Bibliometric Analysis of Research Trends Related to Carbon Sources for Nitrogen Removal in Wastewater Treatment

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Abstract

The low organic carbon content of municipal wastewater weakens the effectiveness of biological nitrogen removal. Here, we review recent trends in carbon sources for nitrogen removal during wastewater treatment. A bibliometric analysis, using the Science Citation Index Expanded database from the Web of Science, was performed to analyze articles published in this field from 2000 to 2019. The major points are the following: (1) the publications from China ranked 1st, followed by the USA and Japan during the statistical period. In terms of research institutions, the Chinese Academy of Sciences was the largest contributor with the most publications. (2) Based on the trend analysis of keywords, future research hotspots are discussed, showing that of the related research on external carbon sources, the research focus on traditional carbon sources (methanol, ethanol, etc.) has decreased. The research focused on alternative carbon sources, such as sludge hydrolytic acidification liquid, fermentation liquid of food waste, agricultural waste, and biodegradable polymers, has increased. (3) New nitrogen removal technologies without external carbon sources, such as anammox is becoming increasingly popular. (4) In the study of nitrogen removal, more attention is paid to the change in the microbial community, and the biological mechanism of nitrogen removal is discussed from a microscopic perspective.

1. Introduction

Nitrogen is a necessary element for all organisms, but excessive nitrogen can lead to water eutrophication. Eutrophication is a major worldwide water pollution issue. It leads to the rapid reproduction of plankton such as algae, the continuous decline of dissolved oxygen in water, the deterioration of water quality, the massive death of fish and other organisms, and the loss of biodiversity of the aquatic ecosystem (Li and Nan 2017; Cook et al. 2018). Water eutrophication not only has a negative impact on ecological health (Hwang 2020), but also poses a threat to human health (Fetahi 2019). The pollution of groundwater by nitrogen-containing compounds, such as nitrate, can affect the safety of drinking water. For example, nitrate converting to nitrite in drinking water can cause methemoglobinemia in infants (Dhamole et al. 2015). Nitrate can also form nitrosamines, which are potentially carcinogenic compounds (Ono et al. 2000). In addition, eutrophication causes harmful algae to multiply in large numbers, and the algal toxins produced by it also have potential threats (Morabito et al. 2018; Le Moal et al. 2019).

Municipal wastewater discharge is a source of nitrogen loads in natural water bodies and is one of the main factors causing water eutrophication (Preisner et al. 2020). With societal and economic development, water consumption and wastewater production has increased. Wastewater is mainly treated by wastewater treatment plants (WWTPs) to reduce environmental harm. Effluent from WWTPs can only be discharged into natural water bodies after reaching a certain standard. This standard is becoming increasingly strict to protect the environment and reduce the nitrogen load of wastewater in the receiving water bodies. Nitrification (Eqs. [1] and [2]) and denitrification (Eqs. [3] and [4]) are biological nitrogen removal technologies commonly used in WWTPs (Zheng et al. 2018). Nitrification is carried out under aerobic conditions. Ammonia nitrogen is first transformed into nitrite nitrogen under the action of ammonia-oxidizing bacteria, and then nitrite nitrogen is further transformed into nitrate nitrogen under the action of nitrite-oxidizing bacteria. The denitrification process is performed under anoxic conditions. Denitrifying bacteria require an organic carbon source as an electron donor and nitrate as an electron acceptor to reduce nitrate into pollution-free N_2. However, the lack of a carbon source is a common problem faced by WWTPs, especially in southern China (Wu et al. 2011; Wang et al. 2021).

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \quad (1)$$

$$2NO_2^- + O_2 \rightarrow 2NO_3^- \quad (2)$$

$$NO_3^- + 2H^+ + 2e^- \rightarrow NO_2^- + H_2O \quad (3)$$

$$NO_2^- + 4H^+ + 3e^- \rightarrow 0.5N_2 + 2H_2O \quad (4)$$

2. Experimental

2.1 Methods

Literature is the embodiment of scientific research results. Bibliometric analysis can systematically evaluate the relative importance of scientific research results in a certain field and indicate the field's direction of development over a period of time (Cimmino et al. 2005). In this study, research direction and progress in the field of carbon sources for nitrogen removal in wastewater treatment were analyzed using a combination of bibliometrics and reviews.

In this study, keywords such as ("carbon source*" or glucose or methanol or ethanol or acetate or BDPs or "biodegradable polymers" or polyhydroxybutyrate or polyhydroxyalkanoates or polycaprolacton or "agricultural waste*" or straw or newspaper or cotton or corncob or "food waste*" or "kitchen waste*" or "Volatile Fatty Acid*" or "VFA*") and ("wastewater treat*" or "waste water treat*" or "waste-water treat*" or "sewage treat*" or "effluent treat*" or "denitrif*" or "nitrogen removal" or "nutrient removal" or "nitrate removal") were used as topic search phrases to acquire from the Web of Science database an index of articles published between 2000 and 2019. A total of 8794 publications were obtained. Microsoft Office Excel 2019 and VOSviewer were used to conduct statistical analyses and visually process the retrieval results.

2.2 Publication outputs and characteristics of journals

Of the 8794 publications, 7796 were articles, accounting for 88.65% of the total publications, followed by proceedings papers (6.14%) and reviews (4.94%). The proportion of other document types was relatively low. As the dominant type of document, 7796 articles were analyzed in the present study. Fig. 1 shows the number of Science Citation Index Expanded publications related to carbon sources for nitrogen removal in wastewater treatment from 2000 to 2019. The annual number of articles published remained stable from 2000 to 2004. From 2005 to 2011, the annual number of published articles increased slowly. However, the growth rate accelerated significantly from 368 articles in 2012 to 881 articles in 2019. With the increase in volume of treated wastewater, as well as stricter effluent nitrogen and phosphorus removal requirements, the requirement of carbon sources for nitrogen removal has also increased. However, the addition of traditional external carbon sources is expensive and greatly increases the cost of WWTPs (Sun et al. 2010). The addition of external carbon sources in the nitrogen removal process of wastewater treatment also increases the consumption of energy and resources. A shortage of resources and energy has attracted extensive attention to this area. Improvement of nitrogen and phosphorus removal, energy conservation, and resource recovery have now become the direction of the development of wastewater treatment technologies. New ideas have been presented on external carbon sources and wastewater treatment technologies that are effective and low-cost.

Table 1 shows the top 10 productive journals in the field of carbon sources for nitrogen removal in wastewater treatment from 2000 to 2019. The top 10 productive journals accounted for 31.94% of the total number of articles. Bioresource Technology had the most publications. Among the top 10 journals, four were from the UK, two from the Netherlands, and one each from Italy, Switzerland, Germany, and the USA. The impact factor and *h*-index (defined as at least *h* articles in a journal that have been cited at least *h* times) are often used to evaluate the influence of a journal in one field. However, Table 1 shows that there is no positive correlation between the influence of journals and the number of publications. Even the rank of the number of articles published by some journals is significantly different from its impact factor and *h*-index ranking. For example, Environmental Science & Technology ranked 10th in terms of publications, while its impact factor (7.864) and *h*-index (52) ranked third. On the contrary, desalination and water treatment ranked high (4th) on the number of articles published, but its impact factor (0.854) and *h*-index (13) ranked last (10th). This shows that the influence and importance of a journal in the field of carbon sources for nitrogen removal in wastewater treatment does not solely depend on the number of articles published.

Table 1
Top 10 most productive journals in 2000-2019

Journal name	TP	TPR (%)	IF (R)	*h*-index (R)	Publishing country
Bioresource Technology	630	1 (8.08)	7.54 (4)	70 (2)	Netherlands
Water Research	402	2 (5.16)	9.13 (2)	81 (1)	UK
Water Science and Technology	254	3 (3.26)	1.64 (9)	23 (7)	UK
Desalination and Water Treatment	181	4 (2.32)	0.85 (10)	13 (10)	Italy
Chemical Engineering Journal	173	5 (2.22)	10.65 (1)	38 (4)	Switzerland
Environmental Technology	171	6 (2.19)	2.21 (8)	20 (9)	UK
Chemosphere	154	7 (1.98)	5.78 (6)	33 (5)	UK
Science of the Total Environment	142	8 (1.82)	6.55 (5)	29 (6)	Netherlands
Environmental Science and Pollution Research	137	9 (1.76)	3.06 (7)	21 (8)	Germany
Environmental Science & Technology	130	10 (1.67)	7.86 (3)	52 (3)	USA

TP, total numbers of publications; TPR (%), rank and percentage of the total publications; IF, impact factor; R, ranking of impact factor and *h*-index

2.3 Publication distribution of countries/territories and institutes

The contributions of different countries/territories and institutes were estimated according to the authors' addresses. Among the total articles, five articles had no author address information, and only 7791 articles with author address information were used to investigate the contribution of countries/territories and institutes. Articles originating from England, Scotland, Northern Ireland, and Wales were re-categorized as falling under the UK. Articles from Hong Kong were included in the Chinese category.

The top five most productive countries and their annual publications are shown in Fig. 2. The total number of articles published by Japan, India, and Spain were roughly the same, and their annual number of publications varied little. From 2000 to 2008, research concerning carbon sources for nitrogen removal in wastewater treatment was mainly conducted in the USA. After 2008, China surpassed the United States in the annual number of publications and has been in the leading position since then. In addition, annual publications increased rapidly after 2012. China's number of publications (428) in 2019 was 4.86 times that of the United States (88) in the same year. This may be closely related to the introduction of a series of policies and regulations by the Chinese government and their intensifying of water pollution control.

Table 2
Top 10 most productive institutes between 2000 and 2019

Institution	TP	TPR (%)	*h*-index
Chinese Acad Sci, China	255	1 (3.27)	48
Harbin Inst Technol, China	182	2 (2.34)	34
Tongji Univ, China	174	3 (2.23)	40
Beijing Univ Technol, China	117	4 (1.5)	35
Tsinghua Univ, China	109	5 (1.4)	26
Univ Queensland, Australia	107	6 (1.37)	47
Zhejiang Univ, China	88	7 (1.13)	26
Delft Univ Technol, Netherlands	83	8 (1.07)	34
Univ Sao Paulo, Brazil	77	9 (0.99)	20
Natl Taiwan Univ, Taiwan	61	10 (0.78)	28
TP, total numbers of publications; TPR (%), rank and the percentage of the total publications			

As shown in Table 2, the majority of the institutes in the top 10 most productive institutes from 2000 to 2019 came from China. Moreover, the top five were all Chinese research institutions, with more than 100 articles, which shows that Chinese research institutions have been prolific researchers in the field of carbon sources for nitrogen removal in wastewater treatment. The Chinese Academy of Sciences has the largest number of publications, and the *h*-index is also the highest, indicating a high publication quality. VOSviewer was used to analyze the top research institutions and their cooperative relations shown in Fig. 3 (containing 98 nodes and 483 links). The Chinese Academy of Sciences has the largest circle size, indicating that it has the largest number of published articles, followed by Harbin Institute of Technology and Tongji University, which is consistent with the results in Table 2. As shown in Fig. 3, the Chinese Academy of Sciences has close ties with the University of the Chinese Academy of Sciences, while the Harbin Institute of Technology has close cooperation with the Beijing University of Technology.

2.4 Author keywords analysis

Statistical analysis of author keywords is helpful for understanding scientific research trends. A total of 35016 keywords in the field of carbon sources for nitrogen removal in wastewater treatment from 2000 to 2019 were detected in this study. During benchmark processing, keywords with the same meaning but different written forms were combined, such as food waste and kitchen waste. The final analysis results for the 30 most commonly used high-frequency words are shown in Table 3. The keywords that appeared 50 times or more were extracted, and the association graph in Fig. 4 (containing 56 notes and 833 links) was drawn using VOSviewer. As shown in Table 3, the keywords "Microbial Community" and "Anammox" ranked only 423rd from 2000 to 2003; however, their ranking increased to 4th and 11th from 2016 to 2019, respectively. Similarly, “Biogas” showed stable and continuous increases. The frequency of these keywords showed a large growth trend, indicating that the research represented by these keywords has gradually become a research hot spot in the field of carbon sources for nitrogen removal.

Table 3
Top 30 frequency of author keywords in 2000-2019

Author's Keywords	**TP**	**R (%)**					
	2000-2019	**2000-2019**	**2000-2003**	**2004-2007**	**2008-2011**	**2012-2015**	**2016-2019**
Denitrification	909	1 (13.39)	1 (24.84)	1 (18.68)	1 (14.48)	1 (13.22)	1 (9.84)
Wastewater Treatment	615	2 (9.06)	2 (8.06)	2 (8.05)	2 (10.32)	2 (9.9)	2 (8.44)
Anaerobic Digestion	234	3 (3.45)	13 (2.4)	9 (2.44)	9 (3.04)	4 (3.32)	3 (4.11)
Wastewater	223	4 (3.28)	4 (5.45)	9 (2.44)	5 (3.9)	3 (3.44)	7 (2.79)
Nitrate	209	5 (3.08)	6 (4.36)	6 (4.17)	3 (4.08)	7 (2.96)	10 (2.25)
Nitrogen Removal	201	6 (2.96)	8 (3.49)	16 (1.87)	10 (2.86)	9 (2.55)	5 (3.43)
Activated Sludge	199	7 (2.93)	4 (5.45)	5 (5.17)	8 (3.47)	8 (2.61)	12 (1.93)
Biodegradation	184	8 (2.71)	10 (3.27)	7 (3.45)	4 (3.99)	5 (3.14)	18 (1.65)
Volatile Fatty Acids	179	9 (2.64)	7 (3.92)	8 (3.3)	7 (3.73)	9 (2.55)	13 (1.86)
Sequencing Batch Reactor	178	10 (2.62)	8 (3.49)	4 (5.32)	5 (3.9)	13 (2.19)	19 (1.54)
Nitrification	174	11 (2.56)	3 (6.1)	3 (6.18)	12 (2.78)	15 (2.07)	22 (1.29)
Carbon Source	168	12 (2.47)	35 (1.09)	15 (2.01)	14 (2.17)	9 (2.55)	6 (2.9)
Microbial Community	160	13 (2.36)	270 (0.22)	423 (0.14)	22 (1.39)	16 (1.96)	4 (3.9)
Microbial Fuel Cell	151	14 (2.22)	N/A	59 (0.72)	10 (2.86)	12 (2.43)	9 (2.58)
Nitrous Oxide	146	15 (2.15)	16 (2.18)	17 (1.72)	15 (1.91)	5 (3.14)	15 (1.75)
Adsorption	146	15 (2.15)	21 (1.74)	20 (1.58)	20 (1.47)	13 (2.19)	8 (2.61)

TP, total numbers of publications; R (%), rank (the percentage of articles in total publications is given within brackets); N/A, none appeared

Author's Keywords	TP	R (%)					
	2000-2019	2000-2019	2000-2003	2004-2007	2008-2011	2012-2015	2016-2019
Biofilm	123	17 (1.81)	10 (3.27)	14 (2.16)	13 (2.69)	19 (1.54)	22 (1.29)
Biogas	100	18 (1.47)	270 (0.22)	44 (0.86)	36 (0.95)	17 (1.78)	13 (1.86)
Nitrogen	99	19 (1.46)	10 (3.27)	13 (2.3)	18 (1.56)	27 (1.19)	29 (1.07)
Kinetics	97	20 (1.43)	25 (1.31)	9 (2.44)	22 (1.39)	18 (1.6)	27 (1.11)
Sewage Sludge	94	21 (1.38)	270 (0.22)	17 (1.72)	22 (1.39)	32 (1.07)	17 (1.68)
Nutrient Removal	93	22 (1.37)	35 (1.09)	22 (1.44)	20 (1.47)	20 (1.42)	21 (1.32)
Anammox	92	23 (1.35)	270 (0.22)	423 (0.14)	49 (0.78)	23 (1.24)	11 (2.15)
Acetate	83	24 (1.22)	18 (1.96)	22 (1.44)	18 (1.56)	23 (1.24)	34 (0.89)
Methane	82	25 (1.21)	35 (1.09)	31 (1.15)	29 (1.13)	23 (1.24)	25 (1.25)
Ethanol	79	26 (1.16)	25 (1.31)	22 (1.44)	16 (1.73)	35 (1.01)	32 (0.93)
Methanol	78	27 (1.15)	13 (2.4)	17 (1.72)	25 (1.3)	41 (0.83)	32 (0.93)
Nitrate Removal	76	28 (1.12)	35 (1.09)	59 (0.72)	42 (0.87)	27 (1.19)	22 (1.29)
Food Waste	76	28 (1.12)	75 (0.65)	59 (0.72)	71 (0.61)	54 (0.71)	15 (1.75)
Methanogenesis	73	30 (1.08)	13 (2.4)	31 (1.15)	36 (0.95)	27 (1.19)	38 (0.82)
TP, total numbers of publications; R (%), rank (the percentage of articles in total publications is given within brackets); N/A, none appeared							

By analyzing the tendencies of the keywords, some conclusions were drawn: the carbon source is an important factor affecting biological nitrogen removal in wastewater treatment processes. External carbon sources are often used to support denitrification. Traditional carbon sources, such as glucose, methanol, and sodium acetate, are still widely used. However, owing to the high cost, researchers continue to explore alternative carbon sources to achieve better denitrification effects and environmental and economic benefits. Alternative carbon sources include methane (biogas) (Kim et al. 2019), sewage sludge hydrolytic acidification liquid (Liu et al. 2020), fermentation liquid of food waste (FW-FL) (Pu et al. 2019), and biodegradable polymers (Xiong et al. 2020). Anammox, a new nitrogen removal method devoid of organic carbon sources that uses ammonia as an electron donor, is becoming increasingly popular. Studying the changes in the microbial community structure during nitrogen removal is useful for explaining the effect of nitrogen removal. In the following sections, the types of external carbon sources and new approaches for nitrogen removal in wastewater treatment are reviewed, and suggestions for future research are presented.

3. Results And Discussion

3.1 External carbon sources

In order to meet more stringent effluent standards, extending and increasing the treatment technologies and processes in a built WWTP can enhance nitrogen removal. However, extending a plant is a huge investment project. External carbon sources can support and strengthen the denitrification process and improve the denitrification capacity of the wastewater treatment technology. Therefore, adding external carbon sources may be a more economical and effective municipal wastewater treatment scheme.

3.1.1 Saccharides

Saccharides such as glucose, sucrose, and fructose are inexpensive, nontoxic, convenient to transport, and easy to use by microorganisms. Glucose and its polymers (such as starch and sucrose) are ubiquitous in municipal wastewater and play major roles in the biochemical mechanisms related to substrate utilization (Shen and Zhou 2016). Therefore, saccharides are suitable as external carbon sources and are increasingly used in WWTPs. The effects of glucose, acetate, and lactate as carbon sources on nitrogen removal in an up-flow anaerobic sludge blanket reactor were compared. This suggests that the NO_3-N removal rate was similar for the three carbon sources, but glucose had the highest specific denitrification rate (Cuervo-Lopez et al. 1999). Since studies have shown that glucose has little influence on anammox bacteria (Zhong and Jia 2013), glucose can be considered as an external carbon source in WWTPs that use the anammox process for nitrogen removal. Furthermore, in anammox-denitrification systems, the effect of the usage of glucose as an additional carbon source in nitrogen removal was explored. The highest denitrification efficiency was obtained when the influent glucose concentration was 56.2 mg/L. However, when the influent glucose concentration was increased to 374.9 mg/L, the anammox activity was inhibited (Qin et al. 2017). On the other hand, it has been reported that when glucose is used as a carbon source, nitrite accumulation and production rates are high during denitrification, which affects the effluent quality (Ge et al. 2012).

3.1.2 Small molecular organics

For a simple structure, easy biodegradation, and low microbial cell yield, small molecular organics are the primary choice of carbon sources for the denitrification process in wastewater treatment. Methanol as a carbon source is widely used. A comparative experiment on the nitrogen removal efficiency with and without methanol addition at the WWTP Zürich-Werdhölzli was conducted. The results showed that compared with the control group (without methanol), the nitrogen removal efficiency was greatly improved by the addition of methanol. The adaptation period for methanol degradation was only a few days, and the process was relatively stable. Based on the influent total nitrogen, the average denitrification efficiency of the methanol addition group was 55%, whereas that of the control group was only 35% (Purtschert et al. 1996). In addition, no negative impact has been reported regarding long-term use of methanol as a carbon source on sludge sedimentation characteristics (Ginige et al. 2009). However, because methanol exhibits high toxicity, poor safety performance, difficult transportation and storage, high cost, and difficult dosage control, other carbon sources have been considered instead.

Ethanol is a traditional carbon source and substitute for methanol. Puig et al. (2007) used ethanol as carbon source. Results showed that the total nitrogen concentration of effluent was 3.0 mg/L (96% N removal efficiency), and the concentration of phosphate was 0.05 mg/L (99.9% P removal efficiency), indicating that ethanol is a promising external carbon source for removing nutrients in wastewater (Puig et al. 2007). Mokhayeri et al. (2008) mainly focused on three common external carbon sources: methanol, ethanol, and acetate. The specific denitrification rates on methanol, ethanol, and acetate were 9.2 mg NO_3-N/gVSS/hr, 30.4 mg NO_3-N/gVSS/hr, and 31.7 mg NO_3-N/gVSS/hr, respectively. Among them, the specific denitrification rate was lower when using methanol, while ethanol and acetate were equally effective carbon sources. However, ethanol is also a dangerous chemical. It is flammable and volatile at room temperature and pressure, difficult to store and transport, and expensive.

The use of acetic acid/acetate as a carbon source has increased in recent years. Chen et al. (2015) studied the effects of several carbon sources, including acetate, on post-anoxic denitrification and biological nutrient removal. Their results indicated that acetate and propionate significantly improved the effect of nitrogen and phosphorus removal, and the removal efficiency, driven by acetate, of total nitrogen and phosphorus reached 93% and 99%, respectively. In contrast, the removal rates of total nitrogen and phosphorus were reduced to 72% and 54%, respectively, using glucose. In the reactors cultured with methanol and ethanol, 66% and 63% of the total nitrogen was removed, and the phosphorus removal efficiency values were 78% and 71%, respectively. Although the effect of acetate is better, its high cost is not conducive to wide application.

Methane can not only be used as a fuel, but also as a carbon source for denitrification by methanotrophs. The denitrification process using methane as a carbon source can be divided into aerobic methane oxidation-coupled denitrification and anaerobic methane oxidation-coupled denitrification. The aerobic methane oxidation-coupled denitrification process means that denitrifying bacteria can use methane oxidation products as substrates for denitrification (Modin et al. 2007). Raghoebarsing et al. (2006) first demonstrated anaerobic methane oxidation-coupled denitrification, a process known as nitrite-dependent anaerobic methane oxidation. During the research on nitrate/nitrite-dependent anaerobic methane oxidation, it was found that using methane as an alternative external carbon source could overcome the barrier of insufficient carbon source in the denitrification process, achieving the goal of high-level nitrogen removal from municipal wastewater (Liu et al. 2019). Methane comes from a wide range of sources, and there are many studies on the use of anaerobic digestion waste to produce biogas (its main component is methane) to achieve treatment and production capacity (Ma et al. 2020; Gao et al. 2021). Compared with methanol, saccharides, and other traditional carbon sources, the utilization of methane does not increase the chemical oxygen demand in the effluent (Cao et al. 2021). However, methane is a flammable greenhouse gas. Using it as an additional carbon source comes with strict requirements for transportation and dosing.

3.1.3 Waste biomass

Owing to the poor safety and/or high price of the previously listed carbon sources, it is necessary to find safe and cheap alternative carbon sources. The new idea of "treat waste with waste" has provided a new direction for researchers. They have looked for alternative carbon sources in materials such as, activated sludge, food waste, straw, corncob, and other biomass wastes. Researchers have conducted studies to examine the use, through bioconversion or direct addition, of these materials as external carbon sources in the denitrification section of wastewater treatment.

Most solids in sludge are organics. The sludge fermentation liquid, which converts solid-phase organic matter into soluble organic matter through biological fermentation, can be used as a liquid carbon source. Li et al. (2020) used the chemically enhanced primary sedimentation sludge supernatant after fermentation as the organic carbon source for biological denitrification and combined it with methanol, sodium acetate, and sodium propionate for comparison. They concluded that the specific denitrification rate of chemically enhanced primary sedimentation sludge supernatant after fermentation was significantly higher than that of methanol, but lower than that of sodium acetate and sodium propionate. The use of chemically enhanced primary sedimentation sludge supernatant after fermentation can considerably reduce the cost of carbon sources. Hu et al. (2020) proved that sludge alkaline fermentation liquid, containing a large amount of volatile fatty acids as a carbon source for biological denitrification, not only improved the removal efficiency of soluble inorganic nitrogen in wastewater, but also reduced the amount and biological availability of the soluble organic nitrogen in the effluent. Nevertheless, there are still two main problems with the process of recovering carbon sources from sludge through acidogenic fermentation: one is the low efficiency (Wang and Li 2016), and the other is the presence of too much nitrogen and phosphorus in the fermentation liquid. The nitrogen and phosphorus need to be removed to improve the availability of carbon sources (Zhang and Chen 2009; Wan et al. 2017).

The worldwide output of food waste is huge and increasing. According to the Food and Agriculture Organization of the United Nations, about 2.2 billion tons of food waste will be produced worldwide by 2025 (Mehariya et al. 2018). Food waste often has a complex composition, high moisture content, high organic matter content, and high degradability (Gao et al. 2020). Moreover, food waste carries a large number of pathogenic microorganisms and can easily rot and deteriorate, breeding mosquitoes and flies and producing unpleasant smells. Dealing with this huge amount of food waste is of great concern to the government and people. Improper treatment can cause environmental pollution and threaten human health. Food waste can be recycled by anaerobic digestion to produce volatile fatty acids and methane (effective carbon source components) to achieve resource utilization (Ren et al. 2018; Wu et al. 2018). At present, there are many studies on the use of FW-FL as a carbon source. Tang et al. (2019) studied the effect of high-temperature FW-FL as an external carbon source on nitrogen and phosphorus removal. Through batch tests, it was found that the soluble and particle components of FW-FL were, as carbon sources, easily biodegradable and slowly biodegradable, respectively. During the long-term operation of a sequence batch reactor, the addition of FW-FL significantly increased the sludge particle size, improved the bacterial metabolic capacity, selectively enriched some functional microorganisms, and considerably improved the denitrification efficiency (approximately 90%). Qi et al. (2021) showed that FW-FL was mainly composed of lactic acid and volatile fatty acids. The nitrogen removal efficiency of FW-FL was slightly lower than that of acetic acid and butyric acid, but higher than that of pure lactic acid and starch. In a full-scale study, the concentration of total chemical oxygen demand in the FW-FL was 6.9–12.8 g/L, and the ratio of total chemical oxygen demand/inorganic nitrogen was 210.5–504.5:1. The removal rate of NO_3-N increased from 52.1–94.2% after the addition of FW-FL, which confirmed the potential of FW-FL as a commercial carbon source replacement in WWTPs.

Industrial wastewater, especially wastewater from the agriculture and food processing industry, contains significant amounts of organic matter. Using industrial wastewater as a carbon source can improve the denitrification effect and treat industrial wastewater simultaneously. It was considered whether three kinds of food processing industrial wastewater (distillery, brewery, and fish-pickling processes) could replace methanol as a carbon source. The nitrate utilization rates of the three industrial wastewaters were 2.4–6.0 gN/(kg VSS·h). Compared with methanol that has lower nitrate utilization rates (0.4–1.5 g N/[kg VSS·h]) and a longer acclimation period, the industrial wastewater has the potential to replace methanol as carbon source (Swinarski et al. 2009). Chen et al. (2013) investigated the applicability of four other industrial wastewaters (potato processing, canola processing, oil refining, biodiesel production by-product [glycerol], and deicing wastewater containing ethylene glycol) for denitrification of a sludge dewatering liquor and compared their applicability with that of methanol and ethanol. Among the four industrial wastewaters, the specific denitrification rate of glycerol was the highest, followed by that of potato processing wastewater. Canola processing and oil-refining wastewater had a lower specific denitrification rate and inhibited nitrification. The adaptation time of microorganisms to ethylene glycol was shorter than that of ethanol and methanol. The disadvantage of industrial wastewater as a carbon source is that it significantly changes the water quantity and quality and its composition is complex. Moreover, the transportation, dosing mode, dosing equipment, cost, and impact on microorganisms should be considered when using it as a carbon source.

Agricultural waste and natural cellulose materials are also potential carbon sources. They have no biological toxicity, and are common, inexpensive sources. At present, there are many studies on agricultural wastes and natural cellulose materials, such as rice straw, wheat straw (Guan et al. 2019), corncobs (Li et al. 2012), and peanut shells (Ramírez-Godínez et al. 2015). Yang et al. (2015) evaluated the carbon release capacity, denitrification potential, leaching elements, and surface properties of eight agricultural wastes through a membrane bioreactor, and discussed the feasibility of using agricultural waste as a solid carbon source and its role in improving denitrification. The results showed that retinervus luffae fructus, corncobs, rice straw, and wheat straw had a high carbon release capacity. Among them, the retinervus luffae fructus, corncobs, rice straw had significant denitrification effects, and total nitrogen removal rates increased from 43.44% (control group) to 82.34%, 68.92%, and 62.97%, respectively. However, some studies have shown that some agricultural wastes release excessive nitrogen and phosphorus and produce chromaticity when used as a carbon source, which causes secondary pollution and increases the difficulty of subsequent treatment (Ling et al. 2021).

3.1.4 Biodegradable polymers

Biodegradable polymers are another hot research topic in the field of carbon sources for denitrification. They are a solid carbon source, similar to agricultural wastes. Biodegradable polymers release carbon sources mainly through Fick diffusion. The released organic substances are mainly short-chain fatty acids, such as acetic acid, propionic acid, and butyric acid (Yu et al. 2020). Compared with agricultural wastes, biodegradable polymers have a higher and more consistent denitrification efficiency and rate, lower dissolved organic carbon release, and higher costs (Wang and Chu 2016). Common biodegradable polymers include polyhydroxyalkanoate, poly-3-hydroxybutyric acid, poly-3-hydroxybutyrate-co-hyroxyvelate, polycaprolactone, polybutylene succinate, and polylactic acid.

Polycaprolactone has been reported to be a good carrier and carbon source for biological denitrification. Polycaprolactone was added in a packed-bed bioreactor and used as both a carbon source and biofilm support for denitrifying bacteria. In stable operation, the average nitrate removal rate reached 93%, and denitrifying bacteria accounted for more than 20% of the total bacteria (Wu et al. 2013). Shen et al. (2016) showed that polybutylene succinate is a carbon source in a packed-bed bioreactor. When NO_3-N loading rate was 0.63 kg $m^{-3}day^{-1}$, the average NO_2-N concentration in the effluent was less than 0.20 mg/L, and the volumetric denitrification rate was 0.60 kg/m^3/day. In the microbial community, *Proteobacteria* was the most abundant phylum, accounting for 89.87%, while *β-Proteobacteria* was the most abundant class. Because most denitrifying bacteria belong to Proteobacteria, it can be inferred that using polybutylene succinate as a solid carbon source promotes the growth of denitrifying bacteria.

3.1.5 Composite Carbon Source

Glucose, methanol, ethanol, acetate, and other chemicals contain only one effective carbon source component, which is called a single carbon source. The composition of wastewater is complex, and the types of organic matter used by denitrifying bacteria are also diverse. Therefore, the simultaneous addition of multiple effective carbon sources may be a feasible way to improve the denitrification efficiency of microorganisms. A composite carbon source is composed of two or more effective carbon source components which are compatible with each other, have no chemical reaction, and have a low safety risk.

Chen et al.’s (2017) results indicated that a nitrate removal efficiency of 97.5% was obtained when acetate and propionate were mixed in a ratio of 33–67%, respectively. The highest nitrate removal efficiency of 92.0% was attained when a mixture of 30% acetate, 60% propionate, 5% butyrate, and 5% valerate was used. This means that different dosage ratios had an impact on the denitrification efficiency. In addition to research on composite liquid carbon sources, agricultural wastes and biodegradable polymers are also research hot spots for composite carbon sources. The carbon release and denitrification properties of a modular composite solid carbon source, PBS-PS-PVA-SA, which was prepared from polybutylene succinate (PBS) and peanut shells (PS) as carbon source materials and polyvinyl alcohol (PVA) and sodium alginate (SA) as carriers (Peng et al. 2021). When PBS-PS-PVA-SA was used as an external carbon source, acetic acid was the main component of the short-chain fatty acids released by the PBS-PS-PVA-SA. The concentration of dissolved organic carbon in the effluent was lower than 20 mg/L.

3.2 New nitrogen removal pathways

Regarding the treatment of wastewater with low C/N ratios, a reasonable external carbon source supplement is necessary to meet denitrification requirements. Accordingly, new nitrogen removal approaches have been introduced to support the denitrification process. These new approaches have low organic carbon requirements or do not even need the addition of external carbon sources (zero organic carbon demand). Currently, the new nitrogen removal approaches mainly include anammox, sulfur/iron-based autotrophic denitrification, shortcut nitrification-denitrification, and simultaneous nitrification-denitrification.

3.2.1 Anammox

The anammox process is a technical progression from the traditional nitrification/denitrification process to autotrophic denitrification. In the anammox process, anammox bacteria use nitrite as an electron acceptor to oxidize ammonium to nitrogen ($NH_4^+ + NO_2^- \rightarrow N_2$) (van de Graaf et al. 1995). The anammox process requires a sufficient nitrite supply. According to different nitrite supply methods, anammox can be divided into nitritation ($NH_4^+ \rightarrow NO_2^-$)/anammox and denitrification ($NO_3^- \rightarrow NO_2^-$)/anammox (Ma et al. 2020). These two processes can complete denitrification with less or without organic matter. First, nitritation/anammox is the mainstream process of anammox. However, limited by the growth of ammonia-oxidizing bacteria, the process is unstable, especially at low temperatures. Recently, denitrification/anammox has been considered as an alternative to mainstream nitrogen removal. Although the denitritation/anammox process requires more organic matter than nitritation/anammox, its nitrite production process is more stable (Ma et al. 2016; Ma et al. 2017).

The WWTP Dokhaven in Rotterdam, Netherlands, adopted a nitritation/anammox process. The total nitrogen removal rates under stable operation at summer temperatures (23.2±1.3℃) and winter temperatures (13.4±1.1℃) were 0.223±0.029 kgN/m^3/d and 0.097±0.016 kgN/m^3/d, respectively. Long-term stability is still the focus of future research (Hoekstra et al. 2019). Li et al. (2019) successfully obtained an enhanced nitrogen removal effect in a municipal WWTP through the in situ enrichment of anammox bacteria. The plant originally removed nitrogen through traditional nitrification/denitrification technology and then upgraded by adding mobile carriers in the anoxic area. They proved that the abundance of anammox bacteria in the biofilm formed on the carrier was higher than that of flocculated sludge. Anammox can be combined with nitrate reduction of the carrier biofilm, which is conducive to nitrogen removal.

3.2.2 Sulfur/iron-based autotrophic denitrification

In addition to heterotrophic denitrification with organic matter as a carbon source, autotrophic denitrification occurs with inorganic carbon as a carbon source. Autotrophic denitrification reduces the formation of downstream adverse byproducts (e.g., trihalomethanes) and sludge production (Di Capua et al. 2019). According to different electron donors, autotrophic denitrification includes anammox, hydrogenotrophic denitrification, and sulfur/iron-based autotrophic denitrification. Hydrogenotrophic denitrification uses H_2 as the electron donor. However, the low solubility, high cost, and safety of H_2 are the main factors limiting the full application of the hydrogenotrophic denitrification process (Tian and Yu 2020).

Reduced inorganic sulfur compounds, such as sulfide, elemental sulfur, and thiosulfate, can be utilized by sulfur autotrophic denitrification as an electron donor to reduce nitrate/nitrite to nitrogen (Cui et al. 2019). Reduced inorganic sulfur compounds are usually treated as environmental pollutants. Sulfur autotrophic denitrification can simultaneously remove nitrogen and reduce sulfide (Manconi et al. 2007). Fu et al. (2020) investigated the sulfur autotrophic denitrification operation performance and microbial community structure when sodium sulfide, elemental sulfur, and sodium thiosulfate were used as electron donors alone. The results showed that the autotrophic denitrification performance was similar for elemental sulfur and sodium thiosulfate. However, denitrification performance was not stable, and nitrite accumulation was significant for sodium sulfide at a high influent concentration (131–156 mg/L). The microbial diversity of sludge was the most abundant with elemental sulfur as an electron donor, followed by sodium thiosulfate and sodium sulfide. H^+ is produced in the process of sulfur autotrophic denitrification, which requires additional limestone to supplement the alkalinity. It also produces a large amount of SO_4^{2-}, which affects the effluent quality (Zhou et al. 2011; Di Capua et al. 2019).

Reduced Fe (Fe^0, Fe^{2+}) is considered an electron donor in the autotrophic iron-dependent denitrification process. Autotrophic iron-dependent denitrification exists widely in natural systems and engineering systems (Laufer et al. 2016). Fe (II)-mediated autotrophic denitrification can remove nitrate and ferrous ions simultaneously, and can be recovered and reused in the form of Fe (III) minerals (Kiskira et al. 2017). Tian et al. (2020) successfully domesticated an autotrophic iron-dependent denitrification system for continuous nitrogen removal from activated sludge, which demonstrated the feasibility of autotrophic Fe (II)-oxidizing nitrate-reducing culture for nitrogen removal. Wang et al. (2020) reported that compared with the control group without electron donors in the influent, the nitrate removal efficiency of an iron-dependent nitrate removal reactor was two times higher and more stable. Nevertheless, the formation of rust hindered the transportation of nutrients in the cells, resulting in the deterioration of the treatment performance of the iron-dependent nitrate removal reactor. Appropriate measures should be taken to avoid the formation of rust or to remove it in a timely manner. Iron sulfides, are mainly in the form of mackinawite (FeS), pyrrhotite ($Fe_{1-x}S$, x=0-0.125) and pyrite (FeS_2), with the simultaneous presence of reducing sulfur and iron. And iron sulfides has a large denitrification capacity, simultaneous nitrogen and phosphorus removal, self-buffering, and fewer byproducts (sulfate, sludge, N_2O, etc.). Autotrophic denitrification with iron sulfides as electron donors is a promising biological nitrogen removal technology (Hu et al. 2020).

3.2.3 Shortcut nitrification-denitrification

The shortcut nitrification-denitrification technology shortens the nitrification and denitrification processes, that is, the technology omits the conversion processes of Eq. (2) and (3): NH_4^+ is only oxidized to NO_2^-, and NO_2^- is directly denitrified to N_2 (Yang et al. 2007). This can reduce the consumption of oxygen and organic matter. The stable accumulation of NO_2^- in the nitrification process is the key to this technology. There are several methods to realize shortcut nitrification-denitrification, such as real-time control to prevent excessive aeration, adding inhibitors to selectively inhibit nitrification, and controlling pH and temperature to make nitrobacteria the dominant bacteria (Wang and Sun 2013). Gao et al. (2009) showed that partial nitrification and total nitrification can be judged by real-time control of aeration and observation of characteristic points on oxidation–reduction potential curve and pH curve. Therefore, the aeration system was controlled to stop immediately after the completion of nitritation, and shortcut nitrification-denitrification was maintained at nitritation ratios (NO_2^--N/NO_x^--N) higher than 96%. In addition, shortcut nitrification-denitrification can also be achieved by adding nitrification inhibitors, including heavy metals, toxic substances, organic compounds, and fulvic acid (Zhang et al. 2000; López-Fiuza et al. 2002; Peng and Zhu 2006). For example, free ammonia and free nitrous acid can inhibit the growth of ammonia-oxidizing bacteria and nitrite-oxidizing bacteria at different concentrations (Sun et al. 2010). Because the concentrations of free ammonia and free nitrous acid in water are related to the pH of the system, controlling the pH of the system can control the growth of nitrifying bacteria to control and realize the effect of shortcut nitrification-denitrification.

3.2.4 Simultaneous nitrification-denitrification

Simultaneous nitrification-denitrification refers to the simultaneous occurrence of nitrification and denitrification in a single reactor. A decrease in total nitrogen can often be observed in the aeration tank of the activated sludge method. A general explanation for this phenomenon is that nitrification occurs on the surface of activated sludge flocs due to oxygen enrichment, and denitrification occurs with less dissolved oxygen inside the flocs. In addition to the activated sludge method, simultaneous nitrification-denitrification also occurs in other water treatment technologies, such as the biofilm method (Bhattacharya and Mazumder 2021). Simultaneous nitrification-denitrification has the advantages of less carbon source and energy consumption, low sludge output, small floor area, and low alkalinity demand (Li et al. 2008). Paetkau et al. compared the sludge characteristics and denitrification performance of a conventional aerated membrane bioreactor (C-MBR) and a low oxygen simultaneous nitrification-denitrification membrane bioreactor. The simultaneous nitrification-denitrification membrane bioreactor system had higher transmembrane pressures, larger activated sludge flocs, more varied concentrations of soluble chemical oxygen demand, and transparent exopolymer substances. The total nitrogen removal efficiency of the simultaneous nitrification-denitrification membrane bioreactor was 80%, whereas that of the conventional aerated membrane bioreactor only reached 31% (Paetkau and Cicek 2011).

3.3 Microbial Community changes

In order to improve the efficiency of the biological nitrogen removal process significantly, in addition to monitoring the effluent index, it is necessary to observe the community structure and diversity of microorganisms in the system. Microorganisms in the denitrification process could be influenced drastically by using different external carbon sources and biological denitrification technologies. The variation in microbial community structures and diversities can reflect the mechanism of nitrogen removal. They may also help explain experimental results.

Xu et al. (2018) investigated the effects of solid organic matter (poly-3-hydroxybutyrate-co-hyroxyvelate/polylactic acid polymer) and liquid organic matter (glucose and sodium acetate) as denitrifying carbon sources on the microbial community. The results showed that the three carbon sources led to different microbial community structures. *Brevinema/ Thauera/ Dechloromonas*, *Tolumonas/ Thauera/ Dechloromonas*, *and Thauera* were dominant in the denitrification system supported by poly-3-hydroxybutyrate-co-hyroxyvelate/polylactic acid, glucose, and sodium acetate, respectively. Among the three systems, the poly-3-hydroxybutyrate-co-hyroxyvelate/polylactic acid system had the largest microbial diversity, and the sodium acetate system had the highest relative abundance of denitrifying bacteria, accounting for the higher denitrification rate of the system. Tang et al. (2018) used lactic acid-enriched FW-FL as a denitrifying carbon source and found that, compared with sodium acetate, sodium lactate, and starch, the addition of FW-FL promoted microbial metabolic activity and community diversity in the activated sludge, and further improved organic matter utilization and denitrification efficiency. The dominant bacteria in the sludge of the system supported by FW-FL were *Rhodocyclaceae* and *Comamonadaceae*, both of which can use refractory organic matter as a carbon source for denitrification.

4. Conclusion

Based on 7796 published articles related to the field of carbon sources for nitrogen removal in wastewater treatment from the Science Citation Index Expanded database, an overview of research on denitrifying carbon sources was formed by bibliometric analysis. Some significant points were identified in this field. Within the statistical time frame, China ranked first in terms of the number of publications, followed by the USA and Japan. Regarding research institutions, the Chinese Academy of Sciences had the largest number of publications, and the top five institutions with the largest number of published articles were all Chinese research institutions, indicating that China is at the forefront in the research field of carbon sources for nitrogen removal in wastewater treatment.

Keyword analysis revealed research hotspots in this field. Traditional external carbon sources, such as glucose, methanol, ethanol, and acetate, and new alternative carbon sources, such as sludge fermentation liquid, FW-FL, industrial wastewater, agricultural wastes, biodegradable polymers, and composite carbon sources containing a variety of effective carbon source components, have been introduced. Different types of carbon sources have unique advantages and characteristics. Appropriate external carbon sources should be selected for practical applications. Four new biological nitrogen removal approaches, including anammox, sulfur/iron-based autotrophic denitrification, shortcut nitrification-denitrification, and simultaneous nitrification-denitrification, are expected to have promising application prospects in the future. In addition, the variation of microbial community structures is an increasingly crucial field in carbon source research, which can clarify the mechanism of the denitrification process supplemented by external carbon sources. It will continue to take efforts to enhance nitrogen removal performance in WWTPs in terms of developing efficient and economical carbon sources, exploring highly efficient denitrification approaches, and developing novel wastewater treatment in the future.

Declarations

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Consent for publication: Not applicable

Availability of data and materials: The datasets generated or analysed during the current study are available in the Web of Science repository [http://apps.webofknowledge.com/].

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Figures

Figure 1

Number of Science Citation Index Expanded publications on carbon sources for nitrogen removal in wastewater treatment (from 2000 to 2019)

Figure 2

The annual publications of top 5 most productive countries in 2000-2019 (TP: total publications)

Figure 3

Collaborative relationships among important research institutions (the size of the circle represents the article's number; different colors represent different clusters; the thickness of connecting link means the number of the collaborative articles)

Figure 4

Analysis of keyword co-occurrence (the size of node represents the frequency of occurrence)