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Review

The r/K selection theory and its application in biological wastewater treatment processes

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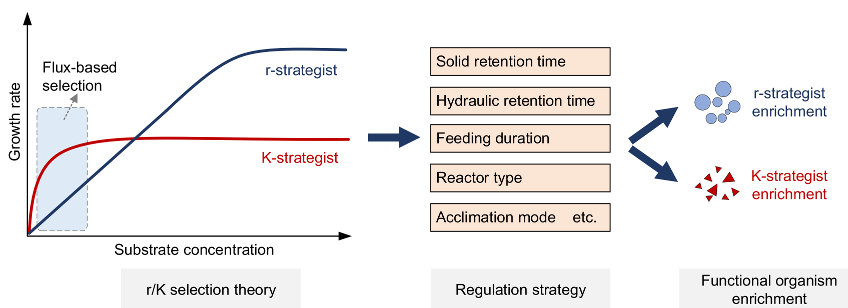
HIGHLIGHTS

- Organisms in wastewater treatment processes exhibit r-/K-properties.
- F/M ratio is the crucial factor affecting the distribution of r-/K-strategists.
- Biofilm systems with different layers can acclimate both r- and K-strategists.
- Substrate flux would benefit the selective enrichment of targeted r-/K-strategists.

GRAPHICAL ABSTRACT



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ABSTRACT

Understanding the characteristics of functional organisms is the key to managing and updating biological processes for wastewater treatment. This review, for the first time, systematically characterized two typical types of strategists in wastewater treatment ecosystems via the r/K selection theory and provided novel strategies for selectively enriching microbial community. Functional organisms involved in nitrification (e.g., *Nitrosomonas* and *Nitrosococcus*), anammox (*Candidatus* Brocadia), and methanogenesis (*Methanosarcinaceae*) are identified as r-strategists with fast growth capacities and low substrate affinities. These r-strategists can achieve high pollutant removal loading rates. On the other hand, other organisms such as *Nitrosospira* spp., *Candidatus* Kuenenia, and *Methanosaetaceae*, are characterized as K-strategists with slow growth rates but high substrate affinities, which can decrease the pollutant concentration to low levels. More importantly, K-strategists may play crucial roles in the biodegradation of recalcitrant organic pollutants. The food-to-microorganism ratio, mass transfer, cell size, and biomass morphology are the key factors determining the selection of r-/K-strategists. These factors can be related with operating parameters (e.g., solids and hydraulic retention time), biomass morphology (biofilm or granules), and operating modes (continuous-flow or sequencing batch), etc., to achieve the efficient acclimation of targeted r-/K-strategists. For practical applications, the concept of substrate flux was put forward to further benefit the selective enrichment of r-/K-strategists, fulfilling effective management and improvement of engineered pollution control bioprocesses. Finally, the future perspectives regarding the development of the r/K selection theory in wastewater treatment processes were discussed.

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Contents

1. Introduction

Environmental bioprocesses have contributed greatly to worldwide pollutant removal for decades (Bodor et al., 2020). With advances in microbiology and engineering, the understanding of how to properly manage functional organisms in environmental engineered bioprocesses for efficient pollutant control is rapidly developing. Nowadays, the properties of many organisms which can be applied for the biodegradation of diverse pollutants have been characterized (Rittmann, 2006). However, to date, we have only scratched the surface of microbial potentials as most organisms and their functions remain unknown. Particularly, facing the stringent discharging standards and emerging pollutants, evolving environmental bioprocesses by properly selecting appropriate organisms and regulating microbial communities is still the key challenge in the foreseeable future. To achieve this, appropriate theories would be necessary to guide engineering practice.

The r/K selection theory (i.e., Andrew and Harris, 1986) can be applied widely to describe two typical types of strategists in natural and engineered ecosystems. The r-strategists can be acclimated in an uncrowded and resource-rich environment (with little competition, e.g., a high substrate supply per capita), which are opportunists; while the K-strategists are non-opportunists, being able to better adapted to a crowded and resource-limited environment and survive close to the carrying capacity (the maximum number of bacteria sustained in a system) (Attramadal et al., 2012; Juteau et al., 1999; Rojas-Tirado et al., 2017). These two strategists show different characteristics in many ways (Table 1). According to the Monod equation and the resource availability (both concentration and loading rate), r-strategists are copiotrophs (eutrophic conditions), i.e., fast-growers possessing a high growth rate and a low resource affinity. In contrast, K-strategists are oligotrophs (oligotrophic condition), i.e., slow-growers with a low growth rate and a high resource affinity (Erbilgin et al., 2017; Oshiki et al., 2016) (Table 1 and Fig. 1).

The distinct properties of r-/K-strategists determine their existence in different ecological niches. Generally, r-strategists survive in unstable environments with dynamic resource variations (disturbances and/or feast-and-famine intermittent supply), while K-strategists would be selected in stable environments with limited resource supply (Cheng et al., 2018). For example, it was reported that K-strategist of stygobites dominated in pristine hyporheic waters, while r-strategist of more tolerant species proliferated in an intermediate level disturbed hyporheic waters (Iepure et al., 2013). Similarly, the disturbance of dairy wastewater discharge led to the enrichment of r-strategists in the stream water and K-strategists in the sedimentary area (Schneider and Topalova, 2014).

In wastewater treatment ecosystems, the identification of functional r-/K-strategists and the selective enrichment of targeted organisms by regulating environmental variables would be a possible strategy to upgrade or develop novel bioprocesses. The enrichment of r-strategists can achieve high pollutant removal loading rates, while K-strategists can degrade the pollutant to low concentrations. This would help to balance the design and operation of bioprocesses efficiently, because diverse microorganisms can enhance the system stability, resilience, and redundancy under disturbance conditions of substrate, temperature, pH, and toxic substances (Kim and Kim, 2006). For example, a high residual ammonium (NH_4^+) concentration could be maintained to enrich r-strategist ammonia oxidizing bacteria (AOB) (Reino et al., 2016). Furthermore, Wu et al. (2016) achieved a high nitritation by maintaining a high NH_4^+ concentration to acclimate fast-growing r-AOB, with the maximum specific growth rate increased from 0.39 to 1.45 1/d and the NH_4^+ half-saturation constant (K_s) increased from 0.51 to 5.23 mg N/L. Nevertheless, so far, the detailed information of the r-/K-properties of organisms involved in the biodegradation of pollutants remains unravelled. Furthermore, how to apply the r-/K-selection theory in managing functional organisms to advance environmental bioprocesses has not been systematically discussed.

In this review, recent findings on r-/K-properties of functional organisms involved in typical wastewater treatment processes including but not limited to nitrification, anaerobic ammonium oxidation, and anaerobic digestion are summarized. The reasons to choose these bioprocesses are because i) they are worldwide used bioprocesses contributing greatly to pollutant removal, and ii) some functional organisms involved in these bioprocesses have already been reported to have r-/K-properties. Besides, the r-/K-properties of organisms responsible for recalcitrant pollutant degradation were also introduced. Subsequently, the crucial affecting factors governing the selection of r-/K-strategists are highlighted. Finally, the potential applications and future perspectives of the r-/K-selection theory in the engineered pollution control bioprocesses are herein addressed and discussed.

Table 1
Characteristic traits of r- and K-strategists (adapted from Andrew and Harris, 1986).

Trait	r-strategist	K-strategist
Maximum growth rate	High	Low
Substrate affinity	Low (high K_s)	High (low K_s)
Efficiency of food conversion to biomass	Low	High
Competitive ability at substrate limitation	Low	High
Resistance to mortality	Low	Variable-high
Tolerance to inhibitory chemicals	Variable-low	Variable-high
Ribosomal RNA operon (rrn) copy number	High	Low

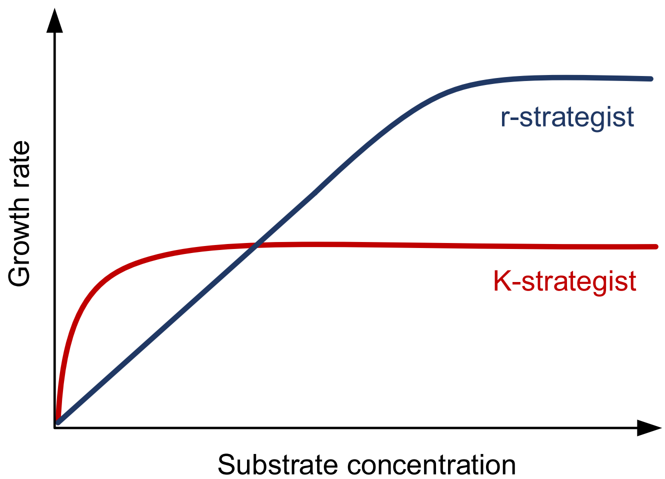

Fig. 1. The growth properties of r-/K-strategists.

2. The r/k selection theory application in biological processes for wastewater treatment

2.1. Nitrification

Nitrification is conducted to oxidize ammonia to nitrite and then to nitrate by AOB and nitrite oxidizing bacteria (NOB), respectively. Nitrifiers of AOB and NOB include both r-strategists and K-strategists (Schramm et al., 1999). Generally, typical AOB such as *Nitrosomonas* and *Nitrosococcus* are considered as r-strategists, while *Nitrosospira* spp. belong to K-strategists. For NOB, *Nitrobacter* spp. and *Nitrotoga* are r-strategists, while *Nitrospira* spp. belong to K-strategists (Dytczak et al., 2008; Gatti et al., 2015; Yu et al., 2020). Compared with r-strategists, K-strategists can be better surviving during starvation and responding to stress or adverse conditions due to their slow reaction rates and low K_s values (Dytczak et al., 2008).

Nitrifiers enrichment are responded to the availability of nitrogen substrates (electron donor) (Park et al., 2017). Theoretically, in domestic wastewater treatment plants (WWTPs), the nitrite concentration is usually quite low, resulting in the acclimation of *Nitrospira* spp. rather than *Nitrobacter* spp. In contrast, when treating ammonia-rich wastewater, a high concentration of nitrite can be accumulated, leading to the enrichment of *Nitrobacter* spp. The nitrite K_s values for *Nitrobacter*, *Nitrotoga*, and *Nitrospira* were reported to be 49–544, 58, and 9–27 μM, while the maximum specific activities were about 4.4–11.3, 1.8, and 1.2–3.3 mg nitrite/g protein·h, respectively (Nowka et al., 2015). Kim and Kim (2006) also reported that the maximum specific nitrite-oxidizing activities of *Nitrospira* and *Nitrobacter* were 10.5 and 93.8 mg/g NOB·h. K-strategists *Nitrospira* had also been found to possess a lower nitrite K_s (0.27–1.1 mg N/L) in comparison with r-strategists *Nitrobacter* (0.39–1.3 mg N/L) (Blackburne et al., 2007; Downing and Nerenberg, 2008) (Table 2).

The r/K properties of AOB and NOB are also reflected in the oxygen (electron accepter) affinity since both are aerobic microorganisms. Due to the lower oxygen half-saturation constant (K_O), AOB have a high O_2-competing ability than NOB (Belmonte et al., 2009). For example, Cao et al. (2017) found that *Nitrosomonas* (r-strategists, AOB) has a higher oxygen affinity (K_O = 0.1–0.3 mg/L) than *Nitrobacter* (r-strategists, NOB), and exhibits a higher μ_{max} of 0.77 1/d (Wiesmann, 1994) at 20 °C than 0.25 1/d (Blackburne et al., 2007) of *Nitrospira* (K-strategists, NOB). However, there are some exceptions and K-strategists NOB may also possess a high affinity for O_2. Especially, the recent discovery of complete nitrification by comammox *Nitrospira* further confirms that NOB may also survive under low oxygen concentrations (Daims et al., 2015; Lawson and Lücker, 2018). *Nitrospira* spp. might be evolved from an anaerobic or microaerophilic origin, and they could be favored under oxygen-limited conditions (Qian et al., 2017).

Denitrifiers, however, are less found to be r-/K-strategists. It was reported that an enrichment of *K*-strategist denitrifier with an abundance of 60% was achieved by a restricted supply of either acetate or nitrite, while

Table 2
Summary of the reported dynamic parameters of AOB and NOB classified as r-/K-strategists.

Organisms	Experiment /Reactor	Substrate	K_S (mg N/L)	μ_{max} (day^{-1})	K_O (mg O_2/L)	Note	Reference
Nitrobacter	SBR-8 L	<100 mg NO_2-N/L	1.2–1.3	–	0.43 ± 0.08	T = 14–40 °C; pH = 6–9; Median volumetric floc size: 44 μm	Blackburne et al., 2007
Nitrospira	SBR-5 L	1000 mg NO_2-N/L	0.9–1.1	–	0.54 ± 0.14	T = 14–40 °C; pH = 6–9; Median volumetric floc size: 66 μm	Blackburne et al., 2007
Nitrobacter spp.	MBR-56 mL	3 mg NH_4-N/L	0.39	0.31	0.0.51	T = 22 °C; HR*T* = 28 min; loading rate = 33 gN m^{-2} d^{-1}	Downing and Nerenberg, 2008
Nitrospira spp.	MBR-56 mL	3 mg NH_4-N/L	0.27	0.28	0.4	T = 22 °C; HRT = 28 min; loading rate = 33 gN m^{-2} d^{-1}	Downing and Nerenberg, 2008
Nitrosomonas (Activated sludge)	SBR-5 L	1 mol NH_4	0.5–5.8	2.0–2.9	0.3–1.4	T = 17–22 °C; HRT = 12 h; SRT = 20 d; pH = 7.5–8.2 Average floc diameter = 123–320 μm, relative abundance of *Nitrosomonas* = 0.004–29.6%	Yu et al., 2020
Nitrospira (Activated sludge)	SBR-5 L	1 mol NH_4	0.5–5.5	1–1.5	0.4–2.0	T = 17–22 °C; HRT = 12 h; SRT = 20 d; pH = 7.5–8.2; Average floc diameter = 123–320 μm, relative abundance of *Nitrospira* = 0.008–0.5%	Yu et al., 2020
Nitrospira spp.	SBR-6 L	40–200 mg NO_2-N/L	0.52 ± 0.14	0.69 ± 0.10	0.33 ± 0.04	T = 22 ± 1 °C; HRT = 0.5 d; SRT = 15 d; pH = 7.5 ± 0.1; Maintaining sustained limiting extant nitrite and dissolved oxygen (0.5–1 mg/L) concentrations	Park et al., 2017
Nitrospira spp.	Batch cultures-0.5 L	–	0.13–0.39	0.45–0.52	–	T = 28 °C; pH = 7.4–8.6; Pure culture	Nowka et al., 2015
Nitrobacter spp.	Batch cultures-0.5 L	–	0.69–7.6	0.39–1.28	–	T = 28 °C; pH = 7.4–8.6; Pure culture	Nowka et al., 2015

r-strategist denitrifier of 70% was enriched with an unrestricted supply of either nutrient (Ginige et al., 2007). Nevertheless, more studies should be conducted to investigate the r-/K-properties of denitrifiers.

2.2. Anaerobic ammonium oxidation (Anammox)

Anammox is a bioprocess in which NH_4^+ is oxidized to N_2 gas with nitrite (NO_2^-) as an electron acceptor. Anammox bacteria include six candidate genera, i.e., *Candidatus* Kuenenia, *Candidatus* Brocadia, *Candidatus* Anammoxoglobus, *Candidatus* Jettenia, *Candidatus* Scalindua, and *Candidatus* Anammoximicrobium affiliated in the phylum Planctomycetes (Oshiki et al., 2016; Zhang and Okabe, 2020). Among these anammox bacteria, *Ca.* Brocadia and *Ca.* Kuenenia show distinguishing r-/K-properties. *Ca.* Brocadia prefers high NH_4^+ and NO_2^- environments due to its low affinities for NH_4^+ and NO_2^- (Zhang et al., 2020). In contrast, *Ca.* Kuenenia has a higher affinity for NH_4^+ and NO_2^-, while the growth rate is lower than that of *Ca.* Brocadia (Zhang et al., 2017) (Table 3). Therefore, *Ca.* Brocadia should presumably be an r-strategist, whereas *Ca.* Kuenenia could be a K-strategist (Puyol et al., 2013; Van Der Star et al., 2008). The simulation of the microbial growth of *Ca.* Brocadia sinica and *Ca.* Kuenenia stuttgartiensis using the Monod equation showed that *Ca.* Brocadia sinica cells more likely overgrow when the NO_2^- concentration is over 80 μM (Oshiki et al., 2016). Several studies have also reported the dominance of *Ca.* Brocadia sinica in bioreactors operated at relatively high NH_4^+ and NO_2^- loading rates (Tsushima et al., 2007; Oshiki et al., 2011; Osaka et al., 2012). Besides, in a partial nitrification, simultaneous anammox and denitrification system, the slight excess substrate at the end of the operational period (6.1 mg NH_4^+-N/L and 8.2 mg NO_2^--N/L), and the insufficient level of substrate during the anoxic phase were also reported to favor the proliferation of *Ca.* Brocadia (Zhang et al., 2019).

Like other r-strategists, *Ca.* Brocadia would be more resisting to adverse conditions. For example, when an anammox reactor was operated under low temperature (room temperature in Shanghai in winter) and was fed with pre-treated real sewage which could be an inhibitor for the deammonification process, *Ca.* Brocadia still could be enriched and was responsible for the anammox process (Liu et al., 2018). On the other hand, due to the high substrate affinity of *Ca.* Kuenenia, when denitrifiers compete with anammox bacteria for NO_2^-, *Ca.* Kuenenia would be favored (Chen et al., 2016).

Apart from *Ca.* Brocadia spp. and *Ca.* Kuenenia spp., other anammox bacteria were less clarified as r-/K-strategies. Liu et al. (2017) reported that the K_S values for NH_4^+ and NO_2^- of *Ca.* Jettenia caeni are comparable to those of *Ca.* Brocadia sinica (Oshiki et al., 2011), but higher than those of *Ca.* Brocadia anammoxidans (Strous et al., 1998) and *Ca.* Brocadia sp. 40 (Lotti et al., 2014). Nevertheless, more detailed information should be provided to further clarify the r-/K-properties of anammox bacteria except *Ca.* Brocadia spp. and *Ca.* Kuenenia spp.

2.3. Anaerobic digestion

Anaerobic digestion is a biotechnology that can convert complex organic pollutants to renewable energy methane (CH_4). During anaerobic digestion, organic pollutants are sequentially degraded by fermenting bacteria and acidogenic bacteria, and finally methanogens utilize acetate or hydrogen and carbon dioxide (CO_2) to produce CH_4. Although the r/K selection theory in anaerobic digestion was less discussed, some microorganisms involved in anaerobic digestion show obvious r-/K-properties (Table 4). For example, the acetoclastic methanogen *Methanosaetaceae* is characterized by a high substrate affinity and a low growth rate (K-strategists). The μ_{max} and K_s of *Methanosaeta* were reported to be 0.001–0.03 1/h and 0.4–1.5 mM (Demirel and Scherer, 2008; Schmidt and Ahring, 1999). In contrast, *Methanosarcinaceae* has a higher growth rate and a low substrate affinity (r-strategists) (Mladenovska and Ahring, 2000). It was reported that the μ_{max} and K_s of *Methanosarcina concilii* were 0.19–0.28 1/h and 5 mM, respectively (Stams et al., 2005). Due to the substrate affinity, *Methanosaetaceae* is often dominant in digesters with a low acetate concentration while *Methanosarcinaceae* is abundant when the acetate concentration is high. For instance, Acharya et al. (2015) reported that the acetate accumulation caused by fast propionate and butyrate degradation could result in the enrichment of r-strategists *Methanosarcinaceae*. In addition to kinetic properties, *Methanosarcinaceae* could also form multicellular aggregates to resist volatile fatty acids (VFAs) inhibition by limiting their concentration inside aggregates with slow diffusion (Acharya et al., 2015).

Hydrogenotrophic methanogens also consist of r-/K-strategists. For example, *Methanobacterium* has a high doubling time and is more adaptable to dynamic environmental conditions, likely to be a r-strategist. This is in line with the fact that *Methanobacterium* was more abundant in sequencing batch reactors (SBRs) under the short solids retention time (SRT) (10 days) condition than *Methanosaeta* (Guo et al., 2022). The high growth rate prevents r-strategists from being washed out when the SRT is short. Similarly, Xing et al. (2021) reported that *Methanobacterium* was enriched with the relative abundance of 74.2% in the SBR at the SRT of 10 days. In contrast, *Methanolinea*, a hydrogenotrophic methanogen with a growth rate of only 0.007 1/h (Imachi et al., 2008), is considered as a K-strategist. Accordingly, *Methanolinea* was found to dominate in the continuous-flow reactor (CFR) with 25 days of SRT but could be hardly detected in the reactor with 10 days of SRT (Guo et al., 2022). In both studies of Guo et al. (2022) and Xing et al. (2021), a distinct result is that r-strategists of methanogens (e.g., *Methanobacterium*) were always dominant under the short SRT conditions, while K-strategists (e.g., *Methanosaeta*) were enriched when SRT was long enough.

Besides methanogens, bacteria involved in anaerobic processes can be characterized as r-/K-strategists as well. *Geobacter*, an electroactive bacterium that can participate in syntrophic methanogenesis via direct interspecies electron transfer, was proposed to be a K-strategist due to its low

Table 3
Summary of the reported dynamic parameters of *Ca.* Brocadia and *Ca.* Kuenenia.

Organisms	Experiment /Reactor	Substrate	K_S (mg N/L)	μ_{max} (day^{-1})	Note	Reference
Ca. Brocadia sinica (90% purity)	MBR-2 L	–	0.48 ± 0.29 (for NO_2^-)	0.17–0.33	pH = 7.0–8.0	Zhang et al., 2017
Ca. Kuenenia stuttgartiensis	MBR-15 L	120 mM ammonium and 120 mM nitrite	0.003–0.04 (for NO_2^-)	0.06–0.084	T = 38 °C; HRT = 2 d; pH = 7.1–7.5; population was shifted from Ca. Brocadia to Ca. Kuenenia; the purity in Ca. Kuenenia increased to 97.6% on day 267	Van Der Star et al., 2008
Ca. Brocadia sinica (Activated sludge)	Batch experiment	90 mg NH_4-N/L and 80 mg NO_2-N/L	0.4 (for NH_4^+) and 1.19 (for NO_2^-)	0.098	T = 25–45 °C; pH = 6.5–8.8	Oshiki et al., 2011
Ca. Brocadia caroliniensis (Flocculent sludge)	Batch experiment	6.5 mM total N concentration	8.96 (for NH_4^+) and 4.83 (for NO_2^-)	0.098	T = 30 ± 0.1 °C; pH = 7.2; Biomass was collected from a granular sludge bed reactor	Puyol et al., 2013
Ca. Brocadia fulgida (Granular sludge)	Batch experiment	6.5 mM total N concentration	7.4 (for NH_4^+) and 5.1 (for NO_2^-)	–	T = 30 ± 0.1 °C; pH = 7.2; Biomass was collected from an MBR	Puyol et al., 2013
Ca. Brocadia sp. 40 (High purity)	MBR-10 L	60–120 mM ammonium and 60–120 mM nitrite	0.038–2.5 (for NO_2^-)	0.21	T = 30 °C; pH = 6.8–7.5; HRT = 1.67 d	Lotti et al., 2014

Table 4
Summary of the reported dynamic parameters of methanogens and bacteria involved in anaerobic processes.

Organisms	Experiment /Reactor	Substrate	K_S (mM)	μ_{max} (day^{-1})	Note	Reference
Methanosarcina mazeii S-6	Upflow anaerobic sludge blanket (UASB)-0.2 L	Acetate	3.6	1.4	HRT = 18 h during the start-up; HRT was decreased stepwise when the effluent concentration of acetate decreased to below 6 mM	Schmidt and Ahring, 1999
Methanosaeta concilii GP-6	UASB-0.2 L	Acetate	1.5	0.77	HRT = 18 h during the start-up; HRT was decreased stepwise when the effluent concentration of acetate decreased to below 3 mM	Schmidt and Ahring, 1999
Methanosarcina spp.	–	2-100 m M Acetate	6.5–24.7	1.06–1.54	T = 50–55 °C; pH = 7.0; Thermophilic strains from full-scale thermophilic biogas plants	Mladenovska and Ahring, 2000
Methanobacterium spp.	–	Formate, H_2/CO_2	–	0.3–3.1	*Methanobacterium bryantii*, *Methanobacterium, formicicum*, and *Methanobacterium ivanovii*	Stams et al., 2005
Geobacter metallireducens	Batch experiment	Benzoate and acetate	–	1.44	T = 30 °C; pH = 6.8; During the retentostat experiment, the growth rate could be as low as 0.48 day^{-1}	Marozava et al., 2014
Geobacter sulfurreducens	Batch experiment	5.5 mM acetate	0.01	0.96–2.16	T = 30 °C; Acetate-limiting conditions with fumarate or Fe (III)-citrate as the electron acceptor	Esteve-Núñez et al., 2005
Clostridium butyricum	Batch experiment	Galactose or glucose	2.5 (for galactose) and 7.3 (for glucose)	–	T = 35 ± 0.1 °C; pH = 6.8–7.0	Park et al., 2018
Lactobacillus casei	Batch experiment	Galactose or glucose	1.4 (for galactose) and 1.9 (for glucose)	–	T = 35 ± 0.1 °C; pH = 6.8–7.0	Park et al., 2018

growth rate (Esteve-Núñez et al., 2005; Guo et al., 2022; Marozava et al., 2014). The effects of operational modes on the selection of r-/K-strategists were investigated and it was found that CFRs could wash out r-strategists but significantly enrich the K-strategist *Geobacter*, with relative abundances ranging from 34.0–72.6% (Guo et al., 2022). This might be because CFRs typically provided continuously low substrate concentrations, benefiting the selection of K-strategists. Besides, the hydrogen-producing bacterium *Clostridium* sp. was suggested to be a r-strategist due to the high growth rate, whereas the lactate-producing bacterium *Sporolactobacillus* sp. was a K-strategist, being energy-efficient even at low substrate concentrations due to its high substrate affinity (Kim et al., 2021). Consequently, the enrichment of r-strategist under high loading rates is beneficial for improving hydrogen productivity. Park et al. (2018) also reported that during biohydrogen production, low concentrations of the initial glucose could provide *Lactobacillus* (K-strategist) a competitive advantage over *Clostridium* (r-strategist), and the different substrate utilization rates as a function of substrate concentration were probably determined by the activities rather than the abundance of the two species.

2.4. Recalcitrant pollutant degradation

The biodegradation of recalcitrant pollutants such as azo dyes, endocrine disrupting compounds and nitroaromatic compounds are challenging not only because many recalcitrant pollutants cannot be efficiently utilized as growth substrates by microorganisms (Nzila, 2013) but also due to the toxicity of recalcitrant pollutants (Martínez et al., 2013). Failure to remove recalcitrant pollutants also negatively affects system performance of waste or wastewater treatment processes. For instance, the release of recalcitrant compounds during thermophilic digestion resulted in a low methane yield (Chen et al., 2018). Therefore, the discovery and identification of microorganisms able to bio-transform recalcitrant pollutants are necessary and urgent.

The r/K selection theory can be applied to distinguish and select species that can degrade recalcitrant pollutants. Generally, due to the low concentration of many recalcitrant pollutants, K-strategists are assumed to play important roles in their biodegradation. For example, Koh et al. (2009) proposed that K-strategist heterotrophs could improve 17α-ethinylestradiol (EE2) biodegradation under low substrate growth conditions. Increasing the sludge age allowed the growth of EE2-degrading organisms, being consistent with the property that K-strategists have a low growth rate (Koh et al., 2009). Later, Ziels et al. (2014) investigated a wide range of initial substrate concentrations and further confirmed that the population selected at a low organic substrate concentration could increase EE2 biodegradation independent of SRT. The highest EE2 biodegradation rate coefficient occurred in the activated sludge configuration with the lowest nitrifying biomass fraction, suggesting that the estrogen was biodegraded by slow-growing heterotrophic bacteria rather than co-metabolized by AOB (Ziels et al., 2014).

The recalcitrant hydrocarbon degradative potentials of the pure bacterial strains *Mycobacterium frederiksbergense* IN53 (K-strategist) and *Acinetobacter* sp. IN47 (r-strategist) were compared (Brzeszcz et al., 2016). Results show that *M. frederiksbergense* IN53 had an advantage over *Acinetobacter* sp. IN47 for degrading hydrocarbon under unfavourable conditions (high hydrocarbon load and soil moisture depletion) (Brzeszcz et al., 2016). Furthermore, in a compost biofilter for toluene degradation, it was found that the *K*-strategists of genera *Pseudonocardia* and *Rhodococcus* dominated, and bacteria of *Rhodococcus* strains could have a better chance of success during bioaugmentation, while the most frequently adopted *Pseudomonas* might be relatively low in number for practical application (Juteau et al., 1999).

The r/K selection theory has been successfully applied to specifically enrich K-strategist for recalcitrant pollutant removal. For example, to enrich a K-strategist *p*-nitrophenol (PNP)-degrading microbial population in an aerobic SBR, Martín-Hernández et al. (2009) adopted a feeding strategy by maintaining long periods under endogenous conditions during the SBR cycle and successfully achieved a high removal percentage of PNP.

On the other hand, some studies also highlighted the importance of coupling r-strategists and K-strategists in recalcitrant pollutant removal. During bioremediation, r-strategists of *P. putida* and *Acinetobacter* could rapidly grow on hydrocarbon contaminants, while K-strategists of *Rhodococcus* and *Mycobacterium* tended to be more successful in nutrient-limited situations (Andreoni and Gianfreda, 2007). Bioaugmentation with both K-strategist, *Mycobacterium*, and r-strategist, *Thauera*, can ensure rapid and long-term bacterial adaptation (Abdelsalam et al., 2020). When composting lignocellulosic waste, it was reported that enzymes secreted by r-strategist microorganisms along with enzymes secreted by K-strategist microorganisms can cooperate to degrade easily accessible fraction and the hard-to-degrade fraction of lignocellulose efficiently (Bohacz, 2018).

3. Key affecting factors

3.1. Food-to-microorganism (F/M) ratio

Since the substrate affinity and the growth rate determine the property of r-/K-strategists, the F/M ratio which describes the relationship between substrate and biomass concentrations is a crucial variable affecting the selection and enrichment of r-/K-strategists (Fig. 2). A high F/M ratio provides unlimited substrates, therefore allowing the growth of r-strategists. In contrast, when the F/M ratio is low, organisms need to compete for limited food, resulting in the dominance of K-strategies.

The F/M ratio can be determined by adjusting substrate or biomass concentrations, and feeding durations, etc. For example, SRT can be used to control the F/M ratio by adjusting the biomass concentration. Theoretically, only organisms that have doubling times shorter than a corresponding SRT will grow fast enough to avoid being washed out. A long SRT can maintain more biomass, resulting in the enrichment of K-strategist with a low F/M ratio, while a short SRT maintains less biomass benefiting r-strategist with a high F/M ratio (Ginige et al., 2007; Yuan et al., 2019). Vuono et al. (2015) observed a shift in community composition for 12- and 3-day SRTs: the composition was altered such that r-strategists were enriched in the system during the 3-day SRT, whereas K-strategists were only present at SRTs longer than 12 days. This shift from K-strategists to r-strategists also corresponded to the loss of ecosystem functions, e.g., nitrification, denitrification, and biological phosphorus removal, for SRTs shorter than 12 days (Vuono et al., 2015).

Shifting the feeding duration is another approach to control the F/M ratio. Applying short feeding durations can promote the substrate uptake at high initial concentrations, and vice versa. This approach was used by Ziels et al. (2014) who fed every 3 to 5 min throughout the 5 h reaction period to ensure a low organic concentration condition, leading to a high-efficient EE2 biodegradation by K-strategists, independent of SRT. Similarly, Guo et al. (2022) reported that the anaerobic CFR with 11 h of the feeding duration enriched K-strategists such as *Geobacter* and *Methanolinea*, while the anaerobic SBR with 10 min of the feeding duration acclimated r-strategists such as *Methanobacterium* and *Mesotoga*.

3.2. Operating mode of reactor

Generally, systems such as membrane bioreactors (MBRs) can maintain constant low substrate concentrations and high biomass concentrations for selecting K-strategists, while plug-flow and SBR systems with a dynamic substrate concentration would select r-strategists (Yu et al., 2011; Rongsayamanontab et al., 2010). In addition, in the MBR system, due to

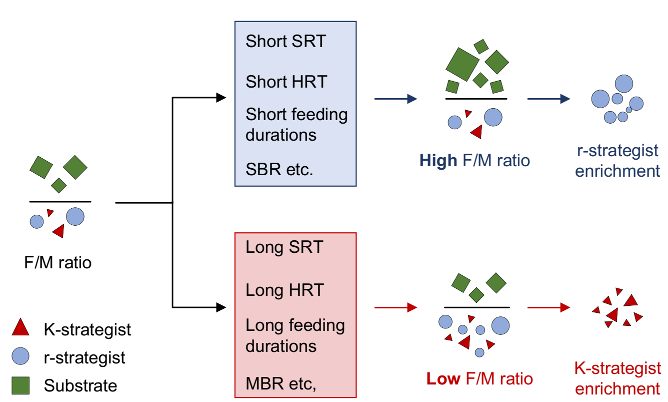

Fig. 2. The strategies to regulate the F/M ratio for enriching r-/K-strategists in wastewater treatment processes.

the high SRT, K-strategists would be easier to be enriched. For instance, in the continuous biofilm airlift reactor with a very low nitrite concentration, the K-strategist *Nitrospira* (59%) dominated rather than the r-strategist *Nitrobacter* (5%); while in the SBR with a relatively high nitrite concentration, *Nitrobacter* (64%) dominated rather than *Nitrospira* (3%) (Kim and Kim, 2006). Terada et al. (2013) also found that halophilic and halotolerant *Nitrosomonas* lineage (*r*-strategist) were more abundant in the SBR (76 ± 4.2%) than in the continuous stirred-tank reactor (CSTR) (38 ± 6.0%), while the CSTR predominantly enriched K-strategist *Nitrosospira* spp. with the relative abundance of 42 ± 1.9% versus 1.4 ± 0.8% in the SBR. Nevertheless, it should be noted that in SBRs, if a long period of low substrate concentrations exist, K-strategists may also be selected, depending on the reaction duration.

On the other hand, even for completely mixed reactors, sometimes system fluctuation may also cause unstable condition in both substrate concentration and the aeration, resulting in the selection of r-strategist (Yu et al., 2011). For example, the transient concentrations of nitrite were 0.05–0.18 NO_2-N/L in the MBR and 0.05–4.4 mg NO_2-N/L in the conventional activated sludge system (Chiellini et al., 2013). Therefore, the MBR system can maintain more stable low nitrite conditions, resulting in the stable enrichment of K-strategists. Furthermore, along with the shift from dynamic conditions to stable conditions, the dominance of r-strategists to K-strategists may also occur (De Roy et al., 2012). It was reported that the uneven dissolved oxygen (DO) distribution in the macro-environment may favor the growth of K-strategist nitrifiers even with a high DO concentration (How et al., 2018).

3.3. Mass transfer

The mass transfer is another factor that may affect the selection of r-/K-strategists. The mass transfer limitation can lead to inhomogeneous substrate concentrations, forming different F/M ratios in the microenvironment. Especially, the thickness of biofilm, granule, and dense flocs plays an important role in controlling the mass transfer and further affecting the detected K_s values within systems, compared with conditions with negligible mass transfer resistances (Blackburne et al., 2007).

Generally, biofilm, granular sludge, and dense flocs can retain slow-growing microorganisms (Regmi et al., 2011). For example, the selection of K-strategist or r-strategist is determined by both concentrations of DO and NO_2^--N and their diffusion limitation within systems (Al-Hazmi et al., 2021). It was reported that within the strictly aerobic reactor, both the heterotrophic competition and dense floc structure reduced oxygen concentrations, therefore favouring the enrichment of the K-strategists *Nitrosospira* and *Nitrospira*, resulting in the lower nitrification rates (Dytczak et al., 2008). In methanogenic systems, *Methanosaeta* species (K-strategists) were commonly found to be the dominant methanogens in many granular systems (Wang et al., 2018; Xu et al., 2018). Integrating suspended flocs and biofilm within one system may allow the co-selection of both r/K strategists (Wu and Yin, 2020). In this integrated system, r-strategists mainly accumulate in suspension or small size flocs while K-strategist is assumed to preferentially dominate in biofilm or granules (Liu et al., 2017).

The layer structure model is often used to describe the interior structure of biofilm or granular systems (Fig. 3). Based on this model, the distribution of r-/K-strategists in biofilm or granules can be further distinguished. Within the biofilm and granules, the high growth rate r-strategist would prefer on the surface while the slow growth K-strategist would grow within the biofilm due to the substrate availability, as dense granules or biofilm possessing steep substrate or oxygen gradients compared to flocs (Liu et al., 2017; Wu and Yin, 2020). Vannecke and Volcke (2015) used this model to explain the spatial distribution of AOB and NOB in nitrifying biofilm reactors: a K-strategist NOB with a high affinity for nitrogen but a low growth rate (K_{NO2} = 0.11 g N/m^3 and μ_{max} = 0.43 1/d) lived beneath another r-strategist NOB with a low affinity for nitrite but a high growth rate (K_{NO2} = 2.93 g N/m^3 and μ_{max} = 1.65 1/d). Schramm et al. (1998) also described a similar spatial distribution for a nitrogen-limited fluidized bed reactor in which a K-strategist related to *Nitrospira moscoviensis*

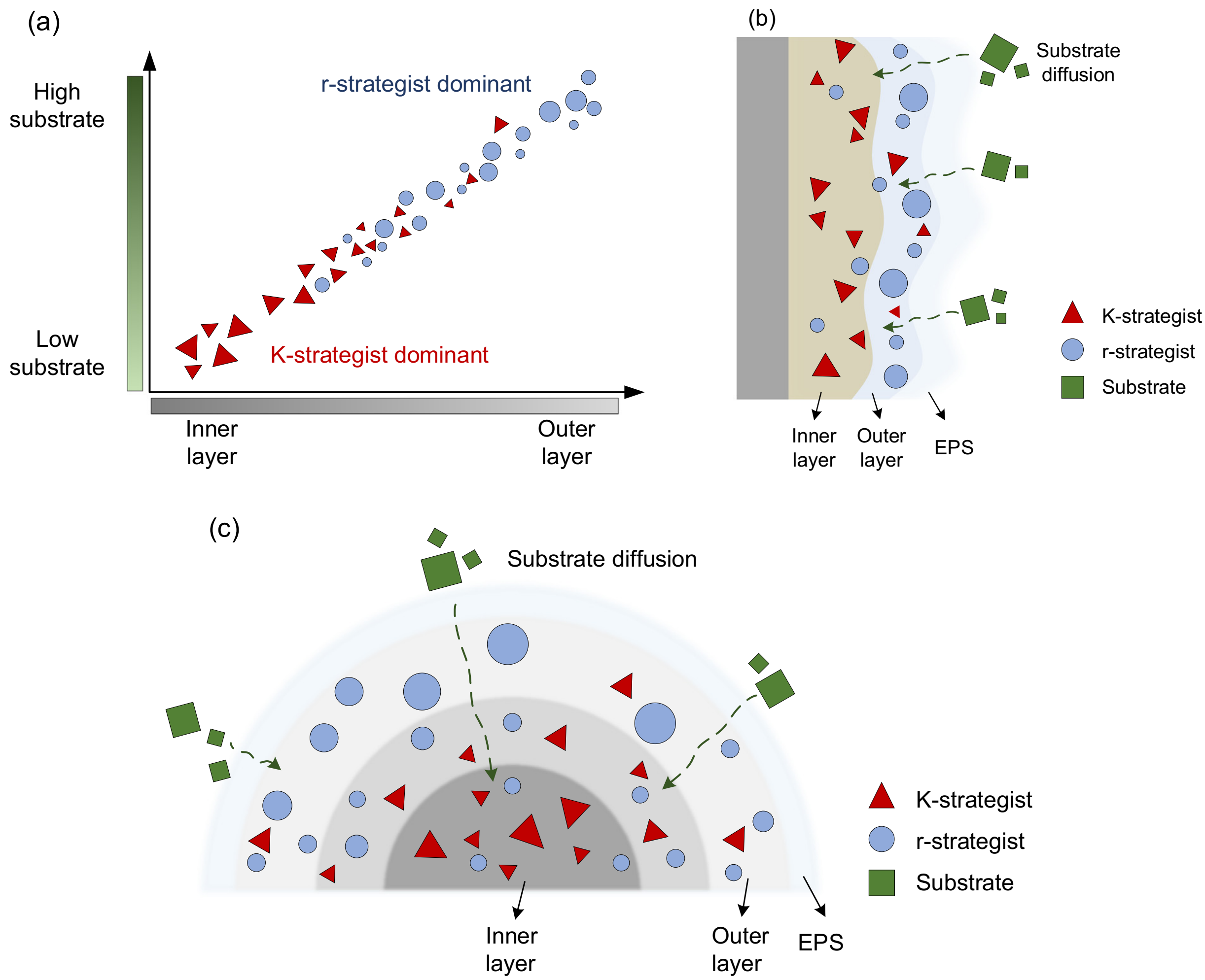

Fig. 3. Distribution of r-/K-strategists under different conditions. (a) The overall distribution tendency of r-/K-strategists; (b) the distribution of r-/K-strategists in the biofilm system, and (c) the granular system.

occurred deep within the biofilm and an r-strategist closely related to *Nitrospira moscoviensis*, survived only at the surface of the biofilm.

The other operational parameters such as extracellular polymeric substances (EPS) and hydraulic retention time (HRT) can also contribute to the selection of specific types of microorganisms within the biofilm or granules (Qian et al., 2021a). Particularly, EPS and some other chemicals may affect the density of the pore size of biofilm or flocs, inducing different diffusion scenarios (Qian et al., 2021a). It is possible that microorganisms growing within biofilm or granules can resist toxic substances or adverse conditions better than those in floc sludge due to the high concentration of EPS and the diffusion limitation (Liu et al., 2021; Yan et al., 2020). This will also favor the growth of some potential K-strategists able to degrade recalcitrant pollutants with low concentrations.

3.4. Cell size, shape, and morphology

The cell size and morphology also impact the r-/K-properties. There are two types of floc forms that exist in activated sludge systems, including dense flocs with floc-forming bacteria dominating and fluffy flocs with filamentous bacteria dominating. Generally, filamentous organisms are acclimated under low substrate concentrations because they have a high cell surface area, which could be classified as the K-strategists. While flocs-forming bacteria have a high growth rate and dominate under high substrate concentrations, which can be classified as the r-strategists. One example is that the filamentous methanogen *Methanosaeta* is a typical K-strategists with the μ_{max} lower than 0.05 1/h (Demirel and Scherer, 2008; Guo et al., 2022; Stams et al., 2005). Similarly, for the commonly applied biofilm systems, it is reasonable to suspect that two types of biofilm morphologies may exist, one is the dense biofilm formed under high substrate concentrations (r-strategist dominant), and the other one is the biofilm formed under low substrate concentrations with distributed channels (like filamentous, K-strategist); and the morphology is also regulated by substrate concentration and diffusion efficiency.

Regarding the cell itself, the cell size can affect the resource utilization efficiency, and further affect their survival strategy (Fig. 4). It was reported that both the metabolic rate and energy consumption for maintenance of organisms increased with increasing cell sizes (Sauterey et al., 2020). Therefore, a trade-off mediated by cell size exists between metabolic and maintenance rates. Organisms with small sizes are better at acquiring energy, but large organisms are more cost-efficient due to lower maintenance requirements. The cell shape also drives spatial patterning. For example, Smith et al. (2017) developed an individual-based model to study the effects of microbial shape in communities and found that round cells preferred the top of the colony, while rod cells dominated the basal surface and edges.

Microbes can additionally adopt an appropriate shape and size to survive, especially under stress conditions such as starvation, oxidative stresses, predation effectors, antimicrobial agents, temperature stresses, osmotic shock, and mechanical constraints (Chien et al., 2012; Gallet et al., 2017; Shen and Chou, 2016). For example, a rod-shaped cell without prosthecae can become small and coccoid, thus saving energy for survival during nutritional scarcity (Young, 2007). Therefore, when characterising

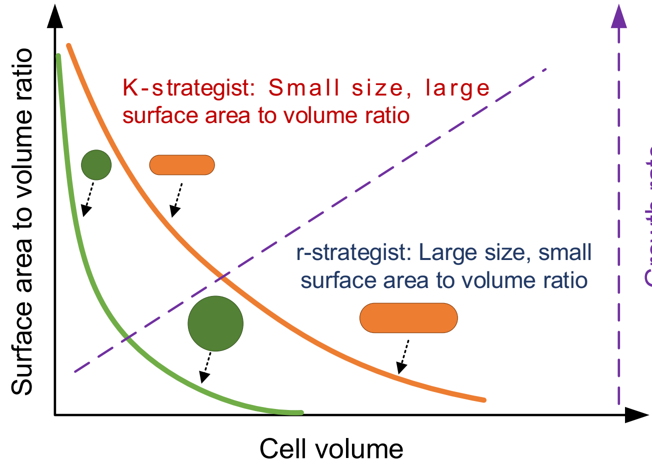

Fig. 4. The proposed effect of cell size on the r-/K-properties and growth rate (adapted from Harris and Theriot, 2018).

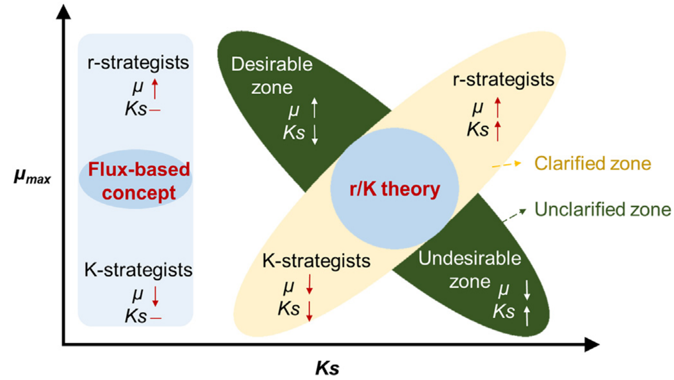

Fig. 5. The proposed types of r/K strategists based on the reaction rate and the substrate concentration.

the r-/K-properties of organisms, the effects of environmental variables on the cell size and biomass morphology should be taken into consideration.

3.5. Other environmental factors

Other parameters also affect the activities of r-/K-strategists. For example, in nitrifying systems, the inorganic carbon limitation might favor *Nitrobacter* over *Nitrospira* even at a low nitrite concentration (Fukushima et al., 2013). Besides, the alternative anoxic/aerobic conditions may favor the r-strategists *Nitrosomonas* and *Nitrobacter* (Yu et al., 2018). Furthermore, predation had an evident influence on the microbial community of nitrifiers, especially the K-strategist, which was more vulnerable to predation than r-strategist during bioaugmentation due to its low growth rate (Yu et al., 2011). From the aspect of inoculum, Terada et al. (2010) found that AOB and NOB in the inoculum might affect the dominance of AOB in a membrane-aerated biofilm reactor, resulting in the attainable degree of nitritation.

Biological processes for wastewater treatment contain mixed organisms and therefore microbial interaction may affect the existence and properties of r-/K-strategists. For example, the cooperation between AOB and anammox may inhibit NOB. In some cases, AOB may produce nitrite for NOB, thus the r-/K-properties of NOB may be related with the existence of AOB. Recently, a mathematical model was applied to describe the competition between r-/K-AOB and NOB (Yu et al., 2020). The result of this model showed that r-AOB and r-NOB were dominant in wide ranges of SRT and DO concentrations in the SBR reactor. In contrast, K-AOB and K-NOB could only be found in very narrow ranges of SRT and DO concentrations due to the competition of r-strategists (Yu et al., 2020). Nevertheless, so far, none of the other studies have investigated the effects of microbial interaction on the selection of r-/K-strategists in wastewater treatment systems.

4. Implications

4.1. Application in the engineered pollution control processes

To comprehensively apply the r/K selection theory in engineered bioprocesses, it is necessary to integrate the above affecting factors. In the study of Wu and Yin (2020), a concept regarding the application of the r/K selection theory was proposed from both the meso-scale and the micro-scale. At the meso-scale, the operational mode should be first taken into consideration. The plug-flow reactor or SBR can be adopted to acclimate r-strategists, while the completely mixed reactor may be adopted for acclimating K-strategists (Guo et al., 2022). At the micro-scale, it is crucial to establish a suitable niche for the spatial distribution of different types of organisms (Wu and Yin, 2020). When considering the layer structure, the biofilm and granular systems can be utilized to acclimate both r-strategists and K-strategists within one system.

From the design viewpoint, a suitable substrate loading rate may be tailored to the practical application. Adopting r-strategists is assumed to benefit the treatment of high-strength wastewater, while acclimating K-strategists with a low loading rate allows the efficient treatment of oligotrophic wastewater. This strategy can be linked with the whole water cycle, i.e., the selective enrichment of r-/K-strategists should match the functional positioning of a specific region or watershed. For example, when a side-stream system is used to treat high-strength wastewater, effluent quality may not be the focus, and in this case, r-strategists should be preferentially acclimated. In contrast, for river and lake systems, enriching K-strategists should be the focus. To simultaneously ensure both high reaction rate and treated water quality (e.g., municipal WWTP), a two-stage process with r-strategists acclimated in the initial stage (r-strategist zone in Fig. 5) followed by a subsequent K-strategist-enriching stage (K-strategist zone in Fig. 5) could be designed. Targeting r-/K-strategists in each stage may balance the loading rate or reaction rate and effluent quality, thereby enhancing the system resilience and redundancy.

For practical engineered systems, the acclimation of r/K-strategists are mainly determined by HRT and SRT, where HRT affects the duration for the utilization of resource while SRT mainly controls biomass concentration and affects the F/M ratio. For example, Qian et al. (2021b) showed that the strong hydraulic selection pressure promoted the niche segregation of AOB and anammox bacteria and the washout of NOB in the granules. The effective retention of the K-strategist Ca. Kuenenia occupying the interior layer of the granules exhibited an SRT 10-fold longer than that of AOB enriched in the aerobic zone (Qian et al., 2021b).

4.2. The relativity of r-/K-strategists

Due to the dynamic and diversity of organisms, r-/K-properties are relative rather than absolute, which may vary under different conditions. For example, compared with *Methanosaeta*, *Methanosarcina* exhibits a high growth rate and a low substrate affinity, tending to be a r-strategist. However, when compared with some hydrogenotrophic methanogens (e.g., *Methanolinea* and *Methanobacterium*) which have high maximum growth rates (Guo et al., 2022), both *Methanosaeta* and *Methanosarcina* belong to K-strategists. Similarly, when comparing denitrifiers with dissimilatory nitrate reduction to ammonium (DNRA) bacteria, denitrifiers should be considered as r-strategists because DNRA bacteria have higher substrate affinity and lower μ_{max} (Jia et al., 2020). As K-strategists, DNRA bacteria can win the competition against denitrifiers when both organisms are subjected to low-nitrate concentrations (i.e., high chemical oxygen demand/N) (Jia et al., 2020).

The relativity of r-/K-strategists may be judged from the microbial interaction and environmental conditions. The detected K_s and μ_{max} values may be inconsistent when using pure and mixed cultures because microbial interactions affect metabolic functions as aforementioned. From the aspect of environmental conditions, for example, it was reported that at a low pH range, polyphosphate accumulating organisms (PAOs) exhibited the K-strategist property, while at a high pH range, PAOs exhibited the r-strategist property (Tu and Chuler, 2013). The substrate concentration and environmental variables are also relative and dynamic. For instance, compared with untreated wastewater, the effluent from WWTP is oligotrophic, while it may still be eutrophic for the receiving rivers or lakes. This can also affect the classification of the enriched r/K strategists.

4.3. The concept of substrate flux

The substrate flux (supply and utilization rates) rather than only concentration may be an important aspect for enriching the r-/K-strategists, especially under anaerobic or anoxic conditions. Kreft et al. (2020) proposed that anaerobic or anoxic organic matter decomposition is carried out through the cooperation among different types of microbes, which could be due to the high flux environments. Winkler et al. (2017) found that the selection of r-/K-NOB not only depended on the substrate concentration, but also the dilution rate, where a low nitrite concentration selected *Nitrobacter* (r-strategist) when the dilution rate was lower than the μ_{max} of *Nitrospira*.

Oxygen as a high redox acceptor, can be supplied with a constant flux, while the measured DO concentration can be maintained at several mg/L for aerobic processes and close to zero mg/L for anaerobic biotechnologies. The achievement of partial nitrification/denitrification is one good example, where the oxygen supply can be controlled based on its stoichiometric relationship with the oxidation of ammonia (Li et al., 2011). In an intermittent oxidation-reduction potential-controlled micro-aeration system for high solids anaerobic digestion, a rapid VFA conversion and CH_4 production were achieved through facultative anaerobes and hydrogenotrophic methanogens under micro-aerobic conditions (Nguyen et al., 2019).

Therefore, here we propose the extended r/K selection strategies based on both substrate flux and concentrations (Fig. 5). If the substrate concentration variation is high, it is the typical r/K selection theory, while if the substrate concentration is quite low with a high supply and utilization rates, still r-strategists may be enriched. Under this condition, we can control the substrate concentration close to zero, which is balanced between the substrate utilization rate and the supply rate. Therefore, when the substrate affinity can be substituted by the substrate flux rate, the r/K selection theory would still work. r-strategists would have a high substrate utilization rate, while K-strategists have a low substrate utilization rate. All these can be classified as the “threshold technology”, where the substrate flux rather than the concentration would be the primary controlling factor.

4.4. Future perspectives

Despite the aforementioned-findings, knowledge gaps still exist in the application of the r/K selection theory in biological processes for wastewater treatment. Although some functional organisms can be classified as r-/K-strategists, there are other (functional) organisms that cannot be simply clustered into these two groups. Therefore, the development of the r/K selection theory is necessary to further extend its applicability.

The establishment of detecting methods of r-/K-strategists is another challenge for the application of the r/K selection theory in engineered bioprocesses. Recently, the ribosomal RNA operon (rrn) copy number has been reported to be a key trait for predicting life strategy under different resource availabilities (Klappenbach et al., 2000; Roller et al., 2016). High maximal growth rates are positively correlated with the number of rrn copies, i.e., r-strategists are supposed to contain more rrn copies than K-strategists (Roller et al., 2016). For instance, the dynamics of average rrn copy number and its correlations with ammonium and phosphate concentrations were shown to reflect the r/K-strategists succession and sensitivity to nutrient disturbances (Dai et al., 2020). Therefore, the detection of the rrn copy number may be a potential way to evaluate the abundance of r-/K-strategists in bioprocesses, which needs further investigation. Nevertheless, more efforts should be made to establish effective methods for the identification and investigation of r-/K-strategists. Specifically, more methods related to bioinformatics, molecular biology, and mathematical modeling are needed. Finally, as microorganisms are mixed flora in wastewater biological treatment systems, whether and how the interaction among microbial populations affects r-/k-properties needs to be unravelled in future studies.

5. Conclusions

Organisms involved in typical environmental bioprocesses for wastewater treatment such as nitrification, anammox, and methanogenesis present distinct r-/K-properties. The F/M ratio, mass transfer, and cell size/morphology are crucial factors determining the enrichment and characteristics of r-/K-strategists. In practical application, integrating both the meso-scale (e.g., operational mode, HRT, and SRT) and micro-scale (e.g., biofilm and granule) aspects can selectively enrich targeted r-/K-strategists in bioprocesses. Overall, although the application of the r/K selection theory in bioprocesses is still in a nascent stage, it can be anticipated that r/K selection-based concepts and technologies will be continuously refined to fully exploit the capabilities of r-/K-strategists.

CRediT authorship contribution statement

Qidong Yin: Investigation, Visualization, Writing – original draft. **Yuepeng Sun:** Writing – review & editing. **Bo Li:** Writing – review & editing. **Zhaolu Feng:** Writing – review & editing. **Guangxue Wu:** Conceptualization, Writing – review & editing, Funding acquisition, Supervision.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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