression of the flag-s protein (EVs-flag-s) had a concentration of 1.58 mg/mL and a size of 394 nm, with a polydispersion index (PDI) of 0.3 (Table 1). The PDI assesses a sample's heterogeneity based on sizes ranging from 0 to 1.0. Given these results and corroborating with data found in the literature, these EVs can be classified as microvesicles [6].

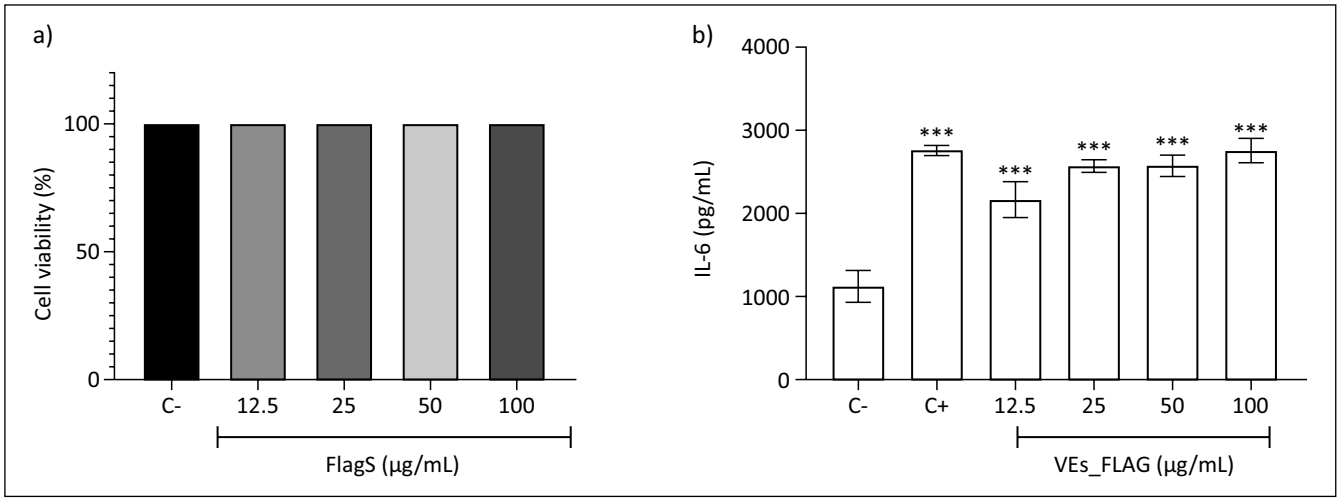
The cell viability of peritoneal macrophages was evaluated through treatment with EVs-flag-s to identify potential cytotoxic effects against these cells. Figure 1 shows the inhibiting cell growth compared to positive and negative controls, indicating that the vesicles could not cause cell changes. Furthermore, flag-s EVs induced IL-6 expression in peritoneal macrophages compared to negative (cell only) and positive (cell + LPS) controls. IL-6 is a pro-inflammatory cytokine produced by immune cells (such as macrophages), so its production indicates a vesicle-stimulated immune response.

We can observe the morphology of EVs-flag-s from transmission electron microscopy (TEM). Figure 2 presents the round shape of the vesicles, surrounded by a lipid membrane. We concluded that we could isolate microvesicles originating from MSCs regarding the physical and morphological characterization. Microvesicles are a class of extracellular vesicles formed from budding from the plasma membrane of cells. They have a diameter ranging from 100 nanometers to 1 micrometer and contain a mixture of lipids, proteins, and cellular material. Growing evidence also suggests that microvesicles may play an important role in cancer, autoimmune diseases, and cardiovascular disease [7].

Table 1. Protein concentration and extracellular vesicle size.

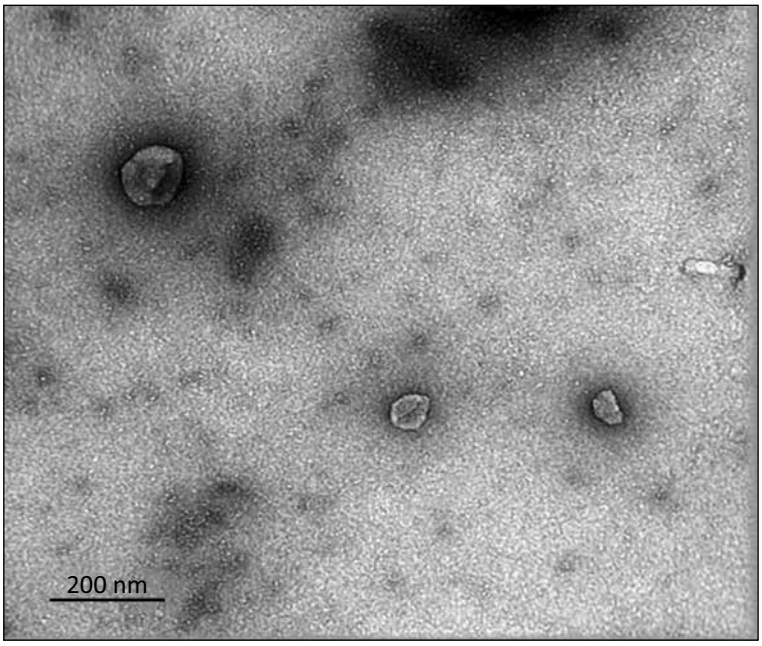
Nanodrop Protein concentration (mg/mL)	**Zetasizer** Vesicle size (d.nm)	**Zetasizer** Polydispersion Index (PDI)
1.58	394	0.3

Figure 1. Cytotoxic activity and assessment of pro-inflammatory cytokine expression.



a) Cell viability of peritoneal macrophages treated with s-flag-EVs; b) IL-6 expression in peritoneal macrophages treated with flag-s VEs.

Figure 2. Morphological characterization of extracellular vesicles obtained by electronic transmission microscopy.



Conclusion

We concluded that the methods could isolate and characterize the extracellular vesicles derived from mesenchymal stem cells. In addition, flag-s EVs produced an essential cytokine for activating the immune system. They did not show cytotoxic effects since there was no inhibition of cell growth or changes in the structure of the macrophage.

Although the vesicle has induced the production of IL-6, many immunological markers still need to be evaluated to confirm the real therapeutic and immunomodulatory potential of extracellular vesicles derived from mesenchymal stem cells. Thus, further research is necessary for evaluating other important cytokine profiles for the immune system and their application in models of chronic diseases, such as cancer.

Acknowledgments

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Cultivation of *Daphnia similis* Species in Standard Conditions

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***Daphnia* is an organism used for ecotoxicity tests to evaluate acute or chronic toxic effects of substances and effluents on an organism's population. *Daphnia* must be cultivated under standard conditions to reproduce themselves. This article highlight the importance of standard *Daphnia* cultivation since unfavorable conditions may impact or interrupt the cultivation progress. The method was based on the NBR12713 standard, which focused on compliance and validation of established cultivation guidelines. The results revealed the adverse effects and impact that *Daphnia* culture might have if they are not in the required conditions.**

Keywords: *Daphnia*. Ecotoxicity. Cultivation.

Introduction

The application of aquatic organisms for ecotoxicological assays has been increasing because some organisms are more sensitive and have a contact surface that allows an analysis of specific compounds, mainly chemicals [1,2]. So, *Daphnia* (*Cladocera* order) is used to analyze and monitor the quality of aquatic ecosystems. Furthermore, they are increasingly applied to ecotoxicological assays because they are susceptible when exposed to toxic compounds at low concentrations [3].

Daphnia reproduces by parthenogenesis, which results in a female population. From the 3rd to the 7th day, they mature for reproduction (depending on the species) [4]. It has an anatomy (Figure 1) that allows easy locomotion through the antennae and an abdominal region that allows food particles to be filtered and conducted to the mouth [5,6].

The two species most used for testing are *Daphinia magna* and *Daphinia similis*, originating from freshwater. They are small (0.5 to 5.0 nm), easier to handle, and favorable for cultivation in laboratory environments. The difference is their life cycle, in which *D. magna* has a longer life cycle than *D. similis* and is more sensitive [7-9]. However, in Brazil, the species most used for testing is *D. similis* since it is recommended for ecotoxicological evaluation in tropical and subtropical climatic countries [3].

These *Daphnia* species applied in ecotoxicological tests allow a study of acute effects (quick responses for organisms exposed to some compound) and chronic effects (responses with extended periods of exposure, which may lead to deleterious effects to organisms, such as immobility). The ecotoxicological tests occur through neonates obtained by reproducing young or adult *Daphnia* (7 to 21 days of age) [6].

This study aims to highlight the importance of standard cultivation of *Daphnia similis*, signaling unfavorable conditions that may impact or interrupt the progress of organism cultivation.

Materials and Methods

We based this study on ABNT NBR:12713 (Aquatic ecotoxicology — Acute toxicity — Test method with *Daphnia* spp.), which specifies a method for evaluating the acute toxicity of liquid samples and chemical substances soluble or dispersed in water for *Daphnia similis* and *Daphnia magna* [10]. Figure 2 presents the standard method flow.

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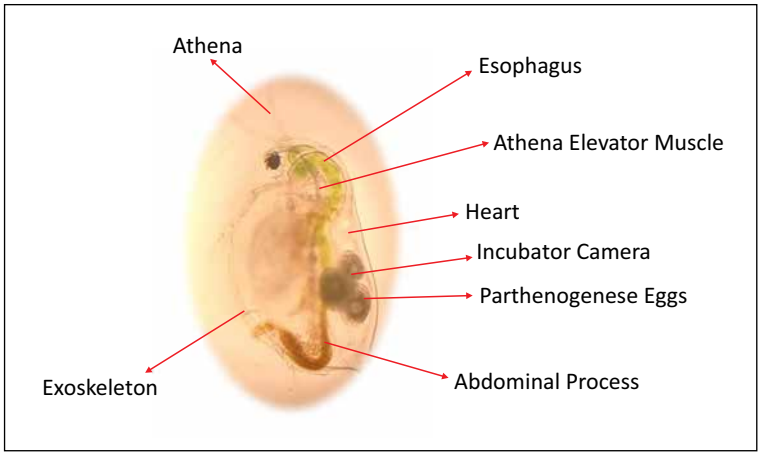
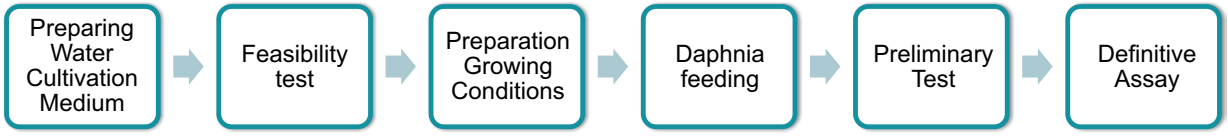
Figure 1. Simplified anatomy of *Daphnia similis*.



Source: Adapted from Oliveira TMN, Kleine T, Vaz C [6]. Photo taken under a microscope.

Figure 2. Applied method flow.



We focused on the tests to achieve standard *Daphnia* (*D. similis*) cultivation, donated by the Environmental Company of the State of São Paulo (ECSSP). The tests were carried out at the Laboratory of Applied Research in Chemistry (LIPAQ), which followed the cultivation method (Figure 3).

Results and Discussion

This article brings the results of cultivating *Daphnia similis* under unfavorable conditions, highlighting the importance of their standard cultivation. We need to adjust some parameters of the culture medium (pH, hardness, dissolved oxygen, temperature, and photoperiod of 12 h to 16 h of light) to maintain the increase and constancy of new cultures. Monitoring these variables in the culture is essential to guarantee that these indicators' alteration will not impact toxicity test performance.

For the maintenance and control of *Daphnia similis* cultures, they must be conditioned in suitable environments, such as temperature (18°C to 22°C) and humidity control at a photoperiod incubator (Figure 4), which simulates day and night (12h to 16h of light) by the ABNT NBR 12713 normative.

However, due to the absence of an incubator to maintain the cultures, since the organism was not cultivated in the laboratory where the present study was conducted, it was necessary to set up a structure to adapt the *Daphnia* to the required conditions. Figure 5 shows a comparative scenario of the conditioned cultures in the incubator of the ECSSP ecotoxicity laboratory and the cultures carried out in LARC.

This condition becomes unfavorable since the organisms are subject to temperature changes by the external environment, which can lead them to death or immobility.

For instance, factors such as temperature, photoperiod, overpopulation of organisms, and

Figure 3. Flowchart method.

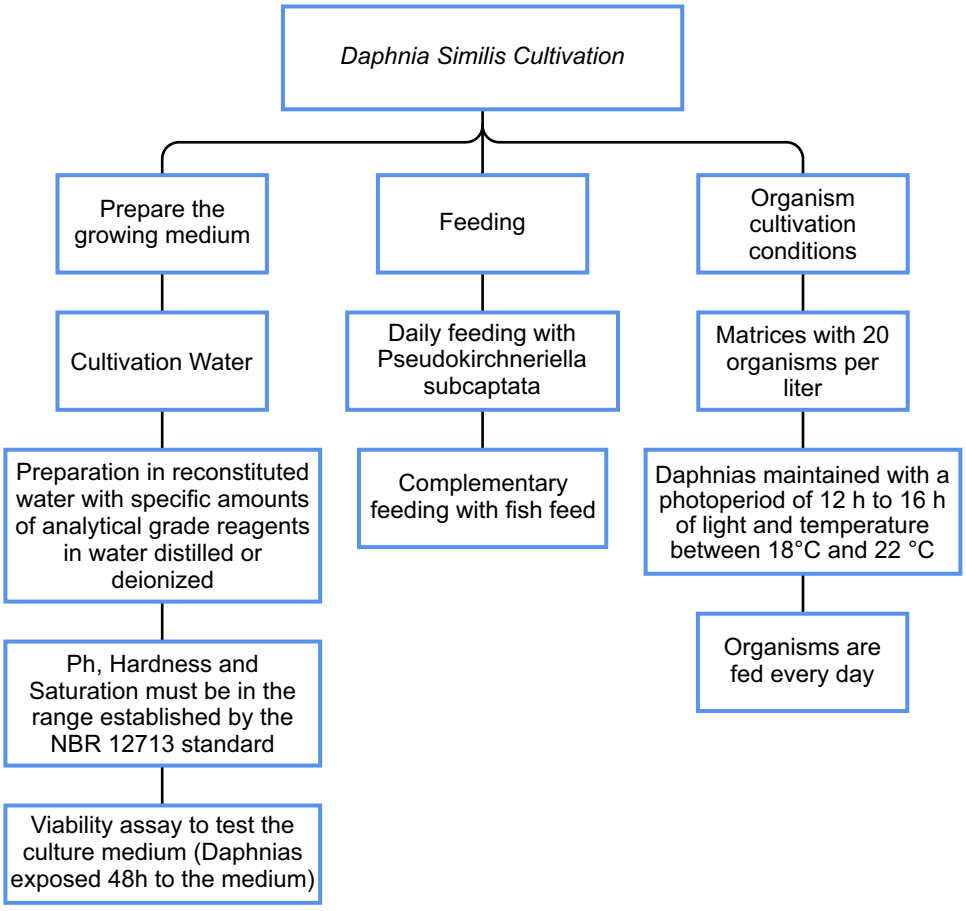


Figure 4. Photoperiod incubator image.

Source: Ethik.

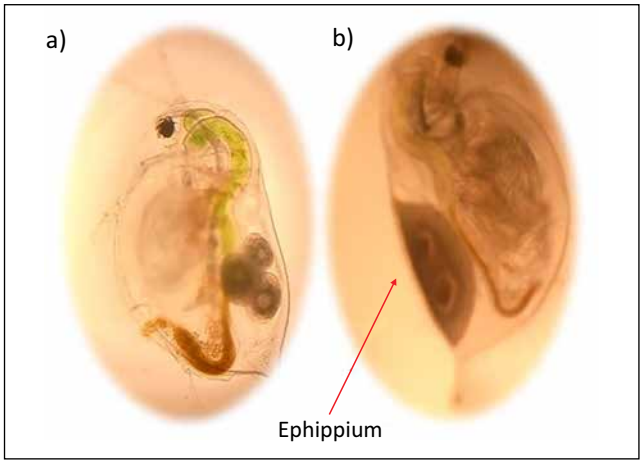
lack or excess of food can influence the cultivation of *Daphnias similis*. This fact can lead to the emergence of males and females with two resistant eggs. These resistant eggs, fertilized by males, are surrounded by a thickening dark color in the incubation chambers of adult females, called ephippium (Figure 6) [4-6]. Therefore, if one or more cases of ephippium occur in a culture, the neonates produced in this culture should not be used, having to discard and reassess the cultivation procedures. For example, we have had a case of ephippium in culture due to the overpopulation of *Daphnia* while testing new growing conditions, such as growing organisms in a container to increase the number of spikes (cultures) [10].

During the *Daphnia* cultivation supervision tests in the laboratory, we observed that not all of them were set when observing dead or immobile

Figure 5. A comparative scenario of crop structure.

Incubator cultivation - ECSSP Cultivation on The Table - LARC Source: Authors.

Figure 6. Comparison between a) *D. similis* with ephippium and b) with parthenogenesis egg.



Source: Authors - Photo taken under a microscope.

Daphnia. Therewith, it was possible to evaluate a change in the pH of the culture medium since it was outside the range established by the standard (7-7.6). We tested this parameter in different scenarios with *Daphnia*:

- Culture containing only reconstituted water;
- Culture containing just algae;
- Culture containing exclusively feed.

These tests were intended to investigate whether the food's pH interfered with the culture's maintenance or whether the problem involved the reconstituted water. However, after 24 hours of observation, the organisms died in all tested cultures. Thereby, we produced a new culture medium to identify if there was any contamination because the organisms continued to die, notwithstanding the pH variations. These tests are still in progress. Therefore, all variables must be in the conditions established for the standard cultivation of *D. similis* since not following may cause negative impacts on the cultivation. Also, it can discontinue the ecotoxicity tests since it needs neonates for their performance and effectiveness. One way to prove this fact is through a study carried out in Germany, which investigated the toxicity of algae organic matter in water reservoirs through bioassays using *D. similis*. Thus, with good cultivation and the emergence of neonates for application in tests, it is possible to determine that the organic matter of algae did not present toxic effects for the species tested under typical environmental concentrations. On the other hand, in non-ideal cultures, it would not be possible to obtain the results with the efficiency and reliability expected from an ecotoxicity assay [11].

Conclusion

This article focused on highlighting the main factors that can interfere in the cultivation of *D. similis*. We observed how sensitive these organisms can be to variations in used parameters (pH, temperature, photoperiod) that must be at ideal intervals to maintain the cultures and allow their reproducibility.

In addition, unfavorable growing conditions, such as overpopulation or temperature variation, can cause ephippium to discard cultures in these conditions, delaying the growth of organisms. Finally, the continuity of the cultures must allow access to neonates to carry out the ecotoxicity tests and, consequently, the studies of toxic compounds.

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Four Different Cryoprotectors in Preservation of *Staphylococcus aureus*

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Preservation associated with the use of cryoprotectants has been one of the most used methods for the long-term storage of microorganisms, mainly because this technique can guarantee the conservation of the original characteristics of the standard strains. The present study aims to evaluate the effectiveness of Skim milk and glycerol as cryoprotectants in preserving the standard strain of *Staphylococcus aureus* ATCC 6538. Preservation was carried out in freezers at -20°C and -80°C, initially only between December from 2022 to March 2023. Viability and purity tests were performed after one day of freezing and after 3 months to evaluate the method's ability to maintain the survival and purity of the microorganism. After analyzing the results, the methods were efficient for preservation, confirming that the use of cryoprotective agents in the freezing of *Staphylococcus aureus* bacteria can be used in laboratory routine.

Keywords: Cryoprotective Agents. Standard Strain. Preservation. *Staphylococcus aureus*.

Introduction

The scientific community has been increasingly concerned with preserving and maintaining biological materials and living microorganisms in the laboratory [1]. This maintenance is essential due to the need to use organisms or specimens for scientific tests, for use in laboratory quality control, and to achieve maximum preservation of cell vitality and the number of viable cells [2]. Therefore, an acceptable preservation method is essential for the best results. This choice is made according to the phenotypic characteristics of the bacteria, the behavior of each species concerning preservation methods, the advantages and disadvantages of each technique, and the maximum time the strains can remain preserved. Therefore, the preservation time defines the classification of maintenance methods, which are short-term (continuous priming), medium-term (preservation in mineral oil, preservation in sterilized water, freezing at –20ºC and drying in silica gel, soil, and filter paper), or long-term (lyophilization and cryopreservation) [3].

However, medium (–20ºC) and long-term freezing methods, if not carried out effectively according to the needs of each microorganism, can cause a deleterious effect, mainly on the cell membrane, and eventually cause cell death, because of the formation of crystals inside the cell. Therefore, it is essential to add cryoprotectants whose action protects against damage caused by freezing [4].

Thus, cryoprotective agents are substances that will be added to the means of suspension and will reduce the physical and chemical stress caused by freezing and thawing the cells, ensuring that the original characteristics of the standard strains are maintained. However, cryoprotectants must have low molecular weight, cellular toxicity, and high solubility in water to achieve this result. These agents include methanol, methyl acetamide, DMSO, glycerol, polysaccharides, mannitol, and skim milk [5,6].

The present work focused on the medium (–20°C) and long-term (–80°C) methods, comparing the efficiency and viability between four different types of cryoprotectants of the bacterial strains of *Staphylococcus aureus* ATCC 6538.

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Ana Verena Pimentel Leal de Moraes Rego. Avenida Orlando Gomes, 1845, Piatã. Salvador, Bahia, Brazil. Zipcode: 41650-010. E-mail: anaverena.com@gmail.com. Study presented in the VIII Scientific and Technological Research Evaluation Seminar and VII Workshop on Integration and Training in High Performance Processing. DOI 10.34178/jbth.v6i2.292.

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Materials and Methods

A strain of the bacterium *Staphylococcus aureus* ATCC 6538 from the Microbiology laboratory was used by the SENAI Institute of Advanced Health Systems (ISI – SAS). These strains were reactivated from the replicate onto Tryptone Soybean Agar (TSA) medium followed by incubation for 24 hours at 37 ± 1°C. After reactivation, the absence of contamination was confirmed from the macroscopic and microscopic visualization with the Gram stain and repeated in a selective medium of Salted Mannitol Agar. Subsequently, another replicate in TSA medium was followed by inoculating the strains for 24 hours at 37 ± 1°C. Before proceeding to freeze, a last repeat was carried out with the addition in the media suspension, the following cryoprotectants: Skim milk 15% in Soy Tryptone Broth (TSB) plus glycerol 20%, skim milk 15% in water, 10% glycerol in TSB and 15% glycerol in TSB. All tests were repeated in 10 tubes and frozen at –20 and –80°C, followed by the viability test after one day of freezing to evaluate frozen strains' activity metabolism. The concentrations of the cryoprotectants were chosen based on the studies by Amorim and colleagues [4] and Becheleni and colleagues [7] with the necessary additions and alterations.

After three months of freezing, the strains were submitted to a new viability test with inoculation in TSA for 24 hours at 37 ± 1°C, with subsequent macroscopic analysis, gram test, and catalase to confirm sample purity.

Results and Discussion

Using Skim milk and glycerol cryoprotectants in freezing and maintaining bacteria *Staphylococcus aureus* proved effective. The glycerol, by decrease the point of freezing and reducing the electrolytes in the non-frozen fraction, has an excellent protective effect becoming the cryoprotectant most used in freezing microorganisms [5]. Skim milk, in turn, acts efficiently in stabilizing the lipid bilayer during freezing and contributes to the stability of cellular enzymes. It occurs due to the presence of lactose and calcium, milk components [5,8,9].

Another critical factor, in addition to the use of cryoprotectants, is to precede the freezing and refrigeration at temperatures between 2°C and 8°C to avoid the thermal shock that could occur due to temperature difference [8].

Conclusion

The four cryoprotectants were able to protect during freezing at –20 and –80°C for *Staphylococcus aureus* bacteria strains for initially three months. This result, therefore, reiterates the use of cryoprotective agents in order to preserve and conserve microorganisms without causingdamage to the morphological, biochemical, and genetic structure of bacteria.

Further studies are necessary to continue evaluating long-term methods so that the results can be validated methods for prolonged use.

Acknowledgments

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Separation of Cellulose Nanocrystals from Bromelia (*Neoglaziovia variegate*) Fibers Using Ionic Liquids Based on Hydrogen Sulfate Anion

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Due to the environmental appeal that has grown in recent years, the use of agricultural wastes and plant fibers to develop new biodegradable materials is increasing quickly. In this context, nanocomposites reinforced with cellulose nanocrystals (CNCs) obtained from Bromelia (*Neoglaziovia variegate*) fibers have stood out as promising materials. Despite CNCs used to be separated from a cellulosic matrix in a good way with inorganic acids, the use of acidic ionic liquids (IL) has been arising as a safer and greener approach. Several authors have proposed the aprotic IL [BMIM][HSO_4] as an excellent alternative media to CNCs separation, and in previous work, we have proven that the cheaper protic IL [2-HEA][HSO_4] is valid for the same purpose. In this work, CNCs were separated from cellulose previously extracted from bromelia through the processing with H_2SO_4, [BMIM][HSO_4], and [2-HEA][HSO_4]. A variety of techniques, like thermogravimetric analysis (TGA), Fourier transform infrared (FTIR) spectroscopy, X-ray diffraction (XRD), electrophoretic light scattering (ELS), transmission electron microscopy (TEM) and atomic force microscopy (AFM) were used to CNCs characterization. The rod-like and spherical nanoparticles showed good thermal stability, and this could allow their incorporation into a polymeric matrix. Nanoparticles isolated with protic ionic liquid showed more excellent crystallinity when compared with nanoparticles with aprotic ionic liquid.

Keywords: Cellulose Nanocrystals. Ionic Liquid. Bromelia. Biodegradable Materials.

Introduction

With the concern and policies aimed at the environment, there has been great worldwide interest in developing technologies enabling products that cause less environmental impact [1].

In this context, vegetable fibers have been widely used as sources of cellulose nanocrystals (CNCs) to act as reinforcements in polymer matrices due to factors associated with cost, rigidity, renewability, abundance, and biodegradability [2-4]. *Neoglaziovia variegate* fibers are an excellent example of the mentioned characteristics and have a high cellulose content [5].

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CNCs are highly crystalline cellulose nanoparticles with a high aspect ratio (L/D) and are very rigid. It is necessary to remove the amorphous region of the cellulose by a hydrolysis reaction, maintaining the crystalline domains to obtain them [4,6]

They can act as reinforcement in polymeric matrices in small quantities, improving mechanical properties [7].

Hydrolysis from sulfuric acid is the best-known and widely used procedure. The method using aprotic ionic liquid to separate the crystalline phase of the amorphous region from cellulose is still little reported in the literature [8-10] and explores microcrystalline cellulose as a source.

However, the use of protic ionic liquid to obtain CNCs from renewable sources has not been reported in the literature. It can be an alternative to the aprotic ionic liquid since it can be synthesized easily, has a lower cost, and is safer, reusable, and less corrosive than inorganic solid acids [10]. Currently, ionic liquids have applications in treating biomass residues [11-14].

In this work, cellulose nanocrystals (CNCs) were obtained from bromelia (*Neoglaziovia variegate*) fibers from processing with sulfuric acid and ionic liquids: (1-butyl-3-methylimidazole hydrogen sulfate (aprotic) and 2-hydroxyethyl ammonium hydrogen sulfate (protic).

Using nanoparticles mixed with polymers has aroused interest in developing polymeric nanocomposites from CNCs, mainly from plant fibers, as reinforcement material for high-performance materials [15].

Other research route at stake for developing more environmentally friendly materials is the study of biodegradable polymers in detriment to those of complex degradation, whose main applications include sectors such as food packaging/ agriculture and the medical field [16-20].

The present study aims to separate and characterize CNCs from *Neoglaziovia variegate* fibers using conventional and alternative ways (ionic liquid processing).

Materials and Methods

Materials

Chemicals

Sulfuric acid (H2SO4), purity 95-99%, was supplied from Vetec and used to prepare the solution 55% (v/v). 1-butyl-3-methylimidazolium hydrogen sulfate ([BMIM][HSO_4]), purity > 95.0%, was supplied by Sigma-Aldrich and used as received without further purification. 2-hydroxyethyl ammonium hydrogen sulfate ([2-HEA][HSO_4]) ~30% (m/m) was synthesized from sulfuric acid and monoethanolamine, purity >90.0%, supplied by Neon.

Fibers

Bromelia fibers were collected in Araci, Bahia, Brazil. After washing, drying, and bleaching processes, the material was used to separate CNCs.

Methods

Separation of CNCs

$H2SO_4$ Processing

Bleached bromelia fibers were hydrolyzed at 50°C for 2 h under mechanical stirring at 1,150 rpm. For each gram of bleached material, 10 mL of H2SO_4(aq) 55% (v/v) was used. After the hydrolysis, 300 mL of water at 8°C was added to the system to quench the reaction. The resulting suspension was centrifuged several times to separate residual acid from precipitate, which was washed with distilled water at 4,400 rpm for 10 min to reach the CNCs dispersion in the water. The turbid suspension containing CNCs was submitted to dialysis for some days until pH 7 was reached [21,22]. Following this step, the purified suspension was stored in a refrigerator and named CNCSA (Cellulose Nanocrystals obtained with Sulfuric Acid).

Ionic Liquids Processing

Hydrolysis methodology was adapted with some modifications from [10]. Note that reaction conditions differ from H2SO_4 processing due to the specific physical features of the ILs, mainly viscosity, and acid strength. The reaction was performed in a becker at 70 C for 1,5 h under mechanical stirring at 580 rpm. For each gram of bleached material, 9 g of each IL was used separately. Following this step, 20 ml of water at 8°C was added to the system to quench the reaction. Centrifugation and dialysis were carried out in the same way described before. The purified aqueous suspensions containing CNCs were stored in a refrigerator and named CNCAPIL (Cellulose Nanocrystals obtained with Aprotic Ionic Liquid) and CNCPIL (Cellulose Nanocrystals obtained with Protic Ionic Liquid), respectively related to [BMIM][HSO_4] and [2-HEA][HSO_4].

Characterization

TEM, AFM, TG, FTIR, XRD, and Zeta potential characterized cellulose nanocrystals.

Transmission Electron Microscopy (TEM) was carried out in aqueous suspension, which was dripped onto 400 mesh copper grids covered with formvar film. Uranyl acetate solution 2 wt% was used as contrast. TEM micrographs were recorded in a JEOL microscope, model JEM-1230, operating at 80 kV, and Tecnai equipment, model G2-20. AFM micrographs were recorded in a Vecco e Cypher microscope, model Dimultimode V e ES Asylum Research. TG was performed in Shimadzu® equipment; model TG-A50 was analyzed from 25 to 800 °C at a heating rate of 20°C min^{-1} under a nitrogen flow of 100 mL min^{-1}. FTIR analysis was carried out using a Shimadzu spectrometer, model IR Prestige-21, transmittance mode, wavelength range of 4000-800 cm^{-1}. X-ray diffraction (XRD) was carried out in a Shimadzu X-ray diffractometer, model XRD-6000, angle range of 5°-50°. Dynamic Light Scattering (DLS) was conducted to determine the Zeta potential in a Zetasizer Nano ZS equipment with water at 25 °C.

Results and Discussion

Transmission Electron Microscopy (TEM)

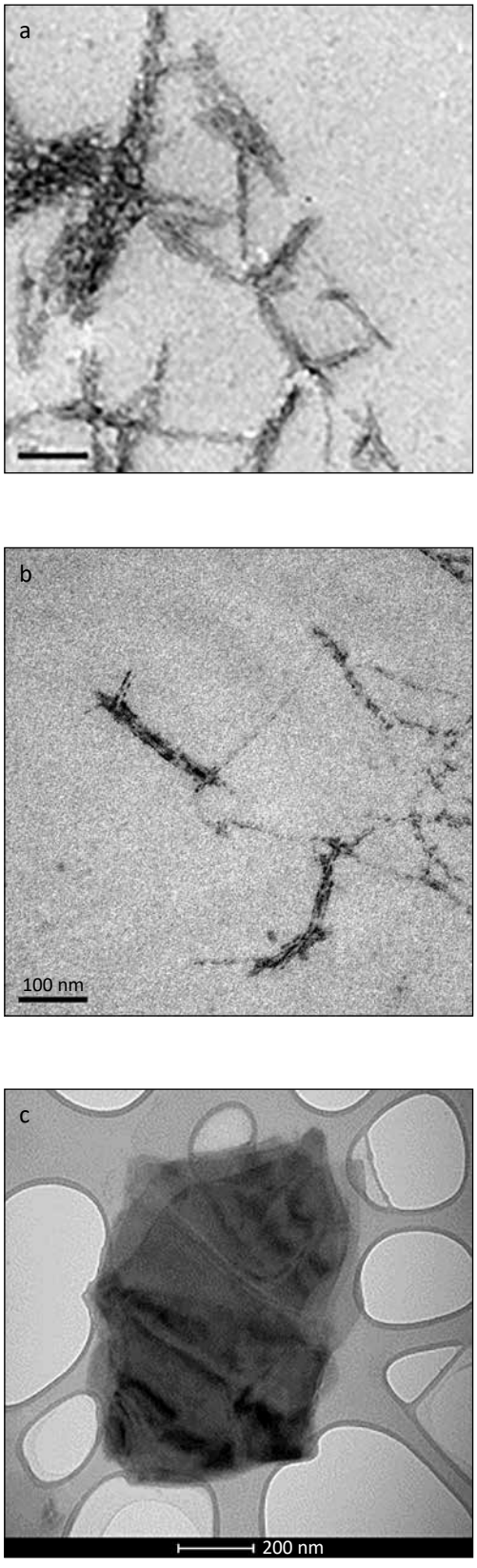
A drop of each CNCs suspension was examined using TEM to confirm the separation of CNCs from bleached bromelia. Figure 1a shows CNCSA as rod-like nanoparticles, confirming them as cellulose nanocrystals. A bundle of particles is formed due to the preferential hydrogen bond formation between single nanoparticles instead hydrogen bonds with the hydrophobic substrate used to cover the cupper grid where the drop was added onto. Several other natural fibers, for instance, licorice, banana, palm; Calotropis procera, Macauba, and gravatar fiber, can provide similar rod-like CNCs [5], [23,24] that make separation of bundles of rod-like CNCs from 'caroá' with an aspect ratio of 16±5. The images of CNCAPIL and CNCPIL, both materials produced by ionic liquid processing, show nanoparticles with different shapes and sizes. While the first ones are rod-like, spherical morphologies were achieved using the protic ionic liquid.

Figure 1b shows that [BMIM][HSO_4] is an efficient medium to separate crystalline domains of cellulose (CNCs) from their amorphous domains under the tested conditions. The particle morphology of CNCAPIL is rod-like, as CNCSA, and all particles are on a nanometer scale, as reported by other authors [8,10]. Despite CNCSA and CNCAPIL being made both of rod-like nanoparticles, in CNCAPIL, the particles are less bonded to each other, which maybe a consequence of big ionic groups added into cellulose by [BMIM][HSO_4] over the reaction. Measurements of length, diameter, and aspect ratio of nanoparticles in image software are better to be done with single nanoparticles than a bundle of nanoparticles, suggesting an advantage for the use of ionic liquid processing [8] also successfully separated CNCS from microcrystalline cellulose using [BMIM][HSO_4] at 120 °C and 24 h of time reaction. The results presented in the current study and those from the literature suggest that [BMIM][HSO_4] improves energy and time-consuming reaction compared to $H2SO_4$ processing.

Figure 1c shows spherical nanoparticles for CNCPIL instead of rod-like morphology of CNCSA and CNCAPIL. The most probable reason to explain the spherical formation pathway under the conditions used for [2-HEA][HSO_4] processing is a self-assembly process of small cellulosic fragments around the rod-like nanoparticles during the drying. According to Lu and colleagues [25], self-assembly decreases particles' surface area, allowing a decrease in their free energy. [2-HEA][HSO_4] is probably inefficient in inserting charged groups on cellulosic chains so that particles can interact by the side by hydrogen bonds.

Table 1 shows the dimensions of CNCs, but CNCPIL measurements were impossible due to the difficulty of identifying the particles individually. On average, particle size for CNCSA was evaluated to be 252 nm in length and 20 nm in width, values which provided a high aspect ratio near to those reported by [24,26].

Figure 1. TEM images of CNCSA (a), CNCAPIL (b) and CNCPIL (c).



Tand and colleagues [10] found that processing of microcrystalline cellulose with [BMIM][HSO_4] at the temperature range of 70°C-100°C led to the formation of rod-like nanocrystals with diameters in the range of 15-20 nm and lower average aspect ratio than 28 (the average found in the current study – Table 1). It means the modifications made to the methodology of [BMIM][HSO_4] and the features of cellulose from bromelia fibers can provide CNCs with an aspect ratio fourfold. The massive importance of the aspect ratio of discontinuous phase (reinforcement) in nanocomposites comes from the percolation network between the phases at the interface, the zone responsible for the mechanical improvements achieved compared to the pure phases [27].

Nanostructures of high aspect ratios can strongly improve the mechanical properties of the matrix due to improvements in mechanical stress at the interfacial zone [28], so both CNCSA and CNCAPIL samples have the potential as mechanical reinforcements in nanocomposites.

Atomic Force Microscopy (AFM)

AFM measurements were carried out to confirm the particle size and morphology by other technique. Figure 2 shows AFM images of CNCs. Although no dissimilarities were observed when compared to TEM images - dimensions and morphology of CNCs were the same for both techniques. Figure 2c provides further details about the CNCPIL sample, which could not be apparent by TEM image: the spherical particles are made of small fragments. This observation agrees with the spherical pathway based on Gallardo-Sánchez and colleagues, and Lu and Lo Hsieh [29,30]. These results suggest that different mediums based on hydrogen sulfate anion allow the separation of different types of CNCs with a wide range of applications.

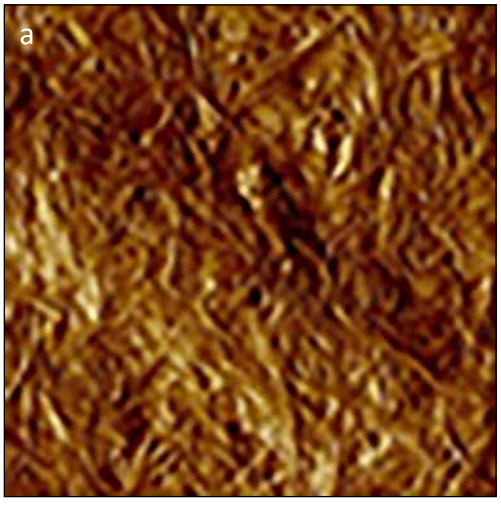
Electrophoretic light scattering (ELS)
It is not enough to separate CNCs from a cellulosic source; they must keep their dimensions on the nanometer scale. ELS measurements

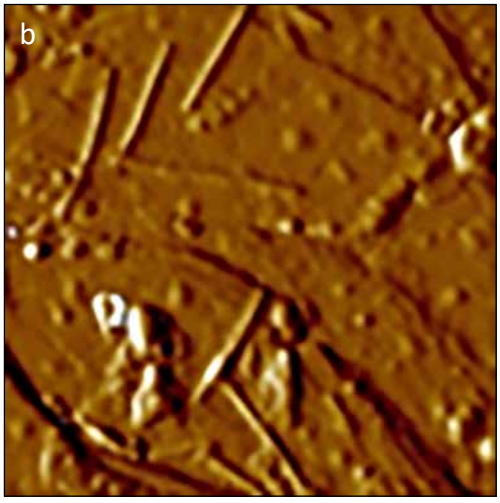
Table 1. Length, diameter, and aspect ratio of CNCs.

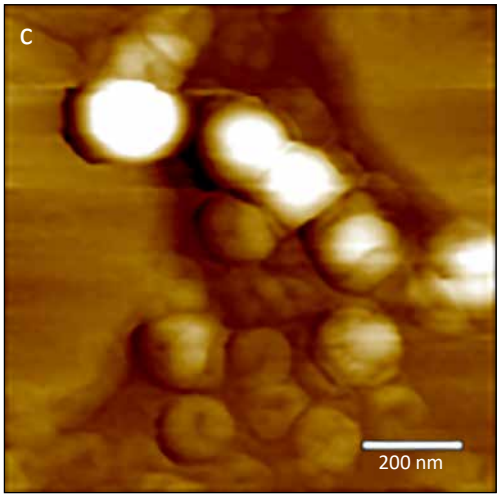
Sample	Length (nm)	Diameter (nm)	Aspect Ratio
CNCSA	252.00 ± 127.94	20.00 ± 14.56	35.09 ± 29.73
CNCAPIL	147.39 ± 60.82	5.55 ± 2.81	27.97 ± 3.87

were carried out to evaluate the stability of suspensions. The negative values of Zeta potential for all samples confirm the presence of negatively charged groups at the surfaces of CNCs over the reaction, such as sulfate and hydrogen sulfate. According to the electrostatic model, the electrostatic repulsion between particles charged with the same signal is the main reason to keep the nanoparticles isolated and stabilized by water molecules. So, as much negative, the Zeta potential is, the more stable the sample is. According to the literature [29], [31] ideal suspensions with Zeta potential higher than 30 mV must be stable so CNCs do not tend to aggregate into more considerable microscopic fragments. Based on the values shown in Table 2, none of the samples meet the stability criteria; however, the values agree with those found by other authors. [29]. CNCPIL was the less stable suspension, and this result deals with the discussion about spherical pathway formation in this sample.

Figure 2. AFM images of CNCSA (a), CNCAPIL (b) and CNCPIL (c).







Thermogravimetric Analysis (TGA)

Thermal degradation of cellulose involves chemical reactions such as depolymerization, dehydration, and decomposition of glycosyl units, processes strongly dependent on the surface area of particles and the charged surface groups. TGA measurements were carried out to evaluate the

Table 2. Potential zeta of CNCs.

Sample	Zeta Potential (mV)
CNCSA	-21.1 ± 0.5
CNCAPIL	-23.5 ± 0.4
CNCPIL	-20.3 ± 0.2

thermal behavior of each sample and understand the influence of each kind of processing (Figure 3). Sample CNCSA was thermally stable until 232°C, with a weight loss of 3 %. DTG profile shows two events: the first at a maximum temperature of 55°C related to humidity loss [32], and the second related to the degradation of sulfated cellulose present on the surface of CNCs. These regions are more susceptible to thermal degradation when compared to the uncharged bulk. Sample CNCAPIL was thermally only until 214°C. DTG profile shows three events: Besides the events related to moisture (below 100°C) and degradation of sulfated cellulose by pyrolysis (214-380°C with maximum degradation temperature until 306°C) [9,32], a third event above 600 °C appears related to carbonaceous matter [24]. This event is absent in CNCSA because of the abundance of sulfated groups over the surface.

CNCPIL was the less thermally stable sample, holding on until 205ºC. DTG profile shows the same three events (at 70-100ºC, 205-389ºC, and 684-773ºC) already discussed for CNCAPIL. Despite the less charged surfaces for CNCPIL, AFM images suggest smaller particles when compared to CNCSA and CNCSAPIL, so small particles tend to have a higher specific surface area, increasing the exposure area over the heating. However, the thermal stability of CNCPIL is enough to allow its incorporation at formulations based on thermoplastic matrices because the most usual range of processing temperature for these materials is around 200°C [33].

Fourier transform infrared spectroscopy (FTIR)

FTIR measurements were carried out to confirm the presence of sulfate groups at the surface of CNCs and their nature of water affinity. Figure 4 shows FTIR spectra of all samples. The signal at 1620 cm^{-1} is related to adsorbed water vapor and is present in all curves, suggesting a hydrophilic nature [10,32]. The signal at 11631 cm^{-1} is attributed to S=O vibration. This double bond belongs to sulfate groups and is present in all curves. All processing methodologies effectively separate and stabilize the CNCs by esterifying their surfaces with sulfate groups.

X-ray Diffraction (XRD)

XRD measurements were carried out to evaluate each sample's crystallinity using the Crystallinity

Figure 3. TGA (a) and DTG (b) profiles of CNCs.

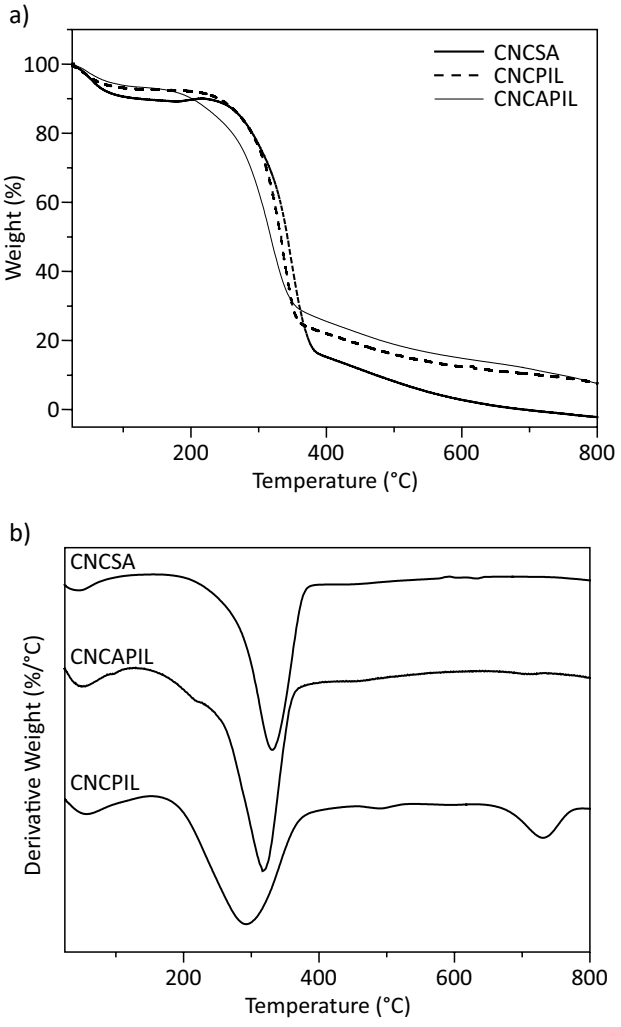
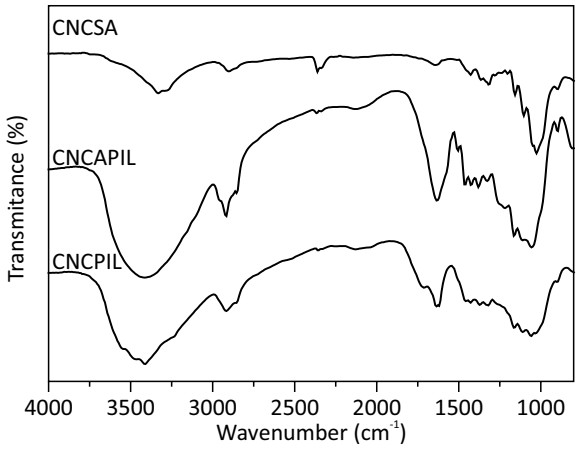


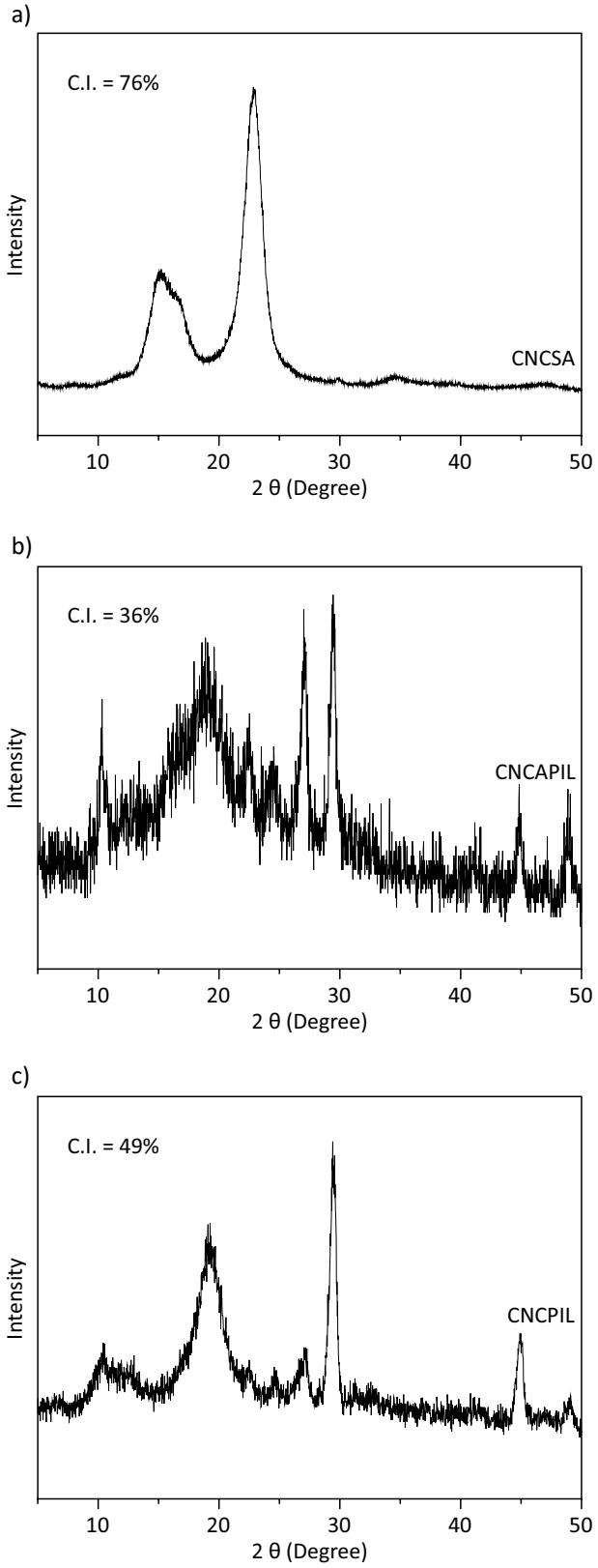
Figure 4. FTIR spectra of CNCSA (a), CNCAPIL (b) and CNCPIL (c).



Index (C.I.) approach, aiming to understand the influence of the processing methodologies employed. Figure 5 presents the results.

Diffraction peaks at 2Θ=15, 22, and 34° ((110), (200), and (040) plans, respectively) are assigned to the suitable cellulose I [34] and are present in the XRD profile of CNCSA. The high intensity of the peak at 22° contributes to the highest C.I. of samples, around 76%. This result suggests the extraordinary ability of the sulfuric acid solution to provide hydronium ions able to break glycosidic bonds from amorphous cellulose and release single CNCs. XRD profiles of CNCAPIL and CNCPIL also exhibit diffraction peaks typical of cellulose I ((110) and (200) plans) [10,35], suggesting that ionic liquid processing methodologies employed were suitable to avoid the cellulose interconversion to unsuitable II types. However, it is essential to highlight the low C.I. calculated for CNCAPIL and CNCPIL because it suggests some degradation and crystalline parts of cellulose, as already noted by Tand and colleagues and Haron and colleagues [10,13]. It could be explained based on the swelling ability of ionic liquids, so their cations can migrate between cellulosic chains, separating them and changing the spatial configuration to some extent. The results of XRD are in good agreement with TGA and FTIR results.

Figure 5. XRD profiles of CNCSA (a), CNCAPIL (b), and CNCPIL (c).



Conclusion

From the use of the sulfuric acid solution, [BMIM][HSO_4] and [2-HEA][HSO_4], it was possible to separate the cellulose crystalline regions of bromelia (*Neoglaziovia variegate*) fibers from the amorphous phase, obtaining cellulose nanoparticles with shaped and spherical shapes, as shown in the TEM and AFM analyzes. The spherical shape obtained from protic ionic liquid can provide important characteristics in new applications that can be studied. Protic ionic liquid has the advantages of being less corrosive than sulfuric acid, capable of reuse, and less costly and less toxic compared to aprotic, being, therefore, a new alternative in obtaining cellulose nanocrystals to be used explored. The CNCSA sample was more thermally stable when compared to the CNCAPIL and CNCPIL samples, showing a higher crystallinity index. CNCPIL also presented a higher crystallinity index when compared to CNCAPIL. The good thermal stability of the three types of CNCs makes it possible to use them in material processing, such as extrusion, and thus could be used as reinforcement to a biodegradable matrix, enhancing the range of possible applications for these materials.

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Environmental Audit in the Solid Waste Collection Process in Military Organizations of the Brazilian Air Force

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This study proposes an environmental audit method for the solid waste collection process in Military Organizations of the Brazilian Air Force and the its improvement. Likewise, performing a comprehension of environmental management in the armed forces (Brazilian Air Force) focusing on managing solid waste and emphasizing the importance of environmental auditing in solid waste collection. This work brings critical social aspects, such as environmental management, to the FAB (Brazilian Air Force, *Força Aérea Brasileira in Portuguese - FAB*). The study is a documental analysis with a checklist application from *in loco* visits for observation, registration, and information collection in the GSAU-SV of BASV (Health Group of Salvador Air Base). We observed that GSAU-SV developed multiple environmental practices to manage its solid waste correctly. However, it does not have a structured Solid Waste Management Plan or disclose its actions. From the analysis of the application of the environmental audit in the GSAU of BASV, we concluded that, despite the non-mandatory nature, the execution of this practice by the Military Organizations must be stimulated.

Keywords: Brazilian Air Force. Solid Waste. Environmental Auditing.

Introduction

Barata and colleagues [1] presented that implementing environmental management in public agencies establishes a new institutional culture. It mobilizes employees to optimize resources, fight waste, and improve work environment quality.

In this context, adopting environmental criteria in administrative and operational activities is a process of continuous improvement that consists of adapting environmental effects to the policy of preventing negative impacts on the environment [2-4].

Maranhão [5] emphasizes the importance of public organizations' participation in the process of socio-environmental responsibility. It encourages the insertion of sustainability criteria in their activities and integrates social and environmental actions with the public interest.

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Therefore, military organizations must implement methods and tools capable of verifying the environmental impacts of their activities on the environment. Moreover, environmental audits have become increasingly frequent since their process culminates in environmental certification for the organizations, which proves their degree of environmental awareness and commitment to society.

The main objective of this research is to propose an environmental audit method for solid waste collection in Military Organizations of the Brazilian Air Force and possible improvements of this process.

Therefore, our specific objectives were performing a comprehension of environmental management in the armed forces (Brazilian Air Force) focusing on managing solid waste, emphasizing the importance of environmental auditing in solid waste collection, and highlighting the importance of environmental auditing in solid waste collection.

This study brings critical social aspects, such as environmental management in the FAB (Brazilian Air Force), and multiple reflection on humanitarian and environmental contributions.

We did a literature review and document analysis, performing a study in the GSAU-SV of

BASV (Health Group that provides health care to active military, reserve, and their dependents from the Salvador Air Base). We applied a checklist from *in loco* visits and observed the areas of collection, handling, and storage of waste, from the sections of the GSAU-SV, with registration and information collections.

Environmental Management in the Armed Forces

The Brazilian armed forces consist of the Army, Navy, and Brazilian Air Force, whose missions are to ensure law and order and preserve the exercise of state autonomy and the indivisibility of the Federation [6].

Maranhão [5] describes that the Armed Forces, despite their constitutional purpose, have been adopting proactive policies towards the environment throughout time, either by developing a mentality embodied in the environmental legislation in force or by operational conditions.

Therefore, aligned with national interests, the Army contributes to preserving the environment through prevention and environmental protection actions. These actions also include keeping an Environmental Management Plan in its space destined for activities in areas under the Army's jurisdiction [7].

Based on the Brazilian Standard (NBR) ISO 14.000, in 2002, the Navy promoted the implementation of the Environmental Management System (SGA) in all land-based Military Organizations (OM) to that achieving legal compliance and the minimization of environmental impacts resulting from their activities [5,8].

This environmental awareness is old, with reports since the time of the Brazilian Empire about preserving waters by controlling pollutants generated by ships [9].

In 1920 there was the first documental record of military legislation caring for the environment through the Ministry of War, about regulating and disciplining the use of natural resources of the Instruction Camps in Rio de Janeiro [10].

Since then, Brazilian legislation has approved Environmental Management Policies that involve planning, execution, and evaluation of environmental patrimony [11].

Environmental Management in the Brazilian Air Force

The Brazilian Air Force (FAB) has always been concerned with environmental management. Resolution RCA 12/-1/2014 has associated sustainability in the military context with project executions, such as in educational activities and actions against deforestation and using solar energy in military properties [12].

The exercise of environmental management, allied with the need to promote the better performance of personnel working in this area, has highlighted the importance of a normative instrument, which brings together all the specific legislation and establishes to users the applicable procedures to each situation [13].

The Air Force Infrastructure Directorate (DIRINFRA) created the Environmental Management Advisory (AGA) to advise on the normatization, planning, coordination, and control of activities related to the environment under the competence of DIRINFRA and its subordinate organizations and to provide measures for sustainability and environmental actions. In this sense, DIRINFRA began the implementation of the environmental management principles in military activities through the creation of the Environmental Management System of the Aeronautics Command (SISGA), approved by Ordinance n°1.447/GC3 of September 19, 2018 [13].

According to the Ministry of Defense Planning for Environmental Management [14]:

- In 2017, the Defense Green Book was implemented to raise awareness of those who are somehow part of the Brazilian Armed Forces and understand environmental preservation practices.
- In 2018, the Environmental Management System of the Aeronautics Command was instituted according to Ordinance No. 1.447/GC3.

- Technical and environmental standards of Ordinance No. 168/GC3 published in BCA No. 019 were approved on February 04, 2019, with instructions on Environmental Control and Management within the scope of the Aeronautics Command. Due to this fact, the Manual for Environmental Practices in Brazilian Air Force Military Organizations was prepared. The manual contains sustainability options for environmental management, such as energy resource management, solid waste, recycling, and bidding.
- In 2020, the Directorate of Infrastructure of the Air Force published OT nº 001/DGA/2020 on the Guidelines about the Declaration of the Military Character of activities and enterprises destined for the preparation and employment of the Armed Forces, followed and approved in 2021 by the Ministry of Defense of the Aeronautics Command.

Solid Waste Management

The National Policy on Solid Waste (PNRS), established by Law 12.305/2010, defines solid waste as all materials, substances, objects, or discarded goods resulting from human activities in society, whose final disposal happens in solid or semi-solid states, as well as gases contained in containers and liquids whose particularities make it unfeasible to launch them into the public sewage system or bodies of water or require solutions technically or economically unfeasible given the best available technology, aiming at reuse, recycling, and treatment of these wastes [15].

The goal of the PNRS starts with the non-generation of waste that encourages the reuse, recycling, and composting associated with the maintenance of green areas and recovery of agricultural areas with educational programs [16].

Solid Waste Management encompasses the strategies and sustainable technological and operational aspects relating to the prevention, reduction, segregation, reuse, packaging, collection, transportation, treatment, energy recovery, and final disposal of solid waste based on current standards and legislation [17].

Strategic decision-making regarding institutional, administrative, operational, financial, and environmental aspects are activities inherent to the waste management of an organization [4].

Solid Waste Management

Waste management improves production yield and reduces costs with raw materials, inputs, reagents, treatment, and final disposal in an organization since the amount of waste generated decreases [4].

Through a Waste Management Program (WMP), public or private establishments must comply with the PNRS law by putting into practice the direction of the waste generated and maintaining the separation of recyclables and organics [18].

Proficient personnel must monitor the final destination of solid waste by determining its characteristics and packaging when necessary for performing the management plan [16].

Despite all the efforts for solid waste management, the PNRS faces challenges due to the low adherence of some organizations in designating their waste, accumulating it in landfills, and without incentive to recycling companies and formalized collectors [19]. The damages caused by the lack of environmental management result in water pollution, air pollution by burning, and problems with animal waste, which affect the environment and population health (20). Correct waste management involves the implementation of the 3Rs of sustainability: reduction at source, reuse, and recycling, to reduce and continuously improve the management system, considering the product life cycle, modifications and innovations in the process, using cleaner technologies [5].

Environmental Auditing

The environmental audit is a process of environmental performance evaluation associated with an organization's environmental management, checking the positive and negative points generated

by the environmental impact. These points are evaluated with criteria through records relevant to the facts, indicating compliance or not, pointing through the PNRS and environmental management procedures for improvements [21].

The audits can be periodic or sporadic and internal or external, as in the case of those contracted by insurance companies [22].

La Rovere and colleagues (2008) compare the environmental audit and the medical examination. For them, the environmental audit is the examination that the doctor (auditor) makes of their patient (organization) to verify health status (environmental performance) and may be applied periodically or eventually in case of suspicion of some dysfunction in the body (organization), being specific to a particular body organ (some sector of the organization) or general (covering all sectors of the organization).

According to ABNT [23], audits can be divided into three parts: the first part or internal, when the organization itself makes the performing; the second part or external, when performed by interested parties; and the third part is also external, when performed by independent audit organizations, usually when there is interest in certification.

Listing internal audits are classified as environmental performance and environmental certification that evaluate the conformities to obtain a system certification established by the organization's standards, having its interest in those matters that affect the economy [24].

As an example of the external audit, legal compliance environmental audits are those applied by governmental legislation, which compulsorily evaluates the situation in which the organization finds itself according to the legislation in force [21].The audits ultimately encourage organizations to follow the guidelines set forth by the Environmental Management Guidelines so that it is a tool for monitoring, and the entire process is documented through a diagnosis made by a technician for evaluation and solutions in cases of environmental damage [25].

Materials and Methods

This research was developed in an OM of the Brazilian Air Force due to the wide range of activities performed by the military that, in many aspects, can be similar to companies or industries in the private sector. This type of research facilitates the understanding of the investigated phenomenon. In this case, a single case with participant observation was chosen due to the nature and magnitude of the phenomenon [26].

For the exploration of the theoretical referential, a bibliographic and documental survey was used, based on the databases: Central Online Content of the Brazilian Air Force, Scielo (Scientific Electronic Library Online), books, scientific articles from websites, scientific journals, and institutional publications, using a set of keywords: military organizations, Brazilian Air Force, environmental management, waste management, solid waste, and environmental audit and for operationalization of the research was structured, from the literature review, visits to the OM facilities, with direct observation of the formal and informal type.

The formal observation included participation in a meeting pertinent to Environmental Management issues. Informally, the handling of waste, the waste storage area, the sectors that produce waste, and the execution of training sessions were observed with the application of a form – Checklist, which consists of a list of requirements in an orderly and systematic manner with closed questions prepared by the researcher and with alternative answers from what was assessed: compliant, non-compliant, and partially compliant. The classification of conformity had as a criterion the normative document the PGRSS (*Plano de Gerenciamento de Resíduos do Serviço de Saúde do* GSAU-SV), without delimitation of severity according to the deviation.

The Military Organization selected for the study was the Salvador Health Group of the Salvador Air Base, which has the purpose of providing health care services, functioning as a polyclinic and 24-hour emergency service to active and reserve military personnel and their dependents of the

Salvador Air Base, with the mission of providing continuous improvement, quality of care and user satisfaction.

Results and Discussion

Environmental Description of the Salvador Health Group (Gsau-Sv) - Salvador Air Base (BASV)

BASV, Salvador Air Base, together with GSAU-SV, Salvador Health Group, previously called ES, Health Squadron, (Health Group that provides health care to active and reserve military people and their dependents from the Salvador Air Base) was created on November 5, 1942, to support anti-submarine warfare actions along the Brazilian coast. The Santo Amaro de Ipitanga field, in which the Salvador Air Base is located today, was built on a site surrounded by small sand elevations interspersed with vegetation over swampy and marshy areas, situated between the Joanes and Ipitanga Rivers, responsible for about 40% of the water supply to the metropolitan region of Salvador. The land belonged to a local farmer, who served as a mediator for the works and authorized the AirFrance airline operation at the Santo Amaro de Ipitanga Aerodrome and its operation permit, granted by the then Minister of Transportation and Public Works.

The Salvador Health Group (GSAU-SV) at BASV develops several activities in three macro health areas: Dentistry, with specialties in dentistry, endodontics, pediatric dentistry, orthodontics, periodontics, and dental prosthesis; medicine, with specialties in cardiology; general surgery; general practice; neurology; ophthalmology, and pediatrics, besides complementary services with pharmaceutical and laboratory assistance in the clinical and radiological analysis, as well as physical therapy, speech therapy, nutrition, and 24-hour emergency service.

From the delimitation of the macro areas mentioned, it was possible to identify that the GSAU-SV produces the following types of solid waste: common solid waste, chemical solid waste, biological solid waste, and sharp solid waste. Thus, the Solid Waste Management Plan (SWMP) must be prepared, updated, and executed, ensuring that all waste is managed appropriately and safely. In the GSAU-SV, there is not a PGRS; however, there is the PGRSS project (Programa de Gerenciamento de Resíduos de Serviços de Saúde – PGRSS) under construction, and within this project, there is a normative document, already in partial execution, which is the PGRSS Action Plan with the description of the PGRSS responsibilities, of operational capacity, organizational chart, generating source, classification of waste generated/ segregation/conditioning, determination of indicators and training of the PGRSS work team in the implementation phase of the activities to be sent to the competent bodies, in order to have an opinion approved and to start the PGRSS.

Audit

Based on the literature review and visits to the GSAU-SV facilities, a Checklist (Table 1) was developed and applied to evaluate conformities, non-conformities, and partial conformities within the Salvador Health Group - GSAU-SV activities.

After the results obtained with the checklist implementation, the conformities, non-conformities, and partial conformities found about the management of solid waste were pointed out. Despite not having an Environmental Policy and having the PGRSS project under construction, it is possible to evidence some strong points about environmental aspects: the existence of an internal procedure for waste sorting discussed, with technical criteria, for example, the fact of performing the sorting with a responsible person.

Legal Requirements

The strengths evidenced were: the existence of a procedure to identify the waste-generating sources with the person responsible for the waste sorting; the procedures make competent people responsible for determining the application of the legal requirements to the environmental aspects. It

Table 1. Ckecklist applied to Salvador Health Group - GSAU-SV.

Requirement Measured	Complies	Not Compliant	Partially Compliant
The collectors are in a good hygienic condition	x		
Collectors are easy to transport	x		
The collectors in the area of preparation and storage of medications in the emergency room have a lid (without manual operation)	x		
There is a segregation / selective waste collection program		x	
There is some employee training concerning waste management			x
There is a PGRS		x	
The GSAU-SV has flowcharts regarding solid waste generation, storage, and location		x	
There are Operational Programs that discipline the collection, handling, and storage of solid waste			x
The waste storage areas have adequate signage, protection systems, and labeling		x	
The GSAU-SV has a control register of solid waste volume generation according to quantity, characteristics, and class			x
There are waste minimization practices and/or programs	x		
There is a labeling system for solid waste generation	x		
There is a sector responsible for the management of solid residues	x		
There is an Environmental Policy		x	
All employees and collaborators know the legislation about Solid Waste Management		x	
All GSAU_SV employees and collaborators are aware of the Environmental Policy		x	

Source: Adapted from references [27-29].

was also found that the waste-generating sources are in good hygiene and are easy to transport, as determined by the current legislation. There is a control of the chemical, biological, and sharp-edged solid waste output (external collection by an outsourced company with an environmental license), containing the date, time of the output, people responsible for the transportation, the typology of the waste, and weighing.

Objectives, Goals, and Environmental Practices to Minimize Solid Waste

There is a PGRSS Commission, formed earlier this year, which works together with the GSAU-SV management to adopt the necessary measures to reduce the amount of waste generated within the sectors of the unit, ensuring good waste management.

Partial Conformities

We evidenced that there is the qualification/training of the hygiene employees regarding the collection, handling, and storage of residues not reaching all the unit professionals. Another point of partial conformities is a register to control the generation of solid waste volumes: biological, regular, and sharps, according to the quantity and characteristics, not covering all the waste produced in the unit.

Non-Conformities

We identified the inexistence of a PGRS, besides the PGRSS, which is under construction, that contemplates the information contained in the norm, likewise all the environmental legislation that is the basis for the issue of Solid Waste in the sector responsible for waste management; the inexistence of an Environmental Policy, as well as the understanding of all professionals of the importance of this policy and the knowledge of the legislation on solid waste management by all military professionals and servers of the unit; the lack of record of training on the themes related to the Management and Management of Solid Waste; inexistence of a segregation program, selective collection, donation of recyclables, as well as the issuing of a final destination certificate by the company responsible for the external collection of chemical, biological and sharp-edged waste, as supported by the norms, RDC and legislation; absence of adequate labeling, protection system and signage in the waste storage area; lack of a solid and critical Environmental Education Program, in accordance with the activities developed by GSAU-SV; the lack of flowcharts of the various types of waste identified since the generation, storage and location of solid waste during the environmental characterization of GSAU-SV.

Suggested Improvements

- Perform classification by class, segregation, and selective collection of solid waste produced by the GSAU-SV, which may be recyclable, through the adoption of composting practices or other viable forms of reuse.
- Environmental Education campaigns must be carried out to manage solid residues and administrative activities adequately. Training must be applied at all levels and for all military and civilian employees.
- Waste flowcharts must be prepared and posted at their respective generating sources.
- Regarding the transportation and disposal of hazardous or non-hazardous solid waste, the unit must request the company responsible for the external collection, besides the Waste Transportation Manifest (MTR), the subsequent return of the Final Disposal Certificate.
- To increase the environmental awareness of the military and civilian collaborators, posters must be made and fixed near the waste collectors, informing their functionalities and recyclability when implemented (Figure 1).

Conclusion

The method for implementing an environmental audit for the solid waste collection process in the Military Organization (GSAU-SV of BASV)

Figure 1. Identification of waste types for segregation of recyclable material source [30].

proved to be an adequate and efficient tool for environmental protection since it has the function of verifying possible violations of environmental standards that result in the minimization of environmental impacts. The tendency is that its use increases increasingly due to the urgent need to put sustainable development into practice, as currently discussed by society.

It was observed that the GSAU-SV of BASV develops environmentally good practices for correctly managing its solid waste. However, it does not have an Environmental Policy or a Solid Waste Management Plan, so essential to improving the control of waste generation directly from the generating source and, consequently, reduce waste, avoid significant environmental pollution and its consequences for public health and the environment, nor does it disclose to its public its actions and/or practices of environmental protection.

Because of the above, and based on the results obtained from the analysis of the application of environmental auditing in the GSAU- SV of BASV, it is also concluded that, despite not being mandatory, the implementation of this practice by Military Organizations should be encouraged. Thus, those organizations that have not yet implemented it should use it because it is a management tool for the preservation and protection of environmental resources, with immediate results, and such an initiative reveals in practice the manifestation of the principles of cooperation, prevention, and environmental information, capable of generating benefits such as meeting regulatory requirements, improving the institutional image and environmental management controls, with the consequent reduction of costs and increase of profits, generating social and environmental responsibility, thus avoiding penalties such as fines, notifications, sanctions and/or lawsuits.

We highlight that the results obtained cannot be generalized because the research strategy used was a single case study, whose conclusions represent only the reality of the Military Organization analyzed. Thus, it is suggested to expand the scope of the research, covering a more significant number of Military Organizations, or even conduct new research to identify other procedures and deepen the environmental auditing techniques applied by a group of Military Organizations.

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Computer Vision-Based Hand Baggage Inspection System

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Object detection is a crucial task in computer vision. It brings two essential tasks, classifying and locating the object in the image or video. This task is performed by specialized human operators, which requires the knowledge and experience for reliable detection. However, the high volume of work often drives the operators to exhaustion, causing a low prevalence of targets. This work aims to build a model using a Deep Neural Network (Darknet) and a single pass object detection method (YOLOv4) for the detection of handguns, shurikens, and blade shavings in hand luggage, using the data set GDXray. The YOLO algorithm model identified different items that could impact security, which could be a tool that makes passenger boarding faster and safer.
Keywords: Object Detection. YOLO. Neural Network. Computer Vision. Deep Learning.

Introduction

Air transport is essential for the economic and social development of a country, and due to the possibility of fast connections, it facilitates the movement of people and goods and boosts commercial activities and tourism [1].

The Brazilian National Civil Aviation Agency (ANAC) was created to oversee civil aviation activities and aeronautical and airport infrastructure. Established in 2005, it began operating in 2006 and is a federal autarchy with a special regime linked to the Ministry of Infrastructure.

In 2021, according to ANAC [2], approximately 62,583,158 passengers were transported. Therefore, searching for processes and tools that help make air travel faster and safer is essential, mainly due to the increased operations in the Brazilian air market. Therefore, it is possible to highlight the large amount of flow of passengers and luggage that has dramatically lengthened the response time of security agents at boarding. Furthermore, due to the importance of this transport, the concerns about air security are severe. Therefore, one of the measures adopted at airports is the X-ray screening of passengers' bags at security checkpoints, being a key component to ensure that prohibited items do not get into airplanes.Currently, this procedure is carried out by inspectors who are specialists in baggage tracking, who, during screening at checkpoints, visually inspect the X-ray images of passengers' bags to decide whether they are harmless or contain prohibited items.

Visual search challenges include:

- Low prevalence of targets,
- Variation in target visibility,
- Searching for a set of unknown targets,
- The possible presence of multiple targets,
- External variables such as job satisfaction.

Computer vision techniques can minimize this problem automatically, quickly, and reliably, enabling the detection and classification of prohibited and potentially dangerous objects for security in passengers' hand luggage. Such a solution can maintain alertness and improve human operators' detection and response time, thus ensuring air transport safety.

According to Butler [3], the ideal system would have a high processing rate, low initial and operational costs, and low rates of false readings (false-positive, false-negative). A false positive reading occurs when the system identifies an object as being dangerous when it is not. This point becomes a problem because these false readings lead to other decision-making that slows down processes, annoys passengers, and

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costs money. On the other hand, the false negative is just as significant as it occurs when the dangerous object is present, but the system cannot detect it. This work compares five primary means of baggage inspection, favoring X-ray systems for their great processing capacity but indicating their high cost for implementation and high false negative rates.

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This work aims to build a model using Darknet and the YOLOv4 algorithm to detect possible threat objects through X-Ray images in the initial screening of passenger luggage. The detection of objects of interest will use the GDXray dataset, a single-pass object detection method to locate and classify revolvers, shurikens, and blade shavings. These objects pose a possible threat to safety when boarding passengers.

Related Literature

Hassan and colleagues [11] present a deep multiscale structure tensor-based framework that can automatically extract and recognize regular and suspicious items, independent of their position and orientation, from multivendor X-ray scans. The proposed framework is unique as it intelligently extracts each object by iteratively picking transactional information based on contours of different orientations. Furthermore, it uses only a single Feedforward Convolutional Neural Network for recognition. The proposed structure was tested on two publicly available datasets containing 1,067,381 X-ray scans and uses the pre-trained ResNet architecture for object recognition.

Akcay and colleagues [12] considered using deep convolutional neural networks with transfer learning for image classification and detection problems in the context of X-ray luggage security images. A pre-trained CNN was used in generalized image classification tasks, aiming to overcome the problem of the limited availability of examples. Using YOLOv2, using images of size 544x544 as input, a mAP of 88.5% was achieved for a six-class detection problem. The same approach, with an input size of 416x416, produced a 97.4% mAP for the two-class firearm detection problem. Akcay and Breckon [13] aim to review computerized X-ray security imaging algorithms by taxonomically fielding conventional machine learning and contemporary deep learning algorithms. The proposed taxonomy subcategorizes deep learning approaches into supervised and unsupervised learning, focusing on object classification, detection, segmentation, and anomaly detection tasks. It also explores well-established X-ray datasets and provides a benchmark of performance.

This project is an alternative to the object detection problem, as we apply a single Deep Neural Network (Darknet) to the complete image. This network divides the image into regions and predicts bounding boxes and probabilities for each region, using the YOLOv4 algorithm that proved to be faster and more accurate than its previous versions.

Materials and Methods

Detection Algorithms

Several methods seek to solve the object detection problem. The Harr Cascade technique was considered the first approach to achieve satisfactory results that could be implemented in real applications. With the rise of Deep Learning, methods based on Convolutional Neural Networks (CNN) were developed, such as R-CNN, Fast R-CNN, SPP-net, and YOLO. Convolutional Neural Networks recognize patterns in data, usually organized in layers, each with weights that are adjusted during the training phase, allowing the network to adapt to the problem and the given dataset. They emerged from studying the brain's visual cortex and have been used in image recognition since the 1980s. In recent years, thanks to the increased computational power, and the amount of training data available, CNNs have achieved superhuman performance in some complex visual tasks.

You Only Look Once: YOLO

YOLO [4] uses Deep Learning and Convolutional Neural Networks for object detection, one of the fastest algorithms being the object detection problem in images. The image is divided into an SxS grid of cells to perform detection. Each of the Cells will predict "N" possible bounding boxes. Most of these bounding boxes will have a very low probability, so the algorithm deletes the boxes below the minimum probability threshold. Bounding boxes with a probability above a threshold are selected to locate the object within the image.

In YOLO, a single convolutional network predicts each detected object's bounding boxes and the class membership probabilities. Unlike classification, which only seeks to predict the class present in the image, object detection, in addition to predicting the class, also needs to identify the object's location in the image, using a Convolutional Neural Network as a feature extractor.

Darknet

YOLO uses a deep neural network called Darknet to implement object detection. This framework is open source, written in the C language, and has GPU support.

Dataset

The data set used in the work is the GDXray [5], provided by the Department of Computer Science of the Catholic University of Chile. The images are organized in a public database that can be used free of charge but for educational purposes only. The database includes five groups of X-ray images: castings, welds, luggage, natural objects, and configurations (Figure 1). In addition, we use 793 X-ray images that can be used for the automatic detection of guns, shurikens, and steel blades to shave.

Proposed Model

The Darknet neural network was implemented to generate embeddings in the Google Collaboration (Colab) environment using a free account with access to the GPU resource. In the accessible version of Colab, the access to GPUs is very limited, and the notebooks run for a maximum of 12 hours.

The pre-processing step involves data standardization and is fundamental for applying Artificial Intelligence techniques. So in this project, we standardized the images in PNG (Portable Network Graphic) format and separated the images into two main sets "Train" and "Test". The Train set contains 713 images that will be used exclusively for network training, while the Test file contains 80 images that will be used exclusively for network testing. After properly separating the dataset, we move to

Figure 1. Sample GDX-ray baggage.

the image annotation stage with their respective classes. We used LabelImg [6], an open-source graphic image annotation tool that supports the task's YOLO, CREATEML, and PASCAL VOC formats. This step aims to help models learn patterns from the data set and use them for future predictions, the work was dedicated to detecting three classes (revolver, shuriken, laminaBarbear), and we used LabelImg to create bounding boxes in the objects of interest, these boxes contain the characteristics of the class, coordinates, height, and width. After annotating the images, we trained and tested the object detector using YOLOv4 and Darknet, performing three training rounds varying the learning rate and momentum of the model. The algorithm was configured with three values for the learning rate (0.001, 0.00261, 0.1), for the momentum (0.9 and 0.949), and the number of epochs in 4700. These parameters have already been used in the literature for training object detection networks using YOLO.

The validation metrics are used to analyze the model's quality, bringing the performance information of the experiments [7]. We use a set of these metrics to gauge how far the model is from perfect detection. In this article, we used the metrics Precision, Recall, F1-score, Confusion matrix, Intersection over union (IoU), and Mean Average Precision (mAP). The Confusion Matrix indicates how many examples there are in the true positive (TP), false negative (FN), false positive (FP), and true negative (TN) groups. Allowing one to easily view how many examples were classified correctly and wrongly in each class. Precision is the metric that evaluates the number of true positives over the sum of all positive values.

$$\text{Precision} = \frac{\text{True Positive}}{\text{True Positive} + \text{False Positive}} \quad (1)$$

It is common to consider the combination of Precision and Recall, also known as sensitivity, as

it measures what fraction of the positives our model identifies [8].

$$\text{Sensitivity} = \frac{\text{True Positive}}{\text{True Positive + False Negative}} \quad (2)$$

The F1-score measure is the harmonic mean of accuracy and sensitivity. This metric evaluates the method's ability to detect positive results successfully.

$$f1 = 2 \text{ x } \frac{\text{precision x sensitivity}}{\text{precision + sensitivity}} \quad (3)$$

Mean Average Precision (mAP) and Intersection over Union (IoU) metrics are popular for checking the performance of object detectors. The IoU compares the bounding boxes with the detected boxes, returning a normalized score. For example, we can calculate the mAP by taking the average of all the average Precision of each class, that is, the average of the area of the Precision-Recall curve of each class.

$$mAP = \frac{1}{n}\sum_{k=1}^{k=n} AP_k \quad (4)$$

AP_k = the AP of class k
n= the number of classes

Result and Discussion

Our study has three possible scenarios that differ in the learning rate and momentum. We have YOLO configured with a learning rate of 0.001 and the momentum term 0.949, providing the confusion matrix in the first scenario (Table 1).

Table 1. Confusion matrix (Scenario 1).

Learning Rate = 0.001	True Positive	False Negative
Handgun	44	4
Shuriken	27	2
Blade shaving	32	3

We modified the training parameters in the second scenario, 0.00261 and 0.9, respectively. Finally, Table 2 presents the confusion matrix.

Table 2. Confusion matrix (Scenario 2).

Learning Rate = 0.00261	True Positive	False Negative
Handgun	44	4
Shuriken	27	2
Blade shaving	32	3

In the third scenario, YOLO failed to converge the training and provided no performance metrics. Training parameters were 0.1 for learning rate and 0.5 for momentum. Table 3 provides metrics for all scenarios. Given the metrics presented by the models, we noticed that the algorithm of the first scenario performs better in the object detection task.

Figure 2 presents a graph demonstrating the evolution of training concerning the number of periods. We also submitted images of luggage that were separated before training to validate the model. Finally, we present the results in Figures 3 to 6.

Conclusion

This work demonstrated the applicability and viability of using YOLOv4 to detect objects in hand luggage at airports, which could be a tool that makes passenger boarding faster and safer, having as a driver the growing demand of the air market that indicates an average growth in an optimistic scenario of 6.14% until 2037. Applying the Deep Learning model provided mAP results more significant than 95% in the two main scenarios (Scenario 1 = 98.79%%, Scenario 2 = 97.62%) (Table 3).

From the analyses, YOLOv4 is a good algorithm for object detection, mainly if we consider the challenges of the visual search, which include a low prevalence of targets, the variation in the target's visibility, the presence of multiple targets, and external variables. Furthermore, despite the need for more significant computational resources, the technique had a performance gain in inference and assertiveness (Figure 2), in addition to the option of applying more effective techniques to the processing on GPUs.

Among the techniques that could not be applied is using other versions of the YOLO algorithm, such as version 5, created in 2020, and version 7 of the same algorithm. These techniques could increase the performance and predictability of the model analyzed in the work. However, studying these algorithms remains a suggestion for exploring future work.

Table 3. Scenario performance metrics.

Taxa de Aprendizado	Momentum	Epocas	Precision	Recall	F1-score	Average loU	mAP
0.001	0.949	4700	0.92	0.99	0.95	73.67%	98.79%
0.00261	0.9	4700	0.94	0.96	0.95	75.24%	97.62%

Figure 2. Training evolution chart.

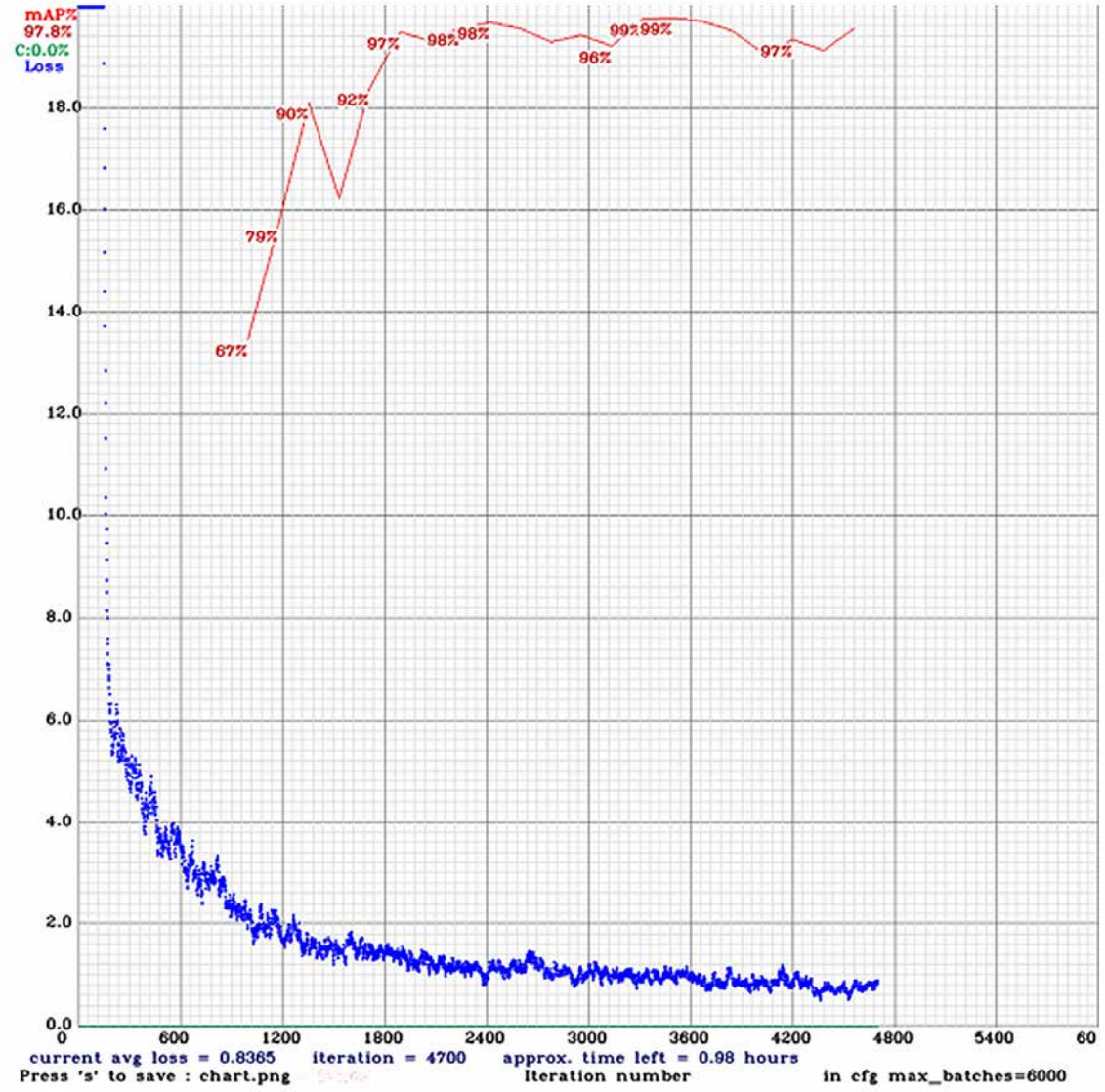


Figure 3. Handgun and shuriken detection.

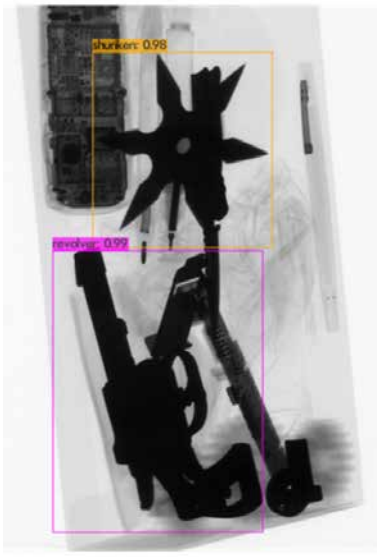


Figure 4. Shuriken and blade shaving detection.

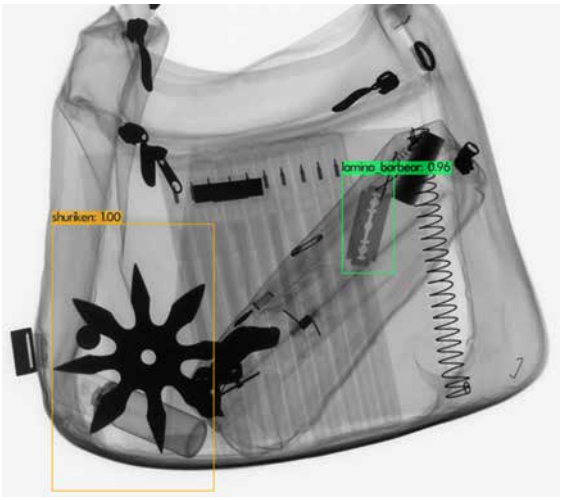


Figure 5. Detection of a blade shaving in a wallet.

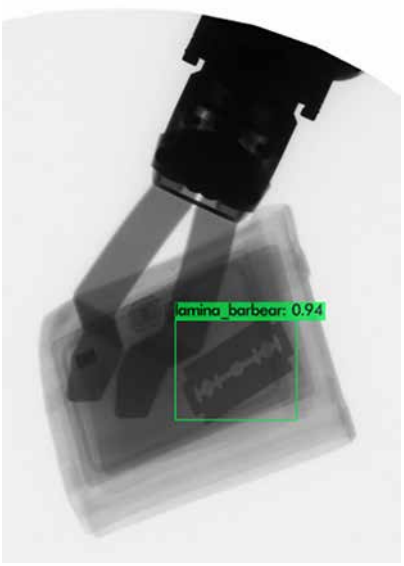
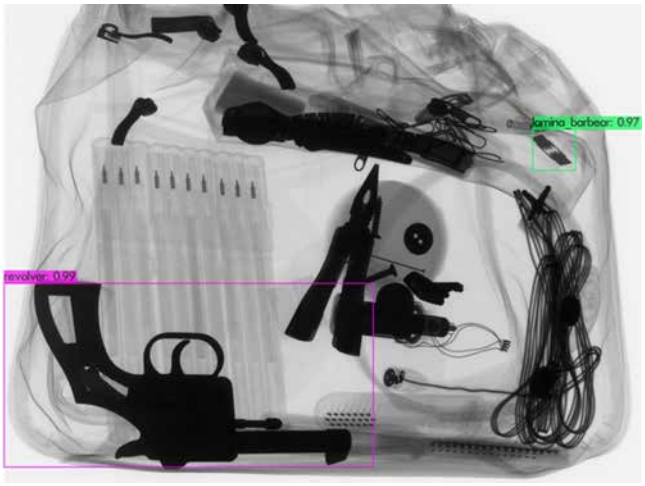


Figure 6. Handgun and blade shaving detection.



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Magnetic Omnidirectional Wheel for Ferromagnetic Surface Cleaning Robots

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Robots for cleaning/inspecting surfaces ferromagnetic are used to guarantee safety and speed. The robot's versatility is by using magnetic fields to obtain adhesion on a ferromagnetic surface. However, there are limitations to the magnetic force and maneuverability of the robot. This article aims to present the development of a magnetic mecanum omni wheel, using additive manufacturing to generate extra magnetic force to assist robots in adhering to ferromagnetic surfaces with high maneuverability. For this, we realized studies about the magnetic arrangement, topological optimization, structural analyses, and practical tests to determine the magnetic force until obtaining the final wheel concept.
Keywords: Robot. Cleaning/Inspecting Surfaces. Ferromagnetic. Magnetic. Mecanum Omni Wheel. Additive Manufacturing.

Introduction

Additive manufacturing (AM) has gained space in the manufacturing environment, becoming an excellent substitute for conventional manufacturing processes. We defined it as a highly automated manufacturing process by adding material, layer by layer, to form a physical part designed on 3D software [1].

One of AM's advantages over conventional processes is its sustainability, as it manufactures parts by adding materials and not removing them. In addition, one of the fields that benefited from additive manufacturing was robotics because of the design's freedom to obtain light and resistant parts, ideal characteristics for robots [2].

One use for robots is for cleaning and inspecting the hulls of ships. The application of robots, autonomous or remotely controlled, facilitates the cleaning, avoiding many risk exposures. These robots often use a central magnet system or suction cups to attach to the hulls and hydro blaster or rotating brushes to clean the surface.

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Cleaning is essential because marine life can grow over the submerged hull surface (algae, barnacles, mussels, corals). As the vessels move around the world, they can cause the introduction of non-native species in different regions, provoking an imbalance in the ecosystem. Additionally, the accumulation of marine files causes an increase in surface roughness, thus increasing the drag force during the vessel movement and, consequently, higher fuel consumption [3].

However, robots usually present linear traction systems based on wheels or continuous tracks. These traction systems present limitations, and maneuvers can take longer to perform the desired movement. For this reason, using omnidirectional wheels on robots, which can move in any direction, could be an alternative.

This work proposes the combination of omni wheels with magnets to be applied in ship hull cleaning robots. The equipment should present better maneuverability and increased fixation on the ferromagnetic surface, being able to reduce the central magnet system. Furthermore, the wheel was designed for additive manufacturing to reduce costs and increase performance.

Omnidirectional Robots

Omni robot has 3 degrees of freedom (translating in two directions and rotating around itself the center

of mass), allowing movement in any direction. In addition, the wheels allow this movement, also called omnidirectional [4].

There are two categories of omni wheel. The first is "conventional" wheels. However, they cannot be considered omnidirectional because there must be at least 2 of them in the structure, and each must have an actuator to reorient them in the XY plane. The other categories are "special drawing" wheels, considered genuinely omnidirectional. Figure 1 shows the two categories and their types [5].

Magnetic Principles

Magnets work by attracting some materials, called ferromagnetic, and this attraction is most potent at their poles. Every magnet has two poles (north and south), and like poles repel each other, different poles attract each other [6]. The force of attraction decreases dramatically with increasing distance between the magnet and the surface.

Using unique magnet arrays, such as Halbach's, creates a strong force of attraction. According to Masi (2010) [7], this array is defined as an organization of permanent magnets (90 degrees out of phase with each other) that maximizes the flux of the magnetic field on one side and minimizes it on the other side, provoking more required magnetic fields.

Materials and Methods

Figure 2 shows the workflow followed during the Project's development. Initially, we studied omni wheels' history, types, applications, and characteristics. Then, we also realized another study about magnetism and joined the two themes.

The omni wheel chosen was mecanum because it is genuinely omnidirectional, compact, and supports high efforts. Another decisive factor for our choice was many projects involving the universal omni wheel with magnetism.

An evaluation was performed to determine which printing process would be used to manufacture the wheel. The chosen process was MJF (Multi Jet Fusion) because the excellent printing speed, surface quality, and dimensional accuracy (±0,2%) make this method very attractive [8]. The material chosen for this printing process was PA12.

Practical tests involving magnets in different arrays seek to better understand the magnetic principles and obtain more reliable results. Therefore, the test was realized using 5x5x5 mm magnets acquired by IMÃSHOP website. According to the company, one of these magnets can vertically sustain a mass of approximately 940 grams in direct contact with the ferromagnetic surface.

After the tests, the final 3D model was made using Solidworks CAD software. Finally, static and topological analyzes were realized by using the Altair Inspire software.

Material Analysis

The material choice was PA12 from the company HP. According to O'Connor, Dickson, and Dowling (2018) [9], this material is one of the most used MJF processes because its melting temperature

Figure 1. Classification of omnidirectional wheels.

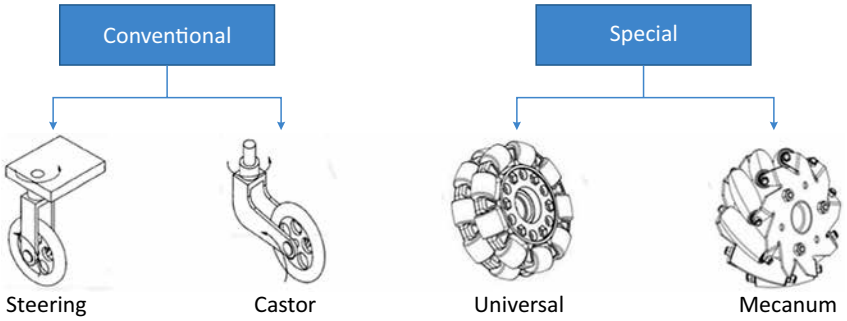
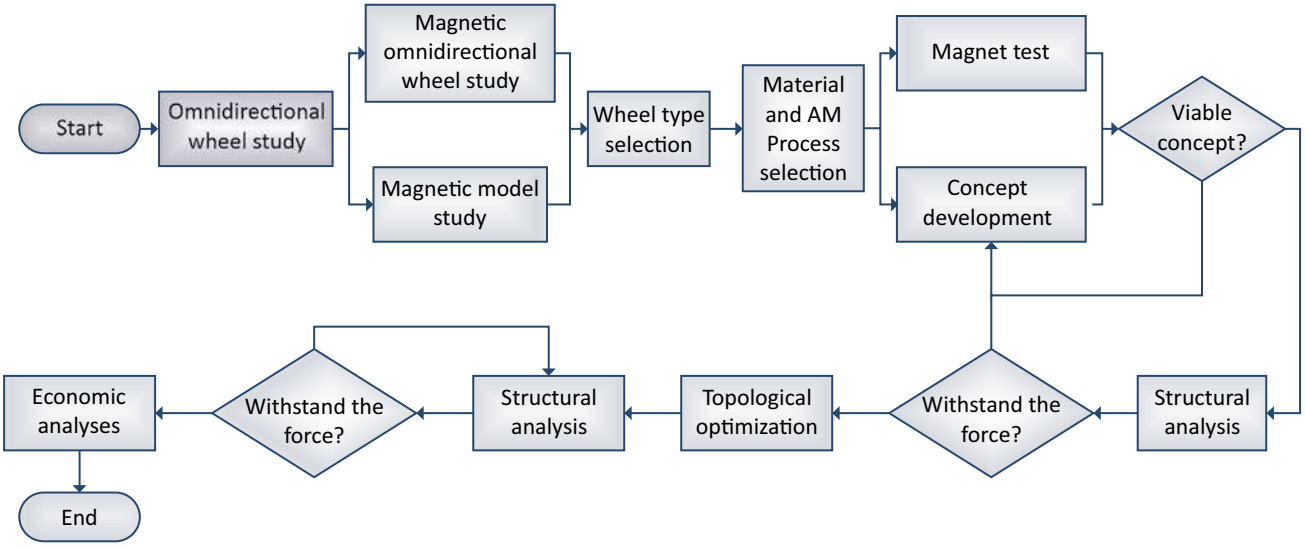


Figure 2. Workflow.



is higher than that of crystallization, and this delay in the recrystallization process during the manufacturing, causes a reduction in the residual stresses and distortions in the piece. However, PA12 is not waterproof, and as the wheel will have contact with the water, a post-process of the piece after printing is required. According to Dizon *and colleagues* (2021) [10], adding a thin layer of epoxy resin, vinyl acrylate, or silicone is sufficient for post-processing.

Another issue is the failure criteria for the material. Since PA12 is a polymer, the failure criterion to be adopted must be based on the deformation. According to Erhard (2006) [11], a good practice for operating with polymers is to adopt the working stress associated with 80% of the yield stress. O'Connor and Dowling (2018) [9] realized the tensile test with PA12 samples by MJF, and the yield region is similar until entering the plastic zone to the failure (Figure 3). The tensile strength is similar to HP's PA12 (50 MPa).

PA12, used by O'Connor e Dowling (2018) [9], was considered similar to HP. Thus, adopting the good practices of Erhard (2006) [11], 40 MPa was considered the maximum stress allowed. Therefore, the combined stress failure criterion adopted was von Mises.

Results and Discussion

Magnet Arrays

A smaller number of magnets were used in the Halbach array to get an increment in magnet force. For that, we developed three arrays to be analyzed in practical experiments. Figure 4 shows: (a) a circular array, (b) a linear array, and (c) an inner linear array that was idealized to be in the inner part of the roll and follow its curvature.

Magnets' Experiments

Experiments were performed with the acquired magnets to test the Halbach array's forces and compare them with its values in the usual array (magnets with the same poles faced the same sides).

For the linear array, experiments considered four values of the gap between the magnet and the surface: direct contact (0 mm), 0.642 mm,1.128 mm, and 1.608 mm. Plates of PLA (polylactic acid) were printed using an AM known as FDM (*Fused Deposition Modelling*) to get these gaps. On the other hand, the circular array just used a fixed value for the gap (1.258 mm) to make a structure for the experiments, and it already had

Figure 3. Stress-strain curve of PA12.

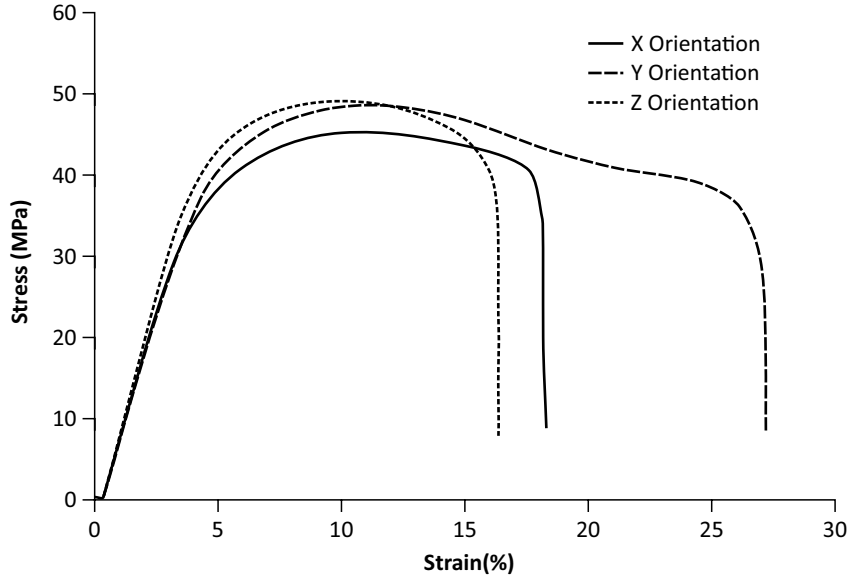
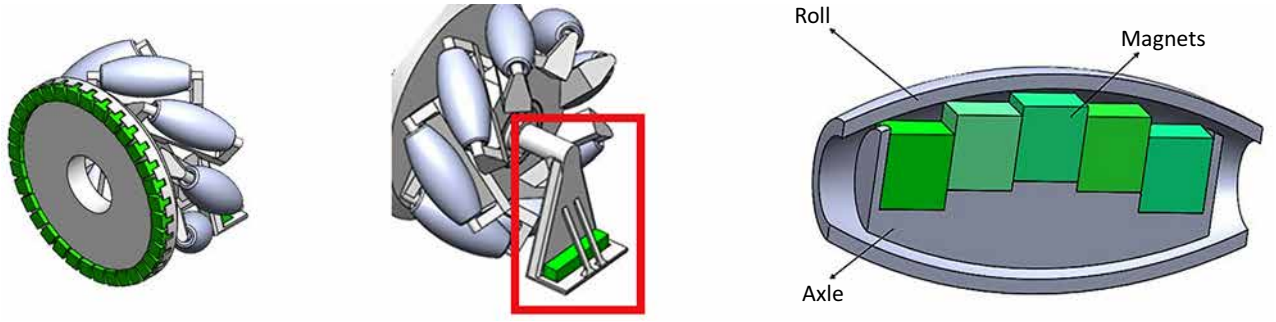


Figure 4. Halbach arrays proposed.



a specific thickness between the surface and the structure.

The experiments were done by binding the magnet arrays on a bottle (Figure 5). The weight of the bottle increases until the attraction force cannot hold, and the bottle falls by putting the water inside the bottle slightly using a funnel with a hose.

After that, the measurements of the system were to get the total mass that the array can hold and, as a consequence, the attraction force.

Figure 6 and Table 1 show the results for linear arrays and circular arrays, respectively.

Final Concept

After the test results and the 3D model, we observed that the magnets would be far from the surface (due to the rollers' shape) in the internal array, and the attraction force generated would be negligible. So, this idea was discarded.

Regarding the Halbach linear array, the distance of the magnets from the surface, combined with the size of the wheel, just three magnets could be used without exceeding the geometric limits of the wheel, making it difficult to move.

The final solution was developed using circular Halbach arrays in the structure. Figure 7 shows the final concept before and after the topological optimization.

It was not advantageous to use magnets around the entire circumference of the wheel because the magnets that were not close to the surface would not generate an attractive force, just increasing the mass of the system. For this reason, a rotating structure

Figure 5. Magnets experiments.

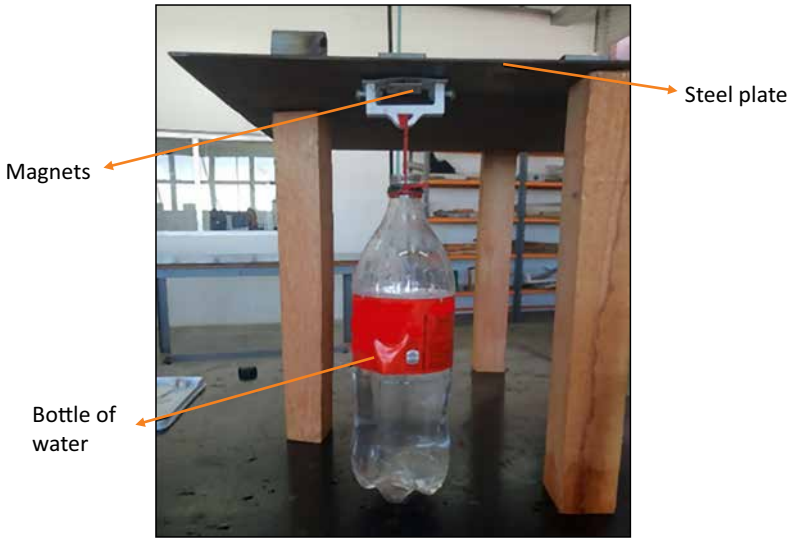


Figure 6. Test results for linear array (regular and Halbach).

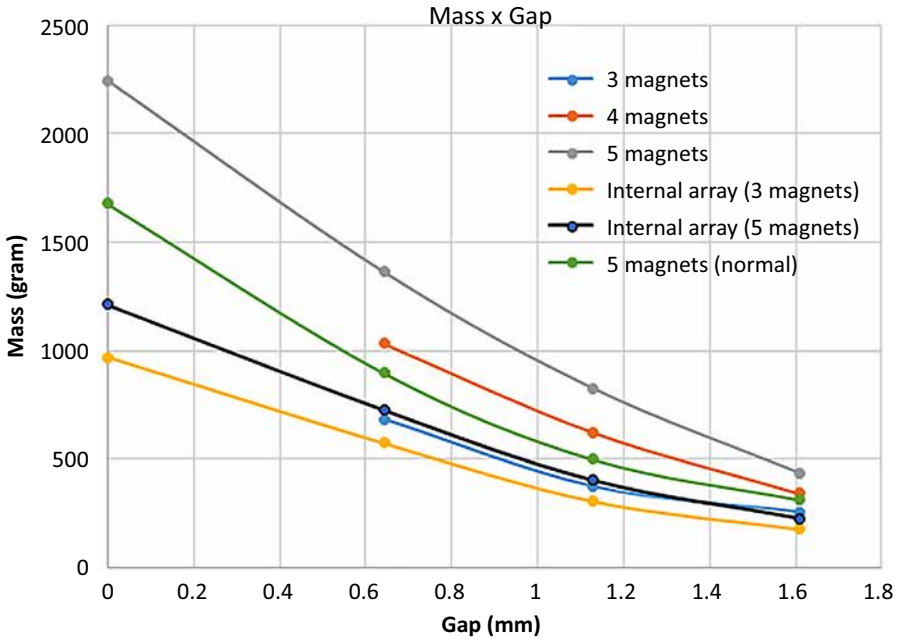


Table 1. Test results for circular arrays (regular and Halbach).

Circular Array	T1 (g)	T2 (g)	T3 (g)	T4 (g)	T5 (g)	Average (g)	Force (N)
Halbach	246	267	242	228	228	242.2	2.376
Normal	190	158	151	150	127	155.2	1.523

(arm) was developed to place seven magnets. Figure 8(a) shows the arm with the magnets in green and the bearings in blue, and Figure 8(b) shows the wheel body.

The static analysis was considered a force of 150 N (¼ of the estimated mass for the robots + attraction force of the magnets). As a result, a safety coefficient of 1.34 for the wheel body was obtained for the roller, 1.46, and a value above 2 for the arms.

Conclusion

Topological optimization reduced the system's total mass by 28% (96.2 grams to 68.92 grams). As a result, the structure in the vertical direction has a mass of 81.52 grams, generating an attractive force of 9.4 N, with seven magnets measuring 5x5x5 mm on each arm.

Future projects can be improved by adding one more row of magnets on each arm, increasing its thickness and/or increasing the size of the magnets with higher attraction force (example: 10x10x10 mm).

The following steps are:

1. Printing the wheel to evaluate clearances and interferences from printing an all-in-one step;
2. The effect of the water on sliding surfaces;
3. The movement of the bearing structure to maintain the magnets in the correct position.

Figure 7. Final wheel concept (a) and the topological optimization (b).

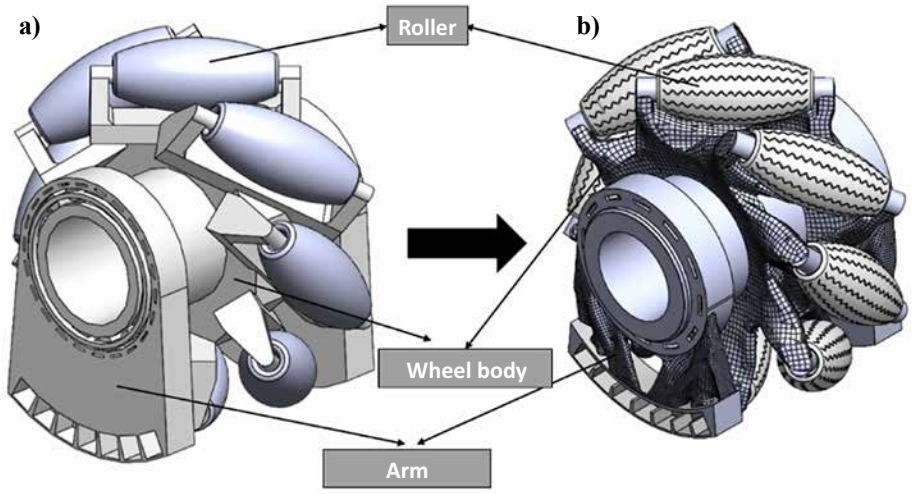
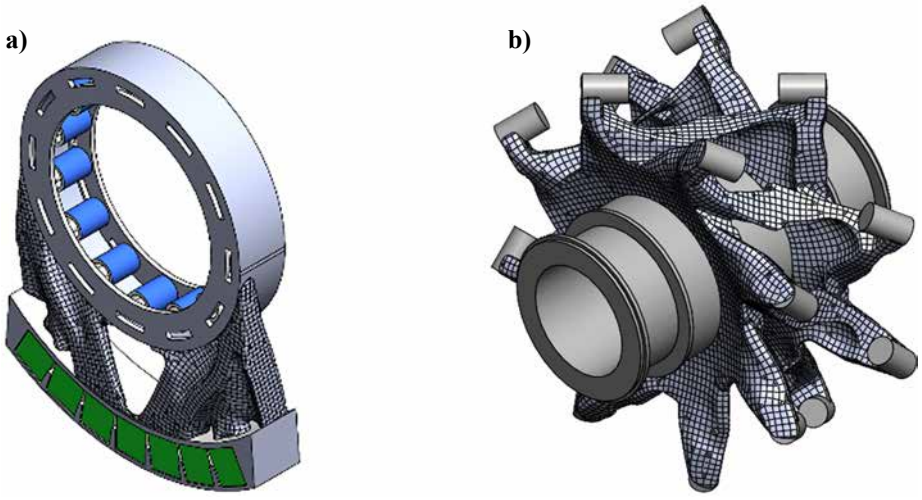


Figure 8. Designed components visualization.



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Industry 4.0 Technological Elements Applied to Steam Distillation

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The essential oil extraction industry is a stimulating target for technology updates in which yield, quality, and energy efficiency are goals to be pursued. This step forward becomes a strong ally in the search for continuous improvement of these key indicators. This work proposes the implementation of Industry 4.0 elements to detect and correct the preferential steam paths (channeling), determine the economic duration of the process, and reduce volatile element losses with efficient condensation. Partial experimental results indicate yield gains more significant than 20% and improvement in product quality indicated by the greater presence of the Citral component in the chromatographic analyses. Furthermore, the experimental results, still ongoing, demonstrate that the design of a plant with sensors, actuators, and process monitoring systems is auspicious.
Keywords: Essential Oils. Steam Distillation. Industry 4.0. Technology Update.

Introduction

Essential oils are natural products extracted from plants (flowers, barks, stems, leaves, roots, fruits, and seeds) with essential applications in industry. Chemically, they are composed of a range of fractions, from the terpenoid family, in a homogeneous mixture, from the most volatile to the heaviest. As a result, they have a pronounced aroma, often pleasing to the human sense of smell. With rare exceptions, their density is lower than water, with a characteristic color for each species varying in intensity [1,2].

Among the various extraction methods, steam distillation is responsible for more than 90% of the worldwide produced volume [3-5].

The steam distillation consists of flowing steam upwards through the aromatic plant (raw material) inside an extraction vessel. Steam has two roles: supply heat to break the cells in which the essential oil is stored (in the plant cells) and carry it until the condenser. The condenser is a shell-tube heat exchanger that liquefies the mixture of steam-essential oil. A separate vessel receives that fluid to isolate the essential oil and the condensed hydrosol. Hydrosol is a stable emulsion of essential oil into the water, hard to break, and has poor economic feasibility. Nevertheless, it is also a market-interest product [1,2,5]. Figure 1 is a sketch of a typical installation with a heat source, extraction vessel, condenser, and vessel for collection and separation (essential oil-hydrosol)

The technological scenario in the EO (Essential Oil) extraction industry via steam distillation brings attractive updating opportunities for yield, quality, energy efficiency, and operational effectiveness continuous improvements [6]. Side by side with technological evolution, environmental performance is increasingly receiving attention. The so-called green extraction brings perspectives and systematization to the initiatives related to sustainable process design [7,8].

For terminology purposes, in this paper, the yield is calculated from the relationship between the extracted essential oil volume (mL) and the raw material mass of fresh raw material (kg). Quality means adherence to specifications and is usually verified via GC – gas chromatography in this industry.

Industry 4.0 is a term used to designate the 4th industrial revolution. It emphasizes the integration of machines with the capability to exchange information and adapt autonomously to market expectations, using its enabling technologies

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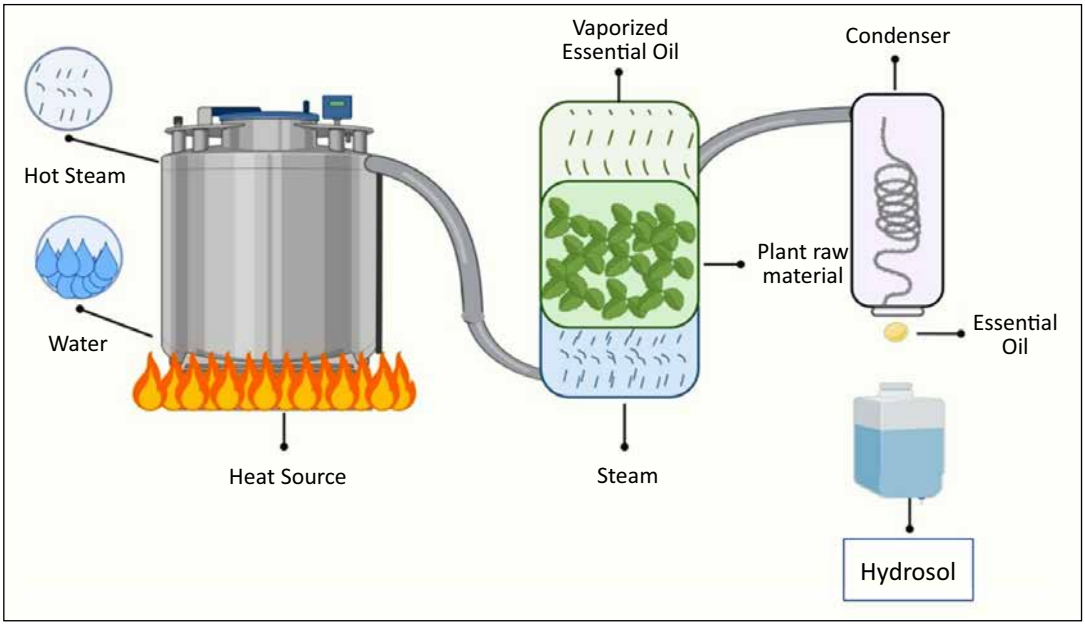
Figure 1. Conventional essential oil steam distillation installation.



and responding smartly to variable and complex demands [9, 10].

Manufacturing is one of the crucial operations within a Supply Chain Organization. Its objective is to meet or exceed performance expectations related to customer service at the right time, quantity, and quality. The technologies brought by wave 4.0 are intended to build capabilities to fulfill business growth and perennity strategies, given the current environment of uncertainties and difficult business predictability [11-13].

Technology update for the essential oil industry embeds innovative instrumentation design for production systems, automatic controls, and process monitoring. The intense dependence on the workforce requires continuous action from the operators to control essential process parameters [14].

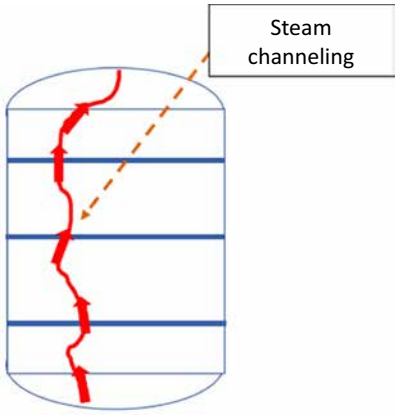
The generation of a process database, its formatting, and analysis enable the investigation to determine good variable correlations and, consequently, create a model. When this model dynamically adapts and proposes improved parameters to the actual process, it is called a digital twin [15]. Moreover, it is a near-real-time digital image of a physical process [10] or, in other words, the cybernetic and physical spaces joining in the manufacturing environment towards optimization [16].

The proposed concept of an intelligent essential oil plant, considered in this work, aims at demonstrating potential benefits arising when proper technology elements are applied, detecting and correcting critical process deviations. These deviations are well-known in the industry, without adequate processes to deal with them. Therefore, this article focuses on three crucial process weaknesses.

The first, channeling, consists of steam paths through the raw material [17] with preferential trajectories (Figure 2). It happens mainly due to the natural anisotropy of the plant medium inside the extractor. This occurrence causes poor yield where the steam flow is less intense and promotes degradation where the aromatic plant is overexposed to heat [1,5,18]. Therefore, channel detection and correction positively impact yield and quality [19].

The second is the determination of the economic extraction duration. This critical process parameter

Figure 2. Steam channeling representation.



also influences both yield and quality. If longer than necessary, light fractions of the extracted essential oil are lost to the atmosphere and overexpose the raw material to high temperatures. If shorter than necessary, extraction is incomplete, affecting yield results [18, 19]. Figure 3 demonstrates how extraction time can impact process results. Instant t1 indicates the maximum slope of the yield curve.

The interval between t1 and t2 presents asymptotic behavior when the yield rate decreases, and the continuity of extraction depends on economic viability; that is, the extracted volume compensates for or exceeds the general production costs. On the other hand, the interval between t2 and t3 represents the time without any economic benefits. In fact, during this period, yield can even decrease once the light fractions can be lost and degradation can take place.

The third is the use of condensation water at a controlled temperature. Usually, extraction industries apply water at room temperature. When chilled water is used, condensation is more efficient, and losses due to evaporation are minimized.

The above-highlighted points are a kickoff for this research field; deepening and exploring such technological subjects to promote a step forward, mainly for small and medium-sized producers who access technology, may become more difficult [5].

There are countless possibilities for digital technology implementation, depending on the operational needs and resources available for investments. However, what is unquestionable is the need for technological upgrading of this industrial branch. Figure 4 shows how precarious some extraction units are.

Thus, the objective of this work consists of a proposal for intelligent digital technologies to improve the extractive processes of essential oils by steam distillation.

Materials and Methods

Figure 5 displays the installation configurations before and after introducing the proposed technological elements, which will be detailed in the text.

Channeling detection and correction, automatic (economic) process duration determination, and condensation water with controlled temperature were the focus of the experiments.

For channeling detection, six thermocouples were installed directly in the raw material, without the thermowell, to obtain quick signal transmission, according to Figure 6.

The module of temperature differences for each pair of thermocouples (TT1 and TT2; TT3 and TT4; TT5 and TT6), when above an adjusted setpoint (to be determined experimentally), indicates that the steam is flowing preferentially through a path (the channeling). When detected, a correction occurs with the stepper motor, with small pulses to re-accommodate the aromatic herb inside the extractor vessel, continuously seeking to suppress the channeling.

For the economic process duration determination, an image processing system was applied. The essential oil–hydrosol mixture is collected and separated in a transparent vessel. So, the camera detects the level it becomes stable, and the extraction continuity would not be economically justified.

Water for condensation was designed to be supplied with a controlled temperature from a chiller.

Figure 3. Yield (Y) vs. extraction duration (t).

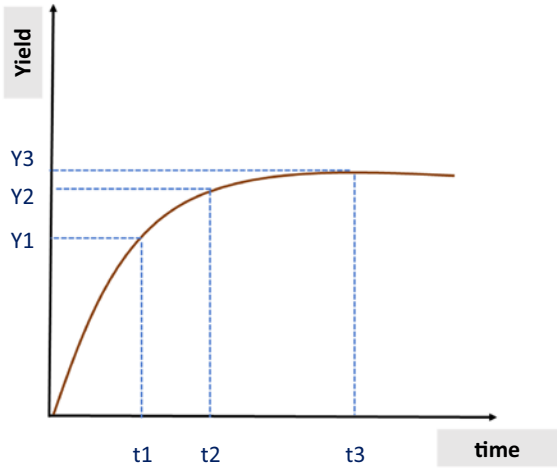


Figure 4. Typical steam distillation installation.

Figure 5. Installation before and after proposed interventions.

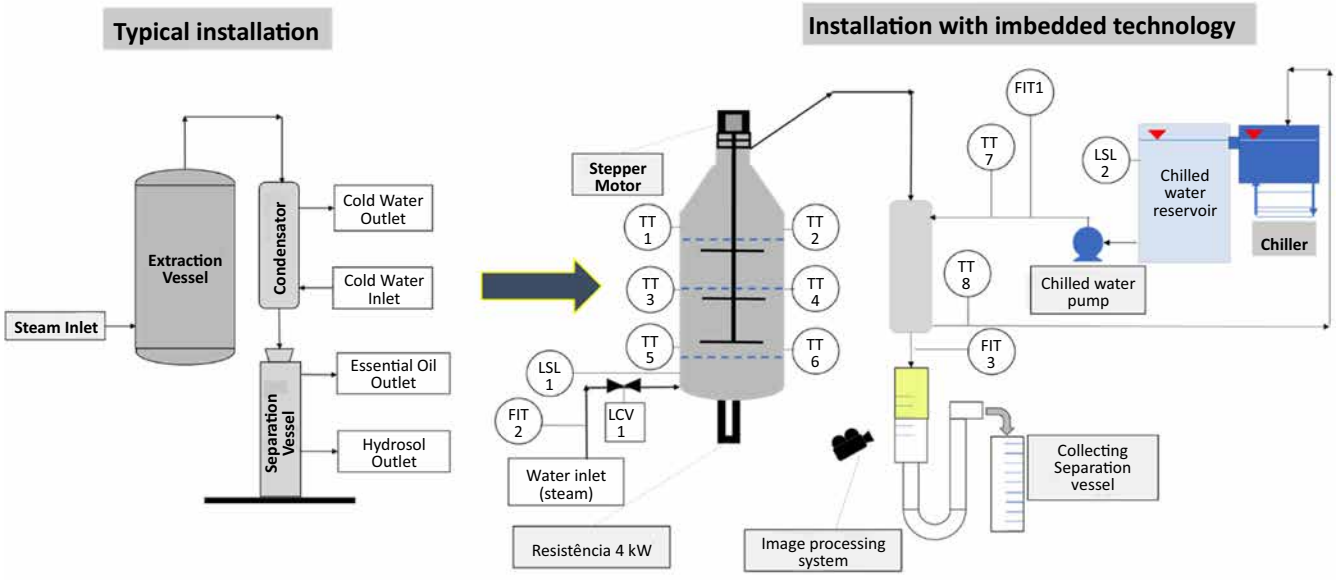
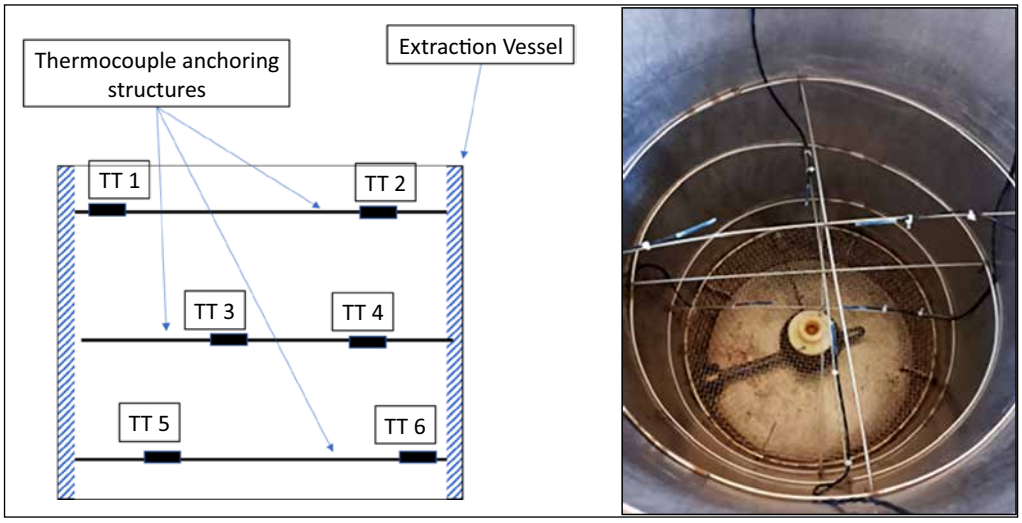


Figure 6. Channeling detection - Thermocouple installation detail.



All these systems were controlled by two PLCs (Programmable Logic Controller) and a SCADA (Supervisory, control, and data acquisition system), and MMI (Man-machine interface).

As a raw material for the experimental phase, lemongrass (*Cymbopogon citratus*) was selected due to the availability of prompt supply. Preparation consisted of drying in the shade for 48 hours and chopping for approximately 20 mm particle size. The essential oil of lemongrass presents Citral as the main component of interest. Therefore, Citral content will be considered a quality indicator. Analysis was performed using chromatography.

Results and Discussion

Partial experimental results indicated progress in process indicators when technological elements are added.

Channeling was detected as expected (Figure 7). The plot of process temperatures TT1 to TT6 indicated differences in channeling evidence. Each pair of thermocouples, at the same level, should indicate approximately the same temperature (TT1 and TT2; TT3 and TT4; TT5 and TT6).

Channeling correction occurs when temperature differences (module) exceed 5 C. The stepper motor was driven to move at a slight angle of 3 to 5 degrees and accommodate the raw material.

The image processing system detected the progress and determined the economic duration of extraction. The decision to conclude the extraction comes from comparing the value of the essential oil extracted in the last period with its operational cost. The processing time, usually 2 hours in the industry, could be reduced to 40 – 50 minutes, meaning a significant capacity increase and energy consumption reduction. Figure 8 illustrates one of the experiments.

Preliminary yield results were 3,7 mL/kg and 4,6 mL/kg, without and with embedded technology), respectively. A sequence of experiments will be performed to confirm that positive trend. Quality-wise, the content of Citral increased from 69% to 76%, detected by gas chromatography, so far confirming that condensation water with controlled temperature is adequate.

Conclusion

The ongoing experimental phase indicates that the objective of exploring technological updates for essential oil extraction via steam distillation can bring yield and quality enhancement, as well as reduce process duration and energy consumption. The continuity of the experiments should confirm this positive trend obtained so far.

Figure 7. Channeling: temperature profile.

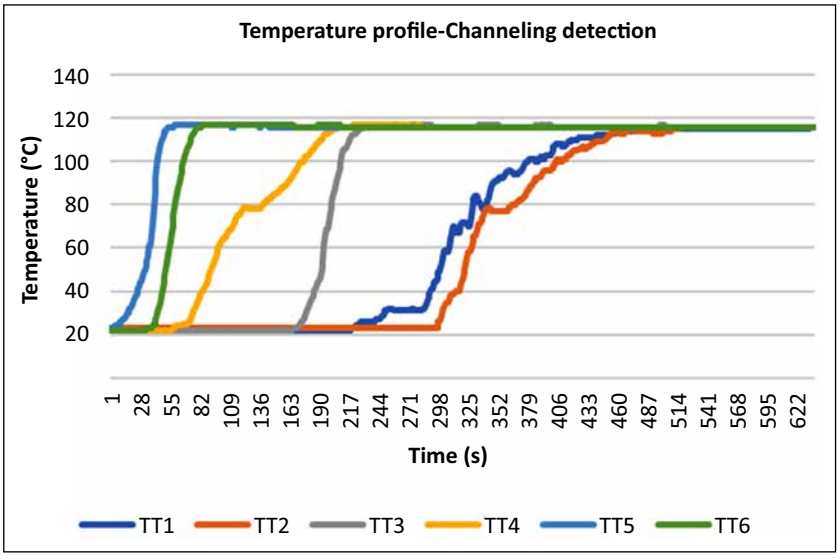
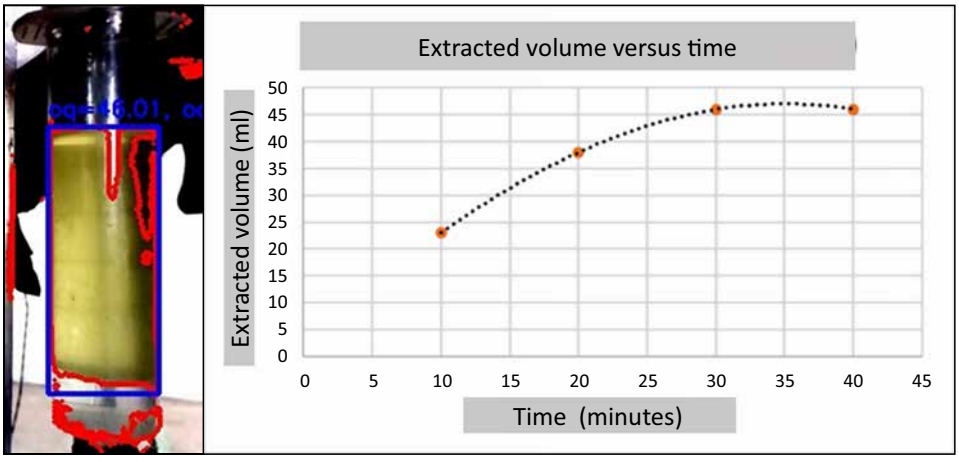


Figure 8. Image processing system determining the extraction endpoint.



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Soiling in Photovoltaic Systems and the Negative Effects of Unpreventive Maintenance

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Soiling in photovoltaic system equipment is one of the main parameters that negatively impacts its performance and useful life. This work presents a case study of dirtiness most frequently found in distributed generation photovoltaic systems and the negative effects of no preventive maintenance. The study was based on 96 maintenance reports from a company in the solar energy segment from May 2021 to May 2022. Among the maintenance performed, 84.37% were preventive, 15.63% were corrective, being found 100% were dust on modules and inverters. This study highlighted the problems caused by the soiling in photovoltaic systems and expects to encourage more preventive maintenance due to the real productivity improvement after cleaning.
Keywords: Solar Photovoltaic Energy. Preventive Maintenance. Efficiency. Soiling in Photovoltaic Modules. Distributed Generation.

Introduction

Installing a photovoltaic (PV) system for solar power generation benefits those investing in this technology. The main advantage is the economy promoted in the energy bill, covering the entire cost of the consumer unit. Another advantage is the possibility of sending energy credits to reduce the consumption of other units within the same state and in the same ownership. In addition, the return on investment is becoming increasingly short, which makes it more interesting [4,11]. Since ANEEL Normative Resolution No. 687/2015 [3], revising resolution 482/2012 [1,2] entered into force in Brazil, the adhesions by PV systems have been gradually increasing in the country, leading to the current number of 1,017,642 consumer units with solar photovoltaic energy installed and connected to the national grid. Of this total, 77.8% are residential, 12.3% commercial, 7.7% rural, 1.9% industrial, and 0.3% public sector [7]. However, to generate energy, reduce self-consumption, and contribute to the planet's sustainability, it is necessary to understand the PV function and continuously follow up this system throughout its operation since preventive maintenance is fundamental to guarantee productivity. This maintenance is also essential to ensure the safety and useful life of the equipment. As the performance level of the equipment in a PV system decreases over time, frequent inspections can help minimize the drops in energy efficiency [10]. The soiling in PV systems is considered one of the main parameters that negatively impacts equipment performance. One definition for soiling is the reduction of effective solar irradiation due to absorption, scattering, and reflection by contaminants present on the surface of the photovoltaic module [6]. The presence of particulates in the Earth's atmosphere occurs in many ways. These particulates, transferred by the wind, come from the soil, pollution produced by industries, automobiles, burning, construction, the presence of animals, and other sources. Thus, the dust and the deposition profile characteristics have a highly regional and seasonal character, specificities that result in the most various percentages of efficiency loss [12].

Regarding the loss of efficiency of photovoltaic modules, soiling is the third most important environmental factor which impacts the power value produced by a solar energy generation system, being inferior only to irradiation and temperature [6]. Although photovoltaic modules are installed with a tilt equal to or close to their local latitude for

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maximum use of solar irradiation throughout a year [5], this tilt cannot be large enough for the modules to be cleaned by rain. Sometimes, the place does not get constant rainfall or has very long dry periods, such as in the semi-arid region. Other times, leftover dirt will accumulate on the module frame and sediment over time, requiring external action. After the dirt has been sedimented, it becomes embedded in the module, making self-cleaning difficult. The soiling can cause irreversible damage to the modules, not just temporary loss of efficiency. There are other impacts on PV energy production, e.g., the mismatch due to dirt inhomogeneity, which is an electrical incompatibility of voltage and current between clean and dirty cells in the same series of modules, generating thermal stress that can contribute to the development of micro-cracks in the cells [6].

Solar inverters are also affected by dirt accumulation. The equipment has an air inlet and an air outlet, making it even more likely that the presence of soiling them. In addition to dust, this kind of equipment is attractive to animals because it offers an environment higher than the ambient temperature. This accumulation forms a barrier and prevents heat exchange between the equipment and the environment, causing an increase in internal temperature and consequently generating a drop in power. It occurs through a defense mechanism of the solar inverter to control the temperature, reducing the production of electrical energy [8]. In this context, this work aims to present, from a case study, the most frequent soiling and the problems caused by the lack of preventive maintenance in photovoltaic systems for solar energy generation belonging to the distributed generation system in the residential and commercial sectors.

Materials and Methods

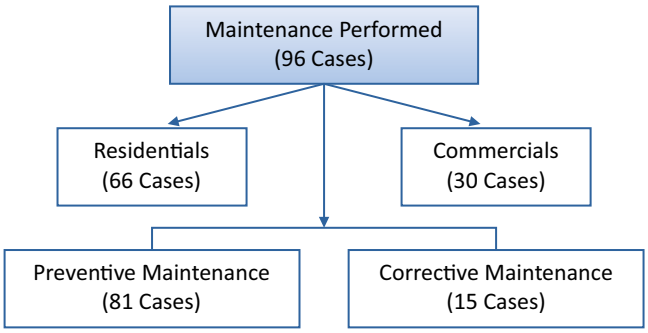
The procedure adopted in this work was the quantitative and qualitative analysis of data provided by a company that has operated in the renewable energy sector since 2016, with more than 1,300 micro and mini generation plants installed in the states of Bahia, Pernambuco, and Piauí. Throughout its operation, this company encountered several situations during maintenance, generating an extensive database that favored elaborating a case study. In total, 96 technical reports of preventive and corrective maintenance performed between May/2021 and May/2022 were available for data collection, classified by the type of consumer, types of soiling, and types of maintenance performed (Figure 1). We obtained the percentage values from this classification for quantitative analysis. In contrast, we did a visual comparison of the photovoltaic systems before and after maintenance for qualitative analysis.

Results and Discussion

Regarding the type of consumer, during the analysis of the 96 reports, 66 (68.75%) were from residential systems, and 30 (31.25%) were from commercial systems. The types of dirt found in each maintenance were tabulated and presented in percentage terms to quantify their frequency in solar energy systems. Table 1 summarizes the data collected concerning the types of soiling on the modules and inverters and how many reports they were mentioned. Dust, for example, was cited in all reports.

Among the maintenance carried out in the period in analysis, 81 (84.37%) were preventive-type, and 15 (15.63%) were corrective-type. Preventive maintenance, in general, is an initiative of the service provider company, which suggests a

Figure 1. Flowchart of the maintenance performed by a solar energy company in this study.



periodicity of twelve months for their customers. In photovoltaic systems, preventive maintenance includes the solar plates and inverter cleaning and a complete inspection of all the electrical components (modules, inverter, string box, and connectors) and mechanical components (supports and fixing structure). As for corrective maintenance, most customers call the service provider to diagnose and repair the problem in their photovoltaic system. Usually, the owner notices that the solar inverter has shut down or notices a substantial drop in energy production. Therefore, it was possible to observe that the accumulation of dirt in systems that receive periodic preventive maintenance is very subtle.

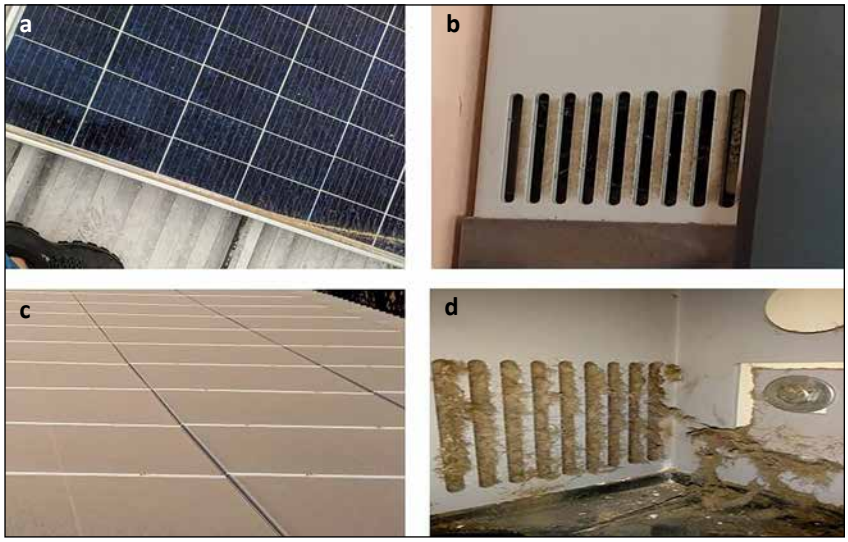
In contrast, soiling is much more severe in systems that do not adopt this conduct. Figures 2(a) and 2(b) show plants that performed maintenance every 12 months. Figures 2(c) and 2(d) present plants that have not performed maintenance for three years.

The inverter monitoring system allows viewing of the electrical voltage, current and power curves over time. Figure 3(a) refers to the curves before maintenance, where it is possible to observe very sharp drops in the system power due to overheating the dirty inverter. Figure 3(b) refers to the curves after maintenance. In this case, the power curve significantly changes, showing the characteristic

Table 1. Frequency of the types of soiling found in the reports.

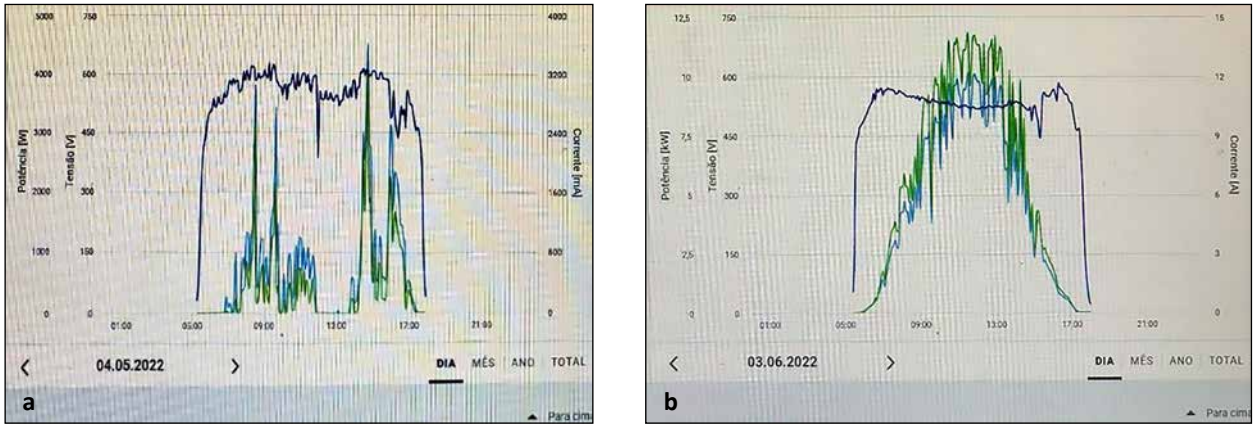
Soiling	Modules	Inverters
Dust	100%	100%
Sand	27.08%	9.37%
Animals	7.50%	5.20%
Limo	33.33%	-
Sheets	4.16%	2.08%
Construction Material	9.37%	3.75%

Figure 2. Comparison between photovoltaic plants.



(a) and (b) with periodic preventive maintenance; (c) and (d) without periodic preventive maintenance.

Figure 3. Power curve plots.



(a) before maintenance; (b) after maintenance. Green: power; Dark blue: voltage; Light blue: current.

bell-shaped format exceeding 10.000W for the peak insulation hours. Therefore, this graph clearly shows an improvement in power production from maintenance.

In the reported cases of corrective maintenance, the most affected item was the photovoltaic inverter, a fundamental part of the use of the energy produced by the modules because if its operation is interrupted, the system stops. The causes for the interruption of the operation of the inverters were the high dust accumulation and the presence of animals on the site. Figure 4 shows inverters damaged in different ways: image (a) is a bird's nest that completely closed off the inverter air circulation, while image (b) shows a frog that managed to enter the inverter, causing an internal short circuit. Image (c) suffered from renovating the owner's house; a lot of construction dirt entered the ventilation to interrupt the inverter operation; finally, image (d) is obstructions caused by excessive leaf litter.

With the popularization and the increasing eagerness to generate their energy, several problems have arisen due to lack of maintenance, as exemplified in this case study. On the one hand, the owners of these plants, technically unaware people with little understanding of the operation and care required to have a power plant, can make an adequate follow-up difficult. On the other hand, some service companies focus only on the installation, leaving the after-sales in second place. In addition, as photovoltaic solar energy generation systems are relatively simple to monitor (e.g., using a mobile application) and require a restricted set of requirements, this may undermine the fundamental importance of preventive maintenance. Nevertheless, this single annual maintenance significantly affects these plants' operation.

Conclusion

From the proposed methodology, it was possible to achieve the objectives of this case study and find the central answers to the research. It was possible to know the most common soiling cases found in a photovoltaic system, how often it appears, and the impacts the soiling causes on equipment (physically) and its operation (efficiency). It was also possible to conclude that systems that receive preventive maintenance within the indicated period have a low accumulation of dirt, lower wear by time and dirt, and good productivity.

The graphics with the inverter performance illustrated another point observed. There was a significant improvement in the rated power of the system, which was very deficient, after carrying out the general maintenance of the solar plant equipment.

Based on the quantitative and qualitative analysis presented in this case study, the value of preventive maintenance to the owner of a solar plant was validated. Besides generating savings by keeping the

Figure 4. Images of drives that required corrective maintenance.

(a) interruption by a bird's nest; (b) interruption by a frog; (c) interruption by building material; (d) interruption by dry leaves.

PV system working correctly, it also guarantees the life of the equipment and safety in the installations.

Further research should deepen the study to include more statistical analysis for future work. It also enables the development of a basic management protocol for residential and commercial PV plants directed to the owners in simple language. It enables and prepares them to take care of their plants, focusing on after-sales: operation and maintenance.

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Decline of COVID-19 Pandemic: Impacts of Vaccination in Brazil

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The COVID-19 pandemic emerged unexpectedly and devastated, surprising the world quickly with the accelerated growth in cases and deaths caused by SARS-CoV-2 infection. As a strategy to face the pandemic, developing safe and effective vaccines fast was a great challenge for the scientific community and the big pharmaceutical companies. However, some factors enabled the production of vaccines soon authorized for emergency use by the WHO. In Brazil, the positive impacts of vaccination did not take long to appear, revealing a drop in the number of cases, mainly in the number of deaths caused by the disease a few months after the immunization campaign.
Keywords: Vaccination. Pandemic. COVID-19. Brazil.

Introduction

The spread of SARS-CoV-2, which originated in Wuhan, China, in December 2019, evolved at an accelerated pace, reaching hundreds of countries in all continents quickly. In March 2020, the World Health Organization (WHO) officially declared a pandemic, the first caused by a coronavirus [1]. Severe acute respiratory syndrome 2 (SARS-CoV-2) has caused, in addition to acute respiratory symptoms, systemic effects, such as neurological, digestive, intestinal, and sensory symptoms [2]. According to the WHO, it is estimated that 15% of the population infected by the virus develops a severe form of the disease requiring oxygen, and 5% require intensive care [3].

The vertiginous growth of severe cases of the disease indicates the lethal potential of SARS-CoV-2, which since January 2020 to date has caused the death of 6.87 million people worldwide, with more than 600 million confirmed cases. Brazil, no different, gained prominence in the world, presenting high numbers, with approximately 700 thousand deaths and 37.14 million cases [4]. Faced with this alarming scenario for public health, authorities, pharmaceutical industries, and the scientific community worldwide have sought to adopt strategies to combat the COVID-19 pandemic. Efficient and safe vaccines stand out as a priority measure to prevent new cases and reduce the lethality rate caused by the virus. The accelerated development of vaccines in times of health crisis was essential for the early start of immunization of the world population since regular vaccine production takes an average of 8 to 20 years [5].The global effort and targeted funding enabled the first COVID-19 vaccines to be produced within months, with approval for emergency use by regulators and the WHO. Despite the speedy process, compliance with the stages involved in the development of a vaccine was maintained: the preclinical phase, developed in animals or *in vitro*; the phase 1 clinical trial, developed in a group of dozens of people to test safety, dose, and immune response; the phase 2 clinical trial, carried out in a group of hundreds of people to confirm the safety data and the immunological response of the vaccine; the phase 3 clinical trial, to prove the drug's safety and efficacy in thousands of people; the approval stage of the responsible Regulatory Agency; and finally, large-scale production. The combination or concomitant conduction of the clinical trial phases justifies the shortening of production time during the pandemic [6], like Pfizer/BioNTech, produced in just 10 months [7].

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In March 2021, just one year into the pandemic, we already had 3 vaccines approved on the WHO emergency use list [5]. However, the moment's urgency alone was insufficient to achieve such success. The production of effective and safe vaccines in a short period was possible due to several factors, including prior knowledge of the SARS virus due to previous epidemics, the sequencing of the SARS-CoV-2 genome, and the use of technological platforms directed to other diseases [8]. Today, are 50 vaccines approved in the world against COVID-19, 183 clinical studies are being conducted, and 199 candidates in preclinical studies, the vast majority, protein subunit vaccines and RNA vaccines [9].

In Brazil, the vaccination campaign started in January 2021, practically a month after the start of most countries, and despite the uncertainties about the adverse effects, making it impossible for the population to accept them completely, these are still statistically insignificant when compared to the benefits generated for vaccination [10]. This review aims to identify and relate the impacts of vaccination with the number of cases and deaths during the COVID-19 pandemic in Brazil.

Materials and Methods

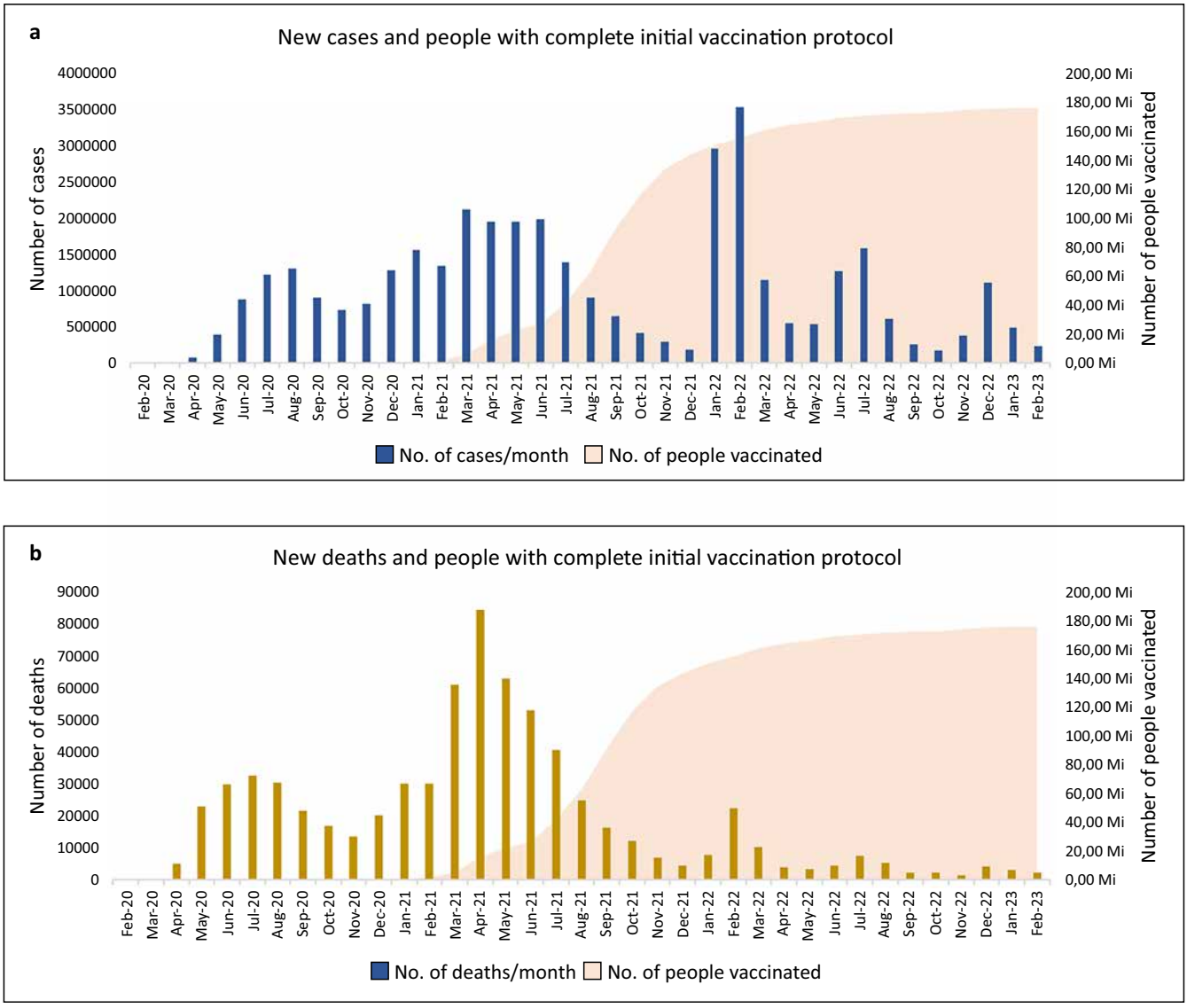
The study is a literature review of the positive impacts caused by vaccination against COVID-19 in Brazil through the observation and relationship of epidemiological indicators. In addition to the active search for related publications on the international Pubmed research platform, a collection was also carried out in the Our World in Data database. The indicator numbers of cases, deaths, and people vaccinated with the complete initial protocol were collected and analyzed, comparing the period from February 2020 to February 2023.

Literature Review and Discussion

Since the arrival of COVID-19 in Brazil, the country has reproduced the accelerated growth of cases and deaths experienced in other countries. Brazil registered its first case in February 2020, and the first death due to SARS-CoV-2 infection occurred shortly after that, on March 12 [11]. Vaccination began in January 2021, almost a year later, and it was already significantly reflected in the alarming numbers caused by the coronavirus [12]. The year 2020 accumulated more than 190,000 deaths and 7.5 million cases. In 2021, it has surpassed the previous one in total numbers, with more than 420,000 deaths and 14.7 million cases. However, for the first time since the outbreak of the first wave, a continuous decline in the number of cases was observed (Figure 1A), mainly in the number of deaths from April to December 2021 (Figure 1B). At that point, Brazil already had 143.4 million people vaccinated with the initial protocol (two doses) completed, equivalent to 67% of the population. In order to achieve herd immunity, that is, to immunize a sufficient number of individuals to contain the spread of the virus and indirectly protect vulnerable people, it would be necessary to have 70% of the population vaccinated [13]. The congruence of these data confirms the responsibility and prominence of vaccinating the stop the COVID-19 transmission chain, resulting in a noticeable reduction in cases and deaths during the last half of 2021 (Figure 1). Studies developed in other countries in the last two years have shown the same effect caused by vaccination. Between 2020 and 2021, vaccination would have prevented the deaths of 14.4 million people in 185 countries [14]. In fully vaccinated populations, vaccination efficacy was observed in 89.1% of infections, 97.2% of hospitalizations, 97.4% of ICU admissions, and 99% of deaths [15]. In Brazil, vaccination would have prevented the daily death of approximately 500 people in the first months of 2021 [16].

Despite the considerable reduction observed in numbers in mid-2021, the beginning of 2022 was marked by a new outbreak and a peak in new confirmed cases (Figure 1A). The second wave, subtly started in November of the previous year by the Gamma variant (P.1 lineage) [17], became

Figure 1. Relationship between the number of people vaccinated and the number of new cases and deaths caused by COVID-19, in Brazil, from February 2020 to February 2023.



(a) Relationship between the number of new cases per month, and the cumulative number of people vaccinated with a complete initial immunization protocol, from February 2020 to February 2023. (b) Relationship between the number of new deaths per month and a cumulative number of people vaccinated with initial complete immunization protocol from February 2020 to February 2023.
Source: Authors (2023)

evident in January and February 2022, with new 2.95 million and 3.53 million cases, respectively.

However, the number of deaths, fortunately, did not follow the same growth rate, with just over 30,000 deaths in the two months, a very different scenario from that observed in the most painful period before vaccination. March and April 2021 stood out as the worst pandemic scenario; they were responsible for 4.06 million cases and more than 145 thousand deaths. The comparison of epidemiological indicators during these two periods (before and after the effects of vaccination) reflects vaccine efficiency, which, despite not preventing infection by the virus and its variants, prevents severe cases and deaths caused by the disease.

Conclusion

The benefits brought by vaccines are unquestionable. The eradication of diseases such as smallpox and polio confirms the importance of vaccination. According to WHO, preventable diseases prevent up to 3 million deaths annually. Likewise, early immunization during the COVID-19 pandemic has demonstrated its positive impacts on a global scale, reducing the severity of the symptoms triggered by the SARS-CoV-2 infection and, above all, preventing the deaths of thousands of people. Currently, at least 186.83 million people in Brazil are vaccinated with at least one dose [4]. The relationship between this index and the reduction in the number of cases and deaths registered in recent months proves the current control of the epidemiological situation in the country and the efficiency of the vaccines used, capable of stimulating and strengthening the immune system of individuals. However, the global interest in interrupting the virus's spread requires mass vaccination adherence. Therefore it is necessary to inform the population of its benefits and impacts to make everyone aware of its importance.

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Comparison and Validation of Cryopreservation Methods of *Candida* Genus Fungi: A Brief Review

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Cryopreservation is a freezing technique that prevents the microorganism's genetic material from carrying out possible reactivations. This method commonly requires cryoprotectants to minimize the possible damage that freezing can cause. The current article seeks to evaluate the efficiency and viability of cryopreservation of fungi from the genus *Candida*, aiming at the best technique. The method was based on searching articles related to the analyzed theme and their ratifications. Thus, with the bibliographic survey, it was possible to conclude the most executable method among the three most used.
Keywords: Cryopreservation Methods. *Candida*. Cryoprotectants.

Introduction

Cryopreservation is fundamental for laboratory research in microbiology, as it keeps microorganisms preserved, allowing the possibility of storing biological material for an extended period until it is necessary to use it [1]. However, the preservation method varies according to the type of microorganism used, which implies no option of standardizing a method for general use. Therefore, it is necessary to analyze the advantages and disadvantages of each type of cryopreservation for different biological materials to determine which is the best for each situation.

Currently, the most used technique is cryopreservation in the ultra-freezer –80°C; being necessary to use cryoprotectants due to the low temperatures. Cryoprotectants are substances added to the medium to prevent severe damage to microorganisms by freezing and thawing them [1]. The protective substance selection depends on which part of the cellular needs protection: external, which not penetrates the cell, or internal, which penetrates the cell [2].

Candida is responsible for 80% of fungal infections in the hospital environment and contributes significantly to bloodstream infections [3]. In addition, these microorganisms have an extensive habitat, like the digestive mucosa and the vaginal mucosa, and can cause infections in the mucous membranes, skin, and systemic tissues [4].

The present study aims to evaluate the efficiency of *Candida*'a genus fungi cryopreservation and compare the methods most used in 3 articles from the current literature.

Materials and Methods

We searched different *Candida*'s genus fungi cryopreservation methods using databases (Google Scholar, Scielo, Pubmed, and Mendeley). After that, we did a spreadsheet with the data collected, filtering about microorganisms, cryopreservation method, and cryoprotectant. Finally, we chose the 3 most relevant articles ("Maintenance of yeast by freezing at – 20°C" by Silva and colleagues (2008) [5], "Update on fungal conservation methods applied to microbiological collections" by Gadêlha and colleagues (2022) [6], and "Evaluation of preservation methodologies for the maintenance of microorganisms of the *Phylum Ascomycota* and yeasts of the genus *Candida* belonging to the Collection of Microorganisms of Medical Interest of INPA" by Oliveira and colleagues [4] (2015).

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Literature Review and Discussion

Cryopreservation is the preservation of microorganisms through freezing at low temperatures, aiming to keep the DNA stable for possible reactivation, with several methods to be carried out, each according to the type of fungus used. Because these are icy environments, cryoprotectants are usually necessary to protect cell structures, reducing the damage that freezing can cause. The formation of intracellular ice crystals, the same as the method, is selected according to the feasibility of the study material. Table 1 presents the microorganisms used, the methods, and the cryoprotectants.

So, reviewing different methods in the same microorganisms allows us to know which technique is more advantageous to be applied according to viability, purity, and growth analysis. Table 1 shows the results of the four different techniques on the same microorganism.

Conclusion

We have evidenced that cryopreservation is a viable preservation technique for *Candida* in the three methods. Furthermore, there were methods with better performances than others. Thus, according to the results indicated in the methods used, we concluded that direct cryopreservation at –70° C proved more efficient and viable since it obtained high growth and purity and was simple to perform and easy to store.

Table 1. Researched articles, methods, and results found.

Article	Method	Results
Yeast maintenance by freezing at –20°C	Cryopreservation at –20°C preceded by 7 days of refrigeration, using flasks containing brain-heart infusion broth with 20% glycerol, incorporated with sterile beads [5].	• High fungal growth; • High conservation; • High recovery; • Easy execution; • Ease of storage [5].
Update on fungal conservation methods applied to microbiological collections	Cryopreservation at –20 °C preceded by 24 hours of refrigeration at 5°C, using cryotubes containing glycerinated broth, seeded with yeast, incorporated with sterile beads [6].	• High fungal growth; • High conservation; • High recovery; • Easy execution; • Ease of storage [6].
Update on fungal conservation methods applied to microbiological collections	Cryopreservation at –80 °C preceded by 24 hours of refrigeration at 5°C and another 24 hours in a freezer at –20°C, using tubes of penicillin containing Sabouraud agar with activated charcoal and performed the fungal culture.	• Medium fungal growth; • Media conservation; • Media recovery; • Easy execution; • Ease of storage [6].
Evaluation of preservation methodologies for the maintenance of microorganisms of the *Phylum Ascomycota* and yeasts of the genus *Candida* belonging to the Collection of Microorganisms of Medical Interest at INPA	Direct cryopreservation at –70° C, using sterile 2 mL microtubes with 0.8 mL of sterile distilled water, 0.05 mL of DMSO dimethylsulfoxide (cryoprotectant), 0.1 mL of glycerol (cryoprotectant), 10 mg of beads (2mm, with orifice) sterile and 100 mg of biomass of fungal isolates [4].	• High fungal growth; • High conservation; • High recovery; • Easy execution; • Ease of storage [4].

Source: Authors.

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Application of Residual Fibers of Polyethylene Terephthalate - PET in Structural Composites: A Systematic Review

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Fiberglass is the most commonly used material in the manufacturing process of wind turbine components, as it meets the basic requirements of the final product. However, as the market for wind turbines expands, industries must keep up with the demands of increasing blade efficiency at a reduced cost. As a replacement for fiberglass, poly (ethylene terephthalate) - PET textile fiber is an option, which has better tensile strength, elongation rate, and low density. However, it has low wettability and poor interfacial bonding with resin matrices. This study aims to perform a literature review of existing scientific studies using PET fiber from the tire industry or not applied as reinforcement in structural composites.

Keywords: PET Textile Fiber. Structural Composites. Wind Turbines. Wind Blades.

Introduction

The way energy has been, since the last century, and has been generated, still in significant volume, brings to the fore the constant search for renewable energy generation methods that do not pollute the environment and are economically and socially beneficial. According to the Global Wind Report (2022) [1], Brazil is the 6th country in the world ranking in terms of installed wind power (with 21.5 gigawatts), and this is a promising market since the economy has been circulating greener technologies driven by renewable and globally competitive energies, which brings us, also, the bias of biodegradability and the reuse of natural and synthetic waste from industries and agribusiness, capable of application in the technological development of greener materials, with longer life cycles and susceptible to recycling [2].

Manufacture wind blades are commonly used fiberglass, which, although highly versatile, presents some intrinsic negative points to the process as the unhealthy during the lamination of the blades and the challenges of increasing the efficiency of the blades since this is related to the increase of its length under a low weight (density of fiberglass equivalent to 2.54 g/cm^3) and cost [3,4]. Therefore, the study regarding the replacement of fiberglass by residual and clean textile fibers of PET-poly (ethylene terephthalate) from the tire industry fills an existing gap around this technological perspective, presenting itself as an innovative idea in the sphere of development of new materials and their application in wind turbines.

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PET Fiber Application

The civil industry is one of the central and oldest holders of the development of composite materials using fibers as reinforcement components in cement matrices since these materials, when applied, tend to improve the initial properties presented by its matrix, especially the structural ones related to traction, bending, and impact.

Moreover, composite materials with fibers, whether synthetic or natural, tend to improve the ratio between the strength and final weight of the material since the strengthening mechanism is associated with the transfer of stress from the fiber to the matrix [4,5]. However, it is noteworthy that natural fibers present a low structural strength response when applied to polymeric or ceramic matrixes, which is not ideal for applications requiring extreme structural stresses. Thus,

synthetic fibers become the most employed when high structural characteristics are required [4].

The study developed by Dias and colleagues (2019) [5] used textile waste from the Vale dos Sinos - RS tire industry. This study aimed to incorporate this waste as fibers in concrete, using it as a promising alternative to sustainable technologies in construction. The tests and validation of the study showed feasibility in the application since the incorporation of textile fiber in the cement matrix reduced localized cracks and improved impact absorption by concrete; however, because it still presents itself as a technological gap in the civil industry, it requires further studies around the application of this composite for more practical application in the sector.

The study prepared by Araujo (2019) [4] applied clean residual PET textile fibers from the tire industry (Kordsa) as reinforcement in a polyester matrix to be later applied as a coating element in civil construction. The results showed an increase in impact strength 10x higher than that established by NBR 15575-3. However, the morphological analysis showed an irregular distribution of fibers as the fiber concentration in the matrix increased, which contributed to the formation of voids and bundles, resulting in decreased adhesion strength between fiber and matrix, and the cited study did not perform any pre-treatment on the fiber. In addition, PET fibers have some drawbacks, such as low wettability and poor interfacial bonding with resin matrices. However, many studies have focused on solving these problems. When subjected to physical or chemical treatments to improve its properties, PET fiber is used as a reinforcement material and produces composites with low weight and high structural strength [6,7].

Teh and colleagues (2004) [8] performed surface treatment with NaOH on PET fibers that were used as reinforcement in an epoxy composite, which, when purchased with the pure resin, showed excellent fracture toughness with only 1% loading since the adhesion between matrix/fiber treated showed excellently. The studies developed by Mao and colleagues (2019) [6] validated a new method of surface treatment on residual PET textile fibers via immersion in a hybrid solution of tetraethylorthosilicate (TEOS)/KH550/polypropylene (PP)-g-MAH (MPP) synthesized (TMPP). The results were highly positive for interfacial properties, mechanical strength (flexural and tensile), and the modified fiber's thermal and physical-chemical properties.

However, the structural performance of fiber-reinforced composites is also affected by the total volume fraction of the fibers relative to the matrix, the distribution of the fiber in the matrix, the length of the fiber strands, and the orientation of the fiber in the matrix [9,10]. Manjunath and colleagues (2019) [7] performed static (flexural tensile and impact) and dynamic (temperature effects, damping properties, storage frequency, and loss modulus) experiments, validated by morphological analyses, on unidirectionally distributed PET textile fiber composites in epoxy resin, evidencing impact resistance and better dynamic mechanical behaviors compared to glass fiber/epoxy composites. Danmallam and colleagues (2015) [11] used PET fibers interlaced with kenaf fiber in a hybrid composite of epoxy resin manufactured via a vacuum infusion process, obtaining improved mechanical properties (bending and impact), morphological (interfacial bonds) and physical (water absorption), enabling the application of the hybrid composite in various sectors that require high structural stresses and exposure to weather such as rain, considering mainly the percentage of fiber/matrix distribution as responsible for the improvements cited.

We did not find studies applying the residual PET textile fibers from the tire industry in the epoxy matrix in the application of wind turbines, either in wind blades or other components. It presents a technological gap amenable to investigation in the renewable energy industry sector.

Materials and Methods

The method aimed to build a systematic review of works that have used PET fiber from, or not, the tire industry as a reinforcement material for structural

composites, improving the performance of these materials and considering the processes inherent to this improvement (chemical or physical surface pre-treatments, fiber distribution, percentage of fibers distributed in the matrix, fiber orientation and distribution of fiber strands. Tables 1 and 2 listed the studies considered in our research sources and keywords.

Table 1. Databases used in the search for scientific articles.

Sources of Research
Science Direct
MDPI
Academic Google

Table 2. Keywords used in the search for articles in the databases.

Keywords
Wind turbines
Wind blades
Structural composites
Composites materials
PET textile fiber
PET fiber

In the Science Direct database, a filter was performed with the keywords in English to obtain the most relevant articles—the selected publications dated from 2018 to early 2022. Figure 1 presents the steps for filtering the publications.

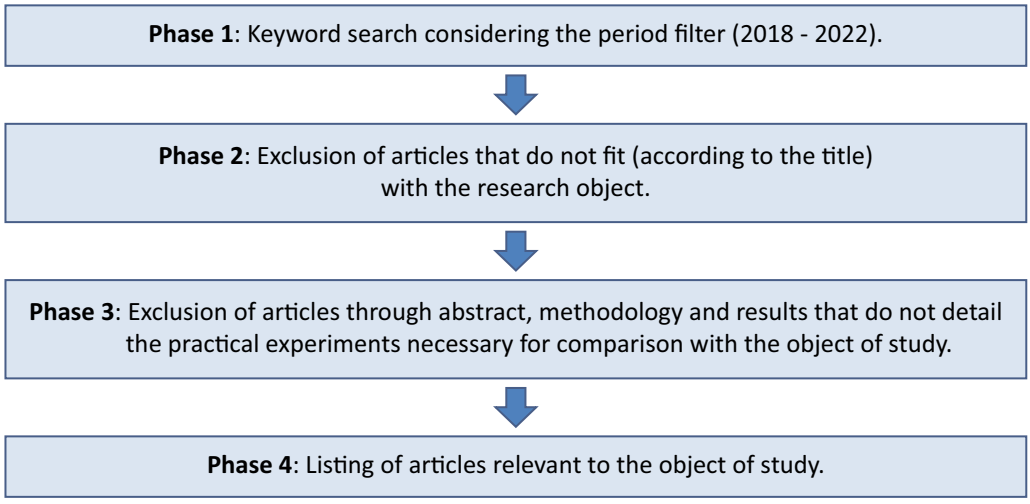
Results and Discussion

Phase 1 ended up performing a relevant filter, using combinations of keywords and Boolean operators, for example, „structural composites AND wind turbine blades“ or „PET fiber AND wind turbines“. When selecting the period (2018 - 2022), the number of articles reduced considerably, mainly in the MDPI and Google Academics databases, since the articles presented in the latter were forwarded to other sites such as Science Direct, Research Gate, or MDPI itself. Table 3 presents the results.

Table 3. Number of articles obtained after the phase 3 analysis.

Sources of Research	Phase 3
Science Direct	23
MDPI	16
Academic Google	7

Figure 1. Exclusion and prioritization phases of the scientific articles.



Four questions (Q) were defined that guided the relevance of the content of each article obtained in the previous phase to supporting the qualitative selection process of phase 4. This relevance was analyzed quantitatively following the logic of assigning values to the answers: "yes" (value 1.0), "in part" (value 0.5), and "no" (value 0). Thus, according to the questions, the higher the value of the selected article, the more its content is in agreement with the research developed.

The questions selected for the study were:

Q1: Does the study use residual PET fiber and epoxy resin for composite structural development?

Q2: Does the study present its results or quotes from the fiber and/or structural composite characterizations?

Q3: Does the study aim to apply the structural composite in the wind turbine industry?

Q4: Does the study define some pre-treatment on the fiber before using it as reinforcement in the polymeric base?

Table 4 presents the results for each article found in the databases, totaling 12 relevant articles.

Table 4. Listing of articles according to the prioritizations defined in phase 4.

Nº	Article Title	Questions					Databases/ Journal
		Q1	Q2	Q3	Q4	Total	
1	Structural optimization of a horizontal axis wind turbine blade made from new hybrid composites with kenaf fibers [12]	0	0	1	0	1	Science Direct - Composite Structures
2	Experimental Study on Mechanical Properties of Natural Fiber Reinforced Polymer Composite Materials for Wind Turbine Blades [13]	0	1	1	0	2	Science Direct - Materials Today: Proceedings
3	Performance analysis of wind turbine blade materials using nanocompWosites [14]	0	1	1	0.5	2.5	Science Direct - Materials Today: Proceedings
4	Development and characterization of PET flakes reinforced polyester resin composites [15]	0	1	0	0	1	Science direct - Materials Today: Proceedings
5	Recycling of thermosetting composites for wind blade application [16]	0	1	0	0	1	Science Direct - Advanced Industrial and Engineering Polymer Research
6	Fiber Orientation Effect on the Behavior of the Composite Materials of the Horizontal Axis Wind Turbine Blade (HAWTB) [17]	0	1	1	0	2	IEEE Xplore
7	Comparative Assessment of Static and Dynamic Mechanical Properties of Glass and PET Fiber Reinforced Epoxy Composites [7]	1	1	0	0	2	Science Direct - Materials Today: Proceedings
8	Experimental and Numerical Comparison of Impact Behavior between Thermoplastic and Thermoset Composite for Wind Turbine Blades [18]	0.5	1	1	0	2.5	MDPI – Materials
9	The Potential of Natural Fiber Reinforced Polymer Composites in Sandwich Structures: A Review of Its Mechanical Properties [19]	0	1	0	0	1	MPDI – Polymers
10	Performance Analysis of Reinforced Epoxy Functionalized Carbon Nanotubes Composites for Vertical Axis Wind Turbine Blade [20]	0	1	1	0.5	2.5	MPDI – Polymers
11	Simulation of Glass Fiber Reinforced Polypropylene Nanocomposites for Small Wind Turbine Blades [21]	0	0.5	1	0.5	2	MPDI – Processes
12	Surface Modification of PET Fiber with Hybrid Coating and Its Effect on the Properties of PP Composites [6]	0.5	1	0	1	2.5	MPDI - Polymers

Conclusion

Few articles were found that used PET fiber in an epoxy matrix, and only the article described in item 7 responded positively to this question. This point highlights the technological gap around using these materials to develop a new structural composite that can be applied in the wind turbine industry, replacing fiberglass in the manufacture of the blade and other components of the system. This project will undoubtedly contribute to advancing new technologies in the sector and assist in future studies involving the proposed theme.

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The Residue of Palm Oil from Frying Acarajé and Circular Economy

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A circular economy is a concept that decouples economic growth from the unbridled use of natural resources, reducing negative impacts on nature and promoting the reuse and valorization of waste and by-products within the scope of sustainable development. The palm oil residue from the acarajé frying can be transformed into other products, avoiding its disposal in nature. This topic has few studies and works. The present article presents the relationship between the circular economy and the reuse of waste in producing another good related to palm oil and acarajé. The method used was bibliographic, descriptive, and exploratory research. Reusing waste aligns with the circular economy, reducing disposal and environmental damage.
Keywords: Sustainable Development. Environmental Damage. Environmental Impacts.

Introduction

Bahia is the birthplace of the foundation of the Brazilian state, whose capital is Salvador. It is a multi-racial state with a cultural diversity of indigenous, enslaved black Africans, Portuguese colonizers, and other immigrants. Among the products originating from African culture is the acarajé, a delicacy made from black-eyed peas pounded and fried in palm oil, which has already become Brazil's material and immaterial heritage.

The acarajé originated from the Gulf of Benin in East Africa and arrived in Brazil through enslaved black people. The word acarajé is the junction of the Yoruba term "akará", which means "fire cake", and "je", meaning "to eat" [1].

The commercialization of acarajé produces many solid and liquid wastes treated as residential waste. Most of it is recyclable; however, its disposal is incorrect, partly due to the lack of an appropriate place. Furthermore, the acarajé 's frying generates an average of 5 liters of waste per day for each seller, and improper disposal causes environmental problems, which can result in the clogging of drainage systems, impact on fauna and flora, production of toxic substances and smelly.

Palm oil has great gastronomic potential and nutritional importance, imparting a peculiar flavor, aroma, and color to the foods. However, after its use, it becomes unfit for human consumption, representing a source of waste that must be appropriately disposed of or reused to produce biodiesel, lubricants, resins, and soap [2].

One of the consequences of population growth is the increase in waste, presented in all human activities. However, some are potentially harmful to the environment, with the ability to threaten life and nature. This fact requires mitigation or elimination of the risk and their effects. Reusing the good for another purpose or recycling the product as a raw material are some of the mitigated actions [3-6].

The industry uses in its production process the linear economy, predominant since the beginning of the Industrial Revolution, having as its principle the extraction, production, use, and later the discarding of the good, generating an accumulation of garbage, which has a distinct decomposition period, increasing considerably dumps and polluting mainly the soil and water sources. Moreover, this

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type of economy is an unsustainable model for the planet's well-being because the linear economy has no provision for reusing generated waste, which is discarded after use [7-9].

In recent years, the Circular Economy (CE) has gained space in governmental discussions and society, becoming a solution for the planet's sustainability. It is a comprehensive concept that generates benefits for humanity, although the intended results are not always achieved [3, 10]. Moreover, the discussion about CE started to have more global evidence in 2012, with several reports by the Ellen MacArthur Foundation entitled „Towards the Circular Economy" [3,7,10, 13,14].

CE or analeptic economics concerns obsolete or useless products that are reused, returned to the economy, or used sustainably and efficiently, avoiding their disposal and the need to explore or extract new resources. In addition, it aims to protect the environment, generate income, create employment, develop new sectors, skills, and capabilities, and make consumers aware of their behavior towards nature.

CE is based on the 4Rs of sustainability, and its model includes the economic, social, and environmental aspects.

Reducing the consumption of goods decreases the emission of pollutants, and the use of resources in a sustainable way produces minimal waste. Reuse means giving new functions to the product, destining them for other purposes, such as using returnable packaging and writing on paper on both sides. Renewing means prioritizing using renewable raw materials in production and sustainable consumption [11,12].

Recycling the product is to separate the classes of products that no longer have utility and reuse, transforming them into something new. This action tends to send the waste to the proper place for its disposal. It is also promising in generating employment, extending the useful life of landfills, reducing soil, air, and water pollution, and helping to make people ecologically aware [11,12].

CE principle encourages the elimination of waste and pollution from the beginning of the product design, keeping it in use for the maximum possible period, which tends to reduce the need to extract new natural resources, avoiding ecosystem destruction. It also maximizes natural assets and prioritizes the flow of goods. This condition tends to minimize waste production at all economic levels, generating innovation in processes and business models, focusing on producing sustainable, intelligent, and integrative goods, which contributes to stimulating economic growth and job creation, especially in the new activities generated. Furthermore, it is a sustainable and applied solution to alleviate environmental impacts because it incorporates recycling and reuse strategies, which is beneficial for nature and biodiversity [7, 12-14].

Recycling and disposal of waste are described in the National Solid Waste Policy (PNRS), Law nº 12,305/2010, which has an easy-to-understand approach to minimizing harmful environmental effects, providing the means of prevention and ways to reduce waste. It also shows the forms of waste management, which has as a tool the reverse logistic in its various stages of production, as well as the responsibilities of individuals and legal entities that produce waste and their management plans [15].

The reuse of waste generated in the frying of acarajé in the production of artisanal soap has been little studied, not finding many academic works on the subject mainly related to the circular economy. The present work aims to verify the relationship between the circular economy and the reuse of palm oil residues from acarajé frying to produce other products.

Materials and Methods

We did a literature review and a description of a particular population to identify the relationship between the variables of circular economy and the generation of waste from palm oil used in frying acarajé [16].

We did bibliographic research in documents already published in physical or virtual form, which allows the collection of information from available sources to serve as a reference in

developing the work [16]. We used keywords such as circular economy, environmental education, waste management, waste generation, waste reuse, waste disposal, and palm oil in the database for the research.

The method steps were as follows:

- Research strategies and keywords definition;
- Checking the information contained in the published works;
- Treatment of the data obtained;
- Information analysis and results obtained.

Results and Discussion

Law nº 12,305/2010 requires companies to be responsible for waste management, taking responsibility in all their production cycles, allowing them to carry out reverse logistics, collecting their products to be reused or reintegrated into their process, and preventing improper disposal by users.

The obligation to minimize the volume of waste generated is on the companies, the consumers, and public service holders, that must adopt practices that allow the reintegration of waste or obsolete materials into the production cycle by installing places to receive these products. Furthermore, the consumer must give an excellent final destination in the previously indicated places, avoiding mixing the generated residues of different natures.

Environmental education has faced many obstacles related to the conscious practices of waste disposal because it represents a new knowledge opposite to what is currently practiced by people, who sometimes find it fashionable, not realizing the danger that the planet is experiencing with the finitude of natural resources.

The Associação das Baianas de Acarajé, Mingau, Receptivo e Similares, ABAM, gives lectures with the associates on the environmental and economic importance of the residues from the palm oil used in the frying of acarajé, explaining ways of collection and disposal and its use as raw material to make soap, which can be sold and generate income for the associates.

Palm oil is one of the most important ingredients in Afro-Bahian cuisine, being used mainly in the frying of acarajé, later becoming unfit for human consumption, constituting a residue capable of causing damage to the environment when discarded incorrectly in the kitchen sink, in the culverts and in the beach sand, which can cause clogging of pipes, contamination of the sewage system and/or rainwater, with the potential to pollute the water table, rivers, streams, beach, fundraising sites water, in the soil and the sand of the beach. Therefore its recycling is equivalent to the management given to the residue.

The residues produced in the frying of acarajé are discarded correctly and may not be used in other production processes. However, in CE, they can have new uses and be valued in a new production cycle, adding value and increasing their life cycle.Transforming the waste into a new product, such as handmade soap, which all the Baianas of Acarajé produce, is a way of using the 4 Rs of sustainability. Artisanal soap can also be manufactured by all people who use palm oil to produce food.

The agreement between the Federal University of Bahia (UFBA) through the Baianambiental project and ABAM made it possible to share knowledge about soap production and its chemical manufacturing process carried out on the premises of the laboratory of the UFBA Institute of Chemistry.

About 25 Baianas de Acarajé of Salvador, selected by the association, participated in activities in the Chemical Institute of UFBA laboratory. However, just five women participated in each meeting, as the laboratory facilities could not house all of them.

The raw material used was the residues from frying acarajé with palm oil. The process used is simple and of low financial cost, having among its components caustic soda or sodium hydroxide (reagent), residues of palm oil used in the frying of acarajé (fat or fatty acid), which needs to go through a process of filtration to remove solid particles, and water. Dyes were used to define the color. Rosemary and vanilla flavorings were used.

The linear economy, the prevailing model in the Industrial Revolution, valued consumption more, and products were thought of only in the production-use-disposal phases. In contrast, CE concepts that products must be reused in a new way cycle, and this attitude contributes to the non-generation of garbage and waste. The use of material many times postpones its disposal, which occurs much later.

CE and the conscious disposal of waste have the same objective. They concern the form of consumption and how the waste generated after using a product is treated. CE is inserted in all stages of producing a good, aiming at the destination of waste and directing its use to a new use before its disposal. It also seeks to use the goods, including products that are little utilized by the owner can serve as a loan for those who need to use them.

The practice of recycling the product, transforming the residue of palm oil from frying into artisanal soap, is a transition from the linear model to the CE, as its raw material is of secondary origin, resulting in a reduction in the environmental impact, especially when compared to the production of industrial soap with the use of other raw materials such as coconut oil, which requires a more complex process for its manufacture.

There are several advantages in the proposal of CE, such as the generation of jobs, innovation in the reuse of waste, and generation of income with the sale of products for reuse in another function different from the one that was designed, as long as the design is thought of in this possibility of use. Furthermore, for companies, it renders visibility in society, showing that their brand is concerned with the environment and biodiversity, revealing that their thinking is not restricted to profitability. This action also served as an instrument of environmental awareness of the people involved in the production of this waste, dissemination, and awareness of environmental risks, as well as encouraging and moving the informal economy, as the handmade soap generated can serve as another product to be marketed by the Baianas do Acarajé or serve as a souvenir to be distributed to customers who buy and consume its products. In this way, it is included in the concept of CE, as the residue of palm oil is transformed into a raw material for the production of another well, positively impacting the environment.

Conclusion

Palm oil is one of the essential ingredients for Bahian cuisine in producing typical and traditional dishes with the know-how, a contribution to the country by enslaved Africans. Frying acarajé leaves a residue of palm oil that must be disposed of consciously to avoid environmental and biodiversity damage. Among the harmful effects of unconscious disposal, there is the possibility of clogging the pipes and contaminating the water table, beaches, rivers, riverside, ponds, and soil.

The reuse of palm oil residues used to fry acarajé in the production of soap constitutes one of the principles of the circular economy, representing an alternative to the disposal of the product. Furthermore, the artisanal soap will be able to generate additional income for the Baianas de Acarajé, who are able to commercialize with the customers that consume the products of its tray, in addition to demonstrating to their customers that they are concerned about the environment.

The production of artisanal soap from the waste generated in the frying of acarajé brings benefits to the environment and biodiversity, reducing the disposal of the product, which is one of the principles of the circular economy.

The PNRS is in line with the principles of the circular economy, helping to encourage the use of a product for the maximum period of its useful life before it becomes disposable waste, removing potential waste that would be discarded to be used in another occupation.

The actions of public entities, consumers, and organizations are fundamental for waste management to be effective in its purpose.

The objective of the work was to verify the relationship between the circular economy and the production of a new product using disposable material, such as the residues of palm oil used in

the manufacture of handmade soap. When disposed of incorrectly, the waste generated causes damage to the environment and can become a serious urban problem. The deficiency in education and environmental awareness, combined with the lack of places for the correct waste disposal, drives some sellers to discard palm oil in the culverts near their point of sale or on the beach sands. This action contributes to contaminating the water table, the soil, and the beaches, which is also extensive for homes that dispose of the kitchen sink due to the lack of someone to collect.

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The Use of Hydrogen in the Production of Fuels and Additives for Internal Combustion Engines

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Currently, energy demand worldwide has inflated the prices of conventional fuels, so it is essential to identify alternative solutions. One such solution involves hydrogen (H_2), which can be used directly as a fuel or an additive for internal combustion engines (ICE). This work aims to review the hydrogen applications related to fuels and/or additives in ignition engines. For instance, hydrated vegetable oil (HVO) can be considered a possible substitute for biodiesel, while synthetic fuels involve a high cost, mainly due to the production process. Furthermore, using H_2 as an additive can offer benefits such as reducing pollutant emissions.
Keywords: Hydrogen. Hydrotreated Vegetable Oil. Diesel. Additive. Synthetic Fuels.

Introduction

The use of fuels to meet population demand across the planet has increased over the years [1]. Under this scenario, it is essential to identify alternative sources of energy for the most varied sectors of society, especially the transport sector. Fossil fuels are still widely used in ICE due to their availability and low cost compared to some biofuels. Nevertheless, fossil fuels also have drawbacks, such as the emission of pollutants and the concern of depletion, and therefore researchers are looking for other solutions [2]. Hydrogen has been indicated as a strategic solution for this scenario. Hydrogen has a higher calorific value, about 120 MJ/kg, than other fuels. Methane, gasoline, and diesel have, respectively, 50 MJ/kg, 44.5 MJ/kg, and 42.5 MJ/kg [3].

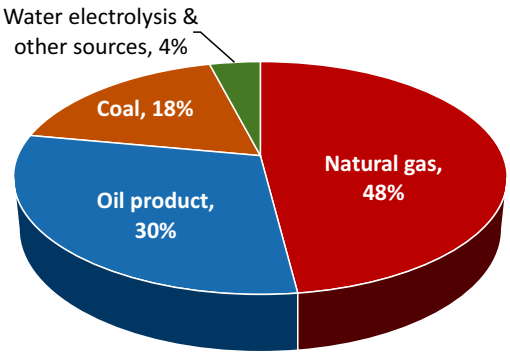
Hydrogen is crucial as it can be produced using both conventional and renewable energy sources. In addition, it has several applications in the transport sector (e.g., in fuel cells) and the industrial sector (in refining and product generation) [4].

Based on the energy source used to produce hydrogen can be divided into three categories, namely: green, blue, and gray [3]. Green hydrogen is considered the cleanest, given that renewable energy sources are used for production. On the other hand, blue and gray hydrogen have similar sources as they can produce hydrogen from fossil fuels. However, the process classified as gray hydrogen uses the capture and sequestration of the generated CO_2 to reduce the environmental impact.

From an environmental point of view, hydrogen should be produced from renewable energy sources, such as solar and wind energy [5]. However, those mentioned earlier have low competitiveness in terms of operating costs compared to extraction methods that use fossil fuels. Figure 1 represents a comparison between the methods commonly used to extract H_2.

The role played by H_2 in the transport sector has been reinforced because of the production of HVO. Furthermore, due to the hydrotreatment processes, this fuel can present better physicochemical

Figure 1. H_2 extraction methods [3].



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properties (e.g., calorific value, cetane number) when compared to biodiesel and Diesel. Furthermore, the search for new fuels has been reinforced as a result of the market oscillations of the oil price in recent years. Therefore, this work aims to present a literature review on the use of H_2 in the production of fuels or its application as a fuel additive in internal combustion engines.

Materialsand Methods

The bibliographic search was carried out in July 2022 using the search engine Periódico CAPES, available at https://www.periodicos.capes.gov.br. The keywords used were: Hydrogen; Hydrotreated vegetable oil; Diesel; Additive; Synthetic fuels.

Articles from the last 10 years were selected so that each one presented contributions to the proposed theme, indicating that it was a literature review or experiments in English. In addition, some articles were selected regardless of publication date due to their scientific relevance to this work.

Results and Dicussion

The transport sector is one of the biggest fuel consumers in the European Union and the rest of the world, presenting a higher consumption than the residential and industrial sectors [6].

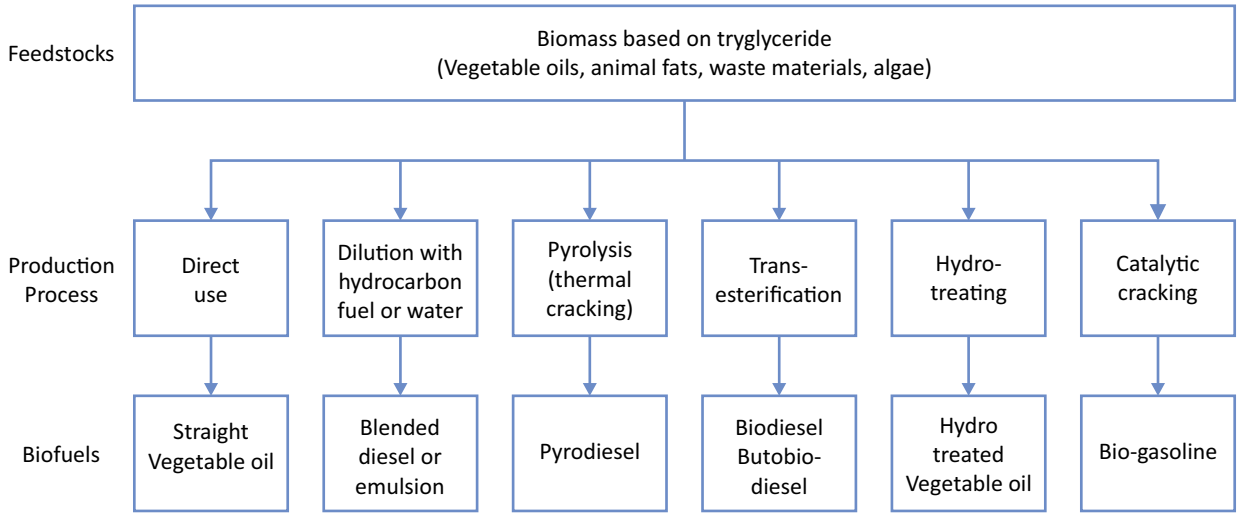
Although biofuels are used without engine modifications, they cost more than fossil fuels. The highest cost for biofuels refers to their raw material [7]. The cost of producing conventional biofuels ranges from US$70 to US$130 per barrel of oil equivalent (boe); while advanced biofuels cost range from US$85 to US$160 per boe [8]. The main biofuels used in compression ignition engines are 1st- and 2nd-generation biodiesel (Figure 2).

Hydrotreated Vegetable Oil

HVO is a bio-based liquid paraffinic fuel that can be used solely or blended in diesel engines [9]. The raw material used in the production process of biodiesel and HVO can be the same. For both processes, vegetable oils, and animal fat are commonly used. The difference in the production of each biofuel occurs in their chemical process. While HVO uses the hydrotreatment process, biodiesel is usually produced via transesterification.

Despite the more frequent use of biodiesel, HVO has advantages in its production. These advantages occur because there is no need to verify the degree of establishment of the raw material for HVO since hydroprocessing results in fully saturated paraffinic hydrocarbons and is not susceptible to oxidative

Figure 2. Production of liquid biofuels from biomass [9].



instability [10]. Moreover, the free fatty acids present in the raw material of HVO are converted into paraffin in the hydrotreatment process, while the transesterification process results in the formation of glycerin. From an economic point of view, paraffin is more attractive due to its ease of commercialization.

In the HVO hydrotreatment process, hydrogen acts by removing oxygen present in triglycerides at high pressure (approx 70 bar) and temperature (between 300 and 400 ºC) [10,11].

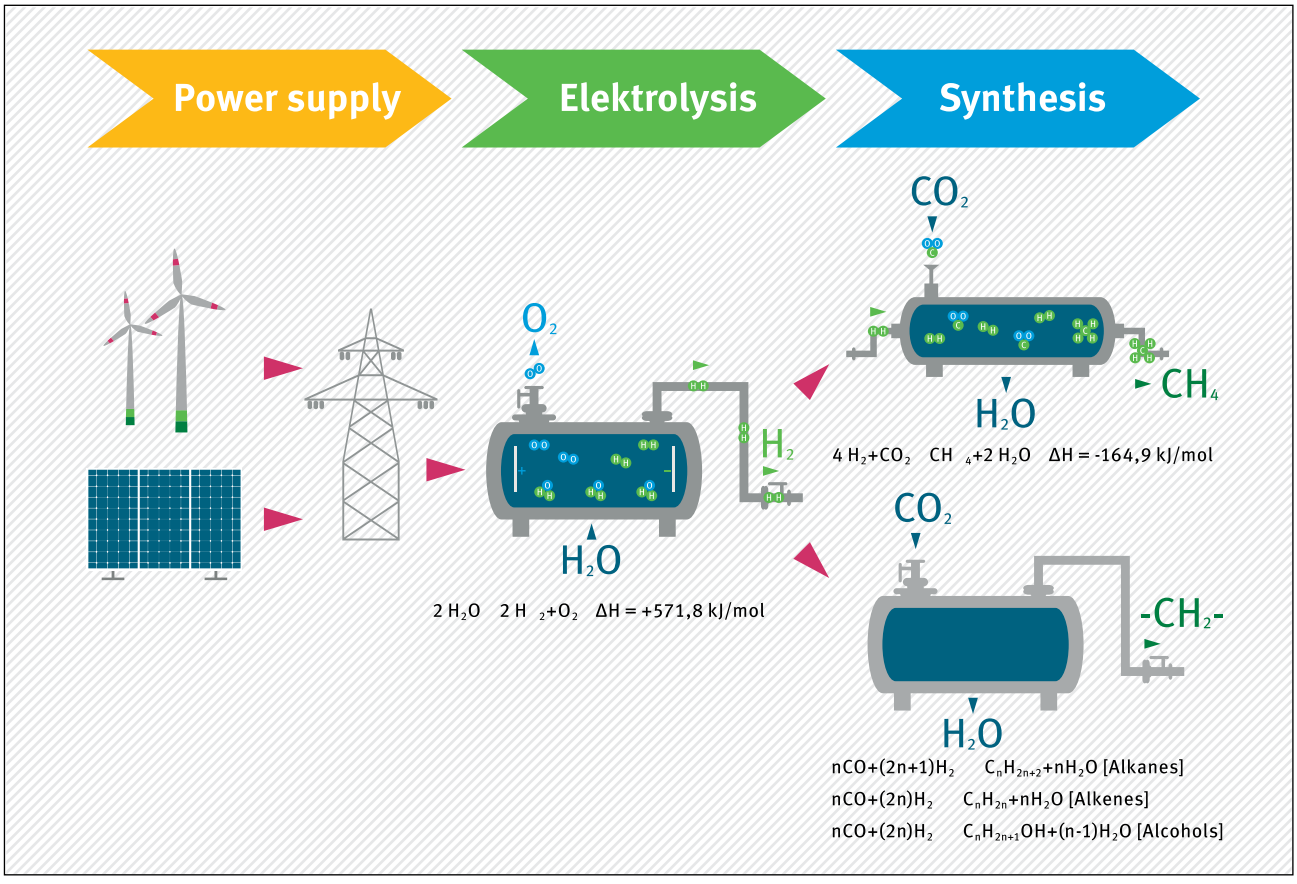
The derivatives of the HVO production process are propane (from the hydrogenation of glycerol), water, and CO_2 [7]. The hydrogen used in the production process can be derived from propane or extracted from other sources, such as renewable and non-renewable resources, microbial production, electrolysis, and other thermochemical processes [3].

According to Waldheim [7], the total cost of producing HVO varies between 600 to 1,100 €/ton (50 to 90 €/MWh). The use of different sources of hydrogen can have a direct effect on the final cost of HVO. Despite the higher value than other fuels, HVO has better physicochemical characteristics that enable better fuel combustion. The cetane number of HVO is higher than the other fuels evaluated (e.g., diesel, ethanol, biodiesel), and it presents good chemical stability in its storage. Other important characteristics are the absence of sulfur content, aromatic components, and ash. This combination of factors provides a complete combustion process and a more efficient catalytic after-treatment process [9].

Hydrogen in the Production of Synthetic Fuels

Synthetic fuels combine hydrogen, hydrocarbons, and an energy source. CO_2 and H_2 can be used to produce liquid fuels, which can be used in the transport sector [12]. This production process,

Figure 3. Simplified scheme of the Power-to-Liquid (PtL) process [13]



called Power-to-Liquid (PtL) (Figure 3), is initiated through the production of H_2, which will later be combined with CO_2, promoting the production of liquid hydrocarbons that can be refined to generate synthetic fuels (methanol, gasoline, and aviation fuel) and also can produce diesel through the Fischer-Tropsch process [13].

Hänggi and colleagues [14] discuss that one of the goals of synthetic fuels is to create a sustainable fuel. According to the source of hydrogen production, the process can present a high-energy loss, which reduces the overall energy efficiency [13]. Synthetic fuels are less effective in energy efficiency than fossil fuels; however, they are an exciting alternative to reducing atmospheric carbon.

Hydrogen as an Additive

The utilization of engines that operated only with hydrogen was tested in 1978, presenting limitations due to the high self-ignition resistance of the fuel [15]. The use of H_2 showed better results in tests on spark-ignition engines. Due to these limitations, several researchers have studied the addition of hydrogen as a fuel additive. The main advantages are the absence of carbon in its molecules, the higher calorific value when compared to diesel fuel, and the reduction of exhaust gases to the environment when used in compression ignition engines. Kanth [16] evaluated the use of hydrogen in a mixture of rice biodiesel and Karanja biodiesel. The experimental setup (Figure 4) had hydrogen supply through a pressure cylinder regulated to enter the diesel engine at a pressure of 2 bar and 7 lpm (liters per minute). A 5.2 kW diesel engine was kept at a constant speed and variable load. They reported a reduction in the specific consumption of fuel, CO, and HC emitted by the engine due to better combustion from flame propagation caused by the presence of hydrogen. However, due to higher combustion pressure and temperatures, NOx emission had slightly increased.

Barrios [17] evaluated the use of hydrogen in a blend of diesel and biodiesel. They reported a reduction in the concentration of fine PM particles emitted by the engine (Figure 5). This result was associated with increased particle oxidation. The latter resulted from higher combustion chamber temperatures and the formation of OH (hydroxyl) free radicals that react with unburned hydrocarbons.

Figure 4. Experimental scheme for hydrogen and biofuels [16].

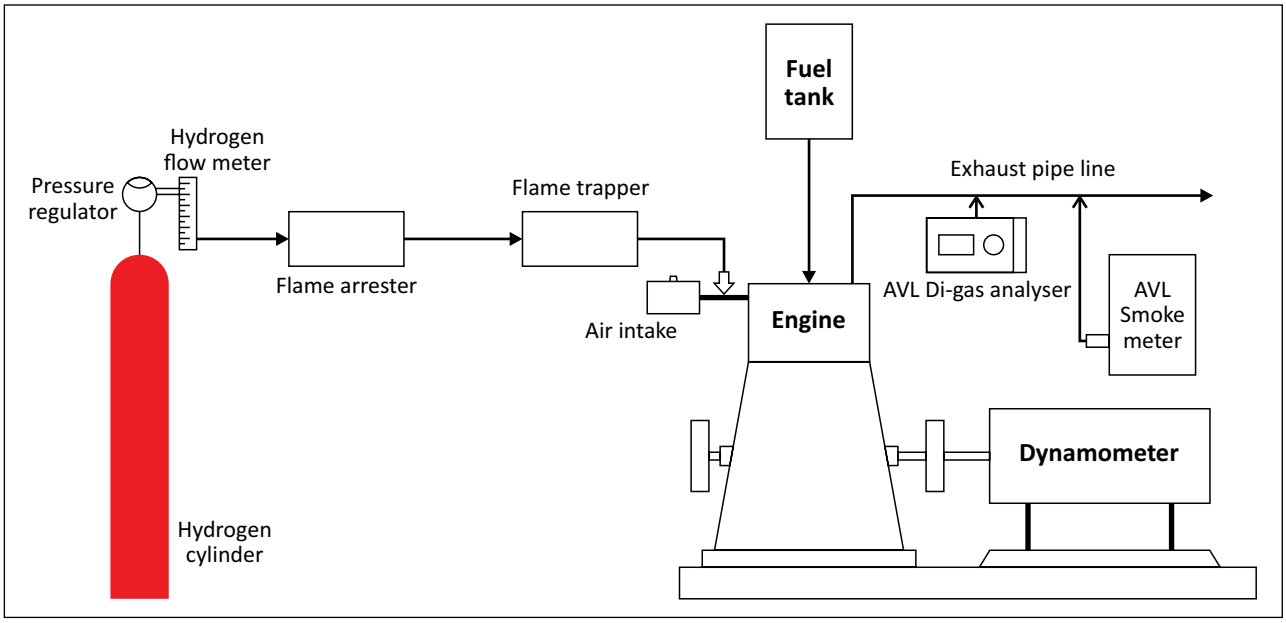
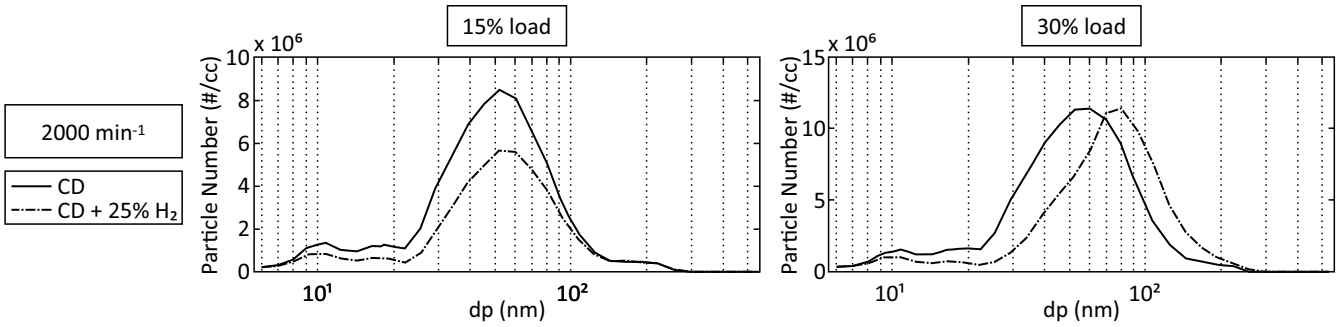


Figure 5. Particle distribution and concentration for blending diesel, biodiesel, and hydrogen [17].



Conclusion

Based on the literature review, one of the challenges for the coming years is the development of new processes and industries for producing biofuels on a global scale, in addition to the development of raw material supply chains for this production, such as biomass. Furthermore, hydrogen has been pointed out as a significant potential in the short- and medium-term scenario as an additive used in conventional fuels and for synthesizing new fuels.

Finally,we summarized the conclusion in topics as following:

- HVO is a possible substitute for biodiesel because it has better physicochemical characteristics that result in better combustion. Despite that, its production cost is a drawback since using hydrogen results in a more expensive fuel than transesterification (resulting in biodiesel) or the production of fossil diesel. Still, more research is regarding the engines' durability before considering a complete replacement of diesel fuel.
- Synthetic fuels are an alternative fuel option as they combine elements such as CO_2 and H_2 in the production process. The main product currently produced is methanol and synthetic gasoline. From an environmental point of view, this fuel presents advantages because of the CO_2 sequestration. On the other hand, it requires more significant investments and fuel production.
- The use of H_2 as an additive in engines was pointed as the lowest cost compared to the others. The use of H_2 can contribute to the reduction of emission pollutants and reduce specific fuel consumption.
- The use of different hydrogen extraction methods has a direct impact on its final associated cost. Although those renewables can be considered to produce the so-called 'green hydrogen,' the H_2 extracted from fossil sources is still the one with the lowest value.

It is expected that in the future, the cost of sustainable fuels to decrease and that technological limitations to be overcome. This combination of factors could provide a cleaner and more affordable fuel.

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Application of Lean Manufacturing Philosophy to Improve Occupational Safety Results in a Mining Company

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The importance of lean manufacturing applications in industrial organizations grows as scientific research points out its main results: productivity increase and waste reduction. When we refer to the direct observation of the work in the safety area, we notice the need for the same efficiency in managing risk scenarios in the activities. The objective of this article is to demonstrate how the Lean Manufacturing philosophy contributes to the understanding and connection of the leaders and also of the executors to the risks associated with the execution of their routine activities in order to mitigate and/or eliminate them. This article was conducted through a case study in a mining company located in Espírito Santo and identifies and analyzes the improvements obtained in process management through the application of the Lean Manufacturing philosophy and its tools, especially FMDS (Factory Floor Management Development System) in the subject of occupational safety. The main result obtained is the reduction of work accidents measured through the Accident Frequency Rate – TRIFR.

Keywords: Lean Manufacturing Philosophy. Safety. Risk Scenarios. FMDS. Accident Frequency Rate – TRIFR.

Introduction

In 2016, the challenge of understanding how safety indicators were managed began in order to seek efficient actions to improve results. Therefore, the research problem of this article is: how can Lean Manufacturing tools help reduce the Total Frequency Rate of Occupational Injuries (TRIFR) in the company that is the object of this case study?

The general objective of this study is to present the use of Lean Manufacturing tools to reduce the work accident rate.

The specific objectives of this work are as follows:

1. Technically confirming the importance of FMDS (Factory Floor Management Development System) for the improvement of the occupational safety indicator,
2. Proving the importance of proactive management for the reduction of occupational accidents,
3. This case study aims to implement improvements that mitigate/eliminate the risks associated with their activities to demonstrate the importance of involving the company's employees.

Literature Review

In the 50', lean manufacturing emerged in Japan through Eiji Toyoda and Onho, where the main objective was to align the best work sequence to add value to the products requested by the customer. Thus, they made it impossible to copy the production model of the American system, which demanded a wide variety of products, thus giving birth to what is known as the Lean Production System or Toyota Production System (Lean Manufacturing / Lean Production).

According to Toledo (2002), lean thinking can be understood as producing more with fewer resources, focusing on customer needs, and being able to offer what they really wanted, generating immediate value to the work and eliminating waste [1].

According to Campos (1996), everything that does not add value in the direct transformation of the product is considered waste, the main ones being: transport, movement, inventory, excessive processing, waiting, rework, and overproduction.

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All of these increase production costs, decreasing the possibility of increasing profits since the customer is unwilling to pay for them [2].

Process Management

For Falconi (2013), achieving good results is one of the most significant sources of human motivation. However, we can fail by not directing the goals correctly due to a lack of technical knowledge, non-compliance with action plans, or circumstances beyond our control [3].

According to Albertini (2016), mapping processes makes it possible to visually represent the activity, generating the opportunity to see the improvements, simplification, and streamlining of processes [4].

According to Liker and Meier (2007), people are visual creatures, and they need to look at their work and be able to quickly detect some abnormality in their process, with well-planned graphics placed on the wall can perform good discussions and raise opportunities in the activities [5].

As the same authors, we can classify eight considerable losses in a process: overproduction, waiting, transport, overprocessing and overcapacity, unnecessary displacement, defects, and failure to use employees' creativity.

The control of the process allows the management of the entire production chain; having indicators that clearly show the reality of the routine in the operations helps in the execution of actions so that the established goals are not lost over the agreed periods. The main point of effective process management is the base (shop floor) involvement in solving problems even when they are somewhat "small'.

Process Improvement

Continuously improving processes is necessary to achieve growth and reach goals and challenges, in addition to the company's competitiveness in the market. For Paim and colleagues (2009), improving processes is necessary for companies to maintain themselves in the market where they operate, which constantly changes [6].

Chiavenato (2000) states that: "change is everywhere: in countries, in organizations, in technology, in cities, in people's habits, in products and services, in time and weather, in everyday life" [7].

During this inevitable constant change, companies seek evolution through creativity and innovation of their operations, relying on the ideas and suggestions of their employees to solve problems raised through process management and maximum use of the employees' abilities and learning capacity. Chiavenato (2000) also points out that creativity and innovation allow the company to remain in perfect harmony with the business world, where creativity provides new ideas and innovation puts them into practice to create a new company [7]. The kaizen circle is a tool used in the pursuit of continuous improvement. It aims to improve the individual capacity and ability of those involved, increase self-confidence and preparedness for problem-solving, and work better in teams because it creates a proactive and integrated environment by expanding the systemic view of the business, according to Kishida (2009) [8].

Factory Floor Management Development System (FMDS)

As an advanced visual management tool, FMDS (Factory Floor Management Development System), developed by Toyota in 2006 in Japan in the context of the company's global expansion, was implemented in Brazil in 2008 at the Indaiatuba plant.

The primary purpose of FMDS is the development of the base employees through exposure and solutions to problems that hinder the achievement of results, in addition to improving communication between leader and subordinate, making daily management a continuous movement of corrections and improvements, reducing losses in the processes.

Since then, many companies seeking to implement the Toyota Production Model have

used the FMDS (Factory Floor Management Development System) tool as a reference in the management of their indicators.

Materials and Methods

This work has an applied purpose and exploratory objectives. According to Gil (1995), applied or practical research arises from the desire to know something to make it more efficient or effective [9].

For the author, exploratory research aims to increase familiarity with the problem under study to make it more explicit or formulate hypotheses.

The main objective of this type of research is to improve ideas or discover intuitions. Regarding procedures, the present work can be considered participant field research since the researcher and the participants are involved in solving a collective problem.

The research can be framed as qualitative and also quantitative. According to Marconi and Lakatos (2008), qualitative research analyzes and interprets the complexity of human behavior. Through qualitative research, it is possible to describe behavior, attitudes, habits, and preferences [10].

On the other hand, for Berelson (1952, p.18), quantitative research is the "objective, systematic and quantitative description of the manifest content of communication" [11].

This work was also done by bibliographic survey, which, through technical-scientific information, at some point in the history of humankind, an individual had the concern to register his knowledge of the subject. As the name implies, the bibliographical survey involves researching bibliographies written by others.

The article is also characterized as a case study whose objective was to apply a research method on the subject, allowing the deepening of knowledge on the theme. This scientific research strategy analyzes a current condition in its actual context, considering the variables that influence it.

The present work was developed and analyzed from January 2017 to December 2020.

Data Analysis

The railcar management at the company that is the object of this case study consists of 12 supervisors responsible for all the maintenance processes of the fleet of wagons of the EFVM (Vitória-Minas Railroad), currently having 439 own employees. Throughout the research, data will be presented to allow a comparison between the previous and current scenarios of the company regarding the risk situation of its activities.

From this case study, it was found that the main bottlenecks that prevented the improvement in the results of the work safety indicators were: The indicator accompanied by TRIFR rate (Total Frequency Rate of Occupational Injuries) did not generate engagement of the base; The employees could not understand what needed to be done to leverage the results; The action plans were only elaborated from the occurrence of accidents; The managers had difficulty in seeing the risks of their routine operations; The management could not direct investments to the principal risks and the Operational procedures did not specify the risks of the activities;

Figure 1 presents the visual demonstration of the equation of treating accidents from the TRIFR rate indicators (Total Frequency Rate of Occupational Injuries). After the results were achieved, a cause

Figure 1. Previous demonstrative equation.

Source: Authors.

analysis was performed to search for non-repetition of the occurrence, that is, a reactive vision, the reaction of the result always happened after an accident and/or event.

Case Study

It was noticeable that the results of the work safety indicators needed to be improved about the other process indicators. Therefore, it was clear that a change in managing such indicators was necessary.

Using the visual management tool FMDS (Factory Floor Management Development System), it was possible to monitor the risks with the highest potential severity at each activity level, bringing more simplicity to the exposure of problems (Figure 2).

Based on the preliminary risk analysis of the management, it was performed in the field the deployment of all the moments that such risk could be materialized, now every employee can see clearly in their operational procedures the risks associated with each step of their work, allowing employees to make improvements to eliminate and/or mitigate the risks of their activities.

Given the clearly and comprehensively mapped risks, management evolved to manage safety indicators proactively. As a result, a ceremony was created to recognize the employees who implemented improvement work, where they changed the risk classification on the visual management board of the FMDS (Factory Floor Management Development System) and the entire management leadership (Figure 3).

Figure 2. FMDS pillar for management safety.

Source: Fellipe Breno Bergamini (2019) (Author).

Figure 3. FMDS weekly follow-up meeting.

Source: Fellipe Breno Bergamini (2019) (Author).

Current Scenario

With the practice of applying the FMDS (Factory Floor Management Development System) tool, a new visual management of safety indicators was elaborated, which allows better results, such as Employees understanding the risks of their activities clearly during their work day; Actions directed to eliminate or mitigate the risks of the activities, in order to prevent accidents from happening; Control measures developed for non-materialization of accidents; Managers with established routines for checking the risks in their operations, allowing to see opportunities for improvement with the performers; Investments directed correctly to the risks with higher probability and severity and Employees making improvements naturally without the need for direction from managers.

Figure 4 follows a visual demonstration of the equation of the treatment of safety results from the mapping of risks and exposure through the FMDS (Factory Floor Management Development System) tool, focusing on the strategy of seeing the problems before an accident and/or unwanted event occurs.

Figure 5 presents the management risk indicator used nowadays, in which each color indicates the severity of the risk, being:

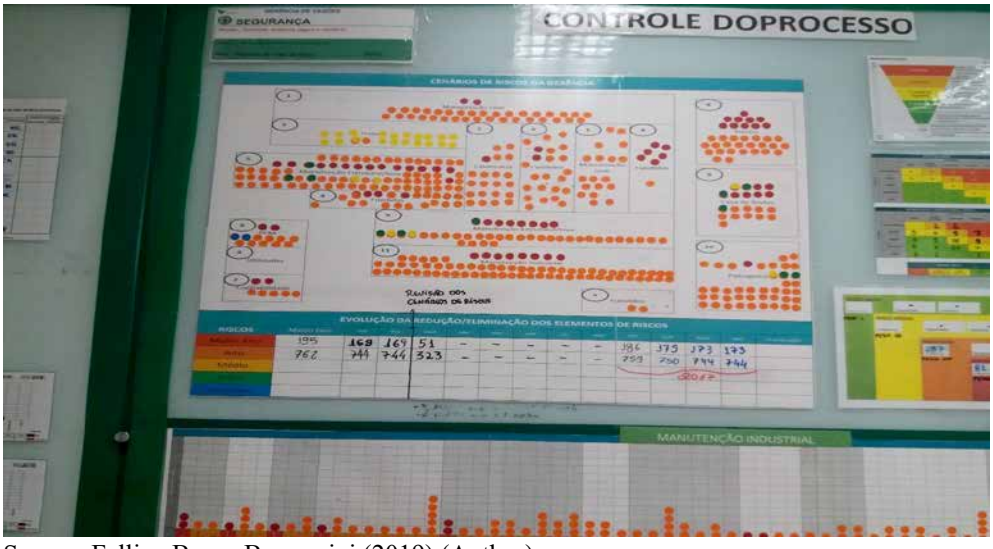
- Red Color: Very high risk;
- Orange Color: High Risk;
- Color Yellow: Medium Risk;
- Color Green: Low Risk;
- Color Blue: Eliminated Risk;

Figure 6 shows the history of the Total Occupational Injury Frequency Rate (TRIFR) indicator used by the company, which shows a significant reduction in accidents after implementing the lean manufacturing philosophy.

Figure 4. Post demonstration equation.

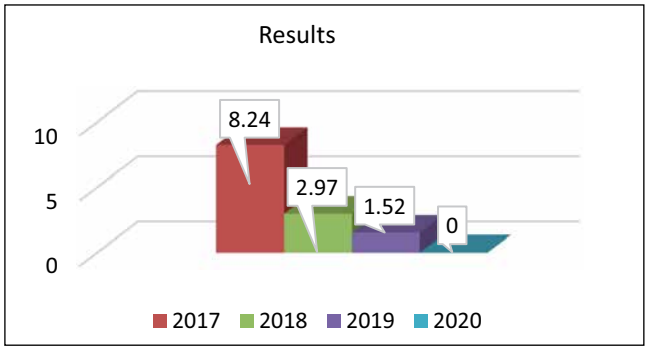
Source:Authors.

Figure 5. Management risk indicator.



Source: Fellipe Breno Bergamini (2019) (Author).

Figure 6. TRIFR rate from 2017 to 2020.



Source:Authors.

Given the research problem: how the Lean Manufacturing tools can help in reducing the Total Frequency Rate of Occupational Injuries (TRIFR), and the general objective of this study which is to present the use of lean manufacturing tools to reduce the rate of occupational accidents, it was found that the lean tools applied most often to eliminate waste and increase productivity also applies in improving the results related to occupational safety, considering the implementation cycle (Figure 7).

Conclusion

Implementing the Lean Manufacturing system tools, especially the FMDS (Factory Floor Management Development System), served as a model for achieving the objective. The methodology, when well adapted, can be used in any business.

With the new visual management model of the safety indicator for the management of freight cars, it is noticeable the involvement of employees in solving the risks of their activities. With this, they begin to be treated proactively, allowing all employees to solve the problems effectively.

Routine management was one of the most challenging points due to the diversity of activities performed, but it is clear that planning and prioritization make it possible to execute. Furthermore, the visual management system helps a lot in self-knowledge and decision-making that no longer stays only at the managerial level but reaches the shop floor where things happen, and people are more prepared to identify the root cause of problems to expose and solve them, making the processes more sustainable.

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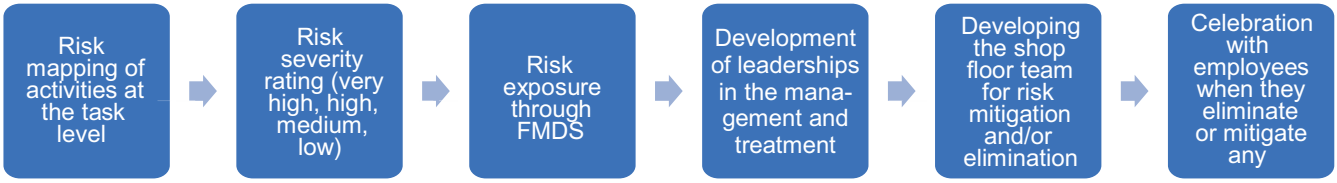
Figure 7. The cycle of the case study.



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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
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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
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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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