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# Investigation of the process stability of different anammox configurations and assessment of the simulation validity of various anammox-based kinetic models

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Over the last 30 years, the successful implementation of the anammox process has attracted research interest from all over the world. Various reactor configurations were investigated for the anammox process. However, the construction of the anammox process is a delicate topic in regards to the high sensitivity of the biological reaction. To better understand the effects of configurations on the anammox performance, process-kinetic models and activity kinetic models were critically overviewed, respectively. A significant difference in the denitrification capabilities was observed even with similar dominated functional species of anammox with different configurations. Although the kinetic analysis gained insight into the feasibility of both batch and continuous processes, most models were often applied to match the kinetic data in an unsuitable manner. The validity assessment illustrated that the Grau second-order model and Stover–Kincannon model were the most appropriate and shareable reactor-kinetic models for different anammox configurations. This review plays an important role in the anammox process performance assessment and augmentation of the process control.

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## 1. Introduction

With the development of the economy of the world, eutrophication has spread fast and led to serious aquatic environmental pollution, especially in the developing countries. The excessively discharged nitrogen can cause many negative impacts, such as eutrophication. Therefore, nitrogen removal shall be processed in a sustainable way, especially for wastewater treatment with a low C/N ratio. With the lower capital investment, less operational maintenance, no additional organic carbon source, 90% reduction of sludge and the limited emission of N<sub>2</sub>O, the anaerobic ammonia oxidation (anammox) process is a new nitrogen removal technology that has gained widespread attention.<sup>1,2</sup> However, despite the increasing interest in the anammox application, there were still many difficulties, such as the low bacterial growth rate and the sensitivity to the environmental factors.<sup>3</sup>

Anammox processes can be conducted in many different configurations. Each process has its own optimized operations and characteristics based on the design of the reactor. However, it is generally known that common reactors have certain shortcomings, and that appropriate operation conditions should be fully investigated to prevent the process from failure without optimized operation.<sup>4</sup> Different reactors have different capabilities of nitrogen removal. Among the applied reactors, the Sequencing Batch Reactor (SBR), Up Flow Anaerobic Sludge Blanket (UASB) and Membrane Bio-Reactor (MBR) were the most popular configurations with different configuration structures that benefit the biomass enrichment in the form of the biofilm or for avoiding sludge wash out.<sup>5–7</sup> The aim of optimizing the operational conditions is to enhance the efficiency of nitrogen removal.<sup>8</sup> The effects of environmental factors at various operational options on the anammox microbial community were also reported.<sup>9</sup> Moreover, the microbial residence time plays a critical role in the taxonomic composition and functional description of the microbial communities.<sup>10</sup>

A large number of previous studies focused on experimental and mathematical methods.<sup>11–13</sup> The process-kinetic models are powerful tools for investigating the performances of different configurations during the anammox process. Appropriate kinetic mathematical models are enabled to assess, control, and mitigate the process inhibition and process optimization.

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These models can be employed to achieve many key objectives involving the optimization of the chemical processes, assessment of the reaction hazards, and the design of emergency relief systems.<sup>14,15</sup> Process-kinetic models enhance the comprehension of the constructed system, and are conducive to the formulation and validation of a hypothesis. The prediction of the system's behaviour at a variety of conditions was developed to reduce the operation risks. However, certain selection criteria and conditions must be obeyed when the proper evaluation and application of the process models were performed for understanding the process or predicting the performance. In order to identify a suitable model, a correct model type shall be selected, and the parameters of the model shall be estimated with the available data. To a great extent, the validity of the studies relies on the model, which is defined by the proper preference of a mathematical model first, and then the validity of the kinetics evaluation. Generally, an evaluation on the process performance is still characterized by the application of the simple kinetics models and methods for evaluating their reactions.

Kinetic models are proved to be a conventional way to estimate and prospect the performances and operations of different processes. It is very important to choose a suitable model relating to the kinetics evaluation of the anammox process assessment. Quantitative process-kinetics can gain insight into the underlying operational strategy with feasibility investigations in both batch and continuous processes. However, it is a pity that most frequently used models cannot fit the kinetic data well. It is necessary for us to know which model is better, and which is perfect in the simulated conditions. Moreover, the optimized operation conditions of the anammox process are also important for the lab scale and full-scale process design.

A majority of previous papers of different configurations (EGSB, UASB, SBR, MBR, and CSTR) were related to discussions of the treatment performance or engineering application. However, only a limited number of articles are available for solving the specific problems of the kinetics evaluation. So far, studies in investigating the anammox process-kinetics with different configurations are relatively limited, and the assessment of anammox performance in different reactors is rare. Thus, the performance of the anammox system in different reactors is reviewed in this paper with regards to validation of the commonly used process kinetic models.

## 2. Process-kinetics approaches

Different reactors used in the anammox process were summarized, and the performance comparison and kinetic simulation were also evaluated. Currently, we are mainly focusing on the application of four kinds of substrate removal models, including the first-order substrate removal model, the Grau second-order substrate removal model, the modified Stover–Kincannon model and the Monod equation, to investigate the anammox process kinetics.

### 2.1 First-order substrate removal model

With regard to the application of the first-order substrate removal model to the bioreactor, the change rate of the

substrate concentration in the completely mixed system may be expressed as follows:<sup>12</sup>

$$-\frac{dS}{dt} = \frac{QS_i}{V} - \frac{QS_e}{V} - k_1S_e \quad (1)$$

Because the change rate ( $-dS/dt$ ) can be neglected under a pseudo-steady-state situation, the equation is derived as:

$$\frac{S_i - S_e}{\text{HRT}} = k_1S_e \quad (2)$$

where  $S_i$  and  $S_e$  are the total nitrogen concentrations ( $\text{g L}^{-1}$ ) in the influent and effluent, HRT is the hydraulic retention time (day),  $k_1$  is the constant of the first-order substrate removal rate constant ( $1/\text{d}$ ),  $Q$  is the inflow rate ( $\text{L}/\text{d}$ ) and  $V$  is the volume of the reactor ( $\text{L}$ ).

### 2.2 Grau second-order substrate removal model

The general equation of a second-order model is described as shown below.<sup>16</sup> By integration and linearization, the above equation can be expressed as eqn (3):

$$-\frac{dS}{dt} = k_2 \left( \frac{S_e}{S_i} \right)^2 \quad (3)$$

By integration and linearization, the above equation is expressed as eqn (4):

$$\frac{S_i \text{HRT}}{S_i - S_e} = \text{HRT} + \frac{S_i}{k_2 X} \quad (4)$$

If  $S_i/(k_2 X)$  is considered as a constant ( $a$ ) and  $(S_i - S_e)/S_i$  is replaced by the substrate removal efficiency ( $E$ ),  $b$  is a constant for the Grau second-order model, the above equation can be represented as a simple one:

$$\frac{\text{HRT}}{E} = a + b\text{HRT}.$$

### 2.3 Modified Stover–Kincannon model

As for the Stover–Kincannon model,<sup>16</sup> initially, the equation was applied to predict the development of the attached-growth biomass in a rotating biological contactor. Later, the model was further modified, and widely used to describe and predict the performances of the bioreactor.<sup>17,18</sup> The original model is expressed, as shown below:

$$\frac{dS}{dt} = \frac{U_{\max} \left( \frac{QS_i}{A} \right)}{K_B + \left( \frac{QS_i}{A} \right)} \quad (5)$$

where  $dS/dt$  is the substrate removal rate ( $\text{kg m}^{-3} \text{d}^{-1}$ ),  $U_{\max}$  is the constant of the maximum utilization rate ( $\text{kg m}^{-3} \text{d}^{-1}$ ),  $A$  is the surface area of the disc ( $L$ ), and  $K_B$  is the constant of the saturation value ( $\text{kg m}^{-3} \text{d}^{-1}$ ). If the volume of the reactor ( $V$ ) takes the place of the surface area ( $A$ ), the Stover–Kincannon model can be modified as below:<sup>18</sup>



$$\frac{dS}{dt} = \frac{U_{\max} \left( \frac{QS_i}{V} \right)}{K_B + \left( \frac{QS_i}{V} \right)} \quad (6)$$

where the Stover–Kincannon model takes  $dS/dt$  as a function of the loading rate of substrate at a steady state:  $\frac{dS}{dt} = \frac{Q}{V} \times (S_i - S_e)$ , so it can be further expressed as shown below in eqn (7):

$$\frac{V}{Q(S_i - S_e)} = \frac{K_b V}{U_{\max}} + \frac{1}{U_{\max}} \quad (7)$$

## 2.4 Monod model

The Monod model was extensively employed.<sup>19</sup> The modified versions of the Monod equation were developed concerning the model anammox process in terms of the oxygen concentration, ammonia concentration and missing alkalinity:<sup>20</sup>

$$\frac{ds}{dt} = \frac{KS_e}{K_s + S_e} \quad (8)$$

where  $K$  is the maximum substrate utilization rate ( $\text{kg m}^{-3} \text{d}^{-1}$ ), and  $K_s$  is the half saturation concentration ( $\text{g L}^{-1}$ ).

## 2.5 Other models used for the anammox-based process

Generally, the anammox bacteria cooperated with other functional bacteria to degrade the wastewater in the applied fields, such as SHARON and anammox process based on the continuity model.<sup>21</sup> The ASM2, ASM2d and ASM3 models were also used to simulate the anammox process, which is enriched from municipal activated sludge.<sup>22</sup> The more complex microbial communities with AOB, NOB DHB and anammox were simulated with complex models, as reported.<sup>23</sup>

## 3. Anammox processes conducted in different reactors

In recent years, the interest in anammox has been increased significantly with more and more articles being published as time has passed, as showed in Fig. 1a. Since 2005, an average of 78 more papers has been published every year. In 2014, 300 papers were published. Fig. 1b shows the number of anammox processes conducted in different reactors, as previously reported. The sequencing batch reactor (SBR) was recognized as the most dominantly employed bioreactor, which was the topic in one third of published papers, followed by the upflow anaerobic sludge blanket (UASB), membrane bio-reactor (MBR), expanded granular sludge bed (EGSB), continuous stirred-tank reactor (CSTR), sequencing biofilm batch reactor (SBBR) and upflow anaerobic fixed bed (UAFB), subsequently. While the number of applied UASBs was two-thirds of that of applied SBRs, both SBRs and UASBs were the most popular reactors conducted for the anammox process. Many advantages of the reactor are summarized in Table 1. The high NLR (nitrogen

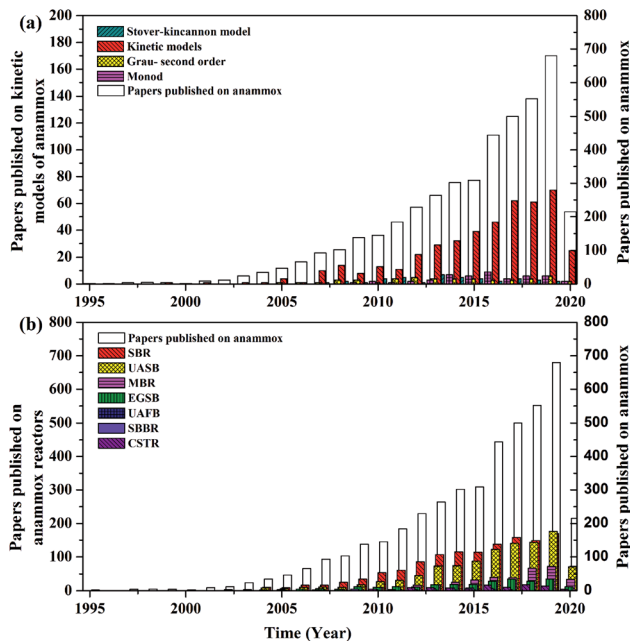


Fig. 1 Published papers in each year: (a) kinetic models used in the anammox process and (b) enrichment reactors. (Web of Scopus, access data: 2020.04.06).

loading rate) and NRR (nitrogen removal ratio), enrichment granular sludge, fast start up and high settleability can be obtained when the UASB or EGSB is applied. However, the MBR (with a long sludge retention time (SRT)) brought a fast startup of the process and a high accumulation rate for anammox enrichment, which was commonly evaluated with special purposes (Table 1). The general comparison of the anammox process conducted in different processes is shown in Table 2. The contents regarding the general process descriptions, applications, advantages and disadvantages, and design criteria are given for each technology based on previous research studies as the referenced applications. The advantages and disadvantages of each reactor constructed for anammox are listed in Table 2. Biofilm, granule and flocculent were the three main kinds of anammox conducted in different reactors, among which the biofilm and granule were the most popular ones. A previous investigation showed that UASB and SBR were the most popular bioreactors that can make granules or keep high anammox biomass. The long retention time of the active sludge is the key factor to influence the successful development of a high-rate anaerobic wastewater treatment. The long retention time of sludge can be realized due to the specific reactor configuration design and fixed biomass in the form of static biofilms, particle-supported biofilms or granule sludge. It was observed that the UASB with upflow can easily change the sludge into granules. The high loading rate of UASB with high removal efficiency was often reported.<sup>7</sup> In contrast, the engineering applications of anammox included more than 100 full-scale partial nitrification/anammox installations worldwide by



Table 1 Anammox processes conducted in different configurations (most popular reactors)<sup>a</sup>

	UASB	EGSB	CSTR	SGBR/SASBR	(G/F) MBR	Nov-BFR	UAFB	MBBR/SBR
Advantage	 <p>High NLR and NRR; enrichment granular sludge; high settleability</p>	 <p>High NLR and NRR; enrichment granular sludge; fast start up; high settleability</p>	 <p>Intensive mixing; ideal model for simulation</p>	 <p>High stability of process enrichment granular sludge; fast start up; high substrate con.; adjust process timing <i>via</i> PLC, convent operation</p>	 <p>High stability of process; fast start up/enrichment; high sludge concentration; high NRR with free cell or/granular sludge; high effluent quality; high degree of automation</p>	 <p>High stability of process; fast enrichment; biofilm culturing; high degree of automation</p>	 <p>High stability of process; fast start up/enrichment; high sludge concentration; high NRR with free cell or/granular sludge</p>	 <p>High stability; fast enrichment; biofilm culturing</p>
Disadvantage	<p>Dead zone areas and substrate inhibition; influent hierarchy; sludge washout; sludge floatation with layer distribution</p>	<p>Costly to operate; sludge washout; sludge floatation consume energy</p>	<p>Low SRT; consume energy</p>	<p>Costly to operate; sludge washout; negative effect on downstream processes; high peak flow may disrupt performance</p>	<p>Costly to operate; membrane cleaning and replacement; process sensitive to sustained peak hour flow; sophisticated operation</p>	<p>Costly to operate</p>	<p>Costly to operate; membrane cleaning; lighter, fluffier sludge flocs</p>	<p>Costly to operate; biofilm fall off</p>

<sup>a</sup> SGBR: static granular bed reactor; SASBR: static anaerobic sludge bed reactor; Nov-BFR: Noven biofilm reactor; MBBR: moving bed biofilm reactor.



Table 2 Performance of anammox process conducted in different reactors<sup>a</sup>

Reactor type	Inoculum sludge	Operation day (d)	T (°C)	pH	Ammonium removal (%)	NLR (kg N m <sup>-3</sup> d <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N influent (mg L <sup>-1</sup> )	NO <sub>2</sub> <sup>-</sup> -N influent (mg L <sup>-1</sup> )	Sludge concentration (VS L <sup>-1</sup> ) or anammox purity (%)	Reference
UASB	Anaerobic granules	400	35 ± 1	7.0	86.5–92.3	6.4	1027	1409	—	46
UASB	Aerobic granules	300	30	7.0	90	0.5	200	220	—	26
UASB	Nitrifying + anammox	>1400	Normal	6.3–7.3	<90	9.5	400–550	575–175	50–60	47
UASB	Nitrifying granules	220	30–33	7.5–8.0	97	0.16	115	130	—	48
UASB	Anammox granules	235	35	7.8–8.0	99.29 (TN)	1.03	458	575	91.2–92.4	49
UASB	AN-GS A-AS	320	–35	7.3–7.9	95	1.25	—	—	—	50
UASB	Anaerobic granular sludge	214/450	35 ± 1	6.8–7	70–99.9	74.3–76.7	–200	240	42–57 g VS L <sup>-1</sup>	7
1 L UASB	anammox sludge	70	35 ± 1	6.8–7.0	93.2 ± 7.1	—	30–70	30–70	—	51
SBR	Synthetic medium	200–600	25	8.0	—	—	—	—	—	52
SBR	AS biomass	320	33	7.0–8.0	99–100	0.43	180	250	—	53
SBR	Anammox	365	36 ± 0.3	7.5–8.2	99.9	1.6	1268	166.14	85.0	6
SBR	Activated sludge	400	35 ± 1	6.7–7.0	60 (TN)	1.0	700	0	—	54
SBR	Anammox	400	35 ± 1	7.5	78 (TN)	0.36	200–250	0	<35	25
SBR	Anammox granules	218	30 ± 1	7.5–8.0	98	0.3	150	150	—	55
2 L SBR	Nitritation–anammox synthetic wastewater	180	33 ± 1	—	85–94	0.18 g N per g VSS per d	500	—	1.8 g VSS per L 18–19% <i>Ca. Jettenia</i> (16.8%) and <i>Nitrosomonas</i> (20.1%)	56
18 L SBR Pa + AN	Raw optoelectronic industrial wastewater	94	37	7.8–8.0	—	10–33 g m <sup>-3</sup> d <sup>-1</sup>	183–200	—	—	57
3 L SBR	0.6 kg N m <sup>-3</sup> d <sup>-1</sup> piggery waste production/no dilution	50 d start up	30	8.1 ± 1	91 ± 10 97	0.5–0.6 g N per L per d	—	NO <sub>2</sub> -N removed/ NH <sub>4</sub> -N removed	274 ± 45% TSS per L and 79% VSS/TSS	58
3 L SBR CANON	Trace N <sub>2</sub> H <sub>4</sub> (4 mg L <sup>-1</sup> ) was added into the influent	80	31 ± 1	—	70 ± 7 (TN)	0.33 ± 0.06 kg N per L per d	150 mg N per L	molar ratio was 1.28 ± 14%	<i>Ca. Scalindia</i> , 3.14 × 10 <sup>9</sup> to 5.86 × 10 <sup>10</sup> copies per g (dry sludge)	59





Table 2 (Contd.)

Reactor type	Inoculum sludge	Operation day (d)	T (°C)	pH	Ammonium removal (%)	NLR (kg N m <sup>-3</sup> d <sup>-1</sup> )	NH <sub>4</sub> <sup>+</sup> -N influent (mg L <sup>-1</sup> )	NO <sub>2</sub> <sup>-</sup> -N influent (mg L <sup>-1</sup> )	Sludge concentration (VS L <sup>-1</sup> ) or anammox purity (%)	Reference
MBBR	Synthetic wastewater 2 L of virgin carrier media	86–121	33.4–35	7.5 ± 0.1	0.57–0.64 ± 0.17	0.03–0.22	348.36–507	0.48–0.77	<i>Ca. Brocadia fulgida</i> shift to <i>Ca. Kuenenia stuttgartiensis</i> AOB1.83–1.4 NOB	60
10 L MBR	Synthetic wastewater		30	6.8–7.5	85% (TN)	1 g N per L per d	12.9 mM	3.4 mM (1.21)	Growth rate of 0.21 d <sup>-1</sup>	5
15 L/8 L MBR	Synthetic wastewater	250	37	7.1–7.5			1680	1680	0.23 day as free cells	61
1.6 L MBR	Synthetic medium	63	30 ± 1	7.0 ± 0.2	82.14	293/1680	840	840	<i>Ca. Brocadia</i> 60%	62
1.8 L GSBF	Effluent of the A-stage of the WWTP	20 °C/1–52 15 °C/60–127 10 °C/134–285	20 and 10	7–7.5	—	50 ± 7 mg N per g VSS per d	0.4 g N per d	50 ± 7 mg N per g VSS per d	<i>Ca. Brocadia</i> 0.35 g TSS/12 g TSS	63
6 L SBBR	Synthetic ammonium-rich wastewater	30	35 ± 1	74		—	470	—	—	64
SBBRs	Synthetic medium	90	35 ± 1	7.0–7.8	88	1.62 kg N m <sup>-3</sup> d <sup>-1</sup>		—	<i>Ca. Anammoxoglobus</i> (DQ317601.1) 40.1%	65
2.6 L FBR	Synthetic wastewater	65	35 ± 0.2	8 ± 0.2		0.05–0.06 kg N m <sup>-3</sup> d <sup>-1</sup> 0.25 kg N m <sup>-3</sup> d <sup>-1</sup>	50 mg L <sup>-1</sup>	50 mg L <sup>-1</sup>	<i>Ca. Kuenenia stuttgartiensis</i>	66
EMBRs	—	50	33 ± 1	8.0 ± 0.3		0.8	—	—	—	67
5 L AnR	Synthetic medium	102	32	7.8 ± 0.3	90	0.30 g N L <sup>-1</sup> d <sup>-1</sup>	1230 ± 61 mg L <sup>-1</sup>	—	—	68

<sup>a</sup> NLR: nitrogen loading rate; anammox purity: the percentage of anammox in the biomass; MBBR: moving bed biofilm reactor; EMBRs: external MBR; FBR: fixed bed reactor; PAN-An R: partial nitrification and anammox reactor; GSBF: granular fluidized bed reactor; SGSR: stirred gas solid reactor.

2014. Most of them were conducted in the one-stage configuration, and the most commonly applied reactors were SBRs, followed by granular systems and MBBRs.<sup>24</sup>

## 4. Characteristic and stability analysis of the anammox process in different reactor setups

Different reactors used for the anammox process are summarized in Table 2. The performance such as the startup time, nitrogen-loading rate, removal efficiency, substrate concentration, and the purification of sludge is described and compared in detail. The most commonly used SBR reactor has an NLR that varied from 0.3 kg m<sup>-3</sup> d<sup>-1</sup> to 1.6 kg m<sup>-3</sup> d<sup>-1</sup> with the highest removal efficiency over 99%.<sup>6</sup> The comparison of the most commonly used UASB and SBR processes with the maximum NRR and startup time is shown in Fig. 2. Generally, the UASB process can obtain a high NRR over 2 kg m<sup>-3</sup> d<sup>-1</sup>. However, that of the SBR reactor was lower than 2 kg m<sup>-3</sup> d<sup>-1</sup> with a shorter time for startup. By comparing the anammox sludge with an organic C content lower than 35%<sup>25</sup> and a content higher than 99%,<sup>6</sup> it was found that the anammox sludge with higher purity can obtain a higher nitrogen removal efficiency. Similarly, the UASB reactors with different purities and operation conditions had relatively large differences in the NRRs, which might vary from 0.5 kg m<sup>-3</sup> d<sup>-1</sup> (ref. 26) to 76 kg m<sup>-3</sup> d<sup>-1</sup>. To date, the highest NRR obtained was 76 kg m<sup>-3</sup> d<sup>-1</sup> when the UASB reactor was fed with substrate of low concentration under short HRT.<sup>7</sup> Other reactors for the anammox process were reported to have different NRRs varying from 0.3 kg m<sup>-3</sup> d<sup>-1</sup> to 1.62 kg m<sup>-3</sup> d<sup>-1</sup> (Table 2).

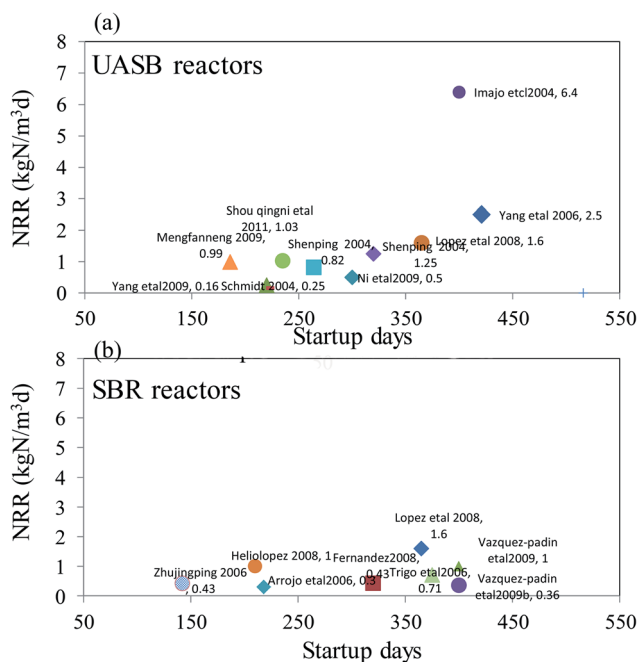


Fig. 2 The start up time and maximum NRR comparison of the anammox process of the most used reactors (a) UASB and (b) SBR.

Over the past twenty years, many studies carried out have focused on the optimization of the anammox process parameters. The relative issues involving the substrate inhibition, temperature effect, organic matter and salinity have been extensively investigated.<sup>27</sup> The drawback of the anammox process with quite low bacteria growth rate can be overcome by MBR, which is more effective than most other reactors for enrichment.<sup>5</sup> A reactor configuration with a high capacity of biomass retention is essential for the anammox process. Recently, a novel process conducted in an MBR with pure free-cell suspension of highly active anammox was successful, with a higher growth rate than the specific maximum growth rate ever reported for the biomass, 0.21–0.23 d<sup>-1</sup>.<sup>5</sup> This strategy has a number of potential capacities in the cultivation of seeding anammox in terms of practical engineering projects.<sup>2</sup> MBR or MBBR and Nov-BFR with carriers can accumulate a certain amount of anammox to keep the process stable. Additionally, inherent with each technology are the advantages and disadvantages of the process, and/or operation and maintenance. Discharging standards are often the primary consideration in selecting a treatment technology.

Anammox processes conducted in different reactors can accumulate different kinds of anammox bacteria following the substrata and operation conditions. Because of its potentials in nitrogen removal during the biological process, the high treatment performance and stability of the anammox process have been constantly studied for engineering application. Most of the lab-scale enrichment anammox bacteria were '*Ca. Brocadia*' and '*Ca. Kuenenia*', which are almost mono-species in the anammox process, illustrating a competition relationship of the anammox species owing to their different substrate affinities. It should be worth noting that under specific physiological conditions, *Ca. Kuenenia* outgrows '*Ca. Brocadia*', resulting in overgrowth when the substrate concentration is low.<sup>28,29</sup> Meanwhile, the ecological roles of the functional microbe and their potentials should be extensively explored in order to attain new perspectives for further design, operation, and maintenance of the process.

Generally, a large number of gas bubbles will be generated in gas tunnels and gas pockets in some high-loading bioreactors inoculated with anammox bacteria of high activity. It is speculated that the gas bubbles entrapped in the gas pockets might be a pivotal factor to cause sludge floatation, especially in the upflow reactor, such as a UASB or an EGSB. After the hollows are filled with gas bubbles, the anammox granules will unavoidably float and be washed out of the reactor, leading to a failure of the anammox process. The granule floatation can be undoubtedly responsible for instability, or even failure of the anammox reactor.<sup>30,31</sup> The existence of this washout obstacle compels the implementation of a large number of studies in pursuit of a feasible way to overcome this obstacle.

## 5. Process-kinetic analysis of different anammox processes

Bibliometric analysis showed that the investigation on the reactor-kinetic models in the published paper was only one-



Table 3 Comparison of the kinetics used for the anammox reactor<sup>a</sup>

Reactor	V (L)	T (°C)	Substrate	Inoculum	NLR (g L <sup>-1</sup> d <sup>-1</sup> )	HRT (d)	k <sub>i</sub> (1/d)	First-order			Grau second-order			Modified Stover-Kincannon			Monod			Y (mg VSS per mg N)	R <sup>2</sup>	Ref.
								R <sup>2</sup>	a (1/d)	b	k <sub>2</sub> (1/d)	R <sup>2</sup>	R <sub>m</sub>	K <sub>B</sub>	R <sup>2</sup>	K <sub>s</sub> (g L <sup>-1</sup> )	M <sub>max</sub> (1/d)	K <sub>d</sub> (1/d)	R <sup>2</sup>			
ILAB	24/20	30 ± 1	Synthetic wastewater	PN-anammox granular	420 ± 30 NH <sub>3</sub> -N mg L <sup>-1</sup>	1.0–0.066	66.9	0.6102	1.09	0.02	2.064	0.9954	22.29	27.25	0.981	0.002446	0.007408	0.0059	0.3511	0.71–0.94	34	
Upflow filter	35		Cultured activated sludge		0.93–7.34	0.08–0.6	0.44	0.172	1.4	0.96	—	0.985	12.4	12	0.979	—	—	—	—	—	69	
ANMR	35		Flocculent anammox sludge		0.107–0.746	0.6–3.0	5.31	0.707	0.11	1.11	—	0.995	7.89	8.98	0.999	0.192	0.169	—	—	—	0.599	35
ANMR	35		Flocculent anammox sludge		115–297/153–313	0.6–2.9	5.305	0.706	0.11	1.11	—	0.9954	8.98	7.89	0.9986	0.192	0.1693	—	—	—	0.599	49
MAR	0.8/0.65	25	Salty synthetic	Cultured anammox sludge	0.08–1.94	0.25–1.0	7.44	0.756	0.06	1.14	—	0.991	6.41	7.37	0.993	0.107	0.952	—	—	—	0.993	11
AUF	35		Cultured activated sludge		270–305	0.08–0.6	—	1.4	0.96	—	—	0.9855	12	12.4	0.9792	—	—	—	—	—	—	12
UABF	35		Anammox granules		288–307	1–0.25 h	—	0.04	1.06	—	—	0.999	35.7	38.1	0.999	—	—	—	—	—	—	36
UABF	35		Anammox granules		475–924	1–0.25 h	—	0.05	1.04	—	—	0.998	20.7	21.6	0.998	—	—	—	—	—	—	37
UASB	1	25	Synthetic wastewater	Preservation treatments-4 + distilled water	50–300/60–300	3–9.62 h	—	—	—	—	—	55.5	56.6	0.99	—	—	—	—	—	—	—	38
UASB	1	25	Synthetic wastewater	Preservation treatments-40 + dimethyl sulfoxide	50–300/60–300	3–9.65 h	—	0.999	1.165	—	—	0.9916	66.7	67.9	0.992	—	—	—	—	—	—	38
UASB	50	37	Synthetic wastewater	Anammox granules	167–278/191–411	0.2–3.2	11.64	0.8043	0.09	1.03	—	0.9985	27.8	27.5	0.999	—	—	—	—	—	—	49
SUASB	8.6	35 ± 1	Synthetic wastewater	Nitrifying sludge and mature anammox granule	5.63	2.5–24 h	—	—	—	—	—	14.8	14.5	0.989	—	—	—	—	—	—	—	70





Table 3 (Contd.)

Reactor	V (L)	T (°C)	Substrate	Inoculum	NLR (g L <sup>-1</sup> d <sup>-1</sup> )	HRT (d)	First-order			Grau second-order			Modified Stover-Kincannon			Monod			Y (mg VSS per mg N)	R <sup>2</sup>	Ref.
							k <sub>1</sub> (1/d)	R <sup>2</sup>	a (1/d)	b	k <sub>2</sub> (1/d)	R <sup>2</sup>	R <sub>m</sub>	K <sub>B</sub>	R <sup>2</sup>	K <sub>s</sub> (g L <sup>-1</sup> )	M <sub>max</sub> (1/d)	K <sub>d</sub> (1/d)			
UASB	1	35 ± 1	Synthetic wastewater	0.16 × 10 <sup>0.24</sup> kg Sm <sup>3</sup> dIwould	14.6							95.29	84.03	0.9758							71
UASB	1	35 ± 1	Synthetic wastewater	Copper(II) recovery		0.4–2.4 h						151.5	157.9	0.73							72
UASB	1	35 ± 1	Synthetic wastewater	OTC recovery		0.4–2.4 h						212.8	228.4	0.746							72
UASB	1.5	35 ± 1	Synthetic wastewater	Re-startup								49.5	56.8	0.985							41
UASB	1.5	35 ± 1	Synthetic wastewater	Re-startup								454.5	528	0.995							41
UASB	1.5	35 ± 1	Synthetic wastewater	Re-startup								384.6	447.7	0.99							41
UASB	1.5	35 ± 1	Synthetic wastewater	Re-startup								476.2	553	0.996							41
UASB	1.5	35 ± 1	Synthetic wastewater	Re-startup								144.9	153.7	0.99							41
UASB	1	35 ± 1	Synthetic wastewater	Single feed	70–266	1.52–2.06 h						15	17.5	0.934							42
UASB	1	35 ± 1	Synthetic wastewater	Multi feed	70–267	1.52–2.06 h						27.5	37.5	0.932							42
UASB	1	35 ± 1	Synthetic wastewater	Activated sludge	0.15–2.8	0.2–3.2	11.6	0.804	0.09	1.03	—	0.999	12.1	11.4	0.999	0.092	0.225	—	—	0.773	16
UASB recycled	50	37	Synthetic wastewater	Anammox granular	0.28–1	<1						27.8	27.5	0.999							49
PN reactor	5	28 ± 2	Reject water wrp	Lab-scale fill-draw reactor								13.9	30	0.96							73
UAF	2.5	36	Reject water wrp	Mesophilic digester of a municipal wastewater treatment plant	0.93–7.34 g L <sup>-1</sup>	2–14.4	0.4395	0.175	1.397	0.964	0.986	12.4	12	0.979							73
UAF	2	30	Synthetic wastewater	Wastewater treatment plant																	12
ILAR	3.8	30 ± 1	Synthetic wastewater	Municipal wastewater treatment plant	(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> 0.39–6.96 g L <sup>-1</sup>	12–21 h						5.77	5.39	0.9572							74

<sup>a</sup> ILAB: internal-loop airlift bio-particle reactor; ANMR: non-woven membrane reactor; MAR: marine anammox reactor; VAF: upflow anaerobic filter; R<sup>2</sup>: correlation coefficient; k<sub>1</sub> and k<sub>2</sub> is the substrate removal rate constant (1/d) of each equation.

tenth of the total. Meanwhile, the Stover–Kincannon model was the most commonly used one, which was followed by the Monod model and the Grau-second model (Fig. 1b). It was confirmed by many studies that the anammox bacteria were very sensitive to operational conditions, such as dissolved oxygen, temperature, pH and composition of organic matters. Unfavorable conditions will largely reduce the activity of the anammox bacteria, and eventually cause a failure in bioreactor performance. Therefore, it is of practical significance to effectively augment the activity of the anammox bacteria in order to achieve a high-rate process under the mainstream conditions, which might cause a large decrease in the specific activity of anammox. Most of the literature reached a consensus in that the process control for anammox is a prerequisite to practically ensure the successful performance under unstable influent conditions.<sup>32</sup>

The common bioreactors have certain shortcomings since different reactors have different optimal operating conditions. For instance, the UASB reactor often encounters unsatisfactory substrate removal efficiency, severe sludge washout, and large dead zone areas. An EGSB is costly in term of the huge consumption of energy and operation cost. In addition, a bio-film reactor has a long sludge age and sporadic sludge flotation. The main objectives of process modeling are to control and evaluate the performance of the process, as well as to optimize the system design and scale up the pilot plant investigations.<sup>33</sup> For example, the concentrations and activities of anammox measured in different reactors could be used in conjunction with models to control the process parameters, involving the influent loading rate and hydraulic residence time in order to

maximize nitrogen removal. To date, process control is widely considered in the literature as an essential factor to guarantee good performance in various bioreactors.

Modeling and parameter identification are subjects with wide ranges, offering a realm of approaches and methods. In many practical industrial projects, most knowledge is available in the form of heuristic rules achieved from rich experience in a variety of processes. Until now, the successful application of anammox in practical projects is still facing huge challenges. The kinetics involves operational and environmental factors, affecting the utilization rates of the substrates. By means of the kinetics evaluation, the optimization on the plant design and prediction of the performance of treatment plants can be conducted,<sup>14,33</sup> and the process models relating to the anammox process can be further discussed (Table 3).<sup>11,14,34–38</sup> Furthermore, the anammox enrichment culture can be significantly promoted because the kinetics can provide a convincing basic recipe for dealing with the operational and environmental factors affecting the performance of substrate removal.<sup>11</sup> It is well known that many mathematical models, such as first-order substrate removal models, Grau second-order substrate removal models, Stover–Kincannon models, and Monod models are widely adopted in the field of wastewater treatment (Table 3). For example, the first-order and second-order substrate removal models are effective for estimating the kinetic constants in anammox processes.<sup>14,39,40</sup> In addition, the Monod model was initially applied to represent the growth of microorganisms, and widely employed to express the degradation kinetics. Moreover, the Stover–Kincannon model is one of the most expensively developed mathematical models for



Fig. 3 Reactor-kinetic validation assessment. (a), N<sub>2</sub> gas production (b),<sup>13</sup> substrate consumption (c),<sup>45</sup> and the model comparison (d).<sup>15,45</sup>





Table 5 Most popular kinetic models used for the activity simulation<sup>a</sup>

Simulation equations	Reference
$q = \frac{S q_{\max}}{K_s + S + \frac{S^2}{K_I}}$	76
$q = q_{\max} \left( \exp\left(-\frac{S}{K_I}\right) - \exp\left(-\frac{S}{K_S}\right) \right)$	77
$q = q_{\max} \left( 1 - \exp\left(-\frac{S}{K_S}\right) \right)$	78
$r = \frac{S r_{\max}}{K_s + S + \frac{S^2}{K_{IH}}}$	79
$r = \frac{S r_{\max} (1 - S/S_m)^n}{K_s + S}$	80
$r = \frac{S r_{\max} (1 - S/S_m)^n}{S + K_s (S/S_m)^m}$	81
$r = \frac{S r_{\max}}{K_s + S} \exp\left(-\frac{S}{k_{ip}}\right)$	82

<sup>a</sup> Where  $q$  is the specific substrate conversion rate constant ( $\text{d}^{-1}$ );  $q_{\max}$  is the maximum specific substrate conversion rate constant ( $\text{d}^{-1}$ );  $K_s$  is the half saturation constant ( $\text{mg N L}^{-1}$ );  $K_I$  is the inhibition constant ( $\text{mg N per L}$ );  $K_{IH}$  is the inhibition constant of Haldane ( $\text{mg N per L}$ );  $k_{ip}$  is the inhibition constant of Aiba;  $r_{\max}$  is the maximum specific activity ( $\text{mg L}^{-1}$ );  $S_m$  is the maximum removal efficiency ( $\text{mg L}^{-1} \text{d}^{-1}$ ).

determining the kinetic constants in immobilized bioreactors. Also, it was reported that the Stover–Kincannon model has been investigated in continuously operated mesophilic and thermophilic upflow anaerobic filters for the treatment of soybean wastewater, paper pulp liquors, and simulated starch wastewater, and the determination of kinetic constants in a packed bed reactor for decolonization.<sup>11</sup>

A detailed comparison of different substrate removal kinetic models for anammox reactors is listed in Table 4. Compared with the various processes conducted in the UASB, MAR, ANMR and up-filters, the result of the first-order simulation showed that the UASB had the highest  $K_1$  of  $11.64 \text{ (d}^{-1}\text{)}$  with  $R^2$  being 0.80, and the up-filter had the lowest  $K_1$  of  $0.43 \text{ (d}^{-1}\text{)}$  with  $R^2$  being 0.439.<sup>12,15</sup> All of the first-order simulations proved that  $R^2$  was low, indicating the poor fitting of the data. While these data simulated by second-order simulation proved a higher  $R^2$ , which was 0.99 or 0.98, respectively. Meanwhile, the modified Stover–Kincannon also gave a high  $R^2$  of 0.98 in a UAF system.<sup>12</sup> In the UASB system, among the most commonly used modified Stover–Kincannon model,  $R_{\max}$  varied from 15 to 476, as reported due to the different operational conditions.<sup>41,42</sup> The difference in the  $U_{\max}$  values was probably due to the different adopted reactor configurations, different treated wastewater characteristics and developed microorganisms in various studies. The Stover–Kincannon model was suitable for the kinetic analysis of a complicated anaerobic process. This is partially due to the simplification of this model without diffusion of the modeling substrate, hydraulic dynamics and other



Fig. 4 SAA kinetic simulation of the EGSB-anammox biomass (a–f)<sup>13</sup> and the different systems of anammox (g), DMX-deammonification system (h), and NF-nitrifying system (i).<sup>43</sup>



parameters, which might be key factors for the reactor performance, but were difficult to measure. The methods of validity assessment between the experiment and prospect can be conducted by the linear regression equation,  $y = x$  (Fig. 3). The modified Stover–Kincannon model and the Grau second-order model are both practical because in the case that  $U_{\max}$  is equal to  $k_B$ , the Grau second-order model can be transformed to the modified Stover–Kincannon model. Thus, the total nitrogen concentration in the effluent predicted by the Grau second-order and modified Stover–Kincannon models may display high correlation with actual measured data. Conversely, the Monod model and the first-order model were proven to be not feasible in many cases. The Grau second-order model and the modified Stover–Kincannon model were both acceptable to predict the total nitrogen concentration in the effluent since their plot lines nearly coincided with the actual line (Fig. 3). The process kinetic models also illustrated that the substrate concentration and granule size should be carefully controlled as the main process parameters. Different types of kinetic models should be used to obtain the appropriate model. For the activity test simulations, most popular kinetic models used for the activity simulation are shown in Table 5. The specific anammox activity (SAA) kinetic simulation of the EGSB-anammox biomass (a–f)<sup>13</sup> and the differences between the anammox (g), DMX-deammonification system (h), and the NF-nitrifying system (i)<sup>43</sup> were compared and verified (Fig. 4). Other simple kinetic ASM1 models for granular biomass floatation were also investigated.<sup>44</sup> Among the kinetic models, the basic Monod equation for the DMX process, the extended Edwards and Luong equation for the AMX process, and the Andrews equation for the NF process were used and validated to be the most appropriate equations for the process.

A simplified kinetic model lacks the capacity of expressing the detailed mechanism, but it can be used to properly describe the main characteristics of a reaction. A good model not only provides a better understanding of the complex biological and chemical fundamentals, but is also fundamental for the process design, start-up, dynamics predictions, and process control and optimization. Obviously, the simplified models with limited variables, which are confirmed suitable for practical engineering applications, were proved to be powerful tools for anammox at low growth rates. Although different kinetic models were investigated to describe the anammox process carried out in different reactors, the modified Stover–Kincannon and Grau second-order models seemed to be the most suitable for describing nitrogen removal.<sup>4,12,37</sup> However, these models still had problems since the limitations of these kinetic models cannot describe cellular metabolism and regulation. Moreover, these models did not give any insight to the variables that could influence cell growth. In addition, these models shall be further modified when the special phase of the process was simulated to get the actual kinetics. More analysis methods and technologies for a more in-depth study shall be extensively carried out with the purpose of improving the understanding of the reaction mechanisms and microbial community dynamics.

## 6. Conclusions

In the review, the process-kinetics of different anammox configurations with process performance was investigated. Among the configurations, SBR, UASB and MBR were the most commonly used and favored for bacterial accumulation. The simulation models of the kinetics were proved as valuable information for process construction and reactor operation. The kinetic model validation proved that the modified Stover–Kincannon model and Graus second-order model could be appropriate for different reactor anammox processes. Rather than the correlation coefficient, the validation revealed that the modified Stover–Kincannon model was more appropriate for nitrogen removal kinetics in most configurations. Based on the simulation of correlated indexes, the activity kinetic models illustrated that the modified Han–levenspiel, Luong and Andrews models were the most appropriate kinetic models for the activity simulation in the anammox process. The appreciate configurations could improve the denitrification efficiency, and contribute to the biomass enrichment in the projects of engineering application.

## Conflicts of interest

There are no conflicts of interest to declare.

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