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Strength characterization of full-scale aerobic granular sludge

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ABSTRACT
For a stable operation, the aerobic granular sludge process requires mechanically strong granules in balance with the shear forces in the reactor. Despite a wide general interest in granular stability, the mechanical strength of both anaerobic and aerobic granular sludge received very little attention. In this study, a high-shear method for strength characterization has been evaluated for full-scale aerobic granular sludge (AGS). Abrasion times up to 90 min showed a stable abrasion rate coefficient (K), while prolonged periods of abrasion up to 24 h resulted in a decrease in abrasion rate. Larger granules have higher abrasion rate than smaller granules. No abrasion was observed at low shear rates, indicating a threshold shear rate for abrasion. Lab-scale AGS showed a lower abrasion rate than full-scale AGS. Incubation of full-scale granules in NaCl led to a decrease in abrasion rate at 25 g L\(^{-1}\) NaCl, but incubation in 50 g L\(^{-1}\) NaCl led to a further decrease for only half of the tested granular sludge samples.

INTRODUCTION
Aerobic granular sludge is a new technology for wastewater treatment with reduced footprint and energy requirements and a good removal rate in comparison to conventional activated sludge processes [1–5]. Granular sludge consists of compact microbial aggregates with excellent settleability in comparison to conventional activated sludge systems [6–8]. For long-term stable operation and sludge characterization, the physical strength of the sludge granules is an important factor.

The strength of granular sludge is a result of both the physical and chemical conditions in the treatment reactor. Detachment forces are key factors for the smoothness, density and porosity of biofilm. Higher shear stress leads to the formation of a denser biofilm [9–12]. Shear forces control the outgrowth of the granules and are increasingly important with increasing growth rate of the microbial population [13,14]. A proper balance between shear and growth is therefore important for promoting a dense and stable granule formation [9,15,16]. External shear forces that are imposed on the granule depend on the type of reactor and their process conditions. The combination of specific forces in the reactor, the wastewater composition, and the resulting composition of extracellular polymeric substances (EPS) add up to the strength of the granules [11,17].

Several methods have been proposed for quantifying and comparing the physical strength of granular sludge, such as measurement in a stirred flask [18] or in a bubble column [19]. However, these methods only reach low average shear rates. These low numbers would result in either low abrasion rate of granules, or a duration of experiments in the order of days. The method that was used in the current study is based on agitation in a stirred tank reactor with standard reactor geometry [20]. The rate of abrasion of granules gives a measure for the strength of the granular sludge, at shear rates in the order of 700–2000 s\(^{-1}\) (at 800–1600 rpm). A standardized protocol for this short-term high-shear method was not yet available. Moreover, the exact mechanism of the breaking-up or abrasion of granules during the strength test is of great importance for the proper interpretation.
of data, and has not been reported in previous studies. Studies on the strength of aerobic granular sludge are very limited and have been performed with either lab-scale or pilot-scale granules [21]. Granules from these smaller scale reactor systems generally receive a more stable and well-defined influent, but these optimal situations and resulting stability are not representative for a granular sludge from a full-scale installation.

For this study, a method was evaluated to quantify the influence of variations in bulk liquid composition, more specific salinity, on the physical strength of granular sludge. Previous studies have shown a negative effect of NaCl on the strength of anaerobic granular sludge [22]. Displacement of Ca²⁺ ions by Na⁺ was suggested to be the major cause for this observation [23]. Also in activated sludge, high sodium concentrations can lead to a deterioration in settling properties [24]. Addition of divalent cations such as calcium and magnesium, in turn, was found to yield higher floc strength and better settleability [25]. For aerobic granular sludge, it was found that the start-up of a reactor with high salinity wastewater can cause problems, although long-term adaptation can lead to stable granules [26,27]. The effect of changing NaCl concentrations on the strength of full-scale aerobic granular sludge has not yet been described in the literature.

The aim of this research was to quantify the strength of aerobic granular sludge from four full-scale aerobic granular sludge (Nereda®) plants. In order to reach this goal, an improved strength characterization method has been evaluated, and the abrasion mechanism of the aerobic granular sludge during this test has been visualized. A detailed protocol is added to allow for future comparative studies.

Materials and methods
Granule collection
Aerobic granular sludge was collected from full-scale Nereda® plants in Utrecht (prototype), Garmerwolde, Vroomshoop and Dinxperlo, all located in the Netherlands. An overview of their influent concentrations is given in Table 1. The average solids retention time (SRT) in the system was 20–50 days. The aeration tanks are aerated with a gas flowrate of 1–2 m³ m⁻³ h⁻¹, resulting in a dissolved oxygen concentration of 1–2 mg L⁻¹.

Lab-scale aerobic granular sludge was taken from a 2.7 L bubble column (5.6 cm diameter), operated as a sequencing batch reactor (SBR), inoculated with Nereda® sludge from Utrecht, Netherlands. The temperature was set at 20°C. The pH was controlled at 7.0 ± 0.1 by dosing either 1 M NaOH or 1 M HCl. The dissolved oxygen (DO) concentration was controlled at 50% saturation. The average sludge retention time (SRT) was 20 days. Reactor cycles consisted of 60 min of anaerobic feeding, 110 min aeration, 5 min settling and 5 min effluent withdrawal. The feed of 1.5 L consisted of 1200 mL demineralized water, 150 mL of medium A, and 150 mL of medium B. Medium A contained 7.785 g L⁻¹ sodium acetate trihydrate (3.66 g L⁻¹ COD), 0.88 g L⁻¹ MgSO₄·7H₂O, and 0.35 g L⁻¹ KCl. Medium B contained 2.289 g L⁻¹ NH₄Cl (600 mg L⁻¹ NH₄⁺ – N), 349 mg L⁻¹ K₂HPO₄, and 136 mg L⁻¹ KH₂PO₄. The combination of these feed streams led to influent concentrations of 366 mg L⁻¹ COD, 60 mg L⁻¹ NH₄⁺ – N and 9.3 mg L⁻¹ PO₄³⁻ – P.

Lab-scale seawater-adapted aerobic granular sludge was taken from a reactor with similar conditions as the above-described reactor. The only difference was a complete replacement of demineralized water in the influent with artificial seawater (35 g L⁻¹ Instant Ocean® sea salts).

Strength characterization
Granules were sieved and washed on a 1.6 mm sieve. The granules were dried (wet weight, WW) by soaking up water from the bottom of the sieve with a tissue. These granules were used for (1) determining the total suspended solids per wet weight (TSS/WW), and (2) the strength characterization test.

(1) A known mass of WW granules was transferred to pre-dried 50 mL Falcon tubes. These were either lyophilized, or dried at 60°C for a minimum of 24 h until completely dry. The mass was measured after drying (TSS), taking into account weight loss of the tubes.

<table>
<thead>
<tr>
<th>Location</th>
<th>COD mg L⁻¹</th>
<th>N-tot mg L⁻¹</th>
<th>NH₄⁺ – N mg L⁻¹</th>
<th>N-Kj mg L⁻¹</th>
<th>P-tot mg L⁻¹</th>
<th>PO₄³⁻ – P mg L⁻¹</th>
<th>Suspended solids mg L⁻¹</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utrecht</td>
<td>707</td>
<td>46.1</td>
<td>64</td>
<td>8.9</td>
<td>5.6</td>
<td>230</td>
<td></td>
</tr>
<tr>
<td>Garmerwolde</td>
<td>506</td>
<td>49.4</td>
<td>39</td>
<td>6.7</td>
<td>4.4</td>
<td>236</td>
<td></td>
</tr>
<tr>
<td>Vroomshoop</td>
<td>797</td>
<td>55.5</td>
<td>55.5</td>
<td>7.92</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dinxperlo</td>
<td>635</td>
<td>59</td>
<td>59</td>
<td>8.06</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
itself without granules. Division of the TSS by WW gives the amount of TSS/WW.

(2) 10 g of WW granules was transferred to a measuring cylinder, and filled up to 500 mL with tap water. These contents were transferred to a stirred tank reactor with standard geometry (500 mL volume: 840 mm diameter, four baffles of 8.4 mm, and a Rushton impeller with 28 mm diameter), and stirred at 800 rpm for 60 min. Afterwards, the reactor contents were sieved over a 200 µm sieve. This filtrate was weighed and divided over 4.50 mL Falcon tubes. These tubes were centrifuged for 10 min at 4200 rpm at 4°C, after which the supernatant was discarded. In case of saline liquid, the pellet was washed and centrifuged several times with demineralized water to prevent precipitation during drying. The centrifuged pellet was either lyophilized, or dried at 60°C for a minimum of 24 h until completely dry.

Quantiﬁcation of strength is derived from the initial amount of TSS and the amount of ﬁne particles (<200 µm) after agitation. Decay of granules into ﬁne particles ($X_f$) is a function of the remaining amount of granules ($X_{nf}$). This gives a ﬁrst-order correlation, which introduces an abrasion rate coeﬃcient ($K$) in Equation (1):

$$\frac{dX_f}{dt} = K \cdot X_{nf}$$

(1)

By solving this differential equation with boundary conditions $t = 0, X_f(0) = 0$, and $X_{nf} = X_0$:

$$\frac{dX_f}{dt} = K \cdot X_{nf} = K \cdot (X_0 - X_f) \rightarrow \ln\left(\frac{X_0 - X_f(t)}{X_0 - X_f(0)}\right) = -K \cdot t$$

$$\ln\left(\frac{X_0 - X_f}{X_0}\right) = -K \cdot t$$

(2)

$X_0$ is the initial biomass concentration (g TSS L$^{-1}$), $X_f$ is the concentration of ﬁne particles after the shear experiment (g TSS L$^{-1}$), $t$ is the duration of the shear exposure (s).

A detailed protocol for strength characterization is described in Appendix A.

**Shear rate calculation**

Calculations of the average shear rates are based on derivations by Sanchez-Perez et al. [28]. The equations for a stirred tank reactor (3) and a bubble column (4) are shown below. $N_p$ is the power number, equal to 6.1 in turbulent ﬂows [29], $\rho$ is the liquid density (kg m$^{-3}$), $d_i$ is the impeller diameter (m), $\mu$ is the dynamic viscosity (Pa s), $N$ is the rotation speed (revolutions per second). For the bubble column equation (4), $\rho$ is the liquid density (kg m$^{-3}$), $\varepsilon$ is the energy dissipation rate (W kg$^{-1}$), $K$ is the consistency index, equal to the dynamic viscosity ($\mu$) in a Newtonian ﬂuid (Pa s), $n$ is the flow index, equal to 1 in a Newtonian ﬂuid, $g$ is the gravitational acceleration (9.81 m s$^{-2}$), and $u_G$ is the superﬁcial gas velocity (m s$^{-1}$).

**Stirred tank reactor:**

$$\gamma = \left(\frac{4N_p\rho d_i^2}{\pi^3 \mu}\right)^{1/2} N^{3/2} = \omega N^{3/2}$$

(3)

**Bubble column:**

$$\gamma = \left(\frac{\rho e}{K}\right)^{1/(n+1)}$$

where

$$\varepsilon = g \cdot u_G$$

(4)

The shear stress proﬁle in a bubble column is rather uniform, so the maximal shear rate is not calculated. For a stirred tank reactor, however, there is a major diﬀerence between the shear at the impeller tip and the average shear rate. Calculation of this maximal shear rate is done by using an equation by Robertson and Ulbrecht [30]:

$$\gamma_{max} = 3.3N^{1.5}d_i\left(\frac{\rho}{\mu}\right)^{0.5}$$

(5)

For a full-scale Nereda® plant, the equation for a bubble column is used. The shear rate in a full-scale Nereda® plant is much lower than in a lab-scale bubble column, due to the size and air ﬂow rate. The lab-scale reactor has an airﬂow of 6 L min$^{-1}$, and a surface area of 24.6 cm$^2$, resulting in a superﬁcial gas velocity of 0.04 m s$^{-1}$. A typical Nereda® reactor has a superﬁcial gas velocity in the range of 0.0015–0.0040 m s$^{-1}$, which is an order of magnitude lower than the typical lab reactor.

**Microscopy and particle size distribution**

The particle size distributions of fresh aerobic granules and the remaining, ﬁltered, granules after the strength test (sieve mesh 200 µm) were obtained using Image Analysis with an Olympus reverse microscope with a magniﬁcation of 7.78× and a Leica Digital Camera, along with its software QWin Pro, version 3.1. An average of four representative pictures was taken of each sample.

**Analytical methods**

Cation concentrations were measured with ICP-OES (OPTIMA 5300DV Optimal Emission Spectrometer, Perkin Elmer). All samples are diluted 10× with 2% HNO$_3$, and measured against three standard samples.
Results

Influence of stirring time, shear rate, and concentration of granules

The influence of the stirring time on the granular sludge abrasion has been determined for granules from the Nereda® prototype installation in Utrecht, Netherlands (Figure 1). During the first 90 min with an average shear rate of 731.1 s\(^{-1}\) (800 rpm), a linear relation can be seen between the natural logarithm of eroded mass and stirring time. However, during prolonged exposure up to 1440 min (24 h), a decrease in the rate of abrasion was measured after 90 min of shearing time.

The abrasion rate coefficient \(K\) correlates linearly with the average shear rate \(\dot{\gamma}\) (Figure 2(a)). At an average shear rate of 285.5 s\(^{-1}\) (400 rpm), a negligible amount of eroded material was observed. For comparison, an overview of average and maximal shear rates in a stirred tank reactor, bubble column, and full-scale Nereda® plants is given in Table 2. The concentration of granules that are added to the strength characterization test have a negligible effect on the value for \(K\) (Figure 2(b)).

Abrasion mechanism

The abrasion mechanism of granular sludge during the shear experiment was tested by exposing a total of 67 granules to an average shear rate of 731 s\(^{-1}\) (800 rpm) during 60 min. Upon abrasion of granules during the experiment, the physical structure remains similar as can be seen in Figure 3. Small chips of granules were observed after 15 min already, and the fraction of these chips increased after 60 min of shear exposure (Figure 3(e, f)).

This visual observation was quantified by means of size distribution analysis before and after the strength characterization test (Figure 4). These results confirm that the majority of particles have a size that is just above this sieving threshold. These small fragments were visually observed to be the shells that were sheared off of the granules, as seen in Figure 3e and f.

Variability of full-scale Nereda® granular sludge

Aerobic granular sludge from four different full-scale Nereda® plants was tested on their strength with the standard test conditions (800 rpm for 60 min). Results of this study and references of other studies are shown.
The abrasion rate coefficient ($K$) of the different Nereda® sludge granules ranged from $(2.68 \pm 0.17) \times 10^{-5}$ s$^{-1}$ to $(7.69 \pm 0.90) \times 10^{-5}$ s$^{-1}$. Freshwater lab-scale AGS had a lower value for $K$ of $(1.78 \pm 0.20) \times 10^{-5}$ s$^{-1}$, and seawater-adapted lab-scale AGS had an even lower value of $(1.17 \pm 0.01) \times 10^{-5}$ s$^{-1}$.

**Effect of granule size on abrasion of full-scale aerobic granular sludge**

Granules with smaller size showed a lower abrasion rate than bigger granules, and this correlation was found to be non-linear (Figure 5). Granules that have a size of >2000 µm show a significantly lower strength than granules that have smaller sizes, whereas the smaller granule fractions did not have a large difference in observed strength.

<table>
<thead>
<tr>
<th>Operation</th>
<th>Conditions</th>
<th>$\dot{\gamma}$ (s$^{-1}$)</th>
<th>$\dot{\gamma}_{\text{max}}$ (s$^{-1}$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stirred tank reactor</td>
<td>200 rpm</td>
<td>91.4</td>
<td>562.3</td>
</tr>
<tr>
<td></td>
<td>800 rpm</td>
<td>731.1</td>
<td>4498.6</td>
</tr>
<tr>
<td></td>
<td>1600 rpm</td>
<td>2068.0</td>
<td>12,724.0</td>
</tr>
<tr>
<td>Bubble column</td>
<td>6.8 mm s$^{-1}$ (1 L min$^{-1}$)</td>
<td>257.6</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40.6 mm s$^{-1}$ (6 L min$^{-1}$)</td>
<td>631.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>67.7 mm s$^{-1}$ (10 L min$^{-1}$)</td>
<td>814.8</td>
<td></td>
</tr>
<tr>
<td>Nereda</td>
<td>1.4 mm s$^{-1}$ (500 m$^3$ h)</td>
<td>116.7</td>
<td></td>
</tr>
<tr>
<td></td>
<td>2.8 mm s$^{-1}$ (1000 m$^3$ h)</td>
<td>165.1</td>
<td></td>
</tr>
<tr>
<td></td>
<td>4.2 mm s$^{-1}$ (1500 m$^3$ h)</td>
<td>202.2</td>
<td></td>
</tr>
</tbody>
</table>

**Table 2.** Overview of calculated average shear rates ($\dot{\gamma}$) and maximal shear rates ($\dot{\gamma}_{\text{max}}$) in a stirred tank reactor, bubble column, and full-scale Nereda® plants at 20°C. Shear rates are calculated for a variety of stirrer speed for a standard geometry stirred tank reactor (rpm), or superficial gas velocity for a bubble column and Nereda (mm s$^{-1}$), with its respective gas flow between brackets.

*Figure 3.* Abrasion of full-scale Nereda granular sludge at 7.78× magnification after (a) 0 min, (b) 15 min, (c) 30 min, (d) 45 min, (e) 60 min, and (f) at 25× magnification after 60 min. Scale bars indicate 1 mm. Arrows indicate hollow granules.
Effect of NaCl on abrasion of full-scale aerobic granular sludge

The short-term effect of NaCl exposure to freshwater-adapted full-scale aerobic granular sludge was determined after incubation of 1 h in NaCl solution, prior to the strength characterization test. Results are shown in Figure 6. At a NaCl concentration of 25 g L\(^{-1}\), the abrasion decreased for all of the full-scale granules. At 50 g L\(^{-1}\) NaCl, granules from Utrecht and Dinxperlo shows even less abrasion, but an increase (relative to

Table 3. Overview of abrasion rate coefficients \(K\) from different granular sludge systems (lower number equals higher strength).

<table>
<thead>
<tr>
<th>Description</th>
<th>(K (10^{-5} \text{ s}^{-1}))</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Utrecht Nereda® plant</td>
<td>2.85 ± 0.10</td>
<td>This study</td>
</tr>
<tr>
<td>Garmerwolde Nereda® plant</td>
<td>5.47 ± 0.14</td>
<td>This study</td>
</tr>
<tr>
<td>Vroomshoop Nereda® plant</td>
<td>7.69 ± 0.90</td>
<td>This study</td>
</tr>
<tr>
<td>Dinxperlo Nereda® plant</td>
<td>2.68 ± 0.17</td>
<td>This study</td>
</tr>
<tr>
<td>Lab-scale acetate-fed aerobic granular sludge</td>
<td>1.78 ± 0.20</td>
<td>This study</td>
</tr>
<tr>
<td>Lab-scale acetate-fed saline aerobic granular sludge</td>
<td>1.17 ± 0.01</td>
<td>This study</td>
</tr>
<tr>
<td>Anaerobic lab-scale butyric acid producing</td>
<td>2451.66</td>
<td>[20]</td>
</tr>
<tr>
<td>organisms</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Aerobic lab-scale nitrifying organisms</td>
<td>101.32</td>
<td>[20]</td>
</tr>
<tr>
<td>Anaerobic lab-scale methanogenic organism</td>
<td>20.08</td>
<td>[20]</td>
</tr>
<tr>
<td>Low-load full-scale methanogenic granules</td>
<td>1.31</td>
<td>[20]</td>
</tr>
<tr>
<td>High-load full-scale methanogenic granules</td>
<td>2.16</td>
<td>[20]</td>
</tr>
<tr>
<td>Full-scale canning wastewater-fed anaerobic granules</td>
<td>1.71</td>
<td>[31]</td>
</tr>
<tr>
<td>Full-scale abattoir wastewater-fed anaerobic granules</td>
<td>15.23</td>
<td>[31]</td>
</tr>
<tr>
<td>Full-scale brewery wastewater-fed anaerobic granules (1)</td>
<td>4.19</td>
<td>[31]</td>
</tr>
<tr>
<td>Full-scale brewery wastewater-fed anaerobic granules (2)</td>
<td>3.88</td>
<td>[31]</td>
</tr>
</tbody>
</table>
25 g L\(^{-1}\) NaCl) in abrasion was measured for granules from Garmerwolde and Vroomshoop.

Exchange of ions in the liquid phase after 1 h of incubation of Garmerwolde granular sludge was quantified by ICP-OES (Figure 7). Sodium was taken up from the liquid by 347 mg g\(^{-1}\) TSS at 25 g L\(^{-1}\) NaCl, and 449 mg g\(^{-1}\) TSS at 50 g L\(^{-1}\) NaCl. Calcium and magnesium both had the same low amount of release at 25 and 50 g L\(^{-1}\) NaCl, at 4.0 mg g\(^{-1}\) TSS and 1.1 mg g\(^{-1}\) TSS, respectively. Changes in concentrations of tin, strontium, lead, manganese, iron and zinc were below the detection limit of 1 mg L\(^{-1}\) (data not shown). Phosphate release during incubation was negligible (<1 mg L\(^{-1}\)).

**Discussion**

**Method evaluation**

Current literature on strength characterization of granular sludge is based on experiments that are either undefined in terms of shear rate [18] or based on experiments that require a long duration [19]. Comparison of quantified strength from different studies is complicated or not possible, because different methods are used for acquiring strength numbers. Some studies have used a bubble column for strength quantification [19,31]. Using this type of reactor, rather than a stirred tank reactor causes different shearing behaviour. A highly turbulent region in a stirred tank reactor induces a maximal shear rate around the impeller, whereas a bubble column does not have this local maximum. Even though a bubble column resembles full-scale practice, the purpose of this strength test is to have a separate and not necessarily similar method of shear exposure.

The results that were obtained in the evaluation of the strength measurement are in line with previous studies, especially Pereboom [20], who used methanogenic granular sludge. A linear abrasion rate over time indicates the negligible influence of detached particles and a linear increase in abrasion rate with increasing shear rate. The increasing biomass concentration did not lead to an increasing abrasion rate, matching the results of Pereboom [20] as well.

The linear abrasion rate during the first 90 min of abrasion and no effect of granular sludge concentration indicates that the particle-particle collisions do not play a role in this test. This can be explained by looking at energy transfer in a stirred tank reactor. Kinetic energy that is put into the system through the stirrer gets dissipated in the bulk liquid throughout a series of eddies. The smallest scale is in the range of Kolmogorov microscale. When the particle size is smaller than these microscales, viscous forces determine the maximum hydrodynamic force, while these are negligible when the particle size is larger, and thereby in the inertial sub-range of turbulence [32]. The size of the Kolmogorov microscales can be calculated according to Equation (6), and depends on the kinematic viscosity (\(\nu\) in m\(^2\) s\(^{-1}\)) and the average dissipation rate (\(\varepsilon\) in W kg\(^{-1}\)) [33]. Equation (7) describes the calculated of \(\varepsilon\), where \(P_0\) is the power number (= 6.1 for turbulent regions), \(N\) is rotational speed (s\(^{-1}\)), \(D_i\) is impeller diameter (m), and \(V\) is the volume (m\(^3\)):

\[
\eta = \left(\frac{\nu^3}{\varepsilon}\right)^{1/4}
\]  

\[
\varepsilon = \frac{P_0 N^5 D_i^5}{V}
\]

In a strength measurement test at 800 rpm in tap water (\(\nu = 1.00 \times 10^{-6}\) m\(^2\) s\(^{-1}\)), the Kolmogorov length equals 4.1 \times 10^{-5} m (= 41 µm). This number is several orders of magnitude smaller than the size of the granules that were used in this experiment of around 10^{-3}–10^{-4} m, indicating a negligible effect of viscous forces on the granule abrasion. The abraded particles will
have a diameter in the range of the Kolmogorov length. However, this size will cause them to follow the turbulent flow, and thereby cause less collisions [34].

After the first 90 min in the abrasion test, no linear abrasion was observed anymore in the test. This could be due to the fact that a relatively high amount of small particles with sizes in the Kolmogorov length are formed. Since these particles will have smaller physical impact upon collision, the overall abrasion rate decreases. However, since the test protocol uses 60 min for quantification, this effect should be negligible.

**Comparison of the strength of aerobic granular sludge to other kinds of granular sludge**

Between different aerobic granular sludge samples in this study, there is a difference in measured strength. Granular sludge from Utrecht and Dinxperlo Nereda® plants have lower abrasion (approx. $2.7 \times 10^{-5}$ s$^{-1}$) than Garmer-wolde and Vroomshoop plants ($5.5-7.7 \times 10^{-5}$ s$^{-1}$). Contributing factors in the Dinxperlo plant can be dosing of iron, and a higher average SRT than other plants. The former leads to higher amounts of phosphate precipitates in the granular sludge, and potentially an increase in granular strength. The lab-scale acetate-fed aerobic granular sludge has higher strength than all full-scale granules ($1.78 \times 10^{-5}$ s$^{-1}$). This is likely due to having a more defined and easily degradable substrate, leading to the selection of a more homogenous microbial population that forms a strong granule (phosphate accumulating bacteria in this case).

Strength characterization has previously been carried out on different types of granular sludge. The aerobic granules from all tested Nereda® plants are weaker than anaerobic methanogenic granules [20,23]. These observations are in line with biofilm being more stable when consisting of bacteria with a lower growth rate [9,35]. However, it was found that slow-growing anammox granules are weaker than the aerobic granular sludge, with no described heterotroph growth was described in their system [36]. This could be due to differences in the ionic composition of the EPS and inorganic compounds within the granules. Batstone and Keller [31] tested the shear resistance of anaerobic granules from different full-scale plants. They found that cannery-fed granules had a low abrasion rate, which was lower than the tested Nereda® sludge from this study. Granules that were fed with protein-rich pig abattoir wastewater exhibited high abrasion in comparison to aerobic Nereda® granules. Anaerobic granules that were fed with brewery wastewater showed an abrasion rate that was similar to the aerobic Nereda® granules. The cannery wastewater has likely more easily degradable substrate than abattoir or brewery wastewater. The higher reported percentage of calcium in this water could theoretically increase the granular strength, but its impact on the overall strength was found to be small in some studies [36,37].

**Heterogeneity of density throughout granules**

Exposure to intensive shear in the stirred reactor during longer time periods than 90 min led to a non-linear abrasion rate (Figure 1(b)). The first-order abrasion rate that was described in Equation (1) therefore only holds for the first 90 min of abrasion. This observation could be the result of a heterogeneity of density throughout the granules. Granules will have a layered structure, conversion of slowly degradable polymeric compounds will be mainly performed by bacteria in the outer shell of the granules. This might have a lower strength than the consolidated (and likely relatively older) inner core of the granule. Measurement of the density and extra-cellular polymers (EPS) within a single granule could give more information on density and EPS differences and their impact on the granular strength.

**Minimal breakage shear rate**

As can be seen in Figure 2(a), at a shear rate of 258 s$^{-1}$ no abrasion takes place from the granular sludge of the Utrecht Nereda® prototype. This indicates the resistance of granules to a certain amount of shear before abrasion [20]. Exposure of granules to shear in a full-scale operation also requires them to resist these forces to prevent washout (Table 2). Properties of the EPS can play a major role in this observation. The alginate-like exopolysaccharides (ALE) that were found in the EPS of the granules are a hydrogel, exhibiting the behaviour of a viscoelastic solid [38]. These properties support the idea that a minimal force is required for breaking the granules, due to their elasticity. This elasticity gives granules some resilience for shear stress without breaking or abrading [39,40].

**Granule size**

Larger granules had lower strength than smaller ones (Figure 5). This could be due to the smaller granules colliding less frequently than their bigger counterparts. The derivation of the previously described Kolmogorov scales can be applied for the effect of granule size on the observed strength. Moreover, the collision impact of bigger granules will be larger since they have higher mass and momentum than the smaller granules [34]. Furthermore, as granules grow larger, the inner core could starve due to diffusion limitation of the substrate.
Hollow cores have frequently been observed in granules. The life cycle of granular sludge is still a topic under discussion, but breakage of larger granules into smaller granules is one hypothesis that is in line with our observations [41].

**Effect of NaCl on strength**

Addition of NaCl to full-scale aerobic granular sludge led to an increase in the granular strength. Addition of calcium and magnesium ions to activated sludge decreases the sludge volume index (SVI) and increases the floc density [25]. An increase in sodium led to the opposite effects in this study, presumably by replacement of calcium which is stabilizing the floc EPS. Leaching of these divalent cations has been discussed to be also detrimental to granule stability [20,23]. ICP-OES analysis indicated that added sodium diffused into the granular sludge but did not lead to a significant release of calcium or magnesium. Therefore the stabilizing effect of these ions on the EPS will not be decreased [38]. In other experiments, the use of EDTA, which actively binds Ca$^{2+}$ ions, indeed led to a decreased strength of granular sludge [21].

The effect of granule size can be of importance in explaining the positive effect of NaCl addition on granular strength [42]. Due to the higher osmotic pressure of saline water than freshwater, an exchange of salts into the granules and water out of the granules can occur. The resulting lower amount of water in the granules can cause shrinking, and an increase in granule density. Smaller granules show a lower abrasion rate than bigger granules (Figure 5). Granules with higher density can similarly have higher strength [21]. This hypothesis needs further analysis, since in the complex heterogeneous non-spherical granular sludge samples we used it was not possible to reliably measure the change in granule volume due to salt addition.

The strength of lab-scale artificial seawater-adapted aerobic granular sludge is higher than of lab-scale freshwater-adapted sludge. Difference in density and adaptation of the EPS likely plays a role in this observation. Higher concentrations of extracted EPS and higher protein contents have for instance been observed in other studies with seawater biofilms [43,44]. Characterization of alginate-like exopolymers from aerobic granular sludge has been described in the literature, but the adaptation of the EPS to seawater should be a major focus for linking this to its strength properties [45].

**Conclusion**

This study has described a method for determining the strength of aerobic granular sludge. This method was tested for granular sludge from four full-scale Nereda® plants, granule size, salinity, lab-grown acetate-fed aerobic granular sludge, both on freshwater and seawater.

Abrasion times up to 90 min showed a stable abrasion rate coefficient (K). Prolonged periods of abrasion up to 24 h resulted in a decrease in abrasion rate. The amount of granules does not impact the value for K. Higher shear rates lead to higher values for K, but a minimal shear rate was required for the start of abrasion. Lab-scale granules exhibited a lower abrasion rate than full-scale Nereda® granules. Incubation of full-scale granules in NaCl led to a decrease in abrasion rate at 25 g/L NaCl, but incubation in 50 g/L NaCl led to a further decrease for only 2 out of 4 tested granular sludge samples.

**Disclosure statement**

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