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SUMMARY

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COVER: *Daphnia magna* is a species of *Daphnia* (a cladoceran freshwater water flea) Vannlopper. Photo: Per Harald Olsen/NTNU. Date: 29 October 2012, 10:18. Author: NTNU, Faculty of Natural Sciences. From Wikimedia. CC-BY-2.0.

Potential Application of Banana Peel Flour in Biofilm

Paloma Amancio Oliveira Sacramento[1*], Ingrid Lessa Leal[1], Tatiana Barreto Rocha Nery[1]
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Agribusiness is a major generator of waste, which generates significant environmental impacts. However, these residues can have several applications, thus having added value. Among all fruits, the banana has the highest rate of consumption in Brazil, consequently producing large amounts of peel. This work aims to get and characterize the banana peel flour for further application in biofilm production. We performed the physical-chemical characterization of the banana peel and the flour by crushing and drying in an oven (60 °C/24 h). Subsequently, we wash and delignification the pulp for its extraction. The results obtained 43% of total fiber in the flour and, at the end of the delignification process, 1.45 g of cellulose. Therefore, the banana peel presents itself as a potential matrix for application in the production of biofilm.
Keywords: Banana Peel. Biofilm. Banana Peel Cellulose.
Abbreviations: BPF: Banana peel flour.

Introduction

Among all fruits, banana is the most consumed in Brazil. According to IBGE data, in 2019, banana production in Brazil was approximately 7.2 million tons, and only 12% have sold as a final product [1]. We use the banana pulp for consumption and discard the peel or utilize it as organic fertilizer or in animal feed, because of the tannin content and the high amount of fiber. However, these residues can add to existing products, becoming a product with higher added value. Fruit leftovers have represented an alternative to the substitution of non-biodegradable polymers.

Using synthetic plastics for food packaging has low-cost, high applicability, and durability. However, these materials are associated with worrying environmental impact because of their extreme resistance to degradation, requiring space for their disposal [2].

Currently, many types of packaging that protect food by the sustainable use of natural resources are being sought through better energy use and lower waste generation. In this sense, there has been a growing development and improvement of biodegradable materials, which decompose quickly, minimizing environmental damage, since we can produce them from renewable energy sources and/or Agro-food waste from food processing industries [3].

These by-products can be used sustainably as raw material for the production of biofilm, reducing the volume of organic waste, and adding value to the chain through better use of nutrients, for example, fibers and bioactive substances with antioxidant potential, which can cooperate with the improvement of the functional characteristics of this type of packaging [4].

Usually, the films should present some characteristics for food packaging: good sensory qualities, good barrier properties, efficient mechanical properties, microbiological, physicochemical, and biochemical stability, absence of harmful components to health, simple technology, low-cost, and non-polluting [3]. Among the materials, those of agricultural origin present an advantage for biodegradable packaging because they are available all year round, are cheaper, and come from a renewable source [2]. This work aims to get and characterize the flour from the banana peel as a potential application in preparing biofilm.

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Materials and Methods

The company BioAlimentos, Camaçari-BA, provided the samples of banana peel to our study. The experiments occurred in the bio-nutrition laboratory of SENAI CIMATEC.

After receiving the shells, we washed them in running water to remove surface impurities, later we sanitized them in a 10 ppm sodium hypochlorite solution for 15 minutes. We used drinking water for rinsing. We dried the banana peels in an oven with forced air circulation (Q314M222, Chemis) at 60°C for 24 hours. Subsequently, we ground them in a household blender to produce the flour.

The infrared moisture balance (MOC-120H; Shimadzu) characterized the moisture content of the banana peel flour (BPF), with the incidence of the heat of 105 ºC, and the decagon (Novasina, Lab Master aw) identified the water activity [5]. The ash content was 5 to 10 g of the sample (capsule), previously heated in a muffle furnace at 550°C. It was cooled in a desiccator to room temperature and weighed. We have repeated the heating and cooling operations until the weight remained constant [5].

Soxhlet extractor determined the lipid content [5]. We weighed 3g of the sample in a cartridge previously prepared with filter paper and cotton, then filled the cartridge with cotton until it covered the entire sample. Then, the extractor was attached to the flat bottom flask previously stared at 105°C. The cartridge was placed into the extractor apparatus. Then, we added enough ether for one and a half Soxhlet and connected the assembly to the condenser. We turned on the heating plate and kept it warm for a long time. Removed the assembly, taking care to remove as much ether from the flask as possible first. Transferred the flask with the extracted residue to an oven at 105°C, holding for about an hour. Then took it to cool in the desiccator to room temperature, weighed with this process, and repeated the operations of heating for 30 minutes in the oven and cooling until the weight was constant [5].

We performed protein determination according to the Kjeldahl Method available in the literature [5]. Weighed 1 g of the sample on tissue paper, then transferred to the Kjeldahl flask (paper + sample). Added 25 mL of sulfuric acid and about 6 g of the catalyst blend and heated on an electric plate in the hood until the solution turned blue-green and free of undigested material (black spots). Heated for another hour and allowed to cool. Immediately connected the flask to the distillation assembly. We dipped the tapered end of the soda into 25 mL of 0.05 M sulfuric acid in a 500 mL Erlenmeyer flask with 3 drops of the methyl red indicator; added to the flask containing the digested sample, via a funnel with a stopcock, 30% sodium hydroxide solution to ensure a slight excess of base. We got heated to boiling and distilled until about 250mL. Titrated the excess 0.05 M sulfuric acid with 0.1 M sodium hydroxide solution using methyl red [5].

For determining the total dietary fiber content, we did appropriate pre-treatments for moisture and lipid content reduction, then measured the volume of the hydrolysate got from the enzymatic treatment. We added 95% of alcohol at 60°C, measured after heating, in a ratio of 4:1 of the hydrolysate volume. Covered the beakers with aluminum foil and let the mixture stand at room temperature for 1 hour for precipitation of the soluble fiber fraction. The crucible, previously prepared and weighed, was placed in a Kitasato attached to a vacuum tube. 15 mL portion of 78% alcohol was passed through the crucibles to redistribute the glass wool. We filtered the alcohol solution containing the hydrolysis residue carefully to not exceed the level of the glass wool during the filtration. We washed the residue with two 15 mL portions of 95% alcohol and two 15 mL portions of acetone; dried the crucibles containing them in an oven at 105°C overnight. Cooled in a desiccator and weighed (P2 for the sample and B2 for the blank). After the evaluating, we determined the protein content in one crucible of the sample and one blank [5].

We performed the quantification of sodium and potassium ions in a flame photometer with wavelengths at 589 and 766 nm; zeroed the measuring scale with distilled and deionized water; and after shaking the samples, we transferred about 40 mL to a clean, dry beaker. The photometer calibrated and zeroed red the samples [5].

For the delignification and bleaching process, 30 g of BPF was used and washed in 2% sodium hydroxide solution (1.2L of solution) at 80°C for 4 hours under stirring using a hot plate. Distilled water at room temperature filtered and washed the sample. We repeated this process 4 times to remove the water-soluble agents and get a cellulosic pulp [6]. Added 300 mL of buffer solution (27 g of solid sodium hydroxide plus 75 mL of acetic acid) into 25.47 g of the BPF, leaving for 10 minutes under constant stirring. Then we added the 1.7% sodium hypochlorite solution at 80°C and kept it at rest for 6 hours. Again, the distilled water filtered and washed the pulp. We dried the pulp in an oven with forced air circulation at 30°C for 24 hours.

Results and Discussion

After the process, the banana peel flour (BPF) became homogeneous, thin, brown, and had approximately 6.50% (Figure 1).

Table 1 shows the values of the physicochemical characterization of the banana peel in natura and BPF, and mean ± standard deviation presents the results.

The moisture contents were 83.78% for banana peel and 5.723% for banana peel flour, differing significantly from each other. However, it is within the standard required by ANVISA, a maximum of 15% moisture content in flours [7]. The water activity content was 0.857% for banana peel and 0.389% for banana peel flour, respectively. Products with water activity above 0.70 allow enzymatic or non-enzymatic reactions, and these cause modifications in the color, flavor, and stability of BPF [8].

The ash contents were 2.72% for plantain peel and 0.24% for plantain peel flour. This content is satisfactory in the banana peel, but the preparation process of the banana peel flour has a significant reduction in mineral content. However, the literature data found amounts of potassium, calcium, magnesium, zinc, among other minerals following this process [9].

The banana peel presents significant amounts of fibers, important for the production of biofilm. We can use them as additives, which help to improve their mechanical properties. These values are also important due to the intake of fiber for the human being to assist in the gastrointestinal tract, besides controlling some chronic degenerative diseases [10]. BPF also presented interesting nutritional results, such as protein and lipid content, and it can also be applied in food formulations.

For application in biofilm production, the literature suggests the reduction of the flour granulometry to improve the film appearance, obtaining a smooth texture and films of small

Table 1. Physicochemical characterization of banana peel in natura and banana peel flour (BPF).

Test	Banana peel	BPF
Moisture (%)	83.78±0.94	5.723±0.06
Water activity	0.857±0.01	0.389±0.01
Ash (%)	2.72±0.11	0.24± 0.02
Lipids (%)	0.35±0.03	10.93 ±0.18
Proteins (%)	2.37±0.36	8.11±0.04
Total dietary fiber (%)	24.49±0.25	43.89± 0.52
Sodium (Na) (g/100g)	<0.0021	0.0128±0.01
Potassium (K) (g/100g)	0.716±0.01	-

Figure 1. Banana peel flour.

Figure 2. Banana peel flour-washing process (A: 1st Wash; B: 2nd Wash; C: 3rd Wash; D: 4th Wash).

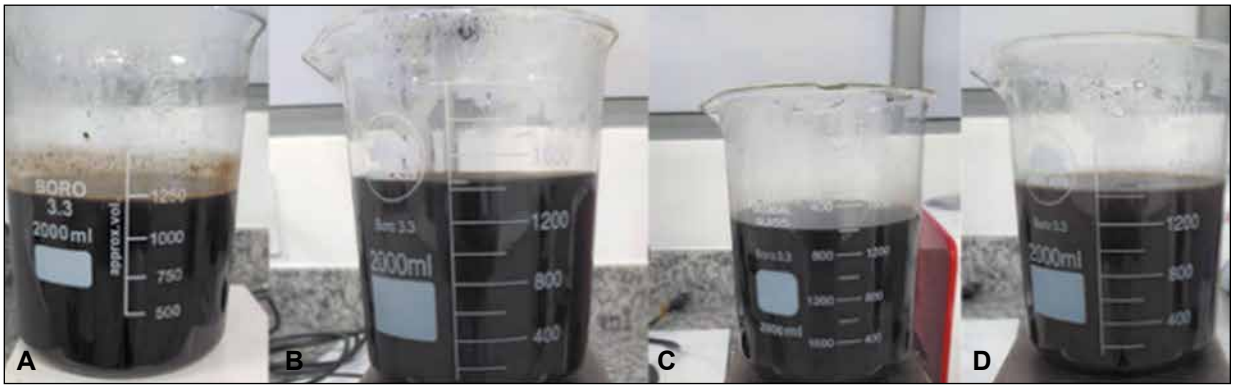
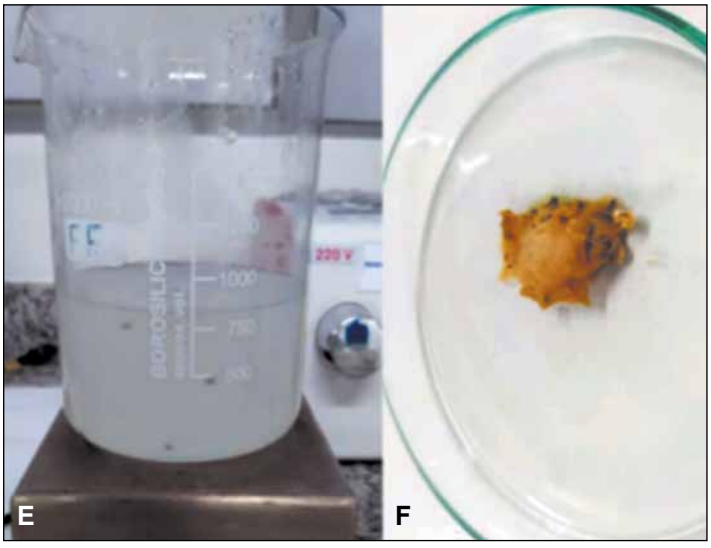


Figure 3. Delignification process (E) and obtaining pulp (F).



thickness. We performed the washing and bleaching phase 4 times for the complete removal of the water-soluble agents. The pulp gets lighter and lighter throughout the washes (Figure 2). We performed the bleaching to remove substances, mainly lignin, which gives a darker color to the pulp (Figure 3).

After bleaching, the BPF was oven-dried [6]. 1.45 g of cellulose was obtained, representing a total yield of 4.83%. Xavier (2018) [11] found values of 23% cellulose in Musa banana peel. Aregheore (2005) [12] reported 25% cellulose in his work.

An important factor is the ripening stage of the fruit. The percentage of cellulose and other components vary according to the ripening stage. In this study, we used peels of ripe fruit, which may explain the low yield of cellulose because during ripening occurs the enzymatic synthesis, proper of the fruit, which causes this natural degradation. We can optimize these values by improving the extraction method. The results obtained suggest that the BPF presents itself as an interesting matrix to get reinforcement material to be applied in films. However, the laboratory tests foreseen in the work plan are under development, and later we will elaborate and characterize the films.

Conclusion

Currently, the scientific field aims at sustainability, reuse, and innovation of what we already have, thinking so, the present study uses the banana peel because this waste has possible for several purposes.

The banana peel flour presented an interesting total fiber composition for cellulose extraction for biofilm production and converted into high-value-added materials. It can also add mechanical resistance to the material developed. Besides, it has several interesting properties to be used in food formulations. Thus, we hoped to obtain a biofilm with properties compatible with the application in the food industry.

Acknowledgments

To my advisors for their corrections and teachings. The company BioAlimentos for giving the samples and the CNPq scholarship for providing the scholarship.

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Evaluation of Microalgae Culture in PA and Commercial Urea Media

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This work aimed to evaluate the growth and cost cultivation of *Chlorella* sp microalgae in PA and commercial urea medium solutions compared to BBM standard medium. We cultivated the microalgae *Chlorella* sp in BBM, PA urea, and commercial urea media, evaluating their growth for 8 days. In addition, we appraised the cost of the culture media considering the quotation of the reagents and the mass used for pilot-scale cultivation (100l). It was possible to observe the similar growth of microalgae with urea PA and BBM. The use of urea PA as a culture media for microalgae has the potential to reduce the cost of the medium by 68%. Thus, the cultivation of *Chlorella* in urea medium represents an alternative to reduce the production costs of biomass from this microorganism.

Keywords: Low-Cost Medium. Urea. *Chlorella*. Microalgae Biomass.

Instroduction

Microalgae biomass has been applied in several processes, such as in the production of food, medicines, animal feed, fertilizers, biofuels, bioplastics, among others. Microalgae, such as *Chlorella* sp, stand out for their biological characteristics and biotechnological applications [1]. The bio-fixation of carbon dioxide makes it possible to apply microalgae in a sustainable remediation process due to the photosynthetic capacity of these microorganisms [2].

In recent years, much interest has been focused on the biotechnological potential of microalgae, mainly due to the identification of several substances synthesized by these organisms [3]. Some species of microalgae, such as species from the genera *Chlorella* and *Arthrospira* (Spirulina) are commercially cultivated due to their industrial applications [1,2].

Several studies progress demonstrated the potential for large-scale production of microalgal biomass, but several challenges persist to turn it economically viable. So, the development of low-cost culture media and more efficient photobioreactors, improvements in nutrient use efficiency, and photosynthetic rate optimization represent some of these challenges [4].

In this context, the development of alternative culture media using urea as a source of nitrogen (N) for the cultivation of microalgae represents an attractive alternative to reduce the cost of production of microalgae biomass [4,5] but it is necessary to evaluate the costs in detail. Some alternatives for cost reduction can already be found in use and have been extensively investigated, such as the use of NPK fertilizer [4] and the cultivation in wastewater [6]. On the other hand, an investigation of nitrogen-only sources is still poorly investigated as an alternative for cultivation [4].

The objective of this work was to evaluate the growth of the microalgae *Chlorella* sp in urea solution (pure commercial reagent) compared to BBM medium, as well as the costs involved in these culture media.

Materials and Methods

The experiments with the BBM, PA, and commercial urea media formulations were performed in parallel. Table 1 and Table 2 show the composition of stock solution and BBM media. We prepared PA and commercial urea solutions at a concentration of 1 g per liter. 180 mL of each culture medium in duplicate was added to an

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Table 1. Composition of stock solution and BBM media.

Reagent	Stock Solution		Media mL per L
	Mass	Vol (mL)	
KH_2PO_4	8.75	500	10
$CaCl_2.2H_2O$	6.25	250	1
$MgSO_4.7H_2O$	18.75	250	1
$NaNO_3$	62.5	250	1
K_2HPO_4	18.75	250	1
NaCl	6.25	250	1
Na.EDTA.2H20 /	2.5	250	1
KOH	1.55	250	1
Trace Metal solution			0.7
H_3BO_3	5.75	500	1
$FeSO_4.7H_2O$ solution and H_2SO_4 (1mL)	4.98	1,000	1

Table 2. Metal Trace solution (BBM media)

Reagent	Stock Solution	
	Mass (g)	Vol (mL per L)
H_3BO_3	2.86	1
$MnCl_2.4H_2O$	1.81	1
$ZnSO_4.7H_2O$	0.222	1
$NaMoO_4.2\ H_2O$	0.39	1
$CuSO_4.5H_2O$	0.079	1
$Co(NO_3)_4.6H_2O$	0.0494	1

Erlenmeyer flask and autoclaved. The inoculum with 20 mL of *Chlorella* sp under sterile conditions and aliquots of the solutions were evaluated by density optical (DO) analysis through absorbance at 570 nm to infer the biomass growth over the 8 days.

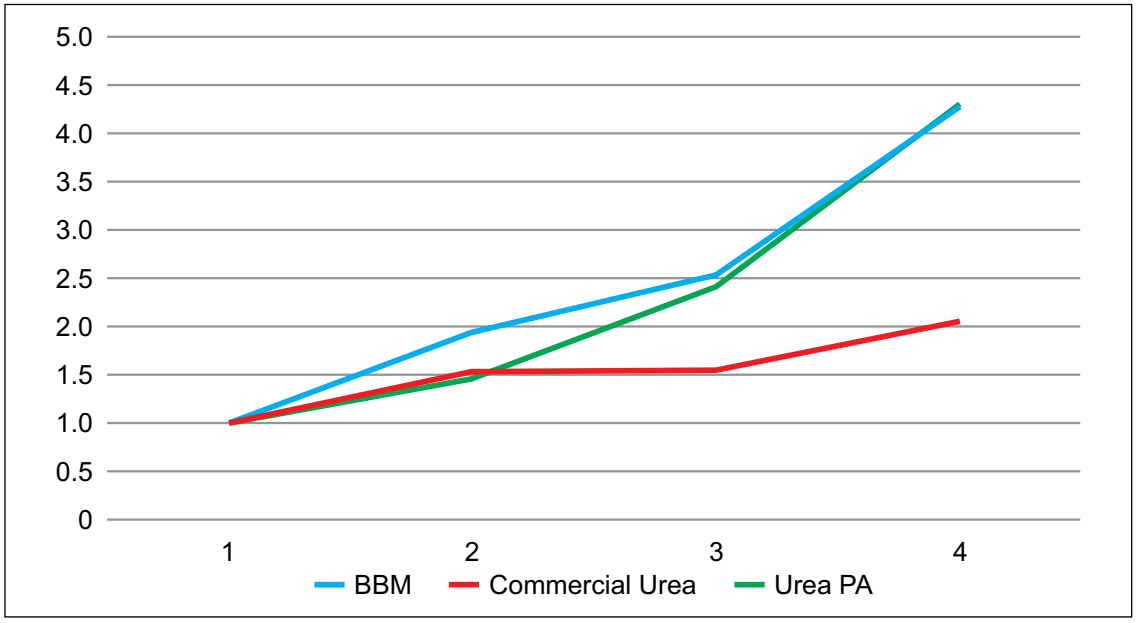
The quotation with suppliers of each reagent used for culture media preparation performed the cost evaluation. After that, we took the arithmetic average of the reagent prices to obtain an average market price and the lower costs. In addition, we calculated the costs based on the reagents' mass values for a culture with a volume of 10O L, both for the BBM medium and PA and commercial urea.

Results and Discussion

Figure 1 shows similar growth rates for cultures with PA and BBM and lowers for commercial urea. Although the cultures show growth over the 8 days, further tests are needed on the quality and productivity of biomass.

Figure 1 shows the growth of algal biomass in urea solution, which indicates the possibility of using urea for other recurrent microalgae species, such as *Arthrospira platensis* (Spirulina). Spirulina is also a type of microalgae enlarged in industry and biotechnological processes [6], and the reduction of its cultivation cost can also contribute to the accessibility to a more sustainable process.

Figure 1. Growth rate (DO/DO0 *versus* time) in culture media BBM and urea.



The urea PA has an average cost higher than the costs of the BBM medium and commercial urea because the suppliers quoted an imported reagent. However, when the minimum costs compared each reagent, the BBM media presented higher costs. Commercial urea had the lowest cost, but biomass growth is lower when compared to BBM culture media. Table 3 presents the costs to carry out a 100 L cultivation with BBM medium, while Figure 2 shows the average cost difference of BBM medium, PA urea, and commercial urea, while Figure 3 presents the cost difference based on the lower price of the same culture media, and Table 4 demonstrate the average and minor costs os cultivation media.

It is noteworthy that there is a high reduction in the average cost and the minimum cost for growing 100 L of culture medium (pilot scale). Depending on the results related to the quality of the biomass generated by urea, it will be possible to reduce by 68% the production cost of microalgae considering the lower cost of reagents (national). Ribeiro and colleagues [3] evaluated the use of nitrogen sources for the cultivation of *Chlorella sorokiniana*. They found that replacing the nitrogen source with urea reduced 65% of the cost compared to the BG11 medium. Thus, these results were similar to the study carried out in this work.

Ramanna and colleagues [6], seeking to investigate the effectiveness of water from domestic effluents compared to nitrogen, observed that the biomass using urea is more viable in terms of quantity and quality of the product obtained. Therefore, in addition to analyzing the quality of algal biomass, it is also necessary to monitor the cultivation with commercial urea for a larger period, to assess whether the cultivation cycle increases or if there is growth inhibition.

Conclusion

In this study, we get partial results for both the growth and the cost of the microalgae cultivation. Taken together, the results presented here indicate that the growth of *Chlorella* sp is similar in the BBM and urea PA and for commercial urea the growth rate is minor. The costs evaluation of the different media indicates that the replacement of the nitrogen source by urea PA can reduce the cost of the medium by 68%. Further studies are needed to evaluate the quality of biomass produced with this low-cost culture media.

Table 3. Example of calculation of the cost of reagents for BBM medium.

Reagent	Mass for 100 L in g	Cost for 100 L (R$)
$MgSO_4.7H_2O$	7.5	0.23
NaCL	2.5	0.07
K_2HPO_4	7.5	0.80
KH_2PO_4	1.75	0.17
$CaCl_2.\ 2H_2O$	2.5	0.14
Na_2EDTA		0.09
$FeSO_4.7H_2O$	0.498	0.02
H_3BO_3	1.436	0.08
$Co(NO_3)_2.6H_2O$	0.0079	0.01
$MnCl_2.4H_2O$	0.181	0.15
$ZnSO_4.7H_2O$	0.0222	0.00
$CuSO_4.5H_2O$	0.0079	0.00
$NaMoO_4.2H_2O$	0.039	0.01
$NaNO_3$	2.5	0.13
H_2SO_4	0.1	6.08
KOH	0.62	0.07

Figure 2. Cost of culture media.

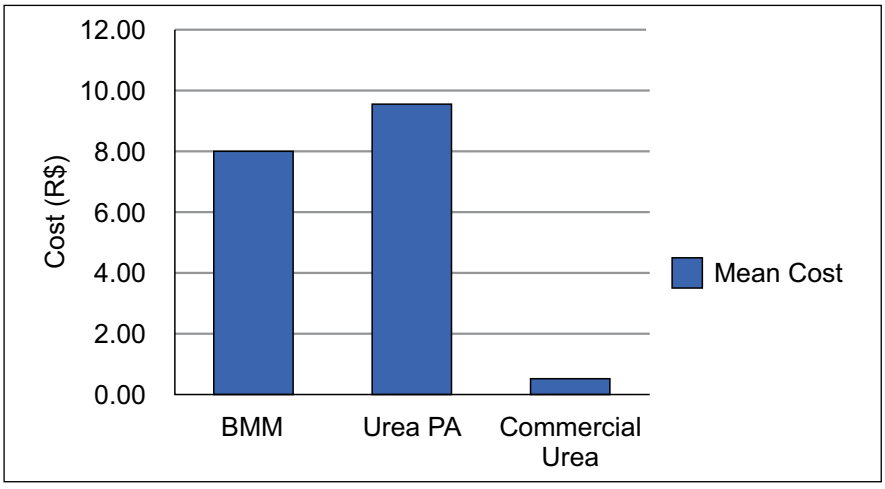


Figure 3. Lower cost of culture media.

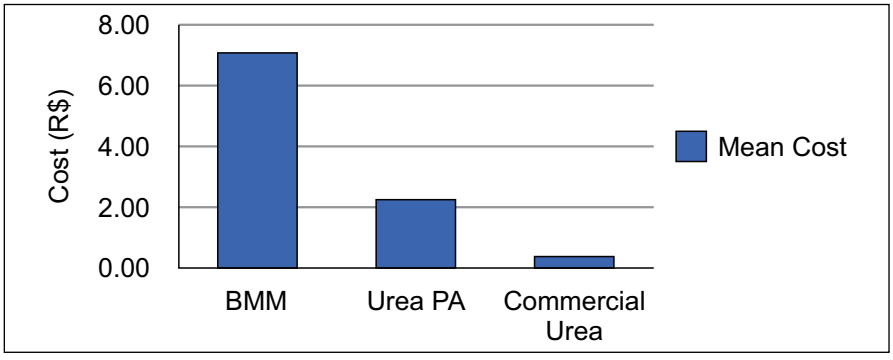


Table 4. Average cost and minor cost of cultivation media.

	BMM	Urea PA	Commercial Urea
Average cost (R$)	8.06	9.55	0.50
Minor cost (R$)	7.06	2.24	0.35

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Essential Oil Steam Distillation: Manufacturing 4.0

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Smart sensors, self-configuration, operational flexibility, and automatic learning, among others, are technological attributes from industry 4.0 appliable to the essential oil extraction by the steam distillation process. These operations are recognized by their simplicity. Nevertheless, lack of automatic controls, process monitoring, and self-adjustment lead to uncontrolled extraction, poor yields, low quality of products. It occurs because of overexposure to high temperatures and overspending resources like energy and water. As far as capacity utilization is concerned, the optimized process is key to planning and managing the production activities.

Keywords: Essential Oils. Technology. Yield. Quality. Industry 4.0.

Abbreviations: EO: Essential Oil(s); AI: Artificial Intelligence; SOP: Standard Operating Procedure,

Introduction

The essential oil industry is a primary supplier to cosmetics, pharmaceutical, and homecare businesses. Besides, the market trend towards natural products justifies the increasing interest in these aromatic substances [1]. Nevertheless, regarding technology updates, there are opportunities [2,3] brought up by the wave called industry 4.0: process integration, intelligent sensors, self-configuration, among others [4].

This paper emphasizes how the "4.0" attributes can deliver significant improvements to the EO industry as well as sets directional propositions for ideas and developments in future research.

Essential Oil Industry

The basic steam distillation processes require a few pieces of equipment, being recognized by their simple configuration and operation. Figure 1 shows a sketch of the steam flowing from the generator to the extraction vessel. It vaporizes and carries the essential oil to be liquified in the condenser and separated by the difference in density [5, 6]. Essential oil floats and is extracted through the upper outlet while the other part of the condensed liquid, called hydrosol [7], is extracted through the bottom of the separation vessel.

These operations handle a broad portfolio of products, processing several types and specimens as feedstock, each of them with its process particularities [8]. The essential oil quality and yield are affected by several conditions like weather, season, rain or irrigation, sun incidence, soil, and plant age [9]. These differences should be detected, compensated, or corrected by the extraction process itself, which is difficult when operating without a proper level of automation. Regular plant floor practices include fixed procedures, times, temperatures, and so on, disregarding specific behaviors of the plant, even when processing the same specimen, different crops, or harvested in diverse conditions.

Main Process Parameters

The yield and quality of the extracted EO are determined by a series of inputs to the process. Regarding the raw material, once the herb from what EO will be extracted is defined, it is necessary to determine the best condition to feed the extractor: fresh, dehydrated and the drying conditions (sun, shadow, drying time, etc.),

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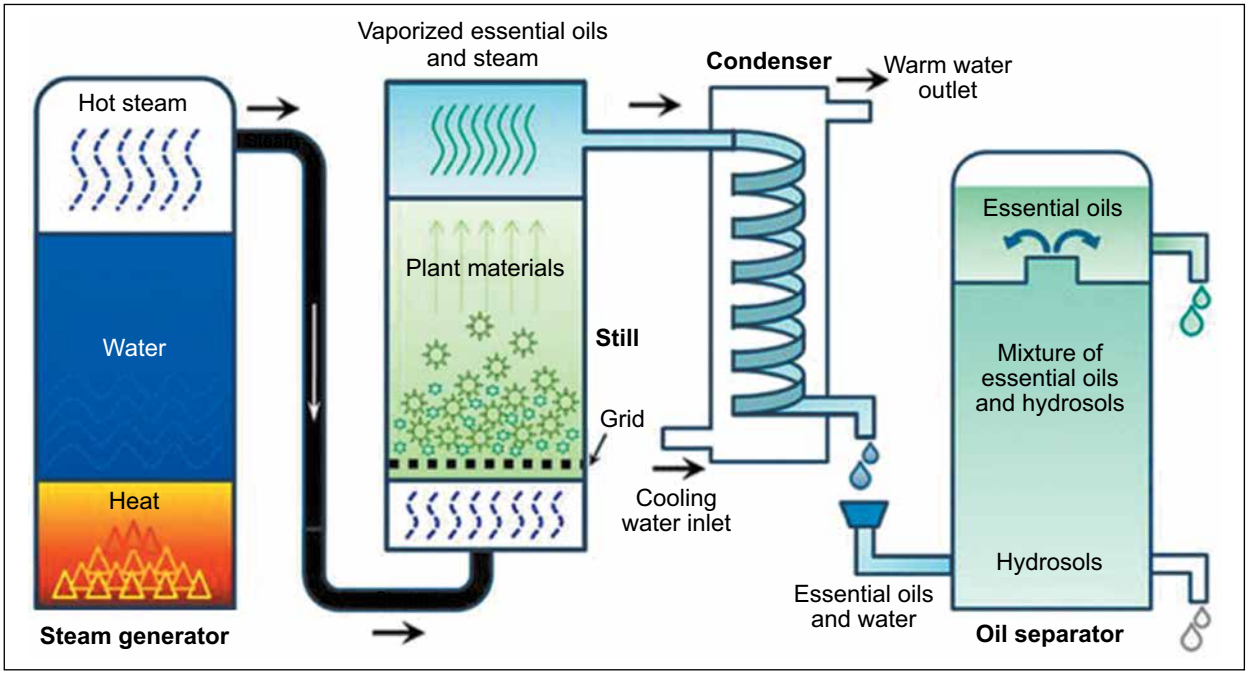
Figure 1. Basic EO steam distillation process.



chopped or not, particle size, and, regarding the process, steam temperature, pressure, and flowrate; cooling water flow rate and temperature [10]. Beyond these basic parameters, during the extraction process, undesired occurrences may happen to require detection and correction. Some of these occurrences are responsible for lowering yield and making product quality poorer. The optimum process time, the relationship between cooling temperature and essential oil yield and quality, the possibility of product degradation issues to be addressed by technology, when properly designed [11].

Undesired and Unknown Process Occurrences

In such non-automated conditions, operators cannot execute accurate process control. If there are no proper corrections during the process, the results are evidenced after the end or, worst, when the customer analyzes the product.

SOP (Standard Operating procedure) or just by historic practices fixed and defined the process duration. However, it would result in several problems such as product degradation because of excessive exposition to high temperature; energy, and water overuse; equipment capacity utilization beyond the optimum required to deliver the production plan, and, eventually, product rejection by the customer. Figure 2 shows an example of product degradation in geranium essential oil.

Possible product degradation root causes could be the excessive process time and the uneven steam stream flowing through the green mass, inside the extractor. Proper smart sensors and actuators can solve these problems.

The relation between condensation water temperature and yield and product quality is another issue. When volatile components are lost, yield decreases, and they are not analytically detected, in chromatography for instance (or detected in smaller quantities) [12].

So, the present paper brings a proposal for the EO industry, adding technological features to contribute to better yield and quality.

The Proposed EO "4.0" Process

The basic process control features to the EO industry would bring significant improvements to this industry segment [13,14]. Figure 3 displays a

Figure 2. Geranium EO degradation.

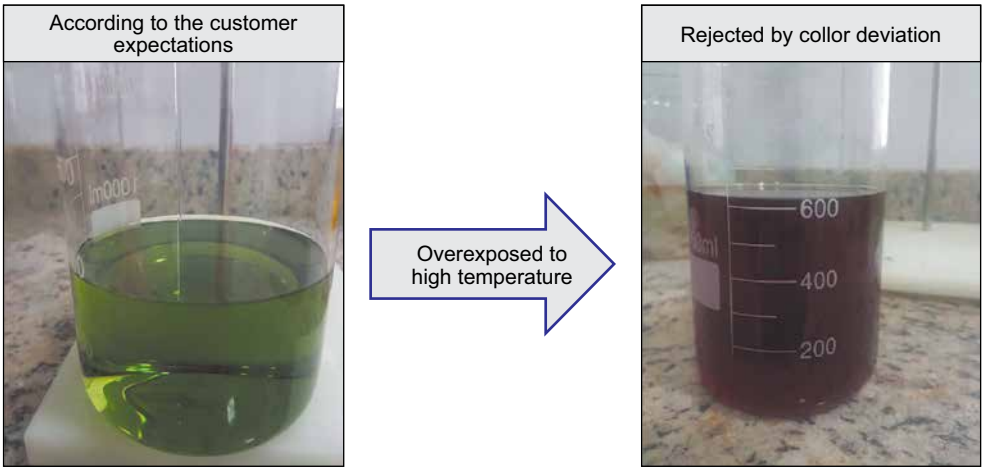
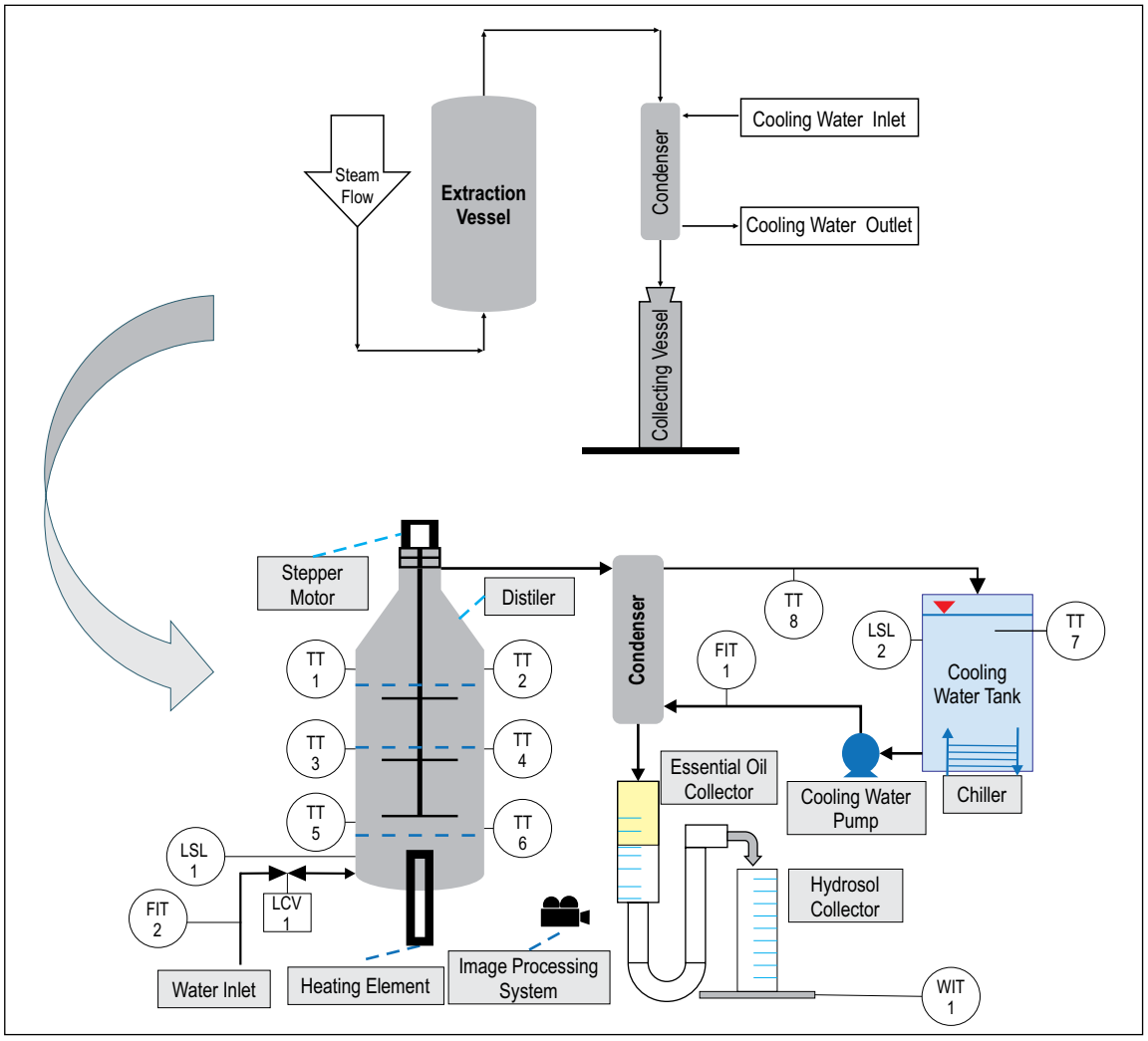


Figure 3. Essential oil plant: current and proposed configurations.



FIT: Flow Indicator and Transmitter; LCV: Level Control Valve; LSL: Level Switch Low; TT: Temperature Transmiter; WIT: Weight indicator/transmitter.

proposal, but it must become clear that the "one size fits all" would not be the case: the design of a "4.0" plant must be made to deliver what is necessary case by case.

Conventional controllers could be applied, nevertheless, the way their installation is designed and executed is the touchstone to detect these undesired process occurrences inside the green mass; the image processing system is a way of detecting optimum process time. AI (Artificial Intelligence) can indicate promising correlations among data and "propose" optimized process parameters, via – for instance – a digital twin [14].

End of Process Discussion

The economic end-of-process time occurs much sooner than the one used as standard practice in the industry. In this specific case, the extraction of lemongrass, determined by the industry SOP, is two hours. Nevertheless, the economic time is 40 minutes (Graphic 1).

It means that the period when the extracted essential oil value is lower than the overall manufacturing expenses or, at least, energy cost, the process should be stopped. Figure 4 illustrates how the optimum end-of-process time could be determined.

Graphic 1. Extraction curve: yield x time.

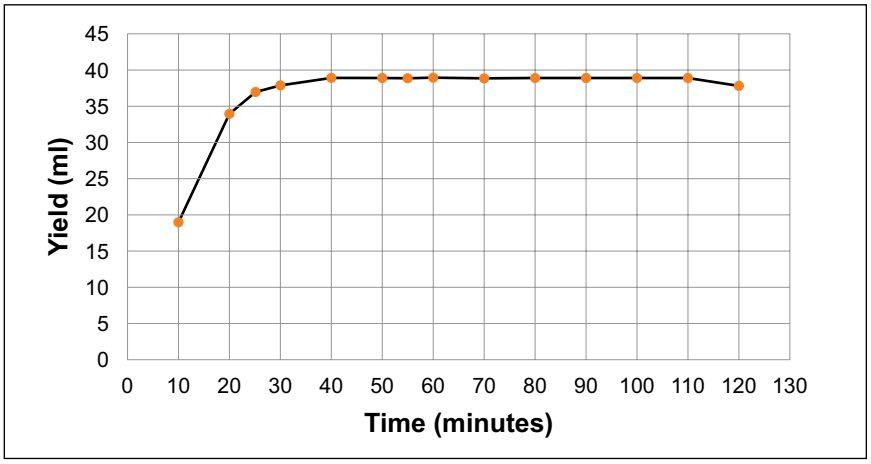
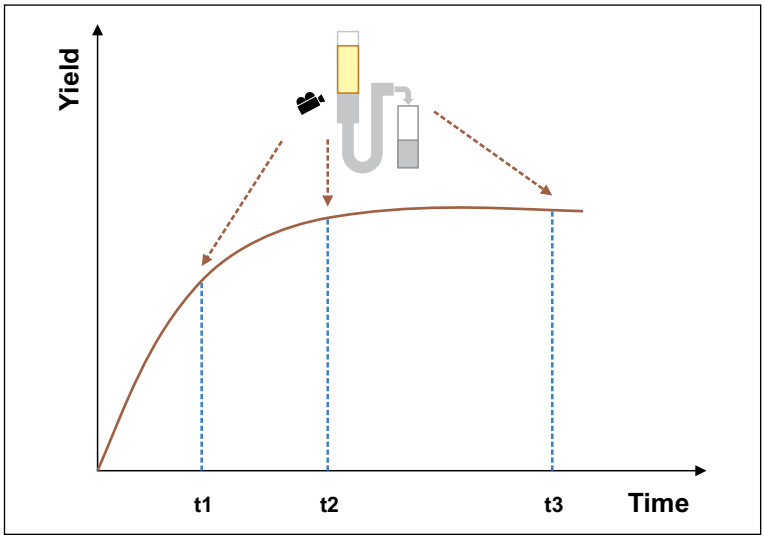


Figure 4. Image processing system.



The period between the start and t1 is the maximum slope, meaning the highest amount of EO should be extracted by the unit of time. In the period between t1 and t2 occurs the point of economical duration, which means, the amount (value) of EO extracted still justifies the continuation. The period between t2 and t3 represents the standard procedure time. However, without effective extraction, meaning a waste of energy, water, and production capacity. Beyond that, this period may cause product degradation due to overexposure to high temperatures or even lowering yield due to evaporation of light fractions.

Conclusion

Yield and quality are attached and are critical to the essential oil process. Technological attributes have the potential to manage process information and enhance steam distillation extraction. It is a must for the EO industry to climb the stairs towards manufacturing excellence. Technology is key to reaching that goal.

Acknowledgments

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Biosurfactants Production by Fermentation Process Using Waste as Substrate – A Patent Search Report

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Biosurfactants are active compounds capable of reducing surface tensions produced by microorganisms, having low toxicity and high biodegradability with possible production from renewable sources. This work aims at presenting a patents search on the biosurfactants production through fermentation, using waste as substrate. The methodology consisted of searching keywords and their blending, using the database of Derwent innovation. The search returned 15 patent registrations filed in the last 8 years. Of the 15, 10 orders are active. After analysis, 3 of the 10 selected patents showed strong adherence to the subject, describing the use of waste as substrates to produce more economical biosurfactants.

Keywords: Patents. Substrate. Fermentation. Waste. Biosurfactant.

Introduction

Biosurfactants are substances synthesized by microorganisms and have amphipathic properties, containing a hydrophilic and a hydrophobic part. They are active compounds with the capacity to reduce surface tension, low toxicity, biodegradable, and from renewable sources productions [1]. Due to the advantage of being more biodegradable when compared to surfactants of chemical origin, they are more appropriate to be used in various applications, such as bioremediation and dispersion in oil spillage; improved oil recovery; removal of oil residues in storage tanks; herbicide, and pesticide formulations; pharmaceutical and cosmetic application [2,3].

However, the production of biosurfactants is limited due to their high cost, low productivity, and the use of expensive substrates. Metabolites produced from cheap, renewable substrates and through economically feasible processes decrease the production costs of biosurfactants [1]. Thus, the economic viability of biosurfactant production at the industrial level is linkage to the cost of the substrate. In this sense, the use of waste or industrial by-products represents an excellent alternative for reducing production costs, especially if this waste has many sources of carbon, which is the principal metabolite for the growth of microorganisms [4].

So, patent research is necessary because it is from it that it is possible to detect technological trends and which regions invest most in each area, which in this article is the production of biosurfactants by fermentation from waste as substrates, thus allowing the identification of relevant technologies, partners, innovations, and investments. And it is from this those patents can help the search for new methodologies and techniques in the production of biosurfactants by economically viable substrates.

Patent

The patent is legal protection over an industrialized invention or creation. It gives the sole right of the inventor or assignee to prevent others from using without his consent. This exclusivity depends on the presentation of documents describing the invention and proving its use [5].

Patents are granted by the State, in which in Brazil, the responsible agency is the National Institute of Industrial Property (INPI). They are granted

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throughout the national territory, with temporary terms depending on the type of patent [5,6]. So, for creation or invention to be patented, it must be either an invention patent, valid for 20 years, that is granted to a technological good that meets the requirements of novelty, inventive step, and industrial application, or a utility model patent, which is conferred on an object of practical use or part of it, is susceptible to industrial application, has a new form and results in a functional improvement of the object, and its validity is 15 years [5,6]. Thus, patents are the instrument for the author or inventor to protect, as a legal right to his invention or industrialize creation [6]. In this context, the objective of this study was to search and evaluate patents related to the production of biosurfactants by a fermentation process using waste as substrates at the Derwent innovation database from Clarivate Analytics.

Materials and Methods

The informational research happened between July 7 and 13, 2021, on Derwent Innovation database from Clarivate Analytics. The search strategy used was the combination of keywords in the field SMART TOPIC, comprehending title, abstract, and keywords, with words previously chosen due to researching patents related to the object of study: production of biosurfactant by bacillus fermentation, using waste as substrate.

Table 1 shows the keywords chosen, and their synonyms or similar technical terms sorted in the columns. The search was carried out in all the languages available in the database, with the word appearing, without restriction, anywhere in the text from 2012 to 2020.

We analyzed the results and the registers with had relevance for the proposed theme were selected.

Results and Discussion

The search resulted in 15 patents registers in the DWII database. After the analysis, we selected 10 relevant patents. Some refer to the use of waste as substrates, and other patents regard the applicability of biosurfactants. Figure 1 shows the patent publishing trends of the 15 relevant patents application selected from 2012 to 2020. We observed that in 2020, 7 patents were registered, that is, half of the 15 patents in the 8 years, demonstrating that the production of biosurfactants through fermentation processes is a very recent technology developed in the last 10 years.

The results were sorted by region and country, and we noticed that China leads the publication of patents in this theme. Brazil presents the registration of one patent.

The main assignees of the patents and their inventors are mostly Chinese groups (Figures 3 and 4). Since there are more than 15 patents filed, we highlight that assignees and top inventors participate in more than one patent.

Among the 15 patents presented, 10 are active and 3 of them point to adherence to the proposed theme, which is the production of biosurfactants from waste as substrates. Among the 3 active patents with strong adherence to the theme, one of them: “Spore strain and application” uses urban waste as a carbon source to produce biosurfactants. This patent concerns a strain of spores that can use urban waste as a carbon source to produce biological

Table 1. Keywords used in the search.

	Synonyms			
Product	Biosurfactant			
Feedstock	Residue	Waste	Bagasse	Lee
Process	Fermentation			
Biocatalyst	Bacillus			

Figure 1. Patents registered.

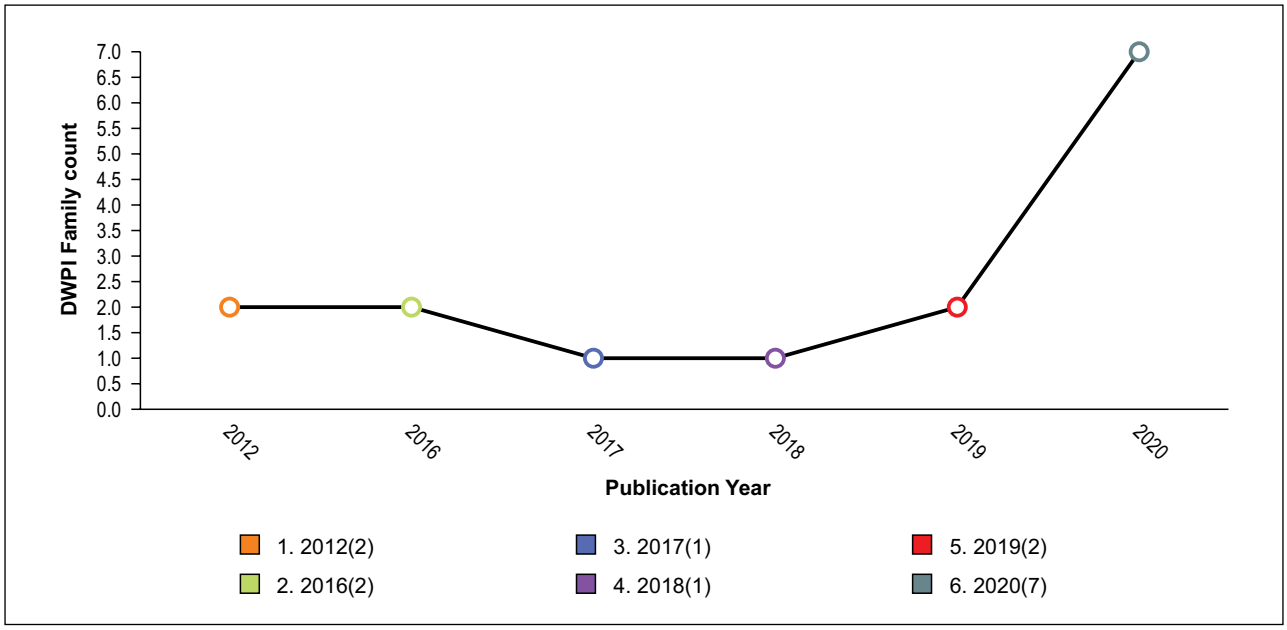


Figure 2. Patents registered by the country.

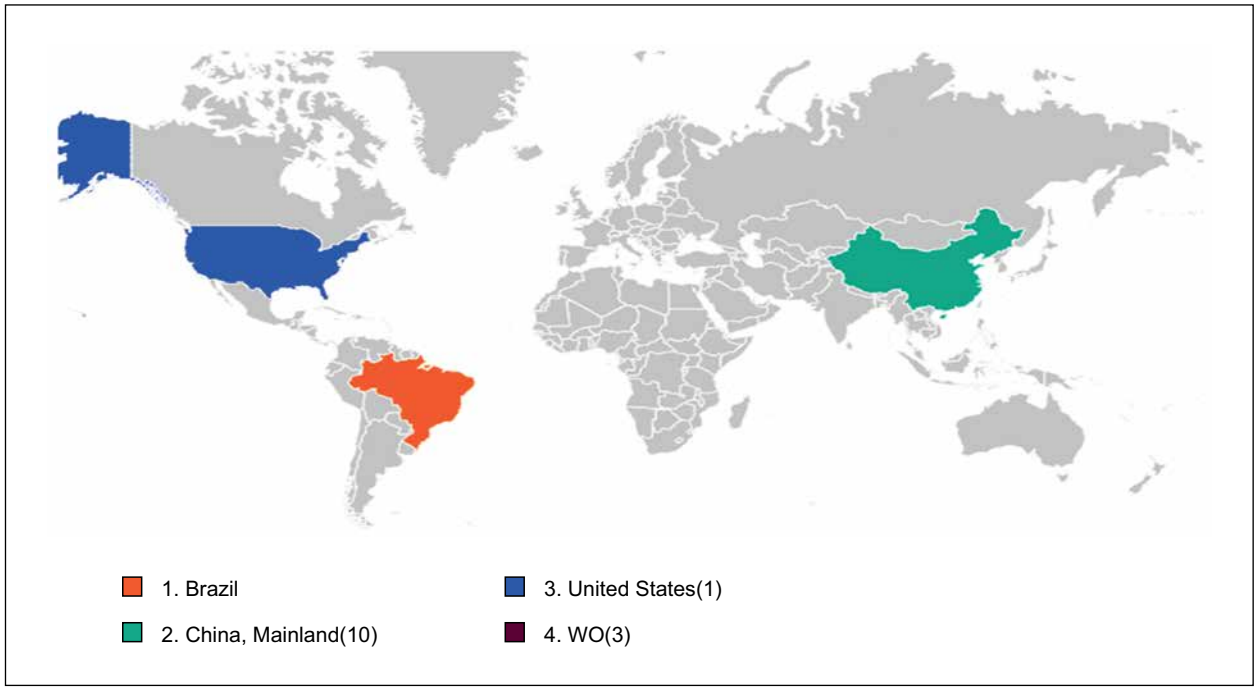


Figure 3. Top Assignees (created 2021-07-13).

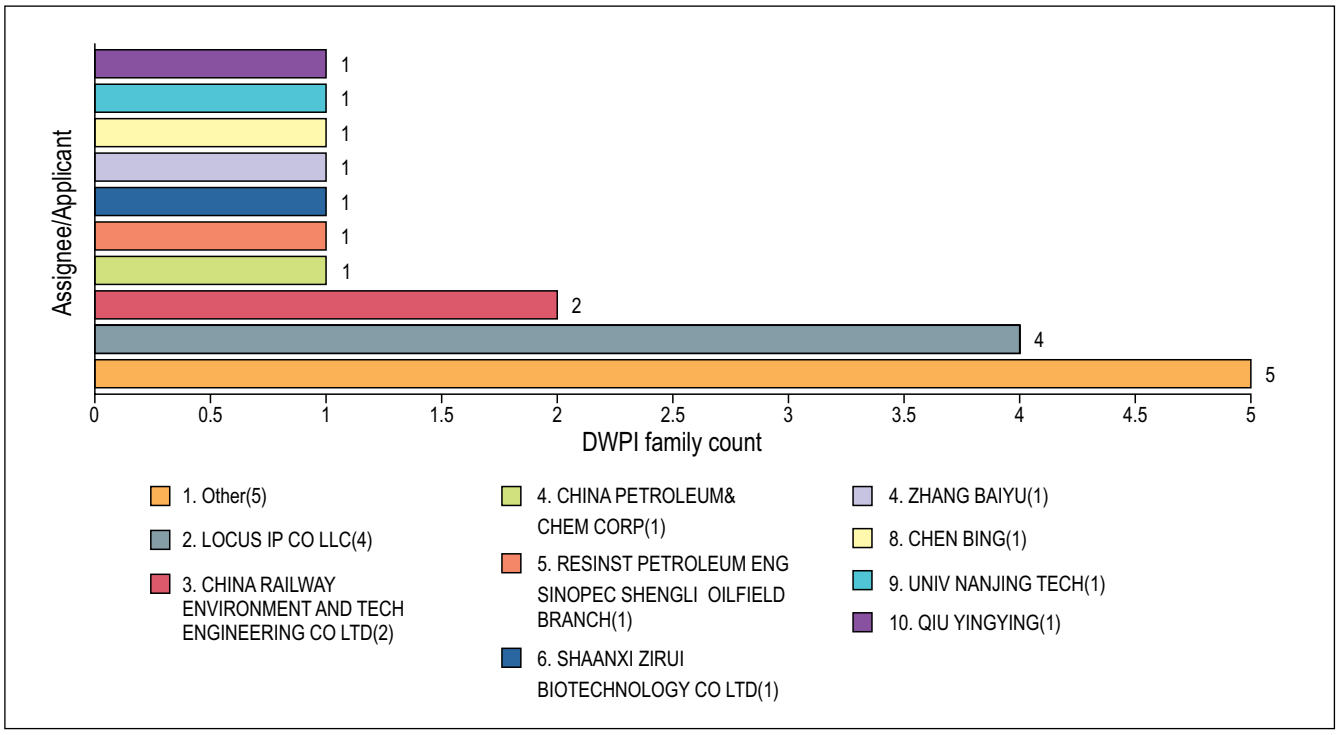
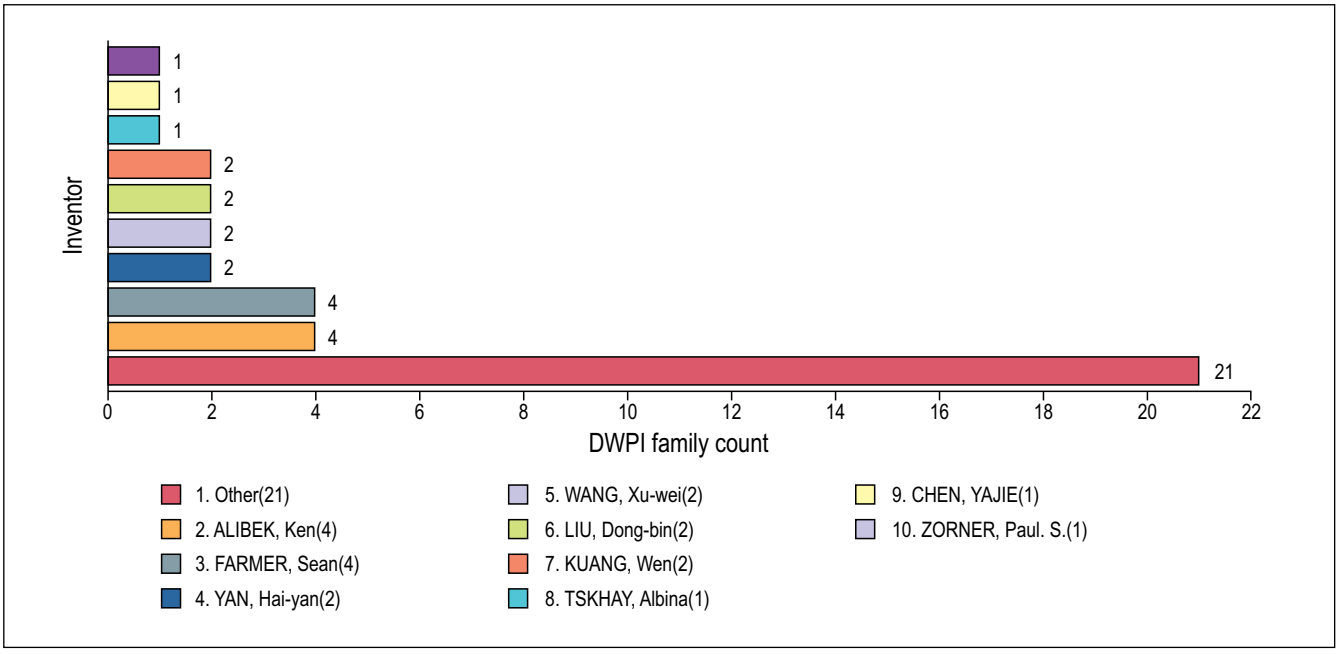


Figure 4. Top Inventors (created 2021-07-13).



surfactants. These crops can promote the growth of tomatoes and corn, exhibiting an adjustment effect and a fertilizing effect. In addition, the invention also claims a new technique for preparing biological surfactant, using urban waste as a carbon source, reducing production costs, and recycling waste.

The second patent: "A method for preparing biosurfactant by fermentation of aquatic product waste" uses aquatic waste, more specifically, fish waste for producing biosurfactants. The method of preparing biosurfactants by fermentation of aquatic waste consists of adding water and hydrological protease to fish residues and thus undergoing hydrolysis to obtain the fish wastewater solution. Subsequently, centrifugation is made, followed by extraction of the supernatant and it is freezing so that lyophilization can be done to obtain fish peptide. After that, the seed culture medium is prepared, the fermentation medium using peptone, and the extraction of biosurfactant. This production from the fermentation of fish waste prevents environmental pollution and increases the yield of the biosurfactants produced when compared to other biosurfactants produced by traditional methods.

Finally, the third patent with adherence to the theme was “Preparing method of lipopeptide biosurfactant” using xylose, an agricultural residue as a substrate to produce biosurfactants.The biosurfactant thrives method involves incubating the G-34 cell of *Bacillus subtilis* and the culture medium understands xylose as the central source of carbon. In addition to xylose as a carbon source, there is the acid hydrolysate of corn cob and corn straw or agricultural by-products. We also used the source of nitrogen for fermentation, which is a cheap biomass residue such as hydrolyzed feather residues or a solution of monosodium glutamate fermentation residues. The fermentation culture medium also includes the acetic acid addition to promote the fermentation of the strain to prepare the biosurfactant. Thus, with the use of this agricultural residue, there is a reduction in the cost of production of lipopeptide biosurfactants.

Thus, these patents show alignment with the proposed theme. The other 7 patents mention areas with great potential for biosurfactants applications. The patent entitled “A preparation method of degrading kitchen waste processing” makes an analogy to the preparation of degrading bacteria - containing in its composition biosurfactant - for the treatment of food waste.

Conclusion

The search resulted in 15 relevant patent documents related to biosurfactant production and its applicability. The patents were published between 2012 and 2020. The participation of the Chinese is relevant. Among the 10 patents selected, 3 mentioned the need for biosurfactants production from economically viable substrates to reduce production costs. Based on the patents presented, to reduce production costs, it is necessary to use economical substrates. And also, 7 patents demonstrated that biosurfactants have economic, environmental, and industrial applicability. It ratifies the need to reduce the costs of their production.

Acknowledgments

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Applications of Metabolities Extracted from Macroalgae to Fight Neglected Diseases in Brazil

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Microorganisms provoke neglected diseases. However, social factors intensified those affected by them. Macroalgae are photosynthetic beings that live in oceans. They are divided into three categories: green, red, and brown algae. These groups are known for having metabolites with various applications in biotechnology and natural products chemistry. The present work aimed to discuss, through bibliographic review, the applications of substances extracted from macroalgae to the treatment of forgotten diseases in Brazil. This research concludes that literature has studies focused on the presence and absence of these compounds, namely its qualitative aspects, in which pharmacological, pharmacodynamic, and reaction mechanisms fields of these bioactive constituents are described.
Keywords: Neglected Diseases. Macroalgae. Metabolites.

Introduction

Algae are organisms with photosynthetic capacity located in aquatic and continental environments. They provide protection and food resources for fish and invertebrates. They are divided into macroalgae (multicellular individuals) and microalgae (unicellular individuals). Three major taxa are: *Rhodophyta*, *Phaeophyta*, and *Chlorophyta*, also referred to as red, brown, and green algae, respectively [1,2].

Therefore, macroalgae are responsible for a large portion of the worldwide produced algae biomass, opening spaces for research development due to its vast production of metabolites and providing relevance to studies regarding pharmaceutical products used to fight infectious agents. Among the metabolites emphasized in previous studies, the caulerpin, a derivative of the *Caulerpa*, seaweeds in the family *Caulerpaceae* and phylum *Chlorophyta*. This compound has antitumor and antimicrobial activities, as the *Spatoglossum schroederi* seaweed, a member of the phylum *Phaeophyta*, which has a bioactive compound (fucoidan) with pharmacological activity. Likewise, the phylum *Rhodophyta* has shown antimalarial activity in literature, these red algae are located in the state of Ceará, reaching the southern limit of the state of Espírito Santo [1,4].

Currently, neglected diseases in Brazil are caused by infectious and parasitic pathogens that endemically affect vulnerable populations in developing countries, which lack positive therapeutic actions such as vaccines and medical diagnosis. These factors can damage communities that already do not have access to health-related resources, preventing them from better assistance and contributing to unequal reality. According to the Fundação Getulio Vargas (FGV), the prediction was that, in February 2021, 27.2 million people in Brazil would be living in poverty, which represents 12.83% of the Brazilian population. Thus, the growing cases of forgotten diseases in Brazil, such as dengue, Chagas disease, tuberculosis, malaria, cancer, leishmaniasis, and others, are inevitable [2,3].

The present work aims to highlight, through a bibliographic review, the management and application of metabolites from macroalgae to fight neglected diseases in Brazil.

Materials and Methods

We did literature research using papers published throughout the last 11 years (2010-2021)

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in the following databases: Instituto Nacional da Propriedade Industrial (INPI), Google Scholar, Scientific Electronic Library Online (SciELO), Periódico CAPES, Science Direct, and Espacenet. During the investigation, we searched for the words "seaweed and neglected diseases in Brazil", "green algae", "red algae", "golden algae", "seaweeds and metabolites", as well as "seaweeds and biopharmaceuticals", using the Portuguese, English, and Spanish languages. All studies were selected according to our subject of interest, and the pre-selected materials consisted of articles, doctoral thesis, and thesis.

Results and Discussion

We gathered 25 scientific materials, including original scientific articles, doctoral theses, masters' dissertations, and patent documents. Due to its continental dimensions, Brazil has a big coastline with potential for finding several species of macroalgae. It facilitates the opportunity of developing new pharmaceutical products for the treatment of neglected diseases and their consequences resulting from the current management methods.

On the other hand, neglected diseases are current for Brazilian citizens, causing a decrease in the well-being in the affected people. In addition, most of the existing drug treatments have demonstrated short-and long-term side effects, such as anticholinergics and opioids used for diarrhea, antimonials employed to treat leishmaniasis, and chemotherapy for various types of cancer [1,(24,25)]. Furthermore, several microorganisms acquire chemical resistance, owing to the prolonged use of treatments and indiscriminate lack of medical control for neglected diseases [3].

The unsuccessful therapeutic initiatives mentioned in the literature incentivates the research community to study therapies and medicines derived from natural resources, as well as the use of inputs extracted from macroalgae.The studies present a reduction of side effects and positive actions against such diseases when compared with the traditional one. Such applications are encouraged by the World Health Organization [4-6].

Dichloromethane and methanol extracts of *Dictyota mertensi* collected at the Itapuama Beach, Pernambuco-Brazil, exhibited 100% of leishmanicidal and cytotoxic activities, inhibiting the growth of promastigotes in the form of *Leishmania amazonensis in vitro*. Inputs from *Lobophora variegata*, *Padina gymnospora*, *Hypnea musciformis*, *Ulva fasciata*, *Ulva lactuca*, and *Caulerpa prolifera* collected from the coast of Ceará, are useful in the inhibition of *S. aureus* and *Salmonella* with the antibiotic-resistance profile. *L. variegata* and *H. musciformis* exhibited antimicrobial activity against *Vibrio harveyi*, which resists six types of industrial drugs, besides being a human and marine zoonotic pathogen.Methanol and ethanol-based raw materials obtained from *Padina gymnospora*, *U. lactuca*, and *C. prolifera* managed to neutralize the dengue virus type 3, an occurrence for the Brazilian health scenario, which seasonally faces outbreaks of dengue and correlated illnesses [7,(20-25)].*Spatoglossum schroederi*, *Udotea flabellum*, and *Gracilaria birdiae* - taken from substrates that originated in the coast of Rio Grande do Norte - showed parasite reduction and antimalarial activity against the *Plasmodium falciparum* species, expressing advantage compared to chloroquine. Other algae species such as *C. glomerata*, *D. dichotoma*, *S. furcellata S. natans*, and *U. lactuca* also have the antispasmodic operation following the action of meroditerpenes, tocopherols, b-tocopheryl-hydroquinone, and d-tocopheryl-hydroquinone. Sesquiterpenes from *L. dendoidrea*, obtained in the herbarium of the Universidade Federal do Rio de Janeiro (URFJ), reacted to leishmaniasis [8]. Fractions of *Prasiola crispa* contain a high leishmanicidal effect with efficiency, aside from having no toxicity to humans [9]. Inputs acquired

through solvent extraction using *S. vulgare*, *P. flagellifera*, *U. fasciata* collected on the coast of Bahia, demonstrated the following percentages of HIV inhibition: 89.92%, 37.18%, and 35.85%, respectively [10].

The genus *Gracilaria* has species with antibiotic activity in their extracts analyzed during *in vitro* studies, which tested their effects on Gram-positive and Gram-negative bacteria, such as *Vibrio cholera*, *Staphylococcus aureus*, *Shigella dysenteriae*, *Salmonella paratyphi*, and *Pseudomonas aeruginosa*. Soxhlet extraction using ethanol and methanol on *Gracilaria debilis*, *G. cervicornis*, *G. corticata*, *G. domigensis*, and *G. debilisi* generate inputs employed as antibacterial agents for multidrug-resistant organisms in severe cases of infections. Whereas *Gracilaria bursa-pastoris* and *Gracilaria* sp., when in contact with methanol, provide citrullinylarginine (Figure 1), a compound with antiretroviral activity regarding the human immunodeficiency virus (HIV), the herpes simplex virus (HSV), and the human papillomavirus (HPV) [11]. However, the reactions involved in these processes are unknown due to the lack of further research on such compounds, thus highlighting the need to assess the toxicity levels produced by prostaglandins, obtained from the mentioned algae through bioassays [12]. These species are found in Brazil from the coast of Ceará to the state of Espírito Santo [13].

Table 1 shows more applications of chemical inputs obtained from macroalgae in neglected diseases.

Conclusion

Based on many social problems surrounding neglected diseases in Brazil, the use of chemical compounds extracted from macroalgae to the direct and indirect treatment of pathologies should be well-studied. Besides, these compounds reduce side effects caused by conventional treatments and improve the well-being of vulnerable Brazilian populations.

Despite many metabolites discovered in marine and coastal macroalgae, the pharmacological and pharmacodynamic descriptions and the mechanisms of the action are still superficially explored to fight diseases.

So, further studies are needed to elucidate the interaction between these compounds and neglected diseases. Therefore, the scientific usage of the marine environment has to be aligned with the current societal sustainable mentality, highlighting the need to provide investments for studies focused on the mechanisms of action of these compounds.

Figure 1. Structure of citrullinylarginine.

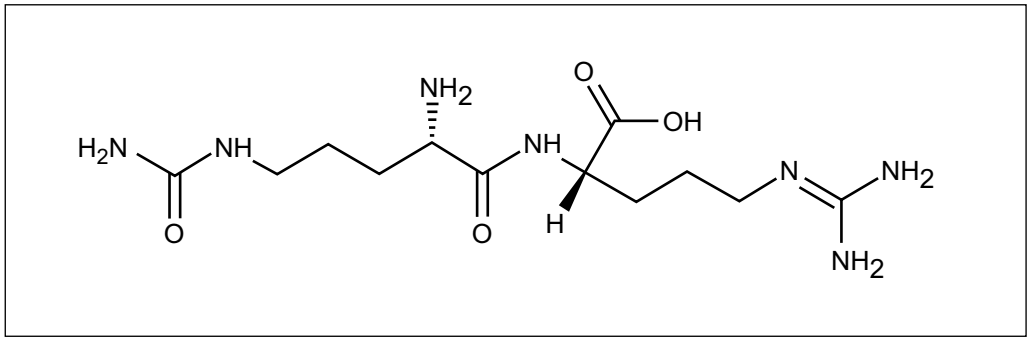

Table 1. Applications of macroalgae chemicals.

Species	Substance	Application	References
Octhodea secundiramea (red seaweed)	Halogenated, monoterpenes and octodens	Schistosomicidal activity	[14]
Byrothamnion triquetrum, *Gracilaria birdia*, and *Gracilaria caudata* (red seaweed)	Sulfated polysaccharides	Antinociceptive action generated by the treatment of neglected diseases and reduction of gastrointestinal lesions caused by antibiotics and acute antidiarrheal drugs	[1]
Dictyota caribea (brown seaweed)	Sulfated polysaccharides (fucoidans), diterpenes, primarymetabolites	*In vitro* macrophage activation for epidermal carcinomas, reduction of sarcoma 180	[15]
Caulerpa lentillifera (green seaweed)	Fucoidans	Apoptosis of sarcoma 180 and increased production of murine macrophages with M1 phenotype for antitumor activity	[15, 16]
Lobophora variegata (brown seaweed)	Polyunsaturated henecosanoid epoxide, apo-13'-fucoxanthinone, phytanyl glyceryl ether	Cytotoxicity against cancer cells and decrease in nitrous oxide responsible for HCT 116 derived inflammations	[17]
Laurencia intricata, *Laurencia obtusa*, *Laurencia microcladia*, *Leishmania mexicana*, *Laurencia scoparia*, *Undaria pinnatifida*, and *Plocamium cartilagineum* (red seaweed)	Diterpene, laurediterpineol, 7-hydroxyllaurane, bromolaurenisol, sesquiterpene, elatol, trichomonicide, β-bisabolene, fucoxanthin, terpenoids, polar fractions florotannins, sterols, alkaloid, and bromophenols	Antitumor activity against T47D cell lines and HIF-1 factors, both related to breast tumor cells. They are also inhibitors of K562 cells, epidermal, cervix, and myelogenous adenocarcinoma derivatives, lung and prostate tumors. They show a significant decrease in the action of helminth parasites and sexually transmitted infections	[8,18]
Gayralia oxysperma, *Monostroma nitidum*, and *Capsosiphon fulvescens* (green seaweed)	Rhamnans, sulfated, manoxylans, and sulfated heteroramnans	Inhibition of gastric tumor cells, Vero cells, human glioma, and herpes activity	[19]
Portieria hornemannii, *Plocamium cornutum*, and *Callophycus serratus* (red seaweed) *Udotea orientalis* (green seaweed)	Pentahalogenated terpenes, oxygenated monoterpenes, bromoficolides, aromatic sesquiterpenes, diterpenes, sulfated polysaccharides, and C-6 galactose	Inhibition of brain, kidney and, colon tumor cells. Antiplasmodic activity against *Plasmodium*, antimalarial, aside from anti-titrypanosomal activity opposed to *Trypanosoma cruzi* amastigotes, antiviral action *in vitro* against DENV-1, DENV- 2, DENV-3, and DENV-4	[20]
Cladophora glomerata, *Ulva prolifera*, and *Ulva lactuca* (red seaweed)	Indoleacetic acid, Indolebutyric acid, kinetin, polysaccharide extracts, and aqueous extracts	Antioxidant and anti-inflammatory actions, pancreatic fat breaking, antibacterial management against *Escherichia coli*	[21-23]

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Water Quality Assessment Using *Daphnia*: A Brief Review

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Due to the need to discover methods that can measure the toxicity level of fresh and marine water quality, this study aimed to present a systematic review of the literature, indicating the culture conditions and toxicological tests used for different *Daphnia* species in the evaluation of water quality. Thus, the applied methodology was a systematic review that identified studies that addressed the application of *Daphnia* in the assessment of water quality. The results obtained from this research consisted of a compilation of articles, which presented the parameters most analyzed in *Daphnia* species, such as mobility and mortality, which can change when exposed to different chemical substances.
Keywords: *Daphnia*. Water Quality. Toxicity.

Introduction

Unfortunately, the environment has been suffering the consequences of the impact of anthropogenic activities over the years, especially the aquatic environment, where numerous chemical compounds are found in it, which in addition to promoting ocean contamination, leaves this environment toxic and harmful to beings that live in it [1]. Therefore, research has been developed in search of methods that can measure the toxicology of these compounds on water quality, and their impact on the aquatic ecosystem, due to the lack of analytical tests that can make such information viable [2].

In this scenario of pollution in the oceans, there are aquatic beings that present a sensitivity to the chemicals that are dumped into the water, in a way that it is possible to detect the level of toxicology of these compounds and how they can affect these beings [2,3]. These organisms are microcrustaceans belonging to zooplankton species, called *Daphnia*, which is considered the oldest model used in bioassays aimed at the toxicology of water quality [4]. It is associated with their behavioral and physiological characteristics, which respond to environmental bioindicators or chemical compounds [3].

Daphnia is a microcrustaceans belonging to the *Cladocera* order, which has species that can be found both in the sea and in freshwater, in the way that, due to their high sensitivity to different compounds, are widely applied in ecotoxicological tests [5]. Thus, the species most used for toxicological tests are *Daphnia magna*, *Daphnia smilis*, and *Daphnia pulex*. They are usually found in freshwater, besides being easily cultivated in laboratories. They respond when in contact with different chemical compounds [5,6].

However, although *Daphnia* species are similar to each other, they have a different level of sensitivity: one species may be more sensitive to some compounds than another [6]. So, in this point, toxicological studies are developed to compare the impact of contaminants in more than one species of *Daphnia* [6]. Therefore, the most observed effect in toxicological tests is the mobility of organisms exposed to chemical substances soluble or dispersed in water [7].

In this context, it is necessary to emphasize that the toxicity tests involving *Daphnia* follow criteria for carrying out the tests of these species, since, according to the CONAMA Nº 430 DE 13/05/2011 resolution, the ecotoxicological tests realized in effluent must use aquatic organisms of at least two different trophic levels [8]. Thus, for the manipulation of *Daphnia* in the laboratory, one of the most used guidelines is the OECD Guidelines for the Testing of Chemicals, whose main objective is to guide the best way in the

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acute toxicity tests of *Daphnia*, providing the necessary information for the cultivation of these beings (culture medium, solutions, test medium, etc), in addition to the ideal time of exposure of the organism to the analyzed substance [9]. In the case of the OECD, the two standards used are OECD 211, which is specifically aimed at *Daphnia magna*, and OECD 202, which can be used for different species of *Daphnia* [9, 10].

Although standards are essential to contribute to effective analyzes in the use of *Daphnia* in the laboratory, it is necessary that the country where the study is performed, has this type of guideline, to ensure that the tests are in the appropriate conditions for analysis [6]. Thus, NBR12713, established by the Brazilian Association of Technical Standards (ABNT) has as its main guideline, specifying a method for evaluating the acute toxicity of liquid samples and water-soluble or dispersed chemical substances, using the species of *Daphnia similis* and *Daphnia magna* [7].

Therefore, this article aims to present a systematic review of literature, indicating the culture conditions and toxicological tests used for the analysis of different *Daphnia* species.

Materials and Methods

We did a systematic review of the literature, focused on studies of the application of *Daphnia* in toxicological tests for the assessment of water quality. The flow of this systematic review is described in Figure 1.

As previously presented, the systematic review of this study was performed in the following steps:

i) Choice of keywords with Boolean operators (and, or): "water quality", "toxicity", "*daphnia* test", "*daphnia*", "characteristics".
ii) Use of databases: Scopus (www.scopus.com), science direct (www.sciencedirect.com), capes (www-periodicos-capes-gov-br.ez68.periodicos.capes.gov.br), web of science (www. webofknowledge.com) and google of scholar (https://scholar.google.com.br).
iii) Search period: 2011 to 2021.
iv) Application of filters, aimed to synthesize the articles found during the search.

The use of Boolean operators (and, or) was important for a more refined result of articles that approached studies of *Daphnia* in toxicological tests, in addition to the use of filters, that served as a facilitator for the convergence of articles that attend the purpose of this study. However, although this method has allowed the acquisition of valid articles for this study, it is necessary to remember that in the current pandemic scenario, the remote access to these databases was not so simple, in a way that, the path used for the access of the databases mentioned previously, was through Google Scholar and Cape's Periodic.

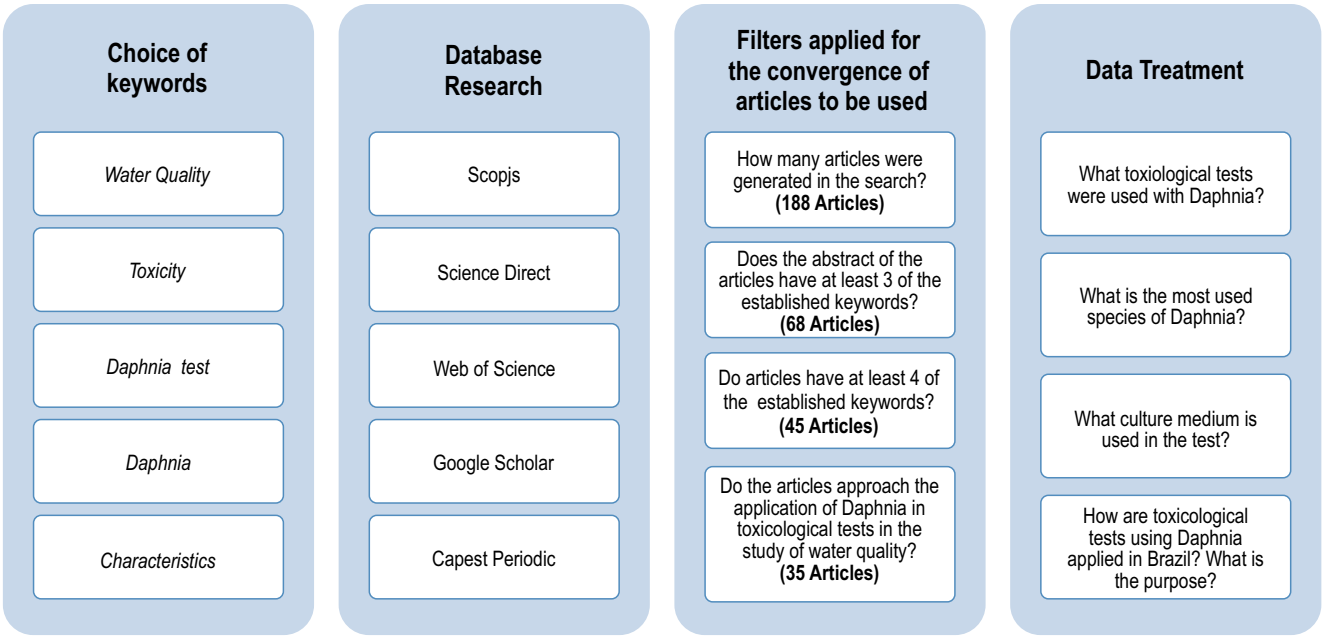
Results and Discussion

From the systematic review, it was possible to compile some articles, comparing the toxicological tests with the use of *Daphnia*, the most used *Daphnia* species, the culture medium used, and the toxicological parameters analyzed (Table 1).

In this study, we could not possible present all the articles found since the number of them was too high for this information space. Thus, six articles are presented that correlate the methods and standards used for it.

From the comparative analysis presented in Table 1, it was possible to detect that most of the explained articles used the *Daphnia magna* species for toxicological bioassays. This data is linked to the fact that *D. magna* is widely used as a bioindicator for the analysis of water quality since it has a short doubling time and high sensitivity [11]. However, *Daphnia similis* also has a significant level of importance when referred to ecotoxicological assessments, for being broadly distributed in the world, as in Europe, North America, and South America [6, 12]. In Brazil, it is frequently used for tests and considered a standard species, due to

Figure 1. Systematic review flow.



its accessibility in temporary, shallow, and turbid lakes [12].

The toxicological tests, in all the studies presented, used the mobility of organisms as an essential parameter for analysis. The immobilization test of these beings indicated for how long of an exposure to the analyzed compound, these organisms tend to lose their mobility [12, 14]. It was analyzed how long these beings exposed to chemical substances survive and what is the necessary age of *Daphnia* to be used for acute toxicity tests [12-14]. In addition, some articles approached the study of *Daphnia* species compared to other trophic levels, such as algae and zebrafish, to measure the sensitivity of these organisms in the presence of thallium and it can be observed that *Daphnia* species are less sensitive than the microalgae analyzed [14].

Due to the use of different species in the articles presented, a table was made with the studied *Daphnia* species, indicating its applicability in toxicological tests and the parameters analyzed for these organisms (Table 2).

We point out that these indicators are associated with the physiological system of *Daphnia*, such as mortality, which is associated with feeding activities determined by filtration and ingestion rates (Figure 1). The filtration rate consists of the volume of medium released per unit of time, in which the filtration process takes place in the thoracic limbs (the activity of the thoracic limbs is measured by calculating their beats per unit of time), generating a stream of water throughout the which food particles are directed to the mouth for ingestion [15]. The ingestion rate corresponds to the number of cells consumed by *Daphnia* in a specific period. It is noteworthy that these two rates can be quantified by measuring the decrease in particle concentration in water over time and by analyzing the material accumulated in the viscera [15]. In this context, the indicators of toxicity to chemical substances were analyzed (Table 2), for different species of *Daphnia* (*magna*, *similis*, and *pulex*). It was observed that all articles analyzed the same parameters, however, mortality was one of the most analyzed indicators, showing a degree

Table 1. Comparison of toxicological studies using *Daphnia* species.

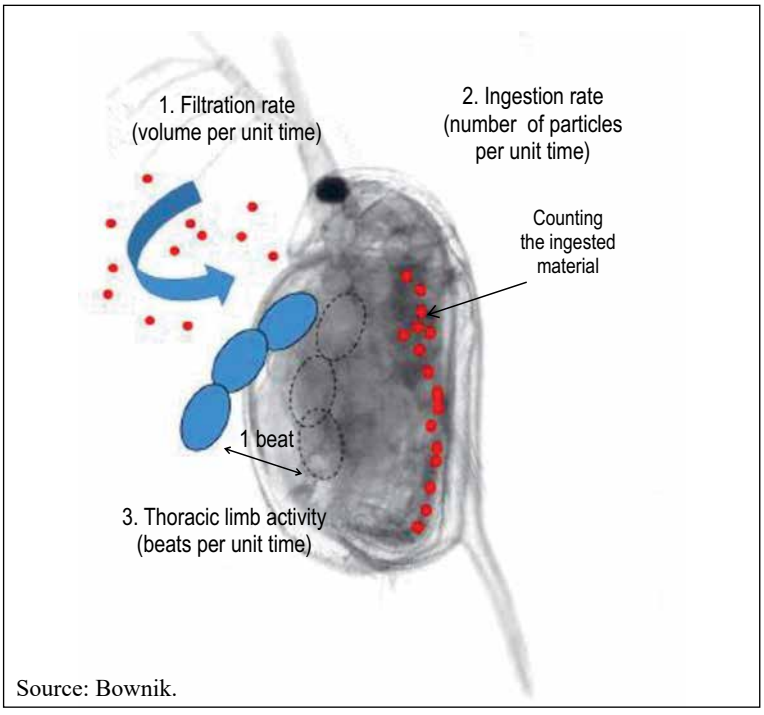
Article Title	Country	Evaluation Site	Year	Article Purpose	Tested Daphnia	Toxicity Test
Advanceents in effect-based surface water quality assessment [1]	Netherlands	Laboratory of the University of Amsterdam	2020	Evaluate the water quality and get analytical answers about specific sources of contamination	*Daphnia magna*	Bioassays performed on dilution series of extracts from all three passive samplers, resulting in nine *in vivo* responses which was immobilized for 48 hours of exposure
Assessing domestic wastewater effluent with a battery of bioassays after treatment with a specific consortium of microalgae and different flocculation methods [11]	South Africa	University of Stellenbosch	2020	To determine the toxicity of supernatants resulting from alum coagulation and chitosan flocculation of algae biomass from wastewater effluent using the bioindicator species	*Daphnia magna*	Five newborns of similar size were selected for each tray compartment. A series of five samples were selected for each solution tested. Mortality was defined as a lack of movement after a gentle nudge after 24 and 48 h
Toxicity of lead and mancozeb differs in two monophyletic *Daphnia* species [6]	Brazil and Portugal	Department of Biology & CESAM, University of Aveiro, 3810-193, Portugal	2019	To compare the sensitivity of two monophyletic *Daphnia* from different climatic areas to two different contaminants (inorganic and organic) to induce negative effects on the ecosystem	*Daphnia magna* and *Daphnia similis*	Newborns of both species between 6 and 24 h were exposed to a range of chemical concentrations for 21 days. For each species, the concentration range was based on its sensitivity to the test chemicals as well as the results of acute toxicity tests
Saxitoxi n-producing *Raphidiopsis raciborskii* (cyanobacteria) inhibits swimming and physiological parameters in *Daphnia similis* [12]	Brazil	Laboratory of Evaluation and Promotion of Environmental Health, Instituto Oswaldo Cruz, FIOCRUZ	2019	To test the effects of a neurotoxic strain of cyanobacterium *Raphidiopsis raciborskii* (CYRF-01) on swimming, activity and physiological parameters of *Daphnia similis*	Tested *Daphnia*	Acute tests were performed to detect the immobilization of *D. similis* during an exposure period of 48 h. The number of dead and immobilized individuals were counted after 30 min, 1, 2, 24 and 48 h after exposure
Protein profiling as early detection biomarkers for TiO_2 nanoparticle toxicity in *Daphnia magna* [13]	Portugal	Nova Lisboa University	2018	Evaluate the toxicity and effects of *Daphnia magna* exposed to TiO_2- NPs, through the response of the protein profile	*Daphnia similis*	The acute toxicity of TiO_2-NPs in *Daphnia magna* was evaluated according to ISO 6341, in which the inhibition of mobility of juveniles *D.magna*, aged between 6-24 h and exposed in 48 h
The acute toxicity of thallium to freshwater organisms: Implications for risk assessment [14]	United Kingdom	University of Plymouth	2015	To assess the acute toxicity of TI(1) to three key trophic species according standardized OECD methods	*Daphnia magna*	Newborns were exposed in triplicate (with 30 animals per treatment) and for 48 h to TI (I) concentrations ranging from 60 to 1200 pg I -1

Table 2. Comparison of applicability of different *Daphnia* species.

Daphnia Species	Application in toxicity	Parameter Analyzed
Daphnia magna [1, 6, 11, 13, 14]	Metal Exposure [1] Exposure to metal lead(Pb) and the fungicide mancozeb [6] Exposure to supernatants resulting from alum coagulation and chitosan flocculation of algae biomass from wastewater effluent [11] Exposure to TiO_2^- [13] Exposure to TI [14]	Mortality [1, 11, 13, 14] Immobilization [6, 11] Reproduction [6, 13]
Daphnia similis [6, 12]	Exposure to metal lead(Pb) and the fungicide mancozeb [6] Exposure to effects of a neurotoxic strain of cyanobacterium *Raphidiopsis raciborskii* [12]	Immobilization [6] Antennae movements, thoracic limbs, post- abdominal claw and heartrate [12]
Daphnia pulex [14]	Exposure to TI [14]	Mortality [14] Immobilization [14]

of sensitivity of species to exposure of these compounds: *Daphnia pulex* > *Daphnia similis* > *Daphnia magna*. These parameters were expressed in different ways in the studies, where the lethal concentration (LC) indicates the value range of a certain compound that can cause the death of *Daphnia*, as well as the effect concentration (EC), that indicates the responses that a determinate compound can induce in these organisms after a time of exposure (Figure 2).

Figure 2. *Daphnia* feeding activity.



Source: Bownik.

It was also identified that among the compounds analyzed in the studies, *Daphnia* species are more sensitive to metals. For example, the study that addresses the exposure of *Daphnia* to lead (Pb), showed that *Daphnia magna* tends to decrease in size compared to *Daphnia similis* [6]. Each *Daphnia* species reacts differently to a certain chemical compound, to the detriment of that, it is necessary that more studies are developed, to mitigate the toxicity of different compounds found in the aquatic environment.

Conclusion

Through the systematic review applied to this study, it was possible to generate a compilation of articles, which provided data for the use of the *Daphnia* species in the assessment of water quality toxicity, from the type of *Daphnia* to be used, to the ideal medium of culture and parameters to be analyzed. Therefore, from these studies, it was possible to detect that the species of *Daphnia magna* and *Daphnia similis* are the most used bioindicators in toxicological tests. This happens since they have a high sensitivity to chemical substances and physiology that allows analyzing the behavior of compounds in these beings. Also, it was possible to identify parameters (mortality, mobility, reproduction) in *Daphnia* that can change in the presence of chemical substances, such as mobility and mortality of these beings, that can indicate the level of toxicity of these chemical compounds.

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Instructions for Authors

The Authors must indicate in a cover letter the address, telephone 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. Also, the protocol letter with the number should be included in the submission article, as well as the name of sponsors (if applicable).

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

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

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

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