A half-century of research on microalgae-bacteria for wastewater treatment

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A HALF-CENTURY OF RESEARCH ON MICROALGAE-BACTERIA FOR WASTEWATER TREATMENT

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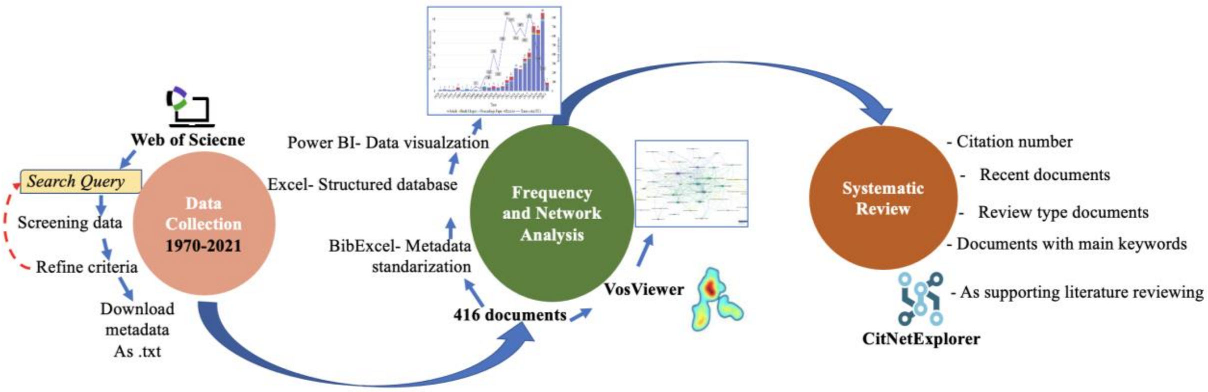
Abstract:

The use of microalgae-bacteria consortia (MBC) for wastewater treatment have received increasing attention because of its high capacity for nutrients and organic matter removal, using sunlight as an energy source, through self-sufficient oxygen production provided by photosynthesis. This review addresses the research hotspots and main challenges of this emerging technology, using bibliometric analysis. The evolution of MBC-related literature from 1970 to 2021 was analyzed, identifying of the main research topics explored, based on the frequency of the keywords. This document develops the following topics identified as research hotspots: **1)** The factors that influence the complex interactions between microalgae and bacteria (e.g., pH, incident light, dissolved oxygen), and the removal mechanisms of nutrients and organic matter, which are still relevant issues still requiring significant research efforts. **2)** The new enhanced pathways of total nitrogen and phosphorus removal, such as short cut nitrogen removal, algammox, and phosphorus luxury uptake, which have been explored during the last decade. **3)** The application of mathematical models to represent the behavior of MBC, as a tool for prediction and optimization of MBC systems. And **4)** The biomass retention technologies, such as biofilms, membrane bioreactors, and granular sludge; that allows the uncoupling of HRT from SRT, a condition required for high-rate wastewater treatment.

Graphical Abstract:



Keywords: Bibliometric analysis, decentralized system, microalgae-bacteria consortia, nutrients removal, wastewater treatment

1. INTRODUCTION

Nowadays, about 80% of the produced wastewater (WW) worldwide is discharged into the environment without prior treatment. In developing countries, this number reached up to 90% in 2010 [1,2]. This situation promotes social, environmental, and public health problems, mainly in rural or small communities [3,4]. In this context, the most widely used wastewater treatment process worldwide is the conventional activated sludge (CAS) [5,6]. However, the application of this technology in small communities could be limited due to the high energy demand associated with mechanical aeration [7,8]. In fact, mechanical aeration in CAS represents between 50 and 90% of total energy demand [9–11]. In addition, CAS entails a high CO_2 footprint and a waste of nutrients (i.e., nitrogen and phosphorous).

Microalgae-bacteria consortia (MBC) based treatment systems are considered a promising bioremediation platform due to the self-sufficient production of oxygen, provided by microalgae in the presence of light (photooxygenation), which reduces the operational costs associated with mechanical aeration [12,13]. This O_2 production supplies the requirements of heterotrophic bacteria (HB) for the oxidation of chemical oxygen demand (COD) and of nitrifying bacteria (NB) for the oxidation of ammonium into NO_3^- during the nitrification process. Meanwhile, CO_2 is produced by HB, which is subsequently used as a carbon source by microalgae and NB [14–16]. Moreover, MBC systems have shown a high capacity for nutrients removal. This is the result of the ability of microalgae to assimilate nutrients, and the mechanisms associated with nitrifying bacteria [17–20].

Use of MBC has the potential to transform the way wastewater treatment is conventionally conceived, by using sunlight as an energy source driving the biological process. Thus, operational costs are reduced, and potentially valuable products could be obtained from wastewater through the further biomass transformation in biofertilizer, biofuels, and/or bioproducts [21]. In addition, when compared to CAS, a reduction of CO_2 emissions is expected, mediated by the capture of CO_2 by microalgae [22–24]. Additionally, the treated effluent from MBC is expected to meet the wastewater discharge and reuse standards [25,26].

However, despite the advances and the knowledge acquired in the past 70 years in the application of MBC, there is still a lack of fundamental knowledge regarding the complex interactions between microorganisms, and substantial challenges associated with biomass separation. In fact, the poor settling properties of biomass is one of the main limitations of the application of MBC [27]. Although some researchers have addressed this limitation by applying different biomass retention technologies (e.g. biomass immobilization, membrane bioreactors, etc.), more research is still required [28].

On the other hand, several works published in the last decade dealing with MBC are devoted to treating different types of wastewaters, and literature on MBC keeps on growing steadily. In this context, a comprehensive analysis of the existing literature seems necessary to understand the current state of this research field, its advances, challenges, and opportunities.

To go beyond a standard state of the art review, the objective of this paper is to quantify and analyze reported research using bibliometric analysis. This tool has proven useful to analyze a scalable body of research literature, providing means for assessing and evaluating output of scientists, collaboration between groups and identification of key or emerging topics [29,30]. Then, this work is intended to provide a deep analysis of available literature dealing with the application of MBC to wastewater treatment, offering an in-depth synthesis of the most challenging topics.

2. MATERIAL AND METHODS

Bibliometric analysis enables the identification of the structure, characteristics, and trends for a given topic by exploring different bibliographic characteristics such as the number and type of documents, countries, institutions, keywords, citation analysis and using descriptive, impact, or/and relational indicators [31,32]. The input data to perform bibliometric analysis in scientific fields is typically provided by referential databases such as Web of Science (WoS). Based on our experience [33,34], a bibliometric analysis procedure was established, which is described in this section, along with the software tools used to generate diverse bibliometric results and trends.

2.1 Data collection

Data were collected up to December 2021, using the WoS Core Collection database, including the SCI-EXPANDED, SSCI, A&HCI, CPCI-S, CPCI-SSH, BKCI-S, BKCI-SSH, ESCI, CCR-EXPANDED, IC. WoS database was selected since it is considered as a source of high-quality scientific data and frequently used for bibliometric analysis [35–37].

2.2 Search strategy

The bibliometric analysis involves a search query (SQ) that includes the use of keywords, boolean operators, and wildcards. In this work, the SQ used was **"TOPIC: (((*alga*$(bacteri* OR activated sludge) AND (consort* OR symbio*) and (*wastewater OR sewage)))) NOT TITLE: (((*hydrogen OR "fuel cell" OR *ferment* OR *ethan*)))"**. Keywords involved in the SQ were searched in the fields title, abstract and keywords of the documents, using WoS database. In addition, the following document types were excluded from the analysis: early access, meeting abstract and editorial material, since they are not expected to provide a significant contribution to our research. The SQ used in this work was developed in order to provide documents related to MBC for wastewater treatment, excluding those that use the algal-based consortia for other purposes, such as the production of biofuels or biochemicals.

2.3 Frequency and network analysis and systematic review

After performing the SQ in WoS, metadata of all documents were obtained in the so-called local database. The following indicators and software were used to perform the bibliometric analysis.

- Bibexcel [38] was used for metadata standardization and the estimation of the local h-index impact indicator for countries [39,40]. The h-index was proposed by Hirsch [41] to characterize the scientific output of a researcher or country. It is defined as follows: "a scientist has index h if h of his/her N papers have at least h citations each and the other (Np - h) papers have ≤ h citations each". Thereby, the importance and broad impact of scientists' or country's cumulative research contribution could be addressed by using both the quantity (total documents) and quality (citation number) of publications. Moreover, the h-index was normalized (NH) as the ratio between the h-index and total documents. The NH ranges from 0 to 1 to describe the influence and impact of publications of each country. A value of 0 means that the country has an h-index equal to 0, i.e., none of its documents were cited. A value of 1 entail that the country has reached the maximum h-index.
- Microsoft Excel v.16.40 and PowerBI® v.2.87.762.0 were used to create a structured database for visualization purposes [42,43]. Descriptive indicators used in this work were the annual distribution of documents and citations, the performance of most productive countries, and normalized h-index (SH).

- VOSviewer v.1.6.16. was used to study the collaborative network of countries, and to elaborate the co-occurrence map of all keywords (author-defined keywords and keyword plus), using a frequency of 3 co-occurrences and a thesaurus file. The co-occurrence keyword map was performed using overlay visualization, with a weight or size of the circle representing the co-occurrence value and the color representing the average publication year. The clusterization in VOSviewer was performed by similarity [44].
- CitNetExplorer was used to analyse cited documents in the database and to facilitate systematic literature by identifying publications that are closely connected in terms of citation [45].

Based on the results provided by bibliometric data, publication trends, keywords co-occurrence evolution, and collaboration network were determined. Then, technical aspects related with the application of MBC for wastewater treatment were discussed.

3. BIBLIOMETRIC ANALYSIS

3.1 Annual distribution of documents and citations

In total, 416 documents were found in the local database, including reports from 1970 to 2021. The documents considered were articles (350 documents, corresponding to 84%), review articles (48 documents, corresponding to 12%), and proceeding papers or book chapters (18 documents, corresponding to 4 %). The distribution of documents by year is shown in Figure 1, along with their number of citations.

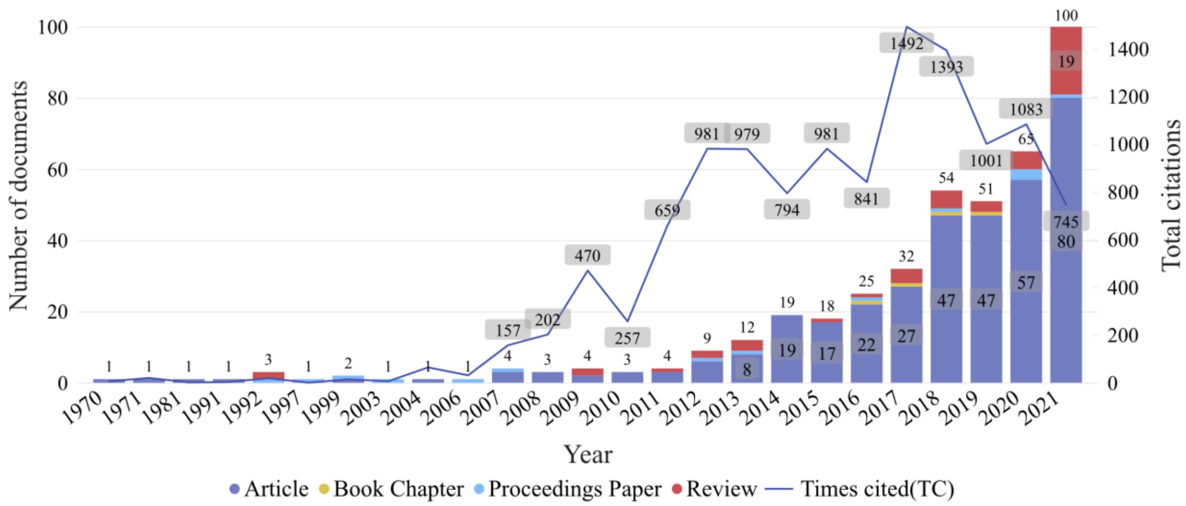


Figure 1. Yearly distribution of type of documents and number of citations dealing with the application of MBC for wastewater treatment from 1970 to 2021

The data presented in Figure 1 shows a growing interest in the study of MBC during the last decade. Three stages can be identified for bibliographic production and knowledge development. The first stage ranges from 1970 to 2012 and involves 40 documents (10 %) with an average annual frequency below 5 documents. Interestingly, the bibliographic production during this period is not continuous, as can be observed in Figure 1, which may be attributed to the use of microalgae in other research contexts (e.g., bioproducts or biofuels). The second stage ranges from 2013 to 2017 and involves 106 documents (25 %). Despite the shorter period involved, the second stage includes 2.7 times more documents than stage 1. The cumulative average growth rate is about 5 documents per year, only including a slight drop in 2015. The third stage ranges from 2018 to 2021. It involves 270 documents, corresponding to 65% of all the documents, showing that the use of MBC for wastewater treatment is becoming an attractive technology, attracting growing research efforts. The average frequency of review production increased from an average of 0.16 documents per year in the first stage to 1.8 in the second stage, and to 8 in the third stage. This increase may be interpreted as the result of a rapid build-up of knowledge on the subject during recent years. Similar trends were obtained by Qi et al. [46], highlighting the growing interest in applying MBC for wastewater treatment.

Several relevant reports that have contributed to the development of MBC-based wastewater treatment were identified by CitNetExplorer, based on their high citation score. The document with the highest citation score was published in 2006 by Muñoz and Guieysse [16], with 875 total citations, with 121 of them from documents included in the local database used in this bibliometric analysis. Review article from Muñoz and Guieysse [16] has contributed to conceptualize the potential use of MBC and photooxygenation for the removal of different pollutants during wastewater treatment. In addition, the application of different operational conditions and bioreactor configurations were discussed, as well as the promising use of microalgae as a feedstock to produce high-value chemicals or biogas. The second most cited article, with 240 citations (75 of them by documents included in the local database), was published in 2012 by Su et al. [47], who investigated the synergistic cooperation between algae (wastewater-born) and activated sludge, at different algae/sludge inoculation ratios. This paper revealed that an algae/sludge ratio of 5:1 showed the highest ammonium and phosphorus removal, assimilation being the main removal mechanisms of NH_4^+ in the consortia.

Several documents published during the third stage also deserve to be highlighted, based on the relevant number of citations received in such a short period of time. The most cited article is the one from Sepehri et al. [48] published in 2020, with 221 citations, reporting that aeration in the conventional nitrification process could be replaced by photo-oxygenation, achieving 100% of NH_4^+ removal at the ratio of 10% of microalgae (*Chlorella Vulgaris*) and 90% of nitrifying activated sludge (w/w) at a low C/N ratio. On the other hand, Su et al. (2012), reported that the removal mechanism of NH_4^+ was mainly governed by biomass assimilation. Therefore, several factors, such as wastewater characteristics and dominant microbial species, could change the mechanisms of removal as well as the optimal ratio of algae and bacteria.

The second most cited article during third stage is the one published by Mohsenpour et al. [49], with 117 citations. The authors reviewed the incorporation and importance of microalgae in wastewater treatment, as well as the cellular and metabolic mechanisms involved in nitrogen, phosphorus and organic matter removal. The third most cited article in the third stage was the one from Renuka et al. [50], with 115 citations. It reviews the potential of microalgae as biofertilizers in agriculture and how cultivation using waste-substrates is emerging as an economically viable strategy. The article also highlighted hat future efforts should be directed to the validation and commercialization of the biofertilizer. The fourth most cited article was the one from Ji et al. [51], with 88 citations. It studies the capacity of coexisting *Chlorella Vulgaris* and *Bacillus Licheniformis* for the removal of nitrogen, phosphorus and COD. Finally, the fifth most cited article corresponds to the published by Zhang et al. [52], with 87 citations, who explored the advances in granulation and how this process is enhanced by MBC (i.e., providing granular stability, improving nutrient removal efficiency, and biomass settleability). These reports and their high citation level provide an insight into the relevance and importance of topics involving MBC systems behavior, such as microalgae/bacteria ratio, granulation, biomass recovery, and valorization.

A comprehensive analysis of the citations showed low values until 2007, when a gradual rise was observed. The highest number of citations occurred in 2017 and 2012, with 1492 and 981, respectively. Unusually, a topic that has been researched for more than 50 years presents the largest number of citations is in the last 10 years. The observed citations evolution could be considered as an indicator of two phenomena: First, the popularization of MBC for wastewater treatment by the end of the first stage, resulting in fast consolidation of knowledge [27,53], and second, the application, improvement, and valorization of biomass from MBC, especially during the third stage [50,54,55].

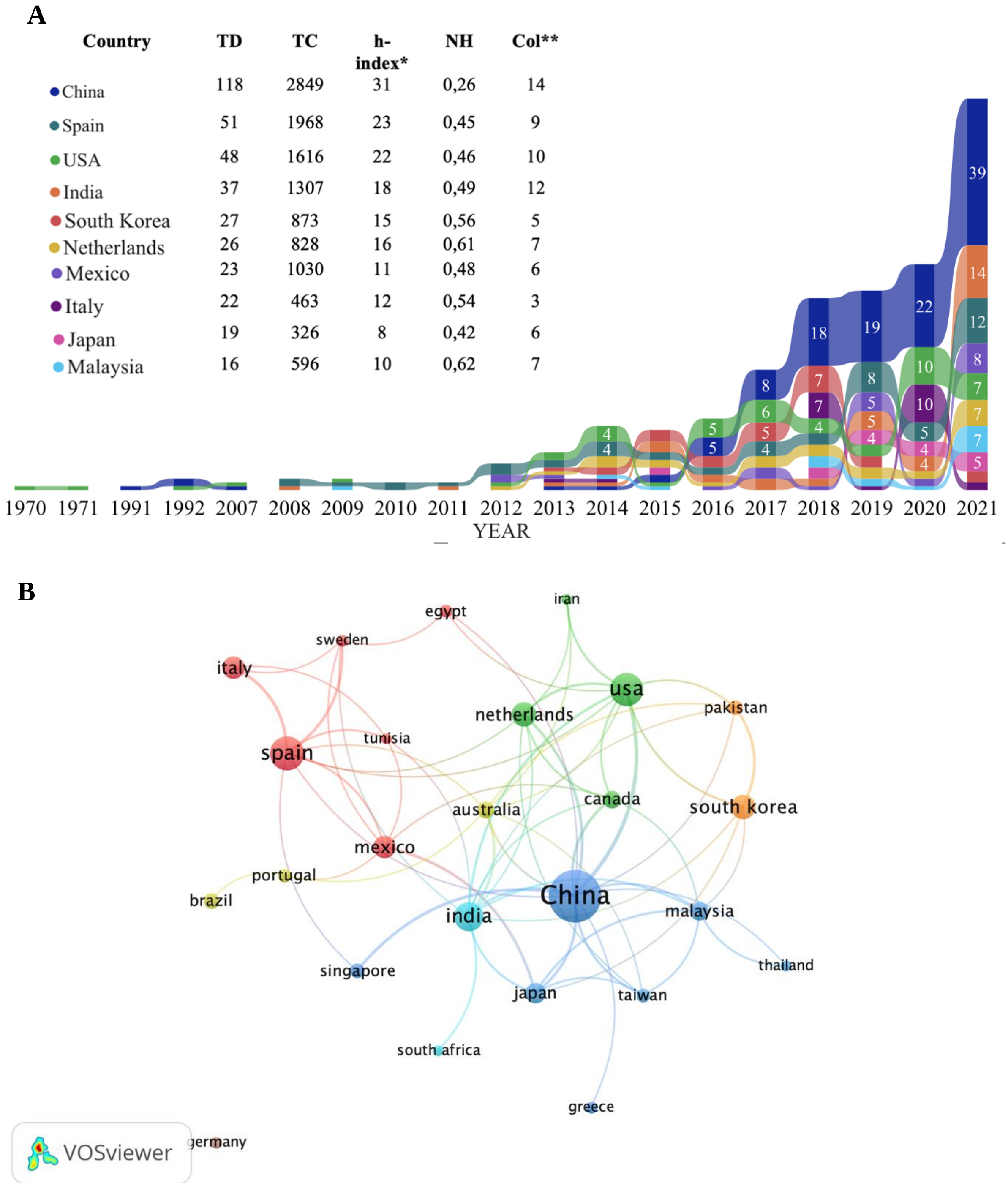
3.2 Most productive and collaborative countries

A total of 60 countries have contributed to the available literature in MBC for wastewater treatment, between 1970 and 2021. Figure 2 **(A)** shows the performance of the 10 countries showing the highest productivities from 1970 to 2021. Information on total documents (TD), total citations (TC), international collaboration (Col), h-index and normalized h-index (NH) is provided. The countries that have the largest contribution are China, USA, and Spain, with 118 (28,3%), 51 (12,3%), and 48 (11,5%) documents, respectively.

However, when considering the most productive countries during 2020 and 2021, few emerging countries appear: Italy with 10 documents during 2020, India with 14 documents during 2021 and Mexico with 8 documents during 2021. Despite China ranking 1st place in bibliographic production from 1970 to 2021, it has the lowest NH (0.26), suggesting a modest impact. On the other hand, HS values for Spain, USA, Italy, and India are 0.45, 0.46, 0.54, and 0.49 respectively. Meanwhile, countries with lower productivity, such as Malaysia, Mexico, Netherlands, and South Korea, have the most significant impact (high NH), probably due to their collaboration with the most productive countries.

The topic of MBC for wastewater treatment is being developed in a collaborative network, as it is shown in **Figure 2 (B)**, where the 25 most productive countries were grouped into 8 clusters, using VOSviewer. All clusters show interconnection, except for Germany, which appears isolated. The closer each cluster and country are located to each other, the stronger their collaboration relatedness. China shows the highest international cooperation, showing interaction with 14 of the 25 countries included in the collaboration network (**Figure 2 A, col**). USA and China have the highest joint collaboration, with 12 documents, followed by Spain-Italy (6) China-Singapore (6), Canada-Sweden (5), and Japan-Mexico (5). A strong collaboration between USA and countries with low productivity but high impact (high NS) is observed (for example with The Netherlands and South Korea) (**Figure 2 B**). Although an interconnected network can be identified, collaboration could be considered low, if the total number of documents and countries contributing to MBC research is considered. Improving the collaboration between countries with different levels of experience and productivity would undoubtedly promote the transfer of knowledge and technology, positively impacting and enhancing research.

The analysis also reveals another interesting aspect of the processes for wastewater treatment involving microalgae: the topics on which each country has focused. China has studied lipid and biomass production using aerobic granular biomass [56–58], biofilm bioreactors [59,60], and membrane bioreactors [61–63]. China was the first country to conduct studies combining membranes and MBC for wastewater treatment, addressing membrane performance and fouling [63,64]. USA has researched the effect of parameters such as biomass inoculation, carbon-nitrogen ratio (C/N), and incident light on MBC-based wastewater treatment [48,65,66]. Italy has focused on photosynthesis and respiration models [67,68], respirometry tests to evaluate the activity of consortia [69,70], mixotrophic metabolism of microalgae [71], and studies involving sewage and outdoor pilot raceways [72,73].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Country	TD	TC	h-index*	NH	Col**
China	118	2849	31	0,26	14
Spain	51	1968	23	0,45	9
USA	48	1616	22	0,46	10
India	37	1307	18	0,49	12
South Korea	27	873	15	0,56	5
Netherlands	26	828	16	0,61	7
Mexico	23	1030	11	0,48	6
Italy	22	463	12	0,54	3
Japan	19	326	8	0,42	6
Malaysia	16	596	10	0,62	7

Figure 2. Contribution of countries on MBC field from 1970 to 2021 (A) Information of the 10 countries showing the highest bibliographic productivity: Total documents (TD), total citations (TC), h-index, NH and international collaboration (Col). * Local h-index calculated using BibExcel. **International collaboration determined using VOSviewer (B) Collaboration network between countries presented using VOSviewer. Each circle represents one country, and its size is proportional to the number of published documents

(TD). Clusters are identified by different colors.

Data shows a low presence of Latin American countries on the revised scientific literature dealing with MBC for wastewater treatment. This region contributed with only 12% of the bibliographic production (50 documents) and included countries such as Brazil, Nicaragua, Bolivia, Chile, Honduras, Argentina, and Colombia. Interestingly, Latin America is a region where the application of MBC for wastewater treatment may represent an alternative to cope with the lack of access to proper sanitation in medium and small communities. One could expect the rapid development of research in this geographic area in the future, which may be enhanced by the opportunities provided by collaborative work.

3.3 Evolution of MBC research for wastewater treatment

The evolution of the research on MBC for wastewater treatment was addressed using all keywords co-occurrence network map over time. CitNetExplorer was used to analyze the cited documents. A total of 1932 keywords were found, which were reduced to 49, using a thesaurus and a minimum of co-occurrence of 5 times, as is shown in **Figure 3**. CitNetExplorer showed how studies on MBC for wastewater treatment dated back more than 60 years. The keywords *"activated sludge", "carbon and nutrient removal"*, *"high-rate algal ponds (HRAPs)"*, and *"photo-oxygenation"* are strongly related to the oldest author cited. Oswald and his research group proposed the use of HRAPs already in 1957 [74]. They proposed for the first time the symbiosis of MBC as a platform for wastewater treatment, reporting COD, nitrogen, and phosphorus removals in the range of 70-90%, 50-90%, and 60-99%, respectively [75–81].

Between 2014 and 2017, the use of MBC was intensified. The emergence of keywords such as *"piggery wastewater"*, *"municipal wastewater"*, and *"domestic wastewater"* shows a speciation of the scientific community towards different technologies depending on the wastewater [82–84]. In the last decade, some strategies have been adopted to address the low settleability of microalgae. This has been identified as one of the most significant limitations of MBC, resulting in unclarified effluents and the need for high HRT and footprints [14,85]. The keywords *"bioflocculation"*, *"biofilm photobioreactor"* [86,87], *"aerobic granular sludge"* [58,88]*)*, *"immobilization"* [89,90], *"membrane photobioreactor"* and *"EPS"* [62,63] refer to reactor configuration, biomass recovery and biomass structure to improve the *"settleability"* of biomass.

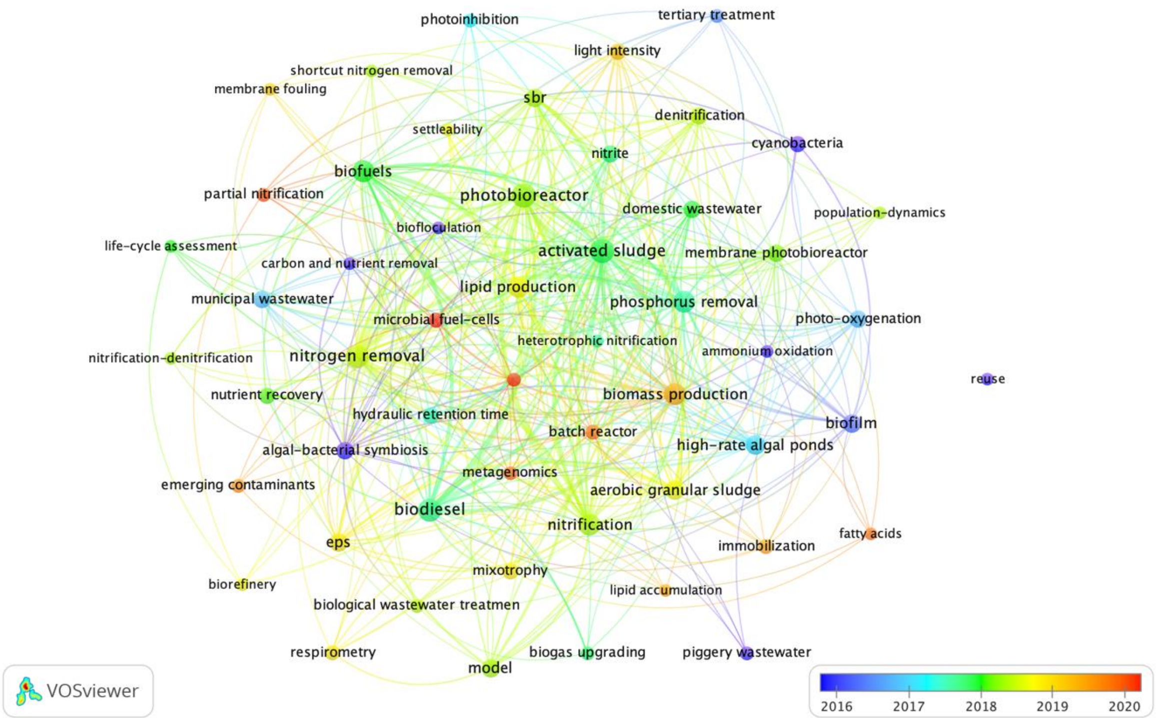

Figure 3. All keyword co-occurrence network map from 1970 to 2021, elaborated using VOSviewer software. Each circle represents one keywords, its size is related to the number of co-occurrences and the color represent the publication year average.

Figure 3 shows how the keywords *"biodiesel", "biofuels", "phosphorus removal"*, and *"nutrient recovery"* reinforce the established structure of the second stage (2013-2017): interactions between microorganisms leading to nutrients removal and biofuels production. This is well fitted with the work published by Olguín [27], with 277 citations at the end of the first stage (Figure 1), where the author defined the dual purpose of microalgae-bacteria based systems to treat wastewater and concomitantly produce biodiesel and chemical products within a biorefinery concept.

The main topics characterizing the current research streams can be identified using the co-occurrence of the keywords for the articles published during stage 3 (2018-2021). Keywords such as "*metagenomic*" [91,92], *"model"* [93–96] and *"respirometry"* [67,69] are related with the efforts oriented to analyze the diversity of microbial consortium, the use of mathematical models describing the complexity of the involved biological processes, and the use of respirometry-based techniques to obtain kinetics parameters of the consortium. In addition, "*membrane bioreactor*"[97,98], "*nitrification-denitrification*" [99], "*shortcut nitrogen removal"* [100], and *"partial nitrification"* keywords are related to emergent bioreactor configurations using physical biomass retention, and nutrient removal mechanisms. On the other hand, keywords such as *"biogas upgrading"* [101,102] *and "microbial fuel cells"* [103] highlight the new directions of the potential use of microalgae, coupling wastewater treatment with other processes, such as CO_2 sequestration and bioelectricity production.

Finally, this bibliometric analysis offers a general perspective of the topics explored during the last 4 years, that were clustered into 4 main categories: microbial interactions, nutrient removal mechanisms, mathematical modelling, and biomass retention technologies. The next sections synthesise the state of the art along these four cross-cutting research streams.

4. INTERACTIONS BETWEEN MICROALGAE AND BACTERIA

Interactions between microalgae and bacteria have been explored for over 70 years [12,81] when terms such as "*photo-oxygenation*" were introduced. As shown in **Figure 4**, the microbial metabolisms coexisting in MBC are photoautotrophic, heterotrophic, and autotrophic [104,105]. The most characteristic interaction between algae and bacteria is the O_2/CO_2 transfer. Microalgae produce O_2 (photo-oxygenation) as a consequence of the photosynthetic process, which is then used by HB and NB to oxidize organic matter and ammoniacal nitrogen, respectively [13,16]. The photo-oxygenation drives, therefore, the availability in the culture broth of O_2, a key electron acceptor during nitrogen and organic matter oxidation [12,106]. Moreover, HB releases CO_2 that is later used by microalgae and AB as a carbon source [14].

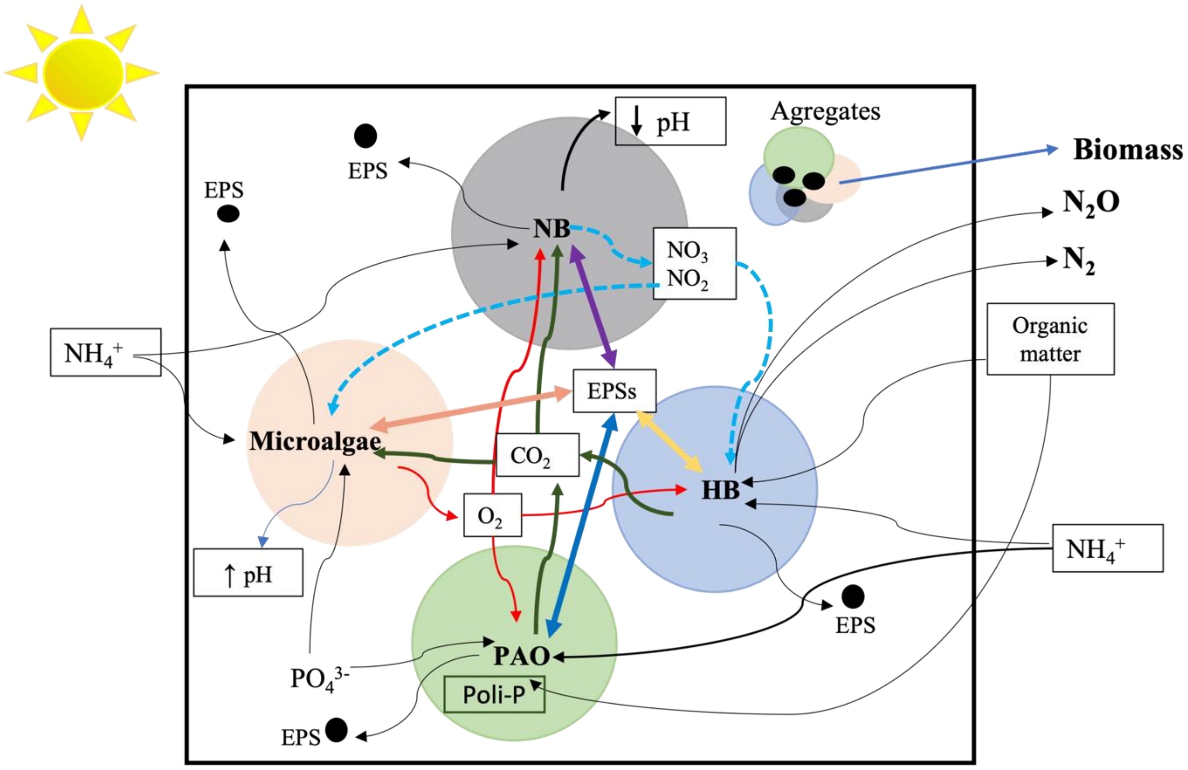

Figure 4. Interactions within MBC. Adapted from Yang et al. [28]

In addition to the above-mentioned O_2/CO_2 exchange, soluble metabolite exchange also plays an essential role in the removal of pollutants. Nitrogen in domestic wastewater is typically found as urea, and ammonia (NH_4^+ and NH_3) and is further oxidized to NO_2^- and NO_3^- by NB. The nitrogen forms that microalga can assimilate are NO_3^-, NO_2^- and NH_4^+. They are catalysed by different enzymatic transformations from NO_3^- to NH_4^+ and finally to the organic nitrogen present in amino acids. The enzymes that act in the nitrogen assimilation process in microalgae are nitrate and nitrite reductase to transform NO_3^- into NO_2^- and into NH_4^+, respectively, and glutamine synthetase to convert NH_4^+ into organic nitrogen [20,107,108]. Nitrate and nitrite reductase enzymes are inhibited at high ammonium concentrations since microalgae prefer NH_4^+ as a nitrogen source due to the lower energy required for its assimilation [53,109]. On the other hand, the activity of denitrifying heterotrophic bacteria has been evidenced under conditions of low O_2 concentration (typically during the dark phase and for low oxygen transfer rate) [60,93,99,100,110]. Phosphorus (PO_4^{3-}) can also be removed from wastewater by both microalgae and bacteria through assimilation in the biomass [111,112].

Cell to cell communication has also been reported as a relevant mechanism for microbial interaction. It involves the intracellular exchange of small signal compounds between bacteria, and the response of microalgae to these signals have been recently also reported in MBC cultures [113]. This cell to cell communication mechanism is normally referred to as quorum sensing, and it is density-cell dependent [114,115]. In MBC, the quorum sensing signals play a crucial role since it can determine the stimulation or inhibition of microorganisms present in the consortium [116–119], the formation of biofilm or aggregates [120–122], the processing and transformation of nutrients [123,124], and lipid synthesis [125,126]. The secretion by microalgae or bacteria of toxic substances, such as algicidal or bactericidal compounds, could be non-beneficial for the culture [21,91,127,128]. For instance, it has been reported that a concentration of 19,45 µg L^{-1} of urocanic acid, excreted by bacteria, negatively affects the activity of microalgae *P. Globosa, P. Donghaiense, H. Akashiwo,* and *S. Costatum* [59]. The secretion of algicidal substances by bacteria are triggered by bacterial cell concentration rather than the detection of microalgae by bacteria [114]. Some reported algicidal compounds are amylase, 2-undecen-1′-yl-4-quinolone, 2-undecyl-4-quinolone indole-3-acetic acid and octadecenoic acid [128]. Moreover, bactericidal substances such as chlorellin, secreted by microalgae, exhibit an inhibitory effect on both Gram-positive and Gram-negative bacteria [21,116,129]. Others compounds reported as relevant for cell to cell communication are indole-3-acetic acid (phytohormone) that stimulate the metabolism of microalgae, enhancing the interaction between microalgae and bacteria [118,130,131]. Likewise, vitamins produced by bacteria such as thiamine pyrophosphate and 4-amino-5-hydroxymethyl-2-methylpyrimidine were found to promote the growth, lipid content, and glucose uptake of *A. Protothecoides* [132]. Moreover, vitamins secreted by bacteria such as B12, B1 and B7 are required for supported the growth of microalgae in the MBC culture [133,134].

Excretion of extracellular polymeric substances (EPS) has been reported as a relevant factor triggering floc formation in MBC, acting as a biological glue, promoting the agglomeration of microalgae and bacteria, and thus improving its settleability [92,135]. The secretion of EPS have also been correlated with quorum sensing acyl-homoserine lactones secreted by bacteria [120,136]. For example, Zhou et al. 2017 [121] reported self-aggregation of *Chlorophyta sp* in 200 µm bioflocs by secreting 460−1000 kDa of aromatic proteins, upon interacting with N-acyl-homoserine lactones.

Gene transfer is another mechanism of microbial that may play a role in the development of MBC. It implies the change on gene expression of individual organisms during the interaction in the consortia. These changes may play a role in microalgae adaptation to changes on environmental conditions [133,137].

Thus, studying the interactions between microalgae and bacteria is of utmost importance to explore and investigate how to better manage their dynamics, and enhance the pollutant removal mechanisms efficiency as well as the quality and quantity of sludge generated during wastewater treatment [118,138].

5. NUTRIENT REMOVAL MECHANISMS

The keywords *"Phosphorus removal"* and *"nitrogen removal"* were identified as relevant terms when analyzing available literature on the application of MBC municipal wastewater treatment (**Figure 4)**. MBC can provide a simultaneous removal of carbon, phosphorus, and nitrogen in a single-step process. Many authors agree that the removal of nutrients provided by

MBC are higher than those provided by algae and bacteria alone [139,140]. Nutrient removal has been approached in different ways. Several mechanisms for the removal of phosphorus and nitrogen in MBC have been identified. Such variety can be considered as an advantage since it provides options or alternatives. However, it also represents a challenge since it involves complexity for understanding, controlling, or simulating the process.

5.1 Phosphorus removal

The mechanisms reported for phosphorus removal in MBC are precipitation and assimilation by microalgal and/or bacterial biomass. Phosphorus precipitation is an indirect removal mechanism induced at high pH values (pH>8), where the pH increase is mainly attributed to the microalgal activity. In this context, some authors have highlighted the importance of Ca^{++} availability in phosphorus precipitation, Mg^{++} playing a minor role in the precipitation process [75,141].

Phosphorus removal through assimilation in microalgae could be limited by unbalanced nitrogen/phosphorus (N/P) ratio in wastewaters. ***Equation 1*** shows the stoichiometric growth of microalgae on NH_4^+, PO_4^- and CO_2, assuming a biomass composition of $C_1H_{1.78}N_{0.12}O_{0.36}P_{0.01}$ [142]. Then, it could be expected that wastewater with an N/P ratio close to 12 would be required for N and P removal without nutrient limitation.

Nonetheless, some authors have observed an optimal N/P ratio between 5 and 12 for nutrient removal, using municipal wastewater [76,142]. The range of N/P can vary since the optimal N/P ratio in microalgae biomass is flexible, according to the cell stoichiometry and strongly depends both on the species of microalgae and their capacity of performing luxury phosphorus uptake [63,143,144]. When the N/P ratio is lower than the optimal maximum for biomass production, phosphorus removal decreases due to nitrogen limitation [79,145,146]. Thus, in MBC systems where the nutrients are shared (Figure 5), since many nitrogen removal mechanisms can take place (e.g., nitrification or ammonia volatilization), it is of utmost importance to guarantee the availability of NH_4^+ for efficient PO_4^- removal [147].

$$CO_2 + 0.12NH_4^+ + 0.01H_2PO_4^- + 0.6H_2O \rightarrow 1.19O_2 + 0.11H^+ + 1C_1H_{1.78}N_{0.12}O_{0.36}P_{0.01}$$
Equation 1

Concerning the microalgae luxury uptake of phosphorus, it has been reported that during light conditions and a preceding phosphorus starvation, the accumulation of larger polyphosphate granules is triggered [148]. The maximum content of phosphorus in microalgae can be up to 3.8 mg P mg^{-1} of dry biomass, about four times the maximum standard content under balanced microalgal metabolism (Equation 1) [149,150]. The most common microalgae performing luxury uptake in waste stabilization ponds and HRAPs have been reported to be *Chlamydomonas*/*Cryptomonas*, *Micractinium*/*Microcystis*, and *Scenedesmus* [149,151]. However, the luxury phosphorus uptake mechanisms must be further explored, considering their complexity.

Another mechanism for PO_4^{3-} removal is the one involving the activity of polyphosphate-accumulating organisms (PAOs), which has not been widely explored in MBC systems (Figure 5.). The accumulation of phosphate through PAOs is achieved in two environments, one anaerobic followed by one aerobic/anoxic. The PAOs uptake and store organic carbon such as acetate and propionate anaerobically as polyhydroxyalkanoate (PHA), with polyphosphate as the energy source [152]. In anaerobic environments, organic carbon decreases while soluble PO_4^{3-} increases. In the subsequent aerobic/anoxic environment PAOs oxidize the stored PHA, which is used as energy for growth and restore internal polyphosphate. During aerobic/anoxic environment, the PO_4^{3-} decreases; through this cyclical process, more P is stored than was released, resulting in the net removal of phosphorus [153,154]. In MBC systems, Huang et al.

[155] studied the effect of algae growth on aerobic granulation and nutrient removal. They observed inhibition of PAOs due to free nitrous acid (FNA) accumulation (6.3–9.8×10^{-3} mg HNO_2-N L^{-1}), obtaining a total phosphorus removal not higher than 44%. Furthermore, PAOs activity may be limited due to the high NO_3^- concentrations typically found in MBC systems, which hamper the occurrence of anaerobic conditions affecting the release of PO_4^{3-} and subsequently the uptake of phosphate [156].

In addition, recent works have reported indicating that a low carbon/nitrogen ratio will provide low organic carbon to HB growth, including PAOs [58,157]. Then, just under some specific environmental conditions, PAOs may play a significant role in phosphorus removal when the MBC system is used [158]. Due to the complex environmental condition where PAOs act as the main actor in phosphorus removal, some researchers have found them playing a role as phosphorus assimilation and growth promoters in microalgae [139,159].

5.2 Nitrogen Removal

During the first and second stages illustrated in **Figure 1**, the most common nitrogen removal mechanisms reported were the assimilation by microalgal biomass, nitrification/denitrification, and NH_3 volatilization. García et al. [160] reported 73% of total nitrogen (TN) removal at HRT of 10 days and 57% at HRT of 4 days. In both systems, ammonia stripping was the dominant mechanism over microalgal assimilation on TN removal. Moreover, denitrification was not considered, and nitrification was identified mainly in the HRAP working at lower HRT (4 days), contributing to NH_4^+ removal. Nurdogan and Oswald (1995) reported 90% of TN removal, which was mainly attributed to volatilization, especially in summer, when pH reached a maximum value of 11. The predominance of ammonia stripping mechanism has generally been found in HRAP systems as a result of high pH, usually higher than 9, due to the activity of microalgae [161,162]. In addition, some authors have highlighted that controlling pH at a lower level by supplying CO_2 can reduce the ammonia stripping in HRAP by favoring the ammonium form. Moreover, reducing inorganic carbon limitation, enhanced nitrogen recovery [26,163–165]. However, Ruas et al. [26] evaluated the domestic wastewater treatment with the supply of synthetic gas mixture (30% v/v CO_2), and the pH was maintained in the system between 6.8 to 7.7. The TN removal obtained was below 40% due to high nitrification activity combined with a lower pH, preventing ammonia stripping. Similarly, Karya et al. [13] reported low TN removal when treating synthetic wastewater using activated sludge and *Scenedesmus Quadricauda* as inoculum (pH-controlled at 7.5). Under the tested conditions, nitrification was the most relevant ammonia removal mechanism (81% - 85%), contributing to the low the TN removal obtained.

Furthermore, pH has indirect effects on the availability of toxic compounds such as free ammonia (FA) and FNA, where the role of pH indirectly determines the magnitude of inhibition by establishing the degree of dissociation of non-toxic NH_4^+ to toxic NH_3. For example, Valdivelu et al. [168] found that *Nitrobacter* was more sensitive than *Nitrosomonas* to FA. For *Nitrobacter*, the growth was completely inhibited at above 6.0 mg NH_3-N L^{-1}, and the capacity to oxidize NO_2^- to NO_3^- was reduced by 12 % at concentration of 6.0-10 mg NH_3-N L^{-1}; meanwhile, *Nitrosomonas* did not experience any inhibition at FA up to 16 mg NH_3-N L^{-1}. Indeed, nitratation process (NO_2^- to NO_3^-) seems to be the more sensitive to ammonia concentrations. Meanwhile, FA can also affect photosynthetic activity. For instance, a FA concentrations of 73 mg NH_3-N L^{-1} in MBC with dominant species of *Chlorella sp*. and *Scenedesmus sp.* can generate 50% inhibition of the photosynthetic activity and photooxygenation [169,170].

Likewise, FNA has a greater impact on *Nitrobacter* by completely stopping the growth process at concentrations of 0.02 mg HNO_2-N L^{-1} and inhibiting the energy yielding metabolisms at concentrations greater than 0.024 mg HNO_2-N L^{-1} [168]. Huang et al. [155] found that microalgae inhibit NB growth by competing for NH_4^+ substrate, which led to an accumulation of NO_2 and FNA (>28 mg HNO_2-N L^{-1}) in the effluent, thus limiting the nitratation and denitrification process. This resulted in low levels of TN removal (40.7% - 45.4%).

The key role of the influent alkalinity regulation to avoid strong inorganic carbon limitation has been recently highlighted in MBC systems [171]. CO_2 is the substrate of the Calvin cycle and strongly determines the microalgal growth rate [48,166,167]. It also determines growth of NB, since it is also their carbon source. Inorganic carbon limitation can affect nitrogen removal and is more often observed for wastewaters characterized by low biodegradable organic carbon contents (e.g., digestate). A low biodegradability will limit HB growth, reducing the CO_2 production [48,157]. Conditions of very low alkalinity results in intense competition for inorganic carbon between algae and nitrifiers (limited growth of photoautotrophic microorganisms), incomplete nitrification (high level of ammoniacal nitrogen in the effluent) and favorable conditions for N_2O production and emissions [171,174–176]. N_2O is a very potent greenhouse gas (GHG), with a global warming potential about 298 times higher than that of CO_2 [173].

A well-known mechanism for the oxidation of ammonium nitrogen by bacteria is complete or partial nitrification (nitritation), which requires further denitrification to achieve an effective total nitrogen removal. New processes have been identified in MBC when nitrogen removal is considered, starting by the end of the second stage (**Figure 1**). Keywords such as *"nitrification-denitrification"* and *"shortcut nitrogen removal"* were the most representative in the same time-lapse. Wang et al. [100] developed a novel shortcut nitrogen removal in a photo-sequencing batch reactor (PSBR) using an HRT of 4 days. Researchers obtained a TN removal of 90% without external aeration. As a result of the high concentrations of FA (2.4 mg L^{-1}) and FNA (0.013 mg L^{-1}) and low O_2, 80% of the TN removal was achieved by nitritation/denitritation with the addition of acetate as an external carbon source (ECS) during the dark period. Other researchers have used methanol as ECS for the same purpose [95]. In addition, Alcantará et al. [177] reported a new anoxic-aerobic photobioreactor process with biomass recycling that provided TN removal in the range 68-79%, when treating synthetic wastewater at an HRT of 2 days. In this system, the authors found that NH_4^+ nitritation was dominant in the aerobic photobioreactor reaching concentrations up to 20 mg NO_2-N L^{-1} at constant pH of 7.7. TN removal was mainly attributed to nitritation in the photobioreactor and denitritation in the anoxic tank. This unusual NO_2^- accumulation and inhibition of NO_3^- production was probably due to the high incident light (400 umol $m^{-2}L^{-1}$), which might have partially inhibited bacterial nitrifiers [178]. Manser et al. [179] proposed the "algamox" process, which consisted of TN removal through the conventional Anaerobic Ammonium Oxidation (anammox) process. The anammox process is based on the anaerobic oxidation of ammonia using nitrite as an electron acceptor, giving molecular nitrogen N_2 as a final product. The algammox process differs from conventional anammox in that the oxygen for NO_2^- production is provided by microalgae [179,180]. The algammox process was carried out in a photo-sequencing batch reactor, alternating periods of light and dark, favouring low DO concentrations, since anammox is inhibited by DO above 1.5 mg L^{-1} [181]. The results showed that ammonia was converted to nitrite during periods of light at a rate of 168 mg NH_4 $L^{-1}d^{-1,}$ and subsequently, an average of 82% of nitrite was reduced to N_2 during the dark (anoxic) period due to anammox activity.

6. MODELLING OF MICROALGAE BACTERIA CONSORTIA

Recently, mathematical modelling has taken an essential role in describing and predicting biological processes such as the MBC system, as we found in the analysis of the frequency of all keywords in Figure 3. Mathematical models are extremely powerful instruments that can be applied for managing existing biotechnologies processes, but also for studying, understanding, and optimizing new processes. Mathematical models have become increasingly attractive since, once calibrated and validated on a long-time scale, they make feasible the investigation of several scenarios and operational conditions. Therefore, the design and operation of MBC systems using models may involve several advantages, such as the reduction of operating costs, the increase in nutrients removal, and the valorization of the nutrients and energy contents of wastewaters.

The classic reference models describing biological processes for waste treatment are Activated Sludge Models (ASMs) [182] and the Anaerobic Digestion Model n°1 (ADM1) [183], which have been widely implemented [184,185]. To date, a large number of models describing microalgae-based bioprocess are available in the literature [186]. However, only a minority of them were developed to represent microalgae and bacteria behavior for wastewater treatment**.** International Water Association (IWA) introduced a standardized notation for models, including the use of a Petersen matrix. This standardized notation could favor the integration of MBC models with those describing existing water resource recovery facilities (ASMs and ADM) [187]. However, among the microalgae/bacteria reported models, there are only 10 that follows this standardized notation [93,94,96,188–195]. **Table 1** summarizes a comparison between the most recent MBC models available in the literature, for wastewater treatment. Some of the proposed models were developed with the purpose of estimating the biofuels/bioproducts production [196–202]. Among these models, only a few implemented both the phototrophic and heterotrophic metabolisms for the algal biomass, such as the ones proposed by Adesanya et al. [203] and Figueroa-Torres et al. [204]

On the other hand, the calibration and validation of mathematical models are crucial steps to produce reliable simulation results [186,205]. However, there is a lack of long-term validation dataset covering yearly seasonal variation (temperature, incident light, etc.) when working with MBC models for wastewater treatment. Only two works involves long-term seasonal study, the works published by Broekhuizen et al. [192] and by Casagli et al. [93].

Table 1. comparison between the most recent algae-bacteria models available in the literature for wastewater remediation

		RWQM1	PHOBIA	Modified RWQM1(D)	Modified ASM3	Bioalgae1	Bioalgae2	ALBA(D)	ABACO
Model structure/characteristics	**Reference**	[189]	[190]	[192]	[195]	[188]	[94]	[93]	**Z**[96]
	State variable (n°)	24	16	24	16	19	19	17	19
	Biological processes (n°)	22	13	22	21	18	18	19	18
	Parameters (n°)	120	75	138	47	94	108	72*	108
	Growth kinetic type	Multiplicative	Minimum	Multiplicative	Multiplicative	Multiplicative	Multiplicative	Multiplicative/ Minimum**	Multiplicative
	Dependence on organic and inorganic carbon	C_{ORG}	C_{ORG}, CO_2, HCO_3	C_{ORG}, CO_2, HCO_3, CO_3^{2-}	C_{ORG}	C_{ORG}, CO_2, HCO_3	C_{ORG}, CO_2, HCO_3	C_{ORG}, CO_2, HCO_3, CO_3^{2-}	C_{ORG}, CO_2, HCO_3
	Considered N-forms	NH_3, NH_4^+, NO_3^-, NO_2^-	NH_3^+, NO_3^-	NH_3, NH_4^+, NO_3^-, NO_2^-, N_2	NH_4^+, NO_3^-, NO_2^-	NH_3, NH_4^+, NO_3^-, NO_2^-	NH_3, NH_4^+, NO_3^-, NO_2^-	N_{org}, NH_3, NH_4^+, NO_3^-, NO_2^- HNO_2, HNO_3, N_2	NH_4^+, NO_3^-, NO_2^-
	Considered P-forms	$H_2PO_4^-$, HPO_4^{2-}	-	$H_2PO_4^-$, HPO_4^{2-}	-	SPO4 (***)	SPO4 (****)	H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}	SPO4 (****)
	Continuity check (mass conservation)	C, O, N, P	(n.s.)	C, O, N, P	(COD, N, P.)	(n.s.)	(n.s.)	C, H, O, N, P, COD	(n.s.)
	Algal biomass composition	$C_{100}H_{232}O_{26}N_{14}P$	(n.s.)	$C_{100}H_{232}O_{26}N_{14}P$	$C_{106}H_{181}O_{45}N_{16}P$	$C_{100}H_{232}O_{26}N_{14}P$	$C_{100}H_{232}O_{26}N_{14}P$	$C_{100}H_{183}O_{48}N_{11}P$	$C_{100}H_{232}O_{26}N_{14}P$
	Bacterial biomass composition	$C_{150}H_{335}O_{13}N_{30}P$	(n.s.)	$C_{150}H_{335}O_{13}N_{30}P$	$C_5H_7O_2N$	$C_{150}H_{335}O_{13}N_{30}P$	$C_{150}H_{335}O_{13}N_{30}P$	$C_{60}H_{87}O_{23}N_{12}P$	$C_{150}H_{335}O_{13}N_{30}P$
	PAR model	Steele	Eilers & Peters	Smith	Poisson	Eilers & Peters	Eilers & Peters	Bernard & Remond	Molina
	pH model	NH_4^+, NH_3, CO_2, HCO_3 CO_3^{2-}, $H_2PO_4^-$, HPO_4^{2-}, Ca^{2+}, H^+, OH^-	NH_4^+, NH_3, CO_2, HCO_3 CO_3^{2-}, H^+, OH^-, $\Delta_{CAT,AN}$	NH_4^+, NH_3, CO_2, HCO_3 CO_3^{2-}, $H_2PO_4^-$, HPO_4^{2-}, Ca^{2+}, H^+, OH^-	-	NH_4^+, NH_3, CO_2, HCO_3 CO_3^{2-}, H^+, OH^-	NH_4^+, NH_3, CO_2, HCO_3 CO_3^{2-}, H^+, OH^-	NH_4^+, NH_3, CO_2, HCO_3 CO_3^{2-}, H_3PO_4, $H_2PO_4^-$, HPO_4^{2-}, PO_4^{3-}, NO_2^-, HNO_2, NO_3^-, HNO_3, H^+, OH^-, $\Delta_{CAT,AN}$, TA	-

	pH growth dependence	-	-	Gaussian law	-	-	CPMI	CPM	CPMI
	Temperature simulation/ growth dependence	-/Arrhenius	-	-/Arrhenius	-	-/Arrhenius	-/CTMI	✓/CTMI	-/CTMI
	Ammonification	-	-	-	-	-	-	✓	-
	DO inhibition	-	-	-	-	✓	✓	✓	✓
	NH_3 inhibition	-	-	-	-	-	-	✓	-
	Gas-liquid mass transfer	O_2	-	O_2	-	O_2, CO_2, NH_3	O_2, CO_2, NH_3	O_2, CO_2, NH_3, evaporation	-
Experimental setup	Reactor type	River environment	Laboratory incubator	Raceway	Column PBR	Raceway	Column PBR	Raceway	Column PBR
	Reactor installation/Volume	Outdoor	Indoor(Lab) /3L	Outdoor/8 m^3	Indoor(Lab)/2 L	Outdoor/1 m^3	Indoor(Lab)/4 L	Outdoor/17 m^3, 1 m^3	Indoor(Lab)/1 L
	Influent	Wastewater discharge	MM	MWW	DSC	MWW	MWW	SWW, DSC	DSC
	Calibration dataset	-	-	365 d	✓ (24 h)	✓ (4 d)	✓ (8 d)	✓ (30 d)	✓ (14 d)
	short-term dynamics	-	-		✓ (24 h)	✓ (4 d)	n.s.	✓ 3, 14 d	✓ (14 d)
	long-term dynamics	-	-	330 d	-	✓ (175 d)	-	✓ (413 d, 189 d)	-
Validation	Sensitivity analysis	✓	✓	-	✓	✓	-	✓	-
	Seasonal analysis	-	-	-	-	-	-	✓	-
	Parameter Uncertainty	✓	-	-	✓	-	-	✓	-
	Confidence intervals for model predictions	✓	-	-	-	-	-	✓	-

Abbreviations: ✓: implemented; (n.s.) not specified or provided in the relative publications; IC: Inorganic Carbon; DSC: Diluted Swine Centrate; MM: Mineral Medium; MWW: Municipal Wastewater; SWW: Synthetic Municipal Wastewater; TA: Total Alkalinity; CTMI: Cardinal Temperature Model with Inflection; CPMI: Cardinal pH Model with Inflection; CPM: Cardinal pH Model. Notes: (D) demonstrative scale reactors (> 5 m^3); (*) not including chemical constants, their temperature dependence, and stoichiometric coefficients); (**) in the ALBA model, only the Monod limitation terms relative to nutrients availability were implemented in the minimum function, while the dependence on inhibitory and environmental factors are multiplied for the minimum term (in the PHOBIA model, all the multiplicative terms considered are included in the minimum function); (***) P limitation term only on algae; (****) P limitation term on algae and bacteria

7. BIOMASS RETENTION TECHNOLOGIES

The bibliometric analysis highlights two factors driving the research oriented towards the development or application of biomass retention technologies:

1. The low settling capacity of microalgae, which still represents a major challenge when they are used as a technological platform, due to the high separation and recovery costs, and the need to guarantee a good effluent quality during wastewater treatment [27].

2. The consolidation of solid retention time (SRT) as a key operational parameter, since it affects the type of microorganisms developing in the system, and consequently the nutrient removal [99,195,206].

As shown in **Figure 3**, the research targeting the development of new reactor configurations has boosted from 2014 onwards, mainly to address biomass retention [62,86]. MBC biomass is typically separated by a secondary settler or by applying the sequential batch operation mode, as highlighted by the keywords *"SBR,"* which showed the highest average frequency in 2018. Indeed, biomass is separated from the liquid phase through gravity sedimentation during a non-aerated or non-mixed phase under SBR operation mode. However, a higher level of automation is required in SBR systems, and separation strongly depends on the biomass aggregation level (flocs). Keywords such as "*biofilm", "immobilization", "membrane photobioreactor", and "aerobic granular sludge"* increased during the last years, thus resulting in new reactor designs (**Figure 3**). De Godos et al. [176] explored a new configuration based on the concept of denitrification-nitrification CAS with a low C/N ratio, internal recycling (NO_3^-), and external recycling (biomass) that allowed the decoupling of the HRT and SRT. The system consisted of two interconnected tanks: an anoxic tank followed by an aerobic photobioreactor inoculated with cyanobacteria-bacteria. Results showed high organic carbon (>95%) and TN (>90%) removal using the photobioreactor alone at HRT of 3.5 days and SRT of 31 d^{-1}, and using the two-stage anoxic-aerobic configuration at HRT 4.5 days and SRT of 20 d^{-1}. Furthermore, the settler system located after the photobioreactor led to an effluent with 25 ± 19 mg L^{-1} of volatile suspended solids, concentration below the European discharge limits. On the other hand, Steen et al. [207] also applied the concept of the CAS, coupling a mixed photobioreactor inoculated with microalgae-bacteria with a settler, providing biomass recirculation to the reactor. The system was able to remove 85% of the applied ammonium load (66 mg L^{-1} d^{-1}) at HRT of 1 day and SRT of 15 d.

Several biofilm-based microalgal bacterial cultivation systems have been reported in recent years, for environmental remediation and biofuel production purposes [208–210]. De Godos et al. [87] engineered an innovative enclosed tubular biofilm photobioreactor inoculated with *Chlorella Sorokiniana* and activated sludge to treat pre-treated swine slurry (7 days of HRT). The system supported removal efficiencies for nitrogen and phosphorus ranging from 94% to 100% and 70% to 90%. The retained biomass was greater than 92% of the generated biomass. Interestingly, *Chlorella Sorokiniana* was dominant throughout the entire experiment. On the other hand, designs such as the rocker system using polystyrene foam as support material [210], the rotating algal disk [211], the rotating rope reactor with spool harvester partially submerged in wastewater [212] and the attached cultivation reactor using glass plate and filter paper as a substratum [213] are among the biofilm-based system's configuration described in the literature. The biofilm approach seems to improve the sustainability and the productivity of the microalgae production systems [214]. Compared to conventional open ponds, enhanced biomass productivities have been shown using biofilm-based microalgal cultivation. For instance, Christenson and Sims [212] reported the tertiary wastewater treatment in an enhanced raceway bioreactor using five rotating biological contactors (RBC), close to the paddlewheel of the bioreactor. The five RBC used cotton cords as biofilm support and were 40% submerged in the reactor. With an HRT ranging from 3 to 6 days, an average removal of TN and total phosphorus were 76 % and 23 %, respectively. The achieved biomass productivity was 5.5 g m^{-2} d^{-1} at laboratory scale and 31 g m^{-2} d^{-1} at pilot scale, values higher than those for conventional raceway ponds. In addition, other studies that explored the configuration of biofilm-based systems have reported productivities up to 302% [208], 697% [215] and 700% [216] of the ones expected for traditional raceway ponds.

Researchers from Brazil have worked with hybrid HRAP systems, consisting of the combination of HRAP with a biofilm unit [217,218]. In hybrid HRAP systems, additional carbon source supplementation (CO_2) was not necessary since biofilm provided better conditions for atmospheric CO_2 capture [217]. In addition, hybrid systems followed by a settling tank provided a biomass production 2.6 greater than that of a conventional HRAP followed by a settling tank. Moreover, biomass harvesting in the hybrid system was facilitated [218]. Recently, attached biofilms in carriers have been explored for MBC [59,60,112]. Gou et al. [59] observed a 90% COD and ammonia removal, reducing the HRT to 12 hours, applying a three-dimensional carrier that improved the efficiency by promoting the mass transfer from the wastewater to the biofilm.

Microalgal-bacterial granules have been lately explored to improve biomass settleability and enhance biomass harvesting. Granules formation depends on several factors, such as the inoculum, the composition and concentration of EPS as polysaccharides and proteins, wastewater characteristics, and environmental factors such as seasonal variability that contributes to the change of incident light and temperature [55,219,220]. For instance, Quijano et al. [219] evaluated the influence of light on EPS and granules formation while treating municipal wastewater, at an HRT of 10 days. Low irradiances levels (< 3800 Wh m^{-2} d−1) promoted the formation of microalgae-bacteria flocs and granules with high settleability (432 m d^{-1}) and relatively high removal efficiencies of TN (60 %) and total COD (89 %). Conversely, phosphorus removal did not experience the same trend at low irradiances, exhibiting removals of 92 % at 6213 Wh m^{-2} d^{-1} and 28% at incident light lower than 3800 Wh m^{-2} d^{-1}. Ji et al. (2020) used microalgal-bacterial granules for synthetic wastewater treatment and reported removal efficiencies of 93 %, 97% and 87% for organic matter, ammonia, and phosphorus, respectively; operating in cycles of 6 hours under non-aeration conditions. The most abundant microalgae found in this system was *Pantanalinema Rosaneae*.

The most recent bioreactor configuration explored for MBC is membrane bioreactors (MBR) [145]. MBRs have been widely explored for aerobic wastewater treatment as an alternative to biomass settling. In MBRs, the cultivation and removal of contaminants are carried out in a reactor, with a membrane system providing complete solids retention and providing a clarified effluent. HRTs as low as 8-24 hours have been reported, with fluxes in the range of 168-360 L m^{-2} d^{-1}, achieving high pollutant removal. In addition, easier operation, amelioration of microbial activity, and better pollutants removal have been achieved in MBR systems inoculated with microalgae and bacteria than in MBR systems inoculated with only activated sludge [62,63]. Furthermore, lower membrane fouling has been reported when operating with microalgae and bacteria consortia, in comparison with a system worked only with activated sludge [98]. In fact, the presence of microalgae in the inoculum is used to improve the biomass quality by transforming the EPS composition and thus improving floc filterability and also decreasing the cake-layer formation in the membrane [63,98].

All these biomass retention technologies have become an essential issue for efficient microalgae valorization. Regardless of the technology used for biomass retention in MBC systems, some researchers have emphasized the importance of carefully selecting the SRT and subsequently the biomass concentration, avoiding growth limitation by self-shading and thus low light availability for microalgae in the reactor [48,206].

8. FUTURE PERSPECTIVES

MBC has been already studied for more than 70 years, and the knowledge consolidation is getting faster. This literature research identified the current trends in the main research streams in MBC for wastewater treatment. The first identified trend is the elucidation of nutrient removal mechanisms in the MBC, under the influence of factors such as incident light, pH, temperature, and dissolved oxygen (DO). Future research studies should not only focus on the conditions that can guarantee the removal of nutrients and organic matter, but also establish the fluxes and symbiotic mechanisms determining process performance. For instance, the environmental and operational conditions that promote nitrogen assimilation over nitrification, and vice versa. In addition, more research is needed in new nitrogen removal mechanisms such as the “shortcut nitrogen removal” or “algammox”. Likewise, more attention should be paid to the removal mechanisms that promote “luxury” uptake of phosphorus by microalgae or the proliferation of PAOs. On the other hand, although MBC has the potential to reduce the direct and indirect emission of CO_2, microalgal-bacterial photobioreactors can lead to the release of NH_3 or the production of N_2O, because of inorganic carbon competition between nitrifiers and microalgae. Thus, these removal mechanisms must further be understood and evaluated together with the potential emissions of GHG.

Mathematical modeling represents an extremely powerful tool for studying, understanding, and optimizing microalgae-bacteria processes. The application of mathematical models must be promoted, since models are especially useful for managing the complexity and nonlinearities arising from the interactions among microalgae and bacteria and physical-chemical processes. involved in nutrients removal mechanisms and GHG emissions. On the other hand, long-term dataset validation must become a standard, to promote reliable mathematical representations that can represent the process over a wide range of potential scenarios and operational conditions.

Low microalgae settling capacity is another issue identified during literature analysis. This issue can hinder biomass separation by settling, which is the most used solid-liquid separation operation in wastewater treatment. Combination of membranes and photobioreactors is expected to bring new opportunities for biomass retention and an effluent clarification. Moreover, the evaluation of biomass properties affecting membrane fouling membrane is key issue. Furthermore, the use of photosynthetic biofilms represents an interesting alternative to facilitate biomass harvesting and valorization. Moreover, the application of different biofilm-based reactor configurations has shown potential for wastewater treatment and the enhancement of biomass productivity. Future works must focus on the strategies and operational conditions of these systems. Finally, more studies are necessary for both membrane and biofilm photobioreactors, optimizing the design and operating strategy and implementing these technologies at pilot and/or full scale.

9. CONCLUSIONS

- The mechanisms involved in the removal of nutrients and COD depend mainly on the interactions of microorganisms and, at the same time, on factors affecting these interactions, such as pH, light, dissolved oxygen, C/N/P ratio. These factors directly impact the chemical equilibria of pollutants and the presence of toxic compounds (e.g., free NH_3, HNO_2), and inhibitors. Further research is needed to assess the influence of these factors on interactions and removal mechanisms.
- Novel nutrient removal mechanisms such as "short-cut nitrogen removal," "algammox," and “phosphorus luxury uptake” have been proposed in the past decade. These could present an alternative to improve nutrients removal during wastewater treatment. However, more research is needed to evaluate the specific conditions in which these mechanisms are predominant.
- The application of mathematical modelling has been explored in MBC systems. This tool presents a high potential for the optimization of MBC systems. However, models need to correctly calibrated and validated.
- Different reactor configurations have been explored to decouple HRT from SRT, and to keep slow-growing microorganisms such as nitrifiers in the reactor. The membrane bioreactor and biofilm photobioreactors have shown high potential, presenting high removal levels of nutrients and organic matter, while facilizing biomass harvesting and reducing systems footprint. These emerging technologies must be thoroughly explored and evaluated, to determine potential full-scale applicability.
- More research efforts must be devoted to assessing the performance of MBC for wastewater treatment at a large scale, including the revalorization biomass and reuse of treated water, along with its socio-environmental and economic implications.
- MBC has been highlighted as a prominent wastewater technology that could compete with conventional systems. However, the main challenges associated are a high footprint, harvesting costs, and biomass processing to produce high-value products.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest concerning the research, authorship, and/or publication of this article

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Author contribution statements

Jineth Arango conceived the structure, wrote the manuscript, designed the SQ and the analysis with permanent feedback from David Jeison. Francesca Casagli, contributed to the structure of modelling section. Olivier Bernard, Francesca Casagli, Raúl Muñoz, and Andrés Donoso contributed to the technical aspects and the critical review of the document, as well as contributed to the structure and conceptualization of the document. All authors provided critical feedback and helped shaping the research, analysis, and manuscript writing.

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