Microalgae as agents for upcycling industrial wastewater: energy patents and scientific literature overview

Microalgas como agentes na reutilização de efluentes industriais: revisão da literatura científica e de patentes

Microalgas como agentes para el reciclaje de aguas residuales industriales: patentes energéticas y reseña de literatura científica

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Abstract
The oil/gas, ethanol, biodiesel and biogas industries generate wastewater known as produced water, vinasse, crude glycerin and methanogenic digester´s based effluents, respectively. Microalgal systems have been suggested as a suitable and adaptable biological approach for the treatment of such wastewaters. The advantage of such an approach is the combination of treatment with the accumulation of algal valuable bioproducts. This systematic literature review aims to analyze scientific and patent production with bibliometric analysis. Another aim is to discuss the algal bioremediation strategies that were adopted by the cited industries for treating their distinct effluents. Data was obtained using scientific papers and patents published between 2000 to 2020 (335 scientific papers and 104 patents). The United States contributed with the largest number of articles, followed by China and Brazil. Meanwhile China, South Korea and the United States are the major owners of patents. This work identified that microalgal based system is successful in bioremediating several pollutants and that glycerol significantly improves algal performance for bioproducts production. Nonetheless, the efficiency of this process is hindered either by the adopted strategy applied before the cultivation stage or after this process. For instance, variables such as nutrient supplementation, turbidity and salinity corrections were identified as pivotal for process performance. Thus, the full-scale process is directly associated to the correct integration of wastewater production and proper management of microalgae systems.
Keywords: Wastewater treatment by microalgae; Industrial wastewater; Microalgal systems.

Resumo
As indústrias de óleo/gás, etanol, biodiesel e biogás geram efluentes denominados água produzida, vinhaça bruta, glicerol e efluentes à base de digestores anaeróbios, respectivamente. Sistemas de microalgas têm sido sugeridos como uma abordagem biológica adequada e adaptável para o tratamento de tais efluentes. A vantagem de tal abordagem é a combinação de tratamento com o acúmulo de bioprodutos valiosos de algas. Esta revisão sistemática da literatura tem como objetivo analisar a produção científica e de patentes com análise bibliométrica. Outro objetivo é discutir as estratégias de biorremediação de algas que foram adotadas pelas indústrias citadas para o tratamento de seus distintos efluentes. Os dados foram obtidos por meio de artigos científicos e patentes publicados entre 2000 a 2020 (335 artigos científicos e 104 patentes). Os Estados Unidos contribuíram com o maior número de artigos, seguidos por China e Brasil. Enquanto isso, China, Coreia do Sul e Estados Unidos são os principais proprietários de patentes. Este trabalho identificou que o sistema baseado em microalgas é bem sucedido na biorremediação de diversos poluentes e que o glicerol melhora significativamente o desempenho das algas para a produção de bioprodutos. No entanto, a eficiência desse processo é prejudicada tanto pela estratégia adotada antes da fase de

cultivo quanto após esse processo. Por exemplo, variáveis como suplementação de nutrientes, correções de turbidez e salinidade foram identificadas como fundamentais para o desempenho do processo. Assim, o processo em escala real está diretamente associado à correta integração da produção de efluentes e à gestão adequada dos sistemas de microalgas.
Palavras-chave: Tratamento de efluentes por microalgas; Águas residuais industriais; Sistemas de microalgas.

Resumen
Las industrias de petróleo/gas, etanol, biodiesel y biogás generan aguas residuales conocidas como agua de producción, vinaza, glicerina cruda y efluentes a base de digestores metanogénicos, respectivamente. Los sistemas de microalgas se han sugerido como un enfoque biológico adecuado y adaptable para el tratamiento de aguas residuales. La ventaja de este enfoque es la combinación del tratamiento con la acumulación de valiosos bioproductos de algas. Esta revisión sistemática de la literatura tiene como objetivo analizar la producción científica y de patentes con análisis bibliométrico. Otro objetivo es discutir las estrategias de biorremediación de algas que fueron adoptadas por las industrias citadas para el tratamiento de sus distintos efluentes. Los datos se obtuvieron utilizando artículos científicos y patentes publicados entre 2000 y 2020 (335 artículos científicos y 104 patentes). Estados Unidos contribuyó con el mayor número de artículos, seguido de China y Brasil. Mientras tanto, China, Corea del Sur y Estados Unidos son los principales propietarios de patentes. Este trabajo identificó que el sistema basado en microalgas tiene éxito en la biorremediación de varios contaminantes y que el glicerol mejora significativamente el rendimiento de las algas para la producción de bioproductos. Sin embargo, la eficiencia de este proceso se ve obstaculizada ya sea por la estrategia adoptada aplicada antes de la etapa de cultivo o después de este proceso. Por ejemplo, variables como suplementación de nutrientes, turbidez y correcciones de salinidad se identificaron como fundamentales para el rendimiento del proceso. Por lo tanto, el proceso a gran escala está directamente asociado a la correcta integración de la producción de aguas residuales y la gestión adecuada de los sistemas de microalgas.
Palabras clave: Tratamiento de aguas residuales por microalgas; Aguas residuales industriales; Sistemas de microalgas.

1. Introduction

The global population reached the mark of 7.7 billion individuals in 2019. An estimative suggests 11.2 billion individuals by the end of the 21st century (Roser, et al., 2019). Such an increase rate may exhaust natural resources such as freshwater. In average, the global annual freshwater consumption is of 4,600 billion cubic meters (bcm) (Liu, et al., 2020). About 20% of this total is used for supporting industrial activities (World Water Assessment Programme, 2014). The energy sector alone, for example, is responsible for the consumption of 75% of this share (Liu et al., 2020). Industries such as oil, sugarcane, textile pulp and paper and pharmaceutical are highly dependent on large amounts of freshwater and, thus, they are major producers of wastewaters (Lv, et al., 2017; Ramlow, et al., 2017). These untreated wastewaters may also compromise other freshwaters bodies after their disposal in the environment (Godfray, et al., 2010; Morée, et al., 2013).

American oil and gas industries alone are responsible for using 3.88 billion cubic meters of water in 2017 (Ground Water Protection Council, 2020). A similar consumption ratio is also common among the ethanol, biodiesel and biogas industries. Therefore, the energy, or fuel sectors, they generate significant amounts of wastewater that need treatment prior to its proper environment disposal. Thus, treatment for such specific effluents is on demand. The goal is to bring about a more sustainable development of the energy sector activities as it will also contribute to improving the circular economy strategy (Nizami, et al., 2017).

Industrial production of fuels often results in the generation of a wastewater rich in inorganic nutrients (Park, et al., 2011). These effluents are therefore suitable for growing commercially valuable microalgae (Posadas, et al., 2014). In addition, microalgae are also known to bioremediate small organic carbon molecules such as volatile fatty acids, dyes and glycerol (Wang, et al., 2016). Several reports show that a microalgal cultivating system is a promising approach for combining bioremediation with upcycling strategy of such wastewaters (Lam, et al., 2012). In the last few years, for instance, the average of commercial microalgal biomass production reached 7.5 million tonnes per year (Mobin & Alam, 2017; Sathasivam,. et al., 2019).

This systematic literature review aims to identify and analyze scientific and patent production with bibliometric analysis to find the main topics, authors, institutions and countries on the subject. It also gathers an overview of the strategies being applied to improve algae cultivation using the different effluents produced by the four main energy or fuel industries. Effluents such as produced water, anaerobic digester effluent, glycerin and vinasse from the oil, biogas, biodiesel and ethanol industries, respectively. It is also part of this research to evaluate the resulting treatment effect on the effluents when they are used for supporting microalgal systems. It is important to Identify and analyze the scientific and patents literature with a bibliometric analysis and to unveil to what extent these strategies are really innovative and, therefore, have led to the creation of intellectual property (patents) or new Science (papers).

1.1 Current scenario

Almost 60% of the total global demand for energy in 2018 was delivered by oil and natural gas (IEA, 2019). The International Energy Agency (IEA) reports that only 10% was provided with the use of biofuels. It is important to highlight that fuel production generates significant amounts of wastewater. For instance, during oil or gas recovery, the oil industry generates an effluent identified as produced water (PW). It consists of a complex mixture containing formation water, injected water and fluids for enhancing fossil fuel recovery from the rock formation (Arthur, et al., 2011; Fakhru'l-Razi, et al., 2009; Neff, 2002). In 2017 alone, the total volume of PW generated in the United States was of 24.4 billion bbl (Ground Water Protection Council, 2020). Typically, about 80% of this volume is reinjected in the rock formation, whether for the purpose of disposal or advanced oil recovery procedures. However, environmental impacts with PW disposal are often reported (Graham, et al., 2017). The produced water has a highly polluting potential risk because of its volume and toxicity. Consequently, some American states require that PW be stored in coated reservoir prior to its proper disposal in order to avoid local soil and groundwater contamination (Oil and Gas Act, 2008).

Biofuels production systems are often considered to be less harmful than the process of obtaining fossil fuels (Perin, et al., 2019). This is true in terms of carbon cycling but a significant amount of wastewater is also produced during the making of biofuels. Ethanol and biodiesel are the most used biofuels in the world. They are currently being produced at a volume of 114 and 47.4 billion liters, respectively (REN21, 2020).

The production of biodiesel generates a significant amount of glycerol (Yang, et al., 2012). Of the total crude glycerol produced worldwide, about 66% comes only from the industrial production of biodiesel (Kaur, et al., 2020). It is estimated that about 4 billion liters of glycerol are generated in this fashion. Glycerol can be used by food, pharmaceutical, personal care and cosmetic industries, but the amount produced highly exceeds the volume required by such industries (Gholami, et al., 2014). Furthermore, the crude glycerol produced by the biodiesel industry contains a low purity level (60-80%) (Kaur, et al., 2020). The required purification for further industrial applications makes its use economically unfeasible (Kong, et al., 2016; Pradima & Kulkarni, 2017). Therefore, several authors report that the future of crude glycerol is attached to its use as carbon supplementation resource for supporting biological process such as microalgal cultivation (Kaur, et al., 2020).

Vinasse is the main by-product of the ethanol industry. This effluent is generated during the fermentation process. In 2018, approximately 1.6 billion cubic meters of vinasse was generated in order to sustain an ethanol production of 108.6 million cubic meter (Renewable Fuels Association, 2019). Currently, vinasse is a major cause of agro-industrial pollution in countries such as Brazil. This is resultant to the fact that vinasse is a complex mixture of recalcitrant materials. The effluent is characterized by a dark brown color, high organic content, low pH (around 3.0 to 4.5) and, at its endpoint disposal, the effluent reaches high temperatures (~90ºC) (España-Gamboa, et al., 2011; Cabello, et al., 2009). After cooling, vinasse is often used as soil fertilizer. In moderation, this application benefits the soil (Cabello, et al., 2009). However, high amounts of vinasse fertirrigation can cause a series of problems such as soil salinization, decreasing seed germination and increased eutrophication of water bodies (Moraes, et al., 2015; España-Gamboa, et al., 2011).

Biogas production via anaerobic bioreactors has developed expressively in the last decade (Xia & Murphy, 2016). Germany stands out with more than 10,000 biogas plants with an annual production of 120 TWh in 2019 (IEA, 2019). The effluent produced by the anaerobic bioreactors are useful as fertilizer because its C, N and P content (Haraldsen, et al., 2011). This effluent direct application on land is dependent on the soil type, crop stage and the season of the year. Therefore, the volume that is generated during the production of biogas is not totally reusable in another process. In addition, the inadequate application on soil can cause potential risks to human health and the environment, as it also contains heavy metals, potential pathogens and recalcitrant organic pollutants (Stenchly, et al., 2017; Nkoa, 2014). Some techniques are employed in order to avoid these problems. It is common to use some sterilization technique or further treatment for also removing nitrogen and phosphorous (Tiwary, et al., 2015; Kiran, et al., 2014). In the last few decades, microalgal cultivation has been used for the bioremediation of nitrogen, phosphorus and some organics from several distinct effluents (Sutherland, et al., 2018).

Microalgae are single-celled and photosynthetic microorganisms. They are able to fix approximately 50% of the global carbon, converting it into biomass that can produce valuable bioproducts such as lipids, carbohydrates, proteins, pigments and vitamins (Markou & Nerantzis, 2013). The first report on the cultivation of microalgae in effluents was made in the 1950s (Golueke & Oswald, 1959). Effluent bioremediation using microalgae has not yet been extensively studied or strategically developed. Most research is mainly focusing on laboratorial experiments rather than the actual development of a bioprocesse. However, the reported results are very encouraging (Choi, et al., 2019; Patel, et al., 2020b). Microalgae-based wastewater treatment systems have shown to have lower capital and energy than several technologies which utilize physical, chemical and biological treatment processes (Craggs, et al., 2013; Moreno-Garcia, et al., 2017). They can be associated to natural disinfection and are more efficient in remedying simple pollutants when compared to traditional wastewater treatment systems (Benemann, 2008; Craggs, et al., 2012). Microalgae are capable of transforming pollutants through mechanisms such as metabolic degradation, bioabsorption, and photobiodegradation (Sutherland & Ralph, 2019). The microalgae Chlorella vulgaris is the most resourceful species for this purpose due to its easy adaptability, high growth rate (or productivity) and ability to assimilate CO_2 at relatively high concentrations (Concas, et al., 2012; Judd, et al., 2015).

The objective of this research is to provide a systematic review of scientific articles and patents that report the use wastewaters generated by the energy or fuel industrial production for supporting microalgal growth systems for commercial use. The goal is compare the different methodologies used for enhancing pollutants removal as well as the best microalgal growth performances when using such effluents. Finally, as a conclusion, it will bring detailed data on microalgal related patents currently expired or under protection of their repositors.

2. Methodology

This systematic literature review aims to identify and analyze scientific and patent production with bibliometric analysis to find the main topics, authors, institutions and countries related to the cultivation of microalgae using industrial waste. Bibliometric analysis can be used to analyze the publications by authors, the most prominent journals, as well as the methodologies used and the conclusions obtained (Durán-Sánchez, et al., 2014; Rojas-Sánchez, et al., 2022). The scientific literature contains important bibliometric analysis such as that by Deconinck, et al. (2020), who perfomed a retrospective technological, economical and environmental microalga harvesting methods. Rodrigues & Junior (2019) mapped patent documents used worldwide in the production of charcoal and filled research gaps on this topic. These researches provide methodological support for this study. This research retrieved the scientific publications from the Web of Science Core Collection (WoS) database and patent families were retrieved from Orbit Intelligence FamPat collection.

The scientific publications present in the WoS were organized with the Science Citation Index-Expanded indexer using the title, abstract and keywords fields. The aim to separately retrieve documents related to microalgae or microalgae biomass combined with produced water, effluent from the anaerobic digester, glycerin, vinasse and their respective synonyms in English; which were combined with the Boolean operators AND and OR. Thus, two individual search strategies were developed. The terms used were (i): "(microalgae biomass OR microalgae OR unicellular algae) AND (vinasse OR vinhoto OR stillage OR glycerol OR anaerobic digestate)" and (ii):"(microalgae biomass OR microalgae OR unicellular algae) AND (" produced water "OR" training water " OR "conata water" OR "oilfield wastewater")". Exclusion criteria are articles that were published before the year 2000 and after the year 2020. The searches were carried out between February 2, 2021 and February 6, 2021, with a total of 853 scientific publications retrieved. Subsequently, a data treatment step was performed to clean up the fields of authors and organizations in order to exclude duplicates and remove documents unrelated to the object being explored. This process reduced the sample to a total of 335 scientific publications.

The Orbit Intelligence was used in the search for patent publications. It was used by completing the title, summary and claims fields. Thus, search strategies were developed aiming to separately recover patents related to microalgae or microalgae biomass combined with produced water, anaerobic digester effluent, glycerin, vinasse and their respective synonyms in English associated with the Boolean operators AND and OR. In addition to the Boolean operators AND and OR, asterisk (*) truncation was used to retrieve documents with the plural words and others with the word root. The two individual terms used were i: "(microalgae biomass OR microalgae OR unicellular algae) AND (vinasse OR vinhoto OR glycerol OR produced water OR conata water OR training water)" and ii:"(microalga* biomass OR microalg* OR unicellular alga*) AND (vinhoto OR stillage OR oilfield wastewater OR anaerobic digestate)". In addition, it should be noted that the documents found were grouped by patent families based on inventions through the Orbit Intelligence FamPat collection. Exclusion criteria are patents publications that were published before the year 2000 and after the year 2020. The searches were carried out between January 6, 2021 and February 7, 2021, with a total of 406 patent families recovered. Subsequently, the data treatment step was performed with cleaning of the inventors and depositors fields to exclude duplicates and removal of documents unrelated to the object being researched, reducing the sample to a total of 104 technological publications.

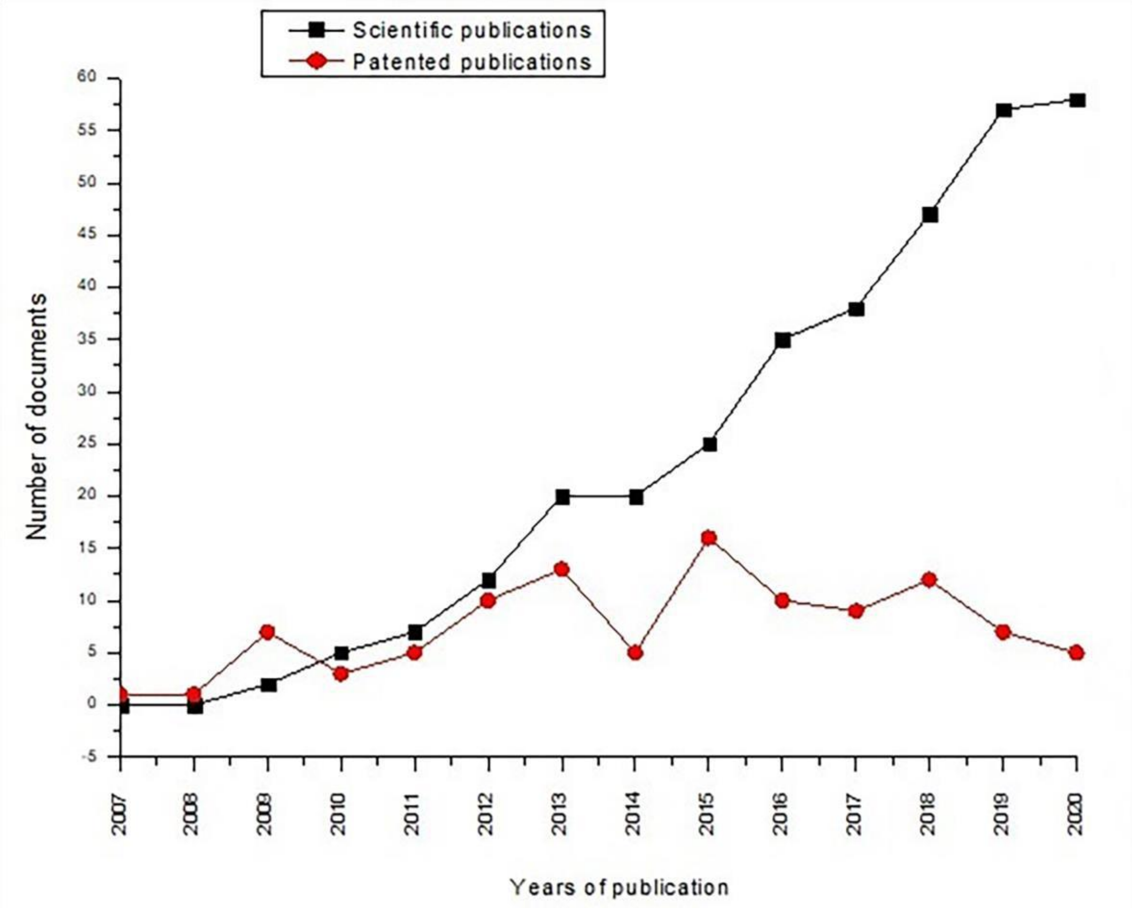
3. Results and Discussion

3.1 Chronological and global analysis

The chronological numbers of documents that were published reporting microalgal cultivation using the described wastewaters (2007 to 2020) are shown in Figure 1. This Figure indicates that the number of patents peaked in 2015 and afterwards progressively declined. The numbers of scientific publication, however, still growing. It was observed two periods of higher scientific publication rates; the period between 2009-13 and 2015-20. The main topic in the first period is the in-depth description of microalgal cultivation methodologies when using effluents. The second phase discusses the production of microalgal bioproduct and the implementation of a zero effluent discharge policy (Schindler, 2006; Brauman, et al., 2007; Grizzetti, et al., 2015; Preisner, et al., 2021).

The number of patents does not show a uniform increase rate (Figure 1). It is observed a modest increase in 2009, followed by a decline in the years 2010 and 2011. The number of published patents started to increase only in 2013. The observed delay on the ratio between scientific paper and patents may be correlated, in this case, to difficulties associated to the application and establishment of industrial algae processes. It is important to highlight that a great number of specialized information is built over non-standardized experimentation. A fast analysis can identify that most of the reported experiments are carried out under their specific condition varying media composition, nutritional supplementation and distinct wastewater effluents. Some of such effluents containing specific toxic pollutants or nutrient conditions. For instance, the produced water generated by the oil industry may vary significantly regarding salts and organics concentrations due to the nature of the rock formation. There are very few practical attempts to standardize such variables before testing these effluents in microalgal systems. Similarly, the effluent of an anaerobic digester may show significant variations on the concentration of ammonia. This variable is very important for microalgal cultivation and standardization is often neglected. Therefore, several variables show significant potential for interfering with microalgal success as an agent for phycobiorremediation.

Figure 1 - Numbers of patented (■) and scientific publications (●) published between 2000-2020.



The figure represents a line graph with the frequency of scientific and patent publications published between the years 2000 to 2020. Highlight for 2019 and 2020 that registered the highest number of scientific articles published. On the other hand, in relation to patents, there was not the same performance. Source: Authors, 2022.

Distinct countries contributed differently to advance the knowledge and knowhow of applied to cultivating microalgae on effluents (Figure 2). The United States contributed with the largest number of articles, followed by China and Brazil. Together, the three countries have about 30% of the total number of registered articles (Figure 2a). It should be highlighted that United States and Brazil stand out as the world main producers of ethanol and biodiesel. These countries detain 83% of the world's ethanol production and 26% of the world's biodiesel in 2020 (REN21, 2020). On the other hand, in 2019, the United States and China are among the six largest oil producers (800 Mt and 210 Mt, respectively) (IEA, 2019). Therefore, it is expected that such countries would try to find alternatives for the volume of wastewater effluents produced by such activities.

Figure 2 - Geographic distribution of the places of origin of scientific articles (a) and the places of patent protection (b).

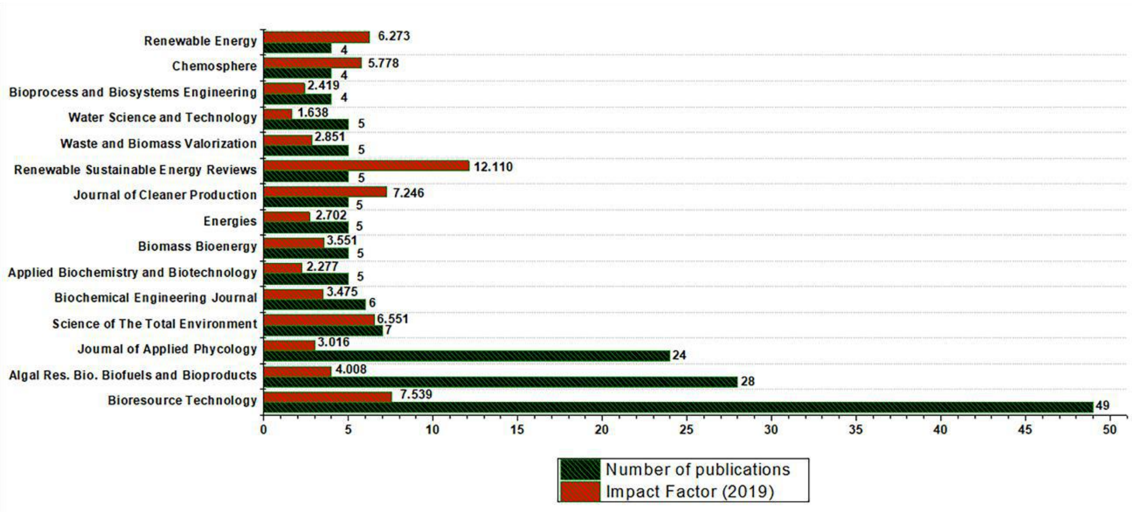
The figure represents the geographic origin of scientific (a) and patent (b) publications in the world. The United States stands out, which appears among the three countries that most deposit patents and publish articles. Source: Authors (2022).

Patent protection of microalgae systems is based on a territorial legislation. China, South Korea and the United States are the major owners of patents (Figure 2b). This provides insights into which country is likely to generate and protect its innovation and technology. Europe is the continent with the highest number of patents, approximately 41%. Asian countries are responsible for 37%. America represented by United States, Brazil and Mexico, mostly, owns 16%. Brazilian scientists publish significant amounts of articles, but this is not true in respect to depositing patents. Data obtained from the Word Intellectual Property Organization (WIPO) show that, in 2020, only 697 international patents applications originated in Brazil. All of them through the Patent Cooperation Treaty (PCT). In the same period, however, there were 275,900 applications for international patents worldwide (WIPO, 2021). Data relating to the PCT are particularly relevant because they indicate the intention to use patents internationally. The lack of knowledge to design an adequate strategy for the protection of Intellectual Property (IP) and limited financial resources to sustain the patents are the main reasons affecting the low Brazilian contributions to the deposit of patents (Pereira & Vasconcellos, 2014).

3.2 Overview of scientific literature

Overall, the articles are distributed within journals with varying impact factors, but 40% of the listed journals has impact factor above 5.0 in 2019 (Figure 3). About 30% of scientific publications were published in Bioresource Technology, a journal with the second highest impact factor listed in the figure, followed by Algal Research Biomass, Biofuels and Bioproducts (28 articles) and Journal of Applied Phycology (24 articles). All three journals account for approximately 62% of the works published in the area. This shows the importance that the scientific community is given to this topic. Bioresource Technology, Algal Research Biomass, Biofuels and Bioproducts belong to the editorial group Elsevier while the Journal of Applied Phycology belongs to the editorial group Springer.

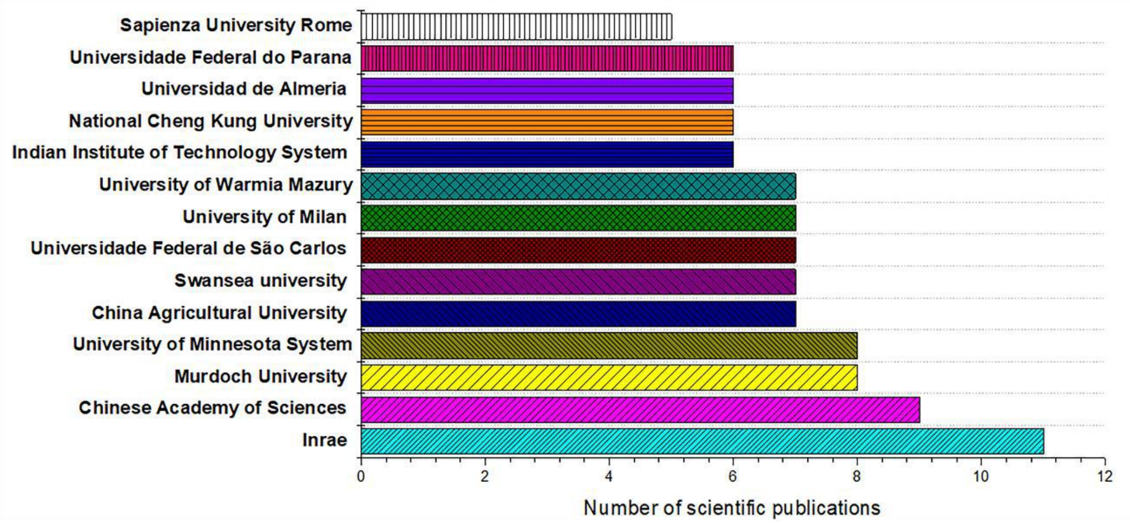
Figure 3 - Top fifteen most productive journals with impact factor.



The figure represents a bar graph about the frequency of published articles and its journals referring to the researched topic. On the Y axis we have the fifteen journals who most publish with this theme and on the X axis the number of articles. Highlight for Bioresource Technology who has already published 49 scientific publications and has the second highest impact factor. Source: Authors (2022).

The main scientific institutions publishing in this area are identified in Figure 4. The highest contributors are National Research Institute for Agriculture, Food and the Environment (INRAE) from France, followed by the Chinese Academy of Sciences from China, the first with 11 articles and the second with 9 articles. Another 9 institutions have at least 5 articles published in the area. These institutions are spread across Australia, the United States, the United Kingdom, Brazil, Italy, Polska, India, Taiwan and Spain. Although France is not among the countries of origin of several articles, INRAE is often an important partner. Seeing in this way, INRAE is the largest number of publications in the area (Figure 4), but France is not one of the most productive countries in terms of number of publications (Figure 2). Thus, France efforts focus on a single institute that has several partners in this area. In the other cited countries research with microalgae is widespread among different institutions. However, it should be highlighted that INRAE administrates eighteen research centers distributed throughout the French territory and they actively foment research in sustainable development and agroecology. It is with such an administrative organization that INRAE gained an international prominence and occupies the second place in the world and the first in Europe in number of publications in agriculture and forestry (INRAE, 2020).

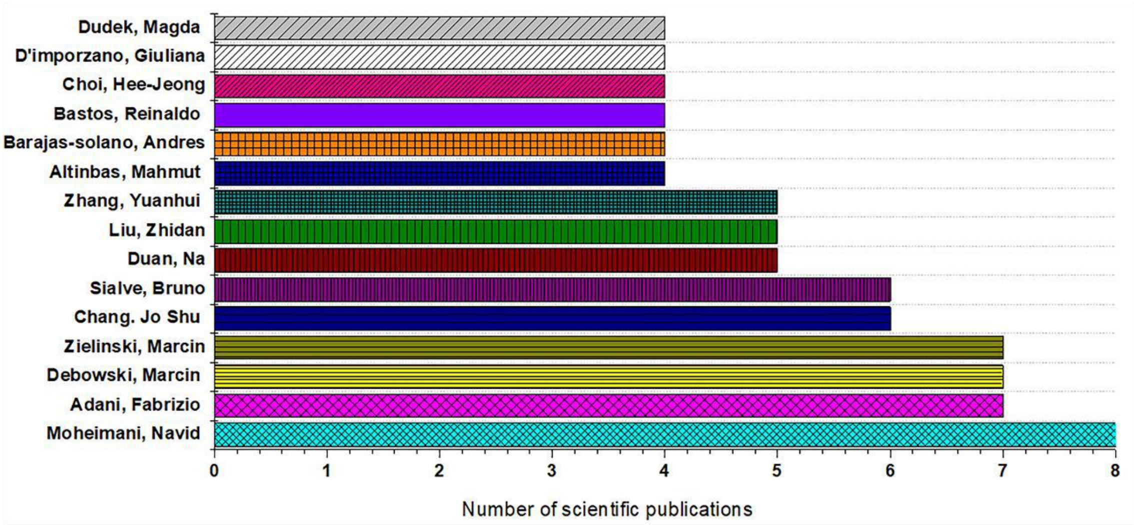
Figure 4 - The most productive institutions associated to the scientific publications.



The figure represents a bar graph on the frequency of published articles and institutions that produce these articles. On the Y axis we have the institutions that most publish with this theme and on the X axis the number of articles. Highlight for INRAE which has already published 11 scientific publications. Source: Authors (2022).

The top fifteen authors are listed in Figure 5. It should be noted that known authors for their work with microalgae may not be cited in this paper. The focus here is data produced with microalgae cultivated using effluents from the industries of the energy or fuel sector.

Figure 5 - Top fifteen authors associated to the scientific publications.



The figure represents a bar graph about the frequency of published articles and his authors referring to the researched topic. On the Y axis we have the fifteen authors who most publish with this theme and on the X axis the number of articles. Highlight for Navid Moheimani who has already published 13 scientific publications. Source: Authors (2022).

The list of the most productive researchers is led by the Australian phycologist Navid Moheimani responsible for the publication of 8 articles, followed by the Italian researcher Fabrizio Adani and the Poles Marcin Debowski and Marcin Zielinski, with 7 articles each. These authors mostly report on different strategies to achieve maximum pollutant removal combined with higher microalgal biomass production. The acclimation of algae along with supplementation with other residues are often applied to favoring algal growth and a better removal of pollutants.

Different types of wastewater are being used for the purpose of associating microalgae biomass and effluent treatment

(Table 1). There are some articles discussing the use of oil produced water as medium for microalgae growth. This is an effluent generated at high volumes. As this effluent may be rich in salts an important emphasis is being given to the marine species *Nannochloropsis oculata*. Ammar et al. (2020) achieved an oil removal above 66% and COD (Chemical Oxygen Demand) above 54% in the diluted produced water using an algae acclimation procedure. Lutzu, et al. (2020) supplemented the produced water with animal waste and obtained a 100% removal of nitrate and ammonia and above 95% for phosphate. Parsy, et al. (2020) used both strategies and achieved a 50-70% COD removal from produced water. Produced water is typically contaminated with organics derived from reservoir hydrocarbons, metals and chemical additives that were intentionally introduced in this environment (surfactants, KCl -Potassium chloride-, methanol, biocides) (Graham, et al., 2017). Several of these compounds are known to be toxic to algae and interfere with phycoremediation.

The wastewater with the best characteristics for microalgal growth is the effluent produced by an anaerobic digester (AD). This wastewater is rich in inorganic nutrients with reasonably lower concentration of organics. Microalgal cultivating systems is a promising strategy to combine further treatment for such an effluent. Thus, microalgal cultivation systems can be easily integrated with anaerobic digestion creating a closed circuit on the treatment process (Chuka-Ogwude, et al., 2020). In this case, the *Chlorella* genus is being the most commonly applied algae. AD effluent pretreatment such as dilution, filtration and / or centrifugation in order to reduce turbidity is often reported.

Total suspended solids cause high turbidity in AD effluent and reduces light transmission. On the other hand, ammonium concentration close to 100 mg N l^{-1} increases significantly algal growth. Higher concentrations are potentially toxic to microalgae (Källqvist and Svenson, 2003). Tao, et al. (2017) applied filtration, centrifugation and 1.5 times dilution with distilled water in order to successfully cultivate *Scenedesmus acuminatus* and *Chlorella vulgaris*. The authors report achieving ammonia and phosphate removal ratios above 95% after these nutritional adjustments. On the other hand, Tan, et al. (2020) promoted algal mixotrophic growth by adding modified starch wastewater to the AD effluent. These authors have also reported ammonia and phosphate removal ratios above 95% and a COD removal between 50-62% for the conditions of 0.5:1 and 1:1 of wastewater with effluent from the modified starch process. AD effluent glucose amendments have also been tested and yielded at least 5 times more algal biomass with significant removal of inorganics (Fuentes-Grünewald, et al., 2021). Microalgae acclimation was also tested on these experiments and this process shows a significant influence in the algal growth performances.

Mixotrophic growth arises when photosynthetic metabolism is supported by the utilization of an auxiliary organic source of carbon. For some species such physiological mode can generate higher biomass yields when compared to autotrophic mode alone (Zhan, et al., 2017). Under mixotrophic conditions, these microalgae can simultaneously absorb inorganic (CO_2) and organic carbon (glucose, glycerol, acetate, etc.) at the same time (Subramanian, et al. 2016). On the other hand, organic supplementation can contribute to 80% of the total costs during microalgal cultivation (Lakshmidevi, et al., 2020). Glycerol, which is a biodiesel by-product, is a cheap alternative and it can be used for this purpose. Studies by Ma, et al. (2016) when formulating a synthetic effluent supplemented with 5 g l^{-1} of crude glycerol or 10 g l^{-1} of hydrochloric acid treated glycerol reported significant algal growth performance along with nitrogen and phosphorus removals ratios between 88-95%, respectively. It was also observed a COD removal ratio of 94-98%. Ren, et al. (2017) cultivated *Chlorella vulgaris* using glycerol and domestic wastewater centrate effluent (the liquid obtained with centrifuged sludge). The authors reported a nitrogen, phosphorus and COD removal ratios above 86%. Chen, et al. (2020) also reported that mixotrophic growth of *Thraustochytrium* sp with crude glycerol resulted in a 185 and 68% increase in biomass and lipids production, respectively.

Vinasse from the ethanol industry is rich in inorganic nutrients and low complexity organics, thus potentially suitable for sustaining microalgal systems (Beigbeder, et al., 2019). The main strategy to develop a microalgal system for treating vinasse is preadapting the cells to the effluent (Sydney, et al., 2019). Vinasse turbidity has been identified as a significant variable affecting algal growth. Santana, et al. (2017) tried to reduce the turbidity effect by diluting or clarifying the vinasse with water or flocculant agents, respectively (Santana, et al., 2017). The dilution strategy favored the growth of *Micractinium* sp., but clarification was more suitable to Chlamydomonas-like species. Demonstrating that culturing strategies are case specific. Treating vinasse in AD has also shown to significantly reduce color and turbidity (Sayedin, et al., 2020).

Some microalgae can switch from autotrophic to heterotrophic or mixotrophic metabolism (Perez-Garcia, et al., 2011; Hammed, et al., 2016). Thus, microalgae can be grown (i) Photoautotrophically, using light as energy source and CO_2 as carbon source; (ii) heterotrophically, utilizing organic substrates as both energy and carbon source; and (iii) mixotrophically, where microalgae perform photosynthesis as the main energy source but both organic compounds and CO_2 are essential sources of carbon (Moreno-Garcia, et al., 2017). Despite the good yields in the mixotrophic mode, the cells show less synthesis of chlorophyll α (Patel, et al., 2020a). This can interfere with the downregulation of the Calvin cycle and the TCA cycle causing a reduction in the photosynthetic efficiency of mixotrophic cells (Grama, et al., 2016; Zhang, et al., 2017; Sim, et al., 2019). Therefore, the sustainable development of a phycoremediation strategy involves improving photosynthetic efficiency, optimized pollutant removal, co-location and the harvesting process.

The construction of facilities nearby to where the effluents are generated (co-location) can significantly reduce costs (Moreno-Garcia, et al., 2017). Strategies for solid-liquid separation after microalgae growth also affects the costs of the entire technology (Yadav, et al., 2021). Thus, the development of new forms of separation is necessary to overcome such economic hurdle. At the moment flocculation has lower costs and energy requirements than membrane filtration (Fasaei, et al., 2018). Therefore, flocculation is suitable for low cost separation, while membrane filtration is suitable for high cost separation but with higher purification. It is ideal for high value-added products. It is also important to assess the concentration of toxic compounds and bacterial contamination for qualitative and quantitative changes in a temporal basis.

Table 1 - List of some articles that report different approaches for obtaining higher biomass/product and nutrient removal ratios within microalgae based systems.

Residue	Algae Specie	Pollutant Removal / Nutrient uptakes	Biomass Production	Methodologies employed	References
Produced water	*Nannochloropsis oculata* and *Isochrysis galbana*	Oil removal above 66% and COD removal above 54% for concentrations of 10%, 30% and 50% in both algae.	Different concentrations of PW (10-50%): 0.31–1.13 g l^{-1} for *Nannochloropsis oculata* and 0.314–1.01 g l^{-1} for *Isochrysis galbana*.	Algae acclimation in increasing concentrations (10-50%) of PW	Ammar, et al., 2018
Produced water	*Picochlorum oklahomensi*	Removal in pure PW and / or combinations of PW with animal waste: 100% nitrate and ammonia; above 95% for phosphate; over 30% for magnesium, calcium, and zinc; and 16% for boron.	Different proportions of PW with animal waste (1:1 and 2:1): 2,4 g l^{-1} and 2,0 g l^{-1}.	Supplementation with animal waste in the PW	Lutzu, et al., 2020
Produced water	*Nannochloropsis oculata*	Removal of N-NH_4 = 3.1 mg L^{-1} d^{-1} - 6.3 mg L^{-1} d^{-1} for concentrations of 10-30% PW supplemented with 5% of the digested liquid. COD removal = 50% - 70% for concentrations of 10-30% PW supplemented with 5% of the digested liquid.	4,8 – 9,3 * 10^7 cells mL^{-1} for concentrations of 10-30% PW supplemented with 5% of the digested liquid.	Adaptation in different concentrations of PW with medium and supplementation of effluent from the anaerobic digester.	Parsy, et al., 2020
Effluent from	*Chlorella vulgaris*	Removal of ammonia and	2,91 g l^{-1} and 8,22 g l^{-1} for	Filtration,	Tao, et al.,

the anaerobic digester	(SAG 211-11b) and *Scenedesmus acuminatus* (SAG 38.81)	phosphate above 95%; removal of COD of 27.6 and 36.1% for *C. vulgaris* and *S. acuminatus*, respectively.	*C. vulgaris* and *S. acuminatus*, respectivelly.	centrifugation and 1.5x dilution.	2017
Effluent from the anaerobic digester	*Chlorella pyrenoidosa*	COD removal: 62-50%; Ammonia removal above 95%; and Phosphate removal above 95% for conditions 1:1 and 0.5:1 for ASW: ADS.	2,59 g l^{-1} and 2,25 g l^{-1} for conditions 1: 1 and 0.5:1 for ASW: ADS.	Filtration and centrifugation. Dilution with modified starch wastewater (ASW).	Tan, et al., 2020
Effluent from the anaerobic digester	*Chlorella vulgaris* and *Scenedesmus obliquus*	Removal of N between 98-134 g $l^{-1}d^{-1}$ for both cultivation and algae modes. Removal of P 0,95-1,89 g $l^{-1}d^{-1}$ in autotrophic cultivation mode for both algae.	1,01 and 0,73 g l^{-1} in the autotrophic mode; 5,51 and 13,83 g l^{-1} in the mixotrophic mode for *C. vulgaris* and *S. obliquus*, respectively.	Strain adaptation at different effluent dilutions. Microfiltration of the effluent, dilution to 2.5% and cultures with autotrophic and mixotrophic (glucose) stages.	Fuentes-Grünewald, et al., 2021
Glycerol	*Chlorella vulgaris*	Removal of N between 92-95%; P removal between 88-95%; Removal of 100% ammonia; and removal of 94-98% COD for both conditions.	1,82 g l^{-1} for 5 g l^{-1} of crude glycerol and 2.92 g l^{-1} for 10 g l^{-1} of pretreated glycerol.	Synthetic effluent with different concentrations of crude glycerol (5 g l^{-1}) and glycerol treated with hydrochloric acid (10 g l^{-1})	Ma, et al., 2016
Glycerol	*Chlorella vulgaris*	Removal of N 89%; Removal of P 88%; Removal of 84% ammonium; and removal of COD 86%.	2,2 g l^{-1} with 1,0 g l^{-1} of crude glycerol	Effluent with different concentrations of crude glycerol	Ren, et al., 2017
Glycerol	*Thraustochytrium* sp.	Glycerol removal above 80%.	15 g l^{-1}	Crude glycerol pre-treated with acid and calcium chloride with maceration liquor from corn and sea salts	Chen, et al., 2020
Vinasse	*Micractinium sp.* and *Chlamydomonas biconvexa*	Removal of 30.8-41.7% nitrate; 5-18% phosphate; and about 5% total carbon removal.	Biomass productivity: 0.17 g $l^{-1}d^{-1}$ for 50% of stillage diluted with *Micractinium* sp. and 0,22 g $l^{-1}d^{-1}$ for 100% clarified vinasse with *C. biconvexa*	Sterilization and dilution or clarification of vinasse	Santana, et al., 2017
Vinasse	*Chlorella vulgaris*	Total carbon removal: 76% and 84% for the scenarios of continuous lighting and reduced lighting, respectively.	Biomass productivity: 0,9 g $l^{-1}d^{-1}$ for the scenarios of continuous lighting and low lighting.	Centrifugation and filtration. Addition of antibiotics or sterilization.	Beigbeder, et al., 2019
Vinasse	*Chlorella sorokiniana*	COD removal: 83%; ammonia removal: 95.3%; and removal of phosphate: 78.3% in digested vinasse diluted 2x precipitated with struvite.	1630 g l^{-1} in digested vinasse diluted 2x precipitated with struvite.	Different dilutions of biodigested vinasse precipitated with struvite (without diluting, 2x, 5x)	Sayedin, et al., 2020

Source: Authors.

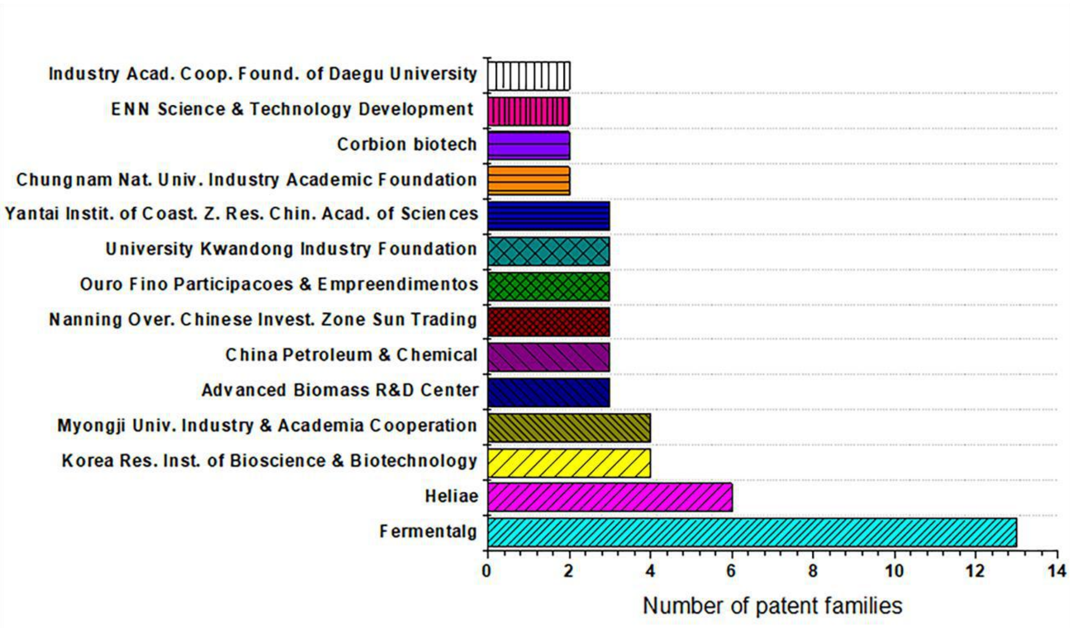
3.3 Patent analysis

This research examined 104 patents and a significant number reports the use distinct liquid supplementation. A ratio of 82 and 7.6% suggests the use of glycerol and vinasse as nutritional amendments for promoting algal growth, respectively. It was also examined patents describing the use of AD effluent for this same purpose (5.7%). Glycerol is attracting the interest of most researchers from either public or private research institutions. The situation of the global glycerol market encourages the search for new applications, since traditional uses of glycerol are not able to absorb the growing production (Monteiro, et al., 2018). In addition, glycerol shows a positive result for not only promoting microalgae growth but also, in several cases, increases cellular lipid accumulation.

Chlorella sp. is the main microalgae reported in the patents (60% of the patents). This is a versatile microorganism and it has been successfully applied for treating all effluents cited in this research. *Chlorella* sp. is probably one of the most widely studied and used algae species (Hong, et al., 2019).

The main depositors of the patents are shown in Figure 6. In total, 73 institutions were identified and 14 institutions are responsible for 51% of the total of submitted patents. For instance, Fermentalg is a leading French microalgae company with the largest number of deposits. This company are responsible for the deposit of 14 patent between 2010 and 2018. Heliae Development, LLC in The United States deposited 6 patents. Korea Research Institute of Bioscience & Biotechnology and Myongji University Industry & Academia Cooperation are responsible for 4 patents each. Fermentalg is a biotechnology company specialized in the production of compounds from microalgae, while Heliae is an american technology company for food and agriculture based on sustainable microalgae products. The formers are the largest stakeholders of technological patents using microalgae. The documents show a wide interest either phototrophic or mixotrophic processes. Korea Research Institute of Bioscience & Biotechnology is a governmental research institute and Myongji University Industry & Academia Cooperation is a private research institution, both are located in South Korea. This country ranks in the third position for the protection of its innovation and technology in this area (Figure 2b). South Korea is responsible for 12% of the total number of deposited patents.

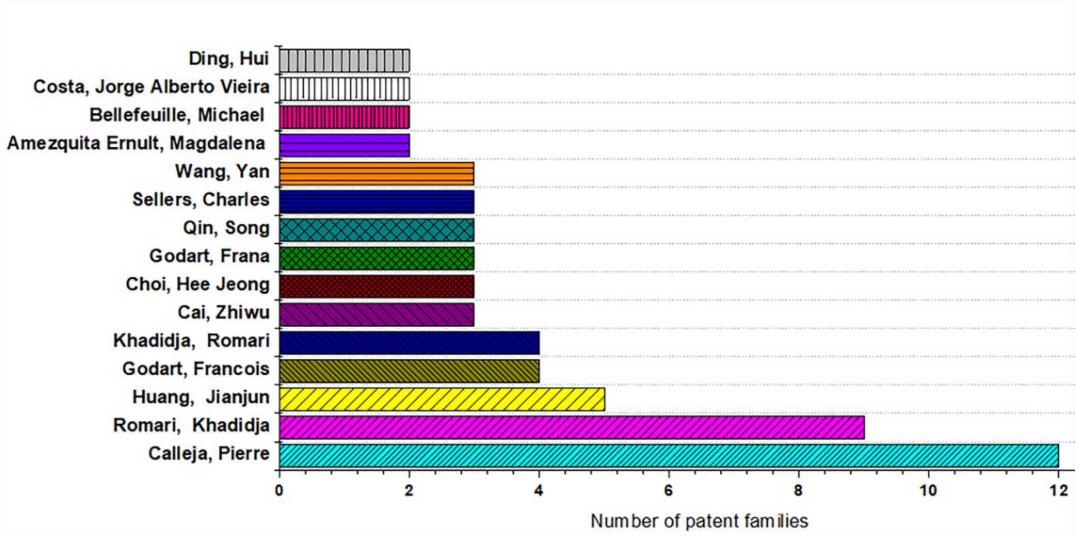
Figure 6 - The most productive institutions associated to the patent origin.



The figure represents a bar graph about the frequency of published patents and institutions referring to the researched topic. On the Y axis we have the institutions that most deposit these patents and on the X axis the number of patent families. Highlight for Fermentalg which has already published 13 patent families. Source: Authors (2022).

The top patent authors are listed in Figure 7. Pierre Calleja, a biochemist and founder of Fermentalg, is responsible for 12 patent families; followed by Khadidja Romari (8 patent families) who was a collaborator at Fermentalg and is currently part of the European Standardization Committee's "Algae or algae based products or intermediates" project. Jianjun Huang deposited 5 family of patents in the organizations Nanning Overseas Chinese Investment Zone Sun Trading and Nanning City Enyuan Health Science & Technology. Eneko Ganuza Taberno, manager of R&D in microbiology at Heliae, is responsible for 5 patent families. François Godart is a biologist and microbiology manager at Fermentalg and has deposited 4 family of patents. All five main authors collaborate or have collaborated at the 15 main organizations shown in Figure 6. It should be highlighted that most of the knowhow reported in these patents are, today, part of the public domain. Table 2 summarize some patents that report the use of effluents from the energy industry in the cultivation of microalgae.

Figure 7 - Top fifteen authors associated to the patented publications.



The figure represents a bar graph about the frequency of published patents and authors referring to the researched topic. On the Y axis we have the authors who deposited these patents and on the X axis the number of patent families. Highlight for Pierre Calleja who has already published 12 patent families. Source: Authors (2022).

Patent EP2093197, for instance, describe acclimation procedure for *Cyanobium* sp., *Phormidium* sp., *Pseudoanabaena* sp., *Amphora* sp., *Monoraphidium* sp. and *Chlorella* sp. Patents CN104556544 and CN209292158 combines a physical with biological step for the removal of pollutants from produced water in order to sustain microalgal growth. Patent KR101549666 describes the use of pig manure anaerobic digester effluent for the cultivation of *Chlorella* sp. An important step in this process is the control of ammonium concentrations. In a more interesting approach, the US20150064761 patent establishes a bioenergy production system with zero residues, addressing factors of what is defined as circular economy strategy. The patent describes the generation of biogas, the use of CO_2, hydrogen sulfide and the effluent from the anaerobic digester as nutrient support supplementation for growing microalgae. Biodiesel is produced with the microalgal lipid and residual biomass can be used for ethanol production. The glycerol formed during oil transesterification is supplemented in the microalgal system for sustaining mixotrophic cultivation. Another interesting work is described in the patent CN108624506. The authors use the co-culture of microalgae and yeasts, specifically microalgae of the genus *Chlorella* sp. or *Scenedesmus* sp. or *Gloeococcuse* sp. for the treatment of AD biogas slurry.

It is a common topic among the patents the discussion of heterotrophic or mixotrophic cultivation strategies in order to increase microalgae biomass productivity. The patent CN108192828 uses a medium formulation containing glycerol from the production of algal biodiesel associated with the hydrolyzed microalgae biomass after lipid extraction. According to the inventors, there is an increase in the biodiesel yield of algae using this approach. Glycerol is also used in association with liquid from the sludge dehydration procedure (centrate) for the cultivation of *Chlorella* sp. (patent CN107177506). During the production of biomass, nitrogen and phosphorus from the sewage are consumed and the cost of treating crude glycerin is, therefore, reduced. The US7989195 patent describes a multiphase culture process for favoring microalgae heterotrophic growth using crude glycerol as the primary carbon source. The growth conditions employed have different phases for increasing cell density, increasing cell size and finally producing and accumulate fatty acids. The patent WO2017 / 184729 describes the use of vinasse from the ethanol industry as a culture medium for microalgae and to obtain a biomass rich in lipids and proteins. The patent CN104561153 describes an approach with filtered vinasse. The patent CN108611276 describes that vinasse associated with activated sludge can generate a large amount of microalgae biomass and reduce treatment costs. The CO2 rich gases generated in the fermentation of ethanol are often used in the microalgae cultivation process.

Table 2 - Patents based on the use of wastewater from energy sector for microalgae systems.

Residue	N° of patent	Algae Specie	Claims	Legal status	Valid till
Produced water	EP2093197	*Cyanobium* sp., *Phormidium* sp., *Pseudoanabaena* sp., *Amphora* sp., *Monoraphidium* sp. e *Chlorella* sp.	10	Granted	2029-02-13
Produced water	CN104556544	No specified	3	Lapsed	2017-04-12
Produced water	CN209292158	*Chlorella* sp., *Scenedesmus* sp., or *Nannochloropsis* sp.	6	Granted	2028-10-08
Anaerobic digestate effluent	CN108624506	*Chlorella sp.* or *Scenedesmus sp.* or *Gloeococcuse sp.* or	10	Pending	2038-05-16
Anaerobic digestate effluent	KR101549666	*Chlorella* sp.	14	Granted	2035-05-13
Anaerobic digestate effluent	US9902977	No specified	22	Granted	2034-10-30
Glycerol	CN108192828	No specified	9	Granted	2038-01-23
Glycerol	CN107177506	*Chlorella* sp.	10	Granted	2037-06-22
Glycerol	US7989195	*Thraustochytrium* sp. and *Schizochytrium* sp.	27	Granted	2028-08-08
Vinasse	CN108611276	No specified	7	Pending	2038-04-16
Vinasse	CN104561153	Cyanobacteria, Green algae, Red algae, Brown algae, Diatoms, Chrysophyta, Xanthophyta, Dinoflagellate, Chara, Euglena, Blue-green algae, *Spirulina* sp., *Chlorella* sp., Grape algae	5	Granted	2035-01-22
Vinasse	WO2017/184729	*Chlorella protothecoides, Chlorella vulgaris, Chlorella sorokiniana, Chlorella saccharofila, Chlamydomonas reinhardtii, Chlorococcum littorale, Platymonas subcordiformis, Anabaena, Nostoc muscorum, Nostoc spongiaeforme, Westiellopsis prolifica, Oscillotoria Miami BG7* and Aphano thee e halophytico.	13	Granted	2037-04-19

Source: Authors.

The sustainability of microalgal processes can be assessed from an economic point of view through the technical-economic analysis (TEA) and through the environmental metrics of the life cycle assessment (LCA). On an industrial scale, the TEA and LCA of the microalgae biorefinery shows that location, harvesting and drying are stages with most economical impact (Bussa, et al., 2021). Under mixotrophic growth, microalgal system costs are also affect the price or availability of organics supplementation (Somers, et al., 2021). Several bioactive compounds can be extracted from the microalgal biomass and requires an innovative business models based on a circular bioeconomy strategy (Awasthi, et al., 2020).

4. Conclusion

The effluent generated by the energy industries, such as produced water, effluent from the anaerobic digester, glycerol from biodiesel and vinasse from ethanol production are generated at high quantities and, often, they are not properly disposed of. Microalgal system is a promising strategy for combining such effluent treatment with the production of valuable bioproducts. An examination of scientific papers and patent indicates the following:

- Dilution of the produced water (oil industry effluent) is often necessary due to high salinity. Nitrogen and phosphorus supplementation is also needed and has been achieved with the addition of another effluent rich in such inorganics. Algal acclimation is important prior to culturing.

- Microalgal culturing can be easily integrated with anaerobic digestion systems. This integration can generate a closed circuit in terms of wastewater utilization. However, it is necessary to monitor the concentration of ammonium and control the turbidity of the effluent. Variably the effluent must undergo a physical or chemical treatment prior to microalgal cultivation.

- Glycerin supplementation in combination with nitrogen and phosphorus often results in high levels of biomass and lipid production. Thus, the combination of distinct effluents from the energy related industries has the potential for supporting large-scale implantation of microalgal systems.

- Microalgal cultivation on vinasse is viable, but the effluent needs a pretreatment for reducing its turbidity and color. The acclimation of microalgal cells in the vinasse-based medium can also increase biomass yields and, consequently, the removal of pollutants.

The scenario for microalgal systems is still challenging, and for such biotechnology to be successfully implemented on an industrial scale, it is still necessary to investigate the integration of distinct effluents within a new concept addressing process sustainability. Future research and development should be conducted on combining low cost microalgae growth medium based on the cited effluents with glycerin supplementation. Optimal concentrations must be researched for each type of effluent. Allied to this, tests with microalgae cells acclimated to these types of culture media should be studied. This may promote a greater accumulation of biomass and bioproducts through mixotrophic growth.

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