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Review

Application of Internal Carbon Source from Sewage Sludge: A Vital Measure to Improve Nitrogen Removal Efficiency of Low C/N Wastewater

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Abstract: Biological nitrogen removal from wastewater is widely used all over the world on account of high efficiency and relatively low cost. However, nitrogen removal efficiency is not optimized when the organic matter has inadequate effect for the lack of a sufficient carbon source in influent. Although addition of an external carbon source (e.g., methanol and acetic acid) could solve the insufficient carbon source problem, it raises the operating cost of wastewater treatment plants (WWTPs). On the other hand, large amounts of sludge are produced during biological sewage treatment, which contain high concentrations of organic matter. This paper reviews the emerging technologies to obtain an internal organic carbon resource from sewage sludge and their application on improving nitrogen removal of low carbon/nitrogen wastewater of WWTPs. These are methods that could solve the insufficient carbon problem and excess sludge crisis simultaneously. The recovery of nitrogen and phosphorus from treated sludge before recycling as an internal carbon source should also be emphasized, and the energy and time consumed to treat sludge should be reduced in practical application.

Keywords: internal carbon source; low C/N wastewater; nitrogen removal; resource recovery

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1. Introduction

Nitrogen pollution in natural water is a serious problem worldwide, especially in developing countries such as China [1,2]. Excessive amounts of nitrogen in receiving water can cause eutrophication, posing potential hazards to animal and human health [3,4]. Thus, it is essential to remove nitrogen compounds before wastewater is discharged to rivers or lakes. Currently, the most efficient and common approach to this endeavor is biological nitrogen removal technology, on account of high treatment efficiency and relatively low cost [5,6]. Generally, the biological nitrogen removal process can be divided into two parts, nitrification, and denitrification, based on the bacteria involved [7,8]. Most denitrification bacteria are heterotrophic and require a biodegradable organic carbon resource such as short-chain fatty acids (SCFAs) as electron donors [7,9]. However, the concentration of organic carbon in municipal sewage is usually very low, resulting in incomplete denitrification, which can affect the nitrogen removal efficiency. To solve the insufficient carbon source problem, conventional external organic carbon, such as methanol and acetic acid, is usually added into wastewater. Obviously, such practices are uneconomic and unsustainable and could increase the operational cost of wastewater treatment plants (WWTPs) and have other side effects, such as producing more greenhouse gas and excess sludge [10,11]. Therefore, it is critical to find other types of carbon sources to replace the external carbon source.

On the other hand, large amounts of waste activated sludge (WAS) are produced in the biological denitrification process. In 2013, 6.25 million tons of dry solids were generated in China, and above 80% of the sludge is improperly dumped because of the difficulty and high cost of disposing sludge [12]. Directly pouring untreated sludge into the environment can cause serious environmental problems, including secondary pollution of natural water and contamination of soil [13]. Meanwhile, the concentration of organic matter in WAS is very high and is a substantial organic carbon source, which has been overlooked in the past [14]. In recent decades, various technologies have already been developed to separate the carbon source from sewage sludge and recycle it to the denitrification part of the biological nitrogen removal process as an additional electron donor to improve nitrogen removal effectiveness. The purpose of this paper is to systematically review and compare the emerging methods for separating internal organic carbon resources from sewage sludge, and to evaluate their performance in promoting nitrogen removal after recycling in the biological nitrogen removal process.

2. Requirement of Carbon Source in Biological Nitrogen Removal Process

Nitrogen elimination in wastewater by biological approaches has been widely practiced around the world since the activated sludge method was inverted about 100 years ago by virtue of its many advantages [15], for example, it is easy to set up and economical to maintain. As shown in Figure 1, during the biological nitrogen removal process, ammonia is oxidized to nitrite by ammonia-oxidizing bacteria (AOB) under aerobic conditions, and further to nitrate by nitrite-oxidizing bacteria (NOB) [16–18].

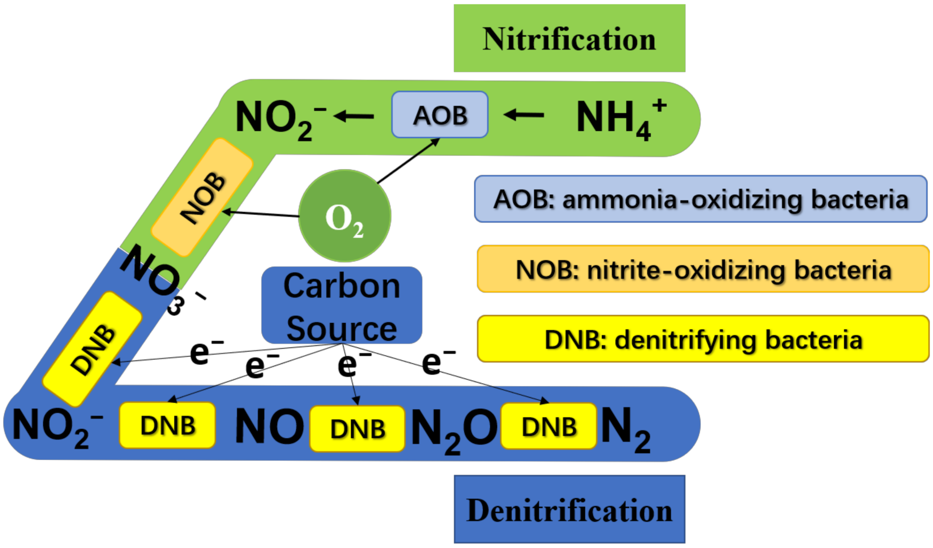

Figure 1. Typical pathway of biological nitrogen removal and involved bacteria.

Denitrification is a denitrifying bacteria (DNB)-mediated process whereby nitrate or nitrite is converted into N_2 via intermediates NO and N_2O in the absence of oxygen. Nutritionally, most denitrifying bacteria are anaerobes and heterotrophs [19]. During the heterotrophic denitrification process, a biological organic carbon source is needed by denitrifiers as an electron donor for oxidizing NO_x-N (NO_2N and NO_3-N) to N_2. The generally recognized pathway is shown in Figure 1.

The carbon source in Figure 1 must be soluble SCOD that is usable to denitrification bacteria, such as volatile fatty acids and other types of small molecular organic acids [20]. Theoretically, the stoichiometric requirement of carbon resource (expressed as COD) for denitrification was 2.86 g and 1.91 g, respectively, when 1 g NO_3-N and NO_2-N was reduced completely to N_2, considering the electronic transmission balance between organic substrates and NO_x-N [21]. Nevertheless, it was reported that the C/N ratio requirements in practice varied with the treatment process or operational condition [22]. The optimal carbon-to-nitrogen (C/N) ratio for nitrogen elimination with various biological nitrogen removal processes and operational conditions is shown in Table 1. As we can see, the requirement of the C/N ratio in practice is usually higher than theoretical requirements and is 3.5 to 11 under various processes and operational conditions. When the C/N ratio is bigger than 10, higher nitrogen removal efficiency is generally obtained [23–25]. Therefore, additional carbon sources should be added to obtain the required C/N ratio for a high nitrogen removal rate. In the following paragraphs of this article, conventional external and internal carbon sources, commonly used by WWTPs, are reviewed in detail.

Table 1. Optimal carbon to nitrogen (C/N) ratio of different processes and operational conditions.

Process	Wastewater Type	Operational Condition	Optimal C/N	Nitrogen Removal Efficiency (%)	References
Anoxic-aeration reactor	Sewage	Anoxic-aeration	5	42.3	[26]
Modified Ludzack-Ettinger process	Industrial *w*/*w*	Anoxic-aerobic-aerobic	7.1	-	[27]
SBR	Synthetic	Anoxic-aerobic	11.0	99.6	[23]
SCBR [a]	Synthetic	Aeration	5	78.4	[28]
Modified MBR	Synthetic	Anoxic-aerobic	9.3	90.6	[29]
Microcosm wetlands	Synthetic	Not given	5	63.83	[30]
SBR	Shrimp *w*/*w*	Aerobic-anaerobic-aerobic	10	99	[24]
SBR	Synthetic	Aeration, DO = 0.5 mg/L.	6.8	50.3	[31]
SBBR [b]	Livestock and poultry breeding wastewater	Aerobic-anaerobic-aerobic-anaerobic	18.9	~96	[25]
PITSF [c] system	Sewage	Intercross real-time control system	4.3	83.2	[32]
SNDPR-SBR [d]	Sewage	Anaerobic-aerobic	3.5	77.7	[33]

[a] SCBR: Suspended carrier biofilm reactor; [b] SBBR: Sequencing biofilm batch reactor; [c] PITSF: Phased isolation tank step feed; [d] SNDPR-SBR: Simultaneous nitrification denitrification and phosphorous removal sequencing batch reactor.

3. Conventional External Carbon Source

Upon initially finding that carbon shortage limited the removal of nitrogen in wastewater, small molecule organics, e.g., methanol, ethanol, and acetic acid, became the most commonly used additional carbon source at WWPTs to improve nitrogen removal [34]. Afterwards, carbohydrates such as glucose and saccharose were also added to sewage to solve the problem. Currently, inexpensive and easily obtained organic matter is increasingly being researched and used as an alternative carbon source to enhance nitrogen removal performance of WWTPs [20].

Because methanol has a very small molecule and is an excellent election donor, it has been widely used as additional carbon to facilitate denitrification. Manoharan et al. [35] found that the treatment of high ammonia and low biodegradable carbon landfill leachate with methanol as an external carbon source obtained excellent nitrification and denitrification performances and achieved complete ammonia removal and the maximum NO_x removal. AbuGhararah et al. [36] compared nitrogen removal efficacy using different kinds of organic sources and found that efficacy was 95–97% when methanol was taken as an external carbon source. Nevertheless, the principal disadvantage of using methanol (its toxicity) limits its expansion. Therefore, other types of small molecule organic compounds such as ethanol, acetic acid and citric acid have been tested and used as suitable substitutes for methanol. Lee and Park [37] took sodium acetate as a carbon source when studying the denitrification of wastewater from coking plants and found that the total removal rate of major soluble pollutants in wastewater by biological denitrification system was over 95%. Since citric acid was taken as an external organic substrate in the biofilm reactor, Artur Mielcarek et al. [38,39] found that it is a particularly effective source of organic carbon in the denitrification process and could prevent clogging of the biofilm reactor, and the total nitrogen removal rate could reach 98.6%. In addition, Iza-bella Kłodowska et al. [40] also clarified that as a promising external carbon source, citric acid could improve the denitrification rate in bioelectrochemical sequencing batch biofilm reactors [40]. When the citric acid production wastewater was used as the alternate carbon source in a pilot scale process, Wenhao Liu et al. [41] showed that the performance of biological nutrients removal was close to the traditional external carbon source (e.g., sodium acetate) and was profitable (4.6 USD/m^3) based on economic analysis. In other articles, authors tried carbohydrates as external carbon sources and compared the result with that of small molecule organics (e.g., methanol, ethanol, and acetic acid), but results concerning which types of carbon source were superior were inconsistent [35,38–44]. This inconsistency might be caused by varying properties of effluence and operational condition. Because of the increasing cost and scarcity of chemical products, research efforts have focused on cheap and abundant industrial wasted organic matter. Three substitute carbon sources in SBR, namely wastewater from sweet food factories, residues from soft drink factories, and residues from dairy factories, were tested by Fernandez-Nava et al. [45], finding that values of the denitrifiers net yield coefficient were higher than those using methanol. Sora Park et al. [46] found that TN removal efficiency could reach 71.7% when modified spent caustic (MSC) was used as an electron donor for denitrification. They explained that this was mainly because MCS would be served as an effective electron donor for denitrification by autotrophic denitrifiers during wastewater treatment. Lee et al. [47] evaluated the feasibility of industrial organic wastes as the substitute external carbon source for denitrification in the biological nutrient removal process. The results showed that the denitrification ability of microorganisms resembled that of using methanol as an external carbon source. Zhu et al. [48] found that corn flour as a carbon source for aerobic denitrification could not only improve the nitrogen removal efficacy but also reduce nitrous oxide (N_2O) emission. Wojciech Janczukowicz et al. [49] explored the potential of fermented wastewater from a butter factory as a carbon source to promote biological nutrient removal. They concluded that fermented butter wastewater could simultaneously improve the quality of phosphate and nitrate in effluent, which may be due to the introduction of fermentation wastewater to increase the content of easily available compounds and the advantage of acetic acid in VFAs that can be used for phosphorus removal and denitrification [49]. In another study, they also proved the feasibility of waste beer as a carbon source to enhance denitrification in an anaerobic sequencing batch biofilm reactor (AnSBBR) [50].

Although conventional external carbon resources added in wastewater could improve nitrogen removal efficacy, inevitable increases in costs of transport and chemical storage facilities plus WAS yield reduced their attractiveness. Thus, finding satisfactory alternatives to conventional external carbon resources is a top priority for wastewater treatment facilities.

4. Internal Carbon Resources

Recently, a growing number of research studies have concentrated on discovering useful internal carbon sources from the main line of a WWTP [51–54]. Gali et al. [55] investigated different internal flows from a WWPT for their availability as internal organic carbon resources in the wastewater treatment, finding that hydrolyzed primary and excess activated sludge were both feasible for denitrification. Figure 2 shows the key aspects of these widely researched and used processes, which use internal carbon sources as electron donors of denitrification to improve nitrogen removal from wastewater. In this scheme, an internal carbon production unit is added to the conventional wastewater biological treatment process. In the internal carbon source production unit, primary or waste activated sludge was treated using various techniques to easily produce used internal carbon by denitrifiers, such as volatile fatty acids (VFAs), the most suitable carbon source for biological nitrogen removal [56,57]. Then, the treated sludge or supernatant was returned to a biological reaction tank to promote denitrification. However, there are still problems about which sludge source to apply and the suitable mechanism for VFAs production [58].

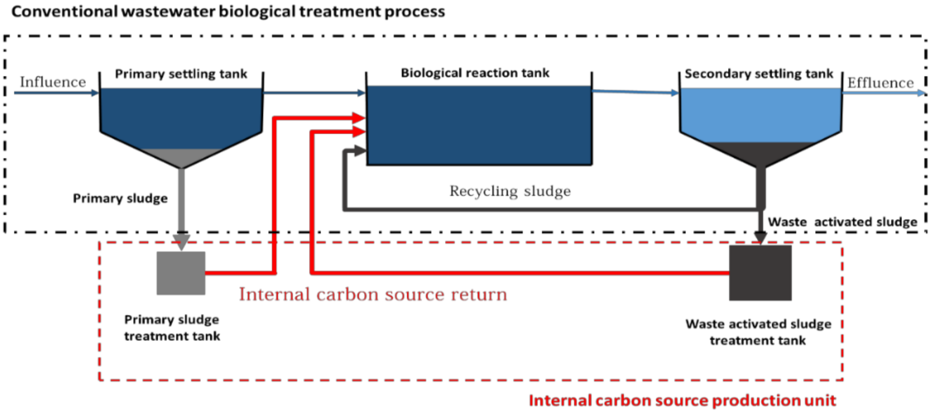

Figure 2. Conventional biological wastewater biological treatment process with an internal carbon source production unit.

4.1. *Techniques Applied for VFAs Production from Sludge*

Numerous sludge treatment technologies to increase VFAs have been developed, mainly including physical processes (such as mechanical treatment, focused–pulsed, and ultrasound) [59–61], fermentation processes (alkaline and acid fermentation) [61–65], and their combination [66,67]. These techniques are used to break sludge floc into a small fraction of soluble COD (SCOD, such as VFAs) [59], or translate long and non-degradable organic substances into short and biodegradable COD [68]. Because the properties of primary and waste activated sludge vary greatly [55], various treatment methods are typically used.

4.1.1. Techniques Applied for VFAs Production from Primary Sludge

Conventionally, ~30–35% of influent COD is removed and settled in primary sludge within the primary settling tank, which may be transformed into a carbon source available to denitrifiers under appropriate conditions [69]. Cokgor et al. [70] investigated the potential of preliminary sludge fermentation to produce easily biodegradable substrates, stating that 22% of the initial volatile suspended solids in the sludge were transformed into soluble biodegradable COD by unbounded fermentation process. However, the production of the biodegradable carbon source might vary with the sludge treatment method. Table 2 summarizes the internal carbon source production of primary sludge under different treatments. Fermentation was one of the technologies most commonly used for dealing with primary sludge among the previously reported methods. Temperature and pH control significantly affected VFA generation by fermentation. Cokgor et al. [70] indicated that the raising temperature (from 10 to 24 °C) resulted in a five-fold increase in VFA production, from 610 to 2950 mg/L. pH adjustment and control had negative impacts on fermentation efficiency, with the initial adjusted pH producing the largest amount of VFAs. However, these results contrast with those of Ahn et al. [71], who found that an increase of pH from 6.6 to 11 in thermophilic (55 °C) conditions improved the system performance. Solid retention time (SRT), recirculating sludge flow rate, and solid concentration were other important influences on fermentation performance. Bouzas et al. [72] investigated their effect in the fermentation elutriation process, finding that maximum VFA production (36 mg HAc L^{-1}) was attained at SRT between six and eight days at the highest waste sludge flow rate of 4.5 and an optimal solids concentration of 23,000 mg/L.

Table 2. Internal carbon source production of primary sludge under different treatment techniques.

Sludge Treatment Technique	Carbon Source Production	References
Thermal-alkaline (pH = 9, T = 55 °C)	0.18 g VFA [a]/g VSS_{COD}	[71]
Fermentation–elutriation (SRT [b] = 6 days, RSFR [c] = 4.5 L/h)	36 mg/L HAc [d]	[72]
Hyper-thermophilic (70 °C, HRT = 2 days) hydrolysis followed by thermophilic (55 °C, HRT = 13 days) anaerobic digestion	76 mg/L VFAs	[73]
Fermentation (T = 24 °C without pH control)	2950 mg/L VFAs	[70]
Thermophilic–mesophilic anaerobic digestion (50–70 °C)	3300 mg/L VFAs	[74]
Fermentation (T = 30 °C without pH control)	0.99 $gCOD/gVSS^{-1}$	[75]

[a] VFA: Volatile fatty acid; [b] SRT: Sludge retention time; [c] RSFR: Recirculation sludge flow-rate; [d] HAc: Acetic acid.

Aside from the advantages above, fermentation under ambient temperature usually has a long SRT because of a long period of initial hydrolysis, the rate-determining step for anaerobic digestion that transforms particulate organic matter into soluble substances [76]. Therefore, some efforts have been made to promote fermentation efficiency by improving sludge hydrolysis rate, such as thermal [71]. Ge et al. [74] used thermophilic-mesophilic pretreatment to improve degradability and the hydrolysis rate of primary sludge; as much as 3300 mg/L VFA was produced within a short SRT. Similarly, implementation of a hyper-thermophilic (70 °C) pre-hydrolysis step could also achieve greater fermentation efficiency [73].

After being treated to produce an internal carbon source, fermented sludge or separated liquid supernatant rich in VFAs can be recycled to a denitrification part as an electron donor. Zhang et al. [75] reported that 85% removal of ammonium and 75% of TN could be attained under optimum conditions, using fermented primary sludge in a continuous system by dealing with sludge dewatering liquors. However, Canziani et al. [77] reported only slight improvement of denitrification capacity caused by the feed of readily biodegradable COD produced from typical municipal primary sludge under psychrophilic (16–20 °C) conditions. This may be because such conditions were not optimal for sludge hydrolysis and acidification, and therefore resulted in small obtainable readily biodegradable COD amounts in treated sludge.

4.1.2. Techniques Applied for VFAs Production from Waste Activated Sludge

Compared to primary sludge, WAS contains higher levels of organic matter and has greater potential as a source of inexpensive organic substrate for internal carbon source production. However, produced after biological treatment of wastewater, WAS is already well stabilized and has a low proportion of easily bioavailable COD. Thus, WAS is relatively difficult to treat in VFA production and a pretreatment technology is usually necessary to improve its bioavailability. Research into methods to treat WAS and promote its production of an internal carbon source are summarized in Table 3. Among all techniques reviewed in the present article, physical process, acid/alkali fermentation processes, and combined processes of fermentation with accelerant addition or calefaction are the most common.

Table 3. Internal carbon source production of waste-activated sludge under different treatment techniques.

Treatment Technique	Treatment Condition	Internal Carbon Resource Production	Nitrogen Removal Performance	References
Mechanical disintegration	Disintegrated with a deflakerfor for 2–15 min	530 mg/L VFA [a]	Denitrification rate was increased by 6.5 mg NO_3-N g^{-1}/VSS h^{-1} compared with the control vessel (30.2 mg NO_3-N g^{-1}/VSS h^{-1})	[59]
Focused-pulsed treatment	Treatment intensity = 28 kWh/m^3	397 mg/L SCOD [b]	Maximum denitrification rate (0.25 g NO_3-N g^{-1}/VSS d^{-1}) was greater than foruntreated WAS 0.05 g NO_3-N g^{-1}/VSS d^{-1}) and methanol (0.15 g NO_3-N g^{-1}/VSS d^{-1})	[61]
Mechanical disintegration	Multi-use rotor driven by a motor with a power of P = 2 kw, revolutions n = 2800 r/min	218.13 mg/L VFA	Denitrification efficiency increased from 49.2% to 76.2%	[78]
Alkaline fermentation	T = 21 °C, pH = 10, t = 8 days	4225 mg/L SCFA [c]	TN removal efficiency increased to 83% compared to the control group, which was 63%	[79]
Alkaline fermentation	T = 20–22 °C, pH = 10, t = 8 days	4189 mg/L SCFA	TN removal efficiency increased to 83.2% with the optimal volume ratio of fermentation liquid to municipal sewage equal to 1/35, compared to the control group, which was 63.3%	[63]
Alkaline fermentation	T = 20–22 °C, pH = 10, t = 8 days	4035 mg/L BOD	TN removal efficiencies of sludge fermentation liquid were much higher than with acetic acid (98.7% versus 79.2%).	[80]
Alkaline fermentation	pH = 10, t = 8 days	2480 mg/L SCFA	TN removal efficiencies of sludge fermentation liquid were much higher than control group (74.7% versus 27.1%)	[81]
Alkaline fermentation	T = 35 °C, pH = 12, t = 8 h	365 mg VFAs g^{-1}VSS 0.66 SCOD mg/mg TCOD [d]	NO_3-N removal efficiency of acid fermentation liquor showed slight differences with that of sodium propionate, and the former had more stable performance than the latter.	[82]
Anaerobic fermentation with nano zero-valent iron addition	T = 20 °C without pH control, t = 4 days, 5.0 g/L nZVI [e]	1307.8 mg/L SCFA	Not given	[83]
Anaerobic fermentation with humic acids addition (SHHA and SAHA)	T = 35 °C, pH = 7.0, t = 9 days, 1.0 g/g TCOD	2741 mg/L SCFA	Not given	[84]
Anaerobic fermentation with alkyl polyglucose addition	T = 25 °C without pH control, t = 4 days, 0.3 g APG/g TSS	2988 mg/L SCFA	Not given	[85]
Anaerobic fermentation with surfactant (SDBS) addition	T = 21 °C, pH = 10, t = 12 days	2056 mg /L SCFA	Not given	[64]
Fermentation with nitrite (NO_2^-)	T = 35 °C, pH = 5, t = 28 days, 0.1 g NO^2-N L^{-1} per day	4500 mg/L SCFA	Not given	[86]
Fermentation with free nitrous acid addition	T = 20 °C, pH = 10,t = 2 days, 1.54 mg FNA [f]/L	370.1 mg/g SCFA	Not given	[87]
Thermal-alkaline pretreated anaerobic digestion	T = 90 °C, pH = 11, t = 10 h	69000 mg/L SCOD	Not given	[66]
Thermal-alkaline pretreated anaerobic digestion	T = 60 °C, pH = 12, t = 12 h	3078 mg/L SCOD, 1096 mg/L VFA	TN removal efficiency within 12h was 51.6% higher than control group, which was 35.6%	[67]
Alkaline fermentation and gamma-ray irradiation	T = 25 °C, pH = 10, gamma-ray irradiation = 20 kGy	2980 mg/L	Max TN removal efficiency with solubilized sludge as carbon source was 51.1%, similar to that of methanol, which was 55.6%	[60]

[a] VFA: Volatile fatty acid; [b] SCOD: Soluble COD; [c] SCFA: Short-chain fatty acids; [d] TCOD: Total chemical oxygen demand; [e] nZVI: Nano zero-valent iron; [f] FNA: Free nitrous acid.

During physical treatment, WAS particles are broken into small pieces and micromolecules are released by membrane breakage and cell fragmentation, resulting from external force. Kampas et al. [59] investigated the potential of mechanically disintegrated WAS for use as an internal carbon source for biological nitrogen removal. The result showed that denitrification rates improved after the addition of disintegrated sludge (36.7 mg NO_3-N g^{-1}/VSS h^{-1}) compared with the control (30.2 mg NO_3-N g^{-1}/VSS h^{-1}) and were even higher than acetate as an additional source (34.7 mg NO_3-N g^{-1}/VSS h^{-1}). Lee et al. [61] evaluated the feasibility of focused-pulsed, and the result showed that VFAs increased 5.1-fold relative to untreated WAS. The batch-test denitrification rate of focused-pulsed-WAS (0.25 gNO_3-N/g VSS d) was higher than that of untreated WAS (0.05 g NO_3-N/g VSS d) and methanol (0.15 gNO_3-N/g VSS d).

Fermentation is an effective means to transform organic matter in WAS into readily bioavailable substrates, such as SCFAs. Usually, the fermentation process under anaerobic conditions can be separated into four stages: solubilization, hydrolysis, acidification, and methane production [76]. All the four phases are influenced by pH. Therefore, the production of VFAs by WAS fermentation would also be affected by acid-base properties. The influences of pH from 4.0 to 11.0 on the hydrolysis and acidification of WAS were investigated and the result indicated that alkaline was the most suitable condition for fermentation [82]. The greatest VFA production (2708.02 mg/L) was achieved at pH = 10, in which acetic, propionic, and isovaleric acids were the three main products. Zheng et al. [68] investigated underlying mechanisms for increasing SCFA production at pH = 10, analyzing microbial community structures in sludge fermentation reactors by using 454 pyrosequencing and fluorescent in situ hybridizations. The results showed that anaerobic sludge fermentation under alkaline conditions increased the number of bacteria involved in hydrolysis and acidification, and reduced the number of methanogens, favoring competition of bacteria over methanogens, thus improving the production SCFA. Yang et al. [82] also discovered that alkaline conditions were suitable for WAS fermentation, with optimal pH = 12. Based on this discovery, many researchers continue to investigate improvement of nitrogen removal performance after fermentation liquid recycling as an internal carbon source to promote denitrification. TN removal efficiency increase from 74.7% to 98.7% was observed after fermentation liquid addition under various nitrogen removal processes and operational conditions [63,79–81]. The effect of various substances used to adjust pH to 10 on VFA production and their performance in enhancing nitrogen removal by added electron donors have also been studied. The results showed that VFA production with the addition of a mixture of $Ca(OH)_2$ and NaOH (1813–1868 mg COD/L) was greater than addition of NaOH or $Ca(OH)_2$ only, which is also economical.

To raise the performance of WAS fermentation, various accelerants were added into the sludge fermentation system. Luo et al. [83] studied the effect of nano zero-valent iron on stimulating the accumulation of SCFAs in the WAS fermentation system via accelerating the solubilization and hydrolysis processes. The result revealed that SCFA production was significantly improved, and fermentation time was shortened compared to not adding nano zero-valent iron, which was because the solubilization of sludge, hydrolysis of solute, and acidification of hydrolysate were all strengthened by adding nano zero-valent iron. The general microbial activity of anaerobic bacteria and relative activities of key enzymes in hydrolysis and acidification of organics were enhanced over those of the control. Liu et al. [84] investigated the influence of humic acids on fermentative SCFA production from WAS, finding that commercially available humic acids significantly improved SCFA production (1.7–3.5-fold). Further study revealed that humic acids added to WAS reacted by improving the solubilization of sludge protein and carbohydrate and the hydrolase activity, enhancing the acidification step. At the same time, this inhibited the activity of acetoclastic methanogens, which caused less SCFA consumption. The influence of nitrite on integrated WAS fermentation and denitrification was researched by Wang et al. [86], and the results indicated that under acidic conditions (pH = 5), nitrite addition enhanced the acidification of WAS. The maximum VFA production was 3.3 times that without nitrite addition. The nitrite addition changed the anaerobic conditions to anoxic, which triggered denitrifiers but restrained methanogens. Thus, VFAs produced by fermentation were mainly consumed by denitrifying bacteria rather than methanogenic bacteria. Similarly, Zhao et al. [87] found that fermentation time was significantly shortened and that SCFA production was obviously improved with sludge pretreatment by free nitrous acid (FNA) for two days. The enhancement effects of thermal-alkaline pretreatment [67] or combination of fermentation and gamma-ray irradiation [60] on VFA production have also been reported (Table 3).

4.2. Techniques Applied for VFAs Production from Sludge

One of the most important problems in using treated sludge as electron donors for denitrification is that large amounts of soluble phosphorus (SOP) and ammonium nitrogen (NH_4-N) are released during sludge treatment, especially for WAS. Chen et al. [62] reported that the concentrations of P and N kept increasing during fermentation at high concentrations and acidic pH (pH = 4.0 and 5.0). Thus, it is important to remove the released P and ammonia from treated sludge before returning as an internal carbon source to improve denitrification. At a laboratory bench, N and P are usually removed by P precipitation and ammonia stripping [80], but the process is expensive and time consuming. Tong and Chen [63] investigated a method of simultaneous recovery of NH_4^+-N and SOP from WAS alkaline fermentation liquid, and Mg^{2+} addition to form struvite($MgNH_4PO_4{\cdot}6H_2O$), which can be used as a slow-release fertilizer. The result showed that Mg/N = 1.80, pH = 10.41, and P/N = 1.16 were optimal conditions for recovering NH_4^+-N and SOP from WAS alkaline fermentation liquid, and the recovery efficiency of NH_4^+-N and SOP was 75.7% and 83.2%, respectively. Additionally, Law et al. [88] reported that N in the anaerobic digester liquor of sludge could be recovered alone by producing FNA. FNA is a biocide that can optimize WWTP bioprocesses in many aspects, e.g., achieving N removal via the nitrite shortcut [89], reducing sludge and N_2O production [90,91], and enhancing toxic metal removal from acidified sludge [92]. Theoretically, there is a strong possibility for combining recovery of N and P (as struvite) with FNA production in the same process because the concentration of N is higher than P in treated sludge [80,81,93], whereas the ideal molar ratio of NH_4^+ to PO_4^{3-} is 1:1 for struvite formation [63].

5. Conclusions

Recycling an internal carbon source obtained from sewage sludge to the denitrification parts of the nitrogen removal process can not only promote nitrogen removal performance but also reduce sludge production of WWTPs. Compared to a conventional external carbon source, the green and sustainable internal carbon source is attracting increased interest. This paper reviewed various techniques for internal carbon source production, from sewage sludge and their application to enhanced nitrogen removal efficiency of low C/N wastewater after recycling as electron donors for denitrification. Among the sludge treatment methods, alkaline fermentation of sludge is an economical and effective means with broad prospects in the future if sludge retention time is shortened to a suitable range. Moreover, N and P are necessarily recovered from treated sludge to prevent the increase of N and P loads in influent. Technology of simultaneous recovery of N and P in the formation of struvite (a slow-release fertilizer) is believed to be the most promising, which can be associated with the production of FNA (an effective additive in WWTPs) from sludge. Although there have been many investigations in the field, the following aspects require researchers' attention in the future:

- How to produce as much as VFA as possible from PS or WAS with short sludge retention time and low cost
- As nearly all research has been conducted in the laboratory with intermittent flow, some important points have not been considered in continuous flow. Such points include the optimal ratio of sludge for producing a carbon source to sludge for recycling back to the main reaction flow, and into which reaction tank or in which period should the fermented sludge be added
- How microorganisms in the reaction tank will be affected after return of the internal carbon source.

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