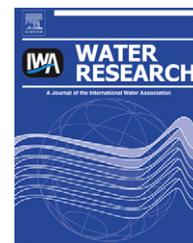




ELSEVIER

Available online at [www.sciencedirect.com](http://www.sciencedirect.com)

SciVerse ScienceDirect

journal homepage: [www.elsevier.com/locate/watres](http://www.elsevier.com/locate/watres)

# Temperature and salt effects on settling velocity in granular sludge technology

M.-K.H. Winkler<sup>a</sup>, J.P. Bassin<sup>a,b</sup>, R. Kleerebezem<sup>a</sup>, R.G.J.M. van der Lans<sup>a</sup>,  
M.C.M. van Loosdrecht<sup>a,\*</sup>

<sup>a</sup> Department of Biotechnology, Delft University of Technology, Julianalaan 67 2628 BC Delft, The Netherlands

<sup>b</sup> Federal University of Rio de Janeiro, Chemical Engineering Program, Rio de Janeiro, Brazil

## ARTICLE INFO

### Article history:

Received 3 January 2012

Received in revised form

28 March 2012

Accepted 22 April 2012

Available online 4 May 2012

### Keywords:

Granular sludge

Settling velocity

Salt

Temperature

## ABSTRACT

Settling velocity is a crucial parameter in granular sludge technology. In this study the effects of temperature and salt concentrations on settling velocities of granular sludge particles were evaluated. A two-fold slower settling velocity for the same granules were observed when the temperature of water decreases from 40 °C to 5 °C. Settling velocities also decreased with increasing salt concentrations. Experiments showed that when granules were not pre-incubated in a solution with increased salt concentration, they initially floated. The time dependent increase in mass and hence in settling speed of a granule due to salt diffusion into the granule was dependent on the granule diameter. The time needed for full salt equilibrium with the bulk liquid took 1 min for small particles from the top of the sludge bed and up to 30 min for big granules from the bottom of the sludge bed. These results suggest that temperature and salt concentration are important parameters to consider in the design, start-up and operation of granular sludge reactors and monitoring of these parameters will aid in a better control of the sludge management in anaerobic and aerobic granular sludge technology. The observations also give an explanation for previous reports which were suggesting that a start-up of granular sludge reactors is more difficult at low temperatures.

© 2012 Elsevier Ltd. All rights reserved.

## 1. Introduction

In activated sludge wastewater treatment processes the sludge-liquid separation in secondary clarifiers is an important step to assure the effluent quality. Activated biosolids however frequently shows poor settling properties, which in turn can potentially harm the process efficiency (De Los Reyes Ill and Raskin, 2002). Recently, interesting alternatives for the conventional activated sludge systems were developed relying on compact and self-immobilized granulated biomass. Examples are for instance Aerobic Granular Sludge (AGS), Upflow Anaerobic Sludge Bed (UASB), as well as expanded

granular sludge bed technology (EGSB) (Beun et al., 1999; Lettinga and Hulshoff Pol, 1991; Morgenroth et al., 1997; Rinzema et al., 1993). One of the most important parameters to select for granular sludge is the settling velocity. By applying short settling times in reactors operated as sequencing batch process, only big and fast settling biomass aggregates are selected, while flocculent sludge is washed out (Beun et al., 2000). As a result, granular sludge technology has a small area requirement due to the absence of large clarifiers and increased sludge content in the bioreactors (De Bruin et al., 2004). The parameters determining the settling velocity of particles and in turn biomass washout are of crucial

\* Corresponding author. Tel.: +31 15 2781551.

E-mail address: [M.C.M.vanLoosdrecht@tudelft.nl](mailto:M.C.M.vanLoosdrecht@tudelft.nl) (M.C.M. van Loosdrecht).

0043-1354/\$ – see front matter © 2012 Elsevier Ltd. All rights reserved.

doi:10.1016/j.watres.2012.04.034

importance to granular sludge technology. The balances of forces for the sedimentation of a spherical particle are depending on the buoyancy, gravity and drag force (Giancoli, 1995). From this relation, the settling velocity is influenced by the viscosity of water, particle size and shape, and the difference between the density of the water and the particles. The density and viscosity of the medium depends on the temperature and solutes present in water. With increasing temperature, the viscosity and density of the water decreases even though the density is not as much influenced as the viscosity. At high temperature, water molecules are more mobile than at low temperature decreasing its viscosity from e.g. 10–40 °C by a factor two (Podolsky, 1994). Salts are dissolved as ions, which enhance the water structure and increase the density of the fluid. The density of ocean water at the sea surface is 1028 kg/m<sup>3</sup>, which is much higher than the density of fresh water (998 kg/m<sup>3</sup> at 20 °C). Since the density of granules is only slightly different from water (reported values vary between 1005 and 1070 kg/m<sup>3</sup>) (Bassin et al., 2011; Batstone and Keller, 2001; Etterer and Wilderer, 2001) changes in the density of the water will have a significant impact on the settling behavior of granules. Many researchers measured, calculated, and compared the settling properties of granular sludge and activated sludge under different process conditions (de Kreuk et al., 2005; Grant and Lin, 1995; Lew et al., 2003; Liu et al., 2008; Nor Anuar et al., 2007). However, no study focussed on the influence of the viscosity and density changes of water due to changes in temperature and ionic strength on the granular sludge settling. This is of special importance during operation of full-scale operation plants, which are susceptible to changes in temperature and in salt content. We measured in this study the settling velocity of laboratory grown aerobic granules under different temperatures and NaCl concentrations. The relevance of considering the physical properties of water for the design and operation of aerobic granular sludge reactors is discussed.

## 2. Material and methods

### 2.1. Density and size distribution measurements

Granules from a lab-scale aerobic granular sludge reactor were sampled for measurement of particle size distribution, dry mass, ash content and granule density. Specific biomass density was measured with a pycnometer and size distribution measurements were conducted by the means of an image-analyzer using the averaged projected surface area of the granules.

### 2.2. Experimental determination of settling velocities at different temperatures and salt concentrations

The settling velocity of granules at different temperatures (5–40 °C) and salt concentrations (0–40 g/L NaCl) was determined in a 3 m long column. The experimental column comprised a double wall for temperature control. Temperature was controlled online by a water bath based on the measurement of the temperature in the column. Laboratory grown granules were collected from a settled sludge bed.

During the settling phase of the sequencing-batch AGS reactor, bigger and denser granules settle faster and occupy the bottom of the sludge bed, whereas small and lighter granules form the top layer of the sludge bed (Winkler et al., 2011a). Granules from top and bottom of the reactor sludge bed were collected and their diameter and density was measured as described in Section 2.1. Henceforth the granules from the top were called small and granules derived from the bottom were called big granules. For the measurement of settling velocities at different temperatures, the column was filled with tap water and temperature was adjusted between 5 °C and 40 °C. For the settling experiments at different salt concentrations (0–40 g/L NaCl), the temperature of the water was kept constant at 20 °C. Moreover, settling experiments for different salt concentrations were conducted a) without pre-incubation b) after 15 min pre-incubation and c) after a day of incubation of the granules in the same salt concentration as used in the experimental setup. For salt and temperature experiments, 5 g of wet granules were placed on a spoon and were released for every experiment in the same manner into the reactor column. The water column height was always kept constant for all experiments. The time necessary to reach half of the column height (1.5 m) was recorded with a chronometer and the settling velocity of granules was determined. Each experiment was carried out 5 times and graphs are based on average values.

### 2.3. Calculation settling velocity

The measured average density and diameter of granules obtained in the settling experiments were used to calculate theoretical settling behavior. For particle Reynolds numbers smaller or equal to 1, Stokes' law was used to calculate the

settling velocity of a particle  $v_s = \frac{g \cdot \rho_p - \rho_w}{18 \cdot \rho_w} \cdot \frac{d_p^2}{\nu_w}$ . For particle

Reynolds numbers in the range  $1 < Re_p < 10^3$ , the sedimentation velocity was calculated by iteratively solving the coef-

ficient of resistance  $c_w(Re_p) = \frac{24}{Re_p} + \frac{4}{\sqrt{Re_p}} + 0.34$  and hence

solving the equations of the stationary sedimentation velocity

of a single spherical particle:  $v_s = \sqrt{\frac{4}{3} d_p \frac{\rho_p - \rho_w}{\rho_w} g \frac{1}{c_w(Re_p)}}$ .

Changes in density and viscosity of water were adjusted according to the temperature and salt conditions as applied in the experimental setup. It was further assumed that inside the granules the same salinity occurred as in the liquid; the granular density was corrected for the increase in density of water at different temperature or salt concentrations. For the theoretical settling behavior at different salt concentrations a density increase due to a granular shrinking of 0.5% per 10 g NaCl was assumed to fit the model.

### 2.4. Calculation salt penetration

The penetration time of a known salt concentration ( $C_L = 41$  g/L NaCl, density 1030 kg/m<sup>3</sup>) into a granule and the resulting specific mass increase and settling velocities over time were calculated. In these calculations, spherical granules were assumed to have equal diameter and composition. Further,

granules were assumed to not influence each other while settling. The chosen diffusion coefficient of salt in solution ( $D_L$ ) was  $9 \times 10^{-10} \text{ m}^2/\text{s}$  and the diffusion coefficient of salt into the granules ( $D_G$ ) was assumed to be  $0.8 D_L$ . For calculations, an initial particle density of  $1010 \text{ kg/m}^3$  and a diameter ( $d$ ) of 1 mm or 3 mm were assumed. Absorption of salt into the particle can be simulated using the diffusion equation, but here the simple standard solution was used. First absorption follows penetration theory, but after a critical time the mass transfer coefficient becomes constant, given by  $Sh = \frac{2}{3}\pi^2$ . This period we call permeation. The salt penetration depth  $\delta = \sqrt{\pi D_L \tau}$  at the critical time  $\tau$  between penetration and permeation was calculated using  $\tau = Fo \frac{d^2}{D_G}$  assuming a critical Fourier ( $Fo$ ) of 0.02. The overall mass transfer coefficient  $K_L = \left(\frac{1}{k_o} + \frac{1}{mk_i}\right)^{-1}$  was derived using an external mass transfer coefficient  $k_o = Sh \frac{D_L}{d}$  (with  $Sh = 2$ ) and an internal (average) mass transfer coefficient  $k_i = 2 \frac{D_G}{\delta}$ . The relative concentration of salt in the granule compared to the salt solution ( $m$ ) was arbitrarily assumed to be 0.8. This assumes that cells and EPS molecules in the granule take up volume leading to a lower volumetric salt concentration in the granules. The mass entering the granule during this period follows from  $\Delta M = K_L A C_L \tau$ . After the penetration period ( $Fo > 0.02$ ), during permeation, the internal mass transfer coefficient  $k_i = Sh \frac{D_G}{d}$  was used with a Sherwood of  $Sh = \frac{2}{3}\pi^2$ . Now the change in mass follows from a mass balance:  $\frac{dM}{dt} = \frac{dVC_G}{dt} = K_L A \left(C_L - \frac{C_G}{m}\right)$ . Combining, the density of the granule follows from  $\rho_G = \rho_{Gi} + mC_L - \left(mC_L - 6 \frac{K_{Lp}}{d} C_L \tau\right) \exp\left(-\frac{K_L}{dm}(t - \tau)\right)$ , with subscript p referring to the penetration period.

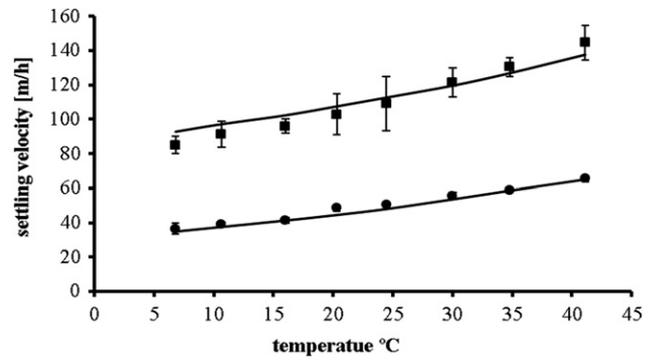
### 3. Results

#### 3.1. Settling velocity at different temperatures

Top (small) and bottom (big) granules with known density, diameter and ash content (Table 1) were used to determine the settling velocity at different temperatures and salt concentrations. The results presented in this work are in alignment with general physical mass and heat transfer theorems. Heat transfer was very quick and results of settling velocities at different temperatures showed a good fit with the calculated settling velocities which were based on the average measured

**Table 1 – Physical properties of small and big granules from settling velocities test.**

Parameter	Small	Large
Ash content %	15	34
Density g/L	1020	1037
Average diameter (mm)	1.5	2.3

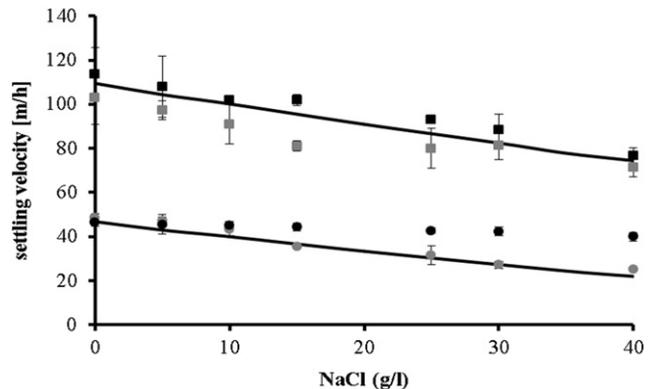


**Fig. 1 – Measured settling velocities and standard deviations at different temperatures for small (●) and big (■) granular sludge particles and the corresponding theoretical settling velocities (lines) based on the measured values in Table 1.**

diameter and density of the granules used in the experiment. Smaller and lighter granules settled slower than bigger and denser granules. Results revealed a two-fold difference in settling velocity for the same granule at 5 °C and 40 °C with values increasing from 84 to 145 m/h for big granules and from 35 to 63 m/h for smaller granules (Fig. 1).

#### 3.2. Settling velocity at different salt concentrations

Opposed to heat transfer, mass transfer of e.g. salt is much slower. For the salt measurements settling tests without a pre-incubation of the granules in the salt concentration in which the experiment was conducted in, most of the granules floated making a measurement of the settling velocity impossible. In order to experimentally show the effect of salt penetration on the density and hence the settling velocity of granules a pre-incubation of 15 min and of one day was chosen. Results showed that the longer the incubation was chosen the faster the granules settled. Theoretical settling behavior of granules at different salt concentrations gave a good fit (Fig. 2).



**Fig. 2 – Measured settling velocities and standard deviations at different salt concentrations for small (●) and big (■) granular sludge particles after incubation in corresponding salt solution for 15 min (grey) and 24 h day (black).**

### 3.3. Time dependant salt penetration

The time dependent increase in density and settling velocity of small and big granules was calculated during incubation in a 40 g/L NaCl solution. Settling velocity of granules increase due to the increase of density caused by the penetration of salt into the granule. Settling velocity of smaller granules is accelerated whereas for bigger granules no settling occurs until 11 min. Only after this time the settling increases linearly due to full salt penetration (Fig. 3A). Fig. 3B shows the time needed for a particle to get as dense as the solution. For large particles this time become in the order of 5–30 min, a relative long time compared to other process times in the operation of granular sludge reactors.

## 4. Discussion

In this study the effects of temperature and salt concentrations on settling velocities of granules were evaluated. Results revealed a two-fold faster settling velocity for the same granules when temperature of water was increased from 5 °C to 40 °C. Measured and calculated settling velocities within this study gave a good fit for fresh water (Fig. 1). Other researchers who measured and calculated settling velocities of aerobic granular aggregates in fresh water also found a good fit of measured and theoretical values (Xiao et al., 2008). In addition, settling velocities were measured at different salt concentration and results revealed as expected in a decrease in settling velocity with increasing salt concentrations. This decrease was however lower than expected from a calculation based on the originally measured density (Table 1). The density of a granule is very sensitive in the calculation of the settling velocity. The first factor to correct for is the salt water inside the granule which also leads to an increased density of the particles. It is simply assumed that the density of the liquid inside and outside of the granule is the same. This correction gave a reasonable fit between theory and data although the slope of the curve in Fig. 2 was too strong leading in an unacceptable fit for salt concentrations higher than 15 g/L. Earlier studies reported a shrinking of alginate gels at increasing salt concentration which could potentially lead to higher granular densities (Moe et al., 1993; Tierney et al., 2010). Since granules are reported to consist of alginate this effect

is likely to have played a role in our experiments as well (Lin et al., 2010). For our theoretical settling velocities we observed that an extra density increase of 0.5% per 10 g/L NaCl yielded a good fit of measured and calculated settling velocities (Fig. 2). The corresponding shrinking of the particle was too small to be measurable with an image analyzer.

In case of the temperature, the main factor influencing the settling velocity is the change in viscosity of the water whereas for salt water the major factor influencing settling velocity is the water density. Temperature and salt have a significant effect on granular sludge settling rate. This is due to the fact that at higher temperature the viscosity of water is decreased hence decreasing resistance while at higher salt concentrations resistance is increased due to the water density increase (Judd, 1970; Thomas and Stevenson, 1973). Certainly when temperature and salt content rapidly vary, like in industrial wastewaters or e.g. due to melt water or storm water in municipal wastewater treatment plants, the effect of these parameters should be considered and appropriate measures need to be taken to prevent process instabilities due to biomass washout. For municipal wastewater during cold winter periods also salt peaks from deicing road salts might influence the settling rate. The concentrations of salts in runoff water can be up to 4 g chloride per liter (Bubeck et al., 1971) and can lead in extreme cases to flotation and washout of the sludge since the diffusion of salt into the granule can take up to 30 min (Fig. 3A, B). Salt events will therefore decrease system functioning not only by toxification of microorganisms (increase in osmotic pressure) (Uygur and Kargi, 2004) but also by washout events due to biomass floating (Fig. 2). For a continuous operation of granular sludge reactors at high salt concentrations of up to 30 g NaCl/L floating has not been reported to be problematic (Figueroa et al., 2008). However, when granular sludge reactors are run in sequencing batch mode, with e.g. 3–4 parallel reactors fed sequentially, the wastewater characteristic can significantly vary in between two influent additions. A strong change in temperature or salt might disturb an even upflow of the influent through the settled sludge bed.

The formation of granular sludge at lower temperatures is hindered by many factors. Earlier research on aerobic granular sludge reactor has experimentally shown that a start-up process at cold temperatures is troublesome (de

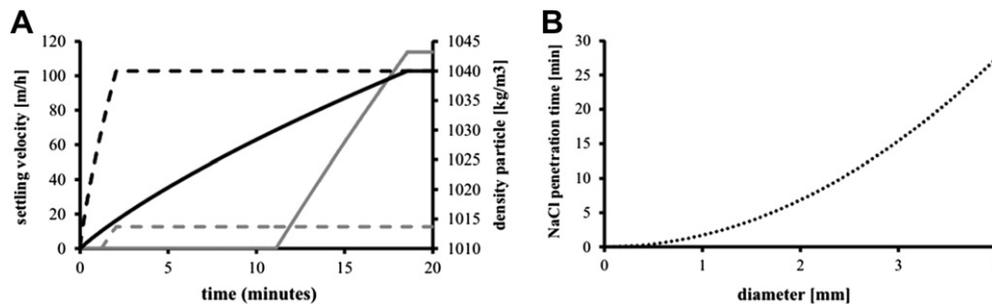


Fig. 3 – A) calculated time dependent increase in density (black) and settling velocity (grey) of a small (1 mm, dashed line) and big (3 mm, solid line) granule during incubation in a 40 g/L NaCl solution. B) Penetration time of NaCl (40 g/L) into a granule as a function of particle diameter.

Kreuk et al., 2005). All microbial processes run slower at lower temperatures (Brdjanovic et al., 1997; Kettunen and Rintala, 1997; Lettinga et al., 2001) which could have limited the granulation at a lower temperature. This research shows that not only microbial factors are hindered at lower temperatures but also that settling velocity is much reduced at lower temperatures (Fig. 1). During a start-up process of a granular sludge system, granular biomass has to be separated from flocculent sludge in order to have selective washout of the latter. Initially granular sludge particles are only small (0.2 mm), which implies that the settling velocity differences between flocculent and granular biomass is small. At lower temperature, this difference is even decreased. The settling velocity of a granule with a diameter of 200  $\mu\text{m}$  and a density of 1010  $\text{kg/m}^3$  will not be higher than 1 m/h regardless of the temperature. At these low temperatures the separation of initial granules and flocs becomes therefore troublesome explaining the reported problematic start-up under cold conditions.

Recently we demonstrated that in aerobic granular sludge vertical segregation of granules occurs based on small differences in settling velocity of the granules and that different types of bacteria grow either in bigger or smaller granules due to physiochemical reasons (Winkler et al., 2011a, 2011b). Granules with a higher density accumulate at the bottom of the sludge blanket. Since the feeding is from the bottom of the reactor, these granules get a higher substrate load and therefore a higher radius, which enhances the segregation effect. Segregation of biomass has been reported earlier and is hence a common occurrence in wastewater treatment based on granular sludge or biofilms (DiFelice et al., 1997; Ro and Neethling, 1994). Selective sludge withdrawal from either top or bottom of the sludge bed can therefore be used as a possibility to control the microbial community structure granular sludge technology. Since we have shown that salt events mainly cause bigger granules to float (Fig. 3A, B) and our previous studies have shown that polyphosphate accumulation organism are mainly located in bigger granules (Bassin et al., 2011; Winkler et al., 2011a), salt events might lead to a significant washout of this functional group and hence to a loss in P removal efficiency. We can conclude that in order to optimize biomass retention within granular sludge reactors a monitoring of a variation in physical water properties like temperature and conductivity (to measure salts) are of importance to understand potential disturbances of the reactor system.

## 5. Conclusion

In this study we showed that the temperature and ionic strength dependent density and viscosity changes of water have great impact on settling velocity of granular sludge. The corresponding slow settling of small granules at decreased water viscosities and increased water densities as caused by a lower temperature can be an important reason for the reported troublesome start-up of granular sludge reactors. Conductivity and temperature measurements can therefore be used as an additional operational factor to stabilize and improve biomass retention in granular sludge technology.

## Symbol list

- $\delta$  = salt penetration depth,  $\delta = \sqrt{\pi D_L \tau}$  [mm]  
 $d$  = diameter particle [mm]  
 $D_L$  = diffusion coefficient (Liquid or Granule) [ $\text{m}^2/\text{s}$ ]  
 $Fo$  = Fourier group (dimensionless time) [-]  
 $k_i$  = internal mass transfer coefficient in penetration period,  $k_i = 2 \frac{D_G}{\delta}$  [ $\text{m/s}$ ]  
 $k_i$  = internal mass transfer coefficient after penetration period,  $k_i = \text{Sh} \frac{D_G}{d}$  [ $\text{m/s}$ ]  
 $k_o$  = external mass transfer coefficient,  $k_o = \text{Sh} \frac{D_L}{d}$  [ $\text{m/s}$ ]  
 $K_L$  = overall mass transfer coefficient,  $K_L = \left( \frac{1}{k_o} + \frac{1}{mk_i} \right)^{-1}$  [-]  
 $m$  = distribution coefficient (solubility salt in granule versus salt solution) [-]  
 $\text{Sh}$  = Sherwood group (dimensionless mass transfer coefficient) [-]  
 $\tau$  = penetration time (during which penetration theory holds) [sec]

## Calculation of settling velocity

$$v_s = \sqrt{\frac{4}{3} d_p \cdot \frac{\rho_p - \rho_w}{\rho_w} \cdot g \cdot \frac{1}{c_w(\text{Re}_p)}} \text{ for } 1 < \text{Re}_p < 10^3$$

$$c_w(\text{Re}_p) = \frac{24}{\text{Re}_p} + \frac{4}{\sqrt{\text{Re}_p}} + 0.34$$

$$v_s = \frac{g}{18} \cdot \frac{\rho_p - \rho_w}{\rho_w} \cdot \frac{d_p^2}{\nu_w} \text{ for } \text{Re}_p \leq 1$$

$v_s$  = sedimentation velocity of a single particle [m/s]

$d_p$  = particle diameter [m]

$\rho_p$  = density of particle [ $\text{kg/m}^3$ ]

$\rho_w$  = density of the fluid [ $\text{kg/m}^3$ ]

$g$  = gravitational constant 9.81 [ $\text{m/s}^2$ ]

$\nu_w$  = kinematic viscosity water [ $\text{m}^2/\text{s}$ ]

$c_w(\text{Re}_p)$  = coefficient of resistivity [-]

$\text{Re}_p$  = particle Reynolds number [-]

## REFERENCES

- Bassin, J.P., Winkler, M.-K.H., Kleerebezem, R., van Loosdrecht, M.C.M., 2011. Relevance of Selective Sludge Removal in Segregated Aerobic Granular Sludge Reactors to Control PAO-GAO Competition at Different Temperatures. Submitted to Biotechnology and Bioengineering.
- Batstone, D.J., Keller, J., 2001. Variation of bulk properties of anaerobic granules with wastewater type. *Water Research* 35, 1723–1729.
- Beun, J.J., Hendriks, A., Van Loosdrecht, M.C.M., Morgenroth, E., Wilderer, P.A., Heijnen, J.J., 1999. Aerobic granulation in a sequencing batch reactor. *Water Research* 33, 2283–2290.
- Beun, J.J., Van Loosdrecht, M.C.M., Heijnen, J.J., 2000. Aerobic granulation. *Water Science and Technology* 41, 41–48.
- Brdjanovic, D., van Loosdrecht, M.C.M., Hooijmans, C.M., Alaerts, G.J., Heijnen, J.J., 1997. Temperature effects on physiology of biological phosphorus removal. *Journal of Environmental Engineering (New York)* 123, 144–153.

- Bubeck, R.C., Diment, W.H., Deck, B.L., Baldwin, A.L., Lipton, S.D., 1971. Runoff of deicing salt: effect on Irondequoit Bay, Rochester, New York. *Science* 172, 1128–1131.
- De Bruin, L.M.M., De Kreuk, M.K., van der Roest, H.F.R., Van Loosdrecht, M.C.M., Uijterlinde, C., 2004. Aerobic granular sludge technology, alternative for activated sludge technology? *Water Science and Technology* 49, 1–9.
- de Kreuk, M.K., Pronk, M., van Loosdrecht, M.C.M., 2005. Formation of aerobic granules and conversion processes in an aerobic granular sludge reactor at moderate and low temperatures. *Water Research* 39, 4476–4484.
- De Los Reyes Ill, F.L., Raskin, L., 2002. Role of filamentous microorganisms in activated sludge foaming: Relationship of mycolata levels to foaming initiation and stability. *Water Research* 36, 445–459.
- DiFelice, R., Nicoletta, C., Rovatti, M., 1997. Mixing and segregation in water fluidised-bed bioreactors. *Water Research* 31, 2392–2396.
- Etterer, T., Wilderer, P.A., 2001. Generation and properties of aerobic granular sludge. *Water Science and Technology* 43, 19–26.
- Figuerola, M., Mosquera-Corral, A., Campos, J.L., Méndez, R., 2008. Treatment of Saline Wastewater in SBR Aerobic Granular Reactors, pp. 479–485.
- Giancoli, D.C., 1995. *Physics*. Prentice Hall, New Jersey.
- Grant, S., Lin, K.C., 1995. Effects of temperature and organic loading on the performance of upflow anaerobic sludge blanket reactors. *Canadian Journal of Civil Engineering* 22, 143–149.
- Judd, J.H., 1970. Lake stratification caused by runoff from street deicing. *Water Research* 4, 521–532.
- Kettunen, R.H., Rintala, J.A., 1997. The effect of low temperature (5–29 °C) and adaptation on the methanogenic activity of biomass. *Applied Microbiology and Biotechnology* 48, 570–576.
- Lettinga, G., Hulshoff Pol, L.W., 1991. USAB-process design for various types of wastewaters. *Water Science and Technology* 24, 87–107.
- Lettinga, G., Rebac, S., Zeeman, G., 2001. Challenge of psychrophilic anaerobic wastewater treatment. *Trends in Biotechnology* 19, 363–370.
- Lew, B., Belavski, M., Admon, S., Tarre, S., Green, M., 2003. Temperature effect on UASB reactor operation for domestic wastewater treatment in temperate climate regions. *Water Science and Technology* 48, 25–30.
- Lin, Y., de Kreuk, M., van Loosdrecht, M.C.M., Adin, A., 2010. Characterization of alginate-like exopolysaccharides isolated from aerobic granular sludge in pilot-plant. *Water Research* 44, 3355–3364.
- Liu, Y., Wang, Zhi-Wu, Liu, Y., Qin, L., Tay, Joo-Hwa, 2008. A generalized model for settling velocity of aerobic granular sludge. *Biotechnology Progress* 21, 621–626.
- Moe, S.T., Elgsaeter, A., Skjåk-Bræk, G., Smidsrød, O., 1993. A new approach for estimating the crosslink density of covalently crosslinked ionic polysaccharide gels. *Carbohydrate Polymers* 20, 263–268.
- Morgenroth, E., Sherden, T., Van Loosdrecht, M.C.M., Heijnen, J.J., Wilderer, P.A., 1997. Aerobic granular sludge in a sequencing batch reactor. *Water Research* 31, 3191–3194.
- Nor Anuar, A., Ujang, Z., van Loosdrecht, M.C.M., de Kreuk, M.K., 2007. Settling behaviour of aerobic granular sludge. *Water Science and Technology* 56, 55–63.
- Podolsky, R.D., 1994. Temperature and water viscosity: Physiological versus mechanical effects on suspension feeding. *Science* 265, 100–103.
- Rinzema, A., Alphenaar, A., Lettinga, G., 1993. Anaerobic digestion of long-chain fatty acids in UASB and expanded granular sludge bed reactors. *Process Biochemistry* 28, 527–537.
- Ro, K.S., Neethling, J.B., 1994. Biological Fluidized-beds containing widely different bioparticles. *Journal of Environmental Engineering-Asce* 120, 1416–1426.
- Thomas, N., Stevenson, T.N., 1973. An internal wave in a viscous ocean stratified by both salt and heat. *Journal of Fluid Mechanics* 61, 301–304.
- Tierney, S., Sletmoen, M., Skjåk-Bræk, G., Stokke, B.T., 2010. Interferometric characterization of swelling of covalently crosslinked alginate gel and changes associated with polymer impregnation. *Carbohydrate Polymers* 80, 828–832.
- Uygur, A., Kargi, F., 2004. Salt inhibition on biological nutrient removal from saline wastewater in a sequencing batch reactor. *Enzyme and Microbial Technology* 34, 313–318.
- Winkler, M.K.H., Bassin, J.P., Kleerebezem, R., de Bruin, L.M.M., van den Brand, T.P.H., Van Loosdrecht, M.C.M., 2011a. Selective sludge removal in a segregated aerobic granular biomass system as a strategy to control PAO-GAO competition at high temperatures. *Water Research* 45, 3291–3299.
- Winkler, M.K.H., Kleerebezem, R., Kuenen, J.G., Yang, J., Van Loosdrecht, M.C.M., 2011b. Segregation of biomass in cyclic anaerobic/aerobic granular sludge allows the enrichment of anaerobic ammonium oxidizing bacteria at low temperatures. *Environmental Science and Technology* 45, 7330–7337.
- Xiao, F., Yang, S.F., Li, X.Y., 2008. Physical and hydrodynamic properties of aerobic granules produced in sequencing batch reactors. *Separation and Purification Technology* 63, 634–641.