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Seasonal variability of biomass density and activated sludge settleability in full-scale wastewater treatment systems

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ABSTRACT

Biosolids sedimentation is a critical component of the activated sludge wastewater treatment process. Seasonal variability of biomass settleability has been previously reported and linked to variable filament content in some studies, but others have reported seasonal variations without changes in filament content. Biomass density (mass per microbial floc volume, not including pore spaces) has recently been shown to vary substantially and to affect settleability in full-scale systems, but its potential role in seasonal variations has not been previously evaluated. Four full-scale activated sludge systems were monitored for density, filament content, and settleability for a year. Biomass density values were significantly higher in warm weather than in cold weather in all plants. Settleability was significantly worse in cold weather in three of the four systems, and the inverse of the buoyant density was correlated with settleability in these three systems. Filament content, on the other hand, exhibited seasonal variability and was correlated with settleability in only one of the four plants. Non-volatile suspended solids content was correlated with buoyant density and exhibited seasonal variability in all four systems. Biomass phosphorus content measurements suggested that seasonally variable enhanced biological phosphorus removal activity affected density in one of the systems. These results suggest that variable density plays a role in seasonally variable settleability in some full-scale wastewater treatment systems, they help to clarify previously unexplained reports of seasonally variable settleability that were independent of changes in filament content, and they provide the basis for development of strategies for improved performance.

1. Introduction

Activated sludge is the most common biological technology used for domestic wastewater treatment in industrialized countries around the world. This process consists of biological reactors, where microorganisms are grown while degrading particulate and dissolved wastes. Separation of the microbial solids produced in these reactors is achieved by sedimentation, with a portion of the settled solids wasted from the system and a portion recycled back to the bioreactors to maintain high solids concentrations and rates of reaction. With wastewater treatment as the largest biotechnology in the world [1], activated sludge sedimentation is likely one of the largest scale solids separation process in existence. However, poorly settling activated sludge biomass remains a common problem, resulting in poor quality effluent, decreased system capacity, increased capital and operating costs, and other problems [2,3]. Excessive growth of filamentous bacteria has been studied extensively and is the most well known cause of poorly settling solids, while other physical parameters, such as floc size and composition, have also been shown to affect settleability [2–7]. Excessive filamentous growth has been attributed to a variety of conditions, including sustained low substrate (low food to microorganisms ratios) and low dissolved oxygen. Numerous treatment designs have been developed and employed to control filamentous bulking [2,3,8]. While previous research has greatly advanced our understanding of biosolids sedimentation and strategies for control of filamentous growth in particular, it has not produced consistent strategies to prevent bulking sludge [3,8].

Seasonal variations in activated sludge biomass settleability have been reported in several studies. The sludge volume index (SVI) is the most commonly reported measurement of activated sludge settleability, with higher values indicative of more poorly settling biomass. Kruit et al. [8] reported 4 full-scale domestic wastewater treatment systems performing enhanced biological phosphorus removal (EBPR) and nitrification/denitrification had lowest SVI values and lowest *Microthrix parvicella* contents in the fall. However, other studies have reported seasonal variability in settleability without concurrent changes in filament content: for example, Andreasen and Sigvardsen [9] surveyed 100 Danish wastewater plants (domestic and industrial) and found SVI

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values were on average higher in winter than summer in both EBPR and non-EBPR systems, but filament content showed little variation. Similarly, Graveleau et al. [10] reported seasonal changes in settleability without observed changed in filament content in a review of settling data from 964 wastewater plants (with unspecified wastewater sources) in France, with average SVI values of 171 and 145 mL/g in the winter and summer, respectively. Wilen et al. [7] reported no seasonal variations in settleability in a full-scale municipal activated sludge wastewater plant with an anoxic reactor for predenitrification, as well as phosphorus removal through precipitation in the primary clarifier. These results demonstrate that seasonal variations in settleability are common in full-scale plants, but not universal, that SVI values are typically higher in the winter than in summer, and that these changes are only sometimes attributable to changes in filament content.

Recent research has demonstrated that variable biomass density (defined as the mass per biomass volume, not including pore spaces) can significantly affect activated biomass settling [11,12]. This is consistent with fundamental physical principles, since the driving force for sedimentation (the gravitational force exerted by the biomass weight) is a linear function of biomass density. Because full-scale activated sludge biomass density (ranging from approximately 1.02 to 1.055 g/mL [11]) is only slightly greater than that of water, small changes in biomass density may have large effects on the net buoyant force, with corresponding effects on settling rates.

Andreasen and Sigvardsen [9] reported that plants performing EBPR routinely experienced improved settling in the absence of changes in filament content and hypothesized that increased biomass density from increased polyphosphate storage was the cause, although density was not measured. It was later demonstrated that increasing polyphosphate content does tend to increase density and improve settleability in laboratory scale [13] and full-scale EBPR systems [11,14], and it was demonstrated that density was variable and correlated with settleability in non-EBPR full-scale systems as well [11]. This work suggested that improvements to settling observed with installation of anaerobic reactors (as well as anoxic reactors where they induce EBPR) may be at least partially due to increased density from increased polyphosphate content via EBPR. Solids residence time (SRT) and non-volatile suspended solids (NVSS) content were also found to be correlated with density in these systems.

Given the extensive use of activated sludge processes and the importance of managing and improving sedimentation in such systems, there is a great need to gain a better understanding of the causes of seasonal variations in activated sludge settling, including the possible role of biomass density. It was hypothesized that (1) seasonal variations in density occur in some full-scale treatment systems, and (2) that these variations are sufficient to contribute to seasonal variations in settleability. The objectives of this research were to determine whether seasonal variations in biomass density, settleability, and filament content occur in full-scale wastewater treatment plants, and to determine factors contributing to observed variations in density. Four full-scale wastewater treatment systems with different configurations were monitored for over a year to address these objectives.

2. Materials and methods

2.1. Wastewater treatment plants

Four full-scale wastewater treatment plants (Table 1) located in central New Mexico, U.S., were monitored for over one year. Two of them were completely aerobic activated sludge plants without anoxic or anaerobic reactors treating domestic wastewater with primarily residential sources (referred to hereafter as conventional activated sludge plants CAS1 and CAS2). The other two were predenitrification (Modified Ludzack Ettinger) systems, which include denitrifying (nitrate reducing) anoxic reactors followed by nitrifying (ammonia oxidizing) aerobic reactors, with an internal recycle linking the two (plants AX1 and AX2). Plants AX1 and AX2 are domestic wastewater plants treating primarily residential sources.

Plant CAS1 was an extended aeration process, as indicated by its relatively long solids retention time (Table 1). Plant AX1 was the largest system included in the study, and it was the only one with primary clarification. Plants CAS2 and AX2 were typically operated at greater than their design capacities and had SRTs of 8 days or less. Samples were collected once per month or more frequently from February 2008 to February 2009 from near the outlet of the most downstream bioreactor for all plants.

2.2. Analytical methods

Total suspended solids (TSS) were by Standard Method (SM) 2540 D, volatile suspended solids (VSS) was by SM 2540 E, total phosphorus was by SM 4500-P B.5, and soluble phosphorus was by SM 4500-P C [15]. Settleability was measured at 22 °C by the dilute sludge volume index (DSVI) as described in Jenkins et al. [2]. Total carbohydrate was by the phenol-based colorimetric method [16]. NVSS was calculated as the difference between TSS and VSS. Non-soluble phosphorus (Pns) was calculated as the difference between total and soluble phosphorus. Samples were kept on ice during transportation. All analyses were performed in triplicate and sample variances were included in the results evaluations.

Biomass density was analyzed as previously described [14]. Briefly, a well mixed activated sludge sample (1 mL) at 22 °C was added to each of a series of test tubes containing solutions with a range of densities bracketing the expected biomass density. These solutions were prepared using mixtures of secondary effluent and Percoll (Amersham Life Sciences, Inc., Arlington Heights, IL), a high density (1.13 g/mL), low osmotic pressure suspension of silica particles. Each mixture was vortexed briefly and centrifuged for 5 min at 1000 rpm. The fraction of biomass located in the top half of the solution was visually determined, and the median biomass density was interpolated based on measurements in the two solutions bracketing the median density. Although this method included visual assessment of biomass separation, it has been demonstrated to be highly reproducible in several previous studies [11–14,17,18]. Because density solution increments were narrow (0.003 g/mL) relative to biomass densities (approximately 1.03 g/mL), any errors in visual readings had only small effects on the calculated average density. Results were highly precise: average standard deviations of triplicate measurements were less than 1% of average buoyant den-

Table 1
Full-scale wastewater plants included in the study.

Plant	Configuration	Design flow (1000 m^3/day)	Average daily flow (1000 m^3/day)	SRT (days)
CAS1	Aerobic (extended aeration)	4.5	3.0	18–30
CAS2	Aerobic	3.8	4.4	6–8
AX1	Anoxic, aerobic	290	230	6–9
AX2	Anoxic, aerobic	13	15	5–8

sity (the difference between biomass density and water density) values.

Filamentous bacteria were quantified by microscopy with digital imaging. Digital images of the sludge samples were taken as soon as possible following physical/chemical analyses discussed above but not longer than 24 h after sample collection, during which time they were stored at 4 °C. Images were taken using an Olympus BX51 phase contrast microscope coupled with an Olympus DP71 color digital camera. Approximately 36 100× magnification phase contrast images were taken of each sample using a wet mount on a hemacytometer slide (Model 3120A Double Neubauer, Hausser Scientific, Horsham, PA). Filaments extending from flocs were traced using a digitizing pad (Intuos 3, Wacom, Ltd., Saitama, Japan), and extended filament lengths were determined by image analysis software (Image J 1.38×, National Institutes of Health, Bethesda, MD). The filament length per dry biomass (m/mg) was calculated from the total measured filament length per hemacytometer volume (m/mL), based on tracing of the digital images, and the dry biomass in this volume, based on the sample biosolids concentration (TSS in mg/L).

2.3. Statistical analyses

Statistical analyses included univariate linear regressions for comparing biomass density with DSVI, NVSS and Pns. Two-sample unequal variance *t*-tests used to compare warm and cold weather variations and linear regression analyses were performed with Excel (Microsoft Corporation, Redmond, WA). The confidence values of linear correlations were calculated using the F statistic. Correlations were considered statistically significant at >95% confidence.

3. Results and discussion

3.1. Seasonal variations

Mixed liquor biomass density values measured over the course of one year varied both temporally and between full-scale wastewater facilities. Warm weather values were significantly higher than cold weather values ($p < 0.05$) in all four activated sludge plants (Fig. 1). Warm weather here refers to June through October, when mixed liquor temperatures in Plant AX1 were greater than 23.5 °C and averaged 26.1 °C, and cold weather refers to December through April, when mixed liquor temperatures in Plant AX1 were less than 21 °C and averaged 20.2 °C, as shown in Fig. 2. Further, Fig. 2 includes the average daily ambient air temperatures for Albuquerque, NM during the course of the study to illustrate an approximate two week lag between sustained changes in ambient temperature and that of the mixed liquor temperature. As noted, all density and settleability measurements were made at 22 °C.

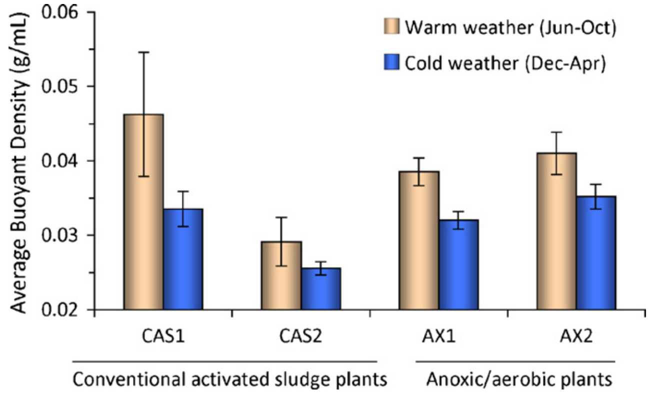

Fig. 1. Warm and cold weather average biomass density values of four full-scale activated sludge plants. Biomass density values were significantly higher in warm months than cold months in all plants ($p < 0.05$).

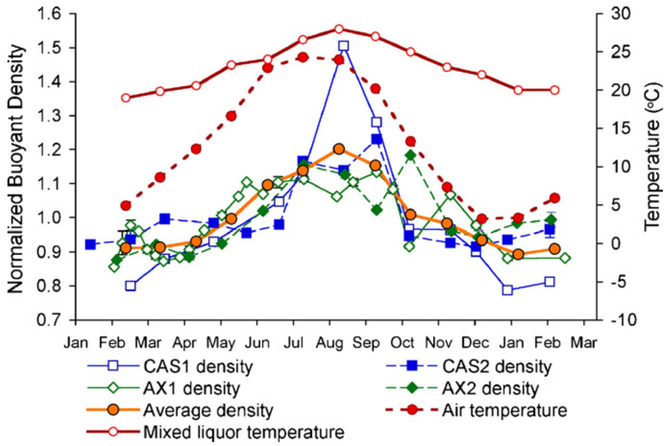

Fig. 2. Temporal variations of biomass density in four full-scale activated sludge systems, and the average of all four plants, showing a consistent cyclic trend of higher density values during warm months and lower values during cold months. Density values have been normalized to yearly average values for each plant to aid in data visualization. Average monthly air temperatures for Albuquerque, NM and mixed liquor temperatures in Plant AX1 are also shown. Error bars (some of which are smaller than the symbols) span two standard deviations calculated from triplicate measurements.

This seasonal variability of biomass density was evident as a cyclic temporal trend in all four activated sludge plants that generally followed mixed liquor temperatures, with higher values during warm temperatures than in cold temperatures (Fig. 2). Maximum biomass density values occurred in the warmest months (July, August, or September) and minimum values occurred in the coldest months (January or February) in all four activated sludge plants. The ratio of maximum (warm weather) to minimum (cold weather) biomass buoyant density values ranged from 126% to 191% across the four plants and the average ratio was 147%. The magnitude of these ratios indicates a strong variability in the driving force for settling. Biomass density was correlated with average ambient air temperatures for the two weeks preceding each sample event for each of the four activated sludge plants (Fig. 3). Ambient air temperatures are shown in Fig. 3 because mixed liquor temperatures were not readily available for plants AX2, CAS1, and CAS2 for the study period. The average ambient temperatures for the two weeks

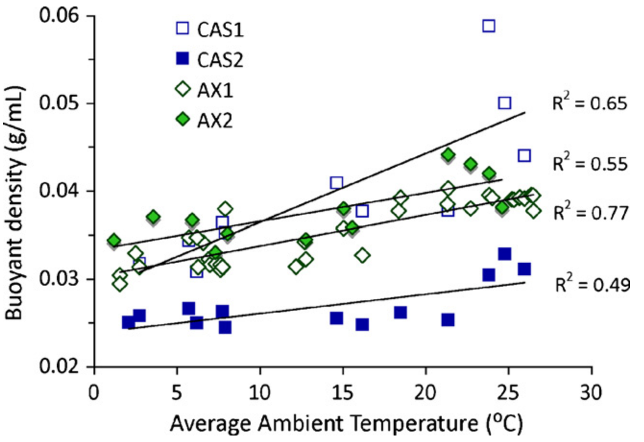

Fig. 3. The relationship between ambient air temperatures (average values in 2 weeks prior to each sampling event) and buoyant density in four full-scale plants over one year. All linear regression correlations were statistically significant ($p < 0.05$).

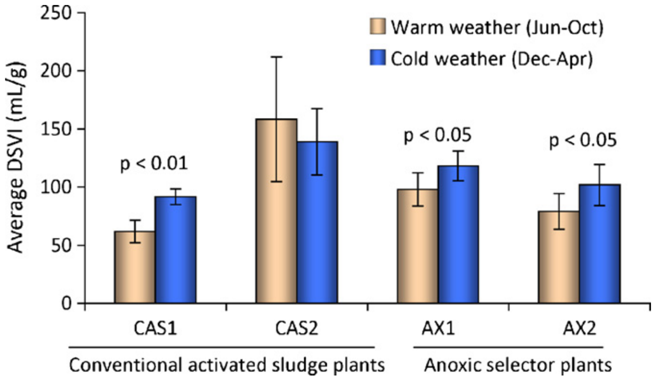

Fig. 4. Warm and cold weather average biomass settleability (as DSVI) of samples from four full-scale activated sludge plants. Settleability was significantly worse in the winter than in the summer in three of the four plants, as shown.

prior to each sample event were well correlated with mixed liquor temperatures in Plant AX1 ($R^2 = 0.72$), and so they were used as a surrogate for mixed liquor temperatures. The four wastewater systems included in this study are located in the same geographic area and they experience similar daily ambient temperatures. Biomass density was also positively correlated with mixed liquor temperature in Plant AX1, the plant for which wastewater temperatures were readily available ($R^2 = 0.66$, data not shown).

Statistically significant seasonal variability in settleability was detected in three of the four activated sludge plants: DSVI values were significantly lower in warm weather (coinciding with higher biomass density values) than in cold weather (coinciding with lower biomass density values) for Plants CAS1, AX1, and AX2 ($p < 0.01$), but this was not true for Plant CAS2 (Fig. 4). Plant CAS2 experienced a grease related upset during the summer of 2008 that apparently negatively affected settling performance (increasing DSVI values), during which time filament content was not substantially changed.

Significant seasonal variation in filamentous bacteria content was only observed in Plant AX1 ($p < 0.05$), where cold weather filament length per mass values were greater than warm weather values. Significant seasonal variation in filament content was not observed in the other activated sludge plants, although Plant AX2 exhibited a nearly significant seasonal change in filament content ($p = 0.07$), with higher filament content in the winter. Across all samples, filament contents ranged from 6.5 to 160 m/mg, which corresponded to "few" to "very common" filaments, according to the Filament Index scale proposed by Jenkins et al. [2]. Filament type was not determined, and so these measurements do not reflect the possibility that changes in filament type affected settleability.

3.2. Filament and density effects on DSVI

The effects of filament content and buoyant density on settleability were evaluated separately by linear regression modeling. There was a statistically significant positive linear correlation between filament content (length/TSS in m/mg) and DSVI ($R^2 > 0.45$, $p < 0.05$, not shown) in only one of the four plants monitored for a full year (Plant AX1). As noted, filament length measurements did not consider possible changes in filament type, and so this does not rule out a possible role of filaments in the observed changes in settleability in the other plants. The inverse of the buoyant density, on the other hand, was significantly correlated with DSVI values in three of the four plants (AX1, AX2, and CAS1, $p < 0.05$). (The inverse of the buoyant density values, rather than buoyant density itself, was evaluated to reflect the phenomenon that as biomass buoyant density approaches zero, settling velocities go to zero and DSVI values go to infinity.) The Plant CAS1 samples exhibited the strongest correlation between 1/buoyant density and DSVI (Fig. 5). This plant also had the poorest correlation between filament content and DSVI ($R^2 < 0.01$) of all plants, possibly because it had the lowest average filament content (14 m/mg compared to an average value of 40 m/mg in all other systems) and the lowest variability in filament content across all samples (average standard deviation equal to 5 m/mg, as compared to an average standard deviation of 38 m/mg in all other systems).

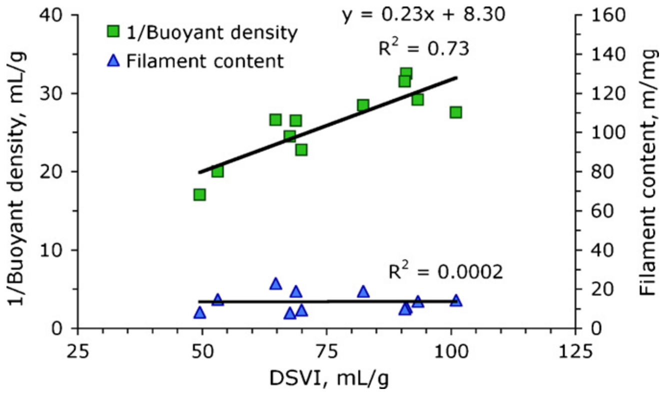

Fig. 5. The relationships between DSVI and 1/buoyant density, and DSVI and filament content in Plant CAS1. The scale of the right *y*-axis spans the range of filament contents measured across all plants, illustrating the relatively low filament content values in Plant CAS1 samples.

3.3. Parameters affecting biomass density

Several components of the activated sludge biomass known to affect density were measured to help evaluate reasons why density was found to vary seasonally, including carbohydrate, NVSS, and Pns (which includes stored polyphosphate). Total carbohydrate was evaluated because it includes stored glycogen, which is stored by organisms known to occur in wastewater treatment systems such as polyphosphate accumulating organisms and glycogen accumulating organisms, and is thought to be of high density relative to bacterial cell mass [19]. Total carbohydrate also includes extracellular polysaccharides, whose effects on biomass density have not been determined. Total carbohydrate was not correlated with biomass density for any of the plants included in this study, nor were any significant seasonal changes in carbohydrate content detected, suggesting that carbohydrate content did not play a detectable role in the observed variability of biomass density.

NVSS was evaluated for its potential contribution to biomass density in part because it includes polyphosphate, which is known to be of higher density than typical bacterial biomass in general [19] and has been shown to increase activated sludge density [11,13]. In addition, increasing NVSS content has been shown to increase biomass density independently of stored polyphosphate, particularly in non-EBPR systems [11], possibly because of the accumulation of metals or other non-volatile, high density inert matter in the biomass. NVSS content was significantly correlated with biomass density both in the conventional activated sludge plants and in the anoxic/aerobic plants (Fig. 6a and b, respectively), consistent with previous results [11]. Furthermore, the slopes of the best fit lines were remarkably similar in each of the four data sets, ranging from 0.045 to 0.049 (g/mL)/(mg/mg).

The reason for the observed variability of NVSS/VSS in the conventional activated sludge plants is not certain. Because these plants did not include anaerobic/aerobic cycling, they are not expected to accumulate excess polyphosphate via EBPR. Consistent with this expectation, the average Pns/TSS values in CAS1 and CAS2 samples were 1.3% and 1.5%, respectively, which were sim-

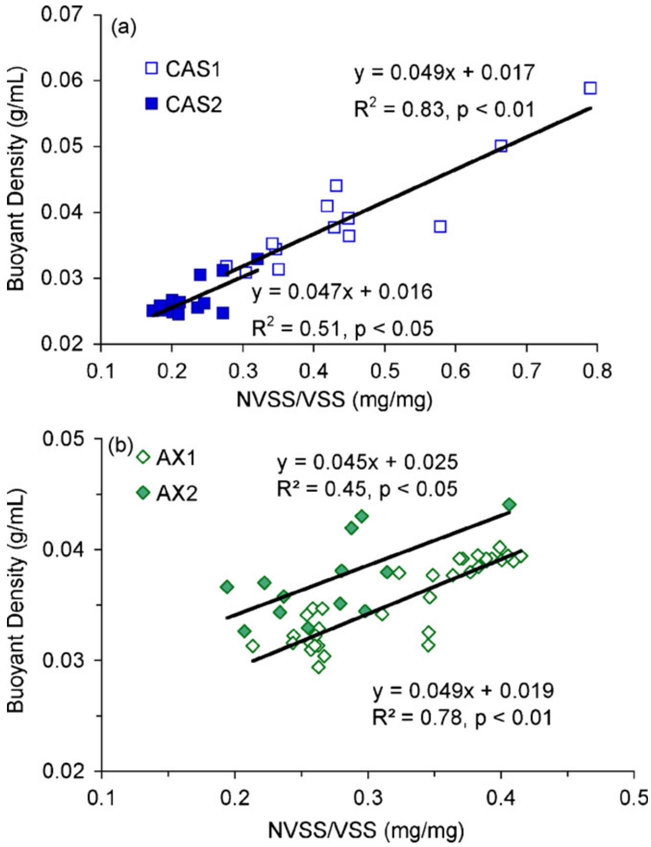

Fig. 6. The relationship between buoyant density and non-volatile solids content as NVSS/VSS for (a) conventional activated sludge plants and (b) activated sludge plants with anoxic reactors. All correlations were significant, as shown.

ilar to typical values suggested for non-EBPR systems (1.5% to 2%, [20]), and there was little temporal variation in these values. The higher Plant CAS1 NVSS/VSS values relative to those from Plant CAS2 (Fig. 6a), may be associated with the relatively high SRT in CAS1, which was an extended aeration system (Table 1). It has previously been reported that increasing SRT tends to increased NVSS in conventional activated sludge systems [11]. Relatively high NVSS contents in extended aerobic systems has been suggested to be due to inert biomass accumulation from high rates of endogenous decay associated with long SRTs [21]. Because warmer temperatures are associated with more rapid biological reactions (including endogenous decay), this explanation is consistent with the observation that NVSS values were higher during warm weather than in cold weather for all four plants (Fig. 7). These higher NVSS values during warm weather are also consistent with the trends in density shown in Fig. 1. It is also noteworthy that neither of the conventional activated sludge systems had primary clarification, and so it is also possible that the relatively high SRTs in Plant CAS1 led to accumulation of more influent NVSS material in the biomass relative to CAS2. However, this latter theory does not explain the seasonal component of NVSS variability (Fig. 7).

As noted, NVSS/VSS was also correlated with density in each of the two anoxic/aerobic plants ($p < 0.05$ for each plant, Fig. 6b). The possible contribution of varying polyphosphate to these trends is evaluated below. Unlike the CAS plant data (Fig. 6a), the two anoxic/aerobic plant data sets were offset from each other. While the reasons for this finding are not known, it suggests that components of the biomass other than NVSS contributed to density and were somewhat consistent across plant samples. For example, glycogen and polyhydroxyalkanoates (PHAs) are known to be of higher density than typical bacterial biomass [19]. Consistent with this hypothesis, average carbohydrate concentrations in Plant AX2 (16 ± 2% of TSS) were higher than those in Plant AX1 (13 ± 2% of TSS). These values could reflect larger quantities of stored glycogen in the Plant AX2 biomass than in the AX1 biomass, yielding higher Plant AX2 buoyant density relative to Plant AX1 for a given NVSS/VSS value.

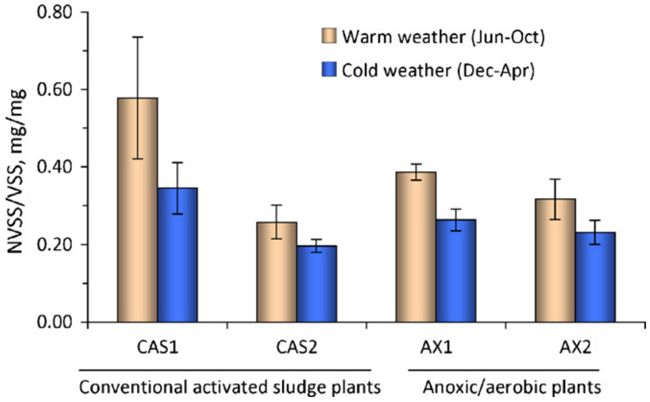

Fig. 7. Warm and cold weather average biomass non-volatile suspended solids contents (NVSS/VSS) of samples from four full-scale activated sludge plants. NVSS contents were significantly higher in warm weather than in cold weather in all plants ($p < 0.05$).

NVSS measurements include stored polyphosphate, which is also known to increase biomass density [11,13,22]. The potential contributions of polyphosphate (as Pns) to NVSS and buoyant density were therefore evaluated. As noted, in the conventional activated sludge plants Pns content varied little and was relatively low (as expected in these non-EBPR systems). In contrast, Plant AX1 and AX2 samples had relatively high phosphorus contents (average Pns/TSS values were 2.5% in both systems, and Pns/VSS values were 3.3% and 3.2%, respectively) compared to the conventional plants and with other non-EBPR systems (which have Pns/TSS values ranging from 1.5% to 2%, [20]). This suggests that these plants had at least intermittent EBPR activity, which can occur when nitrate concentrations are low in anoxic reactors, producing the anaerobic conditions (low nitrate and oxygen concentrations) conducive to EBPR. Such incidental EBPR activity may not be unusual, and in fact reflects the unusual history of EBPR, with its detection in full-scale systems long before they were designed for this purpose [23].

Non-soluble phosphorus content was positively correlated with NVSS and the buoyant density for Plant AX1 (Fig. 8). A positive correlation between these measurements was also observed for Plant AX2, but it was not statistically significant. In addition, the non-

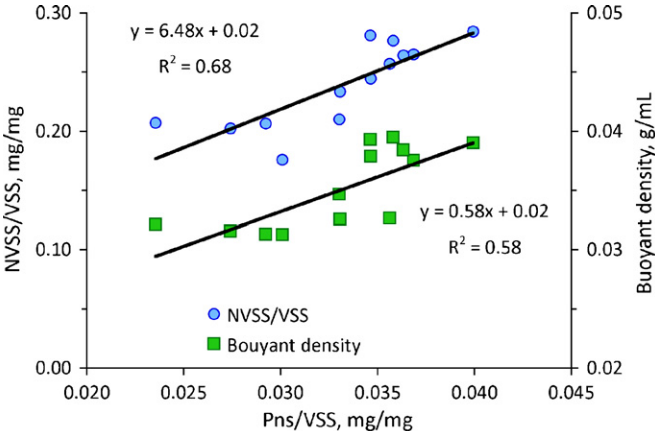

Fig. 8. The relationship between biomass phosphorus content (Pns/VSS) and the NVSS content and buoyant density in Plant AX1 samples.

soluble phosphorus content was significantly higher in the warm months than in the winter for Plant AX1, but not in Plant AX2 (data not shown). These results suggested that seasonal variations in phosphorus content may have contributed to the observed seasonal variations in biomass density in Plant AX1, although this plant was not designed for EBPR. The reason for its apparently increased EBPR activity in the summer (as evidenced by elevated Pns storage) is not known, but it may be linked to higher rates of denitrification in warm weather, resulting in lower nitrate concentrations in un-aerated reactors and more anaerobic conditions. Alternatively, warmer weather could have increased fermentation rates and production of the volatile fatty acids required for EBPR. Regardless, it appears that varying EBPR activity played an important role in the seasonal buoyant density variability of Plant AX1, but not in the other three plants.

Taken together, it appears that the seasonal density variations were linked to changes in NVSS content in all four plants, since NVSS was consistently correlated with density in all systems, consistent with previous research on both non-EBPR and EBPR systems. In the conventional activated sludge plants, variable NVSS may have been due to variations in endogenous decay rates, such as increased rates of decay during warm weather. In one of the anoxic/aerobic plants (AX1), it appears that polyphosphate storage played an additional (or alternative) role, although neither of these plants was designed for EBPR, with increased polyphosphate storage linked to increased density during warmer weather.

4. Conclusions

This research presented evidence that variable biomass density is a significant contributor to seasonal variations in settleability in some full-scale activated sludge systems, providing a potential explanation for previous reports of seasonally variable settleability without corresponding changes in filament content. These results do not contradict or undermine existing knowledge of filament effects on settleability, but simply add to the understanding of factors affecting seasonal variations. Activated sludge biomass density was significantly higher in warm weather than in cold weather in four of four full-scale wastewater treatment systems. Settleability was significantly worse in cold weather than in warm weather in three of these systems, consistent with previous studies finding seasonal variability in many, but not all full-scale systems. Buoyant density values (and their inverses) were correlated with settleability in all three of these systems. The fourth system suffered a grease related upset concurrent with worsened settleability during summer months. Filament content, on the other hand, exhibited seasonal variability and was correlated with settleability in only one of the four plants.

The reasons for the observed variability in biomass density appeared to be related to changes in the NVSS content of the biomass. NVSS content was positively correlated with biomass density in all four systems (with consistent slopes), and it was significantly higher in warm whether than in cold weather in all systems. It is hypothesized that higher NVSS values during warm weather may have been related to greater rates of endogenous decay, but further research is needed on this point. Variable polyphosphate storage appeared to be a significant contributor to seasonal NVSS and density variability in one of the four plants, suggesting that variable EBPR activity played a role in the observed seasonal variability in this plant, although its anoxic/aerobic configuration was not designed for this function.

It appears that both filament content and biomass density play important roles in seasonal variations of activated sludge settleability. This work may provide an explanation for previously reported seasonal variations in biomass settleability without corresponding changes in filament content [9,10]. The demonstration of the role of density in seasonal settleability variations is important because it provides a better fundamental understanding of the activated sludge settling process, and it may lead to strategies for improved operation. For example, generally lower density values in colder weather may be remedied by taking measures to increase density, as by increasing Pns (through improved EBPR performance) and/or NVSS content of the biomass. Furthermore, monitoring of density, along with conventional measurements such as NVSS, Pns, and microscopic monitoring of filaments, may be useful in a more comprehensive diagnosis of seasonal changes in settleability. Further research is required to better understand the causes of seasonally variable density, such as confirmation of endogenous decay effects on NVSS and settleability, and development and verification of specific strategies to counter or capitalize on seasonal variations.

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