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Upcycling glycerin and vinasse by means of microalgae cultivation – Minireview.

Valoração da glicerina e vinhaça por meio do cultivo de microalgas - minirevisão.

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RESUMO: Em 2019 o setor de biocombustíveis biodiesel/etanol geraram como subprodutos o glicerol e a vinhaça em quantidades significativas (476 milhões e 466 bilhões de litros, respectivamente). Portanto, é necessário reutilizar ou tratar essas substâncias para reduzir os seus impactos ambientais. Utilizá-los para dar suporte ao cultivo de microalgas é uma maneira sustentável de valoração e de tratamento desses resíduos. O objetivo desse trabalho é sistematizar os artigos científicos que relatam a utilização dessas substâncias para o cultivo de microalgas e, consequentemente, o tipo de bioprodutos algal obtido. O glicerol pode ser utilizado como uma fonte de carbono em crescimento algal mixotrófico e, por isso, pode aumentar a produção de biomassa. O mesmo processo pode acontecer com várias substâncias de pequeno peso molecular presente na vinhaça, mas, a concentração de nitrogênio e fosforo é que tornam esse efluente mais atraente para o processo de cultivo algal. Embora existam trabalhos científicos descrevendo a utilização dessas substâncias para o crescimento algal, esse trabalho destaca que estes variam significativamente de acordo com os relatos de operação do sistema de cultivo e a sua produtividade lipídica.

Palavras-chave: Microalgas. Bioprodutos. Glicerina. Vinhaça.

ABSTRACT: In 2019, the biodiesel / ethanol biofuels sector generated glycerol and vinasse by-products in significant quantities (476 million and 466 billion liters, respectively). Therefore, it is necessary to reuse or treat these products to reduce their environmental impacts. Using them to support the cultivation of microalgae is a sustainable way of valuing and treating these residues. The objective of this work is to systematize the scientific articles that report their use for the cultivation of microalgae and, consequently, the type of algal bioproducts commonly obtained. Glycerol can be used as a source of carbon in algal mixotrophic growth and, therefore, it can increase algal biomass production. The same process can happen with several small molecular weight substances present in the vinasse, but the concentration of nitrogen and phosphorus is what makes such effluent attractive for the algal cultivation process. Although there are scientific studies describing the use of these substances for algal growth, this work highlights that they vary significantly according to the reports regarding the operation of such culturing system.

Keywords: Microalgae. Bioproducts. Glycerin. Vinasse.

INTRODUCTION

The estimated global consumption of fresh water in 2014 was of 3700 billion m3 (DINIZ et al., 2017, p.821). Most of it was converted into wastewater by industrial activity. The industrial activities best known for contaminating surface waters with effluents are those carried out by the biofuel, sugar-cane, textile, paper and cellulose related industries (LV et al., 2017, p.289; RAMLOW et al., 2017, p.2565). These effluents contaminate surface waters with excessive amounts of organics, nitrogen and phosphorus, which increases the eutrophication and hinder ecosystem processes (GODFRAY et al., 2010, p.812; MORÉE et al., 2013, p.836).

On the other hand, several nitrogen and phosphorus compounds can be used for the cultivation of microalgae. Microalgae are single-celled microorganisms, usually found in salt and fresh water (MOBIN et al., 2019, p.510) and known for fixing atmospheric carbon with the production of oxygen (METZ et al., 2007). For these reasons, they have an important role in the carbon cycling (BOROWITZKA, 1998). They are present in all existing ecosystems, representing a wide variety of species living in a wide range of environmental conditions. Therefore, there are species of microalgae capable of growing in industrial effluents with the advantage of accelerating their bioremediation process. In this way, such effluents become an inexpensive culture medium and can be used on a large scale; reducing the costs of the algal culture system (PIENKOS & DARZINS, 2009, p.431).

Algal cultivation not only assists in the treatment of effluents, but it can generate valuable bioproducts. Algae are already used in industrial production of substances such as proteins, fatty acids, carbohydrates, vitamins and pigments (MORAIS et al., 2015). Today, the production of microalgae biomass is estimated to be around 7.5 million tonnes per year (MOBIN & ALAM 2017, p.510; SATHASIVAM et al., 2019, p.709).

The cultivation of microalgae to obtain these bioproducts requires specific environmental conditions such as the control of temperature (20 a 30°C), light intensity (1000 to 8000 lux), agitation or mixing conditions, nutrient composition (ratio of 106:16:1 to C: N: P) and gas exchanges (oxygen and/or CO2 injection) (ZUCCARO et al., 2020, p.11; SUTHAR & VERMA, 2018, p.141; SHOW et al., 2017). The cultivation process can explore photoautotrophic, heterotrophic and / or mixotrophic metabolism. The cultivation system in ponds or photobioreactors are classified as open or closed systems (ZUCCARO et al., 2020, p.11).

Researchers believe that the mixotrophic cultivation methodmay be a solution for reusing industrial effluents as the culture medium (PATEL et al., 2019, p.245).Mixotrophy is a metabolic capacity of some algae to perform photosynthesis while using simple organic compounds. It was observed that some algae in mixotrophic condition increase their biomass productivity in about 2 to 3 times and by 2 to 10 times in the biomass/lipids ratios (ROOSTAEI et al., 2018). Sajadian et al. (2018) suggested that this would be the best approach for algal lipid production. The most positive side of the mixotrophic process is the possibility of associating the cultivation of microalgae with the treatment of effluents. This association is in line with the principles of sustainability (CHOUDHARY et al., 2017, p.276; MOHAMMAD et al., 2016, p.150).

The global demand for water is expected to continue to increase at a similar rate until 2050. Recently, the biofuel industry accounts for an increase of about 20 to 30% in the use of water (BUREK et al., 2016). Therefore, it is necessary to properly manage their liquid waste in order to avoid environmental impacts. The transformation of these residues into valuable bioproducts and more biofuel, via the biological process, is considered the most adequate and sustainable route (PATEL et al., 2020, p.245). Thus, the recovery of energy accumulated in the liquid waste is considered as an alternative to lower the costs of algal cultivation that serves as treatment prior to its disposal or later use. Finally, such an approach makes microalgae biotechnology economically feasible.

The objective of this work is to systematize the scientific works that report the use and treatment of glycerol and vinasse through the cultivation of microalgae and the extraction of their main products.

MATERIAL AND METHODS

Article assembly was carried out using the "Periódicos Capes", "Scopus" and / or "Science Direct" databases in December 2020. The selection was carried out using the keywords "algal OR microalgal growth AND vinasse" and "algal OR microalgal growth AND glycerin”. Some articles published from 2015 onwards and with an impact factor above 2 (Qualis B1, CAPES) were listed in this research.

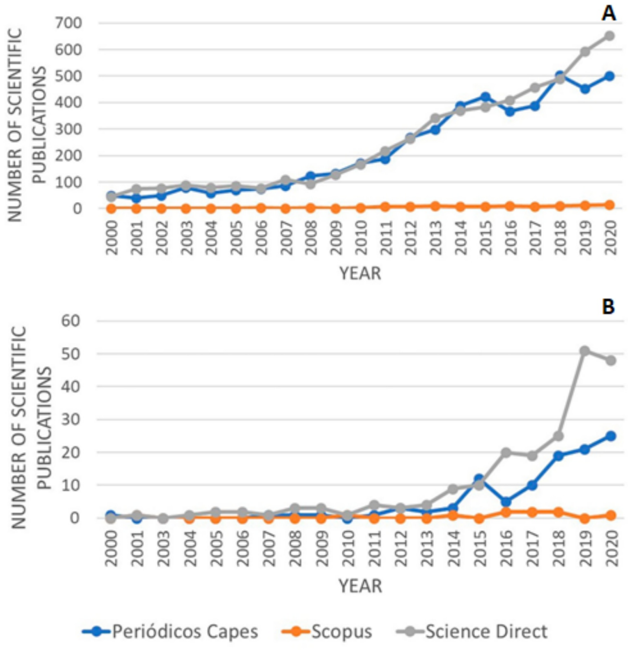
RESULTS AND DISCUSSION

It is important to highlight that the increase in the population will also lead to an increase in the consumption of water and fossil fuels. To get an idea of such an increase, just evaluate the projections for the increase in population size. It is estimated that by the end of this century we will increase from 7.7 to 11.2 billion the number of people (ROSER, 2019). The volume of water demanded for the biofuels sector in 2016 represented approximately 31% of the total global usage of fresh water (IEA, 2016). Therefore, the increase in biofuel production is directly related to a significant impact on water consumption. For this reason, the reuse of industrial effluents is essential for future developments in this area.

The biodiesel and ethanol industry generate large volumes of waste such as glycerol and vinasse, respectively. Therefore, combining the cultivation of microalgae and the treatment of this wastes is an important aspect to protect the industry and environment. Therefore, microalgal cultivation has attracted great attention due to its economic and environmental benefits (UBANDO et al., 2020).

The numeric temporal analysis of the scientific papers published with the keywords combinations cited previously are shown in Figure 1. According to the "Periódicos Capes", "Scopus" and “Science Direct" database, the keyword combination with vinasse generated 105, 10 and 208 publications, respectively. The keyword combination with glycerin generated 4709, 108 and 5202, respectively. Interestingly, the Scopus search algorithm identified the lowest number of hits with the keywords combination used in this research. The Figure also shows that a significant increase in the number of scientific publications occurred from 2010 for glycerin and from 2014 for vinasse. This tendency continued until 2020. Therefore, the interest of using glycerin and vinasse increased simultaneously under the umbrella of the justification for a circular economy and sustainability along with biodiesel and ethanol production.

Figure 1. Temporal analysis of scientific publications using glycerin (A) and vinasse (B) residues in algae cultivation.



Source: Elaborated by authors.

It is also interesting to note that in 2016 Brazil approved the directive law 13.263/2016; which was used for regulating an increase in the addition of biodieselwithin the commercial diesel. Currently, the percentage of biodiesel supplementation is of 13%. The plan is to reach 15% by 2023 (MME, 2020). This increased significantly the volume of biodiesel used in this country once the total of commercial diesel mixture consumed in 2019 was of 5.9 billion liters (EPE, 2019). Glycerol or crude glycerin is a common residue generated during the process of transesterification of oil into biodiesel. Stoichiometric calculations suggest approximately 10% of glycerol is formed compared to the total volume of biodiesel that is produced (MAKRI et al., 2010). In 2019, around 600 thousand tons of glycerol were produced and its total exports reached 283 thousand tons in Brazil (EPE, 2019). The revenue obtained from the export of crude glycerin was of 46.3 million dollars in this period, that is, 52.7% less than what was obtained in 2018. This was due to the higher supply in the market. Thus, there is a consistent drop in the international requirement and price of such a product (EPE, 2019).

Microalgae crops can use glycerol and vinasse differently. For glycerin (or glycerol), the best microalgal biomass production results are those reported by Su et al. (2020, p.73) and Chen et al. (2020). The first authors used *Phaeodactylum tricornutum* grown in F2 culture medium with 0.04 mol l-1 glycerin supplementation (SU et al. 2020, p.73). While Chen et al. (2020) reported the use of acid pretreated crude glycerin with calcium chloride amendment that favored the growth of *Thraustochytrium* sp. Using the semi-continuous cultivation strategy, the results wereimproved to 24.76 g l-1. This biomass contained 56.14% of lipids after 4 cycles (CHEN et al., 2020).

Distinct nitrogen sources can also influence the growth of algae when using such residues (PARANJAPE et al., 2016, p.778). Nitrogen supplementation, however, is commonly achieved by the introduction of another effluent that is commonly rich withsuch substances (REN et al., 2017, p.1130). The metabolic modes as phototrophic, mixotrophic or even heterotrophic cultures were also tested (KATIYAR et al., 2017, p.1359; PODDAR et al., 2018, p.298). The reports show that the results vary significantly according to the species of algae. Genetic modifications were also tested for the overexpression of specific enzymes (SIVARAMAKRISHNAN & INCHAROENSAKDI, 2018, p.532). However, it was not observed a significant improvement in biomass production with this approach, so far. What can be concluded from these works is that glycerol supplementation increases algal biomass productivity, but its success is case specific and correlated with nitrogen supplementation. Therefore, it is possible to identify that glycerin is a feasible additional source of carbon that is capable of stimulating algal growth via mixotrophic metabolism generating higher biomass and EPA productivity (CERÓN GARCÍA et al., 2005, p.297). Apparently, nitrogen and phosphorus supplementation become the limiting factor in this approach (VANEECKHAUTE et al., 2017, p.21). That is why it is highlighted that large-scale cultivation using such effluents can only be carried under supplementation and focusing the perspectives defined by sustainable processes (MAJIDIAN et al., 2018, p.3863).

Ethanol is the most widely used biofuel in the world. Brazil ranks as the second global producer. The cost of this production is, however, the impact cause by the generation of a significant amount of vinasse. The estimated volume of vinasse produced as the result of the 2019/2020 sugarcane harvest for generation of ethanol was of 460 billion liters (CONAB, 2020). This volume is a similar to ten times the volume of Sobradinho Hydroelectric Dam in Bahia State, Brazil. Thus, vinasse is a major cause of agro-industrial pollution in this

Table 1: Comparison of different microalgae crops in residues from the biofuel industry.

Type of effluent from the biofuel industry	Microalgae Specie	Biomass	Bioproducts	Nutrients Removal	Reference
Glycerol	PCH02 (algae similar to *Chlorella sorokiniana*)	1,55 g l^{-1}	L: 27%	51,25% of glycerol	Parajanpe et al. (2016)
	PCH05 (algae similar to *Chlorella vulgaris*)	1,78 g l^{-1}	L:18%	47,43% of glycerol	
Glycerol	*Chlorella vulgaris*	2,15 g l^{-1}	L: 24,1% P:50,7%	94,1% of ammonium; 89,9% of total nitrogen; 88,5% of total phosphorous e 86,3% of COD	Ren et al. (2017)
Glycerol	*Chlorella* sp.	446.50 ± 1.50 mg l^{-1} day^{-1}	L: 34,36%	-	Katyar et al. (2017)
Glycerol	*Synechocystis* sp. PCC 6803	1,8 g l^{-1}	L: 20-25%; Clo:2,8ug/10^{8} cells; Carot: 0,8ug /10^{8} cells;	-	Sivaramakrishnan, & Incharoensakdi (2018)
Glycerol	*Nannochloropsis salina*	0,42 g l^{-1}	L:4,4%	98,7% of glycerol	Poddar et al. (2018)
	Chlorella sp	0,37 g l^{-1}	L: 2,48%	63,5% of glycerol	
Glycerol	*Thraustochytrium* sp. BM2	15 g l^{-1}	L: 41,87%	>80% of glycerol	Chen et al. (2020)
Glycerol	*Phaeodactylum tricornutum*	4,96 g l^{-1}	C: 27%; L:38%; P:13%	-	Su et al. (2020)

Vinasse	*Micractinium* sp. Embrapa|LBA32	1,5 g l^{-1}	C:21,79%; L:2,50%; P:39,62%	It remained practically unchanged in both	Santana et al. (2017)
	Chlamydomonas biconvexa Embrapa|LBA40	2 g l^{-1}	C: 11,71%; L:1,26%; P:39,92%		
Vinasse	*Micractinium* sp. ME05.	1,38 g l^{-1}	L:4,90%	-	Engin et al. (2018)
Vinasse	*Chlorella vulgaris*	0,77 g l^{-1}	C: 36,50%; L:32,82%; P:22,23%	100% of nitrate; 49.93% of COD	Melo et al. (2018)
Vinasse	*Chlorella vulgaris*	0,82 g l^{-1} in continuous lighting	C: 15%; L:9%; P:32%; Clo:1,40 mg g^{-1}; Carot: 0,47mg g^{-1}	76% de of total carbon	Beigbeder et al. (2019)
		0,88 g l^{-1} in reduced lighting	C: 14%; L:3%; P:32%; Clo:1,12 mg g^{-1}; Carot: 0,22 mg g^{-1}	84,4% of total carbon	
Vinasse	*Arthrospira maxima*	3,015 g l^{-1}	C:10,67%; L:11,2%; P:57,04%	81% of COD; 89,2% BOD	Montalvo et al. (2019)
Vinasse	*Botryococcus braunii*	2,25 g l^{-1}	L:13,3%	-	Sydney et al. (2019)
Vinasse	*Chlorella sorokiniana*	1,62 g l^{-1}	C: 17,8%; L:8,9%; P:37,8%	95,3% of nitrogen; 78,3% of phosphorous	Sayedin et al. (2020)

Source: Elaborated by authors. Abbreviations: C: carbohydrates; L: lipids; P: proteins; Clo: chlorophyll; Carot: carotenoids; COD: chemical oxygen demand; BOD: biological oxygen demand.

country. The vast majority are used directly in the ferti-irrigation process. In moderation, it introduces benefits to the soil, such as, increases agricultural productivity, improves physical conditions and the return of nutrients to the soil (CABELLO et al., 2009, p.321; CRUZ et al., 2007). However, the continuous use of vinasse in ferti-irrigation causes a series of problems such as soil salinization, decreased seed germination and increased eutrophication of water bodies (MORAIS et al., 2015; ESPAÑA-GAMBOA et al., 2011, p.1235).

Few studies report the use of crude vinasse as basic medium for growing microalgae. This effluent has a high turbidity and a brown color that makes light penetration difficult and, thus, hinder algae photosynthesis. For this reason, most tests are done with a variable concentration of vinasse diluted in synthetic medium or distilled water. Engin et al. (2018, p.128) and Melo et al. (2018, p.344) used vinasse as a culture medium for microalgae in a dilution of 2-20%. They obtained results of 0.70-1.3 g l-1 of biomass depending on the type of microalgae and the cultivating conditions. Sayedin (2020) reported the use of diluted anaerobically digested vinasse in order to obtain a production performance of 1.6 g l-1. The authors that worked with raw vinasse, managed so by using a pretreatment process prior to the microalgae cultivation. Pretreatment commonly involves clarifying vinasse through filtration, centrifugation or the addition of chemical compounds as flocculants. All these pretreatments have the objective of reducing the vinasse organic loading and turbidity. *Botryococcus braunii* showed a good growth performance in 100% vinasse pretreated with alkali (SYDNEY et al., 2019). Beigbeder et al (2019, p.257) cultivated *Chlorella vulgaris* in centrifuged vinasse and Santana et al. (2016, p.2133) cultivated *Chlamydomonas biconvexa* and Micractinium sp. in 100% clarified vinasse.

Montalvo et al. (2019, p.103) and Sydney et al. (2019) reported the best results with algal biomass production in vinasse (3.01g l-1 and 2.5g l-1, respectively). The authors used different strategies. The first strategy was to dilute the vinasse by 30% and add CaCO3 supplementation. This supplementation not only corrected the pH of the naturally acid effluent to close to neutral, but it also assisted to increase carbon dioxide availability. The second step was to adapt the microalgae to vinasse in a sequential culturing line, first in 10, followed by 20 and 30% of vinasse. All two approaches culminated in a better performance on algal biomass production.

It is important to highlight that for most of the cited works, the accumulation of lipids did not exceed 15% of the produced biomass. This is still a low ratio for commercial production of algal biodiesel. Few reports focus on the productivity of other valuable algal by-products such as proteins, pigments and carbohydrates.

CONCLUSION

High amounts of glycerin and vinasse are currently generated as a result of the increase in the global production of biofuels. Microalgae are able to grow using the two cited liquid wastes, but some challenges still need to be faced. Regarding glycerin (or glycerol), high concentrations can hinder algal growth. Each species of microalgae reacts differently to glycerin supplementation, but at low concentrations and correct nitrogen supplementation it can increase significantly biomass production. Therefore, not only effluent ratios in the medium must be individually tested, by also the correct addition of nutrients such as N and P. The biggest problem faced by the cultivation of microalgae in vinasse is its color and turbidity. Therefore, strategies are needed to clarify vinasse and also to pre-adapt the microalgae. *Chlorella* sppare commonly those that best respond to the use of glycerin and vinasse. No work has yet critically and definitively delimited the operational parameters of algal cultivation systems when using glycerin or vinasse. In order to implement industrial scale algal cultivation, it is still necessary, not only the definitions of system operating variables, but also the clear implementation of the sustainability concept approaches into the process.

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