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# A review on opportunities and challenges of nitrogen removal from wastewater using microalgae

**Dipti Thakur[1], Aditya Kumar Jha[1], Soham Chattopadhyay[2] and Sukalyan Chakraborty[1*]**

[1]Department of Civil and Environmental Engineering, Birla Institute of Technology, Mesra, Ranchi, Jharkhand, India; [2]Department of Bioengineering and Biotechnology, BIT Mesra, Ranchi, Jharkhand, India

**E-mail/ Orcid Id: DP,** diptithakurenvs@gmail.com; **AKJ,** ajha4783@gmail.com;
**SC,** soham@bitmesra.ac.in, https://orcid.org/0000-0002-3797-5333;
**SC,** sukalyanchakraborty@bitmesra.ac.in, https://orcid.org/0000-0002-6702-7238

***Corresponding Author:** sukalyanchakraborty@bitmesra.ac.in

**Abstract**

This study provides information on the occurrence of nitrogenous contamination in surface water, their sources and their negative effects. In addition, the study gives an overview of the possible technical, institutional and scientific methods of various biotechnological approaches of nitrogen removal and their limitations. Microalgae have a high capacity for converting $CO_2$ from the atmosphere into beneficial products, including carbohydrates, lipids, and other bioactive compounds. Microalgae act as biofuels that are sustainable, renewable, and cost-effective. The impact of various environmental conditions on the efficiency of nitrogenous waste removal by microalgal species was studied. Literature related to microalgae-based bioremediation has a large research area with increasing trend. A bibliometric study was undertaken Based on the Science Citation Index Expanded of the Web of Science, to examine the body of knowledge on microalgae generated nitrate removal from wastewater from the year 2011. A global map based on co-authorship and co-occurrence analysis for countries, research areas, authors and institutions is presented based on the bibliometric analysis method.

**Keywords:** Bibliographic analysis, bioremediation, denitrification, microalgae, nitrogenous waste.

## 1. Introduction

Nitrogen (N), the most important nutrient in the biosphere, is an important constituent of several bio-molecules such as DNA and proteins. Nitrogen exists as $N_2$ gas, ammonia ($NH_3$), ammonium ($NH_4^+$), nitrate ($NO_3^-$), nitrite ($NO_2^-$), and in nature and is used as a nutrient by a living organism, which ultimately ends up in wastewater (Blackburne et al., 2008). $NO_3^-$, which is stable in nature, can reduced to $NO_2^-$, an unstable and moderately reactive form, in the presence of carbon source (Appelo and

Postma., 1996). $NO_3^-$ also acts as a conjugate base of strong acid $HNO_3$ and is highly soluble and mobile in nature. It can easily move to groundwater and remain there for a long time and accumulates to a high concentration and contaminates the drinking water (Wellman et al., 2016). Agriculture is the primary source of nitrate generated during the production of artificial fertilizer using the Haber-Bosch process. Animal manure, cars, domestic waste water, failing septic tanks, industrial/sewage effluent, power plants, pet waste, and storm-water runoff, and also add a considerable volume of $NO_3^-$ into the environment (Diniz et al., 2017).

The large untreated fluxes of water contaminated with releasing reactive nitrate to receiving water body are the main contributor of surface or ground water pollution causing depletion of fresh water and ultimately enhances eutrophication, destruction of the ecosystem, making the water unsuitable for human consumption and leads to high energy demand for better treatment (Morée et al., 2013; Taziki et al., 2015). Drinking water with excessive $NO_3^-$ concentration can lead to health problems, especially infants, to cause methaemoglobinaemia by converting the iron in haemoglobin from $Fe^{2+}$ to $Fe^{3+}$, a weaker binding agent for oxygen (Brunato et al., 2003). In humans, $NO_3^-$ is absorbed by the small intestine and transferred to blood (Mensinga et al., 2003). It is rapidly distributed throughout the tissues and breast milk (Lundberg et al., 2004). Other health issues are cyanosis, diarrhoea, tachypnea and vomiting, respiratory problems, birth defects, and formation of carcinogenic compounds and cardiovascular and hypertension (Gupta et al., 2000; Manassaram et al., 2005; Bosman et al., 2009). Reproductive and developmental toxicity was observed in mice (Aly et al., 2010). Toxicity of nitrate is maximum with $LD_{50}$ values of 85 to 220 mg of sodium nitrate per kilogram of body weight was reported in mice and rats (WHO, 1996; Boink et al., 1999).

The overloading of nitrogenous compounds leads to excessive growth of algae, known as algal bloom or eutrophication and damages aquatic ecosystem (Taziki et al., 2015; Ritter et al., 2002). It decreases the oxygen level in the water and makes water cloudy. Some algal blooms release toxins that can cause illness and death of aquatic animals, coral reefs and other organisms like seabirds, Dolphins and many key species (Erisman et al., 2013). Due to these adverse effect of nitrate pollution in the environment, it is necessary to eliminate or decrease its toxicity level. There are many physical and chemical methods for removing nitrogen-containing compounds. These physicochemical methods require high maintenance and cost and create secondary waste, limiting the application of such processes (Rittmann and McCarty, 2012).

Methods such as chemical enhanced adsorption, coagulation, extraction, filtration, membrane filtration and photocatalytic degradation were used conventionally to remove nitrate from the effluent (Sun et al., 2019; Wang et al., 2018). The conventional method of nitrogen removal has several limitations, including membrane fouling sludge formation, slurry formation, partial degradation, formation of toxic by-products, low selectivity of nitrogen species, high operational and maintenance cost.

Microalgae are considered as low-cost and environment friendly alternative for the treatment of nitrate from wastewater. The main mechanism on which microalgae work is biosorption and bioconversion. Microalgae are a group of photosynthetic organisms that can grow very effectively in wastewater. Microalgae can clean and reduce growth media compounds in wastewater (Sydney et al., 2011). With the advancement of technology, photo-bioreactor and high-rated algal pond for microalgae cultivation have been developed for treatment of large quantity of wastewater (Lim et al., 2010).

The bibliometric methodology is a valuable scientific method for employing mathematical and statistical tools to access any given subject's growth and developing tendency. Bibliometric analysis focuses on assessing contributions and partnerships across different nations, institutions, and authors and identifying research hotspots and recognising the impact of journals in this area. Bibliometric analysis give information on publishing kinds, countries/regions, institutions, journals, author keywords and extracts certain research hotspots and outlooks in this subject, allowing scholars to learn more about the topic. This review combines bibliometric study of nitrate removal from wastewater using microalgae with nitrate removal from wastewater strains and other biological processes.

## 2. Various biotechnological approaches of nitrogen removal and their limitations

Di-nitrogen ($N_2$) exists in different oxidation states from -3 to +5 (Watkins et al., 2014). The main process by which converts nitrogen into mobile forms is the nitrogen cycle. Nitrification and de-nitrification are two important pathways for biological nitrate removal. During de-nitrification process, nitrite can be produced, which is influenced by C/N ratio, pH, carbon sources and initial nitrate concentration. The conversions depend on the oxidation-reduction reaction by bacteria, fungi and archaea. The microbial community structure of de-nitrifying bacteria is highly diverse and convert nitrate to nitrite (Cao et al., 2013).

Two types of de-nitrifier carry out biological de-nitrification: autotrophic and heterotrophic (Zhao et al., 2012). Autotrophic de-nitrification uses inorganic carbon sources like carbon dioxide, bicarbonate and sulphur (Ghafari et al., 2008). The heterotrophic de-nitrification process is carried out by organic carbon as a source and organics are required to provide electron donors for catalyzing the reeducates (Lu et al., 2014). Here sodium acetate is used as an external source of carbon, thus increasing treatment cost. It is reported that nitrate can accumulate by anaerobic fermentation liquor as electron donor sources (Yan et al., 2018). Anaerobic fermentation liquor is rich in volatile fatty acid and acidogenic liquid because it is generated from food wastes and sludge (Kim et al., 2016; Yan et al., 2018). This provides forhigh C/N ratio in wastewater (Chung et al., 2014). Hence anaerobic fermentation liquor is promising for the de-nitrification anammox process in nitrogen removal from nitrate contaminated wastewater. Wastewater from different industry contains good organics (Fernández-Nava et al., 2010), which has been used to provide electron donor for nitrate de-nitrification having nitrate removal efficiency upto 97.2% (Kim et al., 2017). Hence nitrite accumulation is mostly affected by types of carbon sources (Du et al., 2017a). De-nitrification provides nitrite and other substrates like ammonium which is supplemented to proceed nitrate removal by anammox process (Du et al., 2019a). A demonstration done by Du et al., (2017b), where 97.8% of $NO_3^-$–N and 94.7% of $NH_4^+$–N were removed from wastewater having $NO_3^-$–N of 69.2 mg N/L and $NH_4^+$–N of 63.6mg N/L.

Implementing heterotrophic and auto-trophic de-nitrifiers provides economical method for nitrate removal from wastewater (Guo et al., 2013). This process is more economically favourable because it requires fewer chemicals, as the alkalinity generated during de-nitrification provides suitable pH for the process (Seifi and Fazaelipoor, 2012). With these advantages of micro-organisms, various studies have reported many heterotrophic bacteria capable of de-nitrification (Huang et al., 2017a). Nitrogen removal performance is excellent in lab scale, but many issues remain towards practical application (Cao et al., 2019; Du et al., 2017a). High concentration of toxic substances affects nitrate reeducates activities (Jin et al. 2012). High demand for external carbon source, high biomass yield, emission of $N_2O$, a strong greenhouse gas, limited pH range, and longer treatment time limits its application on a large scale. The advanced biological process can be used to overcome these problems.

### 2.1. Environmental factors affecting de-nitrification

Different environmental factors such as carbon sources, C/N ratio, pH, and temperature affect the performance of denitrifying bacterial community and nitrate accumulation.

#### 2.1.1. Carbon sources

The heterotrophic organism requires carbon for cell growth and electron donor, which is essential for enhancing the de-nitrification rate. Different carbon sources are ethanol, glucose, glycerol, methanol, and succinate (Cyplik et al., 2012). Various studies reported Glucose as the best carbon for *Anoxybacillus contaminans, Enterobacter cloacae, Serratia marcescens and Bacillus cereus* (Barman et al., 2018; Huang et al., 2017b). Huang et al*.,* (2015) reported 78·86% nitrate removal in 72 hours using sodium acetate for *Zoogloea*. Acetate and succinate have been reported by Yang et al., (2016) as good sources of carbon for *Pseudomonas putida, A. junii*, and *Pseudomonas aeruginosa*. Solid carbon sources like leaf compost, polybutylene, rice straw, and woodchips were also used for denitrification (Ruan et al., 2016). These solid sources of carbon function both as electron donor as well as a substrate for biomass growth reported high variation in biodiversity (Deng et al., 2019). Cotton wool, pine bark, cucumber leaves, and plant based carbon sources serve as low cost and easily available carbon sources (Wen et al., 2010).

#### 2.1.2. C/N Ratio

The carbon to nitrogen ratio is important to measure the electron donor to acceptor ratio. C/N ratio affects cell growth, and denitrification process (Huang and Tseng, 2001). Most heterotrophic bacteria reported to thrive well in the C/N ratio range of 8-10. *Pseudomonas* sp. has a nitrate removal efficiency 98.3%, at C/N ratio 10 (Prasetyo et al., 2018). The highest nitrate removal efficiency was reported at C/N ratio 15 by *Pseudomonas taiwanensis* and *Bacillus* sp. using glucose (He et al., 2018). The range between 2.5 and 5 resulted in a low growth yield of bacteria (Ji et al., 2015). Hence, an optimum C/N ratio is necessary for heterotrophic denitrifiers.

#### 2.1.3. Effect of pH

During the de-nitrification process, nitrate gets depleted, leading to increased pH (Ji et al., 2015). For the de-nitrification process, optimal pH ranges 7-8 (Yang et al., 2016; Li et al., 2018). Huge variation in pH affected the growth of bacteria. pH level above 8.75 or below 6.25 negatively decreases the efficiency of *A. junii* (Ren and Ogden, 2014). Few heterotrophic de-nitrifiers like *Halomonas campestris* and Aeromonas per form well at pH 9 and 11 (Cyplik et al., 2012; Chen et al*.,* 2014).

### 2.1.4. Effect of temperature

Temperature is a major factor in de-nitrification process. High and low temperatures reduce the efficiency of the de-nitrification process. Nitrate removal efficiency of 51.6% at optimum temperature 5°C as achieved by He et al., (2018). Mesophilic, pshycophilic and thermophilic types of de-nitrifier bacteria work at different ranges of temperature (Li et al*.,* 2018). For example: *Enterobacter cloacae* at 37°C, *Bacillus salmalaya* at 35°C *(*Dadrasnia et al., 2017), *Cupriavidus* sp. at 10°C (Srivastava and Mishra, 2018), *Acinetobacter* sp*.* at 2°C *(*Zheng et al., 2018). Mesophilic range of 25–37°C studies reported the most effective range of temperature (Lei et al., 2016; Li et al., 2018).

## 3. Advantages of algal treatment

Algae are photosynthetic organisms and can grow in different aquatic systems such as lakes, ponds, rivers, or even in freshwater. Microalgae represent a group of photosynthetic micro-organisms developed in aquatic habitats and easily convert light energy and inorganic carbon sources into biomass. They can grow alone or in symbiosis with other organisms (Khan et al., 2018). Works on algal-based wastewater technology have been researched since the 1950s (De-Pauw and Van-Vaerenbergh, 1983 and Shelef et al., 1980). Oswals and Goluenke first applied wastewater treatment using microalgae. Different laboratory practical on microalgae shows their potential in synthesize high-value compounds like nucleic acid, phospholipids, and protein, which mostly needs nitrogen molecules obtained from wastewater effluent. Different strains or species have significant biomass productivity and different nitrate removal efficiency and can work at various environmental conditions. Biotreatment with algae in wastewater is more advantageous and attractive due to its capability of converting solar energy into useful biomass. Also algae can treat wastewater and remove excess nutrients, which causes eutrophication. As reported by many authors, the list of algae was compiled for 60 genera and 80species. Among these, the maximum tolerable genera were *Oscillatoria, Euglena, Scenedesmus, Chlamydomonas, Nitzschia, Navicula, Stigeoclonium, and Chlorella* (Palmer, 1969).

Microalgae can convert inorganic nitrogen compounds, including nitrate, nitrite, nitric acid, ammonium, to organic nitrogen found in different biological substances such as protein, energy transfer molecules (ADP, ATP) and genetic material (RNA, DNA) via the process called assimilation. In this process, all eukaryotic algae require inorganic nitrogen only in the form of nitrate, nitrate and ammonium. Nitrate and nitrite undergo reduced enzyme activities. Here the nitrate reducates uses the reduced form of nicotinamide adenine dinucleotide or nicotinamide adenine dinucleotide phosphate to transfer 2-electrons for the conversion of nitrate to nitrite. Nitrate reduction to ammonium is driven by nitrate reductase and reduced ferredoxin. This nitrite transforms into ammonium ions by nitrite reductase involving 6 electrons. Finally, ATP is synthesized, and ammonium is incorporated into amino acid via glutamate dehydrogenase called glutamate synthesis

(Medina et al., 2007). Microalgae tend to prefer ammonium over nitrate and only use it when ammonium gets exhausted. Therefore, wastewater with a high ammonium concentration can be treated using microalgae. Thermodynamically stable nitrate is also an important nitrogen source of microalgae as it induces nitrate reductase activity. The nitrate assimilation process of microalgae depends on the various parameters such as operation factor, types of wastewater, biomass concentration, light wavelength, pH and $CO_2$ concentration (Howarth et al., 2009).

Because of algae's metabolic flexibility, they represent a promising biological system for treating different types of wastewater (Adav et al., 2008). The algal system can treat different types of wastewater like agro-industrial wastes (Phang, 1990), piggery effluent (Pouliot et al., 1986), effluent from food processing factories (Rodrigues and Oliveira, 1987, sewage (Mohamed, 1994), livestock wastes (Lincoln and Hill, 1980), domestic and agricultural wastes (Phang and Ong, 1988) and also remove heavy metals (Hammouda et al., 1995; Cai-Xiao Hua et al., 1995). Among the different wastewater sources, municipal wastewater has high efficiency of production of microalgae (Bhatnagar et al., 2010), because of their efficient fixation of inorganic nitrogen. The efficiency of biosorption and bioconversion mechanism of microalgae varies with the types of wastewater and presence of inorganic carbon in it (Lim et al., 2010). As the biomass increases, the concentration of $CO_2$ decreases. This triggers carbon sequestration from atmosphere to water, also making it a suitable tool for carbon capture from wastewater and its fixation into valuable biomass (Park and Craggs, 2010).

Large scale generation and application of microalgae can be the key to its success.

### 3.1. Advances in micro algal bioremediation

Some technologies are available to compete with conventional technology of wastewater treatment system. These technologies are advancements in the field of algal based bioremediation. One of the most applied and economical technology is the photo-bioreactor. It is important to develop successful technology to achieve targeted biomass production by making algae biotechnology sustainable and economical. The most important parameter depends on the type of reactor used. Their design is based on the type of species we use and mainly the purpose of culture.

#### 3.1.1. Membrane photo-bioreactor (MPBR)

The membrane plate photo-bioreactor is the system or technology of microalgae culture and nutrient assimilation from wastewater effluent. Microalgae culture through MPBR system for obtaining a high level of nitrate removal can be a better option(Luo et al., 2017). The MPBR system combines the flat plate and airlift column with microfiltration and ultrafiltration. MPBR system has desirable control on microalgae retention with strict regulation in both Hydraulic and solid retention time (Honda et al., 2012). This function of MPBR system leads to biomass production almost 3.5 times than PBRs system (Marbelia et al., 2014). Nitrate removal efficiency differs with a different method of MPBR through the cultivation of *Chlorella vulgaris* such as algal biofilm membrane photo-bioreactor remove 64.9% of nitrogen (Gao et al., 2015). Osmotic membrane photobioreactor is designed by (Praveen et al., 2016) for wastewater by high-density *Chlorella vulgaris* cultivation. With two and three days of hydraulic retention time, nitrate removal reached 53% and 66.5%, respectively, using the membrane photobioreactor for the secondary treatment of sewage effluent (Boonchai et al., 2015). Similarly, with six plate membrane photo bioreactor cultivation of *Chlorella vulgaris* under different environmental condition, nitrate removal efficiently reached 57%. The MPBR parameters were analyzed via the standard method (APHA) with a wavelength of 690nm for nitrate (Federation et al., 2005). Light conditions are crucial in microalgae growth and biomass output in this system (Gonçalves et al., 2014). Other species of Chlorella, also remove nitrogen with desirable level. It is reported that *Chlorella zofingiensis* for the piggery wastewater treatment achieved 82.7% N removal (Zhu et al., 2013) and 91% N using *Chlorella vulgaris* in synthetic secondary treated effluent within an MPBR system (Honda et al., 2012). Hence, according to many research methodologies in MPBR, *Chlorella* sp. has been used widely to treat wastewater and produce microalgae biomass (Luo et al., 2017). Overall the MPBR system is concerning different types of wastewater effluent. Also further research has to investigate the nutrient assimilation and pattern of photosynthesis of the species within the system.

**3.1.2. Raceway pond**

Raceway pond is an outdoor system for microalgae cultivation, with water circulation around the recirculation channel or track. This system has been used since the 1950s for mass culture of microalgae and treatment of wastewater effluents. The paddle-wheel in the track prevents sedimentation of the algal biomass. Flow begins from the paddle wheel, where the culture is fed continually during daylight (Ananadhi et al., 2012). Raceway pond is 10 times more cost-effective compared to photo-bioreactor system. In recent investigation, *Chroococcus turgidus* is cultivated at a pilot scale using an open raceway pond under various parameters. Open culture systems are inexpensive, but it can be easily contaminated. It is best at the functional and economical platform for the bioremediation of wastewater effluents. This system acquired 60-80% of nitrogen removal efficiency (Gentili et al., 2017). This open culture system has a better removal rate of nutrients and increases the probability of high biomass production (Arbib et al., 2017). Species like *Chlorophyta* mixed with effluent was grown successfully in the open pond cultivation (Sutherland et al., 2019). Hence, with no use of synthetic chemicals, this system has great treatment efficiency with low energy utilization and high microalgae biomass production. This could be the substitute technology in the place of activated sludge treatment *(*Boshir et al., 2016). To treat inorganic toxins of agriculture, industrial and municipal wastewater, advanced integrated pond system work cooper with the bacterial algal consortium (Wollmann et al., 2019).

**3.1.3. Challenge associated with nitrate removal using microalgae**

Using microalgae for nitrate removal from wastewater is the most promising and potential route and it works as an alternative technology in the place of conventional nutrient removal method (Xie et al., 2018). There are many lab-based microalgae biotechnological research that indicates challenges associated with it. On a larger scale, the efficiency is affected by various parameters like nutrient availability and concentration, algal species present, and light intensity. The production cost of microalgae is high, as they are affected by natural factors such as climate, temperature as well as contamination of other microorganism like ciliates, rotifers and bacteria (Huo et al., 2018). Collection of biomass or solid-liquid separation requires high energy, which affects the economy of the whole technology. Other resistant could be the alkalinity and oxygen generated by microalgae during photosynthesis which limits the bacterial growth. Bacterial growth affects microbial growth. There are several applications of microalgae biomass, but their large scale application still needs further research (Collotta et al., 2019). The uptake of pollutants by microalgae leads to their accumulation in microalgae biomass. If the biomass is not treated properly, it could affect the down-streaming process and become more toxic for the environment (Usher et al., 2014). The presence of various indigenous bacteria and microalgae in biomass makes the handling process complex.

## 4. Bibliographic analysis

To perform bibliometric analysis, a search was conducted on dimensions on 14$^{th}$ of January, 2021. Keywords like – wastewater, microalgae, nitrate removal, challenges, opportunities, environmental factors and strains of microalgae were searched. The result was filtered for the year 2011 to 2021; this yielded 973 articles. The documents were analyzed for types, countries, sources, documents and authors to understand network analysis of citations, co-citation based on the type of analysis. The network diagrams for co-authorship and co-occurrence analysis were performed by VosViewer software (version 1.6.16). In addition to document types, journals covered nitrate removal from wastewater using microalgae publications were studied. After importing data into software, the function starts analysis based on analysis types.

Network visualization, overlay visualization and density visualization graph were plotted (figure 1 (C). In network visualization the bubble shows the weight of the documents (sources, authors and countries). Higher the number of documents, larger the bubble size. In the case of overlay visualization, the blue colour indicates the lowest score of documents and green to yellow indicates the highest score. More than 60% of the article related to microalgae-based wastewater treatment are published in the top 15 journals and top 15 countries that work maximum in microalgae-based nitrate removal in wastewater are listed in table 1. Among these journals, Bioresource Technology is the most productive journal with 127 documents and with total strength of 207 followed by wastewater treatment using microalgae and its challenges. Again, in the case of country-based analysis, China is the top contributing hand in this field, with 668 documents with 900 total link strengths between other countries.

### 4.1. Performance of top most sources

Sources based on citation analyze the best source in the field of microalgae-based nitrate removal from wastewater. The density visualization map represents the link between top 15 sources based on 973 published articles. Bioresources technology is the most highlighted source in this field, with 127 documents and the maximum link strength. In addition, the science of total environment and algal research having documents 144 and 89 respectively. The table-1 represents the list of top most published sources and its link with cited papers.

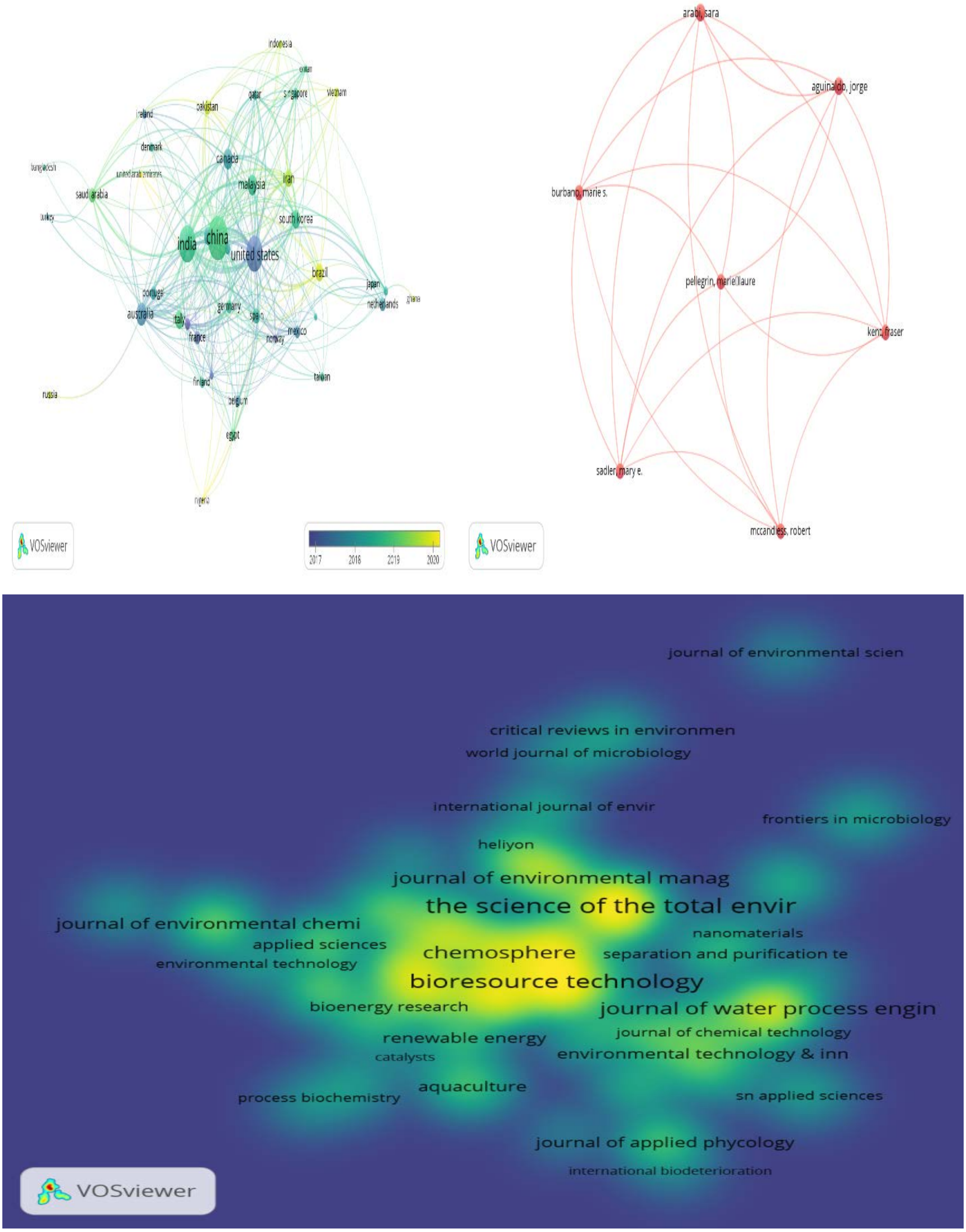


**Fig. 1. Most prolific (a) countries (b) authors and (c) density visualization between the citation and sources**

**Table 1. List of top Journals and Countries.**

| Journals | Document | Total Link Strength | Country | Document | Total link strength |
|---|---|---|---|---|---|
| Bioresources Technology | 127 | 207 | China | 668 | 900 |
| The Science of Total Environment | 144 | 171 | India | 331 | 588 |
| Journal of Water Process Engineering | 89 | 102 | Malaysia | 132 | 383 |
| Journal of Cleaner Production | 68 | 89 | United State | 261 | 356 |
| Chemical Engineering Journal | 75 | 84 | Australia | 143 | 285 |
| Chemosphere | 49 | 72 | Brazil | 62 | 246 |
| Water Research | 75 | 58 | Vietnam | 112 | 220 |
| Journal of hazardous material | 43 | 50 | South Korea | 57 | 219 |
| Environmental Science and Pollution Research | 56 | 44 | Taiwan | 105 | 205 |
| Journal of Environmental Chemical Engineering | 40 | 39 | Italy | 55 | 184 |
| Renewable and Sustainable Energy Reviews | 29 | 37 | Saudi Arabia | 120 | 182 |
| Renewable Energy | 24 | 30 | Spain | 102 | 176 |
| Biomass Conversion and Bio-refinery | 25 | 29 | United Kingdom | 92 | 173 |
| Environmental Pollution | 33 | 29 | Iran | 56 | 131 |
| Environmental Technology and Innovation | 28 | 29 | Pakistan | 60 | 116 |

Based on the list, the map highlighted main sources with yellow colour spot and the least cited paper with a minimum number of documents highlighted with blue colour.

**4.2. Hotspots of microalgae based nitrate removal from wastewater**

The map below visualize the link between the citations based on country. In figure 1(a), it can be seen that China, United States and India are the most prominent countries. Overlay visualization shows United State, Italy, Mexico worked efficiently in 2017 but there is a research gap in year 2018 and 2019 in Italy. In 2019 India, China Malaysia South Korea had a maximum publication in nitrate removal from wastewater using microalgae. In this graph, the yellow coloured cluster indicates publications from Pakistan, Brazil, Iran and Vietnam, working in the field of microalgae based nitrate removal from wastewater from year 2020. The circle size represents the articles' number and the thickness of connecting lines represnts the number of the cooperation articles.

**4.3. Publication Output**

Co-authorship based on the author's analysis is used to analyze the research trend information and helps identify the best and topmost researchers**.** Network visualization web drawing shows the relation of co-authorship based on authors [figure 1 (b)]. In the network visualization map, the bubble shows authors who have the maximum number of articles. The network is based on selected top 10 authors in which the main author in the field of microalgae-based nitrate removal from wastewater are Arabi Sara, Aguinaldo Jorge, Kent Fraser, Pellegrin Sadler, Mary E, Burbano Marie S. as shown in map-3. Among the most prominent authors, the main cluster that indicates the author in the microalgae-based nirate removal is linked with 6 other co-authors and the network line between them shows a total of 16 strong links between other authors.

**5. Conclusion and Future perspective**

The potential of microalgae-based nitrate removal has been apparent for a long time. The recent research field on wastewater using microalgae is very promising. Progresses have been made in developing new technology and strategies to use microalgae-based bioremediation of nitrate removal, especially biomass production. As microalgae utilize nitrogen as a nutrient, it is beneficial for the tertiary treatment of wastewater, but it varies among algal strains and species. It is important to select suitable strains and species of microalgae depending upon the applications. Long term production will improve the economic viability of microalgae biotechnology. Biodiesel based on microalgae is part of a renewable energy system where the choice of algae and its productivity is always a matter of concern for any research worker to produce a fruitful result. Some microalgae do not perform well in wastewater due to unfavourable condition. These strains of algae require genetic modification. Microalgae genetic engineering is made to improve strains' quality to achieve a high growth rate and other value-added product enhancement. Besides that, algae-based wastewater treatment and its potential on a large scale require resources, especially developing cost-effective dewatering, harvesting biomass, screening microalgae strains for high tolerance of pollutants, and controlling environmental parameters. The growth of microalgae depends on the medium in which the culture, achieving a high growth rate of microalgae require organic availability in wastewater, which makes the process cost effective. Hence it is significant to maintain the nutrient concentration for better nitrate removal efficiency and production of algal biomass. Advancement in this technology, such as genetic engineering, shows a promising future in converting challenges into opportunities. Further work is necessary to find out the mechanism and better understanding behind how different strains of microalgae treat wastewater. The promising befit needs realization and demonstration for application of advanced biotechnological tools may render the process environmentally friendly and sustainable in future.

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