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Review article

State of microalgae-based swine manure digestate treatment: An overview

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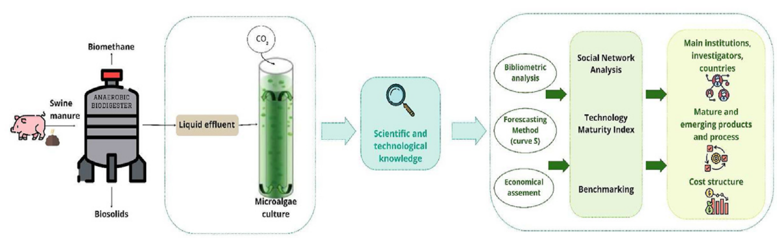
HIGHLIGHTS

- The biometric analysis and SAN provides an overview of the evolution of technology.
- China and the USA are the main players in the use of digestate in microalgae cultivation.
- Biomass and wastewater are trending topics in the microalgal application at the near future.
- Spirulina, Astaxanthin and beta-carotene as the main products based on market worldwide forecasting.

GRAPHICAL ABSTRACT



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ABSTRACT

Global pork production has an annual growth of approximately 2.1%, and its economic and environmental impact are related with the treatment of waste in the production chain. There is little evidence of research advances to generate alternatives for using these wastes. The lack of research related to microalgae cultivation using digestate produced by porcine residues generates negative environmental impact, inadequate and inefficient technologies, low recovery and use of waste and loss of value and competitiveness in the market. The available literature focuses mainly on the treatment of anaerobic digestion liquid effluents for the removal of components, but not on the generation of value-added products. Therefore, there is a need to collect the available information, analyze it and propose other new methodologies. This article presents the information obtained from conducting a systematic review of the literature with a bibliometric and a comparative analysis; achieving an analysis of the temporal and geographical distribution, the main topics, the most influential players, the degree of maturity of the research and different strategies collected for microalgae-based swine manure digestate treatment. In this way, it was possible to capture an overview of the current state of the development of research focused on the use of digestate for the cultivation of microalgae, visualizing important aspects as the evolution of publications, identifying China and USA as the main players in research, biomass and wastewater as potential topics also Spirulina, Astaxanthin and beta-carotene as the main products based on microalgae. Thus, achieving an structure, organized and synthesized landscape of scientific and technological knowledge available for the proposal of investigations that allow the use of anaerobic digestion liquid effluents as cultivation medium for microalgae.

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1. Introduction

Pork is one of the main meats consumed globally, and it represents approximately one-fifth of the total meat production. According to the behavior of recent years, it has been established that this industry had an average annual growth of 2.1% [1] reaching production values close to 100,000 tons. According to what was reported by the USDA, it is expected that after 2021, the production will grow by 6.1% [2]. Globally, the pioneer countries in pork production are China (38.8%), the European Union (23.5%) and the USA (13.1%) [3].

Likewise, according to the Ministero de Agicultura, in 2020 Colombia reached a pork production of 468.880 tons, with an average annual growth of 3.7%, of which the department of Valle del Cauca is the third producer in the country with 71.836 tons The above results in the production of pig manure (feces and urine) of approximately 430.000 tons per year [4].

The waste generated from the pig industry is mainly composed of feces, washing water and food waste, these are characterized for having a high content of nitrogen and phosphorus, as well as traces of heavy metals and hormones [5]. For these, conventional treatment processes focus on water treatment to reduce N and P, but they don't have a pretreatment to generate a by-product with economic potential. Among the most used strategies are solid-liquid separation, aerobic and anaerobic lagoons, composting and anaerobic digestion [6]. The processes that are gaining more relevance are anaerobic digestion, given its ability to eliminate biodegradable organic matter and to generate different useable streams such as biogas, sludge and liquid digestate; and also advanced oxidation processes that are very efficient, promote the mineralization process and require a shorter period of time for the treatment [7]. However, research involving other processes such as ozonation, Fenton's process, photocatalysis, electrochemical oxidation, electrochemical coagulation and UV/hydrogen peroxide are beginning to be considered. These type of pretreatments have gained relevance since they allow that economically profitable by-products are obtained through the transformation of the waste [8].

However, there are still no evident investigative advances to generate alternatives for the integral use of pork waste. After consulting the Scielo and Scopus databases, it was found only a total of 9 articles which were directly related to the study of anaerobic digestion, swine digestate and the evaluation of these residues in Colombia. This suggests a problem since it represents a limited knowledge of the alternatives for the integral use of waste from the pig sector.

There are different alternatives for the utilization of these residues, such as the generation of bioelectricity [9], fertilizers [10], animal feed [11], But there are not many studies regarding its use as a source of nutrients for the cultivation of microalgae [12, 13, 14, 15, 16, 17], thus ignoring the high potential of this effluent since anaerobically treated animal waste contains nutrients such as N and P (NH+4, NO-3, PO3-4) which are suitable for growing alga[which represents a great opportunity as an animal feed, a source of protein or biofuel [14, 15],. In other words, the lack of research related to the cultivation of microalgae using porcine residues, is reflected not only in the generation of a negative environmental impact, but given the inadequate and inefficient technologies, together with the low recovery and use of waste, it also causes a loss of value and competitiveness in the market.

Based on the above, it is proposed to carry out a bibliometric analysis that allows to have a general overview (at an international level) of the most relevant aspects of the cultivation of microalgae as an alternative for the use of pig waste. Furthermore, it is intended to identify the characteristics of the liquid effluent that are necessary to consider pig waste as a potential substrate for the cultivation of microalgae, as well as its possible limiting aspects. Finally, the analysis aims to identify the opportunities of generating value, and its potential to be replicated in different pig farms of other regions of the country. This, thinking about the opportunity to develop new strategies that contribute to the competitiveness of the sector.

In order to determine a strategy for the use of pork waste, through the cultivation of microalgae, as an initial stage, a systematic literature review will identify the most relevant aspects [15]. This is done with the purpose of obtaining an orderly compilation to identify and evaluate data and aspects that can be the basis for answering a research question. In scientific databases, it is possible to find an increasing number of published articles, in the case of ScienceDirect, they publish annually around half a million new records [18]. The fast growth of the number of publications makes a systematic review necessary to avoid dedicating efforts when there is existing research. In addition, this allows the information available to be provided in a clear, reasonable, and impartial manner [19].

Although the performance of a systematic bibliometric review has gained relevance, it is a difficult tool to execute correctly, it is important to ensure that it is systematic, complete, explicit, and reproducible [20, 21]. Therefore, it is necessary to have a clear question, define search criteria and then perform an analysis and selection of the found literature [22]. According to the search criteria, it is possible to find either large quantities or no articles, a factor that may be limiting for the review; that is why it is necessary to play with the criteria so that the search results are good enough and allow a correct analysis [23]. A systematic analysis of the literature regarding the use of porcine residues through the cultivation of microalgae will allow obtaining a combination of techniques that have been effective in existing research, thus enhancing the use of these effluents for the generation of value-added products.

Table 1. Selection criteria.

Variable	Sub Variable	Criterion
Scale of the process	1. Capacity 2. Work Volume 3. Unit processes involved	The documents that propose strategies at a pilot scale or that implement scalable strategies and substrates are chosen
Yield	1. Biomass yield 2. Product yield	The Documents that report yield values for Biomass and product of interest are chosen
Microorganism	Microorganism	Documents that include microalgae and cyanobacteria are chosen
Type of schemes	Feeding strategy	Documents that implement Swine manure digestate as a source of nutrients are chosen.

2. Materials and Methods

2.1. Literature search

The literature review was performed in Scopus, ProQuest, Orbit, and Patent databases. The search criteria were the year of publication (January 1972 to January 2022) and different combinations of keywords and synonyms, having as the main search equation TITLE-ABS-KEY (algae AND swine AND Biomass) In this case, a specific sample size was not determined. However, a minimum of 100 and a maximum of 500 files was established. The sample size depended on the quality of the information obtained.

A documentary analysis was proposed for data collection which allowed obtaining valuable information regarding the topic of interest to create a clear overview of the actual investigation [24]. For this process, the existing and available documents were tracked and inventoried, this was achieved using the databases as an instrument, which yielded the same results, as long as the same search criteria were established. These databases were defined since the largest amount of available literature is concentrated in them [18], by establishing the search criteria, it is ensured that the results were valid and contained the increased amount of information related to the focus.

2.2. Literature selection

For the selection of the sample, a non-probabilistic method was determined since the selection process was not mechanical or by probability, it depended on decisions according to the most relevant criteria for the investigation. Specifically, a discretionary sampling was proposed; this means the selection was made by experts focusing on the quality and not quantity of the information. The above was to avoid leaving out of the analysis some articles that provide relevant ideas and techniques for the approach of the experimental strategies for cultivating microalgae using the liquid digestive. This project involved a non-experimental cross-sectional research design that seeked to analyze the investigation status regarding the topic of interest. It pretended to collect the available information and analyse it, but not to manipulate it. This experimental design allowed us to observe existing situations and propose new ones from these.

Expert researchers, who were previously selected according to their academic training and experience in the subject, were in charge of selecting the most pertinent documents. The scientific article reading technique was taken as a basis for selecting articles, which establishes the best methodology for reviewing the literature. This methodology establishes the following steps: 1) Quick review of the article: look for a general idea (title, sections that compose it, figures and tables). 2) Reading the summary: Identify outstanding facts. 3) Reading the introduction and methodology, identifying context or problematic and relevance and reproducibility, respectively. 4) Reading the results and analysis: determine if these data and facts contribute to building new knowledge and are related to the objectives. 5) Reading of conclusions 6) Review and validation of reference [25]. In addition to these stages, it was important to have in mind that this review should generate questions, guiding questions and critical questions, such as: Does the article focus on the generation of a value-added service products? Are pros and cons mentioned that may occur due to the use of the digestate? Which property of the digestate can be the cause of this? Are the growing conditions adaptable to the research needs? The guidelines being those focused on the information provided by the document, and the critical ones that allow testing the validity of the article in relation to the objective of the current investigation [26]. In this way, as a hypothesis, this research established that in the literature there are experimental strategies for the cultivation of microalgae that meet the criteria mentioned in Table 1.

2.3. Literature analyses

For the analysis of the information, it was determined to use a mixed methodology, which included a qualitative analysis and descriptive statistics and bibliometrics. It was proposed to carry out a bibliometric analysis [27], for which the RStudio®, VOS viewer and Orbit Intellixir software was used as a programming tool to obtain figures and statistics that allow the analysis of the available literature.

2.3.1. Temporal distribution analysis

The Orbit Intellixir software was used as a programming tool to obtain figures and statistics that allow the analysis of the available literature. For the analysis of temporal distribution, the compilation obtained from the Scopus, ProQuest, Orbit and Patent scope databases was entered into the software, from which it is possible to obtain a consolidated number of publications made from the year 1972–2022, making the distinction between articles and published patents.

2.3.2. Geographical distribution analysis

For the analysis of geographic distribution, the publications generated by each country were quantified in order to determine the pioneer

Table 2. Statistical values of the s curve.

	K	a	tm	r	SSE	RMS	MAD	MAPE	SE	ln[MLE]	AICc	R^2	*p*	1%	10%	50%	90%	99%
Protein	165	26,7	2017	0,165	484	4,16	3,62	0,192	4,40	-79,6	166	0,990	1,29E-27	1989	2003	2070	2031	2045
Biomass	534	26,1	2020	0,168	1364	6,98	6,08	0,210	7,4	-94,2	195	0,983	1,71E-24	1993	2007	2020	2034	2048
Wastewater	544	17,5	2019	0,252	1598	8,52	7,30	0,323	9,2	-78,4	164	0,967	3,05E-16	2000	2010	2019	2028	2037
Oil	100	16,7	2013	0,263	144	2,31	2,05	0,381	2,5	-61,0	129	0,981	5,43E-23	1996	2005	2013	2022	2031
Energy	135	18,7	2017	0,235	118	2,17	1,53	0,101	2,3	-55,1	117	0,989	3,00E-24	1997	2007	2017	2027	2036
Average	296	21,1	2017															
Estandar Error	99,9	2,18	1,15															

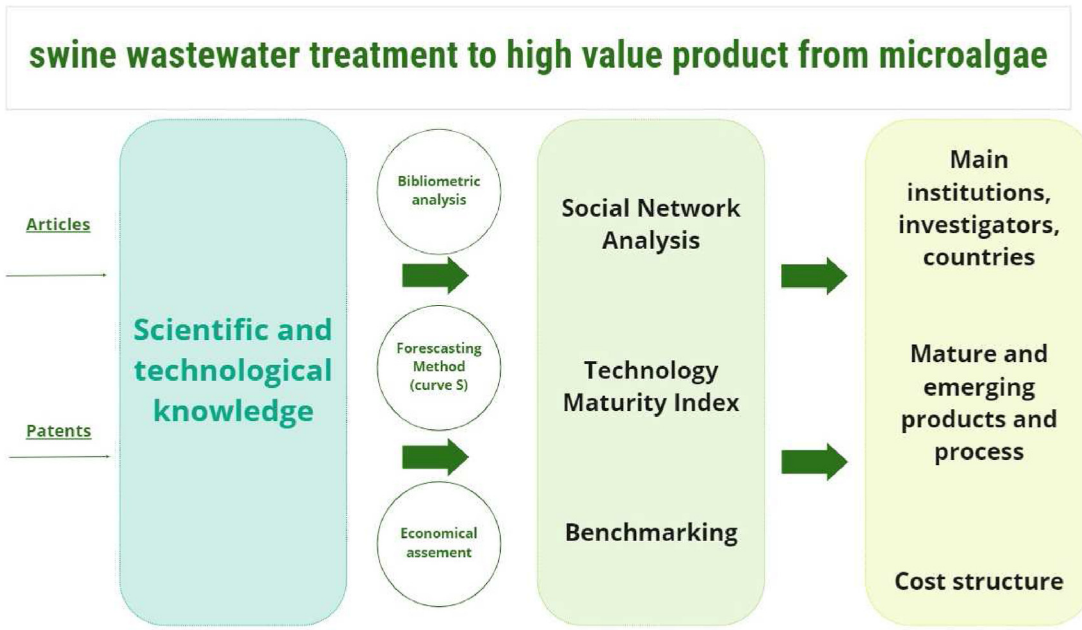

Figure 1. Summary of the implemented strategy.

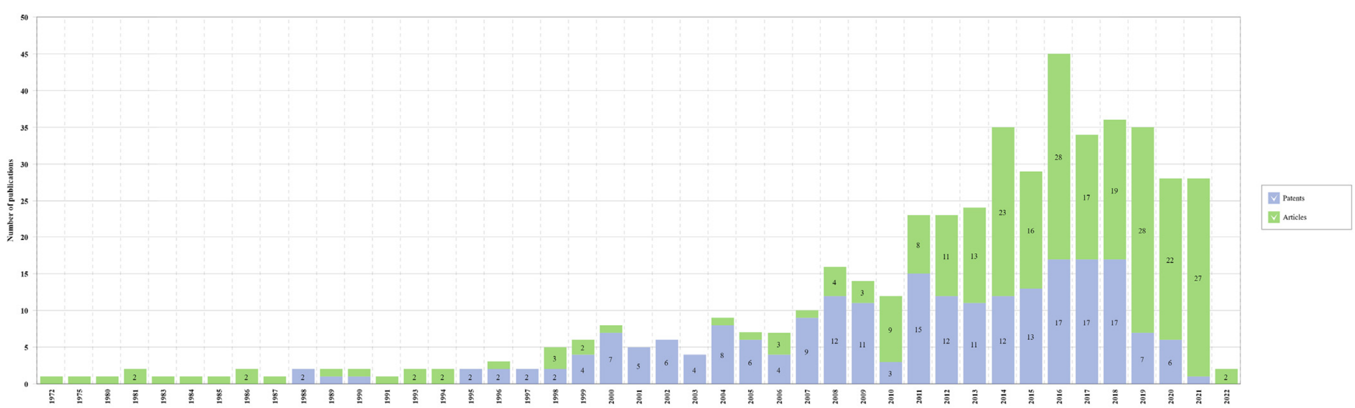

Figure 2. Trend of the number of publications related to the subject of using swine digestate to cultivate microalgae per year from 1972–2022.

countries in the publication of documents related to the research topic. Graphically, a geographical map was proposed in which a color is assigned to each country according to the range of number of publications. Additionally, a temporal distribution diagram was obtained that reports the number of publications for the top 10 countries per year from 2000 to 2022. Finally, a network diagram was proposed taking into account the top 15 countries to establish the relationships between these, according to the collaborations established between co-authors in the publications.

2.3.3. Themes of trend and development

The analyzes carried out were co-occurrences of the most used keywords dividing it into the main clusters. Likewise, the appearance of these keywords over time was analyzed to visualize their growth between the years 2010–2022 and thus identify the potential of each term. Finally, a concept distribution diagram was made, classifying the terms according to their density and centrality to determine the degree of Development of the concepts and their relationship.

2.3.4. Players

Regarding the leading players, the purpose was to evaluate the most active entities in terms of publications related to microalgae cultivation using porcine digestate. First, a diagram was proposed to represent the top 15 entities according to the amount, in which the type of publication, whether patents or articles, is also distinguished. In addition, a diagram was created analyzing the main authors according to the number of publications and their respective affiliations. For this, a graph of the top 15 authors is shown, including their number of publications, the two main institutions to which they are affiliated, the country to which this institution belongs, and the distinction of the entity character, either academic or industrial.

2.3.5. Maturity analysis

For the maturity analysis, an analysis of the life cycle of the research topics was carried out from the S curves. These were created using the Logletlab Plarform in which the data of the number of articles published per year related to each topic was entered. The researched topics were Proteins, Biomass, Water Treatment, Oil and Energy between the years 1990 and projected until 2050. According to the values obtained, the platform calculated the statistical values (R^2, p, K, etc) reported in Table 2, applying the mathematical model referenced in Figure 11, thus obtaining a representation of the Development and degree of maturity of each technology [28].

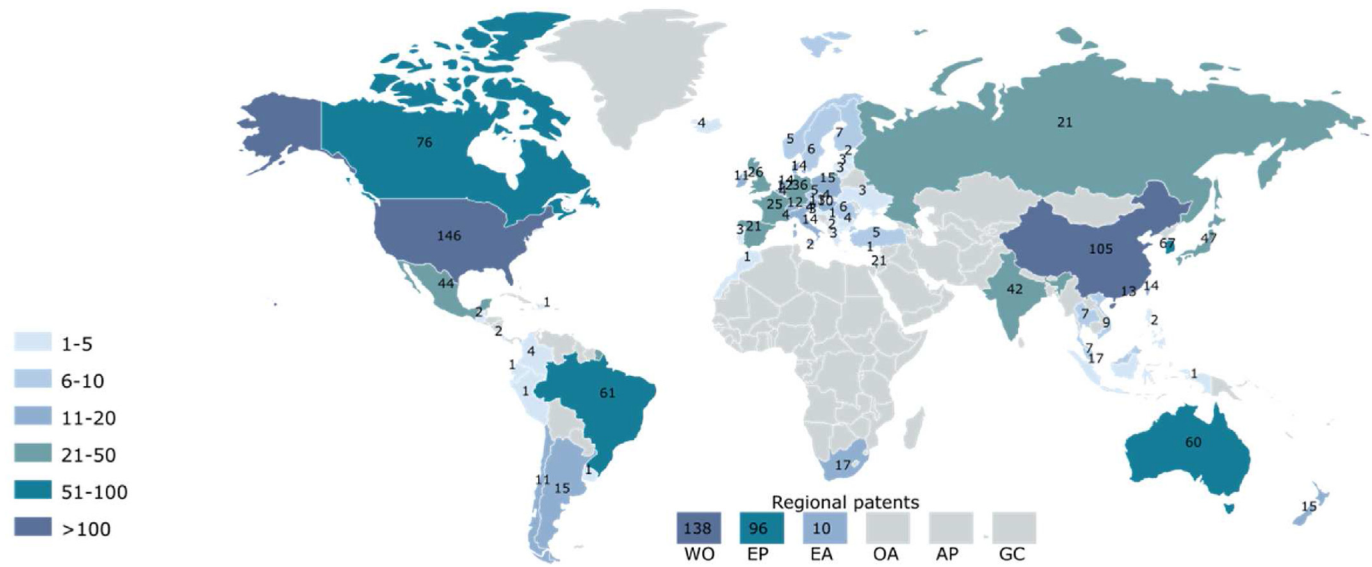

Figure 3. Geographical coverage – Number of publications by country. *WO (World Intellectual Property Organization), EP (European Patent Office), EA (Eurasian Patent Organization), OA (African Regional Intellectual Property Organization), AP (African Regional Industrial Property Organization), GC (Gulf Cooperation Council).

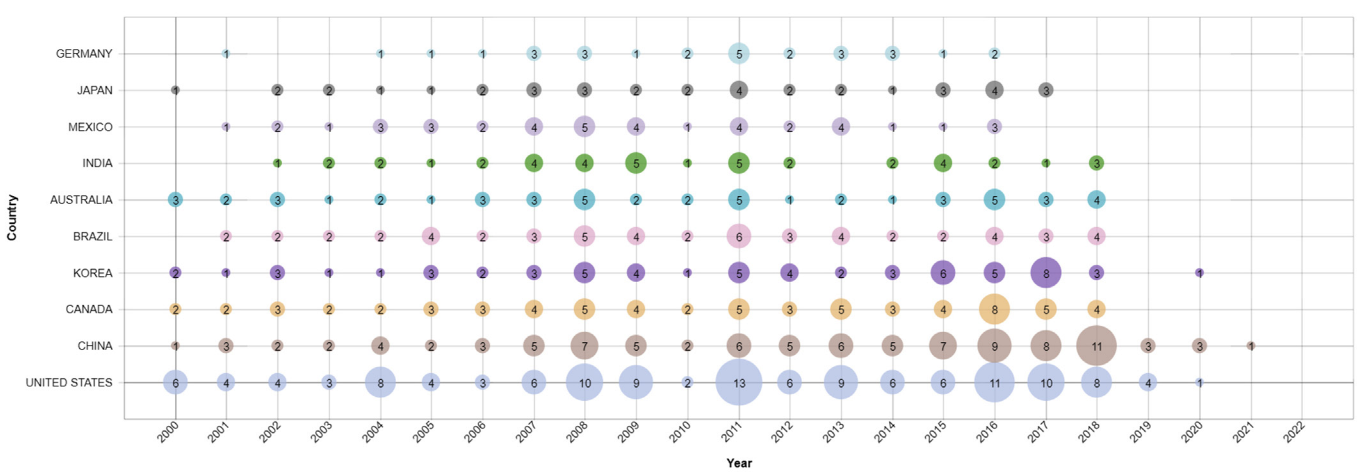

Figure 4. Time coverage – Publications by country.

2.3.6. Financial analysis

A financial analysis was carried out from the market forecast reports products based on micro-algae generated by *The INSIGTH Partners* [29, 30, 31]. From which, it was possible to establish figures of the Market Revenue through the years and its growth rate, the compound annual (CAGR) in the most relevant continents. It was possible to identify the amount of income (USD Million) for the countries with the highest Gross Domestic Product (GPD) value for the different most relevant products in the market.

2.3.7. Comparative analysis

To integrate the results of the studies into an existing theory or derive a new one, two comparative tables were created to look for correlations between characteristics and identify gaps and opportunities [32]. The first table included the criteria substrate, alga specie, digestate pre-treatment and cultivation system to summarise the emerging techniques for the pre-treatment of liquid effluents and the cultivation strategies. The other one integrated the criteria substrate, microorganism, product, product yield, and biomass productivity to summarise the products or services that are of interest for the investigations and the respective yields achieved.

In order to establish a clear link between the proposed methodology and the results shown below, Figure 1 is created to summarize the implemented strategy.

3. Results and discussion

Microalgae are characterized by being microorganisms that adapt quickly and can tolerate a wide variety of nutrients and withstand different growing conditions. Additionally, compared to any other type of agro-industrial cultivation through which a product can be obtained, microalgae stand out because they do not require large areas, and the necessary nutrients and waters are readily available [33]. Despite this, the cultivation of microalgae to generate value-added products such as it is, represents a great concern in economic terms. The production of any product from microalgae biomass should focus on simplifying the process to reduce production and collection costs and make it competitive. Among the important processes to simplify and strategically plan are: the

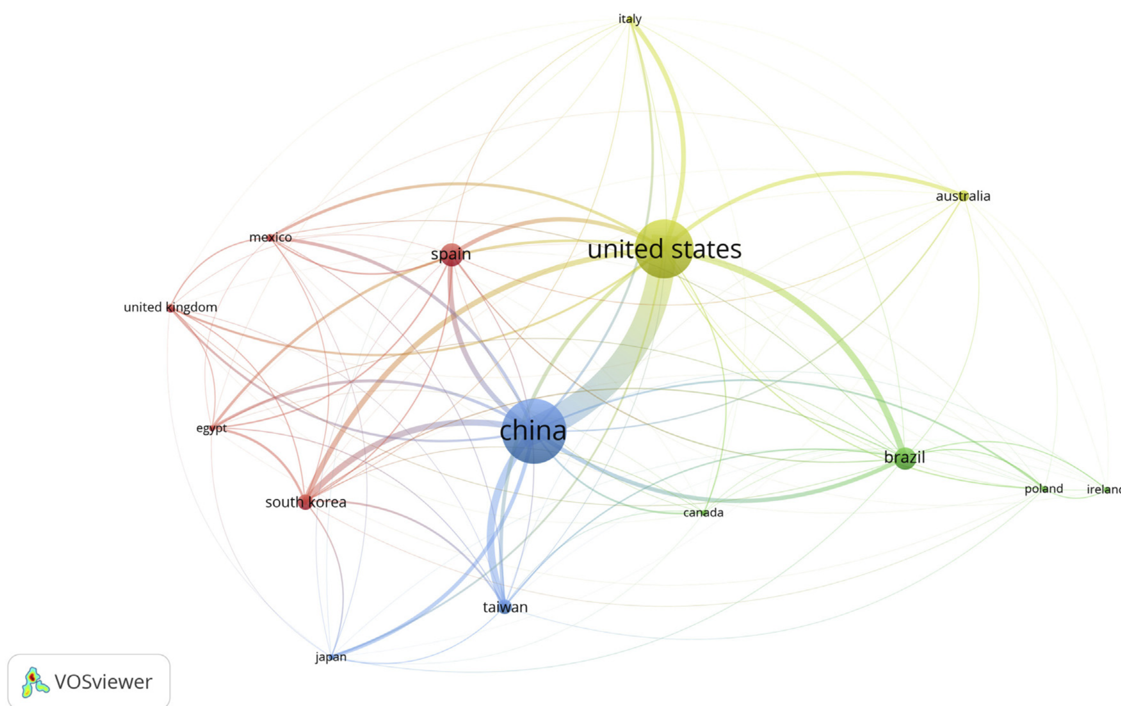

Figure 5. Relations established with other countries.

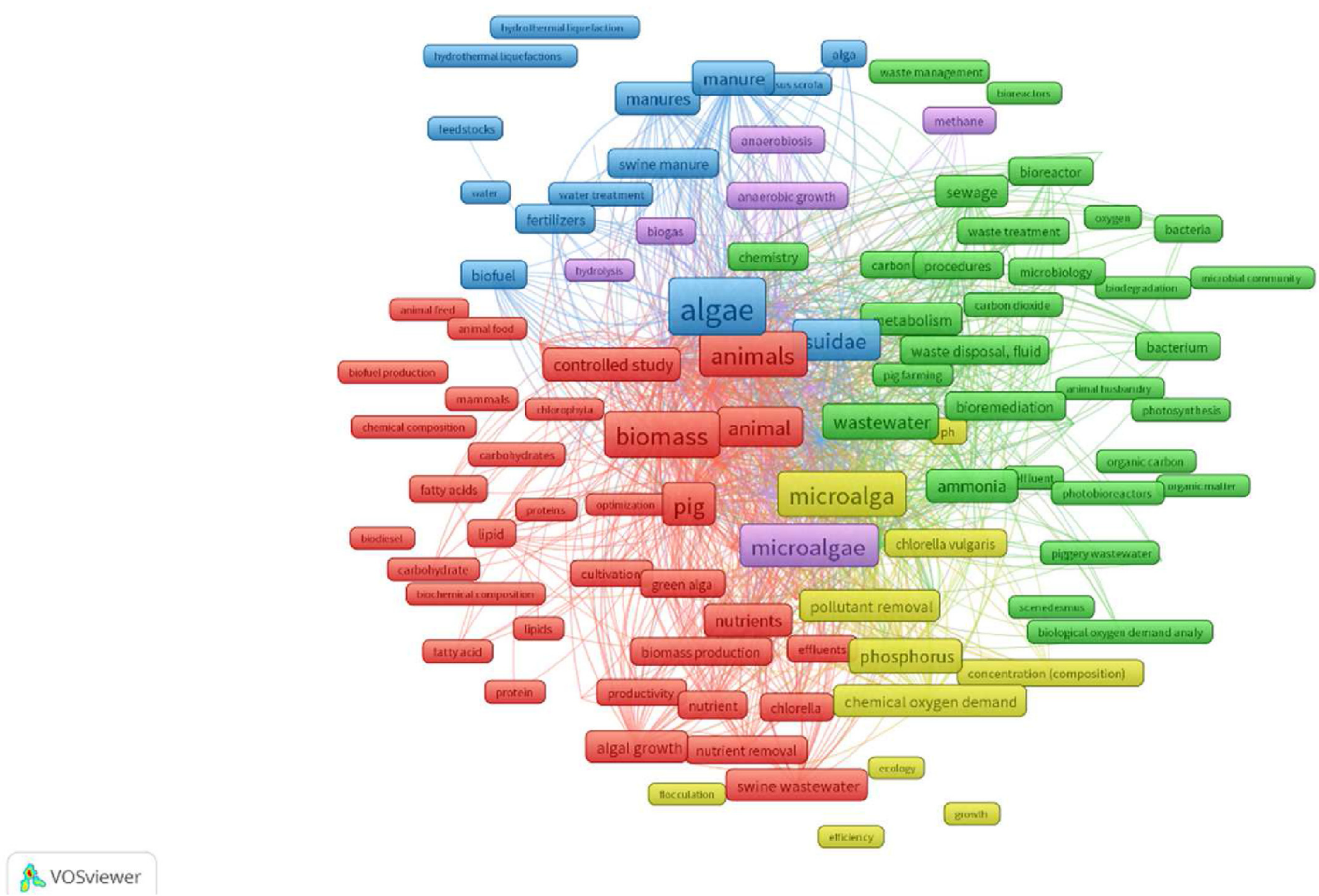

Figure 6. Keyword network.

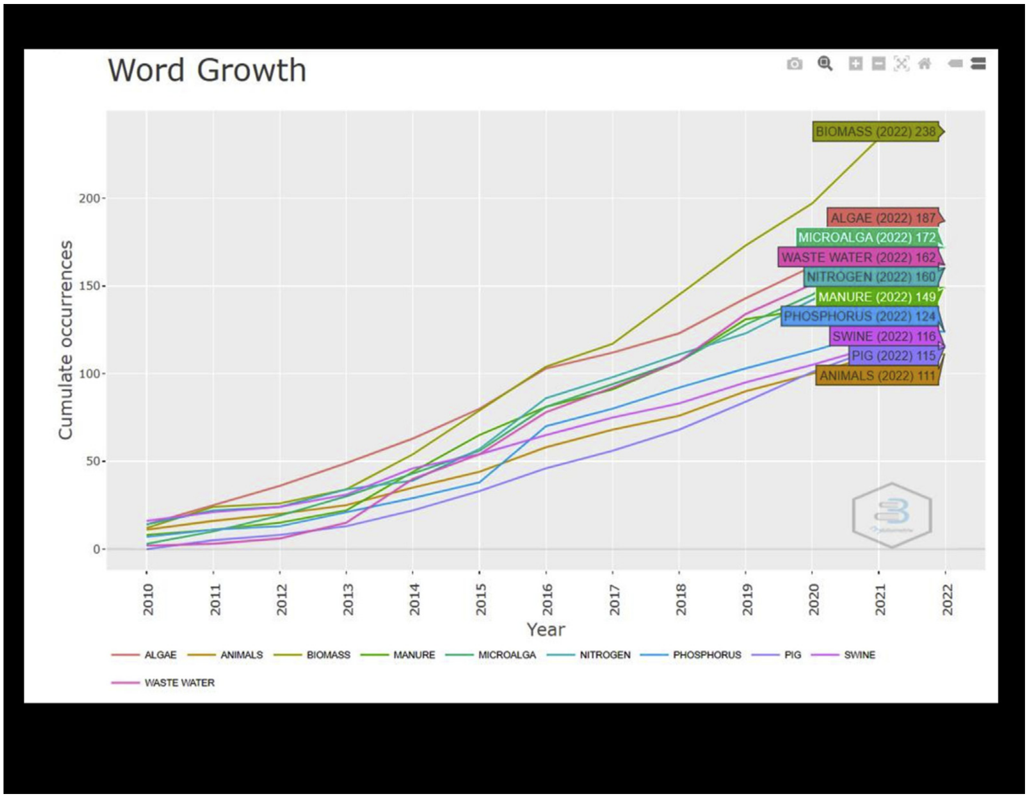

Figure 7. Keyword growth over time.

selection of strains and the Development of the respective inoculum, the design of culture media, energy requirements (light), the feeding strategy and mode of operation of the culture and finally, its recovery methodology [34]. Given the high potential of microalgae to be cultivated commercially, generating value-added products and services, implementing biorefinery strategies and taking advantage of alternative sources of nutrients, the following systematic literature review is planned.

From the result of the literature search, the temporal and geographical distribution, development trend, and research players related to the cultivation of microalgae using swine digestate will be addressed. Then, the maturity of the research topics and the financial status of the different microalgae cultivation processes that are carried out commercially will be analyzed, including the most used technologies and the main products generated. Finally, different algae-based swine manure digestate treatment strategies will be compared and evaluated.

The systematic literature search yielded a total of 2022 articles and 1099 patents, which were filtered according to selection criteria. Thus obtaining a query with a total of 261 articles and 219 patents involving, which involve 1585 authors, 586 affiliations, and 23147 different concepts from which an analysis of strategies for algae cultivation using swine manure digestate was made.

3.1. Temporal distribution

Initially, an analysis of the literature production activity is proposed, the analyzed query has more than 400 documents, of which approximately 74% are located thermally between 2010 and 2021 as can be seen in Figure 2.

From the Figure 2 it is possible to identify that the interest in using swine digestate to cultivate microalgae has been growing continuously representing an average growth rate of 13% for patents and 22% for scientific articles in the last 10 years. Furthermore, it is possible to divide the selected time interval into two stages, in which two different growth rates are reflected. For publications in general, including patents and articles, for the period 1972–2019 an average growth speed of 5 publications per year is determined and for the period 2019–2021 a speed of 29 publications per year.

The above behavior is attributed to the emerging interest of our society in the generation of value-added bioproducts through biorefinery and bioeconomy strategies, which has promoted not only research on issues that can generate bio-based products, but also to open many doors and facilities in terms of publication processes [35, 36].

3.2. Geographical distribution

The analysis of geographical distribution (Figure 3 allows to have a look at the pioneer countries in the publication of documents related to the research topic.

According to Figure 3, it is possible to identify in first place the US in terms of the number of publications with a value of 146 documents, followed by China with 105 and in third place Canada with 76 documents including patents, articles and theses. The special participation of these countries is justified in different situations, in the case of the USA, it is because this country is considered one of the pioneer countries in which the foundations of microalgae cultivation were established since the 1950s, so it plays a leading role in the Development of the production technology of this microorganism [37]. Additionally, within its territories are institutions such as the Hawaii Ocean Science and Technology Park (one of the first occupants of HOST) and the Hawaii National Energy Laboratory (NELHA) in Kona, Hawaii, which has unique systems for Cyanotech production raceways [38].

On the other hand, in the case of China, it is related to the environmental challenges that this country presents in terms of its level of contamination given its high population density; These types of challenges have led them to invest great efforts in the wastewater sector, implementing alternative strategies for the treatment of liquid effluents that in the future will have a great influence in the world [39]. Likewise,

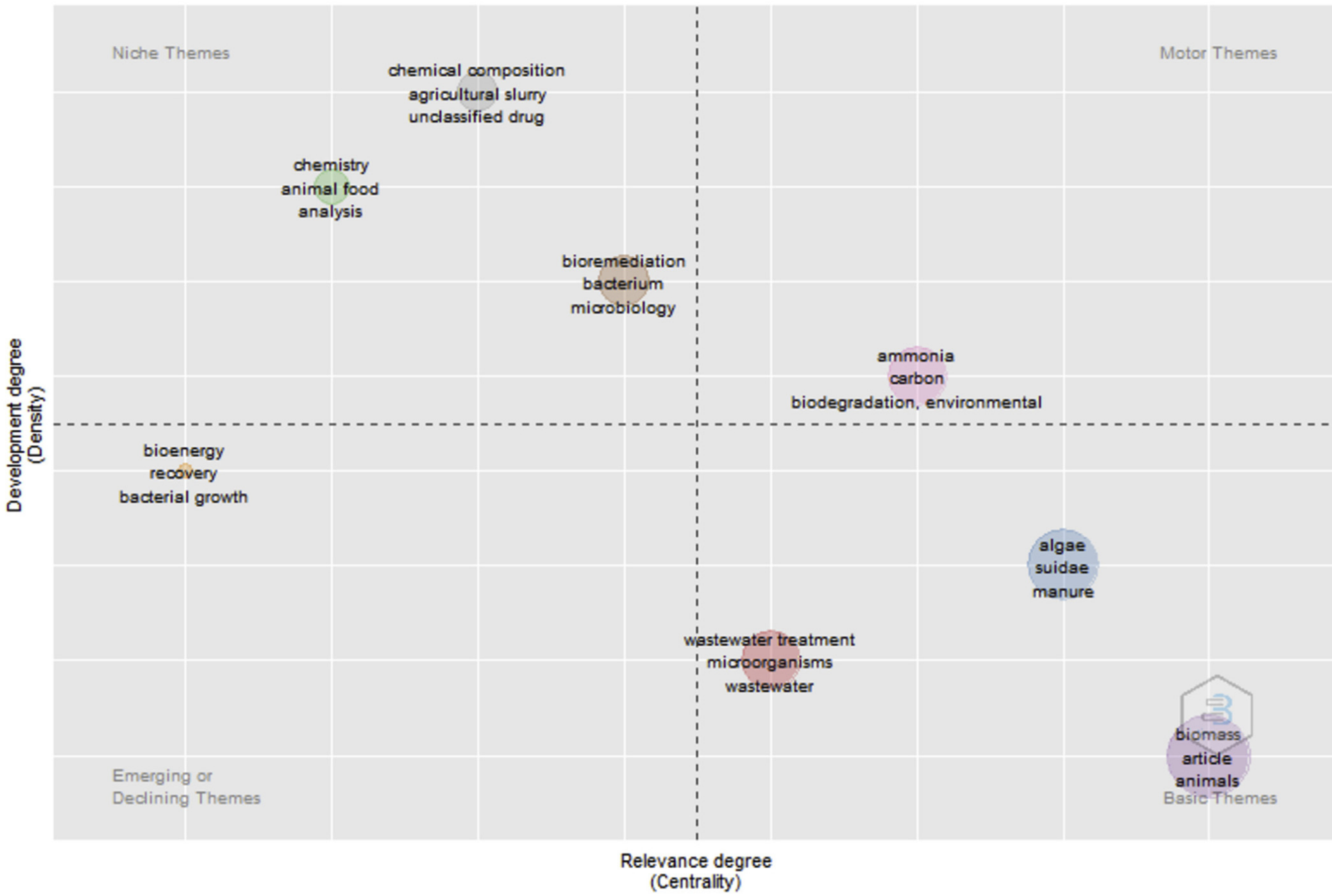

Figure 8. Distribution of key concepts.

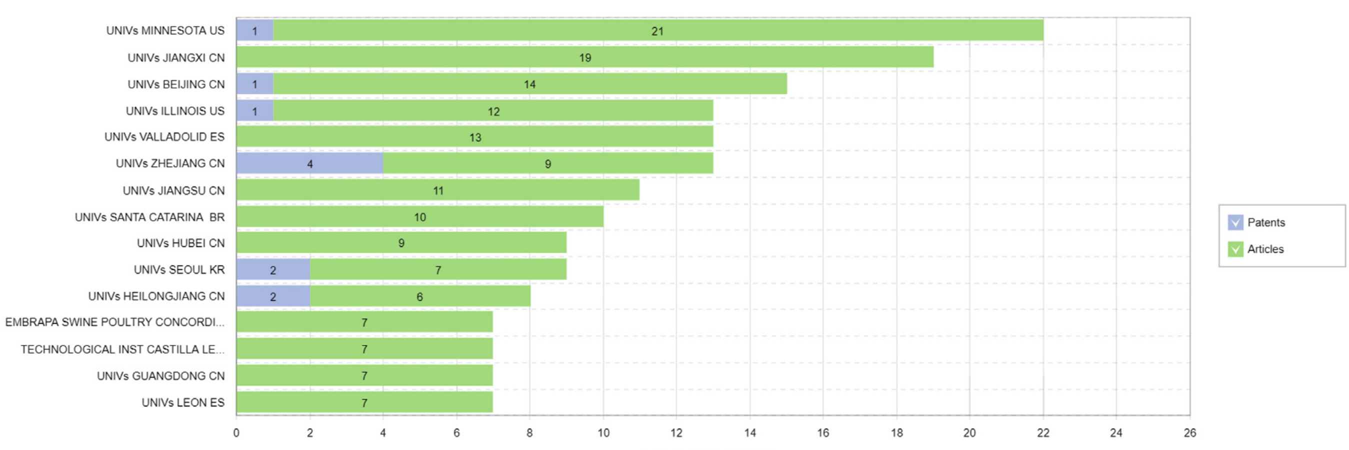

Figure 9. Main entities according to the number of publications.

another reason for China to be one of the pioneering countries in the investigation of the use of porcine digestate for the cultivation of microalgae is due to the fact that China is considered the largest producer of pigs in the world, with more than 55% of the total production, and it is also the largest consumer [40] which is reflected in a high rate of waste generation from this industry that contrasted with its water treatment problems, making this an issue that requires continuous research and Development.

In Figure 4 it can be identified that in recent years, the generation of articles related to the subject of study has been high given that more 3 countries have published an accumulated of at least 70 articles. Regarding the growth of the number of publications per year, there is China with a growth of 32%, Canada 20%, and then the United States with a growth of 17%. It should be noted that the order of the countries according to the number of publications does not correspond to the order of the countries according to growth, this is because in recent years China

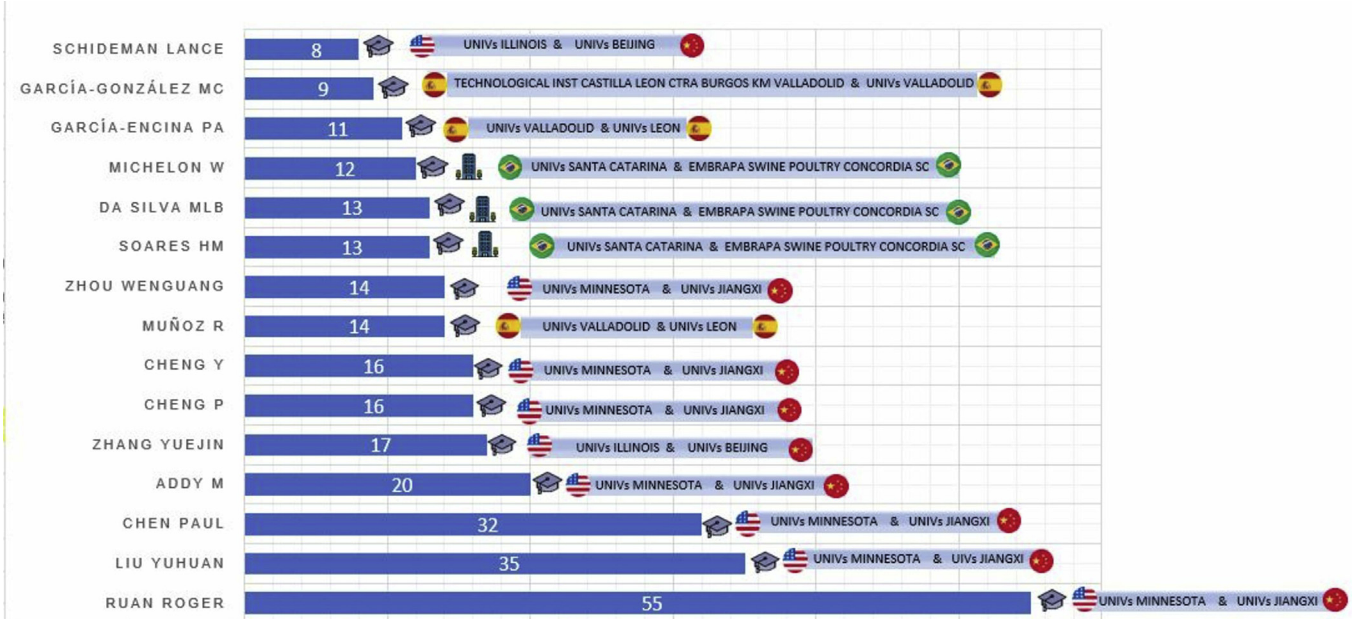

Figure 10. Main authors and their affiliations.

has become a very active and dynamic country in terms of publication of articles and patent application, which puts it in a place with some potential to become the best worldwide player given that its turnover rate is twice that the US.

Figure 5 presents an analysis of the relationships established between the different countries according to the co-authorship of the different publications. The size of the node represents the number of articles published, and the lines simulate the degree of relationship or number of collaborations between the countries, there are as many lines as there are links established in publications. And likewise, using 3 different colors, 4 clusters are established, the first one is yellow, led by the United States, the second is blue, led by China, the third is red, led by Spain, and the second is green, led by Brazil.

3.3. Themes of trend and development of current research

Figure 6 presents an overview of the topics covered by the investigations. For the analysis, a first filter eliminates irrelevant terms, and the words with more than 10 co-occurrences are considered for the figure. Secondly, the size of each word represents the relative proportion of each term to the total number of words, and the different colors represent the clusters in which these keywords were grouped. Among the word cloud it is possible to see that many are related either to the substrate to be implemented, such as wastewater, manure, sewage, ammonia, etc.; or to the product to be generated such as Biomass, biogas, lipids, fertilizers, etc. In general, the word algae prevail with 296 occurrences, Biomass with 288, and wastewater with 200. Additionally, 5 clusters were established: the green led by the wastewater concept, the blue led by the algae word, the red led by the term biomass, lila led by the microalgae aspect and the yellow led by microalgae.

In addition to the number of occurrences of the concepts used as keywords in the publications, it is important to analyze the incremental use of these terms. In Figure 7 you can see the top 10 of words with the most co-occurrence and their growth in the time interval 2010–2021. From this, it is possible to identify the growth focus of research related to the concept of Biomass. Nowadays, the research no longer focuses solely on the treatment of effluents, but it is also of great interest to obtain a product of added value. Additionally, it is possible to identify the preference of the terms between the different synonyms; for example, as the research has been developed, the term algae is used more than microalgae, and the swine concept is the more relevant pig. But the most significant thing is the evident growth of all concepts in general, which is proof of the active growth of research and publications related to the subject of interest.

Finally, regarding the analysis of the concepts involved in the different publications, Figure 8 shows the distribution of the trend topics in cluster determining their degree of Development and their relationship. To analyze the conceptual structure, through the bibliometrix interface, a thematic map is made in which an analysis of the co-occurrence of words is carried out. To plot this graph, the algorithm reviews the titles of all references and highlights additional relevant but overlooked keywords that were not listed by the authors. They are also classified into 4 quadrants according to the type of theme they belong to, whether emerging or declining themes, basic themes, niche themes or motor themes. For the correct interpretation of the figure, the concepts are characterized by their density and their centrality, their density refers to their degree of Development, which is established based on the number of publications, and their centrality indicates their degree of relevance, which evaluates the interaction of the cluster with the rest of the network [41]. The motor themes are characterized by high centrality and density, this means that they are developed and important for the field of research, the emerging themes have low centrality and low density, that is, they are weakly developed. The niche themes have a good internal relationship (high density) but external links of low importance, therefore they have limited importance for the area, and finally the basic themes are characterized by high centrality and low density, that is, they are very important for research but refers to general and cross-cutting issues, with a lot of external relationship but not internal [42]. From Figure 8 it can be determined that the concepts with the highest degree of Development are related to the generation of Biomass, and the one with the least Development is related to the concept of recovery and bacterial growth. Regarding centrality, the concepts that present greater relevance and interaction with the others are related to water treatment, ammonium concentration and biodegradation.

3.4. Players

Another very relevant aspect to analyze is related to the most active entities in terms of publications related to the cultivation of microalgae

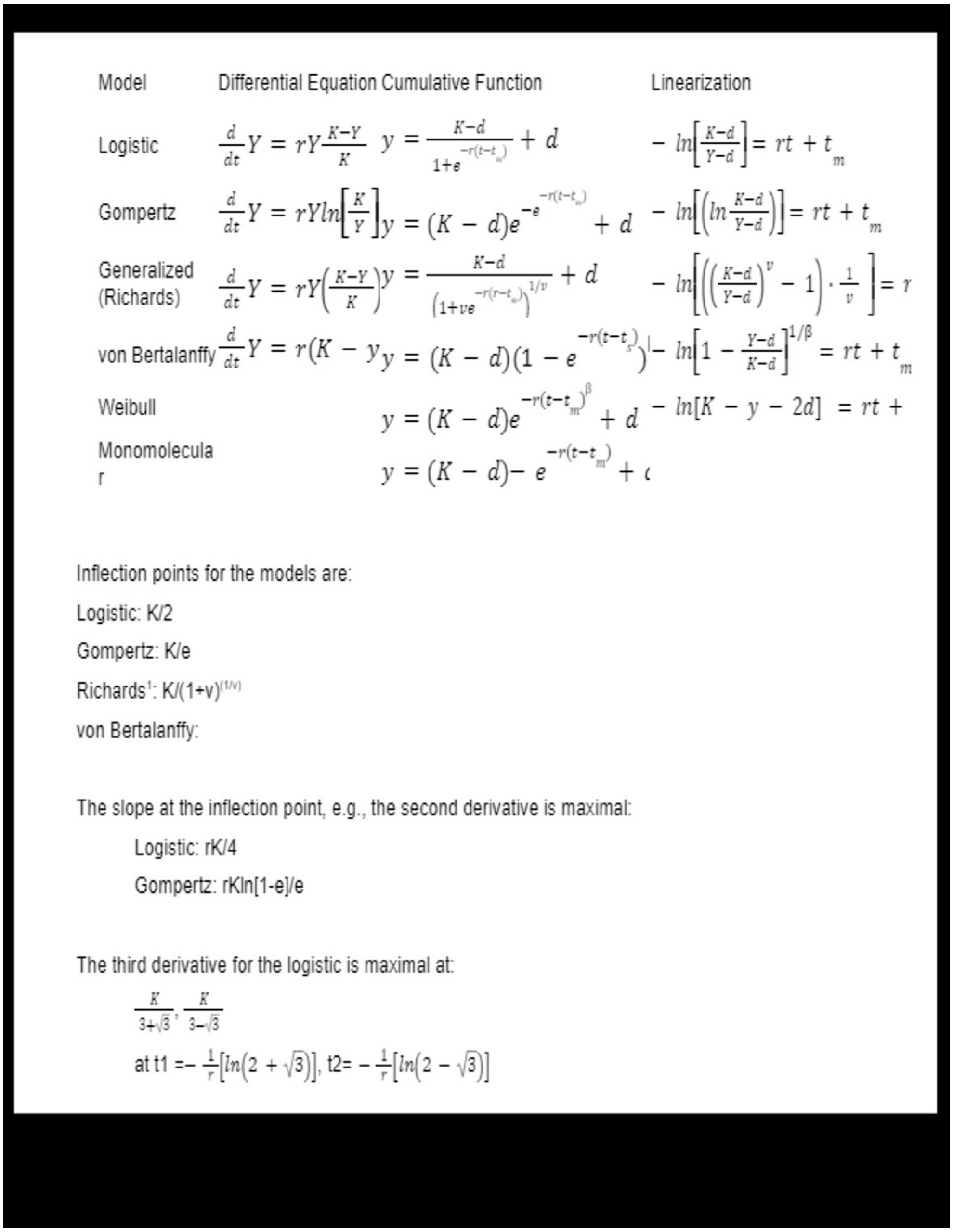

Figure 11. Models applied to the S-curves.

using porcine digestate. In this case, Figure 9 presents the top 15 organizations and their respective publications in terms of articles and patents. From this it is possible to identify that only 40% have applied to patents, being the University of Zhejiang in China the one that occupies the first position in terms of obtaining patents. It is possible to observe the first 4 institutions accumulate approximately 50% of the publications generated by these 15 entities, which gives an indication of the constant publication of documents by these entities and of how centralized the Development of this research.

Figure 10 shows the 15 most relevant authors in terms of patents and articles. Likewise, it allows to identify the institutions to which these authors are affiliated (their top 2 affiliations are shown), the type of institution and the country it belongs. In this way, the 5 most relevant organizations are identified, the University of Minnesota, Jiangxi University, Valladolid University, Santa Catarina University and the EMBRAPA Swine Poultry Concordia organization. In the same way, these organizations, identified as main players, belong to the most participative countries that were mentioned in Figures Figure 3 and Figure 4 and also show the different collaborations mentioned in Figure 5. It is important to highlight that being China and the USA the main generators of literature in this case, for each country the research is centralized only in two universities for the USA and 3 for China, which supports the sectorized nature of the literature. In the same way, when observing the nature of each organization, it is possible to determine that the research is dominated by the academy, according to the affiliations of the authors, only 3 of those presented in the top 15 have a relationship with an

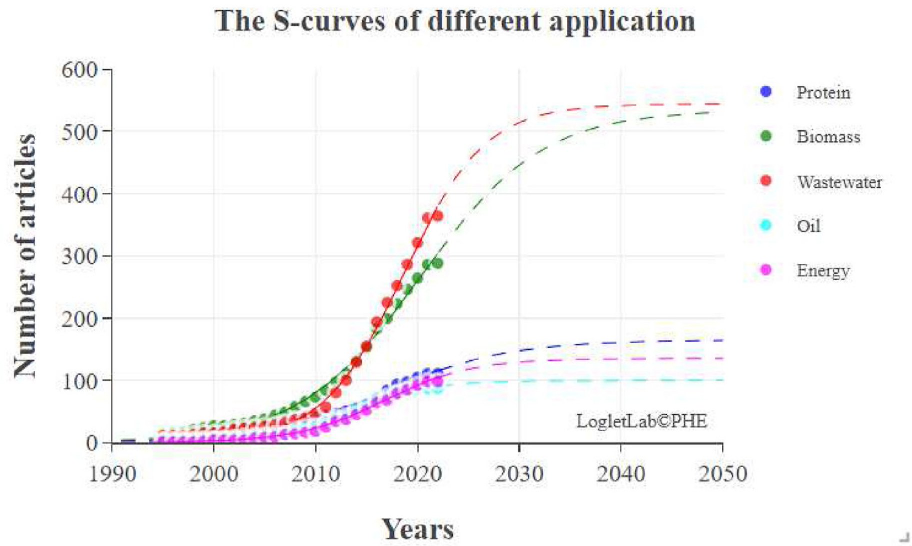

Figure 12. S-curves.

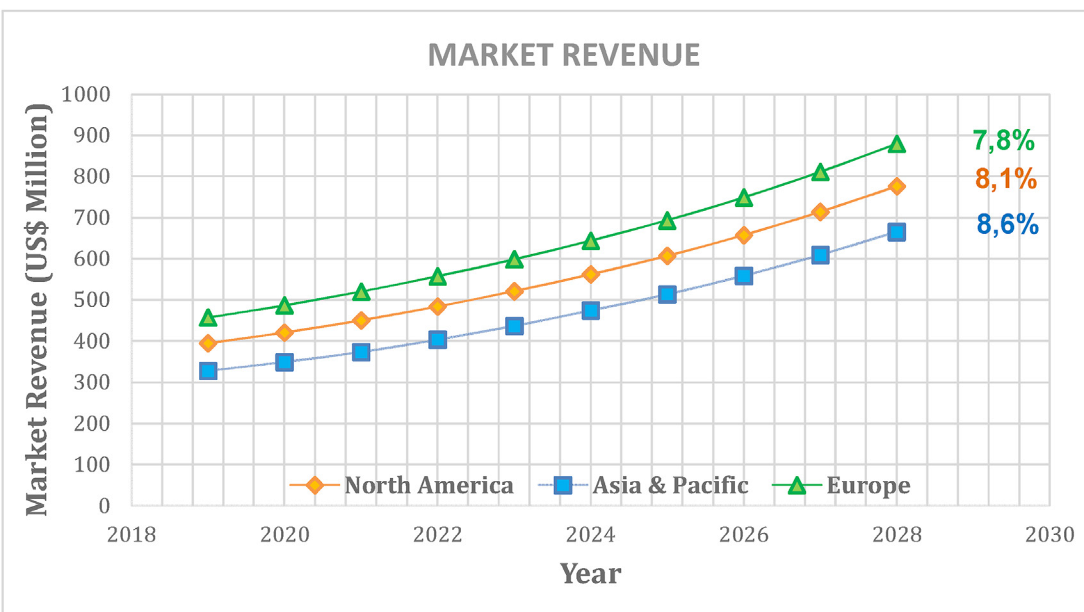

Figure 13. Market revenue 2018–2029 for the three main containers in the microalgae market.

organization, the others are linked to entities and universities, which highlights the role of academia over industry in the Development of research related to the cultivation of microalgae using porcine digestate.

3.5. S curves - maturity analysis

The curve S and the maturity index concepts are used in different knowledge areas. These come from the biological and social sciences and economics and are used for forecasting analysis. Recently, they have had a boom in predicting the technological maturity of processes applied to science and engineering. Obviously, it is a model with its restrictions and advantages [43, 44, 45, 46]. S curves are a tool that allows studying the life cycle of a research, technology or product. This allows to observe how the behavior of this technology or product has been over time and in this way determine the degree of maturity in which it is and have an idea of its future projection [47] The S curve is composed of 4 phases, Emerging stage: Low competitive impact and low presence in products and processes; Growth stage: Highly competitive impact without a presence in products or processes. This technology is known as "pacing technology"; Maturity stage: High competitive impact and product integration. At this stage, the technology is classified as "key/leading technology"; Saturation stage: Loss of competitiveness or the processes will be focused only on cost reduction.

According to the stage of the research, it is possible to determine the right time to make investments and carry out research in that market area [48].

For this research, the S curves were proposed for the main products or applications related to the microalgae cultivation process using porcine digestate. The figure was carried out using the Logletlab tool, which establishes that the Monte-Carlo method applies, and, in its documentation,

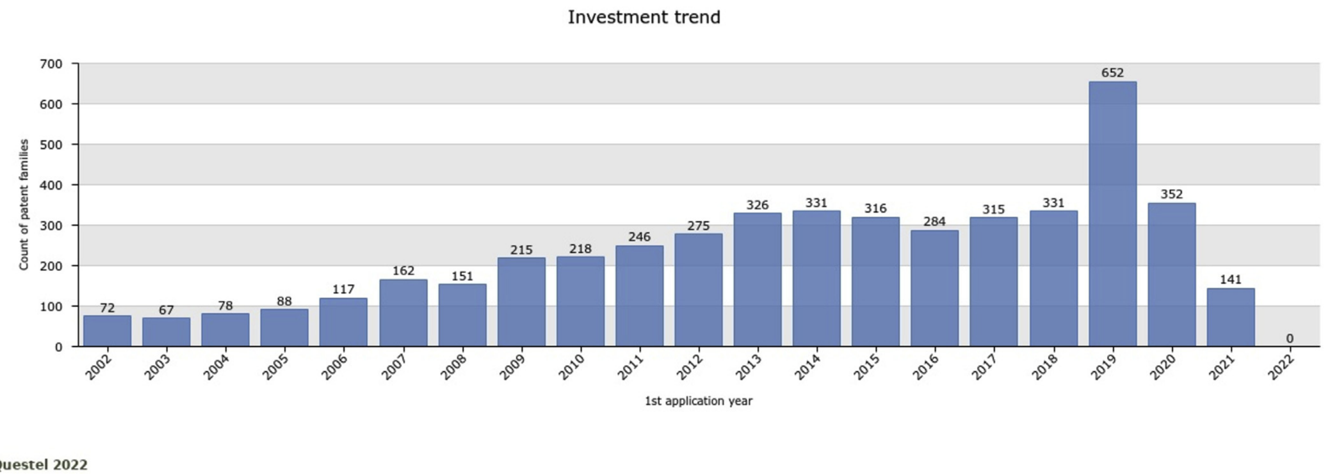

Figure 14. Growth of investments in patents.

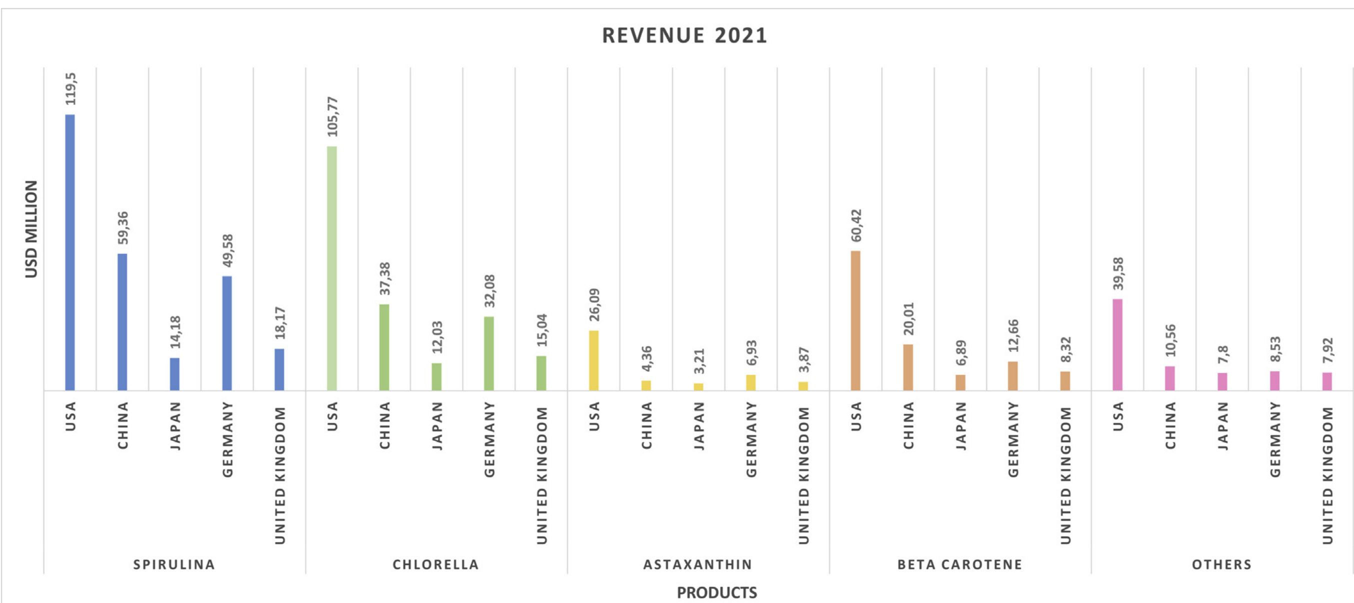

Figure 15. Revenue per product for the most influential countries.

section presents the applied models for the generation of the figure, these are shown in Figure 11 [47, 49] Likewise, Figure 12 shows the figure of S-curves obtained for the trajectory of the products or services related to Proteins, Biomass, Water Treatment, Oil and Energy. This is framed in the time interval from 1990 and projected until 2050.

In addition to the S-curves figure, the Logletlab Platform allows calculating the statistical values obtained from the applied model for the figures, these values are shown in Table 2. From these values it is possible to establish the following. On one hand, according to the values of R2 it is possible to determine the level of adjustment of the curves, an aspect that allows to indicate that all present a correct adjustment, being the protein curve the one that has the best adjustment and the Wastewater curve the one that least adjusts to the model. Likewise, all curves have a p value < 0.05, which is established as the confidence interval, meaning that, for this analysis all these variables are significant.

On the other hand, the K value shown in the table represents the upper asymptote of the curve, which, according to the projection will determine the maximum limit of the number of publications for each topic, being in this case the water treatment that is projected to reach a higher number of publications with a value of 544 by 2050 [50]. In turn, from this K value, it is possible to estimate how the scope of these goals will be over the years. Therefore, in the last columns of Table 2, according to the projection, the years in which certain percentage of this maximum publication limit is reached are shown. For example, in the case of research related to oils, it is planned to reach 90% of the limit by the year 2022, because this focus area is already in the decline stage of the S curve, while for the Biomass and protein, they still have enough boom years to invest in these investigations. Also the percentages show the stages of technology development, making it easier to classify the stage of maturity, as follows: 1%–10% Emergent stage, 10%–50% Growth stage, 50%–90% Maturity stage, 90%–98% Saturation stage, ≥99% Retirement stage [51].

Furthermore, Figure 12 indicates which products and services, the Development of this research area has focused on, with Biomass and water treatment being the topics with the highest number of articles. The foregoing is due, to the environmental problems that arise in our society, which has led us to invest great efforts in issues related to water treatment. However, the great potential of this Biomass has also been identified, so the research has focused not only on performing the water treatment, but also on generating the greatest amount of Biomass

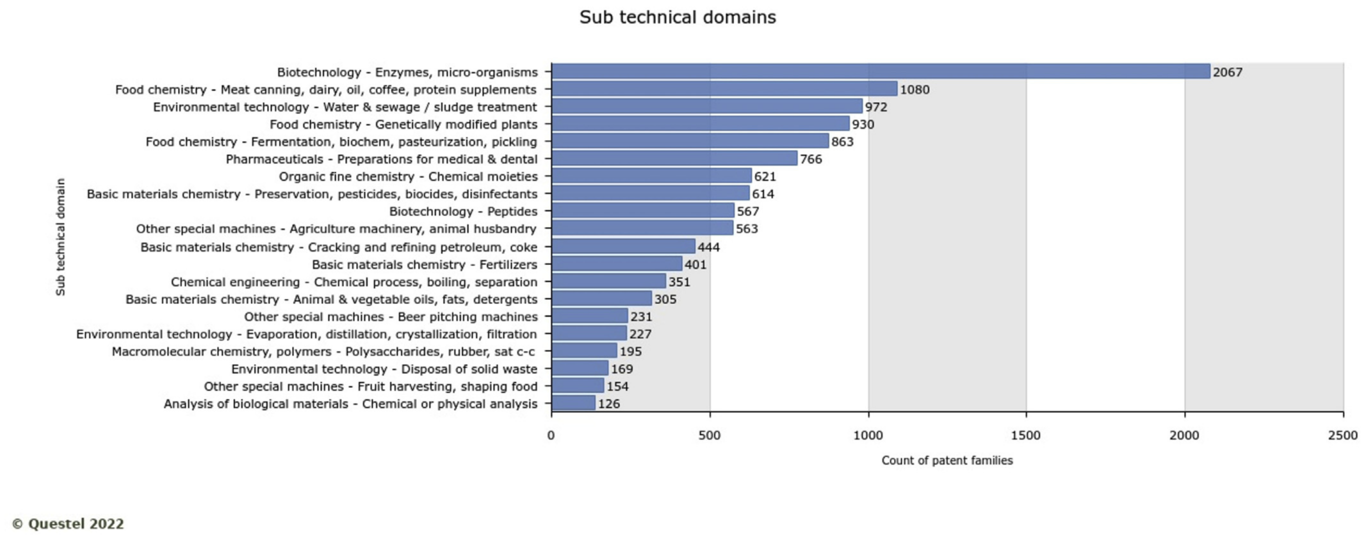

Figure 16. Technical subdomains implemented in the use of microalgae cultures.

possible in this process and achieving a correct advantage taking of this to obtain a more sustainable scheme. In the case of proteins, oils, and energy, it can be observed that the publications are smaller and that even the projections are not very high. This can be considered, since there are still many challenges in the cultivation of microalgae on a commercial scale, and research has focused much on these challenges more than on characterizing and extracting value-added products through the transformation of Biomass. However, it should be noted that this does not mean that the Development of the research does not have this possible focus, only that now water treatment and biomass generation are booming, once these technologies reach a degree of maturity that allows them to reach a saturation stage, the research will focus on the possible products that can be obtained through the transformation of Biomass.

3.6. Financial analysis

In addition to the analysis of the trajectory and level of Development of the different investigations, and giving the interest in generating added value products, below is a financial analysis regarding the income and rates of market growth based on products from microalgae cultivation. According to the report from The *Insight Partners* in its *Microalgae-Based Products Market Forecast* [29, 30, 31]. In Figure 13 the market revenue can be observed in the time interval from 2018 and projected to 2030 for the 3 most participative continents in the market of products based on the cultivation of microalgae. In Figure 13, it is possible to see that Europe is in the first place in terms of the value of US $ Millions per year, however it should be noted that Asia & Pacific id the one that represent a higher growth rate, thus indicating that this continent has an active growth and Development in order to achieve greater intervention in this market in the future.

For the financial analysis of the market for products based on the cultivation of microalgae, it is also of great relevance to analyze the income generated by the most influential countries, making the distinction according to the products marketed. In Figure 13 it can be observed that for the 5 most active countries in the market, the income value for each product of added value is obtained from microalgae. The 5 countries were selected according to the total value of products and services produced within a country in a year (GDP) reported for the year 2020, which represent the following values in US Millions of dollars USA (20,936,600.00), China (14,722,730.70), Japan (4,975,415.24), Germany (3,846,423.93), United Kingdom (2,707,743) [52].

In addition to the Market revenue in US$ Millions, the financial growth of research related to the cultivation of microalgae is also reflected in the investment trend in terms of the number of patents in which it is invested annually. According to Figure 14, it is possible to determine that in the last 20 years, the number of families of patents that have filed their application has increased with a growth rate of approximately 23%, reaching its maximum value in 2019. After this year, a large decrease can be observed due to the pandemic situation that the global society went through.

From Figure 15 it is possible to identify that of the top 5 products obtained from the cultivation of microalgae, spirulina occupies the first place in all countries, while astaxanthin represents the lowest income. Likewise, it is important to emphasize that not necessarily the country that has a higher GDP value is the one that represents a greater number of incomes in terms of microalgae-based products, this indicator is composed of many other products, and for some cases microalgae trade is not the main contributor. For example, Japan occupies the 3rd place in terms of GDP, however when looking at the income in Figure 15 it is possible to realize that Germany and United Kingdom represent higher income even having way lower GDP values.

Also, Figure 15 shows the income generation of each country for the top 5 products generated from the cultivation of microalgae. It is also relevant to identify the main domains of the technologies implemented for the use of the products generated from these crops, the technologies are classified according to the number of patents that apply to each one.

Regarding the technologies implemented for the generation of value-added products from microalgae, it is considered that the investigations have focused on Biotechnology, Food chemistry and Basic materials chemistry. However, the technologies that have great growth potentials such as Environmental technology, Pharmaceuticals, and Organic chemistry are also highlighted.

In turn, in Figure 16 it is possible to identify in more detail the sub technical domains on which, the different investigations that are reported through patent applications, are focused. For the previously mentioned domains the following subdomains are established, Biotechnology (Enzymes, microorganism), Food chemistry (Meat caning, oil and proteins) and Environmental technology (Water, sludge treatment). This allows not only to broadly classify patents according to the different applied technologies, but also to identify in detail what approaches current research has taken and thus be able to determine which of these has the greatest potential to initiate future projects.

Table 3. Cultivation Strategy from various waste substrates and some algal biomass.

Substrate	Alga species	Digestate Pretreatment	Cultivation System	Refs
Anaerobic digestate of piggery effluent (ADPE)	The microalgal consortium was dominated by *Chlorella* sp.	Charcoal filtration, slow feed sand filtration, addition of ammonium chloride	Outdoor growth in raceway ponds (150 L) Two phases (Batch Semicontinuous).	[53]
Pig manure anaerobic digestate effluent	*C. vulgaris FACHB-31*	Filtration, pretreatment with indigenous bacteria	500 mL flasks	[54]
Anaerobically digested piggery effluent (ADPE)	*Chlorella* sp. and *Scenedesmus* sp	Charcoal filtration, slow feed sand filtration	11 m^2 paddle wheel driven raceway pond	[55]
Anaerobic piggery digestate	*Desmodesmus* sp. CHX1 isolated from a local pond treating piggery wastewater	consortium microalgae-bacteria by artificial inoculation with a commercial organic degrading bacteria (mainly Bacillus and Pseudomonas) and nitrifying bacteria (mainly Rhodobacter, Micrococcus, Bacillus, Pseudomonas, Nitrobacter, and Nitrosomonas)	1 L flasks in full temperature light oscillation incubator	[56, 57]
Swine manure wastewaters	*Chlorella sorokiniana* N (18S r DNA, Genbank No. KJ734869)	filtered using multi-layer gauze to remove large particles.	30 L photobioreactor	[57]
ammonia-rich wastewaters	*Micractinium inermum*	microbubble stripping	microbubbles L airlift bioreactors	[58]
Anaerobic digested starch processing wastewater (SPW)	*Chlorella pyrenoidosa*	Settling tank and polyester filter bags	airlift circulation photobioreactor (820 L) and a dynamic membrane reactor	[59]
Nutrient Rich Digestate (NRD) from an AD facility that treats food waste	*Chlorella vulgaris (CCAP 211/11R)* and *Scenedesmus obliquus (CCAP 276/6A)*	membrane microfiltration	800 L Plastic columns Batch	[60]

Table 4. Yield from various waste substrates and some algal biomass (* refers to the fact that this aspect is not reported in the article).

Substrate	Microorganism	Product/Service	Product Yield	Biomass Productivity	References
Anaerobic digestate of piggery effluent (ADPE)	The microalgal consortium was dominated by *Chlorella* sp.	Biomass (Chlorophyll content)	3.0 mg. Cell x 10 -7	19.5 mg L^{-1} d^{-1}	[53]
Anaerobically digested piggery effluent (ADPE)	*Chlorella* sp. and *Scenedesmus* sp.	Microalgal Biomass for animal feedstock	Composition (lipids 6,38%, protein 39,2%, carbohydrate 35,3%, fiber 19,6 essential amino acids 1,24 %)	2.20 ± 0.49 g m^{-2} d^{-1}	[55]
Swine manure effluent	*Rhizoclonium* sp.	Fatty acid (FA)	FA content values of the algal Biomass ranged from 0.6 to 1.5% of dry weight	*	[65]
Swine manure wastewaters	*Chlorella sorokiniana N* (18S r DNA, Genbank No. KJ734869)	Biomass	*	160 mg l^{-1} d^{-1}	[57]
Swine Wastewater	*Chlamydomonas debaryana AT24*	Biomass Biofuel	Estimated 1010–2094 tonnes/year of algal oil	1.21 g L^{-1}	[66]
Anaerobically digested poultry litter effluent (PLDE)	*Chlorella minutissima, Chlorella sorokiniana and Scenedesmus bijuga*	animal feed supplement	protein (39% w/w), carbohydrates (22%) while lipids (<10%)	76 mg l^{-1} d^{-1}	[67]
Wastewater Treatment Plant	*Synechocystis 6803 (cyanobacterial)*	D-Lactate	1.2 g l^{-1}	-	[68]
Anaerobic starch wastewater and alcohol wastewater	*Chlorella pyrenoidosa*	Biomass (Protein and lipids)	lipids productivity of 127.71 ± 6.31 mg L^{-1} d^{-1}	0.58 ± 0.03 g l^{-1} d^{-1}	[69]
Nutrient Rich Digestate (NRD) from an AD facility that treats food waste	*Chlorella vulgaris (CCAP 211/11R)* and *Scenedesmus obliquus (CCAP 276/6A)*	microalgae for animal feed	Protein (0,8 mg/mg) Carotenoids (9 mg/mg)	Chlorella (1621 mg L^{-1} d^{-1}) and Scenedesmus (1591 mg L^{-1} d^{-1})	[60]

3.7. Algae-based swine manure digestate treatment strategies

In addition to the detailed analysis of the current Development of literature related to the research topic, it is important to highlight some details of the strategies implemented in the different studies reported in the literature. The above in order to present a overview of the different techniques applied to these cultivation processes.

All the studies on microalgae cultivation using digestate as a substrate highlight the toxicity of the nutrients and turbidity as the main limitations. According to the systematic review of the literature carried out, as the first comparison criterion, Table 3 summarizes the emerging techniques for the pre-treatment of liquid effluents and the cultivation strategies. From this, it can be noted that most of them include a sterilization or treatment process focused on eliminating pathogens and reducing the ammonia load that characterizes these residues and is considered toxic to microalgae. Also, the last rows of the table mentioned corresponding to studies carried out with the same approach but using an alternative source of nutrients other than the digested porcine. This makes it possible to identify the relevance of this type of cultivation strategy, thus expanding their applicability to a great variety of useable effluents.

For the cultivation of microalgae using digestate as a substrate, the toxicity of the nutrients and turbidity are considered the main limitations. The turbidity of the medium represents a limitation for the reception of light, which directly affects cell growth. At the same time, the high concentration of ammonia in the digestate can be toxic for the cells. Ammonia is a favorable nitrogen source for microalgae, but according to the concentrations and the pH of the process it could be toxic [13]. The toxicity of ammonia for algae is reflected in scenarios like the interruption/inhibition of photosynthesis and the decoupling of phosphorylation electron transport [61]. Among all the proposed pre-treatments, the dilution process should be highlighted, by which the digestate is dissolved in the culture medium, reducing the concentrations of harmful compounds. There are other processes that remove toxic compounds and improve turbidity, such as ozonation or autoclaving, but these result in a significant increase in the cost of the process [62].

Within the culture strategies, the most common is to consider open pond systems or closed systems such as photobioreactors. In commercial terms, closed systems are preferred to keep the process under control and achieve high productivity. However, the cultivation of microalgae in open environments allows to take advantage of natural conditions and focus the process on a bioremediation strategy capturing CO_2 from the environment. But at the same time, it must be taken into account that in open ponds there is a limitation since the necessary cell density is not achieved due to possible errors in the scaling process, or because these systems are very prone to contamination [63]. Of the two types of strategies that stand out in the literature, it is important to clarify that both have pros and cons, the choice is determined according to the availability of equipment and infrastructure in the research and the environmental conditions of the region.

Many of the procedures found in the literature focus on removing the nutrients in the digestate, leaving close to “clean water” to be used for irrigation, in drought areas, as drinking water for animals and as process water in various industries. The most common method for reusing the digestate is as compost, being applied to the soil of the various crops, which is an easy process and allows a quick and easy disposal given the short pretreatment it needs, and the large areas in which it can be distributed [64]. However, this does not focus on the use of this digestate to generate a value-added product or service. This is reflected not only in the generation of a negative environmental impact, but also, given inadequate and inefficient technologies, together with the low recovery and use of waste, also causes a loss of value and competitiveness in the market. Table 4 shows the different added value products that can be obtained according to the literature consulted. The yields achieved both for the generation of Biomass and for the product of interest are also summarized.

In the same way, in the last rows of the comparative table, information is provided on some relevant strategies that implement other alternative sources of nutrients; the use of these different supplements is focused on improving productivity without leaving aside the biorefinery approach. This assumption is done always considering the use of other streams or sub-pipelines to supplement the environment, thus expanding the capacity to use waste and reducing costs [59].

This allows to have an idea of the quantities that can be generated with a biorefinery process based on the cultivation of microalgae and from this to carry out a techno-economic analysis. Among the various products studied, the production of animal feedstock stands out given the protein composition of the Biomass, or the generation of biofuels due to the concentration of lipids that can be concentrated in the cells.

As has been highlighted so far, the cultivation of microalgae using digestate represents a very efficient process given the wide scope for the application of microalgae biomass. Microalgae cultivation systems can be executed as ‘independent’ systems without the need for complex integration of other support systems. Alternatively, on the contrary, they can be easily integrated into the process flow of the biorefinery system, creating a closed circuit with biofactory operation. As reported by Chang S et al. (2018) [33] The most important aspects for the Development of a microalgae culture on a commercial scale are:

- Isolation and selection of the strain
- If desired, the implementation of genetic engineering to work with generativity modified strains
- Strategies to focus the culture promoting the production of Biomass, lipids or the cellular metabolite that is of interest.
- Innovation in the technique and design of photobioreactors or open structures for cultivation
- Biomass recovery methods and the respective extraction of the compound of interest.

4. Conclusions

This paper provides knowledge and information on the strategies currently being developed to cultivate microalgae at an industrial level focused on the generation of value. From the systematic analysis of literature, it is possible to identify China and the United States as the main actors in the Development of research related to the cultivation of microalgae using porcine digestate. This, not only due to their high number of publications, but also to its trajectory in terms of high collaborations, participation of its academic entities and also for its performance in the market, for which an approximate annual income of 370 million US $ are established for China and 450 million US $ for United States, with an estimated growth rate of 8.6 and 8.1 respectively. In terms of the analysis of concepts and trend topics, themes such as Biomass and water treatment stand out.

The relevant assessment is not only due to the number of occurrences and its growth over time, but also given that according to the analysis of curves it shows to be in a stage of development maturity with a promising growth trajectory for the next few years, reaching saturation level approximately in 2050.

Microalgae biomass has enormous potential as a raw material given its robustness and ability to adapt since its metabolism can use the conditions it is subjected to. In addition, the cultivation of microalgae allows the capture of carbon dioxide and the generation of a lower carbon footprint compared to the processes of other products. Among the possible strategies to implement for microalgae cultivation, pig waste liquid effluent is an excellent option, given its composition mainly in terms of carbon source and nitrogen. However, it is crucial to consider its limitations regarding turbidity and toxicity, the first being a limiting factor for the correct penetration of light and the second in terms of the presence of compounds such as ammonia, which inhibit growth.

The studied technologies showed not only the positive environmental impact that it is possible to obtain, but also the viability of different strategies to be scaled at an industrial level and the biorefinery techniques to generate valuable products such as pigments, fatty acids or proteins which could be beneficial from an economic perspective. According to the financial analysis, the market related to the cultivation of microalgae is mainly focused on products such as, spirulina, chlorella, astaxanthin and beta-carotene.

Declarations

Author contribution statement

All authors listed have significantly contributed to the development and the writing of this article.

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No data was used for the research described in the article.

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The authors declare no conflict of interest.

Additional information

No additional information is available for this paper.

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