Intensifying existing urban wastewater Aerobic granular sludge offers improvements to treatment processes

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Hibernating mammals change between active and inactive phases, which is accompanied by functional alterations in their microbiota that support prehibernation fattening or nitrogen recycling. This discovery may lead to potential targets for microbiota-directed therapies for human muscle-wasting conditions.

frogs, gut microbes encode an increased potential for nitrogen salvage (*6*). Using metagenomic sequencing, Regan *et al.* found that the gut lumen of squirrels contains microorganisms with urease genes, which enable the microorganisms to produce enzymes (i.e., ureases) that metabolize urea into carbon dioxide and ammonium. The ammonium is then used by the same microbiota as a source of nitrogen to produce amino acids, some of which are then absorbed by the host. As a result of this process, nitrogen loss during protein catabolism and urea formation is compensated, which counteracts muscle wasting. Although the process of urea nitrogen salvaging has been known in ruminants such as cattle, goats, and sheep (*7*), the identification and molecular delineation of urea nitrogen salvaging in hibernating mammals add another central role for intestinal microorganisms within the coordination of host physiological adaptations.

Muscle wasting is prevalent in humans, for example, during protein malnutrition, which affects millions of people worldwide, especially children in developing countries. The low intake of protein is known to trigger not only muscle wasting but also other health problems, such as various neurological and growth defects, inflammatory episodes, and increased susceptibility for pathogenic infections. Muscle wasting is also prevalent in the elderly owing to age-related muscle loss, which greatly affects quality of life. The exploitation of microbial processes for producing essential amino acids could potentially benefit those whose diets are deficient in protein or those who display impaired food intake or digestion, for example, members of the elderly with sarcopenia (*8*).

The identification of a microbial contribution to urea nitrogen salvaging in hibernating mammals provides potential targets for developing new treatments for muscle wasting and related conditions. The gut microbiota is plastic in its composition and function and can be shaped by external factors such as diet (*9*). Microbiota-directed therapies have already been used successfully for malnutrition (*10*, *11*). These approaches seem promising, especially for treating or preventing muscle wasting, because physiological responses to low-protein conditions similar to those observed in hibernating mammals have been observed in humans, that is, an increase in nitrogen recycling under low-protein conditions (*12*, *13*). The addition of bacteria that produce ureases, for example, from the genus *Alistipes*, which increased in abundance during hibernation in squirrels, could represent potential next-generation probiotics. Successful probiotic supplementation was recently shown when *Lactobacillus plantarum* was administered to malnourished mice, which prevented body weight loss and fostered normal bone development (*14*). However, it is unclear whether *L. plantarum* increased nitrogen recycling. Another approach would be to develop genetically manipulated bacteria, which colonize the human gut, to express urease, thus providing an alternative avenue to increase nitrogen recycling. The feasibility of this approach was recently demonstrated using engineered *Escherichia coli* Nissle to produce enzymes that enabled phenylalanine degradation in patients with phenylketonuria (*15*).

The findings of Regan *et al.* add to previous research demonstrating the microbial function in energy harvest of hibernating mammals. Collectively, these studies highlight the importance of the microbiota for nutritional and metabolic adaptions in mammalian hosts. Muscle wasting is prevalent in those suffering from age-related sarcopenia, protein malnutrition, or prolonged inactivity such as during space travel or hospitalizations related to severe diseases. Because mechanisms for nitrogen salvaging from urea seem to be functional in humans, microbiota-directed interventions for urea recycling could be a potential therapy for treating such conditions. ■

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REFERENCES AND NOTES

1. M. D. Regan *et al.*, *Science* **375**, 460 (2022).
2. A. G. Hindle *et al.*, *J. Exp. Biol.* **218**, 276 (2015).
3. F. Sommer, J. M. Anderson, R. Bharti, J. Raes, P. Rosenstiel, *Nat. Rev. Microbiol.* **15**, 630 (2017).
4. F. Sommer *et al.*, *Cell Rep.* **14**, 1655 (2016).
5. K. Grond, C. C. Kurtz, J. Hatton, M. M. Sonsalla, K. N. Duddleston, *Anim. Microbiome* **3**, 56 (2021).
6. J. M. Wiebler *et al.*, *Proc. R. Soc. London Ser. B* **285**, 20180241 (2018).
7. H. V. Carey, F. M. Assadi-Porter, *Annu. Rev. Nutr.* **37**, 477 (2017).
8. S. Subramanian *et al.*, *Cell* **161**, 36 (2015).
9. L. Schröder *et al.*, *Nutrients* **12**, 3032 (2020).
10. R. Y. Chen *et al.*, *N. Engl. J. Med.* **384**, 1517 (2021).
11. J. L. Gehrig *et al.*, *Science* **365**, eaau4732 (2019).
12. M. Langran, B. J. Moran, J. L. Murphy, A. A. Jackson, *Clin. Sci. (Lond.)* **82**, 191 (1992).
13. M. I. Smith *et al.*, *Science* **339**, 548 (2013).
14. M. Schwarzer *et al.*, *Science* **351**, 854 (2016).
15. M. K. Puurunen *et al.*, *Nat. Metab.* **3**, 1125 (2021).

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WATER TREATMENT

Intensifying existing urban wastewater

Aerobic granular sludge offers improvements to treatment processes

By **M.-K. H. Winkler**[1] *and* **M. C. M. van Loosdrecht**[2]

As the population continues to grow, increasing engineering challenges are associated with the sustainable life cycle of consumption and production of safe, reusable water. The water industry has identified wastewater as a viable and sustainable source for not only quality water, but also for recovering resources while minimizing footprint and energy demand. A recent innovation that targets all of these key issues is the aerobic granular sludge (AGS) technology, which allows for the simultaneous removal (or recovery) of nitrogen, carbon, and phosphate while reducing the footprint by up to 75%.

Today, 55% of the world's population lives in urban areas, and that number is expected to increase to 68% by 2050, making cosmopolitan square footage more arable. As a result, metropolitan wastewater treatment plants (WWTPs) are constrained in space while having to treat higher flow rates (*1*). Additionally, recent regulations demand nutrient removal (e.g., in the US) and recovery (e.g., in Europe), but WWTPs were not designed to easily add on functionalities to accommodate treating increased flows with more-stringent discharge limits. This problem is exacerbated by an aging infrastructure. Amid an economic crisis fueled by the coronavirus pandemic, investment in aging water infrastructure has to remain of prime importance for growth (*2*). The US, for example, was underinvesting in its wastewater systems but has recently passed a massive bill to renew infrastructure (*3*). This pushes for innovative technologies to offer cost- and space-effective treatment solutions.

Granules are a specific form of biofilm structure (see the figure) because they do not grow on a carrier but are self-aggregating,

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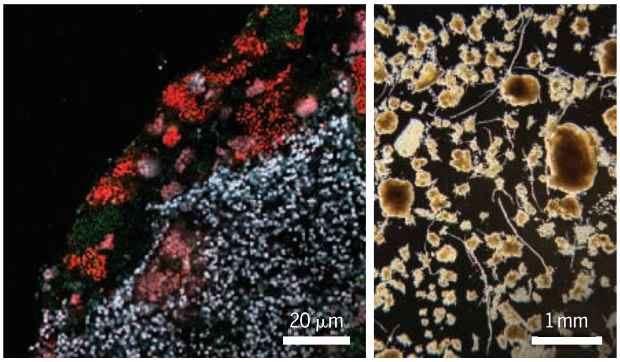
spherical particles with diameters of 0.5 to 2 mm that settle 10 to 15 times as fast as the conventional floc. Flocs can be selectively wasted from the system, enriching for granules and accelerating solid separation substantially. The fast-settling and high-thickening properties of AGS allow integration of the settling process inside only one treatment unit operated at increased solids inventory, thereby bypassing the space-consuming secondary clarifiers and greatly reducing footprint and intensifying reactor operation without energy-intensive recycle flows and mixers (*4*).



A sliced granule of an aerobic granular sludge system (left) includes nitrifiers (red) and denitrifying polyphosphate-accumulating organisms (PAOs) (blue). Granules are shown from a continuous-flow system (right).

In conventional systems, sludge flocs must be pumped across multiple tanks to allow N, C, and P removal, and they are separated from the water in extremely space-demanding clarifiers. Granules consist of a spatially ordered consortium of nitrifiers, phosphate-accumulating organisms (PAOs), glycogen-accumulating organisms (GAOs), and associated microorganisms (*5*). The nitrifiers aerobically oxidize ammonium to nitrate and are confined to grow on the oxygenated granule periphery. The PAOs may grow aerobically but preferentially localize to the granule interior, where they reduce nitrate to nitrogen gas and store phosphate. Together, both functional groups (nitrifiers, PAOs and GAOs) are coupling transformations of C, N, and P to allow water purification. Enhanced biological phosphate removal (EBPR) systems form denser and stronger granules than systems based on ordinary heterotrophs (*6*).

To out-select the fast-growing heterotrophs and promote the growth of slow-growing PAOs and GAOs (that form smoother biofilms), the reactor must be operated with alternating anaerobic and aerobic reactor conditions with influent feeding during the anaerobic period. This task can be accomplished by anaerobically feeding the wastewater directly through the settled granular sludge bed. The concurrent nutrient removal and easy separation of granules allows one to operate the whole process in a single sequencing batch reactor (SBR) at reduced footprint. When three parallel reactors are present, the pre- and posttreatment processes can be operated at continuous flow (CF), as in conventional floc-based processes (*4*).

Nutrient recovery has similar potential for flocs and granules, but granular sludge is special in that it enables 5 to 10 kg of glycoprotein recovery per person per year, which has good economic value in the chemical and agricultural industry (*7*). The high-thickening characteristics of AGS may also be used to produce a phosphorus-rich stream by simple anaerobic holding of the waste granular sludge, offering the promise of P recovery without an anaerobic digester. AGS technology is therefore an attractive alternative for future expansions at many wastewater facilities serving an increasing population with space constraints.

Despite the success story of AGS, which has already reached 70 plants worldwide within the first few years of market introduction, the SBR-based technology does not easily integrate in existing, mostly shallow CF infrastructure that relies on solid separation in a clarifier. This makes retrofitting an existing plant cumbersome without decommissioning. Recently, a high abundance of PAO-based granules was unexpectedly observed at existing, full-scale CF-EBPR plants, suggesting that widespread adaptation of AGS in existing wastewater facilities might be easier than expected (*8*). Therefore, the implementation of a granule-floc separator in CF-EBPR systems to enhance granulation by leveraging an anaerobic phase to select for slow-growing bacteria capable of internally storing carbon (such as PAOs and GAOs) may be a way to integrate AGS technology into CF, which is currently considered the crowning glory for sustainable wastewater treatment (*9*).

A fully aerobic feast-famine regime is sometimes easier to implement in existing infrastructure (*10*, *11*). This strategy can also result in granular sludge, but it needs a stronger selection force for granules than for anaerobic feeding. The main selection pressure used thus far for cultivating granules in CF has been settling velocity based, where fast-settling particles are continuously separated from the slow-settling particles and retained in the system using equipment such as a solids-liquid separator, an external settler, or other designs of similar concept (*12*). These CF configurations can have varying floc-granule separation methods, but many share the common strategy of favoring the growth of GAOs and PAOs in the granules by recycling larger particles to the anaerobic zone from the separator.

Challenges remain in uncoupling floc and granule retention time as an essential requirement to establish granules in CF. Additional challenges include the generation of enough biologically available carbon (through hydrolysis and fermentation) and directing it to select efficiently for storage (GAOs and PAOs) (*13*); sludge aeration and mixing strategies for floc–granular biomass systems; the impact of floc and granule size fractions on diffusive transport (*14*) and microbial competition to manage nitrification and denitrification (*15*); and the integration of resource recovery, especially phosphate and biopolymers, in the water treatment process.

Overall, the paradigm of wastewater treatment to protect public health and improve environmental quality has begun to shift—after a century of municipal wastewater treatment—from flocculent to granular sludge, which offers an exciting opportunity for upgrading existing infrastructure while making wastewater treatment more sustainable. SBR-AGS is the best option for plants that require a major infrastructure upgrade, whereas CF-AGS is best suited for plants that are still in good enough shape to allow a retrofit for intensifying existing infrastructure. AGS technology will be of great importance to the water profession to provide high-quality water in constantly growing urban areas combined with efficient resource recovery. ■

REFERENCES AND NOTES

1. United Nations Department of Economic and Social Affairs, "68% of the world population projected to live in urban areas by 2050, says UN" (2018); www.un.org/development/desa/en/news/population/2018-revision-of-world-urbanization-prospects.html.
2. The White House, "Fact Sheet: The Bipartisan Infrastructure Deal" (2021); www.whitehouse.gov/briefing-room/statements-releases/2021/11/06/fact-sheet-the-bipartisan-infrastructure-deal/.
3. The White House, "Fact Sheet: The American Jobs Plan" (2021); www.whitehouse.gov/briefing-room/statements-releases/2021/03/31/fact-sheet-the-american-jobs-plan/.
4. M. Pronk *et al*., *Water Res*. **84**, 207 (2015).
5. M. K. H. Winkler *et al*., *Appl. Microbiol. Biotechnol*. **97**, 7447 (2013).
6. M. K. de Kreuk, M. C. M. van Loosdrecht, *Water Sci. Technol*. **49**, 9 (2004).
7. S. Felz *et al.*, *Water Res*. **169**, 115291 (2020).
8. S. P. Wei *et al*., *Water Res*. **179**, 115865 (2020).
9. C. Roche *et al*., *Water Environ. Res*. **94**, e1664 (2022).
10. J. J. Beun *et al*., *Water Res*. **33**, 2283 (1999).
11. Z. An *et al.*, *Environ. Sci. Water Res. Technol*. **7**, 1622 (2021).
12. I. Avila *et al.*, *Water Sci. Technol.* **84**, 302 (2021).
13. M. Layer *et al*., *Water Res. X* **9**, 100075 (2020).
14. S. P. Wei *et al*., *Water Res*. **203**, 117514 (2021).
15. B. Nguyen Quoc *et al*., *Water Res*. **198**, 117119 (2021).

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