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COVER: Cyanobacteria, also known as Cyanophyta on water surface.

Challenges in Science, Technology and Innovation after COVID-19

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This special edition of the Journal of Bioengineering and Technology Applied to Health brings a selection of articles presented at the VI International Symposium on Innovation and Technology (SIINTEC). The SIINTEC happens since 2015. The event is annual and promoted by SENAI CIMATEC, Salvador, Bahia, Brazil. The VI SIINTEC was focused on discussing challenges in science, technology, and innovation after COVID-19. We had to make changes and find immediate solutions to keep people together, now this connection provides us the opportunity of having qualified participants from all over the world sharing and building knowledge. The VI SIINTEC occurred in 2020 from October 21 till 23. The main point is providing the opportunity of reuniting the scientific and technological community to discuss innovation, researches, and advances promoted by the pandemic period and draw applicable conclusions to society's new routine.

Manv researchers seek answers and solutions to questions and problems generated in the current pandemic scenario. Vaccine developments to combat the SARS-Cov-2 virus are being carried out by researchers around the world. But also, all scientific development, despite the difficulties imposed by quarantine, remains active. In this first volume of selected articles from VI SIINTEC 2020, research on developments involving the application of lean manufacturing, development of the gamified model, characterization, and application of oils, biomass, and cyanobacteria species will be discussed. Also, Brazilian manufacturers'



presence in the COVID-19 diagnostic products will be discussed as well.

The research presented here makes significant contributions to state-of-the-art presentations, innovative medical products, health innovation initiatives, and a review article. The authors highlighted in their research: the program "Lean Manufacturing in support of the COVID-19 crisis"; Brazil's external dependence on products from other countries; possibilities of using gamification in the construction environment to help workers in the prevention against COVID-19; identification and study of a promising cyanobacteria species for biotechnological applications; composites reinforced with PET fiber residue have advantages in the development of new material sustainable characteristics; Moringa with oleifera Lam oil can be used as a potential source for the manufacture of several industrial products, such as food and cosmetics; thermodynamics of oils of nutritional/cosmetic use; and characterization of Arthrospira sp (Spirulina) biomass growth in hydroponic waste solution.

We wish you all an excellent reading.

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SENAI CIMATEC Contributions for the Strengthening of Brazilian Companies Using Lean Manufacturing in the Combat Against COVID-19

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SENAI CIMATEC (Technology Center at Salvador, Bahia, Brazil) has been in the spotlight in Brazil due to the development of programs and projects to help companies and employees against the pandemic of the Coronavirus-19 (COVID-19). This study presents the project, Lean Manufacturing in the support of COVID-19's crisis, developed by SENAI CIMATEC, in support of the Brazilian enterprises. The methods for the development of the project used case studies. The results achieved thrive to the success of the program and demonstrate how the use of the Lean Manufacturing tools, in a remote access environment, can assist companies in conducting business consultancies.

Keywords: Lean Manufacturing. Industry. COVID-19.

Introduction

The 2019 outbreak of coronavirus disease (COVID-19) had a huge impact on industries around the world. The Business Consultation research [1], published by the National Industry Confederation (CNI), points out the main pandemic problems: the drop in product demand, the difficulty in obtaining consumable goods and raw materials, and the reduction of supply of working capital in the national financial system. CNI also shows that 92% of the consulting industries had negative impacts. Of this total, 40% correspond to companies that had a great impact, 27% a medium impact, and 25% a small impact. The main production was ceased in four out of the ten industries consulted (about 41%) due to the crisis. The situation revealed as serious that actions must be taken to help the Brazilian industry to recover.

Recent world history has demonstrated the importance of Lean philosophy to aid companies

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and their recovery in times of crisis. At the end of the Second World War, the Japanese industry had very low productivity and an enormous lack of resources. Thus, when a Toyota engineer, Taiichi Ohno, was returning from a trip to the United States, in which he observed that supermarkets replaced the goods on the shelves the moment they were sold, he led the development of the Toyota Productive System (TPS) in Japan, which was designed to increase the efficiency and effectiveness of companies, avoiding waste and the main industrial problems. Hence, the pillars of what would later be called Lean Manufacturing were born: a management philosophy developed by Toyota, which called the attention of other companies in the world with the arrival of the 1973 energy crisis. In this period, Toyota's philosophy proved to be an efficient company with its relentless pursuit of waste riddance and consequent increase in productivity [2].

Therefore, in the face of the scenario of the COVID-19 pandemic in Brazil, it became essential to support research and technology centers to help Brazilian companies to mitigate the impacts on industrial productivity. In this regard, SENAI CIMATEC, whose mission is to raise the Bahia and Brazilian industry, decided to present a proposal for a technological solution capable of helping the business community to face this crisis. Considering the present moment and the importance of Lean Manufacturing and the

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presence of SENAI CIMATEC in this scenario, this scientific article shows the following driving question: How to adapt the Lean Manufacturing tools, in face of the COVID-19 pandemic, to help with the companies' productivity? The main objective of this article was to present the practical results of the program carried out by SENAI CIMATEC, to support 40 (forty) companies against the pandemic, using the Lean Manufacturing method as a technological basis.

The specific objectives of this article were:

- 1. Defining the primary lean tools used by the program;
- 2. Explaining the performance indicator "COVID Radar";
- 3. Presenting a detailed analysis of the program created by SENAI CIMATEC to help industries through the use of Lean Manufacturing.

Scientific research can be defined as the search for information elaborated in a systematic, orderly, and rational way, which obeys academic norms, aiming to elucidate a proposed problem and contribute to the extension of scientific knowledge.

According to Yin [3], the investigation must be followed by a research project that aims to link the empirical data to the initial questions of the study in a logical way, which will provide a conclusion.

This scientific paper produces a case study based on the "Lean Manufacturing in the support of COVID-19's crisis", a program developed by SENAI CIMATEC, in support of partner institutions. The program aimed to encourage entrepreneurs in the face of the coronavirus pandemic scenario by raising awareness and guiding actions to mitigate impacts on industrial productivity, using the principles of Lean Manufacturing.

To carry out this program, the following methodological steps were defined:

- 1. Definition of which and how many companies would apply to the program. Twenty companies were approved in the Northeast region and twenty in the Southeast region of the country.
- 2. Definition of the principal lean tools used by the

program. The tools chosen were Value Stream Mapping (VSM), Added Value Analysis, Cell Design, Standard Operating Procedure (SOP), Line Balancing, and the Spaghetti Diagram.

- 3. Development of four technical live streaming programs broadcasted directly from Brazil Model Factory (a test-bed 4.0 laboratory that includes lean technology located at SENAI CIMATEC) (Figure 1) through CIMATEC's YouTube channel. It addresses the following topics: measures to prevent COVID-19 in the industry, common ground between the risks of the pandemic and the waste of production and the use of the Lean Manufacturing tools against COVID-19.
- 4. Technical and statistical analysis on the results of the live streaming programs such as simultaneous viewers, total views, comments, and impressions.
- 5. Remote guidance provided by the institutions' lean consultants. Such guidelines intend to guide companies as they apply lean tools, develop an operational-tactical action plan, and evaluate the results achieved by the 40 companies in the program concerning productivity, inventory / WIP (work in process), defects, and setup time.

Lean Manufacturing is a management philosophy focused on reducing the eight types of waste: transport, inventory, motion, waiting, overproduction, over-processing, defects, and skills. This philosophy has tools capable of helping companies to achieve high productivity gains and waste reduction. In the program "Lean Manufacturing in support of the COVID-19 crisis", some lean tools were chosen for their ability to help companies to preserve and increase their productivity levels in the face of a crisis. The first tool was the VSM (Value Stream Map.

According to Shook and Rother [5], a value flow is every action necessary to bring a product through all the essential product flows. Usually, the VSM is used to understand the product flow in a company, from the supply of raw materials to the final consumer, analyzing bottlenecks, cycle



Figure 1. Brazil model factory.

times, work-in-process, number of operators, and the information flow. The second tool used by the program was added-value analysis. According to Monden [6], added value activities are generally all those that have some value from the customer's point of view.

According to Kumar and Mahto [7], line balancing, the third tool used in this study, is a term that refers to the process of equalizing the distribution of activities among operators of a production line to optimize available resources. Yamazumi is one of its best known and applied approaches from Lean Manufacturing. Focused on balancing resources, this Lean tool is based on two basic elements: continuous flow and high added value [8]. The fourth and final tool, the Spaghetti Diagram, is used to visualize transport and motion. When transport routes are designed, it is easier to find opportunities and reduce waste. The Spaghetti Diagram is usually drawn by hand, over an image of the company's layout [9]. Finally, standardized work allows the definition of procedures that will ensure the work is done correctly, guaranteeing the quality of the processes.

Results

Technical protocols based on the five conception tools were defined and presented in the remote

meetings carried out by the consultants' team to the participating companies.

Technical Protocol 1 (Added Value Analysis)

It is common to redesign industrial layouts when the focus is eliminating waste in the manufacturing environment. It closes workstations and operators to reduce travel and increase productivity. However, in face of the pandemic context, this traditional approach must be evaluated and the minimum distance of 1.5 and 2 meters between employees must be taken into consideration, in addition to the implementation of physical or flow barriers aimed at reducing possible contagious. For Brazil Model Factory, the distances between workstations were maintained and the pathways of each operator were drawn on the floor.

Technical Protocol 2 (COVID-19 Radar)

The creation of the COVID-19 Radar (Figure 2) aimed at the continuous monitoring of key indicators for production during the pandemic. It uses the measurement and presentation structure of the 5S Radar, a commonly known indicator in Lean, to monitor the current position of a company or a specific sector in regards to the effects of the pandemic. The indicator consists of four categories: a) environment hygiene and safety; b) employee

hygiene and health; c) dissemination of information; and d) managing the flow of entry and exit of people. The grades for each category vary from 1 to 2 and are obtained from a questionnaire that must be carried out periodically. To produce a standardized assessment, the following indicators were used: grade 0 must be understood as "not accomplished"; grade 1 as "partially accomplished"; and grade 2 as "accomplished". The average grades of the categories analyzed should be close to grade 2. This means that the company is complying with the recommendations to face the COVID-19 disease.

For the proper monitoring of this and other indicators used by companies, Lean good practices advocate holding meetings and performance dialogues at the beginning and end of shifts. However, due to the pandemic, it is important to rethink such practices and avoid unnecessary gathering of crowds. For the Brazil Model Factory's performance dialogue and KPI's monitoring practices, the number of participants was reduced, pre-determined and the position of each person was signed on the floor, in addition to the incorporation of the COVID-19 Radar as a safety indicator.

Technical Protocol 3 (Line Balancing and Cell Design)

During the pandemic period, the materialization of this concept goes beyond the already traditional productive improvements. The continuous flow application ensures that the production items pass once per workstation and, when combined with the correct operators and the workstations hygiene, reduces the possible contagion points within the production environment. As an example, for Brazil Model Factory, hygiene steps were added to the cylinders before shipping to the customer, ensuring their safety and satisfaction.

Technical Protocol 4 (VSM)

Based on this definition, the VSM shows itself as a tool of great help for companies to adapt to a pandemic such as COVID-19. When carrying out an analysis of the product flow, it is possible to pinpoint all the sectors through which it travels, allowing to trace possible points of contagion among operators. Its use is also evidenced when the information flow is analyzed. At this time,



Figure 2. COVID Radar.

the company must focus on digital information within production, at the expense of physical information, such as printed production orders. If it is not possible to replace physical information with digital information, the focus should be on reducing the number of people who have access to these physical production orders, avoiding contact of several operators with this object.

Technical Protocol 5 (Spaghetti Diagram)

Although the main focus of the Spaghetti Diagram is to verify waste in motion and transport, in the current pandemic scenario it can also be used to check possible contagion points and create independent paths for employees and customers. At Brazil Model Factory, after designing and analyzing the diagram, unique path routes were established for each operator, and marked on the floor, to avoid backflow and contact with other employees.

The practical results of the program were reflected in the companies. Initially, it is worth highlighting the success of the live streaming held with the large participation of employees from the 40 selected companies. The indicators point to 1,135 simultaneous viewers, 4,025 views, 1,508 general comments, and 32,000 impressions. The survey carried out with the participants still shows that 89.65% of the participants said they had their expectations met or exceeded, 86.2% said they were satisfied or very satisfied and 89.65% stated that the content presented during the live streams had relevance for their jobs. Regarding the practical results of the program for the selected companies, these are the results of the remote meetings. The meetings occurred as follows: (i) A total of eleven consultants involved in the project; (ii) Remote assistance to the forty selected companies in the program (20 companies in the Northeast region and 20 companies in the Southeast region); (iii) 220 activities were developed in the action plans for application in companies during and after the end of the guidelines and; (iv) 160 hours were invested in remote meetings, with 1 hour per week for each company (Table 1).

Table 1. Remote meetings results.

Indicators	Results
Productivity increase	35%
Defects reduction	75%
Setup time reduction	20% 30%

Among the results obtained by the online meetings, it was possible to observe very expressive gains, even in a short amount of time. First, there was a 35% increase in productivity in a packaging company after a reduction in transportation, handling, and incidental activities. 75% reduction of defects in a company in the food sector, due to adjustments of an underused machine in production processes. 20% reduction in inventories of a furniture company, after applying the 5S principles. Finally, the program also enabled a 30% reduction in setup time in a company in the print sector, after changes in layout and standardization. It is worth noting that the pilot action lasted for only 1 month, and the results obtained were collected during just one month of action. Expectations are that further gains will be achieved in the long term, which may be presented in future studies.

The program "Lean Manufacturing in support of the COVID-19 crisis", developed by SENAI CIMATEC, presented expressive results in its first pilot. Initially, it is worth highlighting the success and scope of all the live streaming broadcasts, with a significant number of participants. The program also corroborated the importance of adapting and making productive environments safer for industrial operators in the face of the current pandemic. Also, the program was able to adapt Lean Tools for remote learning methods and showed its potential through the expressive indicators of results achieved by the involved companies. Finally, the Lean Manufacturing philosophy reinforces once again how it can be crucial in a moment of crisis.

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Analysis of Brazilian Manufacters in the COVID-19 Diagnostic Products by ANVISA

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In March 2020, the World Health Organization (WHO) declared COVID-19 as a pandemic. Diagnostic and screening tests have been important tools for the clinical characterization of diseases such as COVID-19. This study aimed to analyze the presence of Brazilian manufacturers in the COVID-19 products approved by the Brazilian Health Regulatory Agency (ANVISA). This research used the ANVISA database, in which 329 registered products were identified. We classified them into 4 methods, 3 of which were the object of careful analysis. The results showed Brazil's external dependence on products from other countries, mainly from China. Keywords: COVID-19. Diagnostic Products. ANVISA.

Introduction

The year 2020 presented to the world a new scenario: the pandemic of COVID-19, a disease caused by a novel coronavirus (SARS-CoV-2). Due to the occurrence of this public health emergency on a global scale, the entire social and economic structure all over the world changed. In December 2019, Chinese government officials informed the World Health Organization (WHO) about many cases of unknown pneumonia. On March 11, 2020, WHO announced COVID-19 as a pandemic [1].

The most common symptoms of COVID-19 are dry cough, fever, and tiredness. However, some individuals present different symptoms, such as pain, runny nose, headache, conjunctivitis, sore throat, diarrhea, loss of taste or smell, rash on the skin, or discoloration of fingers or toes. It is noteworthy that about 80% of the individuals affected by the disease do not require hospitalization, but approximately 17% of infected patients develop a severe form of the

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disease, characterized mainly by shortness of breath and pneumonia. According to WHO data, the novel coronavirus pandemic is responsible for the 6th Public Health Emergency of international importance in the last 11 years: 1. H1N1 pandemic (April 25, 2009); 2. The international spread of poliovirus (May 5, 2014); 3. Ebola outbreak in West Africa (August 8, 2014); 4. Zika virus and increased cases of microcephaly and other congenital abnormalities (February 1, 2016); 5. Ebola outbreak in the Democratic Republic of the Congo (May 18, 2018) [1].

Although Italy was the second country in the world to suffer a major pandemic-related impact, shortly after the outbreak started in China, current WHO data show that the countries with the highest number of cases, until July 30, 2020, are the United States, Brazil, India, Russia, South Africa, and Mexico. Concerning the death toll, the countries most affected are the United States, Brazil, the United Kingdom, Mexico, and Italy. In addition to the need to promote social isolation, pandemic control measures initially include the importance of carrying out mass testing of the population [2]. Although WHO indicates that testing is the main tool to contain the spread of the novel coronavirus, a restriction and difficulty of access to diagnostic products are observed worldwide, in addition to the difficulty in pacifying the strategy related to the prioritization of tests and interpretation of the results [3]. Until July 30, 2020, 2,610,102 cases were confirmed in Brazil, with 86,449 deaths.

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On the reference date, there were 1,824,095 people recovered and 694,744 under medical supervision. Until now, Brazil has not been able to contain the spread of the vírus [4].

Around the world, governments have adopted strict rules that restrict personal mobilities and close non-essential commerces in response to COVID-19, but such measures have been causing contractions in the economy. Some countries have managed to implement testing routines for the entire population, while others have restricted testing only to hospitalized people. The detection of SARS-CoV-2 in the population is critical to combat and control the spread of the virus. International health agencies, such as WHO, as well as scientific societies, recommend laboratory tests for patients with suspected COVID-19 as a priority in clinical management and outbreak control. As it is a new disease, diagnostic product segment industries have rapidly presented products capable of responding to emergency demand from the pandemic. Additionally, governments and regulatory agencies around the world have adopted measures to reduce the bureaucracy to the products' registration process [5]. The first step to allow the commercialization of a health product in Brazil is the registration by ANVISA, as indicated by Law 6.360/76 [6]. So, this study aimed to analyze the participation of Brazilian manufacturers in promoting access to diagnosis, from an analysis of the registries of diagnostic products for COVID-19 granted by ANVISA.

Materials and Methods

Structured research was carried out on academic research databases (Scielo; SCOPUS; PUBMED; Elsevier Researchgate; and PlosOne), and the websites of WHO and the Brazil Ministry of Health. The study was conducted in July 2020. The definitions of ANVISA and the Ministry of Health were considered to classify the products into the 4 methods extracted from the documents "Testes para COVID-19: Perguntas e Respostas" [7]; and Boletim COE COVID-19 [8]. Also, the analyses focused on rapid tests, RT-PCR, and ELISA, since these are the main types of diagnostic methods acquired by the Ministry of Health for distribution to the SUS (Unified Health System), under the program "Diagnosticar para Cuidar" [9]. The obtained data of this study occurred by the analysis of diagnosis products approved by ANVISA between May 18, 2020 (date of publication of the first specific kits registration for COVID-19) and July 31, 2020, (a link of the ANVISA website named "Fila Completa de Produtos de Diagnóstico in vitro para COVID-19") [10]. Some registries found on this link did not contain the manufacturer's information. In these cases, a detailed consult was carried out about the registration on the ANVISA website: i) consult of regularized products; ii) consult of health products; ii) consult by the number of processes. Thus, it was possible to verify the manufacturer of the registered product. However, the registration holders are mostly national distributors, which do not serve as a parameter for an analysis of the national industry presence in the market of these products. Also, the same product can be registered in Brazil by different companies, and that the proposed analyses are based on the nationality of the manufacturer in each registry.

Results and Discussion

According to ANVISA, tests related to the diagnosis of the novel coronavirus are categorized as "products for *in vitro* diagnostic use", which includes: "reagents, calibrators, standards, controls, sample collectors, materials and instruments used individually or in combination, with intent to use determined by the manufacturer for *in vitro* analysis of samples derived from the human body, exclusively or primarily to provide information for diagnostic purposes, monitoring, screening or to determine compatibility with potential blood recipients, tissues and organs" [11].

There are many methods to detect SARS-CoV-2. The gold-standard method so far is the

molecular method RT-PCR in which the genetic material (RNA) or "parts" of the genetic material (antigens) of the virus is detected. The serological test is also used, in which the antibodies against the virus (IgM and IgG) are detected in the serum of patients. Among the serological tests, there are rapid tests that use the immunochromatography method, which searches for IgM and IgG antibodies against the virus. The rapid tests are performed through easy-to-use devices and are manual, capable of giving results in up to 30 minutes and without the need for support and laboratory equipment [7]. Although it could test a large number of people and quickly analysis (<15 minutes), this method has serious issues about low sensitivity for ill patients (IgM), which may lead to a misdiagnosis for COVID-19. Besides, these tests are only qualitative for the presence of IgG and IgM and cannot detect the onset of the disease, since the presence of these antibodies can only be detected after the seventh day of infection [12]. According to ANVISA, other serological tests requiring laboratory equipment were also registered, such as the ELISA method (Enzyme-Linked Immunosorbent Assay), which is based on an enzymatic reaction, which makes the antigenantibody reaction visible by a chemical reaction, which turns visible and quantitative; as well as the CLIA method - chemiluminescent immunoassay -, in which the reading of the result is made from the fluorescence formed by the antigen reacts with the antibody, turned visible and quantitative the presence of antigen-antibody [7]. The ELISA test and CLIA are important methods, both in the clinical management of patients with suspected COVID-19 and in the control of the pandemic. These assays, unlike molecular tests, are serological tests that allow for the detection of specific antibodies (IgG and IgM) produced by the immune system in the blood of infected patients or those who have already had exposure to the virus [13].

With the advent of the pandemic, and following the trend of other regulatory agencies, ANVISA published a Resolution of their Board, called RDC 348/2020, which "Defines the extraordinary and

temporary criteria and procedures for the treatment of petitions for registration of medicines, biological products, and diagnostic products in vitro and postregistration change of medicines and biological products due to the international public health emergency arising from the novel Coronavirus". It is emphasized that other resolutions have also been published to facilitate the availability of other products used to combat COVID-19, such as hand sanitizers, masks, ventilators, among others [14]. Until July 30, 2020, ANVISA had published the approval of 329 applications of products for the diagnosis of COVID-19 and rejected the applications of 100. On the same date, the agency was still expecting the Certificate of Good Manufacturing Practices of 19 companies and had placed 126 processes in demand. Among the identified registries, almost 69% refer to rapid tests (immunochromatographic tests) (Figure 1). The total amount of this sort of test registered is higher than the total quantity of the registered tests that use other techniques. For example, the approval of ELISA tests represents 7% and PCR, 15%. Among the tests categorized as "others", there are chemiluminescent immunoassay tests (CLIA) and immunofluorescence tests.

international During health emergencies, the RT-PCR test is the gold-standard method by WHO with a high sensibility and specificity for detecting respiratory pathogens in patients with an acute respiratory infection. Soon after the emergence of the first cases of COVID-19, the presence of SARS-CoV-2 in respiratory samples was detected by RT-PCR and by partial or total sequencing of the viral genome [15]. Another issue about the product registrations at ANVISA is the difference between the quantity of national and international products. One hundred and eighty-eight registries were approved for products with Chinese manufacturers, while only 47 registries have Brazilian manufacturers (Figure 1a). As well, the number of rapid test registries were mainly from Chinese manufacturers (Figure 2b).

Brazil faced many difficulties in providing the necessary inputs for the operationalization of an



Figure 1. Registries of COVID-19-tests diagnosis at ANVISA (July 30th, 2020).

Figure 2. Total test approved by ANVISA. (a) total tests approved per country; (b) total immunochromamatographic tests (rapid tests) per country.



effective mass testing program. This fact is due to the low supply capacity of products from the Brazilian industry and the initial difficulty in acquiring inputs from the United States and China [8]. Many countries have invested in the development and distribution of new tests and their inputs and regulatory agencies have allowed additional flexibility in the registration process to promote the rapid adoption of new methods. However, despite these efforts, the overall testing capacity has not been sufficient to meet current and expected needs in some countries such as Brazil. Several factors contribute to this situation, as follow: lack of available kits or inputs for the tests; a limited number of facilities for testing; scarcity or unequal distribution of consumables and reagents for processing; insufficiency of swabs and personal protective equipment for managing the samples; and absence of certainty on how to interpret or operate the results obtained [16]. The trend repeats if we analyze the PCR method. The participation of Brazilian manufacturers is slightly higher, but still incipient when compared to a large number of Chinese and Korean product registries. Out of a total of 49 approved tests, 16 have Chinese manufacturers, 11 have South Korean manufacturers and only 6 have Brazilian manufacturers (Figure 3). For tests using this method, also a considerable number of products from North American manufacturers were considered as well (7 registries).

We have to highlight that the presence of products from national public laboratories between the manufacturers of Brazilian approved registries. FIOCRUZ had 4 approved products and will produce2PCR tests and 2 immunochromatographic tests (rapid tests). Considering Butantan Institute and Bahiafarma Foundation, the registries have also been found, but as distributors manufactured by South Korean companies. Regarding the participation of the private sector, 24 Brazilian products manufacturers had approved, which shows that there is some capacity to produce tests for diagnostics in the country. The Ministry of Health in Brazil had been distributing exclusive RT-PCR tests and rapid tests until June 2020. This action is part of the program "Diagnosticar para Cuidar", which is included in the national epidemiological and laboratory surveillance strategy for COVID-19. The program intends to perform 46 million tests this year, which would constitute approximately 22% of the Brazilian population being tested. It consists of two strands: "Confirma COVID-19", which will use the RT-PCR method (molecular biology), and "Testa Brasil", which will leverage

the use of rapid tests (serology) in the country to understand the progression of the virus [9]. By the end of June 2020, the Ministry of Health announced that they would start to distribute ELISA Test as part of the program "Diagnosticar para Cuidar". This method allows the detection of antibodies with high accuracy and helps to understand the progression of the virus in the country [17]. There are 28 ELISA tests approved by ANVISA and none of them are from China. Brazilian manufacturers had 9 tests approved, while German manufactures had 9 tests approved.

Thus, Brazil is still working towards the adoption of effective methods for the diagnosis of COVID-19 that collaborate with government strategies aimed at containing the pandemic. It points out a gap that can still be covered by the national industry, which still has low participation in the total number of products approved by ANVISA.

Conclusion

ANVISA has worked to rapidly allow the registration of products for the diagnosis of COVID-19 in Brazil. The agency had made possible the commercialization of 2 methods listed, highlighting that the Ministry of Health has been making use of 3 of them. The number of registries containing Chinese manufacturers is impressive, especially regarding rapid tests

Figure 3. Total RT-PCR tests approved by ANVISA per country.





Figure 4. ELISA tests approved by ANVISA per country.

(immunochromatographic), which reflects the global trend of dependence on China. Despite Brazil is the second country in the number of manufacturers, behind only China, Germany, the United States, and South Korea also have a considerable number of registrations at ANVISA.

Therefore, the analyses performed based on the participation of national manufacturers in the registries of diagnosis products of COVID-19 shows small participation of the Brazilian industry, which can be better used and stimulated by promotional mechanisms to reduce Brazil's external dependence on foreign products, especially products from China.

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A Gamified Model for the Building Site: A Solution to Motivate Construction Wokers in Pandemic Time

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This article aims to present the first results of a study that investigates how construction companies can use gamification to help workers assume appropriate behaviors and motivate construction teams to achieve their production goals. It is an exploratory, empirical, qualitative research, for which the methodological approach is Design Science Research. A closed questionnaire was carried out to construction workers to understand the context of the workplace. This tool aims to investigate motivational aspects, and another questionnaire, with engineers, aims to identify which production problems to address in a proposal for a gamified model. The results show that most workers have a need to be recognized and that the proposed solution can contribute to the continuous improvement of the work environment. The article presents possibilities of using gamification in the construction environment to help workers in the prevention against COVID-19.

Keywords: Gamification. Motivation. COVID-19. Production Control. Construction.

Introduction

In 2020 the world is experiencing a pandemic never imagined. Everyone was preparing for a nuclear war, but it was never thought that a small virus could alter the routine of everyone on the planet. Researchers from all over the world started to look for a vaccine [1,2], which has already been discovered and is in production. The question is: "After the COVID-19 pandemic, will there be others? What will everyday life be like after COVID-19?" Certainly, there will be changes and learning in all sectors that are going to be permanent. More specifically in the Civil Construction sector, many are wondering: "What does the new normal look like?" In the context of Civil Construction, since the emergence of Lean Construction [3], the construction industry has been pursuing the efficient implementation of lean production in

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construction sites. Lean practices are deeply connected to human behavior, and there is no way to separate their effects. Furthermore, at the construction site, projects are complex and involve several intervening factors that work simultaneously, often resulting in unnecessary rework, movement of people, and material handling.

Thus, despite the discussions about lean construction [3-5], the reality of the construction site is still characterized by an environment where communication between managers and workers is deficient, the workforce is rotating and little engaged, the workers perform manual and repetitive work [6] and have a low qualification and education. Furthermore, the diversity of services and the dynamic way in which they take place can make production control a complex activity, and it becomes arduous to meet the goals set in the planning [7].

So, one of the significant challenges during construction is to control its processes so that they occur as planned and, if something goes different from the program, to identify the deviations quickly and make improvements. These actions and their results on a construction site directly involve the performance of workers. Consequently, the importance of investing in improvements in working conditions is evident, since the satisfaction and well-being of workers will reflect in more dedication and commitment to the performance of their duties.

Construction companies generally have few visual mechanisms to inspire, motivate, or instruct workers to do their jobs more effectively, efficiently, and safely [5]. There is a need for structures to facilitate the feedback process to the worker and to make relevant information transparent to them [5]. Little feedback on the work in progress and the performance of the workers involved in the process leads to low engagement at the construction site and makes it difficult to meet production goals effectively [8].

It is in this context that gamification, defined as the insertion of game elements in non-game contexts [9], appears as an innovative idea to improve communication between managers and workers, promoting motivation and engagement. The strategy aims to motivate the worker to perform repetitive tasks, encouraging them to assume the player's behavior. So, how can gamificationstrategiesimproveproductioncontrol and workers' motivation at the construction site? The purpose of this article is to present the first results of a research that investigates, through Design Science Research, how construction companies can use technology to involve and help workers to assume appropriate behaviors and motivate construction teams to achieve their goals, incorporating game elements into applications and processes through gamification.

Gamification

There are many definitions for the term gamification [9-11]. Deterding refers to gamification as adding game mechanisms to everyday life contexts to provide fun experiences and improve the motivation of those involved. It is important to note that, despite the use of game elements, gamified applications are not exactly games. Gamification is usually used to influence human motivation and behavior positively [11,12]. Therefore, several game strategies can be used in gamification, such as Avatars, Teammates, Points, Badges, Leaderboards, Narrative, Performance Charts, Rewards, Voting, Bets, Levels, Missions, Progress Bar, Collaboration, Unlocking content, Rules, Online Store, Missions, Deadline and Feedback [12-16].

Chou [15] states that most systems are designed to facilitate tasks to be performed in the shortest possible time. Therefore, during the design phase, it is necessary to consider that the people who perform these activities have emotions, insecurities, and opinions about what is expected of them, or about how they should achieve their professional goals.

Deterding [17] presents two rhetorics for the conception of a gamification process:

- 1. Rhetoric of chosen architecture that presents human beings with rational and strategic agents and gamification as an information system with incentives. This rhetoric launches gamification as a refinement of existing business practices.
- 2. Humanist rhetoric is based on positive design psychology and virtue ethics. This rhetoric believes that human beings are social, emotional creators of meaning and oriented towards growth. It is directly focused on the well-being of the worker.

Gamification in the Production

In 2012, Korn & Schmidt [16] introduced the first concept of gamification in industrial workplaces. In the motion EAP research project (2013-2016), ways of improving assistance systems in production environments are explored. In parallel, in the same year, Leite [6] began his studies of gamification at the construction site. The research by Korn & Schmidt [18] proposes the implementation of gamification elements as an avatar and uses color-coding to visualize the progression of time, especially for the automobile industry. Similarly, Leite's research [6] uses elements such as points, ranking, and leaderboards to motivate workers to comply with weekly planning goals. Warmelink and colleagues [19], present a Systematic Literature Review (SLR) on gamification in industrial production from 2012 to 2017. SLR was carried out in May 2017. This review results in sixteen studies, twelve of which were developed by Korn & Schmidt [18] and two by Leite [6] and Morêda Neto [8]. This highlights the research gap in the production area.

The Protocol in the Construction Sector

For safe workplace activities during the COVID-19 pandemic, a sectoral protocol was launched to establish recommendations for the application of preventive measures due to COVID-19 in civil construction works [2]. In these environments, there is a concern with the minimum distance between employees, hand hygiene, Personal Protection Equipment, and the tools used by them. Thus, it is essential to raise awareness among this audience through lectures, the establishment of shifts to avoid cluster, and the constant hygiene of the environments, the seats, and other surfaces used by workers.

Usually, some workers have difficulty using the Personal Protection Equipment required by regulatory safety standards. There is also difficulty in communication between the tactical and operational levels at the construction site [6]. Therefore, to overcome these difficulties, gamifying some behavioral rules can serve as an incentive for the worker not to neglect their health in the face of the need to work in a pandemic time. Some examples of the protocols established for the sector: maintaining a safe distance of 1.5m between workers, using a mask, glasses, and face shield; adopting continuous hand hygiene procedures; leaving tools sanitized for the next day; avoid crowding in the intervals; all waste and disposable Personal Protection Equipment's must be disposed of safely, in places indicated on the construction site; participating in lectures on the risk of coronavirus (COVID -19), and measures to prevent contagion, as well as company protocols (Figure 1) [2].

Materials and Methods

Design Science Research was selected as the most appropriate methodological approach for the development of this work because it is used in research wherein the objective is to create, develop, and explore new solutions [20]. In this case, it is an exploratory, empirical, qualitative research to evaluate strategies to improve production control and engagement of construction workers through the creation and implantation of a gamified artifact. The research consists of 4 phases: (1) the understanding phase, (2) the Development phase, (3) the analysis phase, and (4) the Phase of evaluations and reflections (Figure 2).

In the phase of understanding the problem, starting from the bibliographic review carried out in the first stage, the knowledge gap that gave rise to the real problem was identified; for this, an exploratory study was carried out. Contact was made with two companies that practice Lean construction in their construction sites. The two sites were visited to determine the requirements, the plan, and to understand the process for gamification.

Focusing on the problem defined in the previous step, we used data from the research on motivation developed by the research group GCIS-IFBA for the application of questionnaires with the research subjects (supervisors and workers) seeking to better understand the following points:

- 1. What does the worker, the gamification user, expect from the process?
- 2. What would motivate them to participate in gamification?
- 3. What does the company, the environment to be gamified, need?
- 4. What are the desired behaviors to be motivated by gamification? These questions guided the survey of requirements for the gamified solution.

From this knowledge, it was possible to perform the selection of software and hardware to develop the artifact and prepare the first proposal of the model.





Source: Public domain images.





Results and Discussion

Understanding the current situation is the first step towards solving the problem. For this, we carried out an exploratory study to obtain a requirements survey and select the software and hardware to create a model for solving the problem.

The Preliminary Exploratory Study

The exploratory study had two objectives:

- 1. Identify the needs of workers and engineers to insert gamification strategies on the construction site;
- 2. Knowing the characteristics of the workers.

For this, we conducted questionnaires with the target audience that had the following samples: 109 workers answered, 108 of which were male and 1 female. Average age group: 35.76 years. Education: 46.8% did not complete elementary school, 27.5% of them finished elementary school, and 25.7% completed middle school. Composition: 59 professionals, 37 helpers, and 13 servants. The empirical space was the construction site. Regarding the Engineers, we received twenty-six responses, and their experiences ranged from 5 to 35 years. The first question in the questionnaire was: what does work mean to you? And it is aimed at identifying how the construction worker positions himself concerning the work he does.

In Figure 3, 96% of respondents like what they do, and only 3.7% of them find work an unpleasant activity, exercising it for survival. Then, we tried to probe what motivates the worker. Figure 4 presents the results of this question.

Regarding the motivation to do a better job, the majority (51.4%) shows an enormous lack of recognition. It is probably related to the lack of feedback. As already noted in past surveys, the worker never or rarely ever receives a response (feedback) on the achievement of the weekly goals. The next questions were asked to understand the worker's relationship with games. Analyzing the results, 71.6% of workers like to play. At the start, the first ten respondents expressed some discomfort with the question due to their religious line, which perceives games as wrong, and because they did not understand what type of game it was. The researchers were oriented to clarify the issues. After that, the research flowed normally. For those who answered affirmatively to the previous question, a more specific question was asked to find out what they like to play (Figure 5).

Most workers (83.3%) answered sports games, especially soccer. Some also like cards and board games. These data will support the design of a gamification proposal. We can see from the responses that there is a great sense of collaboration among workers, which leads us to believe that this audience will feel motivated to work with collaboration games, giving greater focus to 'competition between teams and collaboration between peers.' To implement the gamification on the construction site, it is necessary to know the level of involvement of the worker with technological resources. For that, we verified if they have smartphones and what applications they use. 45% of workers usually play games using their smartphones. This value corresponds to approximately half of the audience, and because playing using a computer or smartphone is not an essential requirement to participate in gamification, this is quite significant to encourage team play. Thus, it is possible to use the device to give gamification feedback to workers, as half of them are already familiar with the game mechanics (ranking, points, and others) it is believed that this will facilitate the learning of the entire group.

Figure 6 illustrates the workers' responses. We see that 84% of them use WhatsApp, so this tool can be used as a means of communication with the worker during the gamification process at the construction site.

Figure 7 shows the meaning of fun for the worker. For 86.2% of workers, fun is something pleasurable and part of everyday life. And only 0.9% of them do not feel entitled to the fun. Therefore, the majority is prepared to accept a playful activity, and a few could reject it.

They understand that fun is something pleasurable and that it is part of everyday life. They liked the idea of gamification and want to participate. As for the engineers (supervisors): they believe that gamifying is interesting to improve the progress of the work and the motivation of the worker. Only 30% have daily information on worker productivity. Most engineers (96.2%) believe that the idea of giving feedback to workers is a good one and that it would increase motivation. The use of mechanisms that will motivate workers to carry out their activities to their full potential is well accepted. They liked the idea of gamification and would adopt it at work. 65% said their companies want to retain employees.

Figure 3. The job for the worker.



Figure 4. The motivation for working.



Figure 5. What is the games for workers?

If you answered 'Yes', what do you like to play?

78 answers



www.jbth.com.br

Figure 6. App. and social midia used by workers.

Do you have and use Facebook / Instagram / WhatsApp?

109 answers



Figure 7. The worker and the fun.



Proposal for the Gamified Model

A mobile device gamified system will be designed to give feedback to the worker on his performance from the data collected from the monitoring of the services. Rules will be developed, together with the project managers, so that the gamified system can assess the worker's performance. Gamification may use points, avatars, and other game mechanisms to encourage participation and improve performance. Only the worker will know his performance.

A gamified interface presented on a monitor strategically installed in the workplace will show the performance of construction teams and the weekly progress of services. This service will be monitored by the site supervisor.

Working in COVID-19 times, individual controls may be necessary, such as the use of masks, frequent

hand washing, and or use of alcohol, keeping time out regulated by the World Health Organization, and others. This need can be transformed into a gamification rule. The control of these rues can be done by placing a camera in the place available for handwashing. The distance can be measured by an application using the cell phones themselves. The system to be developed must be very flexible in terms of creating rules, so new rules in the event of a pandemic will be easy to implement.

Figure 8 presents a general scheme of the gamified model.

Worker's feedback be presented can through color graphics Green (great), Yellow (reasonable). and Red (bad) already known and used in construction sites, or even by icons such as faces in the same colors (smiles) or other signs that connote performance. Thus, the employee can feel recognized, motivated, and understand the weekly goals, which should improve the team's performance.

Conclusion

The article aimed to present a proposal for a model for the implementation of gamification to improve production control in civil construction. To support this proposal, a field survey was carried out with 109 workers from which it was possible to establish a profile of the target audience. From this, a gamification model was idealized to make the weekly goals more transparent and to motivate workers to meet them at the construction site. The idealized model proved to be flexible enough to allow for the insertion of new rules, as exemplified by the pandemic situation which requires certain behaviors, which the worker can have difficulty assimilating or finding the motivation to comply with them. This research contributes to the continuous improvement of construction sites to provide a more integrated and safer environment for carrying out the work, establishing more effective

Figure 8. General gamification information scheme at the construction site.



Source: Public domain images (Apadted).

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communication between managers and the workforce. Future work will be the modeling and validation of the system with a group of engineers and construction professionals to design the web version.

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Identification and Study of a Promising Cyanobacteria Species for Biotechnological Applications

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The present work aims to evaluate cyanobacteria isolated from a lagoon located in Salvador. The species have a potential for toxin production and growth in a controlled environment suggesting the potential of its biomass for biotechnological application. The species were identified and analyzed for the presence of cyanotoxin-producing genes using molecular methods. The growth kinetics of the microorganism was carried out in the laboratory and the results (productivity of 0.5 g/L) showed that this species can be cultivated under controlled conditions. The sequencing indicated similarity with a *Brasilonema* sp and the PCR products for the detection of cyanotoxins were negative, which makes it possible to use as food or bioactive compound sources. Keywords: *Brasilonema* sp. Biomolecular Methods. Bioprospection. Biocompounds. Bioactives.

Introduction

Cyanobacteria are single-celled microorganization. organisms prokaryotic with photosynthesizers (usually called blue-green algae), and which are present in most ecosystems on our planet [1]. They were responsible for the accumulation of oxygen (O₂) in the primitive atmosphere and for the appearance of ozone (O_2) , which retains part of the ultraviolet radiation. In addition to being important for the production of oxygen, some of them can absorb and fix nitrogen from the air [2,3]. However, some species are potentially toxin-producing, such as cyanotoxins, saxitoxins, and microcystins.

There is still relatively little known about the diversity of cyanobacteria. Numerous special morphotypes are likely to be discovered through bioprospecting, especially in tropical and extreme habitats. The modern combined methodological

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approach, which applies molecular procedures in conjunction with other methods, allows for greater recognition and a more accurate definition of this wider diversity and the differences between existing genders [4]. Thus, sequencing the 16S rRNA gene has been used as a standard genetic approach to delimit genera of cyanobacteria [5],

Despite their long evolutionary history and their essential importance in the different trophic chains, cyanobacteria species represent one of the most recent trends in biotechnology, since they are promising for the sustainable production of products such as food, feed, cosmetics, drugs, chemicals, and chemical fuels and biofuels [6,7].

The growing interest in the study of cyanobacteria is due to its ability to synthesize and accumulate chemical compounds with high added value and its use as a raw material for several products of commercial interest [8]. It is worth mentioning that the cyanobacterium biomass Spirulina (*Artrospira* sp) is approved by ANVISA even for human consumption.

Additionally, the biomass production of these microorganisms has little impact on the environment, the means of cultivation for cyanobacteria are generally cheaper, with low consumption of water and energy in the production process, without the need to use agricultural areas, and normally has a high yield per area [9,10]. Thus, expanding the studies of these raw materials to the field of biotechnology becomes paramount

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in this context, and in this project, it brings the advantage of evaluating and using a promising new species of cyanobacteria as an alternative raw material for industrial application.

The knowledge of the presence of biomolecules in biomass cyanobacteria and its mechanism of action, in addition to the scientific contribution, can be a subsidy for the development of technologies that allow the use of these substances for the production of food inputs, medicines, and cosmetics [11]. Due to the particular characteristics of some cyanobacteria, it is possible to consider that the prospecting of new species and products of these organisms are very desirable and promising initiatives. Several studies on biocomposites extracted from cyanobacteria have highlighted and confirmed their bioactive activities, high protein content for food, among numerous applications [12,13].

The objective of this article was to evaluate a new species of cyanobacteria, *Brasilonema* sp, regarding the potential for toxin production and growth in a controlled environment to infer about the potential for biotechnological application of its biomass.

Materials and Methods

Species Isolation and Molecular Biology Analysis

Isolation of the Species of Interest

To isolate the species of interest, the sample was grown in BBM medium for 10 days, then 10μ L of the sample was collected for inoculation in Petri plates containing BBM agar (2%). After observing the mass growth of microorganisms on the surface of the culture medium contained in the Petri plate, procedures for isolation of the species were performed. These procedures involved repeated pecking and streaking of the species and continuous observations using an optical microscope (Zeiss Axiostar Plus). The species of interest were collected from the plate and resuspended in a sterile liquid culture medium. The cultures were evaluated for the presence of

contaminants species through microscopy. After obtaining the isolated cultures, a fraction of the biological material was inoculated in a BBM culture medium in the liquid state to acquire sufficient cell mass for the identification and DNA extraction steps.

Genomic DNA Extraction

The first extraction step began with cell lysis of cyanobacteria biomass in a microtube, adding 300 µL of TE buffer (Tris EDTA - pH 7.8 for DNA), glass beads (approximately 100 µL), and 300 µL of the phenol/chloroform/isoamyl alcohol mixture (25:24:1) (Roth). Then, the microtubes were placed in a cell disruptor (FastPrep-24[™] 5G, MP Biomedicals). After this procedure, the microtubes were centrifuged for 5 min at 14,000 rpm, and the supernatant was removed and transferred to new 1.5mL microtubes. For DNA precipitation, 100% ethanol was added until completing a volume of 1.5 mL. Then, the microtubes were inverted 10x and centrifuged for 20 min at 14,000 rpm, and the 100% ethanol was discarded. The same procedure was used with 70% ethanol, with centrifugation for 2 min. After the ethanol was discarded, the microtubes were placed in a heating block at 50°C for ethanol evaporation, and then 50 µL of Milli-Q water was added.

PCR Amplification Genes

For each pair of cyanotoxin and cyanobacteriaspecific primers, PCRs were performed in a final volume of 20 μ L. The PCR mixture contained 10 μ L of 2x Red Taq Master Mix (a mixture containing Taq DNA Polymerase, dNTPs, MgCl₂ and reaction buffer at ideal concentrations for efficient DNA amplification by PCR), 2 μ L of a template (DNA extract or ultrapure water), 1 μ L of each primer (16S rRNA primer, cylindrospermopsin, anatoxin, saxitoxin, and microcystin) and 6 μ L of ultrapure water. The template used for the negative control (-) was ultrapure water.

Electrophoresis

The presence of DNA fragments after PCR

was verified by electrophoresis in a 1% agarose gel diluted in TAE buffer. For 30 mL of agarose, 1.8 μ L of the stain Midori Green (Advance DNA Stain, Nippon Genetics Europe) was added to a horizontal gel apparatus (PeQlab). Five microliters of the marker (1 kb DNA Ladder, New England Biolabs) and 10 μ L of PCR products were added to each well. The running buffer used was 1x TAE, and the tension used was 120 volts. The DNA bands in the gel were visualized under UV light in a transilluminator (Phase), and photographs were taken with a Canon camera.

Band Extraction and Sequencing

The bands resulting from PCR amplification were excised from the gel, and the DNA was extracted with the DNA Gel Extraction kit (Monarch) following the manufacturer's instructions. After extraction, the purified DNA was sent for sequencing at GATC Biotech. The results of the nucleotide sequences obtained were compared with the sequences deposited in the BLAST (Basic Local Alignment Search Tool) database.

Species Cultivation and Biomass Productivity

Inoculums Preparation

The Bold's Basal Medium - BBM (freshwater) was used for the growth of cyanobacteria (Table 1). After sterilizing the flasks and the culture medium (autoclaved at 121°C for 30 min), the species were inoculated. After being cultivated in the BBM medium, was used as a pre-inoculum to start the experiments, in a 250 mL flask with 200 mL of medium with 10% inoculum, in duplicate (Figure 1). The cultivation was kept without aeration, under controlled conditions of temperature ($26 \pm 1^{\circ}C$), and with artificial lighting (Figure 1).

Determination of Biomass Productivity

The growth of the species was followed by gravimetric analysis of dry weight for 31 days since the species is filamentous and has a large agglomeration of cells [14].

To determine the dry weight, 200 mL of culture samples from each flask were filtered using glass fiber filters with 0.47 μ m porosity (Figure 2). Before filtering the biomass, these filters were taken to the oven for drying for 24 hours at a temperature of 60°C and weighed. After filtering the biomass, the filters were taken back to the greenhouse under the same conditions as before and then weighed. The dry weight of the biomass was determined by the difference between the weighing values of the filters.

Results and Discussion

The isolation method used in this study was sufficient to obtain the species of cyanobacteria from the environmental sample collected in the lake located in the city of Salvador.

DNA extraction from the isolated strain was carried out, followed by amplification of the 16S rRNA gene and gel electrophoresis, to confirm the presence of cyanobacterium genomic DNA in the extract, before proceeding with PCR and sequencing for cyanotoxins.

The 16S rRNA coding gene was present in the PCR reaction extract, which indicates that the species isolated from the lake is a cyanobacterium. Having obtained this result, an excision of the PCR bands was performed with primers of the 16S rRNA of cyanobacteria (27F/809R) to verify the specie through sequencing.

The results of the sequencing were compared with the database available on BLAST, (Table 2). From the analysis, it was concluded that the isolated strain belongs to the genus *Brasilonema* sp (Identities: 635/716 - 89%). Currently, seven species have been discovered [15,16,17]. Six have been described in Brazil: *B. bromeliae* [15], *B. octagenarum* [16], *B. epidendron*, *B. ornatum*, *B. terrestre* and *B. sennae* [17].

Morphologically, *Brasilonema* presents a development composed of densely compacted fascicles, isopolar filaments, rounded apical cells not attenuated, and isopolar hormonal development [15].

	Stock Solution	Amount /L	mL / half liter
Solution 1	Sodium nitrate (NaNO ₃)	25.00 g	10 mL
	Potassium phosphate (K ₂ HPO ₄ x 3 H ₂ O)	9.80 g	
	Monopotassium phosphate (KH2PO4 x 3 H2O)	17.50 g	
	Sodium chloride (NaCl)	2.50 g	
Solution 2	Calcium chloride (CaCl ₂ x 2H ₂ O)	2.50 g	10 mL
Solution 3	Magnesium sulfate(MgSO ₄ x 7 H ₂ O)	7.50 g	10 mL
Solution 4	EDTA	50.00 g	1 mL
	Potassium hydroxide (KOH)	31.00 g	
Solution 5	Iron Sulfate (FeSO4 x 7 H2O)	4.98 g	1 mL
	Sulfuric acid (H2SO4)	1.00 mL	
Solution 6	Boric acid (H ₃ BO ₃)	11.42 g	1 mL
Solution 7	Zinc sulfate ZnSO ₄ x 7 H ₂ O	8.82 g	1 mL
	Molybdenum trioxide (MoO ₃)	0.71 g	
	Copper sulphate (CuSO ₄ x 5 H ₂ O)	1.57 g	
	Cobalt nitrate (Co(NO ₃) ₂ x 6 H ₂ O)	0.49 g	
	Magnesium chloride (MnCl ₂ x 4 H ₂ O)	1.44 g	

Table 1. BBM Media Composition (CCAC, University of Cologne (http://www.ccac.uni-koeln.de).

Figure 1. Cultivation of the specie in the BBM environment.



The results of the analysis of the PCR products using the primer pairs for the detection of cyanotoxins were negative for the cyanobacterium *Brasilonema* sp. This shows that the cyanobacterium species do not have the

Figure 2. Biomass filtration for gravimetric analysis.



genes that encode the enzymes for the synthesis of cyanotoxins, which makes it possible to use it as a source of bioactive substances.

To evaluate the growth of the species *Brasilonema* sp, the dry weight of the biomass

GenBank	BLASTN Result	Similarity	Nucleotide Sequence	Access year
J30M7XJS014	Brasilonema terrestres train CENA116 16S ribosomal RNA gene	89%	716	2019

Table 2. Identification of the filamentous cyanobacteria isolated by sequencing using the BLASTIN tool.

(g/L) was measured for 31 days (Figure 3). The growth data indicated that this species can be cultivated for biomass production under controlled conditions. The yield was recorded at 0.5 g/L. In a study with the cultivation of Scenedesmus sp, the authors reported a biomass concentration of 0.90 g/L [18]. In another study, the author cultivated the species Spirulina platensis and obtained a maximum biomass concentration of 1.75 g/L [19], and a yield of 0.98 g/L of the species Chlorella vulgaris was observed in cultivation with BBM medium [20]. In comparison with other studies, the value obtained was slightly lower, but the production process can be optimized to increase productivity using other means of cultivation or changing its conditions. Also, the adaptation process of the species in the synthetic medium may have influenced productivity. An interesting aspect of this species is its ability to form agglomerated

colonies that settle at the bottom of the culture vessel, being easily separated from the medium after the culture cycle.

Conclusions

The results of this work demonstrate that the cyanobacteria isolated from a lake located in the city of Salvador-BA are *Brasilonema* sp. As this cyanobacterium species does not have the genes that encode the enzymes for the synthesis of cyanotoxins, its biomass can be used as a raw material in several industrial sectors, including for food purposes. This species can be cultivated under controlled conditions, however, in future works the chemical characterization of the specie's biomass will be carried out, among other studies, to optimize the biomass production and bioactive extraction processes, for its biotechnological applications.

Figure 3. Growth curve of *Brasilonema* sp.



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Properties of Fibrous Composites with Polyester: A Comparative Analysis Between Sisal Fiber and Pet

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Fiber-reinforced composites represent 75% of the application of these materials in several industrial segments. It has the purpose of improving technical characteristics and reducing environmental impact through the use of sustainable raw materials such as natural fibers and other fibers from industrial waste. In this sense, the objective of this work was to study and compare the mechanical properties of polyester composites (PL) reinforced with natural sisal fiber and residues of polyethylene terephthalate (PET) synthetic fibers. Initially, we evaluated the moisture and morphology of the fibers. The composites with PL matrix were obtained and the fiber concentration varied by 1%, 3%, and 5% by weight. In the composites, the mechanical properties under flexion and impact resistance were evaluated. We concluded that the level of reinforcement with sisal fibers did not significantly affect the mechanical properties. However, the PET fiber provided significant improvements in the properties of the composite. Thus, the composites reinforced with PET fiber residue have advantages in the development of new material with sustainable characteristics.

Keywords: Sisal Fiber. PET Fiber. Polymeric Composites. Mechanical Properties.

Introduction

Levels of carbon oxide emissions into the atmosphere have become increasingly alarming over the years. This situation becomes worrying due to the accumulation of these gases, identified as one of the main aggravating factors of global warming. This problem led to the development of many studies on new materials with higher renewable potential. Composites reinforced with natural fibers and textiles emerge as alternatives to reduce environmental impact in a scenario in which new materials with improved properties reduce environmental damages [1].

The class of materials that best suits the requirements described and with a wide range of applications are composites. Composites are materials made up of two or more phases on a

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macroscopic scale, obtained from the mixture of two or more different materials, whose mechanical properties must be superior to those of the constituent materials. To achieve the desired properties in a composite material, each element (matrix, reinforcement, and interface) must have appropriate characteristics. Thus, its properties will depend on the characteristics of its constituents, the relative concentration, the characteristics of the interface, the efficiency in the preparation and transformation processes to ensure the best morphology in the final composite. [2,3].

Polymeric composites reinforced with fibers have wide applications in the industrial and civil construction sectors. Composites are materials that present advantages in their use, as they allow for obtaining particular properties from the coalescence of different materials, whereas this junction results in new applications [4].

Technologically, the significant composites are those in which the dispersed phase is in the form of fibers. These materials combine high strength and stiffness with low weight, as the reinforcement mechanism is associated with the transfer of tension from fiber to the matrix. In this context, this work aimed to evaluate the

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properties of polyester matrix (PL) composites reinforced with two sustainable fibers, sisal fiber and polyethylene terephthalate (PET), and to compare the mechanical properties obtained in composites reinforced with each one of them. Sisal fiber has received particular attention in Brazil, as it corresponds to about 70% of the commercial production of all solid fiber in the country. Sisal fibers are rigid, inflexible, and have superior strength compared to other natural fibers, in addition to good durability. They consist of cellulose, hemicellulose, lignin, pectin, wax, and water-soluble substances, the first three compounds being responsible for the physical and mechanical properties of sisal fiber [5].

The second fiber studied in this work is PET fiber, a polymer widely used because it is a polyester of great relevance in the industrial context. The application of PET fibers as a reinforcement in automotive components has high prominence due to the excellent mechanical properties and thermal stability intrinsic to the fiber. Academic and industrial interest has guided the development of extensive studies on the crystallization of PET, which is induced by the orientation of its polymer chains, giving rise to structural evolution of practical implications in the optimization of fiber properties. In addition to its triclinic crystalline form, PET forms a mesomorphic phase, which has an intermediate structure between the crystalline and amorphous parts of the polymer [6,7].

Materials and Methods

The composites were made using two types of fibers as reinforcement:

- a. Sisal fiber (Figure 1a), originated from the rope making process of companies located in the city of Conceição de Coité-BA and PET fiber (Figure 1b).
- b. PET fiber is an industrial waste generated during the process of obtaining fabrics used in tire reinforcements. This fiber was made available by a factory from the city of Camaçari-BA. The choice of which fiber is due to the opportunity to use industrial waste for the development of new materials, reducing the environmental impact caused by their disposal.

The matrix used accelerated, thixotropic, and low viscosity unsaturated polyester

Figure 1. Fibers used. (a) sisal fiber; (b) PET fiber.





resin. In general, it is a resin indicated for the manufacturing of composite parts by open molding in the processes of Hand Lay Up and Spray Up.

Fiber Humidity Content

Oven drying was performed to evaluate the humidity. Samples of sisal fiber and PET subjected to temperatures of 80, 90, 100, and 110°C in a SOLAB model SL-102 greenhouse with air circulation and renewal. The humidity was related to the mass of the samples and the measurement of this parameter was performed every 60 minutes until the mass stabilized. There is following the relation of the equation used to calculate the humidity in percentage:

$$humidity (\%) = \frac{(wet mass-dry mass)}{wet mass} X \ 100\%$$

Fiber Morphology

Morphological analyzes were performed using scanning electron microscopy (SEM). To carry out the analyzes, the sisal and PET fibers underwent a carbon deposition process on the surface. The procedure was performed with the Denton Vacuum equipment model Desk V. The scanning electron microscopy was performed with the equipment of the brand Jeol model JSM-6510 LV.

Composites Manufacturing Process

The composites were manufactured in a silicone mold following the dimensions required by the ASTM D638-14 tensile strength test standard. The proportion of 5% by volume of the catalyst was used for the polyester resin and 12 minutes for curing. The composites were obtained with 1%, 3%, and 5% by weight of the fibers. These percentages were defined by experimental observations since higher concentrations would make the molding of the specimens unfeasible in the mold used. After de-molding, the samples

were exposed to air for 24 hours until the resin curing process was completed

Then, the specimens were separated, stored, and cataloged according to the compositions.

Mechanical Property of Composites

To evaluate the mechanical properties of the composites obtained with the different fiber formulations, mechanic tests of flexion and impact resistance were performed on pure resin and composites reinforced with sisal and PET fibers. The mechanical tests were performed at the Mechanical Testing Laboratory at SENAI CIMATEC. To determine the impact resistance, an Instron machine, model CEAST 9050, with a 2.7 J hammer and IZOD configuration, without a notch, was used, following the ASTM D4812 standard. The flexural properties were obtained through the 3-point test following the ASTM D790-17 standard on the universal testing machine Emic Model DL 2000, and the data were processed using the Tesc Software.

Results and Discussion

Fiber Humidity Content

Table 1 shows the humidity for the sisal and PET fibers as a function of the temperature used in the greenhouse.

The results show that the sisal fiber has the highest percentage of moisture at all temperatures used. This behavior was already expected, considering that it is a vegetable fiber with hygroscopic characteristics, in addition to having hydroxyls and other polar groups [8,9]. The PET fiber samples showed relatively low humidity values. The presence of moisture in polyethylene terephthalate resins is critical mainly in its molten form as it can change the intrinsic viscosity of the polymer by changing the forming conditions under temperature [10]. Therefore, in applications where high levels of humidity are not required, PET fibers can be used without prior drying.

Sample	Temperature (°C)	Test time (hours)	Humidity (%)
Sisal fiber	80	4	7.71
	90		6.55
	100		7.16
	110		7.25
PET fiber	80	4	1.36
	90		1.49
	100		1.60
	110		2.04

Fiber Morphology

Through the micrographs represented in Figure 2a obtained by SEM, it is possible to verify the longitudinal surface of the sisal fiber. This fiber has an angular or almost cylindrical section, with a diameter of 100 μ m to 200 μ m and superficial residues with parenchymal cells distributed equally along with the fiber. These residues impair the interaction of the fiber with the matrix in a polymeric composite [11].

Figure 2b shows the micrograph of the PET fiber, a smooth surface with small surface defects. In other studies, the cause of these surface defects or incrustations on the surface of the fibers is not precisely defined. They can arise during the

process of obtaining the fibers or in some drying process [12,13].

Impact Resistance

Figure 3 shows a comparative graph between the results obtained for impact resistance in composites reinforced with both types of fibers.

There was a significant positive effect on this property when added to PET fibers. However, the same increase could not be observed with the addition of sisal fibers, possibly due to the presence of moisture, since moisture generates voids and bubbles during molding. Also, the manual distribution in the manufacture of the samples does not allow for a







Figure 3. Impact resistance in composites reinforced with sisal and PET.

homogeneous distribution of the fibers, possibly causing points of fragility in the composite. The addition of 5% PET fiber resulted in an increase of more than 300% in this property. The fibers acted as intensifiers of fracture toughness by the impact. These can show that there is good compatibility in the interface between polyester matrix and PET fibers since the chemical structures present in the matrix and reinforcement composition have an affinity for being of similar families. Similar results were obtained by other researchers, who observed an increase in the impact resistance of the composites with the increase in the content of incorporated PET fibers [14].

Flexural Properties

Figure 4 shows the results of the flexural properties of composites with sisal and PET. Composites with sisal presented elastic moduli larger than composites with PET. A considerable increase in the modulus of elasticity when adding vegetable fibers has been observed in other studies, varying its maximum values also depending on the type of vegetable used [15]. The elastic modulus is an indirect measure of the stiffness of the materials as natural fibers have a greater stiffness than the polymer (composites expected to show an increase in this property) [16].

The increase in the volumetric fraction in the two types of fiber led to a reduction in flexural strength (Figure 4b). The presence of voids due to failures in the molding step can justify this fact. The voids act as stress concentrating points in the composite, which leads to non-performance and rupture of the material when subjected to stresses lower than those caused by the flexural rupture of the pure polyester matrix [17]. Other factors may also have influenced the result, such as the presence of moisture in the fiber and lack of compatible additives, which hinders the adhesion between the phases resulting in a weak interface. However, although the behavior of the samples regarding the flexural strength of the composites was similar, the composites reinforced with PET fibers obtained great result, which helps to confirm that the interaction at the interface between the polyester matrix and the PET reinforcement has more synergy than at the interface with the matrix and the sisal fiber.

Conclusion

PET and sisal fibers from industrial waste can be used as reinforcement to obtain polyester composites. A 5% concentration of PET fiber results in a significant increase in the impact resistance values of the composites. The



Figure 4. Flexural properties: (a) elastic moduli; (b) flexural strength.

presence of moisture in the sisal fiber may have hindered the adhesion between the matrix and the reinforcement. It also provides the formation of voids during molding.

The flexural strength of the composites reduced in the presence of the evaluated fibers, being lower for higher concentrations of fiber. This behavior can also be associated with the formation of voids in the specimens and poor adhesion between the phases. However, morphological analysis of the interface should be performed for better conclusions.

Finally, composites reinforced with sisal and PET fibers have advantages in the development of new sustainable material since they satisfy demand from society for the search for materials composed of renewable raw materials and waste, reducing the environmental impact caused by disposal.

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Nutritional Quality of Moringa oleifera Seeds and Physicochemical Properties of Its Crude Oil

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This study aimed to evaluate the centesimal composition of *Moringa oleifera* Lam seeds and identify the physicochemical properties of crude oil extracted from *Moringa oleifera* Lam seeds. The values found for the centesimal composition showed that the seeds are constituted by 41% of crude oil, 32.40% of proteins, 4.20% of ash, 19.54% of carbohydrate, and 2.86% of moisture. These results indicate that *Moringa oleifera* Lam seeds are a potential source for health and diet food products. Compared with oils from *Moringa oleifera* Lam seeds grown in other countries, the physicochemical characteristics found in the oil are in agreement with data from the literature. Therefore, *Moringa oleifera* Lam oil can be used as a potential source for the manufacture of several industrial products, such as food and cosmetics.

Keywords: Moringa oleifera Lam. Seeds. Vegetable Oil. Physicochemical Properties.

Abbreviations: ANVISA: Agência Nacional de Vigilância Sanitária; TGA: thermogravimetric analysis; FTIR: Fourier transform infrared spectroscopy.

Introduction

Moringa oleifera Lam (*Moringa oleifera* Lamarck) is a species that belongs to the Moringaceae family, popularly known as Moringa, White-lily, or Okra of quina [1]. Originally from northern India, *Moringa oleifera* Lam is widely distributed in different tropical regions of the world and was introduced in Brazil in 1950. Due to high adaptability in nutrient-poor soils, this specimen was spread in several regions worldwide, such as the Brazilian northeast [2,3].

Moringa oleifera Lam is considered to be one of the most cultivated plants in the world and as an adult, it can produce annually 3 to 5 tons of seeds per hectare [4]. Several health benefits were reported as a result of supplementation with Moringa oleifera Lam seeds [5]. The mature seeds are rich in lipids, which represent between

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22% and 42% of their total weight [6]. The oil extracted from the seeds of Moringa oleifera Lam has a fatty acid profile similar to olive oil, and has been considered one of its substitutes as a functional food. Additionally, high-stability frying has enhanced its use as cooking oil in different regions of the world [7,8]. Moringa oleifera Lam oil has also been used as a raw material for the manufacture of cosmetics and biofuels [3]. However, due to the lack of studies in the literature on the nutritional quality and safety of Moringa oleifera Lam, the Agência Nacional de Vigilância Sanitária (ANVISA) banned the marketing and use of products containing Moringa oleifera Lam in Brazil. The industrial application of vegetable oils requires an analysis of seeds conservation status, as well as the physicochemical properties of oil extracted [9]. However, detailed information on the centesimal composition and physicochemical properties of oil extracted from Moringa oleifera Lam seeds is lacking.

In this study, the centesimal composition seeds (lipids, proteins, ash, carbohydrates, and moisture) and physicochemical properties (acid value, free fatty acid content, iodine value, saponification value, peroxide value, density, kinematic viscosity, and refractive index) of oil extracted from *Moringa oleifera* Lam seeds were

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studied. Also, the extracted oil was analyzed by Fourier transform infrared spectroscopy (FTIR) and thermogravimetric analysis (TGA/DTA).

Materials and Methods

Moringa oleifera Lam Seeds

The seeds of *Moringa oleifera* Lam were collected in Tiradentes University, located in Aracaju, Sergipe, Brazil. The seeds were removed from the pod, selected, ground in a domestic blender, and sieved to reach a particle size of 60 mesh.

<u>Centesimal Composition of Moringa oleifera Lam</u> <u>Seeds</u>

The centesimal composition of *Moringa oleifera* Lam seeds was determined by moisture, ash, lipids, proteins, and carbohydrate contents, according to the Official Methods of Analysis of AOAC International [10]. The determinations were performed in triplicate and expressed as mean values and standard deviation.

Extraction of Moringa oleifera Lam Oil

The oil from 50g of ground *Moringa oleifera* Lam seeds was extracted with n-hexane using a Soxhlet apparatus for 8 h at 68°C. Then, the solvent was removed from oil using a rotary evaporator (IKA, RV 10 Control). Finally, the oil was ovendried at 60°C to completely remove all residual solvent traces.

Characterizations of Moringa oleifera Lam Oil

The physicochemical properties: acid value, free fatty acid content, iodine value, saponification value, peroxide value, density, kinematic viscosity, and refractive index, were determined according to standard analytical methods recommended by Official Methods of Analysis of AOAC International [10]. Fourier transform infrared spectroscopy (FTIR) spectrum of *Moringa oleifera* Lam oil was recorded on a Cary 630 FTIR spectrometer (Agilent Technologies, Germany). The spectrum was measured in a spectral range from 4000 to 650 cm⁻¹ and at a spectral resolution of 4 cm⁻¹ and 32 scans. Thermogravimetric analysis was carried in Shimadzu DTG-60H simultaneous DTA-TGA apparatus out in synthetic air. The *Moringa oleifera* Lam oil (5 mg) was heated from 25 to 600°C at a rate of 10 °C min⁻¹ with a gas flow rate of 50 mL min⁻¹.

Results and Discussion

<u>Centesimal Composition of Moringa oleifera Lam</u> <u>Oil</u>

The nutritional value of seeds depends on the centesimal composition of dry mass, especially the content of proteins. Therefore, the centesimal composition of Moringa oleifera Lam seeds after grinding and sieving is presented in Table 1. As it can be seen, the nutritional composition of Moringa oleifera Lam seeds (on a dry basis), was $2.86\% \pm 0.3$ for moisture, $14.20\% \pm 0.2$ for ash, $41.00\% \pm 0.2$ for lipids, $32.40\% \pm 0.8$ for proteins, and $19.54\% \pm 1.3$ for carbohydrates, which are in agreement with previous studies reported [11,12]. The small variability may be due to different local growing conditions (type and pH of soil) and climatic conditions (temperature and time of year). In short, these results suggest that Moringa oleifera Lam seeds are a source of nutrients that can be used to supplement diets.

Characterizations of Moringa oleifera Lam Oil

Table 2 summarizes the physicochemical properties of *Moringa oleifera* Lam oil extracted and reported by different authors. The acid value of *Moringa oleifera* Lam oil was 21.41 mg_{KOH}/g, similar to acid values reported in the literature [3,8]. The high acidity index obtained indicates that oil has undergone hydrolysis of triacylglycerol chains,

Centesimal composition	Determined values	Abiodun and cols. (2012) [11]	Bridgemohan and cols. (2014) [12]
Moisture (%)	2.86 ± 0.3	4.7	5.4
Ash (%)	4.20 ± 0.2	4.1	3.7
Lipids (%)	41.00 ± 0.2	45.8	38.2
Proteins (%)	32.40 ± 0.8	28.0	37.2
Carbohydrates (%)	19.54 ± 1.3	18.3	15.5

Table 1. Centesima	l composition	of Moringa	oleifera	Lam seeds.
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Values are mean \pm SD of triplicate determinations.

Table 2. Physicochemical properties of oil extracted from Moringa oleifera Lam seeds.

Properties	Determined values	Bhutada and cols. [15]	Pereira and cols. [13]
$\overline{\text{Acid value }(\text{mg}_{\text{vou}}/\text{g})}$	21.41 ± 0.86	26.22	20.50
Free fatty acid (mg_{VOI}/g)	10.70 ± 0.86	13.11	10.25
Iodine value $(gL_2/100g)$	72.64 ± 1.24	75.06	70.70
Saponification value (mg_{KOI}/g)	181.99 ± 2.62	172.16	179.40
Peroxide value (meqO ₂ /kg)	3.98 ± 0.26	ND	5.40
Density at 15°C (kg/m ³)	894.5 ± 5.51	240	907.20
Kinematic viscosity at 40°C (cSt)	40.32 ± 0.28	ND	39.10
Refractive index	1.45 ± 0.04	ND	ND
Water content (%)	0.35 ± 0.02	ND	ND

Values are mean \pm SD of triplicate determinations.ND = not determined.

releasing free fatty acids. The determination of iodine index is a useful measure for understanding the degree of oil unsaturation and is directly related to their oxidative stability [14]. The average value obtained (72.64 gI₂/100g) is not significantly different from values reported by other authors [13,15]. Saponification index was 181.99 mg_{KOH}/g, within the expected range for vegetable oil seeds or saponifiable animals (180 and 200 mg_{KOH}/g) [13]. The peroxide value was 3.98 meqO₂/kg, which indicates high resistance to oxidation of *Moringa oleifera* Lam oil [16].

In the evaluation of physical properties, refraction index was 1.45, whereas density at 15° C amounted to 894.5 kg/m³ and kinematic viscosity at 40°C was 40.32 cSt. These values are consistent

with the requirements of the standard for vegetable oils [17]. The content of water in oil determined using Karl Fischer method amounted to 0.35%. This low moisture content may be related to seed storage or interaction with the solvent during the oil extraction process. The variations observed in Table 2 can be attributed to the different climatic conditions of plant cultivation, stage of ripeness and time of seed harvest.

Figure 1 shows FTIR spectrum used to analyze the main functional groups present in the oil extracted from *Moringa oleifera* Lam seeds. The absence of a peak after 3000 cm⁻¹ indicates very low concentrations of impurities contained in hydroxyl groups (OH), such as free glycerol and water [18], which corroborates the moisture data



(0.35%) obtained in this study. There is a wide band from 2922 to 2850 cm⁻¹, due to the symmetrical and asymmetric elongation of the C–H group, attributed to the fatty acids present in the oil [19]. In the region between 1770 and 660 cm⁻¹, there are intense bands attributed to the elongation of the C=O bond, a characteristic carbonyl group of proteins and fatty acid structures. The peak in the region 1460-1370 cm⁻¹ indicates the presence of C–C. The peaks at 1160 and 721 cm⁻¹ are assigned to C–O bonds and the asymmetric deformation of CH₂ group present in fatty acids, respectively [19]. The presence of these groups suggests the potential of crude oil from *Moringa oleifera* Lam for the manufacture of several bioproducts, such as food and cosmetics [3].

The thermogravimetric curve and its derivative (TGA/DTA) show the volatilization and/or thermal combustion of 100% of the mass of the oil in three thermal stages (Figure 2). The first stage, starting at approximately 30°C and finishing at 181°C, where a loss of mass of 0.38% is associated with the volatilization of the water contained in the oil. The second stage occurred between temperatures 181 and 405°C with weight loss equal to 93.03%. In

this stage, the greater decomposition indicates the decomposition of fatty acids in the oil, for example, oleic acid, which has a boiling point of 360°C. The third stage is associated with the decomposition of the existing impurities in the oil constituents, showed a weight loss of 6.54% at temperatures between 405°C and 500°C [20]. In short, thermal analysis shows the potential of *Moringa oleifera* Lam oil for frying due to its high thermal stability.

Conclusion

In this study, the centesimal composition of *Moringa oleifera* Lam seeds and physicochemical properties of oils from *Moringa oleifera* Lam seeds were identified. In *Moringa oleifera* Lam seeds the main components were lipids (41%) and proteins (32.4%). Thus, *Moringa oleifera* Lam seeds may be considered to be a good and safe source to supplement daily nutrient needs could help to prevent many diseases. The physicochemical properties of oil from *Moringa oleifera* Lam seeds were also determined following extraction either with n-hexane. The physicochemical properties





of crude oil from *Moringa oleifera* Lam seeds showed that it can be a source of fatty acids for food and cosmetics production.

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Thermodynamics of Oils of Nutritional/Cosmetic Use: *Bertholletia excelsa*, *Cocos nucifera*, and *Pterodon emarginatus* Vogel

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This paper contains the results of a new experimental study about the temperature effect on density and ultrasonic velocity for Brazil nut (*Bertholletia excelsa*), coconut (*Cocos nucifera*), and sucupira (*Pterodon emarginatus* Vogel) oils. Isentropic compressibilities and isobaric expansibilities were computed from the experimental magnitudes as a function of temperature. Halvorsen's model (HM), and Collision Factor Theory (CFT) were selected for the prediction of these properties due to their wide range of application and easy computation. An accurate response was observed, despite the use of several simplifications as molecular group contribution procedures for estimation of theoretic criticals points of the fatty acids and the complex nature of the studied fluids. Keywords: Thermodynamic Properties. Vegetable Oil. Theoretical Model. Estimation. Functional Group Contribution.

Introduction

Thermodynamic properties and phase equilibria data are the most critical parameters required in the design of equipment and processes, as well as predictions and simulations in food technology. Knowledge of these magnitudes as a function of operation magnitudes is of practical interest to the industrial manufacture of fats and oils. Thermal and mechanical procedures applied in the oil industry are closely related to their temperature and pressure dependence. Despite their economic importance, no systematic projects of consistent thermodynamic properties of fats and oils have been developed until now, and data for these kinds of compounds is relatively lacking. Different works published previously report data compilations of physical and thermal properties of fats and oils [1-5], but this information is not systematic, it is dispersed and many physicochemical properties have not been studied adequately. The fat and oils are biological, and they are not simple or

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one-molecular-structured. Their thermodynamic properties are strongly dependent on double-bound presence and position into the original molecular structure, chain length, nature of fatty acids, and molecular package of esters into solvents. Among the above indicated thermodynamic properties of solvents, volumetric and ultrasonic magnitudes have proved especially informative in elucidating the complex molecular interaction into the liquid phase. These values are of real core interest for direct industrial applications and theoretical predictive model development. The oils studied here have in common, in addition to growing economic importance, applications in food, medical or cosmetic uses, an enormous potential into emerging areas (nutraceuticals, phytotherapeutics, among others), and, at the same time, a severe gap in terms of thermodynamic data disposable into open literature. Fats and oils generate triglycerides, three molecules of fatty acids joined to a glycerol molecule. The chain length of the fatty acids and their organization on the glycerol backbone vary greatly, although in most of the edible oils it is with 16 and 18 carbons. The triacylglycerol is often considered the main chemical structure when developing estimative studies on the thermophysical properties of oils.

The Rackett equation, described from the Halvorsen method (HM) [6], was tested for density estimation. This method requires the critical

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properties of the fatty acids and considers their composition as input. The Collision Factor Theory (CFT) used the Wada method [7-9] for estimation of ultrasonic velocity of the enclosed fatty acids and the evaluation of their collision factors chosen for prediction of ultrasonic velocity. So, the tested models offer accurate results of the studied thermodynamic magnitudes, despite geometrical simplifications and the use of estimated critical dimensions by the Constantinou-Gani group contribution method [10].

Materials and Methods

The oils (cold-pressed quality), supplied by usual local providers, were stored in sunlight protected form and constant humidity and temperature in our laboratory. They were analyzed to determine their fatty acid compositions, the procedure being described earlier [11,12]. The average molar mass was computed as follows:

$$M_{oil} = 3 \cdot \left(\sum_{i=1}^{N} x_i \cdot M_i \right) + 2 \cdot M_{CH_2} + M_{CH}$$

xi the molar fraction and Mi the molar mass of each fatty acid without a proton, N the number of fatty acids found by analysis, and MCH2 and MCH are the molar mass contributions of glyceride molecule residue. The variation in the composition between different samples affects mainly the mono and polyunsaturated fatty acids, the change in molar mass being lower than ± 1 g mol⁻¹. The molar mass and fatty acids composition are gathered in Table 1. Densities and ultrasonic velocities were measured with an Anton Paar DSA-5000M vibrational tube densimeter and sound analyzer, with a resolution of 10⁻⁵ gcm⁻³ and 1 ms⁻¹. Apparatus calibration was performed periodically following vendor instructions using Millipore quality water and ambient air at each temperature. Accuracy in the measurement temperature was better than $\pm 10^{-2}$ K through a temperature control device that applies the Peltier principle to maintain

isothermal conditions during the measurements. The experimental and disposable literature data of the oils at 298.15 K are gathered in Table 1.

Results

The measured physical properties were correlated as a function of temperature using Eq. 2:

$$P = \sum_{i=0}^{N} A_i T^i$$

where P is density (gcm⁻³) or ultrasonic velocity (ms⁻¹), T is the absolute temperature in Kelvin, and Ai is the fitting parameters. N stands for the extension of the mathematical series, optimized through the Bevington test. The fitting parameters were obtained by the unweighted least squared method applying a fitting Marquardt algorithm. The root means square deviations were computed using Eq. 3, where z is the value of the property, and nDAT is the number of experimental data.

$$\sigma = \left(\frac{\sum_{i=1}^{n_{\text{DAT}}} (z_{\text{exp}} - z_{\text{pred}})^2}{n_{\text{DAT}}}\right)^{1/2}$$

Fitting parameters of Eq. 2 and the root mean square deviations (Eq. 3) are gathered in Table 2. In Figures 1a, 1b, and 2a, the temperature trend of density, ultrasonic velocity, and isentropic compressibility (computed by the Newton-Laplace equation from density and ultrasonic velocity) are gathered. These figures show a decrease in density and ultrasonic speed when the temperature rises due to a sharp lowering of efficiency in terms of molecular structuration into the bulk phase of triacylglycerol. Progressively higher molecular kinetics by heightening temperatures and steric hindrance of heavy molecular structures produces a growing difficulty in packing molecules. As observed in Figure 1a, different collections of density data for Brazil nut and coconut oil are disposable in the open literature. As far as we

Oil	Molar Mass (gmol ⁻¹)	Fatty Acids Composition (mass%)					
		Palmitic (16:0) 13.0		Linole	eic (18:2) 37.2		
Brazil nut	876.16	Stearic (18:0) 3.3		Linole	enic (18:3) 2.8		
		Oleic (18:1) 42.3		Araqu	ídic (20:0) 1.4		
		Miristic (14:0) 0.1		Linol	aic (18.2) 10.2		
		Palmitic (16:0) 4.7	,	Linol	2nic (18.2) 19.2		
Coconut	882.38	Palmitoleic (16:1) 0	.3	Gado	leic $(20.1) 0.7$		
		Stearic (18:0) 1.3		Eruc	vic (22.1) 0.1		
		Oleic (18:1) 65.3		Eruc	210 (22.1) 0.1		
		Palmitic (16:0) 5.5		Linala	nia(18.2)200		
Sucupira	878.26	Stearic (18:0) 2.0		Araquidic (20:0) 0.7 Gadoleic (20:1) 0.3			
		Oleic (18:1) 15.5					
		Linoleic (18:2) 46.0					
Oil	Exp. Dens.	Lit. Dens. Exp.		. Ultra. Vel.	Lit. Ultra. Vel.		
	0.915286	0.91462 ^a (293.15 K)			NA		
Brazil nut		0.876960 ^b (293.15 K)		1449.86			
		0.911°					
		0.9107 ^d (310.97 K)					
		0.919-0.937° (288.15					
		K)					
Coconut	0.919272	0.910 ^f (293.15 K)		1408.81	NA		
		0.9148 ^g (288.15 K)					
		0.02h(202.15 K)					
		$0.95^{\circ}(295.15 \text{ K})$					
		0.909 ⁱ (293.15 K) 0.909 ⁱ (313.15 K)					
Sucupira	0.926582	0.909 ⁱ (293.15 K) 0.909 ⁱ (313.15 K) N A		1456.30	NA		

Table 1. Molar mass, fatty acids compositions and experimental and literature data of density (gcm⁻³) and ultrasonic velocity (ms⁻¹) for the studied vegetable oils at 298.15 K

^aCeriani and colleagues (2008), ^bGonçalves and colleagues (2014), ^cMuniz and colleagues (2015), ^dNoureddini and colleagues (1992), ^eBailey (2005), ^fLafont and colleagues (2015), ^gSajjadi and colleagues (2016), ^hKalam and colleagues (2016), ⁱQiu and colleagues (2016).

know, our new data on the density of sucupira oil are the first to be published. For Brazil nut oil, the three available collections are those of Ceriani and colleagues (2008), Gonçalves and colleagues (2014), and Muniz and colleagues (2015) [13-15].

Only the first author shows an analysis as a function of temperature (only six experimental points). All of them gather lower values than our experimental measurements, Gonçalves and colleagues (2014) show an unrealistic value (4.5% of deviation

Table 2. Parameters of Eq. 2 for 288.15-323.15 K and the corresponding root mean square deviations (Eq 3).

ρ/(gcm ⁻³)						u/(m	s ⁻¹)			
Oil	Ao	Aı	A2	A3	σ	Ao	Aı	A2	A3	σ
Brazil nut	1.283313	-2.279389e-3	5.162837e-6	-5.560350e-9	1.540e-10	3754.551308	-15.344744	0.036297	-3.608038e-5	9.231e-2
Coconut	1.225855	-1.533320e-3	2.372553e-6	-2.276184e-9	0.574e-11	5601.527929	-33.106210	0.091952	-9.417774e-5	0.306e-2
Sucupira	1.183274	-1.154745e-3	1.382130e-6	-1.330648e-9	0.436e-11	3105.584507	-8.832402	0.014672	-1.207929e-5	2.428e-2

from our data) far away from the other literature collections. The other references, Ceriani and colleagues (2008), and Muniz and colleagues (2015), are slightly lower than our collection (0.5% of deviation), but with a similar trend in terms of evolution with temperature. For coconut oil, many references would be found, but only the first of them gathers values as a function of temperature. Noureddini and Qiu's works [16,17] are coincident with our experimental data. The other collections show extreme deviations from our measurements [18-20]. Such differences are probably related to the difficulty in identifying the origin of raw material for oil production, the industrial process applied to obtain the oil, and the purity grade of the oil used in these earlier studies. There is no information in these works if the used oil is of refined or virgin nature, or about the conservation procedure or possible applied purification pre-treatments. No values were found in the open literature for the ultrasonic velocity of these oils. Our new collections of ultrasonic velocities will also be the first ones to be published. In Figure 1b, our experimental data of ultrasonic velocities are gathered. Analogously, the ultrasonic velocity shows a decreasing trend with rising temperatures, with a similar trend for Brazil nut and sucupira oil. The experimental measurements for coconut oil are presented only at the range 293.15-323.15 K due to the high gelling point of this oil. As expected, the isentropic compressibilities increase when the temperature rises for the three oils due to the inverse relation of this magnitude with density and ultrasonic velocity.

A frequently applied derived quantity is the temperature dependence of volumetric properties,

which is expressed as isobaric expansibility or thermal expansion coefficient (α). The data reported in the literature currently shows only values of the thermal expansion coefficients both of pure compounds and its mixtures, showing the relative changes in density, calculated through (- $\Delta r/r$) as a function of temperature, and assuming that a remains constant over the temperature range. As in the case of pure chemicals, these oils can be computed by the following expression:

$$\alpha = -\left(\frac{\partial \ln \rho}{\partial T}\right)_{P,x}$$

Figure 1a presents the strong temperature dependence of density. An isobaric expansibility of the Brazil nut (*Bertholletia excelsa*), coconut (*Cocos nucifera*), and sucupira (*Pterodon emarginatus* Vogel) oils attend to this relation (Figure 2b). As observed, they all amount to negative values, but only with sucupira oil the isobaric expansibility diminish almost linearly. Both other oils show maxima at the studied temperature range (294.15 K for Brazil nut oil and 306.15 K for coconut oil).

Constantino and Gani [10] developed an advanced group contribution method for critical point estimation of covalent molecules based on the UNIFAC molecular groups. This procedure allows for a second-order level of contributions, overcoming the limitation of traditional group contribution models that cannot distinguish isomers or resonance structures. This method was applied to obtain the critical point of the fatty acids enclosed into the studied oils and then used in the prediction density method that was indicated above. The observed deviations, when

compared with the database, are negligible. The physical property packages used in powerful chemical simulators typically rely on generalized equations for predicting properties as a function of temperature, pressure, among others. In the last few years, despite the success of developing several procedures of density estimation for pure compounds or mixtures, only a few of them may be of practical application and high accuracy for fats and oils. One proposed correlation that holds promise for application to oils is the Rackett equation of state, as well as the modification by Spencer and Danner. The procedure proposed by Halvorsen and colleagues using these equations has proven to be accurate, only requiring critical magnitudes for the enclosed fatty acids and their composition into the oil. If these magnitudes are not known, they must be estimated as indicated above. The procedure of Halvorsen is described as follows:

$$\rho = \frac{\sum x_i \cdot M_i}{R \cdot \left(\frac{\sum x_i \cdot T_{ci}}{P_{ci}}\right) \cdot \left(\sum x_i \cdot \beta_i\right)^{1 + (1 - T_r)^{2/\gamma}}} + F_C$$

where r is the oil density, xi is the mole fraction of fatty acids into that oil, Mi is the molar mass of each fatty acid, R is the universal constant of gases, Pci is the critical pressure of each fatty acid, and Tr is the reduced temperature. The b parameter is the compressibility factor for the original equation of Rackett (Zc) or an acentric factor-dependent parameter if we use the modified Rackett equation (ZRA). Kay's rule is used to compute the pseudocritical temperature, and FC is a correction factor proposed by Halvorsen that depends on the oil structure backbone. Table 3 shows the root square deviations for density values by Halvorsen's model (HM) *versus* experimental data at 298.15 K.

Ultrasonic velocity has been systematically measured in recent years, but this kind of data is still extremely scarce for fats and oils. The experimental data were compared with the values obtained by the Collision Factor Theory (CFT) [7-9], which is dependent on the collision factors among molecules as a function of temperature:

$$u = \frac{u_{\infty} \left(\sum_{i=1}^{N} (x_i \cdot S_i) \right) \left(\sum_{i=1}^{N} (x_i \cdot B_i) \right)}{V}$$

where $u\infty$ is 1600 m/s, Si is the collision factor of each fatty acid, Bi is the molecular volume of each fatty acid, and V is the molar volume considering each oil a mixture of fatty acids attending to the composition (Table 1). The collision factors (Si) were estimated using the Wada method, and the molecular volume by Bondi contribution model for each fatty acid. The deviations for the CFT method are gathered in Table 3.

Table 3. Root mean square deviations (g/cm³) for Halvorsen model (HM) density prediction and deviations (m/s) for the Collision Factor Theory (CFT) ultrasonic velocity prediction at 298.15 K. **Conclusion**

Oil	Halvorsen	Collision Factor
	Model	Theory
Brazil nut	0.015165	3.07
Coconut	0.053932	3.57
Sucupira	0.023490	5.07

This paper contains the results of a new experimental study about the effect of temperature on density, and ultrasonic velocity for Brazil nut (*Bertholletia excelsa*), coconut (*Cocos nucifera*), and sucupira (*Pterodon emarginatus* Vogel) oils due to their rising economic value and potential in emerging areas as nutraceuticals and phytotherapy. We summarized our conclusions following the points: The tested methods Halvorsen's model (HM), and the Collision Factor Theory (CFT) showed the accurate capability of prediction of the measured magnitudes at the range of application,

Figure 1. Dependence of temperature of experimental (a) density and (b) ultrasonic velocity of Brazil nut (*Bertholletia excelsa*), coconut (*Cocos nucifera*), and sucupira (*Pterodon emarginatus* Vogel) oils and available open literature data.



Source: By authors.

Figure 2. Dependence of temperature of experimental (a) isentropic compressibility and (b) isobaric expansibility of Brazil nut (*Bertholletia excelsa*), coconut (*Cocos nucifera*), and sucupira (*Pterodon emarginatus* Vogel) oils.





despite different simplifying assumptions, the use of estimated critical dimensions by molecular group contribution approach, and the complex nature of the studied fluids. A review of the physical properties of Brazil nut (*Bertholletia excelsa*), coconut (*Cocos*)

nucifera), and sucupira (*Pterodon emarginatus* Vogel) oil revealed a critical gap in terms of accurate and quality thermodynamic data. The measured experimental collections of density and ultrasonic velocity contribute to a better characterization of these vegetable oils and increase the disposable data for theoretical works and modeling/simulation of macromolecules.

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Characterization of *Arthrospira* sp (Spirulina) Biomass Growth in Hydroponic Waste Solution: A Review

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The use of Spirulina (*Arthospira* sp) as a food source has stood out for being a potential source of proteins, fatty acids, vitamins, and others. However, the cultivation medium for its production is relatively expensive. Then, an alternative culture medium is necessary for reducing costs. So, hydroponics drainage effluent, a solution rich in nutrients, represents an alternative source of substrate for microorganism cultivation. Thus, this work aimed to characterize the biomass of *Arthospira* sp grown in an alternative culture medium based on hydroponic effluent through a systematic review. We noted a trend to reduce biomass growth and bioactive compounds due to an excess of iron and copper in the effluent. The study concluded that it needs to supplement the residual medium with a synthetic medium for its use to obtain biomass for commercial utilization. Keywords: *Arthrospira* sp. Hydroponic Waste. Drainage Medium. Biomass Composition. Microalgae.

Introduction

The use of microalgae and cyanobacteria as a food source, both for humans and animals, has stood out for being a potential source of proteins, unsaturated fatty acids, vitamins, minerals. pigments, enzymes, antibiotics, and other biologically active metabolites [1,2]. However, the potential associated with these microorganisms depends directly on the variables related to the growth of biomass. Therefore, it is necessary to control the pH, light, temperature, and nutrients. Nutrients are supplied to the microorganism by a solution containing nitrogen, phosphorus, potassium, some salts, and metals in lower concentrations, which is designated as a culture medium and represents the most expensive cost for the production of biomass [3].

The hydroponics technique uses a nutrient solution that feeds the plants replacing the soil.

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However, this solution discarded periodically, generating a drainage solution rich in nutrients [4].

Spirulina (Arthospira sp) is the cyanobacteria (blue-green alga) most used in animal and human food due to the high protein content in biomass (up to 60% of dry weight) and the high nutritional value associated with vitamins, minerals, and acids fatty content, biological activity, and easy digestion [5,6]. This algae species has been approved by ANVISA (Brazilian Health Surveillance Agency) for human consumption. Due to the existence of different Spirulina strains, they can adapt to extreme environments (alkaline environments, saline environments, high temperatures, and light, among others), and their cultivation and consumption have popularized in many parts of the world [5]. Thus, an alternative medium based on hydroponic waste for the cultivation of cyanobacteria Arthospira sp becomes an interesting opportunity to save costs and avoid wastewater disposal.

The objective of this work was to determine the proximate profile (carbohydrate, protein, lipids) of the biomass of *Arthospira* sp cultivated in alternative culture media based on hydroponic effluent through a systematic review to identify the effects of low-cost culture media on biomass composition.

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Materials and Methods

The keywords used for the research in this study as follow: Microalgae, hydroponics, aquaponics, tomatoes, comparison, alternative media, cultivate media, NPK, Spirulina, Biomass composition, *Arthrospira*, aquaculture, by which 78 combinations were made. The scientific database used was ScienceDirect with no limitation on publication time and restricted to individual articles. The keywords were searched both in the title field and within the entire text. A total of 49 articles were found on the ScienceDirect search platform considering the following exclusion criteria:

- No publications with not present full articles available;
- Adherence to the objectives of informational research.

Results and Discussion

After applying systematic review criteria, 19 articles were selected, and part of these articles was used to characterize the proximate profile of *Arthospira* sp biomass. Evaluating the articles, studies that use the exactly hydroponic wastewater for *Arthospira plantesis* or *Arthospira* sp cultivation was no found in this search.

The studies found that uses culture media with a proximate composition to the hydroponics or/and aquaponic effluent for *Arthospira* sp cultivation were those based on aquaculture residues (Table 1) [7,8]. Thus, we included other microalgae species, specifically *Chlorella* sp in the search, because was reported in the literature the cultivation of this species with the hydroponics drainage effluent as a growing medium (Table 2).

Analyzing the effects of these alternative culture media in the growth of microalgae with studies carried out for *Arthospira* sp and observing the similarities and differences in the effect on the biomass composition (in terms of carbohydrate, protein, and lipids profile), it was identified some points to serve as arguments in the Spirulina biomass characterization cultivated in a residual hydroponic medium.

All experiments involving the hydroponic and aquaculture wastewater founded excellent results for the use of residual effluents. However, there is a need to complement this solution with other micronutrients to obtain good results in the production of biomass and by-products of high added value. Also, in some of the selected works, the use of a pre-treatment process of residual effluent was observed to eliminate the presence of microorganisms that could negatively influence the microalgae development and also reduce or increase micronutrient levels, which may have inhibitory or catalytic effects.

Thus, the high presence of metals like Fe and Cu, reduction or excess of essential nutrients, and other events can interfere in the growth of Spirulina through alterations in the metabolism or reducing its activity [2, 7, 15].

After the evaluation of each selected work, it was possible to verify the approximate profile (carbohydrates, proteins, lipids) and compounds with high added value with reduced productivity expectation if used raw residual effluent. However, if this effluent is supplemented or treated for contaminants removal, a yield better or equivalent to that of synthetic culture media such as Zarrouk's solution can be obtained [16].

Besides, it is possible to infer that the biomass composition of *Arthospira* sp cultivated in hydroponic effluent will be similar to that reported by Bertoldi and colleagues [1], who studied the cultivation of *Chlorella* sp in this alternative medium. The same inference could be made related to the results obtained by Cardoso and colleagues [8], who evaluated the growth of Spirulina in aquaculture wastewater medium. In both studies, we noticed that biomass with a similar composition to that observed for cultivation in the Zarrouk medium can be obtained.

Hultberg and colleagues [4] reported a normal or subpar amount of carotenoids, bioactive with high added value, depending on stress conditions and hydroponic waste with a possible reduction **Table 1.** Studies that present alternative media for Arthospira growth based on hydroponic, aquaponic, or aquaculture cultivation residues.

Ref.	Culture Medium	Main Results
[7]	Aquaculture Wastewater	The algae grew well in fish water with a specific growth rate of $0.026 \text{ h} - 1 (0.623 \text{ day} - 1)$ and a doubling time of 28 h. These growth parameters compare favorably with those reported in the literature, indicating the adequacy of the cultivation of <i>S. platensis</i> in fish waters.
[8]	Aquaculture Wastewater	The best results of <i>Spirulina</i> sp LEB18 growth parameters - crops that used wastewater aquaculture - were obtained in experiments with 25% and 50% Zarrouk supplementation. In these experiments, the maximum biomass concentrations were equal ($p < 0.05$) to the control test without the addition of residual aquaculture water. Also, the highest yield rates and the maximum growth coefficients in these two experiments were about 1.7 and 1.8 higher, respectively, than the results obtained in the control cultivation. Although, the experiments using 75% Zarrouk supplementation showed a lower maximum biomass concentration than the control. The fatty acid composition of the biomass in the control, and with 25% supplementation of Zarrouk medium was observed in both experiments with the same profile. The content of unsaturated fatty acids in the treatment with 25% Zarrouk was high.
[9]	Confectionary Wastes	In this study, <i>A. platensis</i> grew very well in the residual confectionery effluent during the different experimental runs of the 22 CCD (Central Composite Design). The seaweed can use sugar present in the effluent. As expected, the results ensured the adequacy of the use of <i>A. platensis</i> for growth in residual confectionery effluent. Besides, the biochemical composition of the biomass was 20% higher than the control after 12 days. O, β carotenes (mg L1) was doubled. The levels of proteins, lipids, and carbohydrates increased by 20%, 22.2%, and 22.3%, respectively. However, chlorophyll decreased by 12% as the biomass became pale and lost its typical blue-green color.
[10]	Zarrouk Synthetic Medium with Added Glycerol	The addition of glycerol to <i>Spirulina</i> sp (LEB 18) stimulated cell growth (3.00 g L-1 of biomass, 0.72 g L-1 d -1 of maximum productivity) and protein production (69.78% w w-1), obtaining the best results at 0.05 mol L-1. The substrate application had a significant effect on the composition of fatty acids. The increase in unsaturated fatty acids, mainly oleic acid, and the reduction in saturated fatty acids, principally palmitic, were proportional to the increasing in the concentration of glycerol in this ambient.
[11]	Formulation of a Low-Cost Medium	This investigation was carried out with the principal objective of providing a simple and inexpensive environment, and our results indicate that the newly formulated solution RM6 is on the same level as Zarrouk's solution about the performance of <i>Spirulina</i> sp when evaluated in terms of chlorophyll, protein, or dry biomass.
[12]	Dairy Farm	Wastewater can support the growth of <i>Arthrospira platensis</i> for the production of biodiesel. A. <i>platensis</i> shows a wide range of temperature tolerance; however, 30° C was considered more suitable for the production of lipids. Change in light intensity from 160 to 300 μ moL / m ² / s during the intermediate exponential phase favors the production of saturated fatty acid in <i>Arthrospira platensis</i> .

Table 2. Studies that present alternative media for *Chlorella* growth based on hydroponic, aquaponic, or aquaculture cultivation residues.

Ref.	Species	Culture Medium	Main Results
[1]	Chlorella vulgari	Residual Hydroponic Solution	The cell density in the cultivation of the residual hydroponic solution with 25% residue and 75% deionized did not present any significant difference about the control (BBM). The other crops (SHR and SHR50) were significantly lower than the control crop but did not show any statistical difference between them. The cultivation of <i>Chlorella vulgaris</i> in residual hydroponic solution represents an attractive option for the development of nutritional supplements due to the high protein content and biologically active substances present in the cell biomass obtained in the process. The residual hydroponic solution and its respective dilutions (SHR50 and SHR25) proved to be a good option as an alternative way of the environment in the cultivation of <i>Chlorella vulgaris</i> , enabling the recycling of this residue sustainably.
[4]	Chlorella vulgari	Artificial Drainage Solution and Spent Drainage Solution*	This study showed that microalgae have the potential to reduce nutrient concentrations in the production drainage solution in a hydroponic greenhouse. We observed significant reductions in nitrogen and, in particular, in phosphorus concentration.
[13]	<i>Chlorella</i> spp	Co-Cultivation with Tomato in a Hydroponic System	In the EHS (eco-hydroponic culture), the production of microalgal biomass and the production of agricultural biomass was increased by aerating, by the photosynthesis of the algae, and by fertilizing C of the crop root respiration and exudation, respectively. In particular, the co-use of nutrients in the nutrient solution by microalgae and culture can increase the efficiency of nutrient use and minimize the discharge of nutrients.
[14]	Platymonas subcordiformis	Aquaculture Wastewater	It was possible to couple the removal of nitrogen and phosphorus from wastewater to algae biomass and the production of biofuels. <i>Platymonas</i> <i>subcordiformis</i> kelp can remove 87%-95% nitrogen and 98%-99% phosphorus in sole aquaculture wastewater. The algae biomass was 8.9 times greater than the initial level.

*Solution used in commercial greenhouse cultivation of tomato.

of up to 50% of these substances, considering the case of the wastewater having an excess of metallic compounds such as Fe, Cu, and Mo, there may be a reduction in biomass and proteins [15,17].

Conclusion

We observed that all experiments involving the hydroponic and aquaculture wastewater as culture media founded optimistic results for the use of residual effluents enriched with other synthetic culture media. A trend to reduce biomass production and bioactive compounds mainly when the microorganism suffers the effects of the excess of some micronutrients such as iron and copper, which is associated with the use of wastewater was observed.

Thus, analyzing the articles makes it possible to infer that the biomass of *Arthospira* sp will be composed of biomass with protein yield and concentration of carotenoids similar to the standard Zarrouk medium if the effluent is added as a complement in the culture medium. This study brought some insights from literature about the feasibility of the use of hydroponic, aquaponic, or aquaculture cultivation.

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Authors must indicate in a cover letter the address, telephone number, fax number, and e-mail of the corresponding author. The corresponding author will be asked to make a statement confirming that the content of the manuscript represents the views of the co-authors, that neither the corresponding author nor the co-authors have submitted duplicate or overlapping manuscripts elsewhere, and that the items indicated as personal communications in the text are supported by the referenced person.

Manuscripts are to be typed as indicated in Guide for Authors, as well as text, tables, references, legends. All pages are to be numbered with the order of presentation as follows: title page, abstract, text, acknowledgements, references, tables, figure legends and figures. A running title of not more than 40 characters should be at the top of each page. References should be listed consecutively in the text and recorded as follows in the reference list, and must follow the format of the National Library of Medicine as in Index Medicus and ""Uniform Requirements for Manuscripts Submitted to Biomedical Journals" or in "Vancouver Citation Style". Titles of journals not listed in Index Medicus should be spelled out in full.

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

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*First and last name with a sequencing overwritten number. Corresponding author(s) should be identified with an asterisk; Type 10, Times or Arial, single space. Running title of not more than 40 characters should be at the top of each page. References should be listed consecutively in the text. References must be cited on (not above) the line of text and in brackets instead of parentheses, e.g., [7,8]. References must be numbered in the order in which they appear in the text. References not cited in the text cannot appear in the reference section. References only or first cited in a table or figures are numbered according to where the table or figure is cited in the text. For instance, if a table is placed after reference 8, a new reference cited in table 1 would be reference 9.1 would be reference 9.

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- □3. The entire manuscript (including tables and references) must be typed according to the guidelines instructions.
- □4. The order of appearance of material in all manuscripts should be as follows: title page, abstract, text, acknowledgements, references, tables, figures/graphics/diagrams with the respective legends.
- □5. The title page must include a title of not more than three printed lines (please check the guidelines of each specific manuscript), authors (no titles or degrees), institutional affiliations, a running headline of not more than 40 letters with spaces.
- □6. Acknowledgements of persons who assisted the authors should be included on the page preceding the references.
- \Box 7. References must begin on a separate page.
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