Anammox bacterium 'Candidatus *Kuenenia stuttgartiensis*': a review

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Anammox bacterium 'Candidatus *Kuenenia stuttgartiensis*': a review

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Abstract. Anaerobic ammonium oxidation (Anammox) process is a type of biological nitrogen removal technology which is known to directly convert ammonium and nitrite to nitrogen gas. The freshwater 'Candidatus *Kuenenia stuttgartiensis*' anammox under the phylum of Planctomycetes is used to study the parameters that affect the anammox development and the metabolic pathways alongside the associated enzymes. These observations were made using state-of-the art techniques for detecting anammox bacteria based on their small-subunit ribosomal RNA genes, functional genes and unique reaction pathways. This review systematically summarizes up-to-date studies on the parameters affecting the growth of the anammox bacteria and metabolic networks driving anammox bacterial anabolism and mixotrophy beyond genome-based predictions. The *K. stuttgartiensis* survives in summer and winter conditions besides in the aerobic zones (dissolved oxygen >2 mg/L), which consequently contribute to better nitrogen removal in the wastewater treatment. Furthermore, the *K. stuttgartiensis* utilizes the Wood–Ljungdahl pathway to directly assimilate extracellular formation instead of oxidising it completely to CO_2 prior reassimilation.

1. Introduction

Anaerobic ammonium oxidation (anammox) is being implemented in wastewater treatment system for nitrogen removal as excessive nitrogen compound in the wastewater may induce eutrophication, which consequently promotes the growth of green algae and cyanobacteria [1-2]. Anammox process was introduced to oxidize ammonium into dinitrogen gas by consuming nitrite as electron acceptor under anaerobic conditions [3]. Anammox is recognized as the energy-saving technology compared to conventional nitrification–denitrification due to short-cut pathway of nitrogen removal in wastewater treatment system [4]. The key advantages of anammox over conventional process are reduction of energy consumption for oxidation of soluble organics and ammonia via aeration, terminates the carbon source addition for nitrite reduction heterotrophically and deduces the volume of sludge produced from the treatment process [5].

Anammox process is mediated by the chemolithoautotrophic bacteria affiliated with a monophyletic group in the phylum Planctomycetales [6]. To date, 15 species of anammox have been successfully deciphered in wastewater treatment plants, freshwater and marine environment which categorized into six Candidatus genera [7]. Since, a classical pure culture of anammox species is

challenged to be obtained, though all have the taxonomical status of Candidatus, which are classified into six different genera: (1) *Kuenenia*, represented by *Kuenenia stuttgartiensis* [8]; (2) *Brocadia* (with four species: *B. anammoxidans, B. fulgida*, *B. sinica*, and *B. caroliniensis*) [9-12]; (3) *Anammoxoglobus*, represented by *A. propionicus* [10]; (4) *Jettenia* with three species reported (*J. asiatica*, *J. moscovienalis* and *J. caeni*) [13-15]; (5) *Scalindua* including five species (*S. brodae, S. sorokinii, S. wagneri*, *S. profunda* and *S. rubra*) [16-19] and (6) *Anammoxomicrobium*, represented by *A. moscowii* [15].

The first genome sequence of the anammox model organism that was used to determine the detailed analyses of substrate conversions in bioreactors was Candidatus *Kuenenia stuttgartiensis* (hereafter, *K. stuttgartiensis*), which eventually lead to the discover of hydrazine [3,8, 20-21]. The species *K. stuttgartiensis* was recognized as being capable to survive and develop during winter and summer despite being grown in the aerobic zones (i.e., dissolved oxygen >2 mg/L) [22]. Furthermore, the *K. stuttgartiensis* (27.6%) that was developed in the micro-granule structure had succeeded to achieve the nitrogen removal efficiencies up to 71.8 ± 9.9% [23]. This shows that the *K. stuttgartiensis* may survive at different temperature while simultaneously resulting in excellent nitrogen removal efficiency within the smaller structure of granules. Additionally, *K. stuttgartiensis* possesses a complex network of regulatory systems to confer cells with the ability against Zn (II) toxicity. These systems include functions related to substrate degradation and Zn (II) efflux, chelation, DNA repair, protein degradation, protein synthesis, and signal transduction, respectively [24].

Anammox is one of the key biogeochemical processes in marine and estuarine environments, hence studies on natural habitats have forced us to reassess the global nitrogen cycle [22,25]. Extensive amazing and fascinating results have been reported regarding *K. stuttgartiensis* ubiquity in wastewater treatment, owing to fast evolving molecular approaches that primarily focus on small-subunit ribosomal RNA (16S rRNA) and functional genes in a culture-independent manner [26]. The distinct characteristics of *K. stuttgartiensis* have piqued the interest of many researchers around the world, who are working to improve the performance of the anammox in the nitrogen removal process. Therefore, beyond the genome-based predictions, this review aims to summarise the parameters affecting *K. stuttgartiensis* development and the metabolic networks driving anammox bacterial anabolism and mixotrophy.

2. Parameters effect on Candidatus *Kuenenia* development

2.1. Nitrogen loading rate

According to Ma *et al.* [27], successful anammox sludge enrichment may be achieved through the sequencing batch reactor (SBR) by feeding the synthetic wastewater containing ammonium and nitrite concentration 280 mg N/L and 360 mg N/L, respectively. After 270 days of cultivation, the specific anammox activity (SAA) of the reactor was stabilized at approximately 0.31 ± 0.01 g N/g VSS d by the *K. stuttgartiensis* as the dominant anammox bacteria (81 ± 9%) among the total bacteria based on fluorescence in situ hybridization (FISH). The higher the concentration of ammonia and nitrite in the SBR medium, simultaneously longer stabilization period required by the *K. stuttgartiensis*. Moreover, the enhancement of the nitrogen loading rate by decreasing the HRT and increasing the ammonium and nitrite concentration enables the *K. stuttgartiensis* along with *Candidatus* “Brocadia” anammox community to be developed [28-29].

The enrichment of the *K. stuttgartiensis* bacteria in the anammox sludge may be achieved by varying the nitrogen loading rate along with the parameters of the reactor. This shows the adaption of the targeted anammox with the nitrogen loading rate consumes time in conjunction with the reactor configuration, parameters and the selection of the seed sludge. This subsequently causes industrial applications of anammox to be limited and challenging due to a long start-up period owing to extend doubling times of anammox bacteria (2.1–25 days) [27, 30-32].

Whilst, Izati *et al.,*[33] study utilizing upflow anaerobic sludge bed (UASB) reactor seeded with municipal returned activated sludge (RAS) able to detect the *K. stuttgartiensis* after 270 days though. The concentration of ammonium and nitrite were 52.02 and 62.42 mg N/L, respectively in the synthetic

wastewater. Furthermore, the target of the study was to observe anammox micro-granular biomass enrichment performance. Thus, the nitrogen loading rate for the reactor was low compared to [27] for the feeding concentration. The anammox granules size distribution was between 253.2 to 465.7 μm for 75% of the total sample volume. The polymerase chain reaction (PCR) analyses have proven the *K. stuttgartiensis* is the second major genera in mature anammox sample whilst *Candidatus* "Brocadia" as predominant in the inoculum. This study proves the *K. stuttgartiensis* may be cultivated in the micro-granular by introducing low level concentration of ammonia and nitrite. Additionally, the niche of the seed sludge plays a crucial role for the development of the *K. stuttgartiensis* bacteria.

The enrichment of anammox sludge in different configuration of reactors consumes approximately 270 days to develop the matured anammox granules. The *K. stuttgartiensis* able to be the dominant bacteria in sample sludge with the nitrogen loading rate of ammonium to nitrite ratio of 0.7 to 0.8. Anammox bacteria requires ammonium as the electron donor and nitrite as an electron acceptor [34]. Therefore, sufficient amount of ammonium and nitrite should be supplied for the development of the *K. stuttgartiensis* in conjunction to carry out the anammox process in the anaerobic condition [35].

2.2. Seed sludge

In order to achieve quick start-up of anammox, the appropriate selection of seed sludge is crucial, which consequently may lead to the wide practical application [36]. Anammox biomass, notably anammox granule, is the most frequently used inoculum or seed sludge for anammox reactors, as it has several advantages over flocculent anammox sludge [7]. However, the utilization of solely anammox bacteria as an inoculum hinders the implementation of the anammox technology to treat nitrogen-rich wastewater systems [36]. Therefore, a mixture of more than one type of sludge is considered as the best inoculum to nurture anammox bacteria and enhance the anammox process [37].

The anammox granule that commonly used as the inoculum for the reactor start-up usually consist of *K. stuttgartiensis* as the dominant anammox bacterium [38-39]. Moreover, the *K. stuttgartiensis* can be dominant anammox species in the enrichment cultures using inoculum from different types of wastewater treatment plant activated sludge such as being sourced from landfill leachate treatment plant, municipal sewage treatment plant and monosodium glutamate (MSG) wastewater treatment plant, respectively [40]. Consequently, this demonstrates the potential of *K. stuttgartiensis* to be determined in the seed sludge sourced from the activated sludge and anammox granule. Additionally, the start-up period of the reactor is primarily influenced by the type of seed sludge selected [37]. However, the seed sludge consisting of anammox bacterium namely *K. stuttgartiensis* was found to be able to initiate the anammox process in approximately 10 to 20 days [36-37, 41]. Furthermore, different configuration of reactors utilising various options of biomass as the inoculum also may affect the start-up period. Therefore, the seed sludge selection for the anammox process is mostly focused on the maturity of the granules and the anammox bacterium consortium [41].

Many studies are being conducted to determine the appropriate seed sludge for the rapid start-up of the anammox process. However, the type of anammox bacterium in different seed sludge consequently affects the nitrogen removal efficiency in conjunction with the parameters for the reactor operation like temperature, pH and dissolved oxygen. This is because seed sludge sourced from different areas like marine, brackish and freshwater may consist of different anammox bacterium consortium according to its preferable condition to survive [42]. Table 1. summarizes the type of seed sludge used for anammox start-up resulting in the discovery of *K. stuttgartiensis* as the developed anammox bacterium.

Table 1. The type of seed sludge used for anammox start-up.

Reactor Type	Seed sludge	Start-up period (days)	N-removal efficiency (%) achieved at the end of start-up	Microbial analysis	References
Air-lift reactor	From a pilot-scale PN-anammox reactor.	94	31.28	qPCR: 8.47 x 10^{10} (floc) & 1.68 x 10^{10} (granule) (copies/gVSS)	[43]
Expanded granular sludge bed reactor	Preserved anammox consortia	20	Ammonium: 94 and nitrite: 99	Metatranscriptomic analysis: Candidatus *Kuenenia stuttgartiensis* (dominant)	[41]
Upflow anaerobic sludge blanket reactor	Municipal returned activated sludge	55	85	qPCR: Candidatus *Brocadia* (0.46 %) and Candidatus *Kuenenia* (0.24 %) relative abundance	[33]
Sequencing batch reactor	Mixed activated sludge (aerobic sludge, anaerobic sludge, simultaneous partial nitrification, anammox and denitrification (SNAD) sludge and anammox sludge)	14		qPCR of AnAOB 53 sequences: Candidatus *Brocadia*, Candidatus *Jettenia* and Candidatus *Kuenenia*	[36]
Anaerobic sequencing batch reactor	Anammox sludge and activated sludge	10	80	qPCR: Candidatus *Brocadia* and Candidatus *Kuenenia*	[37]
Anaerobic membrane bioreactor	Conventional activated sludge	125	20	FISH: Candidatus *Brocadia anammoxidans* and Candidatus *Kuenenia stuttgartiensis*	[44]
Rotating flat-sheet membrane bioreactor	Anammox activated sludge	16	Ammonium: 90 and nitrite: 90	FISH: Anammox bacteria 90% of total bacteria	[45]

2.3. Temperature

The most crucial parameter for the biological wastewater treatment plant especially for the microbial growth is the temperature. According to Oshiki *et al.* [46], the temperature range between 30 to 40 °C is been recognized as the optimum temperature for the growth of anammox bacteria. However, in many countries the average temperature is quite lower than this range. Moreover, it is a big challenge and may not be cost effective for the wastewater treatment plant to maintain an optimum temperature for anammox process. Thus, many solutions are being studied to overcome this problem, therefore, the best possible may comply for wider application of anammox technology [47].

The growth temperature preferred by the *K. stuttgartiensis* bacterium is between 25 to 37 °C, whilst the optimum temperature is 37 °C [48-49]. Additionally, in a study Arumugham *et al.,*[50] succeeded in determining the *K. stuttgartiensis* bacterium at the temperature range of 29.6 to 32.0°C at the domestic wastewater treatment plant. This indicates that the *K. stuttgartiensis* bacteria is able to survive below the optimum temperature within the open wastewater treatment system. The temperature condition reflexes upon the nitrogen removal process of the anammox because relatively high ammonia removal rate was achieved under the mesophilic temperature conditions of 30 to 40 °C [32,51]. Therefore, many studies have studied upon different temperatures in order to enhance nitrogen removal [52].

According to Ma *et al*. [52], the temperature drops from 30 to 16 °C has resulted in a drop in the nitrite and ammonium removal efficiencies from 94.35% and 92.81% to 92.31% and 78.45%, respectively. The drop in temperature subsequently resulted in the production of nitrate in the anammox UASB reactor as the ammonium removal decreased at 16 °C. This is because at the low temperature in conjunction with the high dissolved oxygen, the potential for nitrite-oxidizing bacteria (NOB) and activity is higher compared to ammonia-oxidizing bacteria (AOB)[53-54]. However, the *K. stuttgartiensis* genome survived at low temperature and succeeded in achieving stable anammox operation after 28 days [26]. Furthermore, the NOB was inhibited at low temperature by intermittent high strength feeding (IHSF) strategy. Therefore, the anammox community was predominant in the treatment compared to NOB and AOB was not significantly affected by the temperature change [26]. This shows the *K. stuttgartiensis* able to survive at low temperature and enable to carry out the anammox process without inhibition from NOB by ISHF the strategy.

3. Anammox mechanism

The application of a combination molecular techniques on *K. stuttgartiensis* to explore key enzymes and processes involved in anammox catabolism revealed hydrazine and nitric oxide as volatile intermediates in the anammox bacterium [55-57]. In the central of the anammox cell is surrounded by a unique ladderane lipid membrane, in where the nitrite is initially reduced to nitric oxide before combining with the ammonium and yield hydrazine. Lastly the hydrazine oxidizes into dinitrogen gas [58]. Then, a pH gradient is created over the anammoxosome membrane when hydrazine is oxidised, which fuels the generation of adenosine triphosphate (ATP) [59].

The anammoxosome, which is recognized as the specialized intracellular organelle is responsible for the reactions contributes for the energy conservation [60-61]. Additionally, the anammoxosome of *K. stuttgartiensis* contains membrane-bound respiratory complexes for electron transport chain, including complex I, ATP synthase and an NAD+: ferredoxin oxidoreductase (RNF)[62]. These nitrite reductase, hydrazine synthase (HZS) and hydrazine dehydrogenase (HDH) are soluble enzymes that is not membrane-bound in the anammoxosome and responsible for anammox catabolism [60,63].

According to de Almeida *et al*. [64], the most easily detected soluble enzymes on the anammoxosome of *K. stuttgartiensis* were HDH and HZS with high expression of proteins. Meanwhile, the candidate nitrite reductase in few studies, namely nitrite reductase NirS was hardly detectable, as expected from its low transcription levels [8,65]. The HDH is also recognized as an octaheme protein connected to hydroxylamine oxidoreductase (HAO) from the aerobic ammonium-oxidizing bacteria

[66-68]. There were nine more HAO-like octaheme proteins besides HDH, which were encoded in the *K. stuttgartiensis* genome and was determined in the proteome [64].

The HAO is established as the alternative catalyst for the hydroxylamine oxidation. However, in anammox species namely the *K. stuttgartiensis* generates nitric oxide using nitrite reductases as the catalyst encodes cytochrome cd_1-containing (cd1-NIR) [8,65,69]. In order to convert ammonium into nitrogen gas using nitrite as the terminal electron acceptor in the anaerobic condition, the anammox bacteria utilizes the oxidative power of nitric oxide to carry out the process [57]. Therefore, the anammox bacteria community oxidizes the hydroxylamine into nitric oxide through encoded octaheme HAO as the catalyst for the process [70]. However, each anammox species has diverse nitric oxide generation pathways. Thus, it was suggested that nitrite reduction to nitric oxide might be a feature that evolved after the core anammox catabolism was already in place.

The *K. stuttgartiensis* have revealed the anammox bacteria conserve energy and grow by using nitric oxide as its terminal electron acceptor in the anaerobic condition for ammonium oxidation [71]. Besides that, nitrate production was inhibited and the sole end product is nitrogen gas. This was determined using the comparative transcriptomics and proteomics techniques. The *K. stuttgartiensis* suppresses the transcription of proteins involved in nitric oxide production and nitrite oxidation during the development of nitric oxide-dependent ammonium oxidation [57]. It is proven that anammox bacteria is not responsible for producing any nitrous oxide, although enormous quantities of nitric oxide are supplied. This concurrently shows the anammox bacteria consumes nitric oxide in natural and manmade ecosystems to produce harmless nitrogen gas instead of the greenhouse gas nitrous oxide. As a result, anammox maintains the regulation of nitric oxide and nitrous oxide emissions, which are two key chemicals in atmospheric chemistry.

The ATP synthesis in anammox bacteria is commonly catalyzed by the ATPase-1. In *K. stuttgartiensis* has four membrane-bound ATPases encoded in its genome: the H^+-dependent F_1F_O type (ATPase-1, kustc3787–3796) and two types (ATPase-2, kuste4592–4600; ATPase-3; kustc0572–0579) that lack the δ subunit but have one or two extra membrane subunits (AtpQ, kuste4594 and kustc0574; AtpR, kuste4595) instead [8,64,72-73]. The ATPase-2 and ATPase-3 are thought to be Na^+-pumping ATP hydrolases (N-ATPase) other than that a prokaryotic V-type ATPase (ATPase-4, kuste3864–3871) is also encoded [74]. According to the previous research, ATPase-1 was recommended to be the primary ATP synthase in *K. stuttgartiensis* and the migratory profiles of the ATPases analysis have confirmed those findings [75]. Most of the N- and V-ATPases subunits which were not vary as ATPase-1, able to recover as high-molecular-mass complexes and subcomplexes, but their expression was much lower than ATPase-1. It should be highlighted that the existence of N-ATPases was only hypothesised based on genetic analysis because their protein expression had never been determined before [74]. This shows *K. stuttgartiensis* capable of producing energy through ATPase-1 besides recovering high-molecular-mass by utilizing N- and V-ATPases.

The experimental research and genomic evidence have determined that the anammox bacteria are more adaptable than originally envisaged. This study revealed substitution electron donors to ammonium, such as formate, acetate, and propionate for energy conservation with nitrite or nitrate as electron acceptors [10,76]. Besides that, the *K. stuttgartiensis* also utilizes ferrous iron as electron donor meanwhile iron and manganese oxides as electron acceptors [8,10,18,60]. This shows the *K. stuttgartiensis* is capable of substituting the electron donor for ammonium and electron acceptors for nitrite or nitrate with many other alternatives.

Intriguingly, it's been discovered that these organic substrates are entirely oxidised to carbon dioxide rather than being directly incorporated into cell biomass, implying that anammox bacteria maintain their autotrophic existence [72]. Therefore, a study was conducted upon the central carbon metabolism of a planktonic *K. stuttgartiensis* through time-series ^{13}C isotope tracing, metabolomics and isotopically nonstationary metabolic flux analysis (INST-MFA) [55]. According to previous studies [8,72], the Wood-Ljungdahl route and pyruvate:ferredoxin oxidoreductase (PFOR) would fix carbon dioxide (CO_2) on a regular basis, resulting in rapid labelling of acetyl-CoA and pyruvate, followed by

phosphoenolpyruvate and other downstream metabolites. In the TCA cycle and gluconeogenesis, acetyl-CoA and pyruvate are predicted to enter to generate biomass precursors.

The planktonic *K. stuttgartiensis* on the other hand, lacks the citrate synthase necessary to initiate the oxidative TCA cycle. As a result, it's hypothesized that primary precursor metabolites like succinyl-CoA and alpha-ketoglutarate are synthesised in a reductive manner, resulting in high ^{13}C-labelling of oxaloacetate, succinate, and alpha-ketoglutarate. [72]. Alternative biomass precursor is predicted to be generated in *K. stuttgartiensis* via gluconeogenesis and the pentose phosphate pathway [8]. There are several acyltransferase candidates that exist as substitutes for citrate synthase in *K. stuttgartiensis.* For instance, genes annotated as (R)-citramalate synthase (KSMBR1_RS19040) responsible for isoleucine biosynthesis and redundant copies of 2-isopropylmalate synthase (KSMBR1_RS18315 and KSMBR1_RS10820) [71,77].

The isotopically nonstationary metabolic flux analysis (INST-MFA) was performed by fitting measured, time-resolved metabolite mass isotopomer distributions from ^{13}C-formate tracer experiments to an isotopomer network model in order to resolve the metabolic pathways used for the biosynthesis of sugar phosphates in *K. stuttgartiensis* [78]. The ^{13}C-formate tracer analysis demonstrated that a significant fraction of fructose 6-phosphate was present as M+ 1 mass isotopomers, which was unpredicted as gluconeogenesis produces mostly M+ 2 mass isotopomers [71]. As a result, different routes for the generation of fructose 6-phosphate may exist. According to the genome annotation of *K. stuttgartiensis*, the hexulose 6-phosphate synthase and 6-phospho-3-hexuloisomerase (KSMBR1 RS05220 and KSMBR1 RS18790, respectively) are recognised as primary enzymes of the ribulose monophosphate (RuMP) pathway, a formaldehyde assimilation mechanism in many methylotrophic bacteria [79]. Furthermore, these mechanisms utilize a hexulose 6-phosphate intermediate to fix formaldehyde to fructose 6-phosphate. Therefore, these processes and the undiscovered formaldehyde dehydrogenase, were demonstrated in this study could explain the abundance of M+ 1 pentose and hexose phosphate isotopomers found during ^{13}C-formate labelling [71].

In the reverse Wood–Ljungdahl pathway, the acetyl-CoA will be oxidized in order to label the formate as a pathway intermediate. However, in the study utilizing the *K. stuttgartiensis* genome, the formate remained unlabelled and the result was that the reverse Wood–Ljungdahl pathway was not involved in acetyl-CoA oxidation to CO_2 [71]. In contrast, the ^{13}C-labelling of TCA cycle and gluconeogenic metabolites has taken place to a much lesser extent compared to results from ^{13}C-formate tracing. The multiple cycles of the oxidative TCA cycle using *Si*-citrate synthase instead of *Re*-citrate synthase may possibly explain the observed unique patterns for TCA cycle metabolites. The finding of the *Si*-citrate synthase gene candidate in the *K. stuttgartiensis* genome, indicates a low abundance bacterium in the bioreactors side population may have oxidised the acetate. However, the low relative isotope abundance of *K. stuttgartiensis* proteins revealed by ^{13}C-acetate labelling, combined with the *Si*-citrate synthase activity proved by ^{13}C- and ^{2}H-acetate tracer experiments, shows that *K. stuttgartiensis* was unable to use acetate as an electron donor or carbon source in situ. Therefore, the study suggests that *K. stuttgartiensis* utilizes the Wood–Ljungdahl pathway to directly assimilate extracellular formate instead of oxidising it completely to CO_2 and then reassimilation.

4. Perspectives and further research

Anammox process is recognised as the key step in the biogeochemical nitrogen cycle and has been established as a novel treatment technology for removing nitrogen from wastewater in a sustainable way. There are various anammox species in the environment, however, the *K. stuttgartiensis* was selected to be studied in detail. This is due to the feasibility of *K. stuttgartiensis* to survive in summer and winter conditions besides in the aerobic zones (dissolved oxygen >2 mg/L). Additionally, the microbial detection methods for anammox bacteria and metabolic networks drive anammox bacterial anabolism and mixotrophy beyond genome-based predictions. Therefore, the genomics investigation on *K. stuttgartiensis* is being explored globally, with the goal of revealing new anammox-specific genes, enzymes and reactions involved in anammox catabolism.

Moreover, the ATP synthase of *K. stuttgartiensis* was explained thoroughly. The substitute of electron donors for anammox instead of ammonium was discovered, for instance, like formate, acetate, and propionate for energy conservation, along with nitrite or nitrate as electron acceptors. The organic substrates in the anammox are entirely oxidised to carbon dioxide rather than being directly incorporated into cell biomass, implying that anammox bacteria maintain their autotrophic existence. The study has revealed that *K. stuttgartiensis* utilizes the Wood–Ljungdahl pathway to directly assimilate extracellular formate instead of oxidising it completely to CO_2 and then reassimilation. In conclusion, the *K. stuttgartiensis* anammox bacteria is able to contribute greatly to wastewater treatment for nitrogen removal by developing the bacteria at suitable parameters. Thus, *K. stuttgartiensis* able to carry out catabolism and anabolism in the wastewater efficiently.

The anammox bacteria, *K. stuttgartiensis* is unique as compared to other species in the genera due to its capability to survive at different environmental condition besides various substitution for electron donor. As a result, by promoting *K. stuttgartiensis*, the adaptation speed of the anammox to surrounding environment in actual wastewater may be improved towards achieving stable nitrogen removal performance in the system. . This review reveals the important connection between the reactor parameters and the development of *K. stuttgartiensis* as well as the changes in the mechanism process.

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